POPULATION DYNAMICS AND IMPACT OF HELMINTH PARASITES ON SOME FISHES OF RIVER GOMTI IN LUCKNOW, UTTAR PRADESH

THESIS

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BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
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IN

APPLIED ANIMAL SCIENCES

Submitted By

ANITA SINGH

M.Sc.

Under the supervision of

Dr. SUMAN MISHRA

Assistant Professor

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY

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(A CENTRAL UNIVERSITY)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025 (U.P.), INDIA

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DEDICATION

First of all I will dedicate this study to our Almightly God, who gave me strength and knowledge for my everyday life.

To my beloved parents for their understanding and for their overwhelming, support, morally and financially.

To my supervisor Dr. Suman Mishra for continuously inspiring me.

To my loving husband who gave me help and companionship during the compilation of this thesis.

And

To my dear son, whose smiling face gave me renewed vigour to give my best.

DECLARATION

I hereby declare that the thesis entitled "**POPULATION DYNAMICS AND IMPACT OF HELMINTH PARASITES ON SOME FISHES OF RIVER GOMTI IN LUCKNOW, UTTAR PRADESH**" submitted to the Department of Applied Animal Sciences, School of Biosciences & Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow by me for the award of degree of the Doctor of Philosophy in Applied Animal Sciences is an outcome of my original work and the outcome of my own efforts under the supervision of Dr. Suman Mishra, Assistant Professor, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. It has not been submitted in part or full to this or any other University for the award of any other diploma and degree.

ANITA SINGH

CERTIFICATE

This is to certify that the thesis titled "**POPULATION DYNAMICS AND IMPACT OF HELMINTH PARASITES ON SOME FISHES OF RIVER GOMTI IN LUCKNOW, UTTAR PRADESH**" submitted by **ANITA SINGH** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulations -1999 as amended in 2008/2010/2013 and it is fit for submission and is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the university.

Date: Supervisor

Dr. SUMAN MISHRA

Head of the Department

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ANITA SINGH

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS & SYMBOLS

%	Percentage
<	Less than
>	Greater than
±	Add or subtract
µ	Micron
cm	Centimeter
dH_2O	Distilled water
dL	Deciliter
fl	Femtoliter
g/dl	Gram per deciliter
gm	Gram
H_2SO_4	Sulphuric acid
Hb	Haemoglobin
HCL	Hydrochloric acid
Hct	Haematocrit
MCH	Mean cell haemoglobin
MCHC	Mean Cell haemoglobin concentration
MCV	Mean cell volume
Mg	Milligram
mL	Milliliter
N	Normality
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
ºC	Degree Centigrade
PCV	Packed cell volume
Pg	Pico gram
RBC	Red blood cell
SEM	Standard error mean
SL	Standard Length
Temp	Temperature
TL	Total Length
W	Weight
WBC	White blood cell
$\bar{X}$	Mean
µm3	Cubic micron

Chapter 1

General Introduction

Introduction

Fishes represent a major group of organisms that are typically cold blooded and found abundantly in sea and freshwater. Fishing is the main source of employment and income for the majority of people. Fish are an important resource for humans worldwide, especially as food, being an excellent source of proteins. Commercial and subsistence fishers hunt fish in wild fisheries or farm them in ponds or in cages in the waterbodies (lakes, rivers, oceans etc.). Fish are also caught by recreational fishers, kept as pets, raised by fish keepers, and exhibited in public aquaria. Fish have played a role in culture through the ages, serving as deities, religious symbols, and as the subjects of art, books and movies.

People consume about 146.3 million tons of fish and nearly 20.9 million tons are used as animal feed that helps to produce other forms of proteins. Fish protein represents about 25% of the total animal protein consumed by the world's population. In addition to protein, fish also contains carbohydrates, vitamins, iron, calcium and other mineral salts.

Millions of human beings suffer due to hunger and malnutrition, and in order to meet the demand of food partly, fishes can be used as alternative source of food. The nutritive and medicinal value of fish has been recognized from times immemorial as fishes are an excellent source of protein and vitamins like A, D, E, K and B_{12} besides being rich in calcium, iron, phosphorous and iodine for human diet. Traditionally the fish is considered as the "protein for poor man's diet". It costs much less in comparison to its food value. It is an almost zero-carbohydrate food, good for diabetics and other such patients. Fish also contains poly unsaturated fatty acids which are known to provide protection against cardio vascular diseases. Fish flesh and fish oils are considered to be essential for the prevention of coronary heart disease.

Economic Importance of Fishes

The fishery industry plays an important role in the economy of India as, besides providing employment opportunities, it is a source of nutritional food and foreign exchange. Millions of people are employed in fishing industries and depend upon

fisheries for their livelihood in various ways. In addition to those who directly catch the fishes for marketing; there are equally large number of people engaged in subsidiary industries like preservation, canning, transport, refrigeration and in the manufacture of fish products and bye-products. There are a variety of bye-products of fishes which are of great economic value; such as fish oil which is edible and used for industrial purposes (body oil); fish liver oil which contains vitamin A and is of immense medicinal value; fish manure which is a very good concentrated organic manure for agricultural crop; and fish meal which contains appreciable quantities of nitrogen and phosphate and is used as manure or feed for pig, cattle and poultry. Other important commercial byproducts of commercial value include: Ising glass which is a good substitute for gelatin; fish glue, leather soap, fish flour, and fish sausages. Apart from these, fishes are used for ornamental purposes and forms a popular sports and game, several species of fishes (*Puntius sophorus*, *Rasborada nicotious*, *Channa oriantalis* and *Mystus vittatus*) are known larvicidal in nature and are used in biological control of malaria and filarial diseases.

Thus, fishes are of immense importance not only as food but for their multiple commercial benefits. However, the fishes suffer from a variety of diseases, both parasitic and non-parasitic diseases which pose a severe constraint to fishery industry/pisiculture. They cause a variety of problems. The diseased fishes are short of their life, weak and unable to lead a normal life. Moreover, sometimes, the diseases make them sterile. The tumors and sores from diseases also make the fish unfit for sale.

The various diseases of fishes may be grouped into three types.

1. Nutritional Diseases

Such diseases may fall under three main categories: a.) Those arising due to under nutrition. b.) Those arising due to dietary deficiency or imbalances in the major components of food. c.) Those arising from toxic effect of the diet.The commonly occurring nutritional diseases in fishes are pin heads (enlarged head and slender body), Lipoid hepatic degeneration disease (Yellow brown liver), Vitaminosis A (Vitamin A imbalance), Vitamin B6 deficiency (Pyridoxine), Pantothenic acid deficiency (Gill diseases) and Vitamin C deficiency (spinal deformities).

2. Diseases caused by parasite and pathogens

Several diseases in fishes are caused by bacteria (infectious dropsy, cotton mouth, tail rot, fin rot, columnaris and vibriosis); viruses (Koi Herpesvirus Disease, Viral Haemorrhagic Septicaemia Virus VHSV, Infectious haematopoietic necrosis IHN); fungi (dermatomycoses, gill rot and deep mycoses) and parasites. The parasitic diseases in fishes are caused by protozoans (ichthyophthiriusiasis. Costiasis and trichodinasis); flukes (Diplostomid metacercaraie, black spot disease, blood worm disease); cestodes (Bothriocephally disease, caryophyllosis, eubothriosis) and parasitic nematodes (Ascaridatoses, cucullanellosis, spiruratoses).

3. Diseases due to intrinsic causes

Among these distinctions can be made whether they are caused due to different environmental factor:

- Due to alteration in H^+ concentration (pH) of water.
- Due to asphyxia caused by depletion in DO content in water.
- Due to chill and cold following drop in temperature of water.
- Due to indigestion and constipation following intake of food available in water.

Parasitic Infection and Impact on Aquaculture

Fishes are the predators –prey pyramid within fresh water system and therefore tend to be infected by a considerable range of parasites which may occur in large members. Parasitic diseases of fishes are very common all over the world and are of particular importance (Robert and Janovy, 2000) as the parasitized fishes are usually of poor food value. Because of their deleterious effects, parasites often cause serious outbreaks of disease in cultured fish populations. Although compared with the viral and bacterial diseases of fish, tapeworm-induced mortality is relatively of minor importance, but there are some cestode species that can seriously affect cultured and wild fish populations (Hoole, 1994). The presence of dense populations of fish kept in particular environmental conditions may also favour certain parasite species, with the result that the parasite population increases to a very high level. The study of these

parasites and diseases caused by them is therefore an important aspect in aquaculture, but fish pathology and parasitology has generally been a neglected field in the Asia and far eastern countries (Gopalakrishnan, 1968). Unfortunately, very few works have been initiated on the effect of parasitism on the host animals particularly in freshwater fishes although in this region the fish culture has been making rapid progress and creating conditions favourable for fish disease. The study of parasitology is thus very important from the point of view of fishery management, fish yield and to check the spread of human and animal disease for which fish act as a carrier (Srivastava, 1975).

Helminths are an important group of parasites which occur in the adult stage usually in the vertebrates host, practically invading every organ system of the host; and in the larval stage in the invertebrate hosts. Once the helminth parasite reaches its residence in the defective host, its primary concern is to secure nourishment. To fulfill this objective, the helminth usually choose a position where digested or semi digested food is abundantly supplied. The parasites and its effects usually adversely affect the marketability of the commercially produced fish. Thus, parasitic infection in fish especially by helminths is a public health concern, especially in areas where raw or smoked fish is consumed (Hoffman & Bauer, 1972; Paperna, 1996). Some parasites are transmitted directly from fish to fish, such as some ectoparasitic protozoa and skin/gill flukes, however, they often involve a free-living phase found in the water or tank substrate. This is called a direct life-cycle. Other larger parasites often have complex life-cycles involving two or more hosts, including a fish. This is called an indirect life-cycle. It is important to know this information, as it will affect on the treatment methods and determine the success or failure when treating parasites/parasitic diseases.

Helminth parasites of fishes and their life cycles

Among the parasites that infect teleostean fishes, helminths represent the largest and important group. No other group of vertebrates has such a diversity of helminth species and some of the helminth groups like monogeneans are unique to fish. It is estimated that there are more than 30000 helminth species parasitizing marine and freshwater fish (Williams and Jones, 1994) and some of them are known to be the agents of serious fish diseases or may represent an important public health problem.

The word 'helminths' was first used by Aristotle (384–322 B.C.) for some of the worms found parasitic in animals (Hugot *et al*., 2001). Helminths, as parasites in general, do not represent a monophyletic assemblage since under that term members of phylogenetically not related phyla are included, i.e., Platyhelminthes ("flatworms") comprising cestodes, monogeneans and digeneans; Nematoda ("roundworms"), previously placed in the phylum Nemathelminthes (or Aschelminthes); and Acanthocephala ("thorny-headed worms").

Among the helminths, monogeneans are mostly ectoparasites of fish with relatively high host specificity. Buchmann and Bresciani (2006) assumed that many fish hosts including freshwater ones could harbour at least one unique monogenean species. Apart from being hosts to less harmful adult digeneans, fish may also be infected with metacercarial larval stages, which are the main agents of fish diseases (Paperna and Dzikowski, 2006). Most of the cestode orders (except Cyclophyllidea, Diphyllobothriidea, "Mesocestoidea" and Tetrabothriidea) have members that can infect fish (both Chondrichthyes and Osteichthyes) as adults. The number of species of nematodes infecting fish is relatively low compared with their terrestrial counterparts, but is still quite high (Molnár *et al*., 2006). A large number of nematodes of piscivorous birds, mammals or reptiles infect fish during their larval stages. Among approximately 1100 species of acanthocephalans (Golvan, 1994), nearly onehalf parasitize as adult in the intestine of bony fish (Teleostei), especially in Cypriniformes (Nickol, 2006).

Fish helminths with their mostly complex life cycles may also represent excellent models for the solution of a number of theoretical questions, including host-parasite relationships including host manipulation, biology, ecology, zoogeography and phylogeny of these parasites and their hosts (Williams and Jones, 1994).

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Trematodes

Trematodes (flukes) have small, flat, leaf-like bodies with oral and ventral suckers and a blind sac-like gut. They also lack a body cavity and have a tegument for body covering. Those flukes which remain confined to external surface of the body of fishes such as gills, skin, and fins and complete their life cycle on a single host are called Monogenetic trematodes. Monogenetic trematodes could be considered as one of the most prevalent parasitic agents affecting skin and gills of fishes leading to impairment of breathing. (Snieszkoand Axelored, 1980; El-Nobe, 2003).

On the other hand, those flukes which are confined to internal organs like stomach, intestine, liver, gall bladder, heart etc. and complete their life cycle on two hosts or more hosts are called Digenetic trematodes. Fish work as the second intermediate host in the life cycle of trematodes. Humans and fish – eating mammals and birds may function as final hosts liable on trematode species involved. People become infected with trematodes if they eat raw or undercooked freshwater fish and sometimes brackish water fish having active metacercariae. When the fish tissue is broken down through digestion, metacercariae are released into the small intestine, where they excyst and migrate to the suitable internal organs of the host and develop to adult trematodes. Fertilized eggs are released into the environment with feces of host and the eggs may reach water sources such as ponds, lakes, streams or rivers. Each egg contains miracidium, and when eaten by snails, themiracidium is released in the intestinal tract of the snails. The miracidiumpenetrates the rectal wall and develops into a sporocyst, which asexually generatesrediae. The rediae in turn, migrate to the digestive gland of the host snail where they asexually produce cercariae. Cercariae released from the snail and penetrate the skin of the fish, encyst as metacercariae in the muscles of the fish.

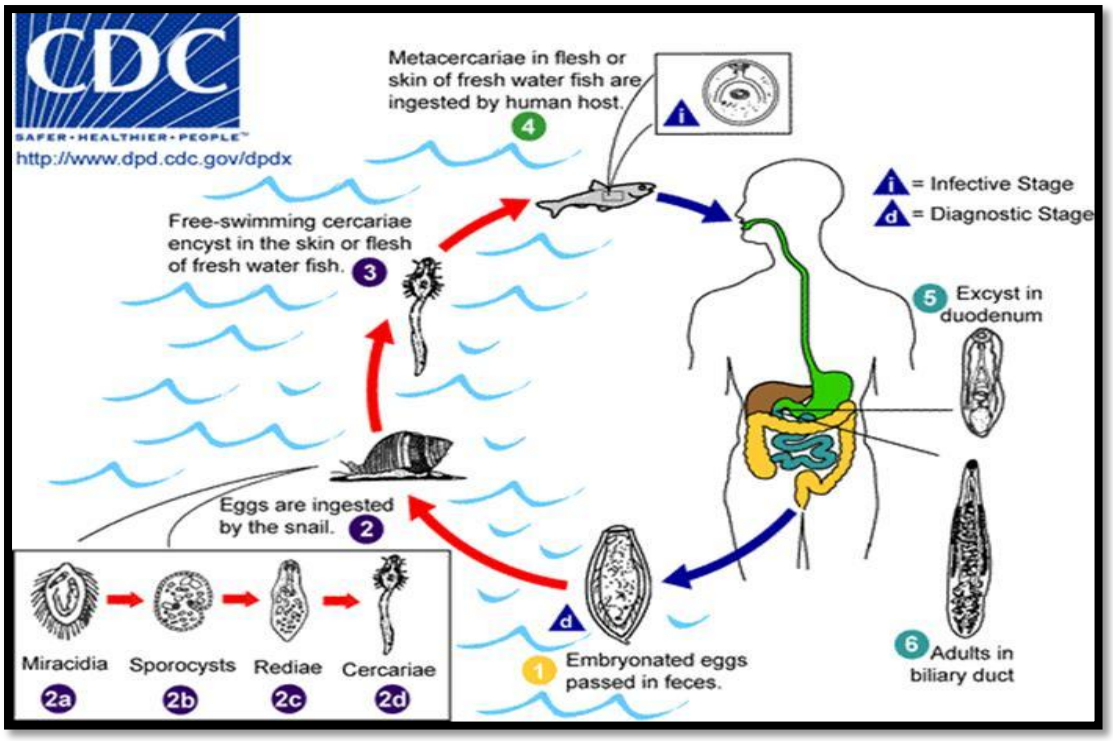


Fig .1 The Life cycle of *Clonorchis sinensis*as a representative for fish borne zoonotic trematode
Source:(modified from http:www.dpd.cdc.gov/dpdx)

Cestodes

Cestodes (tapeworms) have long flat ribbon-like bodies with scolex and numerous segments. They lack a body cavity and have a tegument for body covering. They do not have a gut and all nutrients are taken up through the tegument. In general life cycle of cestode parasites, the adult tapeworm lives in the intestine of a definitive host and releases eggs which pass out in the host faeces. If the eggs reach water, they hatch and release a free-swimming stage which may be eaten by a crustacean. Within this crustacean intermediate host, procercoid larvae are developed and if the infected crustacean is ingested by small freshwater fish the procercoid larva is released and develops into a plerocercoid larval stage, which is infective to the definitive host (fish).

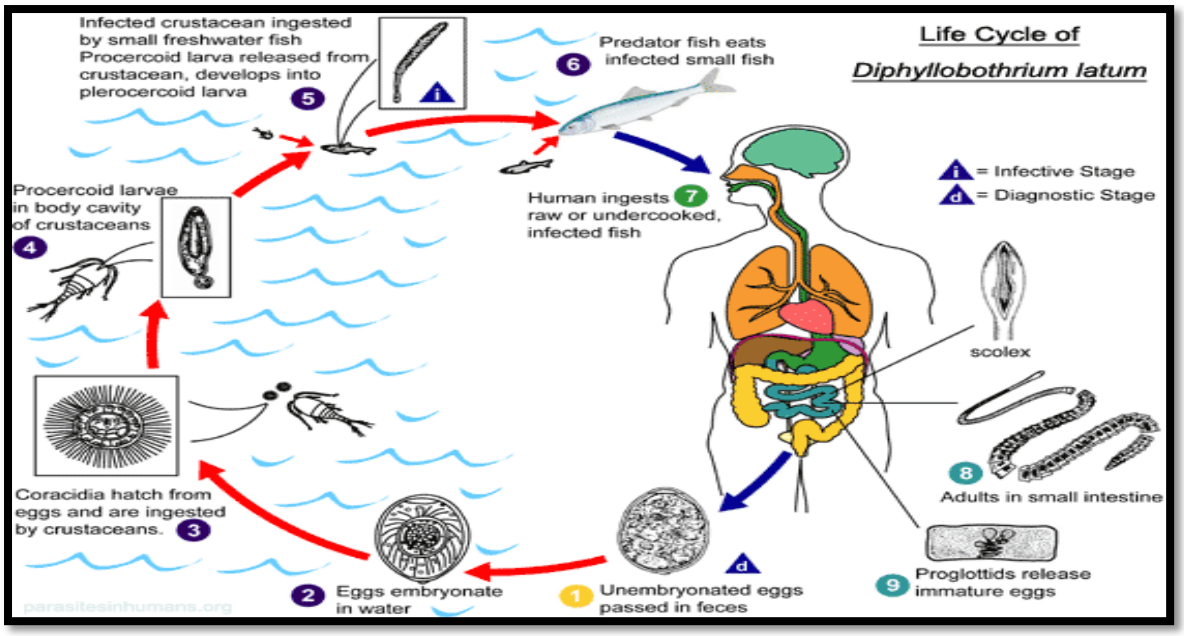


Fig No.2 Life cycle of *Diphylobothrium latum* as a representative for fish borne zoonotic cestode
Source: modifeied from
http://www.cdc.gov/parasites/diphyllobothriumlatum/biology/htm.)

Nematodes (roundworms)

Nematodes (roundworms) have long thin unsegmented tube-like bodies with anterior mouths and longitudinal digestive tracts. Adult worms form separate sexes with well-developed reproductive systems. They are a diverse animal phylum inhabiting a very broad range of environments. Anisakids nematode parasites (larval stage) are found in marine fish and are responsible for human infection. Adult anisakids are present in the stomachs of the marine mammalian definitive hosts. Eggs produced by female worms pass in the faeces and embryonate in the ocean waters. Larvae hatch from the eggs, enter small marine micro-invertebrates and develop into third stage larvae. When the crustacean is eaten by a fish, the larvae are released and pass through the gastrointestinal tract, then enter the mesenteries, muscle. If the infected fish is eaten by humans, the larvae may enter the tissue of intestine and cause disease.

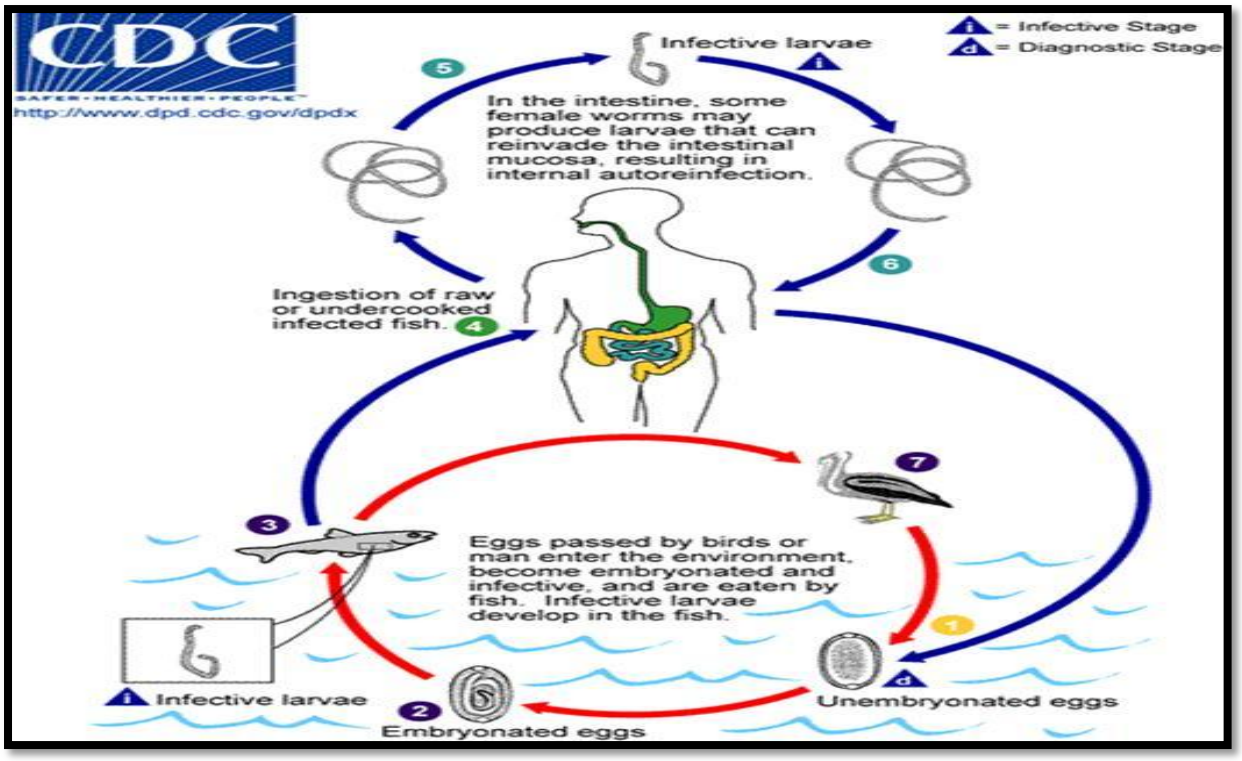


Fig. no. 3 Generalized life cycle of an nematode from a fish
Source: (modified from http:www.dpd.cdc.gov/dpdx)

Acanthocephalans

Acanthocephalans are also called thorny headed worms or spiny headed worms and are characterized by the presence of an eversible proboscis armed with spines, which it uses to pierce and hold on to the gut wall of its host. Acanthocephalans have complex life cyclesinvolving at least two hosts, which may include invertebrates, fishes, amphibians, birds, and mammals. Acanthocephalans require a vertebrate animal as a definitive host and arthropods as an intermediate host. Fish usually are the final host for aquatic acanthocephalans, and microcrustaceans (amphipod, copepod, isopod or ostracod) are generally the intermediate host. Intermediate hosts are infected by eating eggs eliminated in the feces of parasitized fish. An egg will hatch in the intermediate host releasing an acanthor (larvae) that penetrates the gut and develops intoan acanthella/cystacanth (larvae). The life cycle is completed when a fish eats a parasitized microcrustacean and the adult worm develops in the alimentary tract of the fish host. In some cases, fish are the second intermediate host as well as the final host.

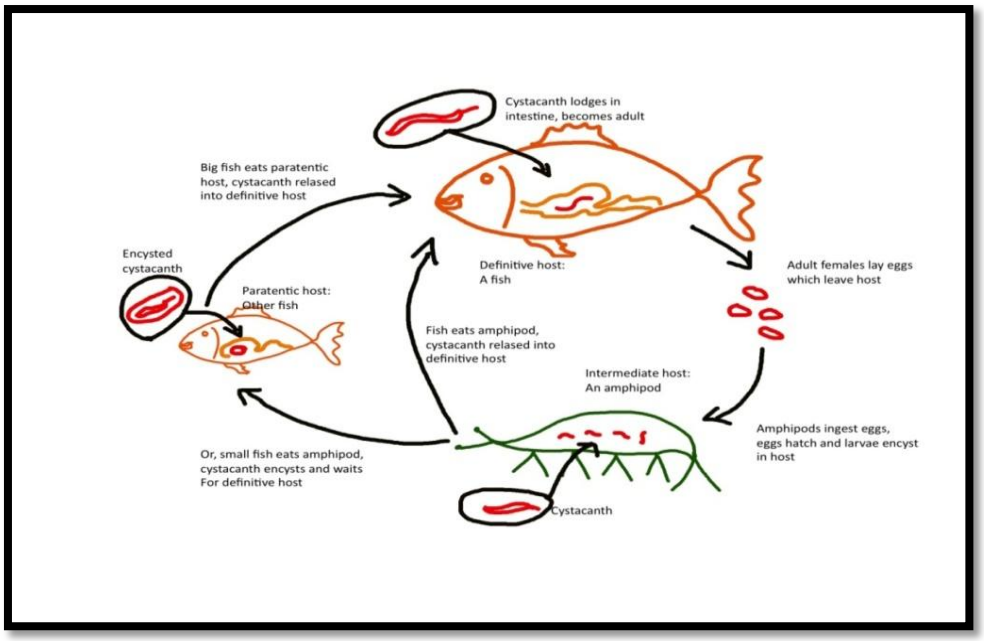


Fig No.4 Generalized life cycle of an acanthocephalan from a fish
Source: (https://increasingdisorder.wordpress.com)

Effects of helminth parasites on fish Health

During the infection by helminth parasites, the basic pathological condition recognized in fishes, are wound repair, inflammation, haemorrhage, necrosis, hyperplasia, ulceration, anaemia etc. The patho-physiological damages caused by different helminth parasites are briefly outlined below.

Effects of infection by Trematodes

The pathological consequences of trematode infection in fishes are normally considered to not cause disease even when their numbers are high. Maximum parasites are, however potentially pathogenic. Due to the trematode infection the sub mucous membrane of intestine of the host shrinks and the mucous membrane and villi are extensively damaged. The villi are erupted at certain regions of the intestine. The surface epithelium becomes flattened which leads to a complete damage to lamina propria and oedema of sub mucous membrane of the intestine resulting fibrosis associated with hyperplasia and metaplasia. The dilation of blood vessels, degeneration of intestinal folds, epithelial necrosis, vacuolation of submucous cells all of which lead to the degeneration of various layers of the intestine are evident in helminth parasitosis (B. Laxma Reddy and G. Benarjee, 2013). According to Pardeshi

et al (2012), the infection in the liver by the helminth parasite causes disturbances in the vital functions of the glands. These disturbances may directly affect the chemical nature of the infected tissue by lowering or increasing the important molecules which plays important role in metabolism.

Effects of Infection by Cestodes

Due to the cestode infection, pathological effects are exhibited as ruptured serosa layer, strong inflammatory edema and vacuolization in tunica muscularis and lamina propria, shortened and irregular shaped villous processes with blunt tips and breakage and separation of villous processes with large spaces. Chronic infection with *C. striatus* has been reported to cause damaged submucosal layer and thinning and fusion of villous processes (kaur *et al*., 2014). Ruhela *et al*. (2006) reported formation of pyknotic epithelial cells in mucosa, vacuolization, separation of muscular layers, rupturation of serosa and shortening and truncation of villi in the intestine of *C. batrachus* experimentally infected by *Procamallanus*. They also caused functional disturbances, blood and metabolic changes, retarted growth and weight loss. Maximum infections found in the intestine of fish.

Effects of Infection by Nematodes

Nematodes, or roundworms, infect many different species of aquacultured and wild fish. Small numbers of nematodes often occur in healthy fish, but high numbers cause illness or even death. Nematodes are severe parasites on many fishes. When the fishes are maximum infested with the nematode parasites, they tend to show losses in their weights. The nematodes and their larval forms can penetrate in the tissue of various organs and due to this causing severe tissues damage and destruction of cell of the organs (Fatima, 1988; Fatima and Bilqees, 1989). The common symptoms of the infection by nematode parasites includes inflammation, haemorrhage, necrosis, hyperplasia, ulceration, oedema, encapsulation etc.

1. Effects of Infection by Acanthocephalans

Acanthocephalan parasites are mainly found in intestine of fish. They cause tissue damage, inflammation of tissue, erosion of layers of intestine, necrosis, hyperplasia,

ulceration, oedema etc. Spiny headed worm penetrates their proboscis in to the intestinal wall of definitive host and cause intestinal disease.

Significance of the study

Fish is the primary source of quality protein for humans in many parts of the world, especially in developing countries (Dick and Choudhury, 1995) and parasites present a continual and unacceptable threat to the well-being and economy of millions of people as well to domesticated, farmed and wild animals in all parts of the world. In this context, the importance of fish parasites is related directly to the economic importance of the fish species that they may infect.

India is among the 17 megadiversity countries (Mittermeier et al., 1997) and hosts as many as 55 families of freshwater fish (Teleostei) (Froese & Pauly, 2012). For the last few decades, fish (both Chondrichthyes and Osteichthyes) have been extensively used as a protein rich diet for human consumption in the Indian subcontinent and thus contribute substantially to its economy. It is estimated that about 10 million tons of fish are required annually to meet the present-day demand of fish proteins in India compared to an actual annual production of only 3.5 million tons (Shukla and Upadhyay, 1998).

Parasites infecting fish in natural waters may not be detrimental to fish, but do affect its quality as food for human consumption. Furthermore, some fish parasites are zoonotic. These problems are compounded when fish are produced under farming or aquacultural practices where mass mortalities frequently occur. Parasites therefore cause great economic loss and it is important to generate more information about them to augment approaches to fishery management programmes.

The basis of any parasitological studies is the identification of parasites (systematics) followed by their biology and ecology. Since very scant studies have been conducted regarding the prevalence and other ecological aspects related to the helminth parasito-fauna of food fishes of the River Gomti in Lucknow, the present study was undertaken with this basic approach in mind. With the knowledge gained through this study, insights could be made on the helminthes parasites and their effects on the fish health, thereby making it possible to obtain economic gains through practicing

suitable prevention or treatment methods for parasites that infect the hosts in large quantities.

Aims and Objectives

Keeping the economic and social importance of fish parasitology in view, the present study describes a programme of research undertaken to obtain quantitative data on the helminth fauna and their effects on the morphological and physiological parameters of some popular freshwater food fishes of River Gomti in Lucknow. This study broadly investigates the prevalence of helminth infection in the selected fishes and other parameters of population dynamics viz., monthly variation, the intensity of infection, abundance of infection, effects of length, weight and sex on the burden of infection. The effects of heavy helminth infection on the haematological parameter of the fish as well as biochemical components of fish muscles infected with helminth parasites was also studied.

The present work was conducted to achieve the following objectives:

1. To study the population dynamics of helminths parasites of some common food fishes of river Gomti in Lucknow.
2. To investigate the relationships between prevalence of helminths parasites and body weight, body length and sex of the fishes.
3. To investigate the alterations in haematological parameters of the fishes infected with helminth parasites.
4. To investigate the alterations in biochemical parameters of the fishes infected with helminth parasites.

The work embodied in this thesis has been organized into the following chapters:

Chapter 1.General Introduction and objectives of the study.

Chapter 2. Deals with Review of Literature.

Chapter 3. Describes the study of population Dynamics of helminth parasites in selected freshwater fishes.

Chapter 4. Deals with study of the relationship between prevalence of helminth infection and length, weight and sex of host fish.

Chapter 5. Deals with the study of haematological parameters of helminths infected freshwater fishes.

Chapter 6. Deals with the study of biochemical parameters of helminths infected freshwater fishes.

Deals with the references.

Appendix

Appendix deals with paper published in journals, articals/ chapters published in books, paper presented in conferences/symposia/seminars, participation in training programs/workshops, conferences/symposia /seminars attended, membership of professional bodies/ organizations and published two research paper.

Chapter 2

Review of Literature

Review of Literature

Studies on the infestation of helminth parasites in different freshwater food fishes in the Indian subcontinent and the effects of these infections on the different morphological and physiological aspects of the host fishes are limited and fragmentary although a considerable number of works have been carried out in other parts of the world. In this context, the literature related to the present study is briefly reviewed below.

Murad and Mustafa (1988), in their study on the catfish, *Heteropneustes fossilis* (Bloch) parasitized by metacercariae of *Diplostomulum sp.*, investigated 90 specimens of the fish and from a pond at Aligarh and reported infection in 40% of the population. In the study, the changes on the blood characteristics of the catfish parasitized with the larval helminths were analyzed. The various blood parameters considered were erythrocyte count, haematocrit, haemoglobin concentration, means corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total and differential leucocyte-thrombocyte count, and erythrocyte sedimentation rate (ESR). The authors concluded that of and reported that the amount of RBC, PCV, Hb, MCV, MCH, MCHC were reduced in infected fishes as compared to the control fishes whereas the level of TLC was found to be increased in the infected test fishes.

Martins *et al.* (2004) reported that the Parasite infection in fish species *Leporinus microcephalus* provoked significant reduction ($P<0.05$) in hematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration and lymphocyte percentage. On the other hand, a significant increase ($P<0.05$) of WBC was recorded in the circulating blood of infected fish species *Leporinus microcephalus.*

Akinsanya and Otubanjo (2005) studied a total of 360 randomly selected specimens of *Clarias gariepinus* Clariidae) collected from Lekki Lagoon for the intestinal helminth parasites. The study reported low prevalence and worm burden with 17 specimens being reported to be infected with intestinal helminth parasites showing a 4.72% prevalence of parasites. They reported three cestodes: *Polyonchobothrium clarias*, S. *tocksia pujehuni* and *Wenyonia acuminata*, and a nematode,

Paracamallanus cyathopharynx the tested fish species. In their study, the prevalence of helminth infection showed no significant difference between the both the sexes of *Clarias gariepinus*, but they concluded that the parasite prevalence was related to the length and weight of the specimens. The fish samples studied by them were observed to show negative allometric growth and smaller samples recorded higher helminth infection.

Ahmed *et al*. (2007) studied the prevalence of internal helminth parasites in the digestive tract of some freshwater fishes collected from various freshwater ponds in the vicinity of Lahore. The study investigated a total no. of 43 fishes which included 11 *Heteropneustus fossilis fish,* 16 *Mystus vittatus fish* and 16 *Channa striatus* fish. The study reported that out of a total of 39 fishes were infected with helminth parasites in which, while 100% *H. fossilis* were found to be infected with nematode parasites, only 37.5% of *M. vittatus* were bearing the nematodes. In case of *C. striatus,* 56.25% fish were found to be bearing acanthocephalans, 25% cestodes and 5% nematodes. The mean intensity of infection was recorded as 14.36 in *H. fossilis,* 6.5 in *M. vittatus* and in *C. striatus,* 3.33 for acanthocephalans, 2.4 for nematodes and only 1.0 for cestodes per infected fish. The low prevalence and parasite intensity of cestodes was attributed to the fact that the ponds were not permanent ones and dried when rainwater was not available, thus disturbing or blocking the cestode lifecycle.

Shah *et al*. (2009) studied the impact of helminth parasitism on fish haematology of the fishes of Anchar Lake, Kashmir. They have found that the fish fauna viz., Schizothorax spp. and Cyprinus spp. inhabiting the lake were infected with cestode, trematode and acanthocephalan parasites, either singly or in mixed populations. Their results showed a mean significant decrease in haemoglobin level from 9.39±0.18-7.39±0.14 g % in Cyprinus spp. and 10.57±0.23-7.62±0.13 g% in Schizothorax spp. They also recorded a decrease in the RBC counts in summer season viz. from 2.07±0.03-1.66±0.05 (×106 mm3) in Cyprinus spp. and 2.32±0.02-1.69±0.04 (×106 mm3) in Schizothorax spp. However, their study reported a significant increase in WBC counts, with a mean increase from 1.58±0.16-3.93±0.33 (×104/mm3) in Cyprinus spp. and 1.56±0.10-2.76±0.27 (×104 mm3) in Schizothorax spp. in the summer season. Further, a well-marked increase in eosinophils was also observed by these workers in all the helminth-infected fish fauna. The entire study revealed that

the intensity of helminth infections is responsible for altering the haematology of fish hosts and that there was a seasonal relationship of infection with the haematological alterations.

Vankara *et al*. (2009) studied two important food fishes of Andhra Pradesh viz. *Macrognathus aculeatus* (Bl.) and *Mastacembelus pancalus* (Lac.) heavily infected with the metacercariae of *Clinostomum mastacembeli Jaiswal*, 1957. In the study, the histopathological changes caused due to encystment of the metacercariae of C. mastacembeli in the esophagus of these freshwater eels were observed. Out of the 72 fishes evaluated by these workers, 57 were found to be infected with the trematode parasites. In their study, they found that the histopathology of the esophagus of the infected fish was noteworthy showing extensive damage to all the esophageal layers of the infected host fishes.

Dhole *et al*. (2010) conducted a survey of helminth parasites in freshwater fishes from Marathwada region, MS, India. They collected fish samples from Osmanabad, Aurangabad, Latur and Nanded in Marathwada region and examined them for helminth parasites included the three classes i.e. Cestode, Trematode and Nematode. During the study, a survey was carried out on 879 freshwater fishes which included *Mastacembalus armatus, Clarias batrachus, Wallagu attu and Channa punctatus* from various places of Marathwada region. The study reported that 487 fishes were found to be infected and a total of 689 helminth parasites were isolated belonging to seven genera of helminth parasites among which four belonged to cestodes, two were trematodes and one was a nematode. The study established that the *M. armatus* fish was one of the most heavily fish species as compared to the others.

Eldeen *et al*. (2010) conducted studies on the haematology of healthy and helminthes infected rabbit fish (*Siganus rivulatus*). The blood samples were collected after fish catch, and then subjected to biochemical investigations which included haematological estimations such as haemoglobin (Hb), packed cell volume (PCV), and erythrocyte counts. Their results revealed a decrease in packed cell volume (PCV), haemoglobin (Hb) and red blood cells count of helminthes infected fishes as compared to control one.

Onyedineke, *et al.* (2010) reported 60.6% prevalence of helminth infection and an infection rate of 59.15% in their study on the helminth parasites of some freshwater fish of river Niger at Illushi, Edo state, Nigeria. They collected 71 fish samples that belonged to 14 species (*S. eupterus, S. clarias, C. nigrodigitatus and C. kingsleye, T. galilaeus, T. zilli, A. nurse C. citarius, H. vitatus, M. rume, L. cubie, D. aegycephalus, L.niloticus*). The gills, stomach and sometimes muscles were found infected. Parasites were more prevalent in the fish length range of 10 – 30cm. In *C. kingsleye*, parasites were most prevalent in fish of standard lengths 14- 17cm and 34-37.9cm. There did not appear any specific trend in the parasite prevalence in *S. eupterus, S. clarias, C. nigrodigitatus and C. kingsleye* with regards to the weight of the fishes.

Yadav *et al.* (2010) conducted an ecological study on the infections by the digenetic trematode parasites in one of the important food fish *Channa punctatus* collected from River Gomti at Lucknow. The study examined a total of 359 host fishes, out of which 223 were found to be infected with trematode parasites. The study also reported the seasonal changes in prevalence, intensity and relative density patterns of digenean trematodes infection in *Channa punctatus* during the their one-year long study. They concluded that the digenean trematodes are important helminth parasites of *Channa punctatus*.

Anarse *et al*. (2011) conducted a study to investigate the effect of innate factors and other characteristics such as season, temperature, humidity, age and sex of the host on the distribution of cestode parasites in the host fish *Trygonzugei* from Ratnagiri district (M.S.), India. They recorded a total of 212 cestode parasites from 360 fishes. The collected parasites were of only one genera i.e. *Tetragono cephalum* Shipley, They summarized the data incidence, intensity, density and index of infection of cestode parasites with effect of environmental factor. They concluded that the population dynamics of *Trygon zugei* infected by *Tetragono cephalum* was very high in summer followed by winter.

Fartade *et al.* (2011) conducted a study on the biochemistry (protein, glycogen, lipid) of Ptychobothridean parasites in the fresh water fish *Mastacembalus armatus*. They reported that the percentage of lipid was higher in the parasites as compared to protein and glycogen content. They also estimated that the lipid content was very high in parasite which is 25.07mg/gm as compared to in the host *M. armatus* (13.44mg/gm).

Jadhav *et al*. (2011) carried out extensive work on the incidence of helminth parasites in freshwater fishes from Sina Kolegoan Dam, Osmanabad. They collected a total 286 samples from different sites of Dam, Osmanabad during the month of June 2008 to May 2010. They isolated 289 helminth parasites from the collected fish samples. The parasitic species collected from freshwater fishes included cestodes (*Senga* spp., *Circumonco bothrium* spp.), trematodes (*Azygia* spp., *Isoparorchis* spp.) and nematode (*Camallanus* spp.). the study reported the results of investigations on the extensive damage caused by the helminth parasites on the fish economy.

Jawale *et al.*(2011) carried out a biochemical studies of *caryophylidean* tapeworms in fresh water fish *Clarias batrachus*. They estimated the quantitative values of protein, lipids and glucose in parasite and compared it with the host intestine. They concluded that the percentage of lipid is higher in the parasites as compared to the protein and glycogen content and also that the parasites were taking advantage of the host and absorbing most of the host nourishing materials..

Ali *et al.* (2012), in their study, made a comparative investigation on the haematological and biochemical parameters of healthy and monogenean infected common carp fish *Cyprinus carpio*. The study reported marked alterations in the haematology and biochemistry of the infected fish. The changes in the the total RBC count, haemoglobin content, haematocrit (PCV), ESR and total leucocyte count (TLC) as 2.68±0.04 × $10^6/mm^3$, 9.50±0.14 gm/100 ml, 27.50±0.08%, 3.92±0.48 mm/hr and 11.12±0.13 × $10^3/mm^3$ respectively in healthy fishes and 1.92±0.3 × $10^6/mm^3$, 7.35±0.42 gm/ml, 20.14±0.81%, 4.11±0.52 mm/hr and 12.25±0.08 × $10^3/mm^3$ respectively in the monogenean-infected fishes. The study concluded that increased TLC and DLC values may be associated with the defense mechanism and immunological response against the infection be monogenean parasites in the host. The study also reported elevated levels of serum transaminases in the infected host fish which are markers of liver functions.

Dar *et al.* (2012) conducted an 18 months survey on the infection of helminth parasites in coldwater fishes of Ladakh from November 2007 to April 2009. A total of 93 fishes belonging to two species viz., *Schizothorax plagiostomus* and *Diptychus maculatus* were collected from different sites of Suru river, Kargil. They reported two

helminth groups, i-e. Acanthocephala and Nematoda and reported a total of 2 helminth species viz., *Neoechinorhynchus yalei Datta,* 1936 and *Rhabdochona himalayai Fotedar & Dhar,* 1977. They observed that out of 93 hosts examined, 31 were infected with 43 parasites being recovered in total. The overall prevalence, mean intensity and abundance was found to be 33.33%, 1.38 and 0.46 respectively. Relationships between prevalence of helminth infection and sex and size of host was also analysed in this study. According to these workers, the helminth infection showed no significant relationship with sex of hosts, however, it showed mostly significant relation to size of host.

Kaur *et al.* (2012) carried out a study on the effect of digenetic trematode *Clinostomum complanatum* on the histopathology and haematology of *Nandus nandus* collected from the Lower Lake of Bhopal and local fish markets. The study reportedthe total haemoglobin content, number of erythrocytes, granulocytes and lymphocytes as 5.79±0.57g/100ml, 1.266±0.049 x $10^6/mm^3$, 71.59±4.37 x $10^3/mm^3$and 54.93±5.39 x $10^3/mm^3$ respectively in the control fish specimens of *Nandus nandus,* whereas the *N. nandus* fish infected by *Clinostomum complantum* showed a decrease in the total haemoglobin as 3.51±0.43g/100ml, the number of erythrocytes as 0.878±0.059 x $10^6/mm^3$and an increase in the number of granulocytes and lymphocytes viz., 95.21±4.01 x $10^3/mm^3$ and 77.45±3.91 x $10^3/mm^3$ respectively.they also reported several histological alterations in the infected livers of the host fish.

Kaur *et al.* (2012) reported the prevalence of different helminth parasites of freshwater murrel, *Channa punctatus* which were collected from local fish markets of Bhopal. The study examined a total of 75 samples of *Channa punctatus* for infection by the helminth parasites and reported recovery of two species of digenetic trematodes (*E. heterostomum* and *Clinostomum complanatum*) and one species of acanthocephalan (*Pallisentis* sp.) from different organs of the host fish. *E. heterostomum* was found to be the dominant species (prevalence: 28.0%) followed by *Pallisentis*sp. (26.6%), while *Clinostomum complanatum* showed minimum prevalence of infection (20.0%).

Malathi *et al.* (2012) studied the haematological parameters: RBC, WBC count, haemoglobin content and haematocrit, MCV, MCH, MCHC of two species of the freshwater fish *Channa punctatus* and *C. striatus* (Bloch) collected from freshwater bodies of Cauvery delta in and around Thanjavur. They observed 40 specimens of each of these species and reported the range of different haematological parameters as RBC: 3.29×10^6 to 3.42×10^6/cumm; WBC: 4.3×10^3 to 4.6×10^3/cumm; Hb: 9.37 to 10.48g/dl; PCV: 34.42 to 36.14%dl; MCV: 104.62 to 105.67 μm^3, MCH: 24.09 to 28.48 Pg/dl; MCHC: 27.22 to 29.99 g/dl. They observed that these parameters reflected the physico-chemical conditions of the habitat of the fish.

Mofasshalin *et al*. (2012) conducted a study on the parasitic infection of Indian major carps collected from Rajshahi, Bangladesh. They examined a total of 480 host fishes, of which 370 fishes were found to be infected by 4 protozoans (*Trichodina* sp., *lchthyophthirium s*sp., *Apiosoma* sp. and *Chilodonella* sp.), 2 monogeneans (*Gyrodactylus* sp. and *Dactylogyrus* sp.), 2 crustaceans (*Argulus* sp. and *Larnaea* sp.), 1 digenean (*Fellodistomum* sp.) and 1 nematode (*Camallanus* sp.) parasitic species. Among the isolated parasites *Fellodistomum* sp. was found most prevalent, while *Chilodonella* sp. was found as the lowest in number. They reported that infection and infestation rate of parasites varied with fish size and season and was found to be higher in the post-monsoon and winter periods (November-March), when fish are most susceptible to parasites.

Deshmukh *et al*. (2013) conducted studies on the prevalence of cestode parasites in freshwater fishes *Mastacembelus armatus, Mystus seenghala, Wallago attu, Channa punctatus, Channa gachua, Channa stratius, Clariasbatrachus, Cirrihana mrigala,* collected from different localities of Nanded region. Out of the 438 fishes investigated, 200 were infected by cestode parasites belonging to *Senga, polyyonchobothrium, Proteocephlus, Bothiocephalus,* and *Gangesia,* species. Their results showed that adults were more infected than juveniles and males were more infected than females. They reported that the prevalence of infection rate was high in summer (February to May) and monsoon (July to October) seasons and low in the winter season (November to January).

Omeji *et al.* (2013) conducted a comparative study on the endoparasitic infestation in *Clarias gariepinus* fish collected from earthen and concrete ponds in Makurdi, Benue state, Nigeria. The study reported that male fish *Clarias gariepinus* collected from the earthen ponds had more percentage of parasitic infestation (64.29%) than the female fish (57.69%) whereas in the concrete ponds, female fish had higher percentage of parasitic infestation (22.73%) than the male (16.67%). It was observed that bigger fishes of weight class between 750-849g were more infested than the smaller counterparts of less than 149g from both ponds. In the earthen pond, the highest percentage intensity of infestation (0.83%) was recorded in fish with the weight class between 750-849g while the lowest was recorded in fish with weight class between 250-349g. on the other hand, the highest percentage intensity of infestation (0.42%) was recorded in fish with the weight class between 750-849g while the lowest was recorded in fish with weight class less than 149g.

Shahi *et al*. (2013) gave a first report of blood parasites in fishes of four taxa namely *curvifrons cyprinus carpio, carrasius carassius, schizothrax* and *triplophysa marmorata* captured from River Jhelum and Anchar Lake in Kashmir. The study reported the haematological investigations in fishes infected with *Babesioma* and *Trypanosoma.* The study recorded a significant decrease ($p>0.05$) in the haemoglobin value and packed cell volume in the infected fishes in comparison to the control fishes. A significant reduction ($p<0.01$) in red blood cell count was observed in the infected fish as compared to healthy one.

Amare *et al.* (2014) conducted a work from November, 2010 and August, 2011 in Lake Lugo (Hayke), northeast Ethiopia, with the aim of determining the prevalence and identifying the species of internal parasites from freshwater fish. A total of 412 randomly selected fishes comprising of 225 *Oreochromis niloticus*, 72 *Clarias gariepinus* and 115 *Cyprinus carpio* were examined for internal helminthes parasites. An overall prevalence of 47.8% (197/412) internal parasitic infestation was assessed. Statistically significant difference at $p<0.05$ was noted in the prevalence of internal parasites among the different species, length and weight of fish. However, the difference between sexes was not statistically significant at $p>0.05$, but the prevalence was a bit higher in females (48.31%) than males (47.44%).

Amaechi (2015) carried out a study on the prevalence, mean intensity and abundance of endoparasites in 250 specimens of two cichlids, *Oreochromis niloticus* and *Tilapia zilli*, from Asa dam, Ilorin, North Central Nigeria during from February, 2013 to August, 2013. They reported two digenetic trematode parasites belonging to two different species viz., *Euclinostomium heterostomum* and *Clinostomum tilapiae*. The highest prevalence was recorded in *O. niloticus* (35.9%) infested by *C. tilapiae*, while the highest mean intensity was recorded in *T. zilli*. There was no relationship ($p > 0.05$) between parasite burden and fish size (length and weight). In this study, the male fish were found to be more heavily infected than the female fish.

Kaur *et al.* (2014) studied the variations in haematological parameters of *Channa punctatus* under influence of parasitic infection. Their study showed that during the course of investigation, the total haemoglobin content, number of erythrocytes, granulocytes and lymphocytes to be 6.52±0.43g/100ml, 2.44±0.22 x $10^6/mm^3$, 123.0±5.09 x $10^3/mm^3$and 68.0±4.4 x $10^3/mm^3$ in the non-infected specimens. In the individuals of *Channa punctatus* infected by cestode parasite, the total haemoglobin recorded was 3.70±0.60g/100ml and the number of erythrocytes was 1.28±0.37 x $10^6/mm^3$. In their study, these workers have also reported increase in the number of granulocytes and lymphocytes as 142±6.1 x $10^3/mm^3$ and 87.4±3.9 x $10^3/mm^3$ respectively.

Khalil *et al.* (2014) studied the haemoglobin contents in *Heterpneustes fossilis*, and reported that the range of haemoglobin percentage in uninfected fishes was 2.8-6.2% and in infected it was 2.2 – 6.2%. The average percentage in uninfected fishes was 4.77±1.02 and in the infested fish it was 4.18±0.71.

Kumar (2014) investigated the prevalence of infection in sampled species of *Labeo* and *Channa* from Bareilly district. They examined a total of 360 samples of *Channa* and *Labeo* collected from different sites of Bareilly region during the months of July 2007 to June 2009. The study reported a collection of 246 trematode parasites from the host fishes. These trematodes belonged to six species, in which three species were ectoparasites (*Paramazocraes, Mazocraeschauhani, Yogendrotrema*) and rest of the three species (*Allocreadium, Dactylostomum, Genarchopsis goppo*) were endoparasites.

Emre *et al*. (2014) in their study on the helminth parasites reported that the sex of the host was influenced by the parasitic burden. The prevalence of the infection was found to be higher in the male fishes as compared to the female fishes. A total of forty-two male fishes were found to be infected by the parasites. The overall prevalence of infection was 84% and 54.1% for male and female fishes respectively. They also reported that the larger sized fish were more heavily infected than the smaller fish.

Okoye *et al.* (2014) studied the prevalence, mean intensity, abundance and seasonality of parasites of fish from the natural freshwater tropical Agulu Lake (natural, freshwater tropical lake), in southeast Nigeria. In the study, a total of 1191 fish specimens belonging to four families (*Cichlidae, Bagridae, Hepsetidae and Channidae*) were collected and examined for the parasites. The study was reported 11 species of parasites comprising metacercariae of three digenetic trematodes, one cestode, five nematodes and two acanthocephalans.

Pilla *et al*. (2014) studied the biochemical compositions of muscle and liver of normal and infected fish of *Lutjanus johni* off Visakhapatnam Coast. Proteins, lipids and carbohydrates in muscle and liver tissues were studied with respect to different seasons. The study reported that the level of Lipids were elevated, (10.27±0.58mg/g,), while protein content declined 74.60mg/g, (±2.61) in the infected liver tissues as compared to the normal fish (78.29±1.86mg/g). Some fluctuations were also observed by these workers in total carbohydrate content in the infected fishes.

Hassan *et al.* (2015) reported the biochemical changes in muscles and liver of Koshar Fish (*Epinephelus summana)* in relation to helminth infection. In the study, carried out in Jeddah, Saudi Arabia, a total of 102 *E. summana* were collected randomly from the Red Sea, Jeddah coast during the period of March to September 2014. Helminthes parasites were detected in the liver, intestine and stomach of the infected fish. Total protein and carbohydrates in muscles and liver were also quantified using spectrophotometer. Their results showed that the total muscle protein of fish and total carbohydrates of both muscles and liver decreased significantly in infected fish. They reported that intestinal trematodes had higher effect on the muscles protein rather than stomach trematodes, whereas intestinal nematodes had a low effect on muscles protein than the stomach nematodes. They

attributed this decrease might be due to the weakened ability of the intestinal absorption by the infected fish as the digested food is consumed by the parasite.

Kumar *et al*. (2015) studied the distribution of protein, lipid and carbohydrates in muscle and liver of infected and normal *Upeneus vittatus*, a marine species found in the coast of Bay of Bengal, Visakhapatnam. It is observed in the study that there were significant differences in the Lipid content of infected and normal fish. Lipid levels were observed to be more in the fish with infection as compared to the non-infected fish.

Nnabuchi *et al*. (2015) studied the effects of parasites on the biochemical and haematological indices of some clariid catfishes from Anambra River, Nigeria. A total of 360 fish species (231 *C. gariepinus* and 129 *C.anguilaris*) were subjected for investigations on their infection status by parasitic organisms. The study reported that parasites recovered from the fish belonged to protozoans (*Trichodinaacuta* and *Epistyli s*sp.), cestodes (*Polyonchobothrium clarias* and *Monobothriode woodlandi*) and nematodes (*Rhabdochona congolensis* and *Procamallanus laeviconchus*), with a total prevalence of 42.1% and mean intensity of 4.15 ± 1.57. They also studied the haematological manifestations of the infected fishes which showed marked decrease in the content of haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells. However, in their study, the infected fishes had higher content of white blood cell (WBC) than the uninfected ones. The study also reported that there was a significant ($p<0.05$) negative correlation between parasite intensity and condition factor. Hepatosomatic index of the fishes increased with increase in parasite intensity and the fishes infected with *Trypanosoma* showed emaciation, dullness, respiratory distress, loss of escape reflex, mild ascitis and paleness of the gills. Post-mortem examination of the infected fish revealed paleness of the internal organs (liver and kidneys) and slight congestion of spleen. Further, in the study, the haematological examinations of infected fish revealed significant decrease in erythrocytic count, haemoglobin and packed cell volume but significant increase in total leucocytic count accompanied with neutrophilia and eosinophilia.

Sultana and Salam (2015) identified ten species of helminth parasites i.e. *Neopecoelina saharanpuriensis, Mesolecithal linearis, Asymphylodoratincae,*

Genarchopsis bangladensis, *Ditestolepisdiphana*, *Senga* sp., *Neoechinorhynchustylosuri*, *Pallisentis* sp., *Ascardia* larvae., *Gnathostoma spinigerum* from *Channa punctatus* of Savar area. They reported majority of the parasites from the intestines of the infected fish. The overall prevalence of infection of the parasites was 32.5% and the mean intensity was 1.46±1.29. Both the prevalence (34.61%) and mean intensity (1.5±1.21) were observed to be higher in male fishes than the female fishes (28.57% and 1.375± 0.99 respectively) in this study. The prevalence (13.75%) and mean intensity (1.64± 0.67) of trematode parasites were found to be the highest among the parasites groups. Both the prevalence and mean intensity were also reported to be the highest in the intermediate length (10.1-15.1cm) and weight (21-31gm) groups of the fishes.

Masarat (2016) investigated the helminth parasitic loads in the freshwater fish *Cyprinus carpio*. The study reported the recovery of three species of helminth parasites viz., *Adenoscolex, Bothriocephalus* and *Acanthocephalus* from *Cyprinus* sp. The prevalence, intensity and abundance were calculated in relation to sex, length-group and LWR of fish. It was observed in the study that the larger sized fishes were highly affected by the helminth parasites as compared to small sized fishes. Thus the negative allometric growth was observed among infected fish. Male fishes were found to show the highest infection as compared to the female fishes.

Chapter 3

Study of Population Dynamics of Helminth Parasites in Freshwater Fishes.

Introduction

In recent years, an ecologic approach has been emphasized in order to understand the basic principles of parasitology, because the concepts of ecology in the study of parasites have been ignored in the past. This becomes especially important in the context that the relationship between the parasites and their hosts may be viewed as "ecological" because the host provides the environment in which the parasites lives, and as such the host's environment, the open sea or ocean, affects the parasites through its host. Furthermore, this becomes quite apparent to believe, rather confirm, that the parasite, be it an endo-parasite or ectoparasites, synthesizes its enzymes for digestion or other metabolic function from the constituents provided by the host's tissues, particularly the endo-parasitic digenetic trematodes and tapeworms. The food, pre-digested by the host, is absorbed via ultramicroscopic microvilli and other microscopic structures present on or immediately beneath the body surface of the Trematodes or Cestodes and used within their body.

Of course, the ecology of parasites is not only limited to meet their nutritional requirements but also equally interesting to study the ecologic factors that influence the distribution of parasites and the parasite density that is, in all, the parasite population dynamics.

Helminth parasites of Fish

Fishes live in aquatic medium; many parasites are presence in this aquatic medium. So fish are infected with many parasites. These are typically divided into two groups: ectoparasites and endoparasites. Ectoparasites are live on the outside of a tropical fish host (including the gills, mouth, skin and fin surfaces); and endoparasites, which live in the tissues, blood and/or organs (Intestine, Air bladder, Liver, Muscles). Endoparasites or helminths parasite are multicellular, bilaterally symmetrical animals having three germ layers. Most helminth parasites carried by fishes are Nematodes, Acanthocephalans, Cestodes and Trematodes.

The Helminth parasitess, in the adult stage are usually found in vertebrate hosts, while the larval stage generally inhabit the invertebrate hosts. The parasite prevalence, density of infection and intensity infection depend on many factors like parasites and

its life cycle, host and its feeding habits and the physical factors of water body where the fish inhabit. Population dynamics is necessary to provide data for the prophecy of integrated methods to achieve the regulation of numbers of harmful parasites (Kennedy, 1974 and 1976).

Many trematodes attach themselves to superficial parts of the host as ectoparasites. But many others penetrate in to the body and settle down in one of the internal organs as endoparasites. Those flukes which remain confined to external surface of the body of fishes such as gills, skin, and fins and complete their life cycle on a single host called Monogenetic Trematodes. On the other hand, those flukes which are confined to internal organs like stomach, intestine, lever, gall bladder, heart etc. and complete their life cycle on two hosts or more hosts are called Digenetic Trematodes. Digenean are common, asympytomatic infections in fish. About 1700 species of adult digeneans infect fish. Metacercariae are even more common than adults. Digeneans are common in fish.

The nematodes are another important helminthic group infecting fishes. They have elongated bodies tapering at both ends and lack segmentations and suckers. Fish are either intermediate are final hosts for nematodes. About 650 species of Nematode parasitize fish as adults and many others use fish as intermediate hosts while Nematodes are common in fish. The adult form of nematode occur in almost all fishes, generally in the intestine while larval Nematodes may be found in connective tissues, body cavity or muscles. These Nematodes are very important as they cause pathological changes in fishes, due to which the production of fishes is effected through the increases in their mortality rate.

Tapeworms and their allies are invariably endoparasitic which lives in freshwater fish, and attain maturity only in the alimentary canal of the vertebrate animal. Adult Cestode is white flattened, segmented worms that inhabit the intestine. With a complex life cycle that required one are two intermediate hosts, Cestode are relatively more common in fish. Fish can be an intermediate host, definitive host or both. Some Cestodes are the most damaging parasites to viscera of fish and decrease nutritive value of fish, if present in muscles.

Acanthocephala are a thorny-headed worm or spiny-headed worms. These parasites found in all vertebrates but are more common in birds or fishes. Acanthocephalans require a vertebrate animal as a definitive host and arthropods as an intermediate host. Fish usually are the final host for aquatic acanthocephalans and microcrustaceans are generally the intermediate host. Intermediate hosts are infected by eating eggs eliminated in the feces of parasitized fish. An egg will hatch in the intermediate host releasing an acanthor that penetrates the gut and develops into an acanthella/ cystacanth. The life cycle is complete when a fish eats a parasitized microcrustacean and the adult worm develops in the alimentary tract of the fish host. In some cases, fish are the second intermediate host as well as the final host.

The helminth worms are wide spread though the intensity of infection may differ from time to time or place to place and produce a wide variety of direct effects undermining the welfare of man and the animal he is associated to smaller or greater extent. The extensive role of parasites and their economic effects are solely responsible for the development of the helminthological research.

Selected Food Fishes of River Gomti, Lucknow of Uttar Pradesh

Fishes are aquatic vertebrates that are naturally cold blooded. They are abundantly found in the sea water and in freshwater. Fishes are a good source of protein, vitamins like A, D, E, K, B12, minerals like calcium, iron, phosphorous and iodine, omega-3-fatty acids. Fish oil contains omega–3–essential fatty acids which are necessary for the proper functioning of the brain, heart and immune system (Hohn, 1999). Millions of human beings suffer due to hunger and malnutrition. In order to meet the demand of food partly, fishes can be used as alternative source of food. *Channa punctatus* (Bloch), *Mystus tengra*, *Mystus vittatus* (Sykes), *Mystus bleekeri*, *Mastacembalus armatus*, *M. pancalus*, *Wallago attu* (Schn), *Heteropneustes fossilis* (Bloch), *C. striatus*, *Labio bata*, *Labio rohita*, *Clarias batrachus* (Linn) *and Anabas testudineus* are one of the common freshwater fish species of Uttar Pradesh, abundantly found in River Gomti.

Channa punctatus (Bloch, 1793) is a freshwater snake – headed spotted murrel belonging to the family Channidae of the order Channiformes and has accessory respiratory organs that help the fish to survive in inhospitable situations. *Channa*

punctatus can very well be termed as 'lean' fish because of its very low lipid content throughout the year. It is a carnivorous fish, voraciously a predatory to small fishes and fries. It has great economic importance because it is eaten as food and has good commercial value. *Heteropneustes fossilis* (Bloch) is an indigenous air-breeding catfishes belonging to the family Heteropneustidae of the order Siluriformes. It is very popular and highly valued of the cat fishes in India. It is omnivorous in nature. It is not only recognized for its delicious taste and market value but is also highly venerated for nutritional and medicinal aspect. The species has very high content of iron (226 mg per 100 g) and fairly high content of calcium in comparison to many other freshwater fishes. *Mystus vittatus* (Bloch, 1794) commonly known as Asian striped dwarf catfish is a freshwater fish species belonging to the Bagridae family and order Siluriformes. *Mystus vittatus* is an important target fish for small-scale fisherman. This small, indigenous fish species has a good demand as a food fish in fish markets as it has high nutritional value in terms of protein, micronutrients, vitamins and minerals. Recently it has also got its entry in ornamental fish markets of India and has been reported to have moderate export price too. *Mastacembelus pancalus* (Lac.) is belonging to the Mastacembelidae family. These fishes are considered to be the most delicious fish in Uttar Pradesh due to the tenderness of their muscles. About 80% population is poor in the country and they depend on small size fish for their daily supply of animal protein as they are available at reasonable price. They inhabit a good number of metazoan parasites. These parasites situated in this fish as a metacercarial stages are known to cause yellow grubs in the muscles and other organs of the fish and make them inappropriate for human consumption and *Wallago attu* (Bloch and Schneider, 1801) is known as the freshwater shark belonging to the Siluridae family of the order siluriformes. *Wallago attu* or Padhan'', as it is known locally in India, has been studied extensively in India as well as in the rest of the Asian countries for its helminth parasites. *Wallago attu* is a fast growing catfish species. This fish is an excellent food fish, famous for rapid growth and rich in nutritional quality of its flesh (Goswami & Devraj, 1992; Lilabati & Viswanath, 1996). It is also popular as a sport fish and recently has also made its entry in ornamental fish markets.

Keeping all the above perspectives in mind, the present study has been undertaken with a view to obtaining quantitative data on prevalence and other population

dynamics of helminth fauna of five freshwater fishes, particularly *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu* consumed by the rural population and commonly available in abundance all-round the year in the freshwater in and around River Gomti, Lucknow of Uttar Pradesh.

Materials and Methods

Fishes was collected from different regions of River Gomti, in Lucknow and maintained in the laboratory under ideal conditions. The weight and length of each animal was recorded prior to experimentation.

Study Area:

Different sites of the Gomti River where food fishes are captured such as Daliganj, Hanuman Setu, Pakka Pul, Nishatganj Pul, Gaughat and Gomti barrage were visited for obtaining the animals for study.

Survey and collection of food fishes:

Fresh and preferably live specimens of five common food fishes i.e. *Channa punctatus*, *Heteropneustus fossilis*, *Mystus vittatus*, *Mastacembalus Pancalus*, and *Wallago attu* of all sizes and weights were obtained from different sites such as Gaughat, Pakka Pul, Daliganj, Hanuman Setu, Gomti barrage, Nishantganj Pul in river Gomti at Lucknow in the years 2013 and 2014. The specimens were brought to the laboratory alive in a small container with water and maintained in glass aquaria. The fishes were identified using standard keys and acclimatized to standard laboratory conditions before being subjected to parasitic, haematological, and biochemical investigations.

Dissection of the fishes and collection of helminthes parasites:

The host fish was anaesthetized by clove-oil or by cutting at the neck region. A slit was made on ventral side near the genital pore or anal region and the fish was opened towards the head up to the opercular region. Before dissection, the Total Length (TL), Standard Length (SL), and weight (w) of the fishes were recorded using stainless steel scale and weighing machine respectively. Each fish was dissected with fine scissors used to make an incision and the sex was determined after examining the reproductive organs.

The surface of the visceral organs and body cavities and serous membranes were examined for encysted larvae and parasites by using hand-lens. Thereafter all the

organs were removed carefully and intact from the body and kept in petridishes with 0.9% saline (NaCl) solution. After separating the internal organs viz., stomach, intestine, liver, kidney and spleen each of the organs and the body cavity were examined individually for infection by parasites. The stomach and intestines were carefully opened by an incision and were then shaken to dislodge the parasites that might remain attached to the lining of the epithelium by their head ends. The epithelial layers of the stomach and intestine were scraped with a scalpel to remove any parasite that might remain attached to the layers.

The collected parasites were then washed with fresh physiological saline solution. Sometimes larger helminths were visible with naked eyes lying in the body cavity with their heads buried in the intestines and were quickly isolated using forceps. The parasite number and place of their attachment were also recorded during the experiment.

Fixation and preparation of helminthes parasites:

Fishes showed different stages of helminth parasites as cyst, larvae, and adult forms of Trematode, Tematode, Cestode and Acanthocephalan. Digenean Trematodes and Acanthocephalan parasites collected from the different organs were fixed in AFA solution (alchohol-85 ml, formaline-10ml and acetic acid-5ml) by keeping for 12-24 hours depending on the parasite. Each parasite individually was kept on a plain slide, covered with coverslip and slight pressure exerted on the coverslip to press the specimen slightly and finally kept in coupling jar filled with AFA solution for fixation.

Trematodes

The fixed trematode parasites were thoroughly washed with water and then transferred to descending grades 70% alcohol, 50% alcohol and 30% of alcohol for 5-10 minutes in each grade. Staining was done with Borax Carmine for 5-10 minutes and the specimen was checked from time to time because thicker parasites usually take more time to absorb the stain. Two drops of acid alcohol in 70% alcohol was then added to remove excess stain. Then the specimen was dehydrated using different grades of alcohol: placed in 30% alcohol then transferred to 50% and 70% alcohol

then in 90% and finally in absolute alcohol for 5-10 minutes each grades. Dehydrated parasites were cleared in xylene for 5 minutes and then specimen was kept on a clean slide and mounted in DPX, covered with the help of clean coverslip. The prepared specimen was examined under the microscope and identified using standard keys (Yamagutti, 1958).

Cestodes

Cestodes were collected in normal (0.9%) saline and then washed with hot water. The individual specimen was cut into different regions viz. the scolex, immature and gravid segments and individually placed on a clean slide and pressed with another slide by exerting pressure till the parasite became flattened. The slides bearing the parasite (flattened) were tied together with a thread and kept in coupling jar filled with Carnoy's fixative (75ml ethyl alcohol+ 25ml glacial acetic acid). The specimen was then transferred to grades 70% alcohol, 50% alcohol, 30% of alcohol for 5-10 minutes in each grade. After dehydration the specimen was stained with Borax Carmine for 5 minutes. The specimen was checked from time to time because thicker parasites usually take more time to absorb the stain. Two drops of acid alcohol in 70% alcohol was used to remove excess stain. Then the specimen was dehydrated through ascending grades of alcohol: placed in 70% alcohol for 15-20 minutes then transferred to 90% and 100% alcohol (2 changes) with 10 -20 minutes in each grade. Finally, the specimen was cleaned in xylene (5 minutes) and kept on a clean glass slide and mounted in DPX. The prepared slide was observed under microscope and identified using standard keys (Yamagutti, 1961).

N**ematodes**

Nematodes were collected in normal physiological saline, then washed with water and fixed in 70% alcohol. Nematode larvae were preserved in a solution of 5 parts of glycerine and 95 parts of 70% alcohol. Larger nematodes were fixed in formoalcohol and small amount of glycerine, i.e., 70 parts of 70% alcohol, 10 parts of 10% formalin and 5 parts of glycerine. Nematodes were commonly preserved in a mixture of 70% alcohol and glycerine with the ratio of 1:1. These were cleared in lactophenol. Preserved nematode was kept on a clean slide in a drop of glycerine and a drop of lactophenol was added on to the specimen and finally covered with a glass coverslip.

The prepared slide was observed under microscope and identified using standard keys (Yamagutti, 1961).

Acanthocephalan

The fixed Acanthocephalan parasites were thoroughly washed with water and then transferred through descending grades of alcohol viz., 70% alcohol, 50% alcohol and 30% alcohol for 5-10 minutes each. Staining was done with Borax Carmine for 5 minutes with checking of the specimen from time to time because thicker parasites usually take more time to absorb the stain. Two drops of acid alcohol in 70% alcohol was then added to remove excess stain. Then specimen was dehydrated using different grades of alcohol placed in 30% alcohol then transferred to 50% alcohol, 70% alcohol, 90% alcohol and finally in absolute alcohol for 5-10 minutes each. Dehydrated parasites were cleared in xylene for 5 minutes and then specimen was kept on a clean slide. A drop of DPX was put over the specimen covered with the help of clean coverslip. The prepared slide was observed under compound microscope and identified using standard keys (Yamagutti, 1963).

Identification of parasites:

As the study involved investigations on population dynamics of the infected by helminths, the parasites were identified only up to the class level (Trematoda, Cestoda, Nematoda and Acanthocephala) by the method of Yamaguti, Vol. I, (1958), Vol. II, (1961), Vol. III, (1961) and Vol. V (1963).

Population studies of Helminth parasitic infection in host fishes.

To study different parameters of parasitic infection in the selected food fishes i.e. *Channa punctatus*, *Heteropneustus fossilis*, *Mystus vittatus*, *Mastacembalus Pancalus*, and *Wallago attu*. The different parameters of population dynamics such as incidence of infection, intensity of infection and density of infection was recorded and calculated as given below by following Margolis *et al*., (1982).

Prevalence:

It is also denoted as incidence of infection in the host (fish). The Prevalence was calculated by the formula:

$$\text{Prevalence} = \frac{\text{No. of Host infected}}{\text{Total Host examined}} \times 100$$

Intensity of Infection:

This is the mean number of parasites found in the infected host (fish). It was calculated by using the following formula:

$$\text{Intensity of infection} = \frac{\text{No. of parasites collected in a sample}}{\text{No. of infected host}}$$

Density of Infection:

It is also known as abundance of the parasites in the host (fish). It was measured by the formula:

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

Results

A. Population Dynamics of Helminth Parasites in Fresh Water Fishes

The present study has been designed to obtain quantitative data on helminth fauna of freshwater fishes commonly consumed, as well as available in abundance, all-round the year in the freshwater in and around River Gomti, Lucknow of Uttar Pradesh. The fish specimens were brought in the laboratory alive, identified, maintained in glass aquaria and acclimatized to standard laboratory conditions before being dissected for parasitic investigations.

a. Prevalence of total helminth parasites in the selected fresh water fish

A total of 1491 fishes belonging to genus *Channa punctatus*, *Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu* were dissected for parasitic examination, out of which, 293 fishes were found to be infected with different helminth parasites. A total of 156 out of 455 *C. punctatus*, 58 out of 394 *H. fossilis*, 36 out of 263 *M. vittatus*, 25 out of 227 *M. pancalus* and 18 out of 152 *W. attu* were found to be infected with the helminth parasites. The highest prevalence (34.28%) was observed in *C. punctatus*, followed by *H. fossilis* (14.72%), *M. vittatus*, (12.28%), *W. attu* (11.84%) and *M. pancalus* (11.01%) (Table 1).

Table 1: Overall prevalence of infection in the selected hosts.

Examined host fishes	Total no. of host examined	Total no. of infected host	Prevalence (%)
Channa punctatus	455	156	34.28
Heteropneustes fossilis	394	58	14.72
Mystus vittatus	263	36	12.28
Mastacembalus pancalus	227	25	11.01
Wallago attu	152	18	11.84
Total	1491	293	19.65

b. Microhabitat Distribution of the Helminth Parasites in the selected freshwater fishes

The parasites collected from different body parts of fishes were belonged to four major classes of helminth parasites i.e. Cestoda, Trematoda, Nematoda and

Acanthocephala. The Cestodes parasites were collected from intestine only in all the fishes. Trematode parasites were collected from liver, stomach, intestine, body cavity of *C. punctatus*, Muscles of *H. fossilis*, swim bladder of *M. vittatus*, Oesophagus, body cavity, intestine, swim bladder, stomach of *M. pancalus* and swim bladder of *W. attu*. Nematode parasites were collected from intestine, air bladder and body cavity of *C. punctatus* and intestine of *H. fossilis*, *M. vittatus* and *W. attu*. Acanthocephalan parasites were collected from intestine of *C. punctatus* and *M. vittatus* only. Alimentary canal of the host was found to be highly infected with helminth parasites, followed by swim bladder and body cavity.

Table 2: Microhabitat distribution of the helminth parasites in the selected fresh water fishes.

Examined Host Fishes	Examined Parasites	Infected organs
Channa punctatus	Cestode	Intestine
	Trematode	Liver, Stomach, Intestine, Body cavity
	Nematode	Intestine, Swim bladder, Body cavity
	Acanthocephala	Intestine
Heteropneustes fossilis	Cestode	Intestine
	Trematode	Muscles
	Nematode	Intestine
Mystus vittatus	Cestode	Intestine
	Trematode	Swim bladder
	Nematode	Intestine
	Acanthocephala	Intestine
Mastacembalus pancalus	Cestode	Intestine
	Trematode	Oesophagus, Body cavity, Intestine, Swim Bladder, Stomach
Wallago attu	Cestode	Intestine
	Trematode	Swim bladder
	Nematode	Intestine

c. Studies on the overall prevalence of different groups of helminth parasites in selected fresh water fishes

In the present study of two years (2013 and 2014) all the four major groups of helminth parasites i.e. Cestoda, Trematoda, Nematoda and Acanthocephala were found in *C. punctatus* and *M. vittatus* only. In *H. fossilis* and *W. attu* Cestode, Trematode and Nematode parasites were found while Acanthocephalan parasite was not observed at all. While in *M. pancalus*, only Cestode and Trematode parasites were found while Nematode and Acanthocephalan were absent.

The prevalence, abundance and mean intensity of different group of parasites in selected fresh water fishes is depicted in Table 3. In *Channa punctatus*, the most prevalent (16.04%) parasite was found to be Acanthocephalan, followed by Trematode (12.08%), Nematode (4.39%) and Cestode (1.7%). However, Trematode parasites were found to be most abundant (Abundance: 0.37) and the least abundant parasitic group was found to be Cestode (Abundance: 0.01) parasites.

In *Heteropneustes fossilis*, Trematode parasites were found to be the most prevalent (9.89%) and abundant (0.52). The Trematode group was followed by Nematode (Prevalence: 2.79%; Abundance: 0.03) and Cestode (Prevalence: 2.03%; Abundance: 0.02). Acanthocephalan was not found at all in *H. fossilis*.

In *Mystus vittatus*, Trematode parasites were found to be the most prevalent (6.84%) and abundant (0.17). It was followed by Nematode (Prevalence: 2.66%; Abundance: 0.03), Acanthocephalan (Prevalence: 2.28%; Abundance: 0.03) and Cestode (Prevalence: 0.76%; Abundance: 0.007) parasites.

In *Mastacembalus pancalus* Trematode parasites were found to be the most prevalent (8.37%) and abundant (0.21), followed by Cestode (Prevalence: 2.64%; Abundance: 0.02). Nematode and acanthocephalan parasites were not found at all in these fishes during the study period.

In *Wallago attu*, Trematode parasites were found to be the most prevalent (4.60%) and abundant (0.11), followed by Cestode (Prevalence: 3.94%; Abundance: 0.03) and Nematode (Prevalence: 3.28%; Abundance: 0.03) parasites. Acanthocephalan parasites were not found at all in *W. attu.*

The highest prevalence (3.94%) of Cestode parasites was observed in *W. attu* and least (0.75%) in *M. vittatus.* Trematode parasites were found highest (12.7%) in *C. punctatus* and least (4.60%) in *W. attu*. Nematode parasites were found maximum (4.61%) in *C. punctatus* and least (2.66%) in *M. vittatus.* It was not found at all in *M. pancalus*. Acanthocephalan parasites were found in *C. punctatus* (16.9%) and *M. vittatus* (2.28%) only. It was not found at all in *M. pancalus, H. fossilis* and *W. attu.*

Table 3: Prevalence, Abundance and Mean Intensity of different group of parasites in selected fresh water fishes

Species	No of fish examined	No of fish infected	No. of parasites	Isolated parasites (Class)	Prevalence %	Intensity	Density
Channa punctatus	455	8	9	Cestode	1.7	1.12	0.01
		55	170	Trematode	12.08	3.09	0.37
		20	32	Nematode	4.39	1.6	0.07
		73	168	Acanthocephala	16.04	2.30	0.36
Heteropneustes fossilis	394	8	8	Cestode	2.03	1	0.02
		39	208	Trematode	9.89	5.33	0.52
		11	14	Nematode	2.79	1.27	0.03
		00	00	Acanthocephala	00	00	00
Mystus vittatus	263	2	2	Cestode	0.76	1	.007
		18	45	Trematode	6.84	2.5	0.17
		7	8	Nematode	2.66	1.14	0.03
		6	8	Acanthocephala	2.28	1.33	0.03
Mastacembalus pancalus	227	6	6	Cestode	2.64	1	0.02
		19	48	Trematode	8.37	2.52	0.21
		00	00	Nematode	00	00	00
		00	00	Acanthocephala	00	00	00
Wallago attu	152	6	6	Cestode	3.94	1	0.03
		7	17	Trematode	4.60	2.42	0.11
		5	6	Nematode	3.28	1.2	0.03
		00	00	Acanthocephala	00	00	00

B. Monthly Variation in Helminth Infection in *Channa punctatus*

a) Monthly variation of total helminth infection in *Channa punctatus*

Month wise prevalence of the helminth parasites was also recorded in both years of the study and shown in Table 4. During 2013, the maximum prevalence i.e. 80.0% was recorded in the month of April, while the least prevalence i.e. 20.8% was recorded in the month of November (Figure 2**)**. The intensity (8.0) was recorded maximum in August whereas minimum intensity (1.41) was reported in March (Figure 3). In the present investigation, highest density i.e. 2.0 was recorded in the month of August whereas lowest density of 0.45 was recorded in November (Figure 4).

During 2014, the highest percentage of helminth infection (45%) in fish was observed during the month of July and the least prevalence of the parasite (7.14%) was recorded in November (Figure 2). The maximum intensity of helminth parasites in fish *C. punctatus* was recorded as (4.44) in the month of July and minimum (1.33) in the month of December (Figure 3). The highest density of parasites (2.0) was observed in July whereas the minimum density of infection was recorded as 0.14 in the month of November (Figure 4).

b) Monthly variation of different groups of helminth parasitic infection in *Channa punctatus*

The fish *C. punctatus* were found to be heavily infected by helminth parasites belonging to the classes Cestoda, Trematoda, Nematoda and Acanthocephala. In the year 2013, a total of 248 helminth parasites were collected from the 102 infected fish of *C. punctatus*. The monthly variation of different groups of helminth parasitic infection in C*hanna punctatus* in the year 2013 was recorded and shown in Table 5.

The maximum prevalence (10%) of Cestode parasites in fish *C. punctatus* was found in the month of March whereas the minimum prevalence (5%) was recorded in July. The fishes were found to be free of infection with Cestode parasites for most months of the year viz., January, February, April, May, June, August, September, October and November (Figure 5). In the months of March, July and December (maximum intensity of infection =1.0) the fish were found to be infected by the

Cestode parasites only (Figure 6). In fish *C. punctatus*, the density of Cestode parasitic infection was recorded at peak (0.1) in the month of March whereas minimum density of infection i.e. (0.05) was recorded in month of July (Table 5) (Figure 7).

In case of infection by Trematodes, the maximum prevalence (45%) in *C. punctatus* was recorded in the month of April whereas the minimum prevalence (8.33%) was recorded in the month of November (Figure 5). The infection was not reported in the months of May and June, 2013. The maximum intensity of infection (9.75) was found in month of August whereas the minimum intensity of (1.0) was observed in the month of March (Figure 6). The density of infection with Trematode parasites was found highest (1.95) in the month of August whereas minimum density (0.12) of Trematode infection was reported in the month of November (Table 5, Figure 7).

The prevalence of Nematode parasites in fish *C. punctatus* was recorded highest (25%) in month of July whereas minimum prevalence (4.16%) of the parasites was recorded in month of November (Figure 5). The infection was not reported during the months of January, February, April, May, August, and October. The maximum intensity of infection (1.0) was observed in month of March, September and December (Figure 6). The maximum density of infection (0.35) was recorded in month of June whereas the minimum density of infection (0.05) was recorded in months of March and September (Table 5, Figure 7).

Studies on the prevalence of Acanthocephalan parasites in fish *C. punctatus*, showed that it had highest prevalence (35%) in the month of April and least (5%) in the month of August (Figure 5). The maximum intensity of infection (9.0) was observed in the month of December whereas minimum intensity of infection (1.0) was recorded in the months of July and August (Figure 6). The maximum density of infection (1.12) of acanthocephalan parasites was recorded in the month of December and least density of infection (0.05) was observed in the month of August (Table 5, Figure7).

The monthly variation of different groups of helminth parasitic infection in C*hanna punctatus* in the year 2014 was recorded and shown in Table 6.

In the year 2014, a total of 215 helminth parasites were collected from the 50 infected fish of *C. punctatus*. Overall Incidence of infection in fish with Cestode, Trematode, Nematode and Acanthocephalan parasites was found to be 1.86%, 6.51%, 3.72% and 13.0% respectively. The fish were found to be highly infected with Acanthocephalan parasites and least with Cestode parasites.

The maximum prevalence (8.3%) of Cestode parasites was recorded in the month of December whereas the minimum prevalence (5%) was recorded during the month of March, April and July (Figure 5). The infection of Cestode parasites was not reported in the months of January, February, May, June, August, September, October and November. The maximum intensity of Cestode infection (2.0) was recorded in month of April and minimum intensity of infection (1.0) was recorded in months of March, July and December (Figure 6). The maximum density of infection (0.10) was recorded in the month of April whereas the minimum density of infection (0.05) was recorded in months of March and July (Table 6, Figure 7).

The maximum prevalence (15%) of trematode parasites in fish *C. punctatus* was recorded in the month of July and minimum (5%) in the months of February, March and April. The infection of trematode parasites in fish was not reported in the months of June, August and November (Figure 5). The maximum intensity of trematode infection (6.6) was recorded in the month of July and minimum intensity (1.0) was recorded in the months of April and September (Figure 6). The maximum density (1.0) of trematode infection was observed in the month of July and minimum density of infection (0.05) found during the month of April (Table 6, Figure 7).

The prevalence of Nematode parasites in fish *C. punctatus* was recorded highest (10%) in the month of April whereas minimum prevalence of Nematode parasites (4.7%) was recorded in the month of October. The infection of Nematode parasites in fish was not reported during the months of May, July, September, November and December (Figure 5). The maximum intensity (2.0) of Nematode infection was recorded in the months of January, February, March and October whereas the minimum intensity of infection (1.0) was recorded during the months of June and August (Figure 6). The maximum density of infection (0.15) was recorded in the month of April and minimum density of infection (0.05) was recorded in the month of June (Table 6, Figure 7).

The maximum prevalence (25%) of Acanthocephalan parasites in fish *C. punctatus* was recorded in the month of July whereas the minimum prevalence (7.1%) of parasites was found in the months of August, September and November (Figure 5). The maximum intensity (4.0) of infection by Acanthocephalan parasites was recorded during the month of September whereas the minimum intensity of infection (1.0) was recorded in the month of December (Figure 6). The maximum density (0.95) of infection by Acanthocephalan parasites was found in the month of July and least density of infection (0.08) was observed in the month of December (Table 6, Figure 7).

Table 4: Monthly variation of total helminth parasitic infection in *Channa punctatus*.

Month	Study Period: 2013						Study Period: 2014					
	No. of host examined	**Total no. of host infected**	**No. of parasites collected**	**Prevalence %**	**Intensity**	**Density**	**No. of host examined**	**Total no. of host infected**	**No. of parasites collected**	**Prevalence %**	**Intensity**	**Density**
January	20	09	17	45	1.88	0.85	20	05	08	25	1.6	0.4
February	20	08	15	40	1.87	0.75	20	04	12	20	3.0	0.6
March	20	12	17	60	1.41	0.85	20	07	15	35	2.1	0.75
April	20	16	37	80	2.31	1.85	20	08	15	40	1.87	0.75
May	20	06	14	30	2.33	0.70	20	05	12	25	2.4	0.60
June	20	07	12	35	1.71	0.60	20	03	06	15	2.0	0.3
July	20	13	26	65	2.00	1.3	20	09	40	45	4.44	2.0
August	20	05	40	25	8.00	2.0	14	02	03	14.2	1.5	0.21
September	20	05	14	25	2.8	0.7	14	02	05	14.2	2.5	0.35
October	20	10	16	50	1.6	0.8	21	05	09	23.8	1.8	0.42
November	24	05	11	20.8	2.2	0.45	14	01	02	7.1	2.0	0.14
December	16	06	29	37.5	4.83	1.81	12	03	04	25.0	1.33	0.33

Table 5: Monthly variation of different groups of helminth parasitic infection in *Channa punctatus* in 2013

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2013	Cestode	20	09	00	00	00	00	00
	Trematode			05	08	25	1.6	0.4
	Nematode			00	00	00	00	00
	Acanthocephala			04	09	20	2.25	0.45
Feb. 2013	Cestode	20	08	00	00	00	00	00
	Trematode			05	08	25	1.6	0.4
	Nematode			00	00	00	00	00
	Acanthocephala			03	07	15	2.3	0.35
Mar. 2013	Cestode	20	12	02	02	10	1	0.1
	Trematode			03	03	15	1	0.15
	Nematode			01	01	5	1	0.05
	Acanthocephala			06	11	30	1.8	0.55
Apr. 2013	Cestode	20	16	00	00	00	00	00
	Trematode			09	27	45	3	1.35
	Nematode			00	00	00	00	00
	Acanthocephala			07	10	35	1.42	0.5
May 2013	Cestode	20	06	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			06	14	30	2.33	0.7
Jun. 2013	Cestode	20	07	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			03	07	15	2.3	0.35
	Acanthocephala			04	05	20	1.25	0.25
Jul. 2013	Cestode	20	13	01	01	5	1	0.05
	Trematode			03	15	15	5	0.75
	Nematode			05	06	25	1.2	0.3
	Acanthocephala			04	04	20	1	0.2
Aug. 2013	Cestode	20	05	00	00	00	00	00
	Trematode			04	39	20	9.75	1.95
	Nematode			00	00	00	00	00
	Acanthocephala			01	01	5	1	0.05
Sep. 2013	Cestode	20	05	00	00	00	00	00
	Trematode			02	06	10	3	0.3
	Nematode			01	01	5	1	0.05
	Acanthocephala			02	07	10	3.5	0.35
Oct. 2013	Cestode	20	10	00	00	00	00	00
	Trematode			06	11	30	1.83	0.55
	Nematode			00	00	00	00	00
	Acanthocephala			04	05	20	1.25	0.25
Nov. 2013	Cestode	24	05	00	00	00	00	00
	Trematode			02	03	8.33	1.5	0.12
	Nematode			01	02	4.16	2	0.08
	Acanthocephala			02	06	8.33	3	0.25
Dec. 2013	Cestode	16	06	01	01	6.25	1	0.06
	Trematode			02	08	12.5	4	0.5
	Nematode			01	02	6.25	2	0.12
	Acanthocephala			02	18	12.5	9	1.12
Total		240	102	102	248	42.50	2.43	1.03

Table 6: Monthly variation of different groups of helminth parasitic infection in *Channa punctatus* in 2014

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2014	Cestode	20	05	00	00	00	00	00
	Trematode			02	03	10	1.5	0.15
	Nematode			01	02	05	02	0.10
	Acanthocephala			02	03	10	1.5	0.15
Feb. 2014	Cestode	20	04	00	00	00	00	00
	Trematode			01	03	05	03	0.15
	Nematode			01	02	05	02	0.10
	Acanthocephala			02	07	10	3.5	0.35
Mar. 2014	Cestode	20	07	01	01	05	01	0.05
	Trematode			01	04	05	04	0.20
	Nematode			01	02	05	02	0.10
	Acanthocephala			04	08	20	02	0.40
Apr. 2014	Cestode	20	08	01	02	05	02	0.10
	Trematode			01	01	05	01	0.05
	Nematode			02	03	10	1.5	0.15
	Acanthocephala			04	09	20	2.2	0.45
May 2014	Cestode	20	05	00	00	00	00	00
	Trematode			02	05	10	2.5	0.25
	Nematode			00	00	00	00	00
	Acanthocephala			03	07	15	2.3	0.35
Jun. 2014	Cestode	20	03	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	01	05	01	0.05
	Acanthocephala			02	05	10	2.5	0.25
Jul. 2014	Cestode	20	09	01	01	05	01	0.05
	Trematode			03	20	15	6.6	01
	Nematode			00	00	00	00	00
	Acanthocephala			05	19	25	3.8	0.95
Aug. 2014	Cestode	14	02	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	01	7.1	01	0.07
	Acanthocephala			01	02	7.1	02	0.14
Sep. 2014	Cestode	14	02	00	00	00	00	00
	Trematode			01	01	7.1	01	0.07
	Nematode			00	00	00	00	00
	Acanthocephala			01	04	7.1	04	0.28
Oct. 2014	Cestode	21	05	00	00	00	00	00
	Trematode			02	03	9.5	1.5	0.14
	Nematode			01	02	4.7	02	0.09
	Acanthocephala			02	04	9.5	02	0.19
Nov. 2014	Cestode	14	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			1	02	7.1	02	0.14
Dec. 2014	Cestode	12	03	01	01	8.3	01	0.08
	Trematode			01	02	8.3	02	0.16
	Nematode			00	00	00	00	00
	Acanthocephala			01	01	8.3	01	0.08
Total		215	54	54	131	25.11	2.42	0.60

C. Monthly Variation in Helminth infection in *Heteropneustes fossilis*

a) Monthly variation of total helminth infection in *Heteropneustes fossilis*

Month wise prevalence of the helminth parasites in *Heteropneustes fossilis* was also recorded in both years of the study and shown in Table 7. In 2013, the maximum prevalence (25%) was recorded in the month of June while the least prevalence (10%) was recorded in the months of January, April and October (Figure 8). The maximum intensity (6.75) was recorded in July whereas minimum intensity (1.0) was reported in the months of March, October and December (Figure 9). In the present investigation, the highest density (1.35) was recorded in the month of July whereas lowest density (0.10) was recorded in October (Figure 10).

In 2014, the highest percentage of helminth infection (33.33%) in this fish was observed during the month of July and April and the least prevalence of the parasite (10.0%) was recorded in January and June (Figure 8). The highest intensity (6.0) and density (2.0) of helminth parasites was recorded during the month of July whereas the minimum intensity (1.5) and density of infection (1.1) was recorded in the months of January and February respectively (Figure 9 and 10).

b) Monthly variation of different groups of helminth infection in *Heteropneustes fossilis*

The fish *H. fossilis* were found to be heavily infested by helminth parasites belonging to classes Cestoda, Trematoda and Nematoda. In the year 2013, a total of 102 helminth parasites were collected from the 33 infected fish of *H. fossilis*. The data of monthly variations of different groups of parasites in the year 2013 have been depicted in Table 8. The overall incidence of infection with cestode parasites in *H. fossilis* was found to be 1.79%, with trematode parasites 9.41%, with Nematode parasites 3.58%, while the infection with Acanthocephalan parasites in the fish was not reported in this year. Again, it was observed that the fish were highly infected with Trematode parasites and minimum with Cestode parasites.

The maximum prevalence (5.2%) of cestode parasites in fish *H. fossilis* was found in the month of March whereas the minimum prevalence (4.3%) was recorded in September and October (Figure 11). The fishes were found to be free of infection

with Cestode parasites for most of the months in the year 2013 *viz.*, January, February, April, June, July, August, October and December. In the months of March, May, September and November maximum intensity of infection (1.0) with Cestode parasites was recorded in the fish **(**Figure 12). The density of Cestode parasitic infection was recorded highest (0.05) in the months of March and May whereas minimum density of infection (0.04) was recorded in months of September and November (Table 8).

The prevalence of Trematode parasites in fish *H. fossilis* was recorded highest (25%) in month of June whereas the minimum prevalence (4.3%) was recorded in months of September and November (Figure 11). The maximum intensity of infection (12.0) was found in month of November whereas the minimum intensity of (1.0) was observed in the month of March and October **(**Figure 12). The density of infection with Trematode parasites was found highest (1.35) in the month of July whereas minimum density (0.05) of Trematode infection was reported in the month of March and October (Table 8).

The prevalence of Nematode parasites in fish *H. fossilis* was recorded highest (10%) in the month of August whereas minimum prevalence (4.3%) of the parasites was recorded in months of September and November (Figure 11). The infection was not reported during the months of January, February, March, June and July. The maximum intensity of infection (2.0) was observed in month of April and May whereas the minimum intensity of (1.0) was observed in the month of August, September, October, November and December (Figure 12). The maximum density of infection (0.10) was recorded in months of April, May and August whereas the minimum density of infection (0.04) was recorded in months of September and November (Table 8).

The fishes were found to be free from the infection of Acanthocephalan parasites in the year 2013.

The data on monthly variations of different groups of parasites in *H. fossilis* in the year 2014 have been depicted in Table 9. In the year 2014, a total of 106 helminth parasites were collected from the 25 infected fish of *H. fossilis*. Overall prevalence of infection in *H. fossilis* with Cestode, Trematode and Nematode parasites was found to be 2.3%, 10.52% and 1.75% respectively. The fishes were found to be free of

infection with Acanthocephalan parasites in the year 2014 also. The fish were found to be highly infected with Trematode parasites and least with Nematode parasites.

The maximum prevalence (10%) of Cestode parasites was recorded in the month of February whereas the minimum prevalence (6.6%) was recorded during the months of April, August and September (Figure 11). The infection of Cestode parasites was not reported in the months of January, March, June, July, October, November and December. The highest intensity of Cestode infection (1.0) was recorded in months of February, April, and September **(**Figure 12). The maximum density of infection (0.10) was recorded in the month of February whereas the minimum density of infection (0.06) was recorded in months of April, August and September (Table 9).

The prevalence of Trematode parasites in *H. fossilis* was recorded highest (33.3%) in the month of July and minimum prevalence (5%) was recorded in the months of January and February (Figure 11). The maximum intensity of Trematode infection (6.0) was recorded in the month of July and minimum intensity (2.0) was recorded in the month of January (Figure 12). The maximum density (2.0) of Trematode infection was recorded in the month of July and minimum density of infection (0.1) observed during January (Table 9).

The prevalence of Nematode parasites in *H. fossilis* was recorded highest (6.6%) in the month of March whereas minimum prevalence of Nematode parasites (5%) was recorded in the months of January and May (Figure 11). The infection of Nematode parasites in fish was not reported during the months of February, April, June, July, August, September, October, November and December. The maximum intensity (2.0) of Nematode infection was recorded in the month of May whereas the minimum intensity of infection (1.0) was recorded during the months of January and March **(**Figure 12). The maximum density of infection (0.50) was recorded in the month of January while minimum density of infection (0.06) was recorded in the month of March (Table 9, Figure 13).

The fishes were again found to be free of infection with Acanthocephalan parasites in the 2014.

Table 7: Monthly variation of total helminth parasitic infection in *Heteropneustes fossilis*

Month	Study Period: 2013						Study Period: 2014					
	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density
January	10	01	04	10	4	0.4	20	02	3	10	1.5	0.15
February	14	02	03	14.2	1.5	0.21	10	02	11	20	5.5	1.10
March	19	02	02	10.5	1.0	0.11	15	03	10	20	3.3	0.67
April	20	02	08	10.0	4.0	0.4	15	02	13	13.3	6.5	0.87
May	20	03	11	15.0	3.66	0.55	20	03	13	15	4.3	0.65
June	20	05	18	25.0	3.6	0.9	10	01	06	10	6.0	0.60
July	20	04	27	20.0	6.75	1.35	15	05	30	33.3	6.0	2.0
August	20	04	07	20.0	1.75	0.35	15	02	04	13.3	2.0	0.27
September	23	03	04	13.0	1.33	0.17	15	02	05	13.3	2.5	0.33
October	20	02	02	10.0	1.0	0.10	09	01	04	11.1	4.0	0.44
November	23	03	14	13.0	4.66	0.61	15	00	00	00	00	00
December	14	02	02	14.2	1.0	0.14	12	02	07	16.6	3.50	0.58

Table 8: Monthly variation of different groups of helminth parasitic infection in *Heteropneustes fossilis* during 2013

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2013	Cestode	10	01	00	00	00	00	00
	Trematode			01	04	10	04	0.4
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2013	Cestode	14	02	00	00	00	00	00
	Trematode			02	03	14.2	1.5	0.21
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2013	Cestode	19	02	01	01	5.2	1	0.05
	Trematode			01	01	5.2	1	0.05
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Apr. 2013	Cestode	20	02	00	00	00	00	00
	Trematode			01	06	05	06	0.30
	Nematode			01	02	05	02	0.10
	Acanthocephala			00	00	00	00	00
May 2013	Cestode	20	03	01	01	05	01	0.05
	Trematode			01	08	05	08	0.4
	Nematode			01	02	05	02	0.10
	Acanthocephala			00	00	00	00	00
Jun. 2013	Cestode	20	05	00	00	00	00	00
	Trematode			05	18	25	3.6	0.9
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jul. 2013	Cestode	20	04	00	00	00	00	00
	Trematode			04	27	20	6.75	1.35
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2013	Cestode	20	04	00	00	00	00	00
	Trematode			02	05	10	2.5	0.25
	Nematode			02	02	10	1	0.1
	Acanthocephala			00	00	00	00	00
Sep. 2013	Cestode	23	03	01	01	4.3	01	0.04
	Trematode			01	02	4.3	02	0.08
	Nematode			01	01	4.3	01	0.04
	Acanthocephala			00	00	00	00	00
Oct. 2013	Cestode	20	02	00	00	00	00	00
	Trematode			01	01	05	01	0.05
	Nematode			01	01	05	01	0.05
	Acanthocephala			00	00	00	00	00
Nov. 2013	Cestode	23	03	01	01	4.3	01	0.04
	Trematode			01	12	4.3	12	0.52
	Nematode			01	01	4.3	01	0.04
	Acanthocephala			00	00	00	00	00
Dec. 2013	Cestode	14	02	00	00	00	00	00
	Trematode			01	01	7.1	01	0.07
	Nematode			01	01	7.1	01	0.07
	Acanthocephala			00	00	00	00	00
Total		223	33		102	14.79	3.09	0.45

Table 9: Monthly variation of different groups of helminth parasitic infection in *Heteropneustes fossilis* during 2014

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2014	Cestode	20	02	00	00	00	00	00
	Trematode			01	02	05	02	0.10
	Nematode			01	01	05	01	0.50
	Acanthocephala			00	00	00	00	00
Feb. 2014	Cestode	10	02	01	01	10	01	0.10
	Trematode			01	10	10	10	01
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2014	Cestode	15	03	00	00	00	00	00
	Trematode			02	09	13.3	4.5	0.6
	Nematode			01	01	6.6	01	0.06
	Acanthocephala			00	00	00	00	00
Apr. 2014	Cestode	15	02	01	01	6.6	01	0.06
	Trematode			01	12	6.6	12	0.8
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
May 2014	Cestode	20	03	00	00	00	00	00
	Trematode			02	11	10	5.5	0.55
	Nematode			01	02	05	02	0.1
	Acanthocephala			00	00	00	00	00
Jun. 2014	Cestode	10	01	00	00	00	00	00
	Trematode			01	06	10	06	0.6
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jul. 2014	Cestode	15	05	00	00	00	00	00
	Trematode			05	30	33.3	06	02
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2014	Cestode	15	02	01	01	6.6	01	0.06
	Trematode			01	03	6.6	03	0.2
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Sep. 2014	Cestode	15	02	01	01	6.6	01	0.06
	Trematode			01	04	6.6	04	0.2
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Oct. 2014	Cestode	09	01	00	00	00	00	00
	Trematode			01	04	11.1	04	0.4
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Nov. 2014	Cestode	15	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Dec. 2014	Cestode	12	02	00	00	00	00	00
	Trematode			02	07	16.6	3.5	0.58
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Total		171	25		106	14.61	4.24	0.61

D. Monthly Variation in Helminth infection in *Mystus vittatus*

a) Monthly variation of total helminth infection in *Mystus vittatus*

Month wise prevalence of the helminth parasites in *M. vittatus* was also recorded in both years of the study and shown in Table 10. In 2013, the highest prevalence (30%) was recorded in the month of May while the least prevalence (5.88%) recorded in the month of August (Figure 14). The maximum intensity (3.0) was recorded in August whereas minimum (1.0) was reported in months of February, June, July, and November (Figure 15). In the present investigation, highest density (0.6) was recorded in the month of May whereas lowest density (0.06) was observed in November (Figure 16).

In 2014, the highest percentage of helminth infection (27.27%) in *M. vittatus* was observed during the month of December and the least prevalence of the parasite (10%) was recorded in the months of March, May and October (Figure 14). The infection was not reported during the months of January, September and November. The maximum intensity and density of helminth parasites in *M. vittatus* was recorded as 4.0 and 0.40 during March and July respectively whereas the minimum intensity and density of infection was recorded as 1.0 and 0.2 in the months of December and May respectively (Figure 15 and 16).

b) Monthly variation of different groups of helminth infection in *Mystus vittatus*

The fish *M. vittatus* was found heavily infested by different classes of helminth parasites *viz.* Cestoda, Trematoda, Nematoda and Acanthocephala. In the year 2013, a total of 33 helminth parasites were collected from the 19 infected fish of *M. vittatus*. The overall Incidence of infection with Cestode parasites was found to be 0.71%, 8.57% with trematode parasites, 2.14% with nematode and Acanthocephalan parasites. It was observed that, the fish were highly infected with Trematode parasites and minimum with Cestode parasites.

The data of monthly variations of different groups of parasites in the year 2013 have been depicted in Table 11. The maximum prevalence (6.66%) of Cestode parasites in *M. vittatus* was found in the month of July whereas the fishes were found to be free of infection with cestode parasites for most of the months in 2013 *i.e.* January, February, March, April, May, June, August, September, October November

and December (Figure 17). In the month of July intensity of infection (1.0) with Cestode parasites was recorded maximum (Figure 18). The highest density of Cestode infection (0.06) was recorded in months of March and July (Table 11, Figure 19).

The prevalence of Trematode parasites in fish *M. vittatus* was recorded highest (20%) in May whereas the minimum prevalence (5.88%) was recorded in August. The infection was not reported in the month of June and November (Figure 17). The maximum intensity of infection (3.0) was found in the months of March, August and September whereas the minimum intensity (1.0) was observed in the months of February and July (Figure 18). The density of infection with Trematode parasites was found highest (0.5) in the month of May whereas minimum density (0.06) of Trematode infection was reported in the month of July (Table 11, Figure 19).

The prevalence of Nematode parasites in fish *M. vittatus* was recorded highest (12.5%) in February whereas minimum prevalence (6.25%) of the parasites was recorded in November (Figure 17). The infection was not reported during the months of January, March, April, August, September, October and December. The intensity of infection (1.0) was observed at peak in February, May and November (Figure 18). The maximum density of infection (0.12) was recorded in month of February whereas the minimum density of infection (0.06) was recorded in month of November (Table 11, Figure 19).

In fish *M.vittatus*, the prevalence of acanthocephalan parasites was found highest (12.5%) in the months of March and June and least prevalence (6.66%) was recorded in September (Figure 17). The intensity of infection (1.0) was observed in the month of March, June and September (Figure 18). The maximum density of infection (0.10) of Acanthocephalan parasites was recorded in the month of March and June and least density of infection (0.06) was observed in the month of September (Table 11, Figure 19).

In the year 2014, a total of 30 helminth parasites were collected from the 17 infected fish of *M. vittatus*. Again, the overall Incidence of infection in fish with Cestode, Trematode, Nematode and Acanthocephalan parasites was found to be 0.81%, 4.87%, 3.25% and 2.43% respectively. The fish were found to be highly infected with trematode parasites and least with Cestode parasites. The data of monthly variations of different groups of parasites in the year 2014 have been depicted in Table 12.

The highest prevalence (10%) of Cestode parasites was recorded in the month of February. The infection of Cestode parasites was not reported in the months of January, March, April, May, June, July, August, September, October November and December (Figure 17). The highest intensity of Cestode infection (1.0) was recorded in month of February (Figure 18). The density of infection (0.10) was recorded at peak in the month of February (Table 12, Figure 19).

The prevalence of Trematode parasites in fish *M. vittatus* was recorded highest (20%) in month of July and minimum (7.6%) in the month of April (Figure 17). The infection of Trematode parasites in fish was not reported in the months of January, May, August, September and November. The maximum intensity of Trematode infection (4.0) was recorded in the month of March and minimum intensity (1.0) in December (Figure 18). The maximum density (0.4) of Trematode infection was recorded in the month of March and minimum (0.09) during the month of December (Table 12, Figure 19).

The prevalence of Nematode parasites in fish *M. vittatus* was recorded highest (9.09%) in the month of December whereas minimum prevalence of Nematode parasites (6.6%) was recorded in the month of July (Figure 17). The infection of nematode parasites in fish was not reported during the months of January, February, March, May, August, September, October and November. The maximum intensity (2.0) of nematode infection was recorded in the month of July whereas the minimum intensity of infection (1.0) was recorded during the months of April, June and December (Figure 18). The maximum density of infection (0.13) was recorded in the month of July and minimum (0.07) in the month of April (Table 12, Figure 19).

The prevalence of Acanthocephalan parasites in fish *M. vittatus* was recorded highest (11.1%) in the month of August whereas the minimum prevalence (9.09%) was reported in the month of December (Figure 17). The maximum intensity (2.0) of infection by Acanthocephalan parasites was recorded during the months of May and August whereas the minimum intensity of infection (1.0) was recorded in the month of December (Figure 18). The maximum density of infection (0.20) by Acanthocephalan parasites was found in the months of May and August and least (0.09) was recorded in the month of December (Table 12, Figure19).

Table 10: Monthly variation of total helminth parasitic infection in *Mystus vittatus*

Month	Study Period: 2013						Study Period: 2014					
	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density
January	08	01	02	12.5	2.0	0.25	05	00	00	00	00	00
February	08	02	02	25	1.0	0.25	10	02	03	20	1.5	0.30
March	08	02	04	25	2.0	0.50	10	01	04	10	4.0	0.40
April	08	01	02	12.5	2.0	0.25	13	02	04	15.3	2.0	0.31
May	10	03	06	30	2.0	0.60	10	01	02	10.0	2.0	0.2
June	08	01	01	12.5	1.0	0.13	12	02	03	16.6	1.5	0.25
July	15	02	02	13.3	1.0	0.13	15	04	06	26.6	1.5	0.40
August	17	01	03	5.8	3.0	0.18	09	01	02	11.1	2.0	0.22
September	15	02	04	13.3	2.0	0.27	09	00	00	00	00	00
October	12	01	02	8.3	2.0	0.17	10	01	03	10.0	3.0	0.30
November	16	01	01	6.2	1.0	0.06	09	00	00	00	00	00
December	15	02	04	13.3	2.0	0.27	11	03	03	27.2	1.0	0.27

Table 11: Monthly variation of different groups of helminth parasitic infection in *Mystus vittatus* during 2013

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2013	Cestode	08	01	00	00	00	00	00
	Trematode			01	02	12.5	02	0.25
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2013	Cestode	08	02	00	00	00	00	00
	Trematode			01	01	12.5	01	0.12
	Nematode			01	01	12.5	01	0.12
	Acanthocephala			00	00	00	00	00
Mar. 2013	Cestode	08	02	00	00	00	00	00
	Trematode			01	03	12.5	03	0.37
	Nematode			00	00	00	00	00
	Acanthocephala			01	01	12.5	01	0.12
Apr. 2013	Cestode	08	01	00	00	00	00	00
	Trematode			01	02	12.5	02	0.25
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
May 2013	Cestode	10	03	00	00	00	00	00
	Trematode			02	05	20	2.5	0.5
	Nematode			01	01	10	01	0.1
	Acanthocephala			00	00	00	00	00
Jun. 2013	Cestode	08	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			01	01	12.5	01	0.12
Jul. 2013	Cestode	15	02	01	01	6.66	01	0.06
	Trematode			01	01	6.66	01	0.06
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2013	Cestode	17	01	00	00	00	00	00
	Trematode			01	03	5.88	03	0.17
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Sep. 2013	Cestode	15	02	00	00	00	00	00
	Trematode			01	03	6.66	03	0.2
	Nematode			00	00	00	00	00
	Acanthocephala			01	01	6.66	01	0.06
Oct. 2013	Cestode	12	01	00	00	00	00	00
	Trematode			01	02	8.3	02	0.16
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Nov. 2013	Cestode	16	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	01	6.25	01	0.06
	Acanthocephala			00	00	00	00	00
Dec. 2013	Cestode	15	02	00	00	00	00	00
	Trematode			02	04	13.3	02	0.26
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
	Total	140	19		33	13.57	1.73	0.23

Table 12: Monthly variation of different groups of helminth parasitic infection in *Mystus vittatus* during 2014

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2014	Cestode	5	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2014	Cestode	10	02	01	01	10	01	0.10
	Trematode			01	02	10	02	0.2
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2014	Cestode	10	01	00	00	00	00	00
	Trematode			01	04	10	04	0.4
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Apr. 2014	Cestode	13	02	00	00	00	00	00
	Trematode			01	03	7.6	03	0.23
	Nematode			01	01	7.6	01	0.07
	Acanthocephala			00	00	00	00	00
May 2014	Cestode	10	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			01	02	10	02	0.2
Jun. 2014	Cestode	12	02	00	00	00	00	00
	Trematode			01	02	8.3	02	0.16
	Nematode			01	01	8.3	01	0.08
	Acanthocephala			00	00	00	00	00
Jul. 2014	Cestode	15	04	00	00	00	00	00
	Trematode			03	04	20	1.3	0.26
	Nematode			01	02	6.6	02	0.13
	Acanthocephala			00	00	00	00	00
Aug. 2014	Cestode	09	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			01	02	11.1	02	0.2
Sep. 2014	Cestode	09	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Oct. 2014	Cestode	10	01	00	00	00	00	00
	Trematode			01	03	10	03	0.3
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Nov. 2014	Cestode	09	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Dec. 2014	Cestode	11	03	00	00	00	00	00
	Trematode			01	01	9.09	01	.09
	Nematode			01	01	9.09	01	.09
	Acanthocephala			01	01	9.09	01	.09
	Total	123	17		30	13.8	1.7	0.24

E) Monthly Variation in Helminth Infection in *Mastacembalus pancalus*

a) Monthly variation of total helminth infection in *Mastacembalus pancalus*

Month wise prevalence of the helminth parasites in *Mastacembalus pancalus* was also recorded in both years of the study and shown in Table 13. In 2013, the maximum prevalence (20%) was recorded in the months of April, August and December while the least prevalence (8.33%) was recorded in the months of May and October (Figure 20). The infection was not reported during the months of June, September and November. The maximum intensity (4.5) was recorded in December whereas minimum intensity (0.1) was reported in the months of April and July (Figure 21). The highest density (0.90) was recorded in the month of December whereas lowest density (0.17) was recorded in months of July and October (Figure 22).

In 2014, the highest percentage of helminth infection (25%) in fish was observed in the month of April and the least prevalence (10.0%) was recorded in February and May (Figure 20). The infection of helminth parasites was not reported in the months of March, June, August and November. The maximum intensity and density of helminth parasites in *M. pancalus* was recorded as 3.67 and 0.92 during the month of April whereas the minimum percent intensity of infection (1.0) was recorded in the months in January, February and October and the minimum density of infection (1.1) observed in February (Figure 21 & 22).

b) Monthly variation of different groups of helminth infection in *Mastacembalus pancalus*

The data of monthly variations of different groups of parasites in the year 2013 have been depicted in Table 14. The fish *M. pancalus* were heavily infected by helminth parasites which belonged to the classes Cestoda and Trematoda. In the year 2013, a total of 31 helminth parasites were collected from the 14 infected fish of *M. pancalus*. The overall Incidence of infection with Cestode and trematode parasites in fish *M. pancalus* was found to be 2.5% and 9.1% respectively. The infection of Nematode parasites and acanthocephalan parasites in this fish was not reported in this year. A higher infection of trematode parasites was observed in *M. pancalus.*

The maximum prevalence (10%) of Cestode parasites in fish *M. pancalus* was found in the month of April whereas the minimum prevalence (8.3%) was recorded in July. The fishes were found to be free of infection with Cestode parasites for most months of the year viz. January February, May, June, August, September, October November and December (Figure 23). In the month of March, April and July maximum intensity of infection (1.0) with Cestode parasites was recorded (Figure 24). The density of Cestode infection was recorded highest (0.10) in month of April whereas minimum density of infection (0.08) was observed in July (Table 14, Figure 25).

The prevalence of Trematode parasites was recorded highest (20%) in months of August and December whereas the minimum prevalence (8.3%) was recorded in months of May, July and October. The infection was not reported during the months of June, September and November (Figure 23). The maximum intensity of infection (4.5) was found in month of December whereas the minimum intensity of (1.0) was observed in the month of April and July (Figure 24). The density of infection with Trematode parasites was found highest (0.9) in the month of December whereas minimum density (0.08) of Trematode infection was reported in the month of July (Table 14, Figure 25).

The fishes were found to be free of infection with Nematode and Acanthocephalan parasites in the year 2013.

In the year 2014, a total of 23 helminth parasites were collected from the 11 infected fish of *M. pancalus*. The data of monthly variations of different groups of parasites in the year 2013 have been depicted in Table 15. The overall incidence of infection in fish with Cestode and Trematode parasites was found to be 2.8% and 7.47% respectively. The fishes were found to be free of infection with Nematode and Acanthocephalan parasites in the year 2014 also.

The maximum prevalence (11.1%) of Cestode parasites was recorded in the month of October whereas the minimum prevalence (8.3%) was recorded during April. The infection of Cestode parasites was not reported in the months of February, March, May, June, July, August, September, November and December (Figure 23). The maximum intensity of Cestode infection (1.0) was recorded in months of January,

April and October (Figure 24). The maximum density of infection (0.11) was recorded in the month of October whereas the minimum density of infection (0.08) was recorded in April (Table 15, Figure 25).

The maximum prevalence (16.6%) of Trematode parasites in fish *M. pancalus* was recorded in April and minimum prevalence (10%) was recorded in the months of January, February and May. The infection of Trematode parasites was not reported in the months of March, June, August, October and November (Figure 23). The maximum intensity of Trematode infection (5.0) was recorded in the month of April and minimum intensity (1.0) was recorded in the months of January, February and September (Figure 24). The maximum density (0.83) of trematode infection was recorded in the month of April and minimum (0.1) during the months of January and February (Table 15, Figure 25).

M. pancalus were found to be free of infection with Nematode and Acanthocephalan parasites in the consecutive year 2014 also.

Table 13: Monthly variation of total helminth parasitic infection in *Mastacembalus pancalus*

Month	Study Period: 2013						Study Period: 2014					
	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density
January	10	01	02	10	2.0	0.2	10	02	02	20	1.0	0.20
February	10	01	04	10	4.0	0.4	10	01	01	10	1.0	0.10
March	11	02	03	18.1	1.5	0.2	07	00	00	00	00	00
April	10	02	02	20	0.1	0.2	12	03	11	25.0	3.6	0.92
May	12	01	04	8.3	4.0	0.3	10	01	03	10	3.0	0.30
June	07	00	00	00	00	00	08	00	00	00	00	00
July	12	02	02	16.6	1.0	0.17	08	01	02	12.5	2.0	0.25
August	10	02	03	20.0	1.5	0.30	08	00	00	00	00	00
September	10	00	00	00	00	00	08	01	01	12.5	00	0.13
October	12	01	02	8.3	2.0	0.17	09	01	01	11.1	1.0	0.11
November	06	00	00	00	00	00	09	00	00	00	00	00
December	10	02	09	20.0	4.5	0.90	08	01	02	12.5	2.0	0.25

Table 14: Monthly variation of different groups of helminth parasitic infection in *Mastacembalus pancalus* in 2013

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2013	Cestode	10	01	00	00	00	00	00
	Trematode			01	02	10	02	0.2
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2013	Cestode	10	01	00	00	00	00	00
	Trematode			01	04	10	04	0.4
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2013	Cestode	11	02	01	01	9.09	01	0.09
	Trematode			01	02	9.09	02	0.18
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Apr. 2013	Cestode	10	02	01	01	10	01	0.1
	Trematode			01	01	10	01	0.1
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
May 2013	Cestode	12	01	00	00	00	00	00
	Trematode			01	04	8.3	04	0.33
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jun. 2013	Cestode	07	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jul. 2013	Cestode	12	02	01	01	8.3	01	0.08
	Trematode			01	01	8.3	01	0.08
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2013	Cestode	10	02	00	00	00	00	00
	Trematode			02	03	20	1.5	0.3
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Sep. 2013	Cestode	10	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Oct. 2013	Cestode	12	01	00	00	00	00	00
	Trematode			01	02	8.3	02	0.16
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Nov. 2013	Cestode	06	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Dec. 2013	Cestode	10	02	00	00	00	00	00
	Trematode			02	09	20	4.5	0.9
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
	Total	120	14		31	11.6	2.21	0.25

Table 15: Monthly variation of different groups of helminth parasitic infection in *Mastacembalus pancalus* in 2014

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2014	Cestode	10	02	01	01	10	01	0.1
	Trematode			01	01	10	01	0.1
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2014	Cestode	10	01	00	00	00	00	00
	Trematode			01	01	10	01	0.1
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2014	Cestode	07	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Apr. 2014	Cestode	12	03	01	01	8.3	01	0.08
	Trematode			02	10	16.6	05	0.83
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
May 2014	Cestode	10	01	00	00	00	00	00
	Trematode			01	03	10	03	0.3
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jun. 2014	Cestode	08	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jul. 2014	Cestode	08	01	00	00	00	00	00
	Trematode			01	02	12.5	02	0.25
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2014	Cestode	08	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Sep. 2014	Cestode	08	01	00	00	00	00	00
	Trematode			01	01	12.5	01	0.12
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Oct. 2014	Cestode	09	01	01	01	11.1	01	0.11
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Nov. 2014	Cestode	09	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Dec. 2014	Cestode	08	01	00	00	00	00	00
	Trematode			01	02	12.5	02	0.25
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
	Total	107	11		23	10.28	2.09	0.21

F) Monthly Variation in Helminth Infection in *Wallagu attu*

a) Monthly variation of total helminth infection in *Wallagu attu.*

Monthly variations in helminth parasites was recorded in both years of the study and shown in Table 16. In the month of March, maximum prevalence (25%) was recorded while the least prevalence (14.28%) was recorded in the month of December (Figure 26). The fishes were found to be free of infection with helminth parasites for most months of the year *viz*. January, May, June, July, September and November. The maximum intensity (2.0) was recorded in April whereas minimum intensity (1.0) was reported in the months of February, March, August, October and December (Figure 27). In the present investigation, highest density (0.33) was recorded in the month of April whereas lowest density (0.14) was recorded in December (Figure 28).

In 2014, the highest percentage of helminth infection (33.33%) in fish was observed during the month of December and the least prevalence of the parasite (10.0%) was recorded in June (Figure 26). The infection was not reported during the months of January, August, September and November. The maximum intensity and density of helminth parasites in fish *W. attu* was recorded as 3.0 and 1.0 during the month of December whereas the minimum intensity of infection (1.0) was recorded in the months of April, May and July and minimum density of infection (0.17) was recorded in the months of May and July (Figure 27 and 28).

b) Monthly variation of different groups of helminth infection in *Wallagu attu*

The data of monthly variation of different groups of helminth parasitic infection in *Wallagu attu* has been shown in Table 17**.** The fish *W. attu* were heavily infested by helminth parasites belonging to class cestoda, trematoda and nematoda. In the year 2013, a total of 8 helminth parasites were collected from the 7 infected fish of *W. attu*. The overall Incidence of infection with Cestode, Trematode and Nematode parasites in *W. attu* was found to be 2.85%, 4.28% and 2.85% respectively. The infection of Acanthocephalan parasites in fish was not reported in this year. It was observed that, the fishes were found to be highly infected with Trematode parasites as compared to the other groups.

The maximum prevalence (20%) of Cestode parasites in fish *W. attu* was found in the month of February whereas the minimum prevalence (12.5%) was recorded in March. The fishes were found to be free of infection with Cestode parasites for most months of the year viz., January, April, May, June, July, August, September, October November and December (Figure 29). In the month of February and March, intensity of infection (1.0) with Cestode parasites was recorded in the fish. In fish *W. attu*, the density of Cestode parasitic infection was recorded at peak (0.20) in February whereas minimum density of infection (0.12) was recorded in March (Table 17).

The prevalence of Trematode parasites in fish *W. attu* was recorded highest (16.6%) in months of April and October whereas the minimum prevalence (12.5%) was recorded in March. The fishes were found to be free of infection with Trematode parasites for most months of the year viz., January, February May, June, July, August, September November and December (Figure 29). The maximum intensity of infection (2.0) was found in month of April whereas the minimum intensity (1.0) was observed in the months of March and October. The density of infection with Trematode parasites was found highest (0.33) in the month of April whereas minimum density of Trematode infection (0.12) was reported in March (Table 17).

The prevalence of Nematode parasites in fish *W. attu* was recorded highest (16.6%) in month of August whereas minimum prevalence (14.2%) of the parasites was recorded in December (Figure 29). The infection was not reported during the months of January, February, March, April, May, June, July, September, October and November. The highest intensity of infection (1.0) was observed in month of August and December. The maximum density of infection (0.16) was recorded in month of August whereas the minimum density of infection (0.14) was recorded in December (Table 17).

The fishes were found to be free of infection with Acanthocephalan parasites in the year 2013.

Monthly variation of different group of parasites in *Wallago attu* in 2014 has been shown in Table 18. In the year 2014, a total of 21 helminth parasites were collected from the 11 infected fish of *W. attu*. The prevalence of infection in the fish

with Cestode, Trematode and Nematode parasites was found to be 4.87%, 4.87% and 3.65% respectively. The fishes were found to be free of infection with Acanthocephalan parasites in this year. The fish were found to be highly infected with Cestode and Trematode parasites as compared to others and least infected with the Nematode parasites.

The maximum prevalence (20%) of Cestode parasites was recorded in the month of April whereas the minimum prevalence (10%) was recorded during the February. The infection of Cestode parasites was not reported in the months of January, March, May, June, August, September, October and November (Figure 29). The intensity of Cestode infection (1.0) was recorded in months of February, April, July, and December. The maximum density of infection (0.20) was recorded in the month of April whereas the minimum density of infection (0.1) was recorded in February (Table 18).

The prevalence of Trematode parasites in fish *W. attu* was recorded highest (16.6%) in month of December and minimum prevalence (10%) was recorded in February. The infection of Trematode parasites in fish was not reported during the months of January, April, May, June, July, August, September and November (Figure 29). The maximum intensity of Trematode infection (5.0) was recorded in the month of December and minimum intensity (2.0) was recorded in the month of March. The maximum density (0.83) of trematode infection was recorded in the month of December and minimum density of infection (0.25) during March (Table 18).

The prevalence of Nematode parasites in fish *W. attu* was recorded highest (16.6%) in the month of May whereas minimum prevalence of Nematode parasites (10%) was recorded in the month of June. The infection of Nematode parasites in fish was not reported during the months of January, February, March, April, July, August, September, November and December (Figure 29). The maximum intensity (2.0) of Nematode infection was recorded in the month of June whereas the minimum intensity of infection (1.0) was recorded during the months of May and October (Figure 30). The maximum density of infection (0.2) was recorded in June while minimum density of infection (0.14) was recorded in October (Table 18, Figure 31).

The fishes were found to be free of infection with acanthocephalan parasites in the year 2014 also.

Table 16: Monthly variation of helminth parasitic infection in *Wallago attu*

Month	Study Period: 2013						Study Period: 2014					
	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density	No. of host examined	Total no. of host infected	No. of parasites collected	Prevalence %	Intensity	Density
January	05	00	00	00	00	00	05	00	00	00	00	00
February	05	01	01	20	1.0	0.20	10	02	04	20	2.0	0.40
March	08	02	02	25	1.0	0.25	08	01	02	12.5	2.0	0.25
April	06	01	02	16.6	2.0	0.33	05	01	01	20.0	1.0	0.20
May	06	00	00	00	00	00	06	01	01	16.6	1.0	0.17
June	05	00	00	00	00	00	10	01	02	10.0	2.0	0.20
July	06	00	00	00	00	00	06	01	01	16.6	1.0	0.17
August	06	01	01	16.6	1.0	0.17	06	00	00	00	00	00
September	05	00	00	00	00	00	07	00	00	00	00	00
October	06	01	01	16.6	1.0	0.17	07	02	04	28.5	2.0	0.57
November	05	00	00	00	00	00	06	00	00	00	00	00
December	07	01	01	14.2	1.0	0.14	06	02	06	33.3	3.0	1.0

Table 17: Monthly variation of different groups of helminth parasitic infection in *Wallago attu* in 2013

Month	Name of Parasite	Class of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2013	Cestode	05	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2013	Cestode	05	01	01	01	20	01	0.2
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2013	Cestode	08	02	01	01	12.5	01	0.12
	Trematode			01	01	12.5	01	0.12
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Apr. 2013	Cestode	06	01	00	00	00	00	00
	Trematode			01	02	16.6	02	0.33
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
May 2013	Cestode	06	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jun. 2013	Cestode	05	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Jul. 2013	Cestode	06	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2013	Cestode	06	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	01	16.6	01	0.16
	Acanthocephala			00	00	00	00	00
Sep. 2013	Cestode	05	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Oct. 2013	Cestode	06	01	00	00	00	00	00
	Trematode			01	01	16.6	01	0.16
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Nov. 2013	Cestode	05	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Dec. 2013	Cestode	07	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	01	14.2	01	0.14
	Acanthocephala			00	00	00	00	00
	Total	70	07		08	10	1.14	0.11

Table 18: Monthly variation of different groups of helminth parasitic infection in *Wallago attu*. in 2014.

Month	Class of Parasite	No. of Host Examined	Total No. of Host infected	No. of Host infected	Total No. of Parasites collected	Prevalence	Intensity of infection	Density of infection
Jan. 2014	Cestode	05	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Feb. 2014	Cestode	10	02	01	01	10	01	0.1
	Trematode			01	03	10	03	0.3
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Mar. 2014	Cestode	08	01	00	00	00	00	00
	Trematode			01	02	12.5	02	0.25
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Apr. 2014	Cestode	05	01	01	01	20	01	0.2
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
May 2014	Cestode	06	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	01	16.6	01	0.16
	Acanthocephala			00	00	00	00	00
Jun. 2014	Cestode	10	01	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			01	02	10	02	0.2
	Acanthocephala			00	00	00	00	00
Jul. 2014	Cestode	06	01	01	01	16.6	01	0.16
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Aug. 2014	Cestode	06	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Sep. 2014	Cestode	07	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Oct. 2014	Cestode	07	02	00	00	00	00	00
	Trematode			01	03	14.2	03	0.42
	Nematode			01	01	14.2	01	0.14
	Acanthocephala			00	00	00	00	00
Nov. 2014	Cestode	06	00	00	00	00	00	00
	Trematode			00	00	00	00	00
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
Dec. 2014	Cestode	06	02	01	01	16.6	01	0.16
	Trematode			01	05	16.6	05	0.83
	Nematode			00	00	00	00	00
	Acanthocephala			00	00	00	00	00
	Total	82	11		21	13.41	1.90	0.25

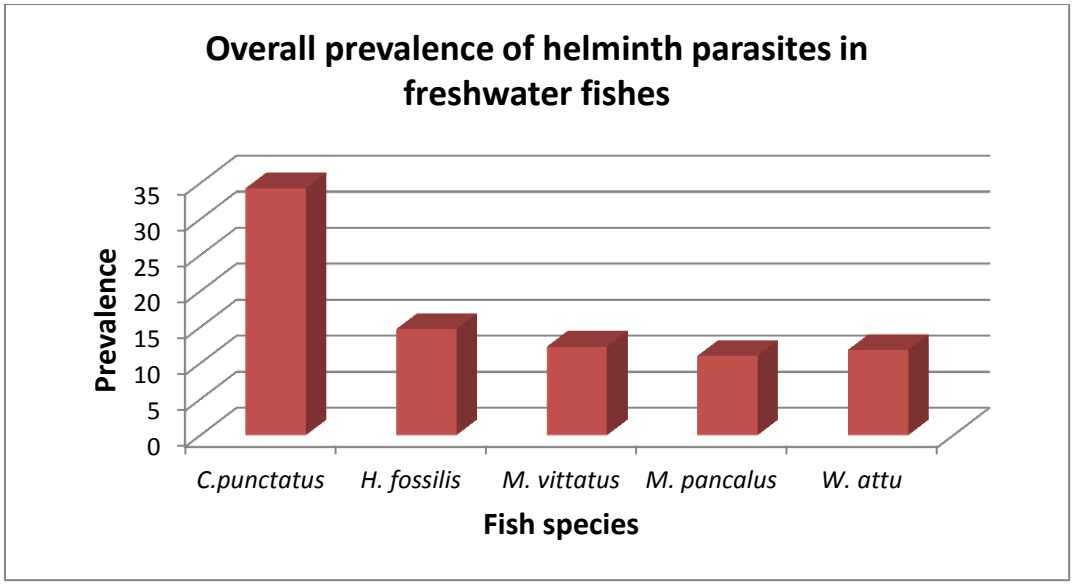


Figure 1: Overall Prevalence (%) of Helminth Parasites in freshwater fishes during, January 2013 to December 2014.

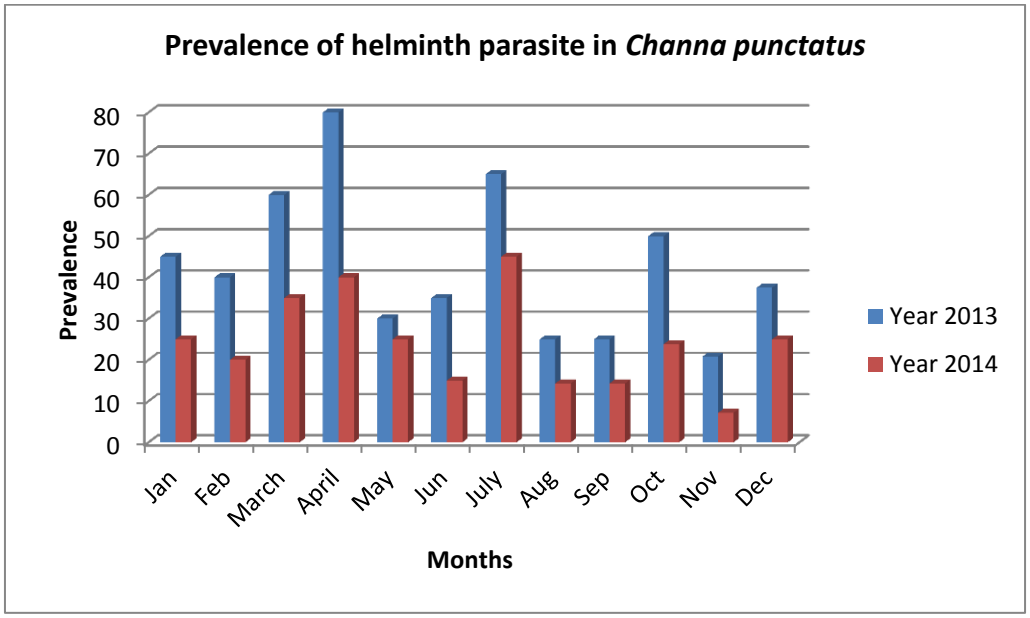


Figure 2: Prevalence (%) of Helminth Parasites of freshwater fish *Channa Punctatus* during, January 2013 to December 2014.

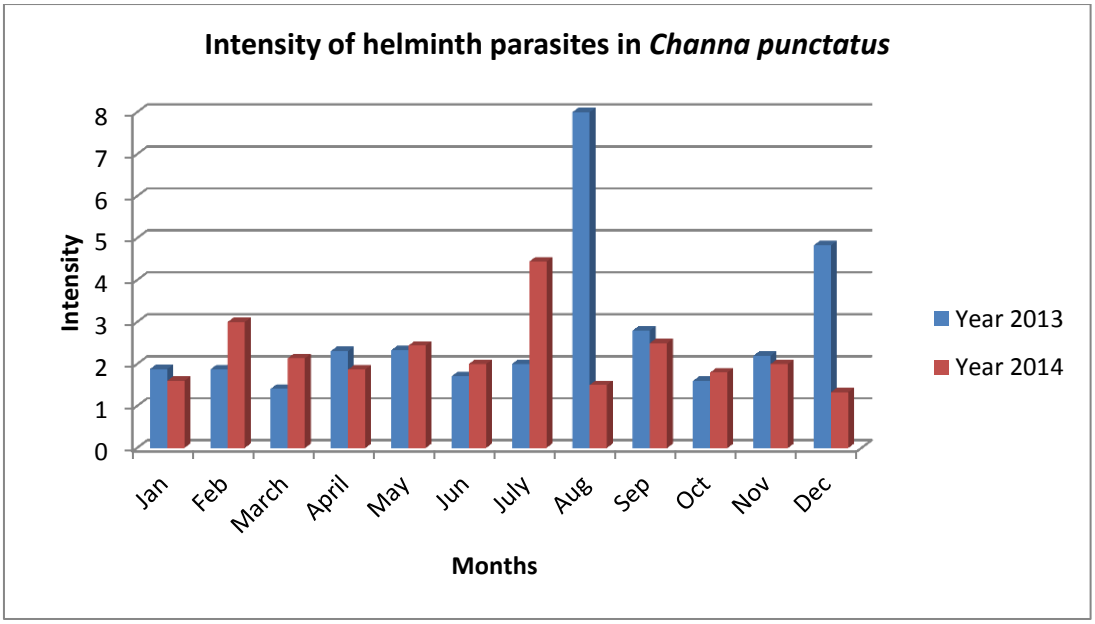


Figure 3: Intensity (%) of Helminth Parasites of freshwater fish *Channa Punctatus* during, January 2013 to December 2014.

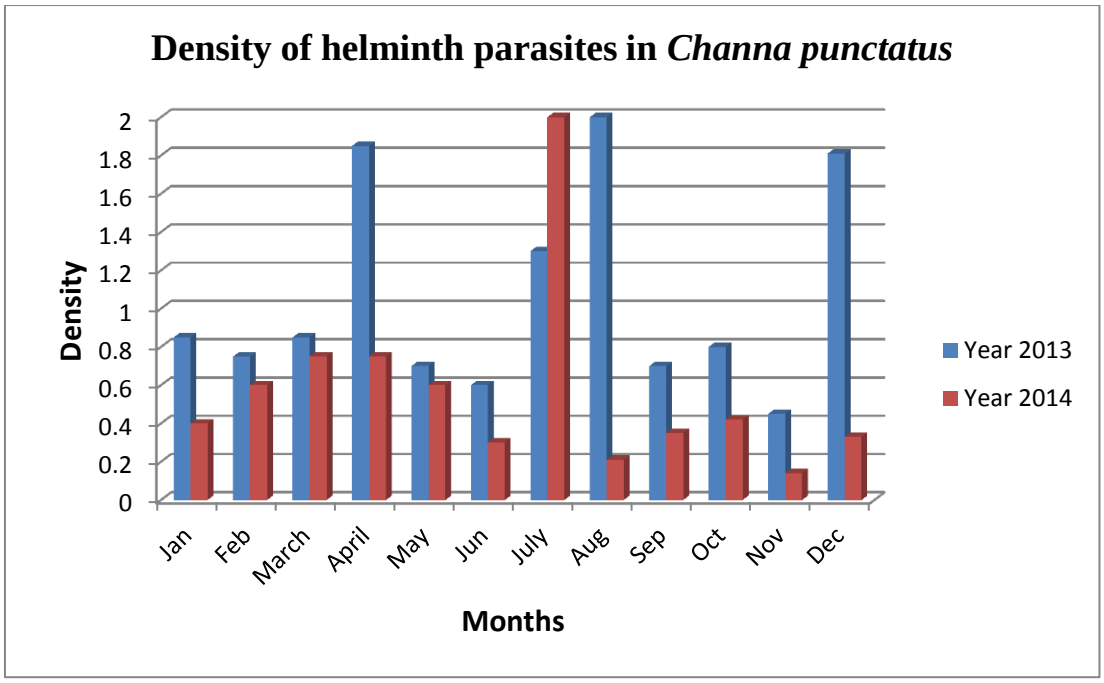


Figure 4: Density (%) of Helminth Parasites of freshwater fish *Channa Punctatus* during, January 2013 to December 2014.

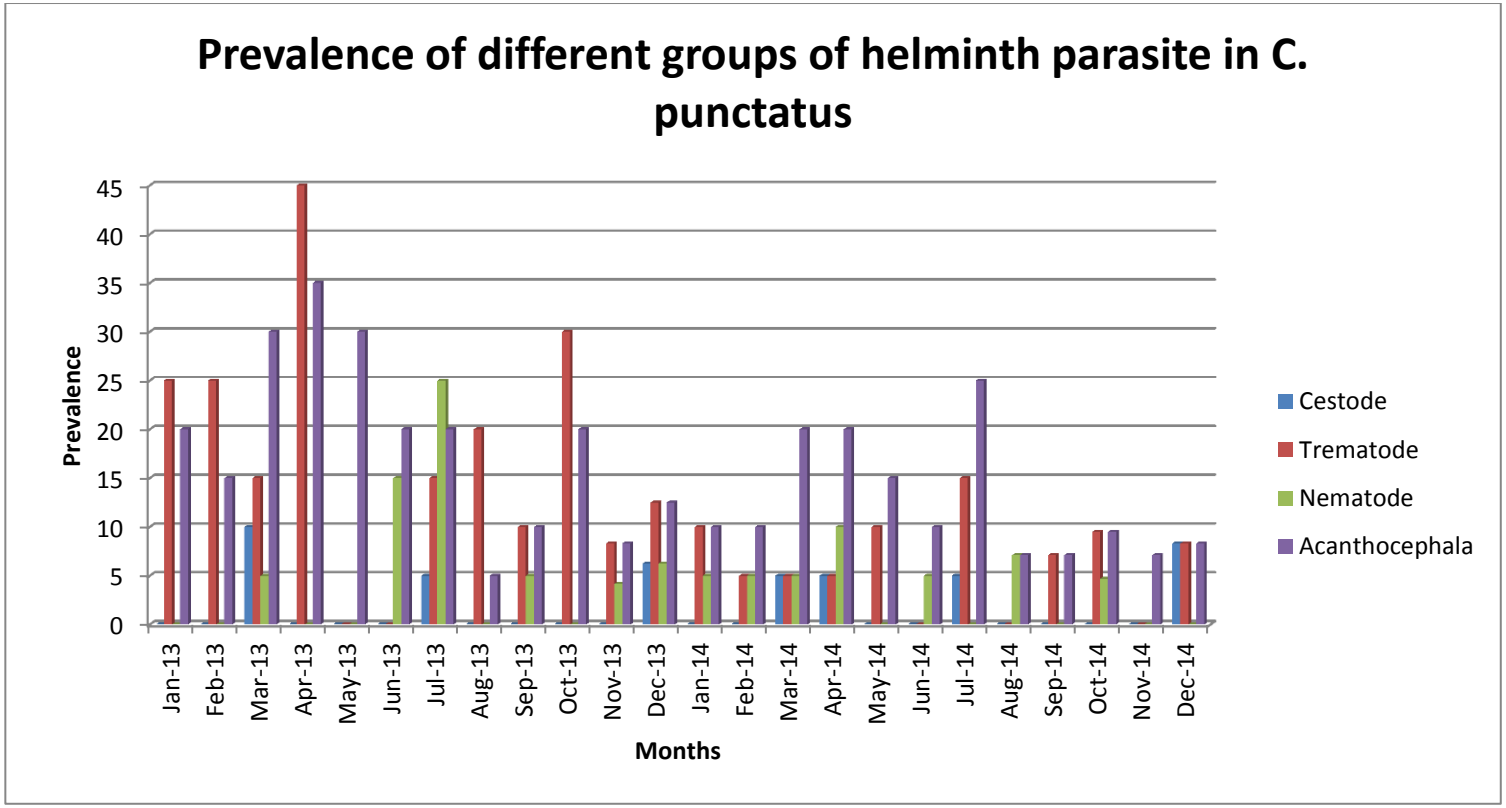


Figure 5: Prevalence (%) of different groups of helminth Parasites of freshwater fish *C. punctatus* during, January 2013 to December 2014.

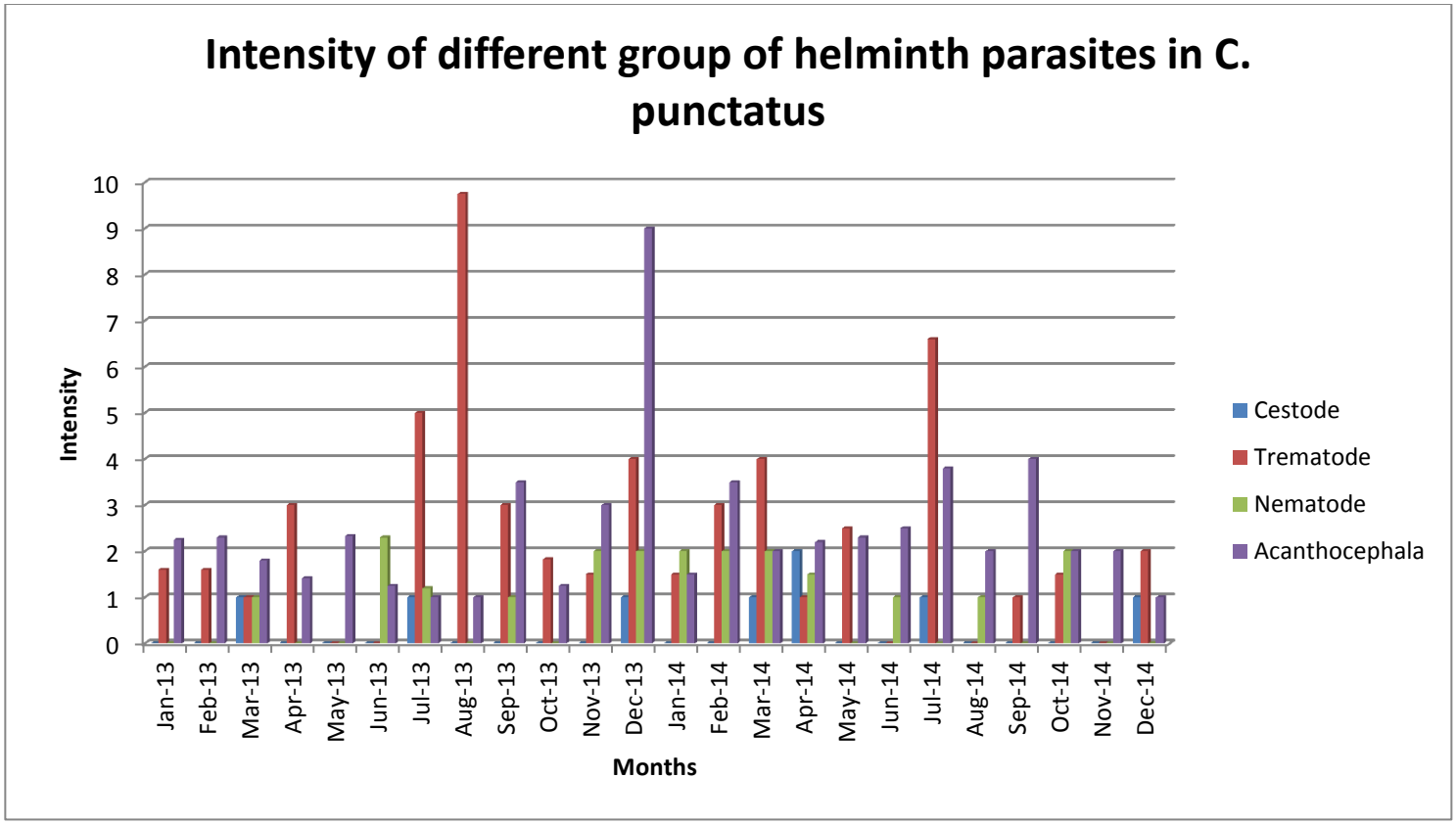


Figure 6: Intensity (%) of different groups helminth Parasites of freshwater fish *C. punctatus* during, January 2013 to December 2014.

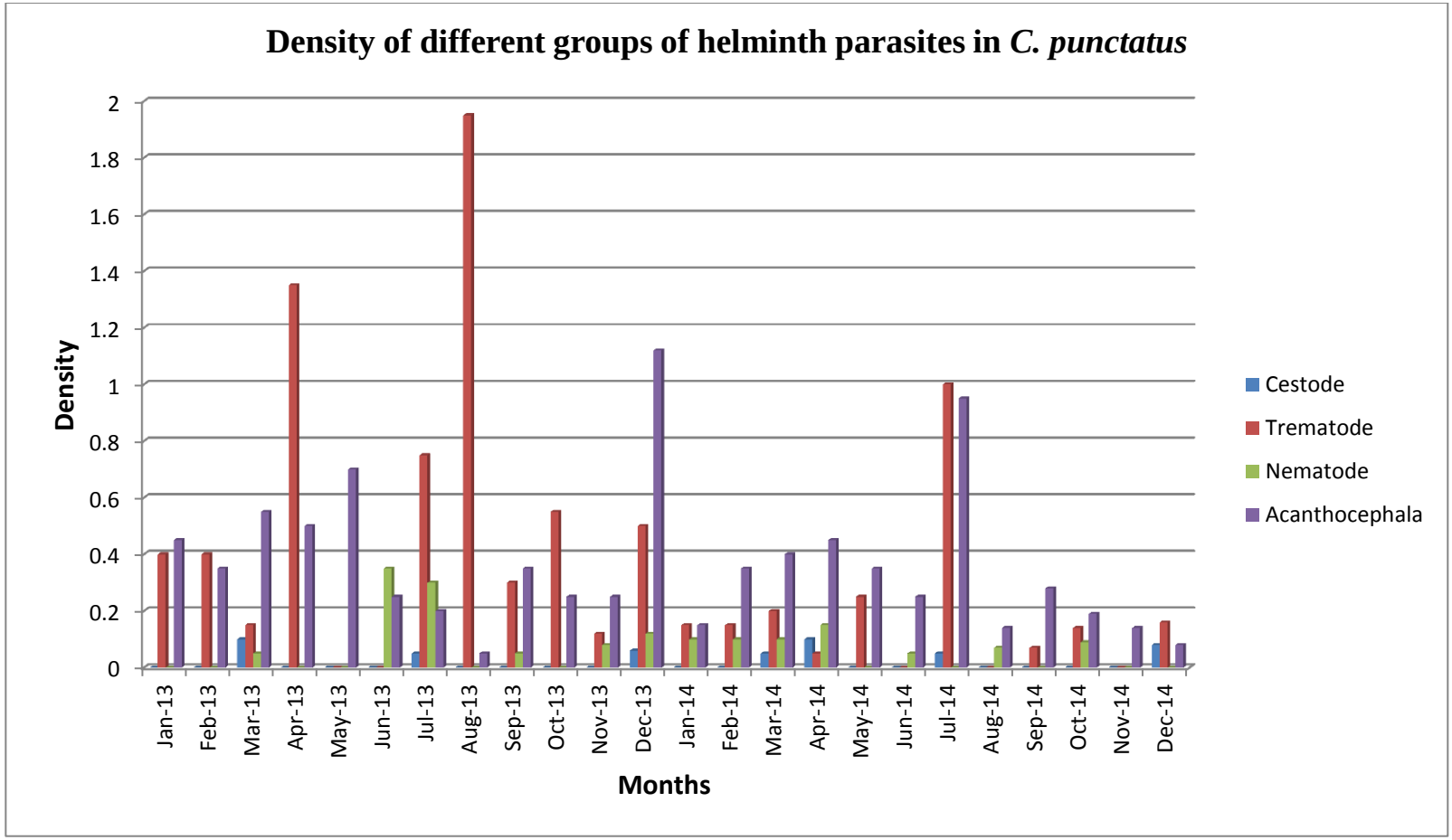


Figure 7: Density (%) of different groups of helminth Parasites of freshwater fish *C. punctatus* during, January 2013 to December 2014.

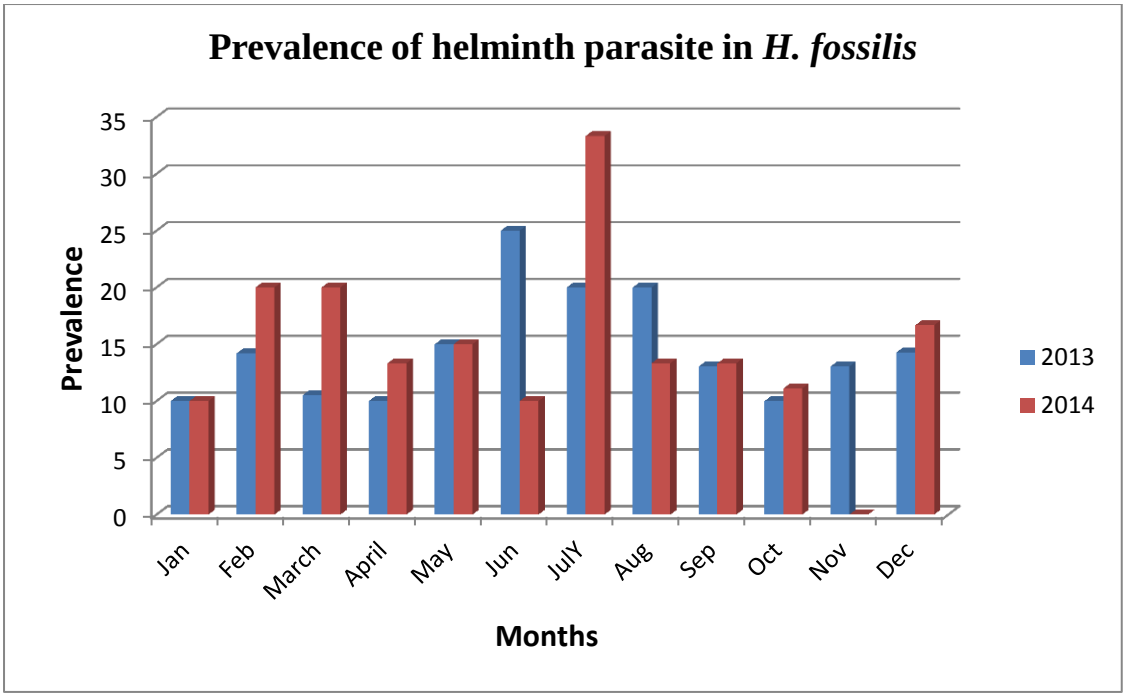


Figure 8: Prevalence (%) of Helminth Parasites of freshwater fish *Heteropneustes fossilis* during, January 2013 to December 2014.

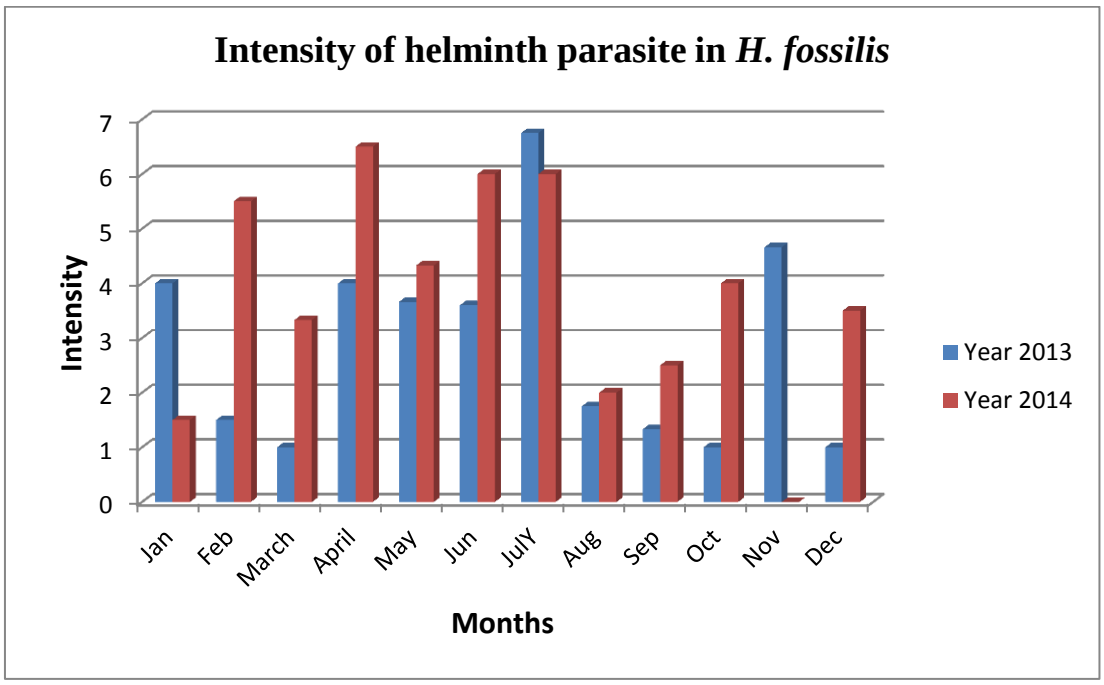


Figure 9: Intensity (%) of Helminth Parasites of freshwater fish *Heteropneustes fossilis* during, January 2013 to December 2014.

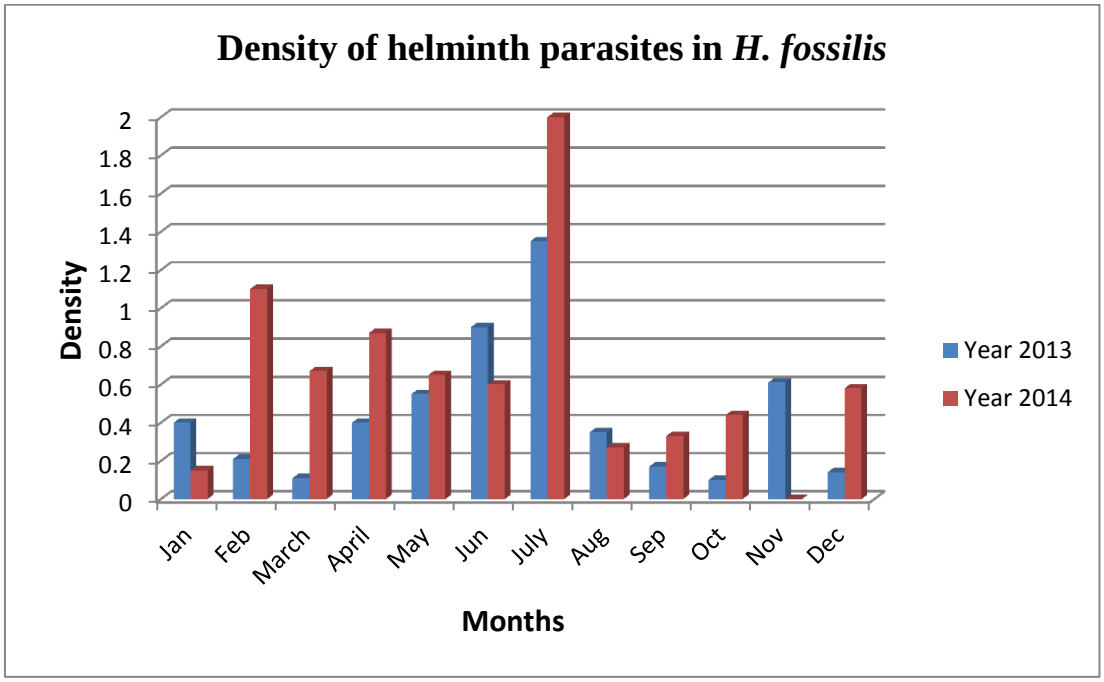


Figure 10: Density (%) of Helminth Parasites of freshwater fish *Heteropneustes fossilis* during, January 2013 to December 2014.

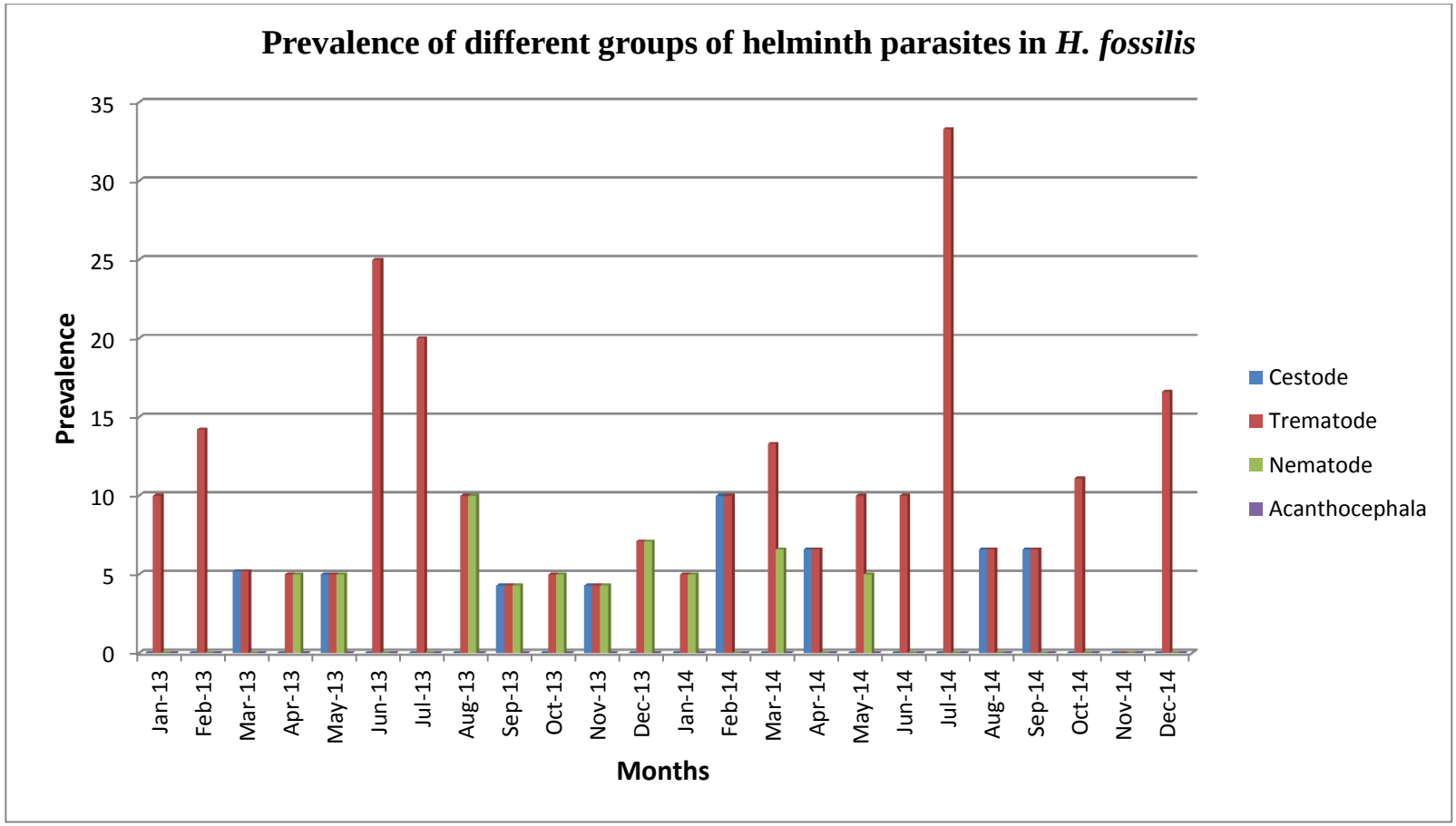


Figure 11: Prevalence (%) of different groups of helminth Parasites of freshwater fish *H. fossilis* during, January 2013 to December 2014.

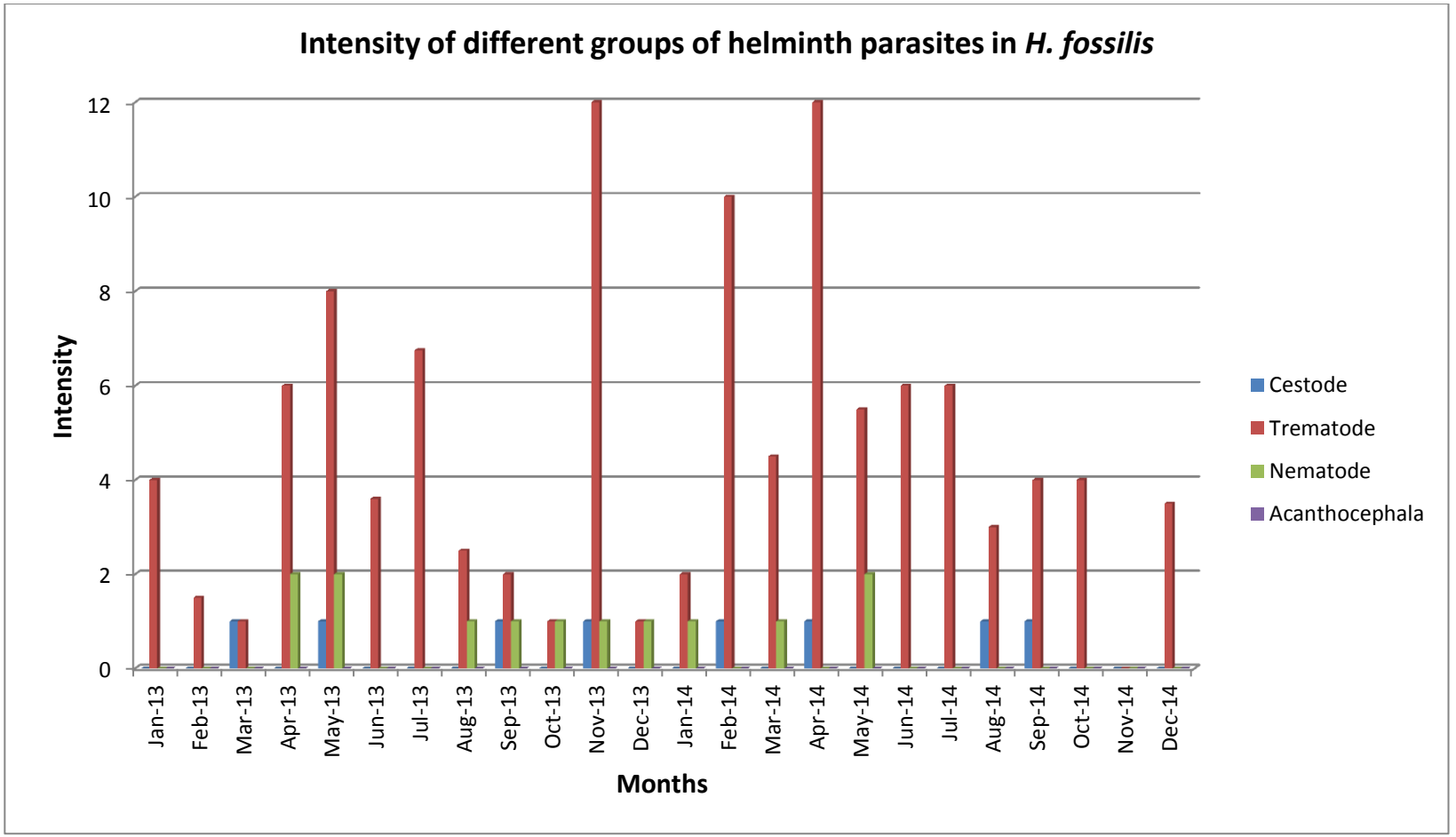


Figure. 12: Intensity (%) of different groups of helminth Parasites of freshwater fish *H. fossilis* during, January 2013 to December 2014.

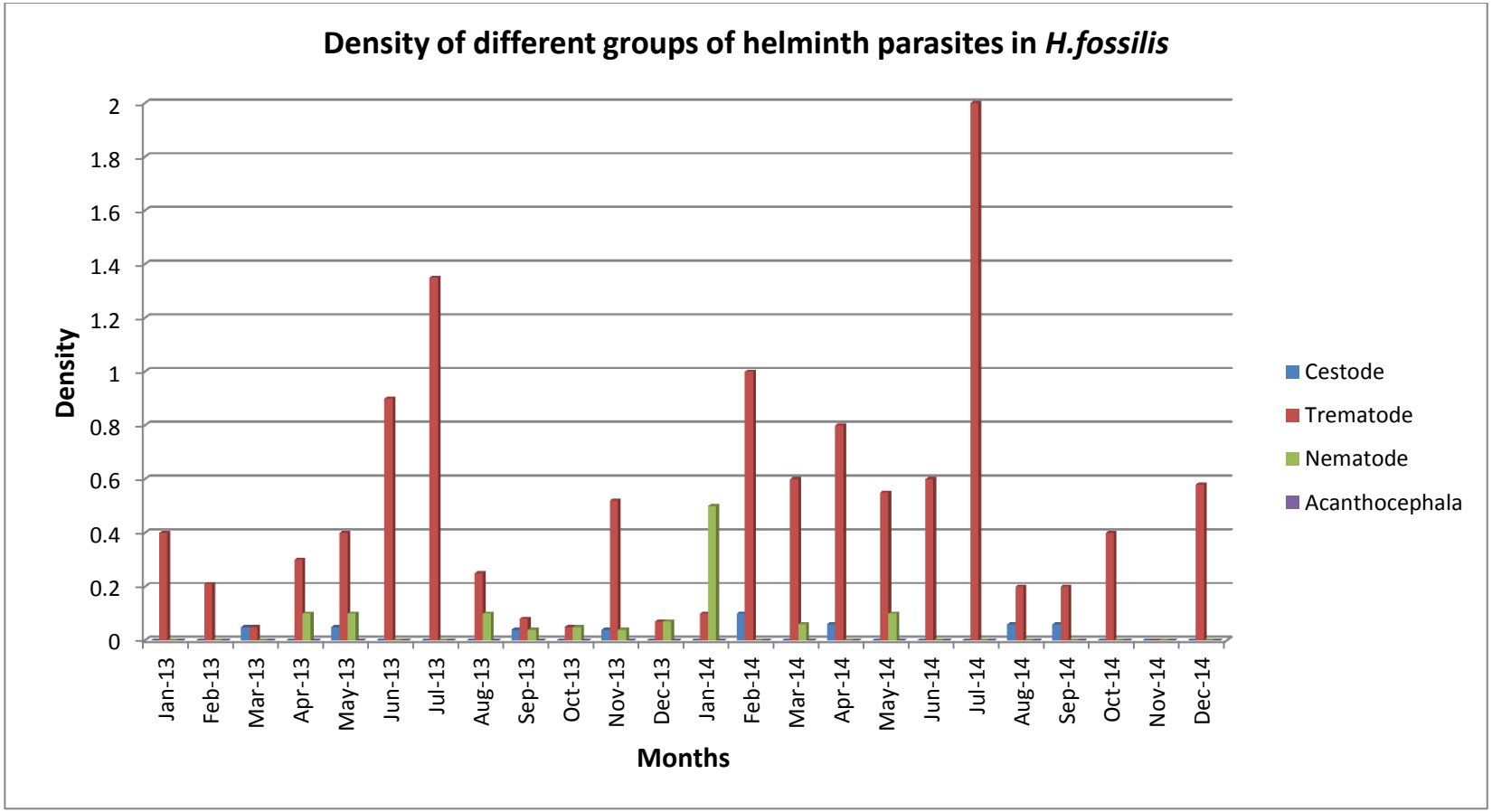


Figure 13: Density (%) of different groups of helminth Parasites of freshwater fish *H. fossilis* during, January 2013 to December 2014.

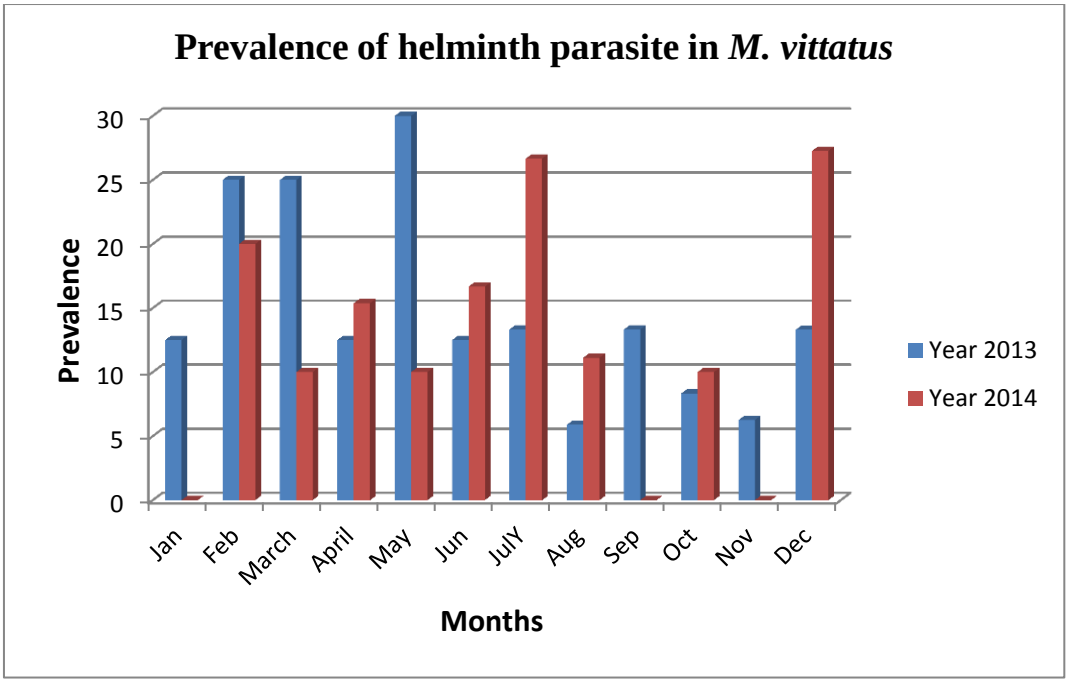


Figure 14: Prevalence (%) of Helminth Parasites of freshwater fish *Mystus vittatus* during, January 2013 to December 2014.

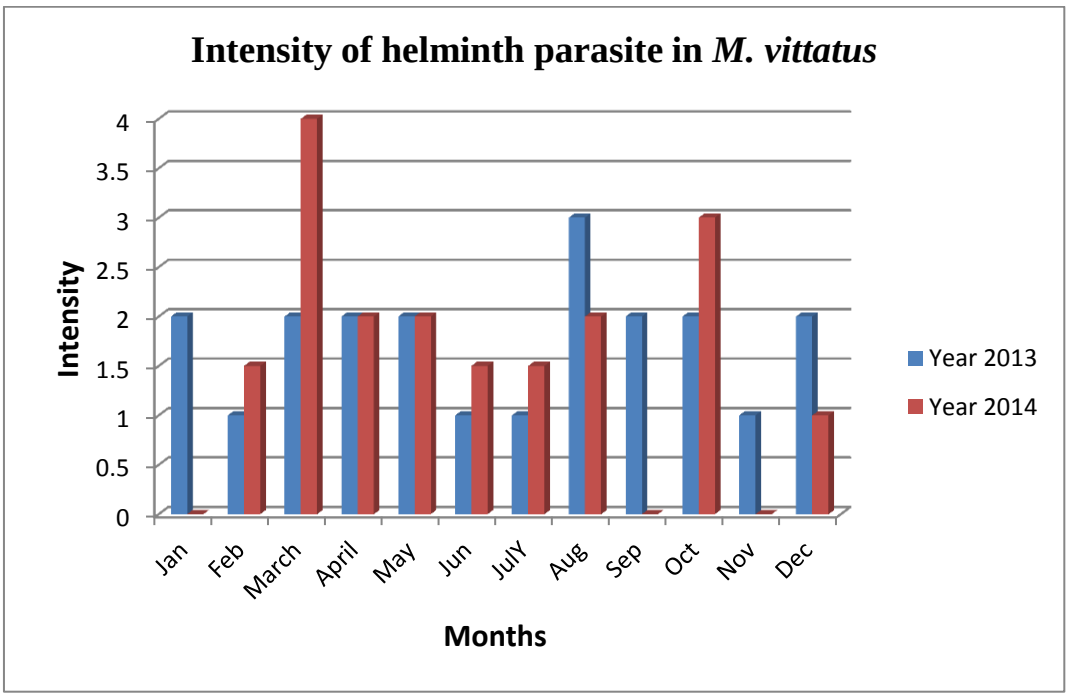


Figure 15: Intensity (%) of Helminth Parasites of freshwater fish *Mystus vittatus* during, January 2013 to December 2014.

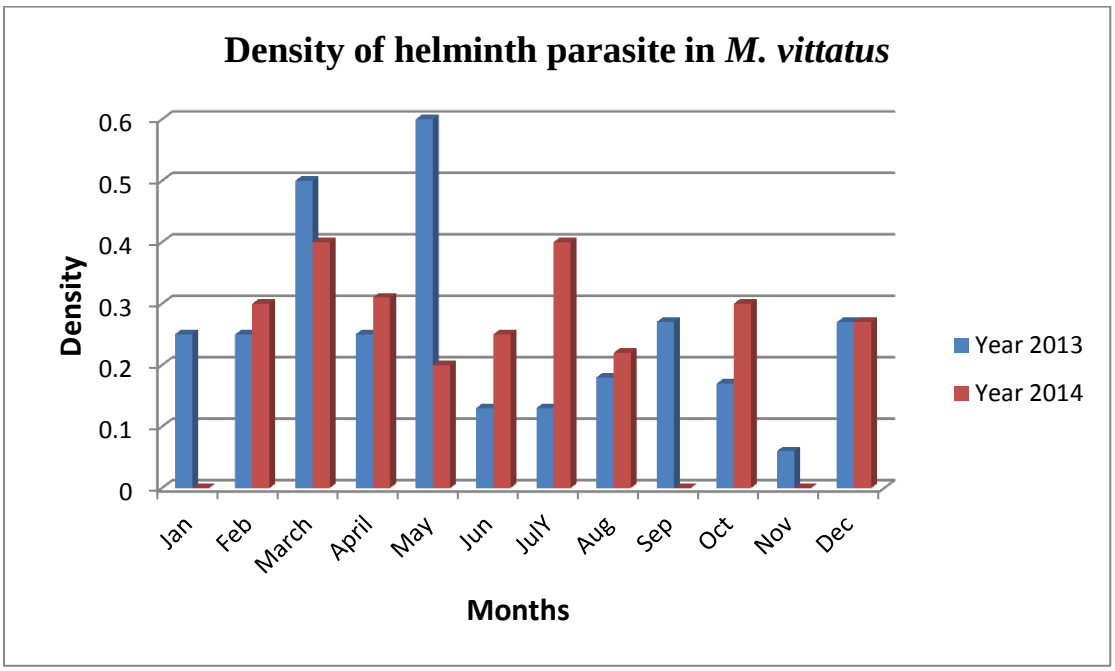


Figure 16: Density (%) of Helminth Parasites of freshwater fish *Mystus vittatus* during, January 2013 to December 2014.

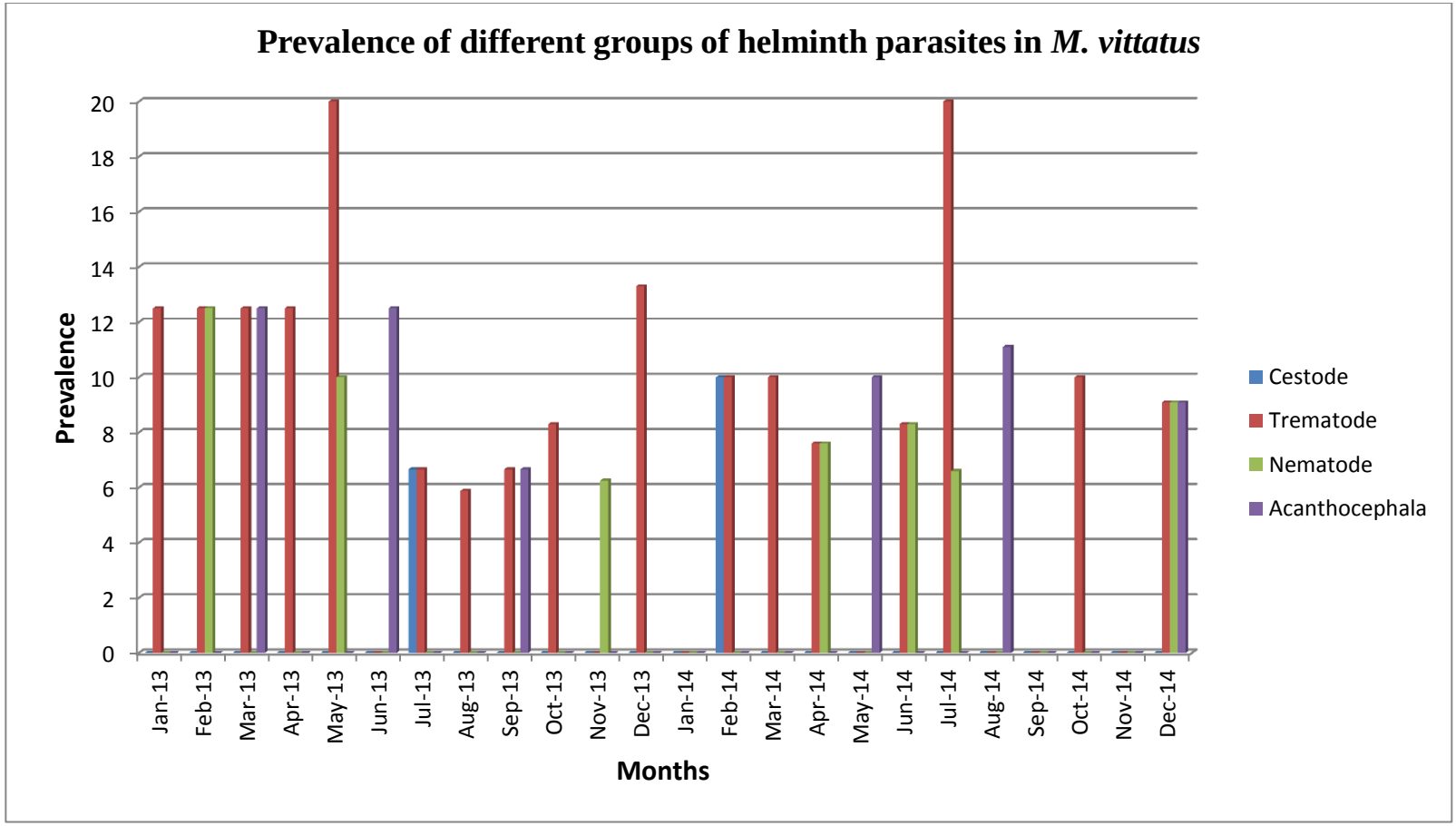


Figure 17: Prevalence (%) of different groups of helminth Parasites of freshwater fish *M. vittatus* during, January 2013 to December 2014.

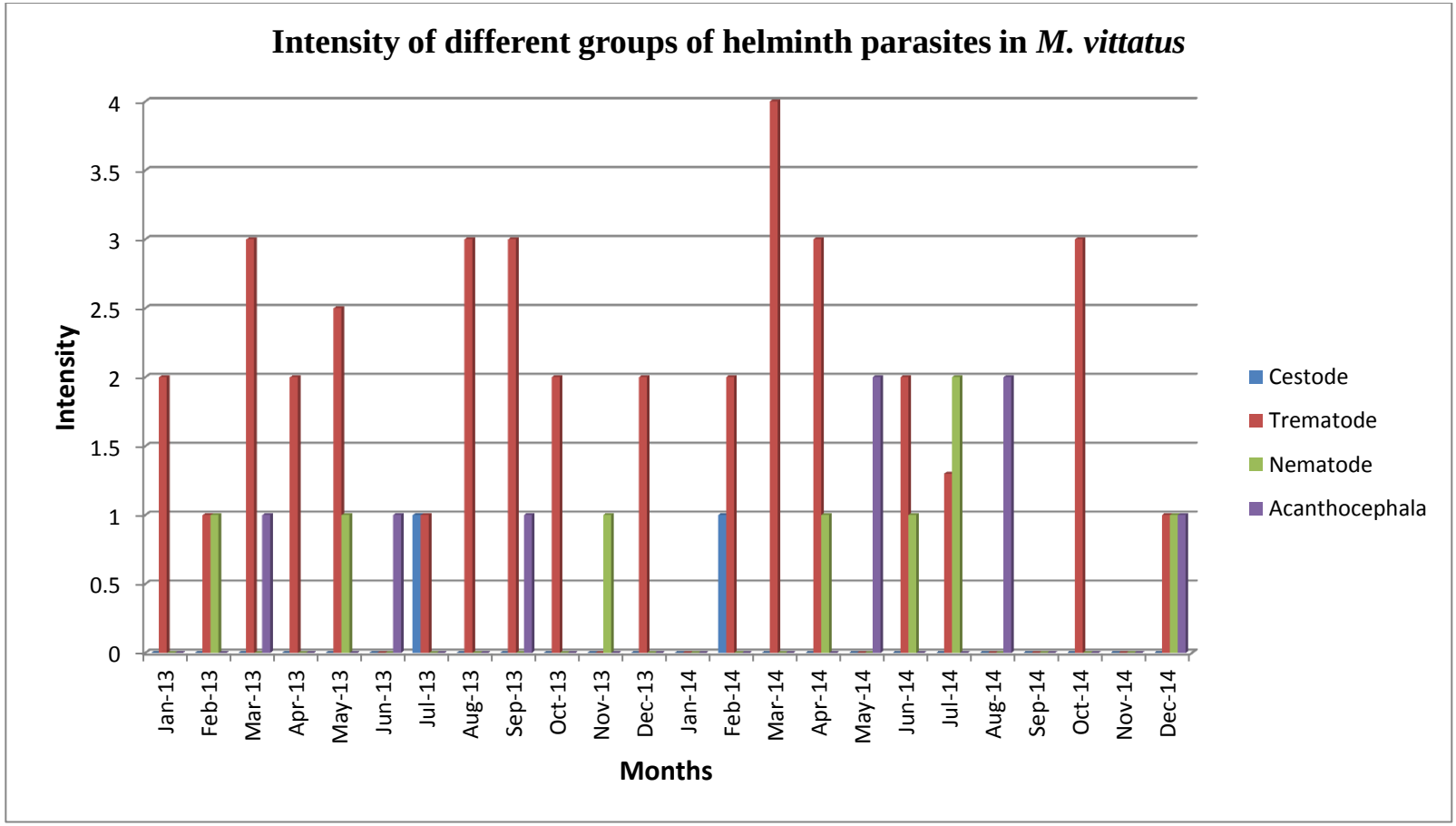
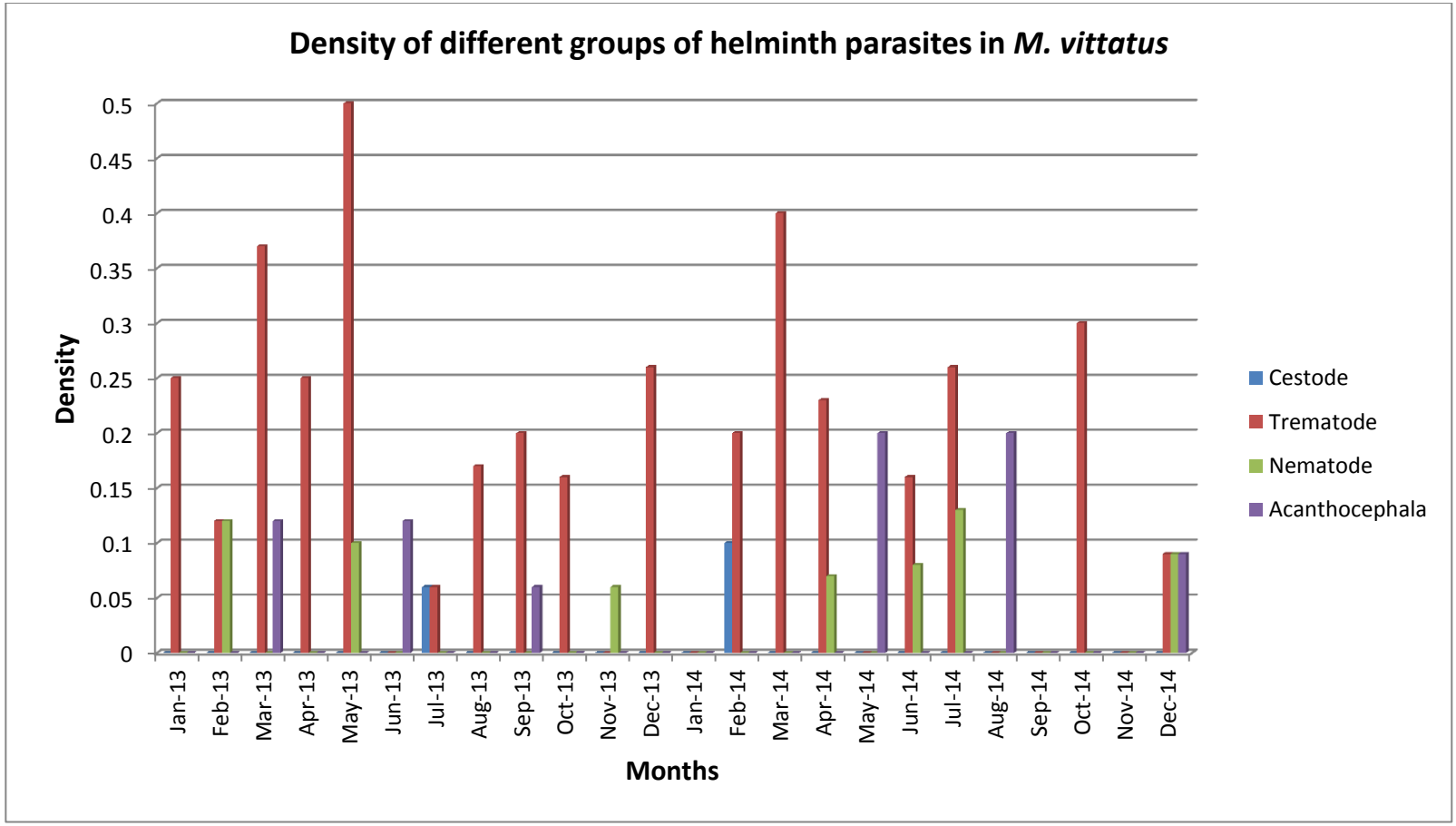


Figure 18: Intensity (%) of different groups of helminth Parasites of freshwater fish *M.vittatus* during, January 2013 to December 2014.



2014.

Figure 19: Density (%) of different groups of helminth Parasites of freshwater fish *M. vittatus* during, January 2013 to December.

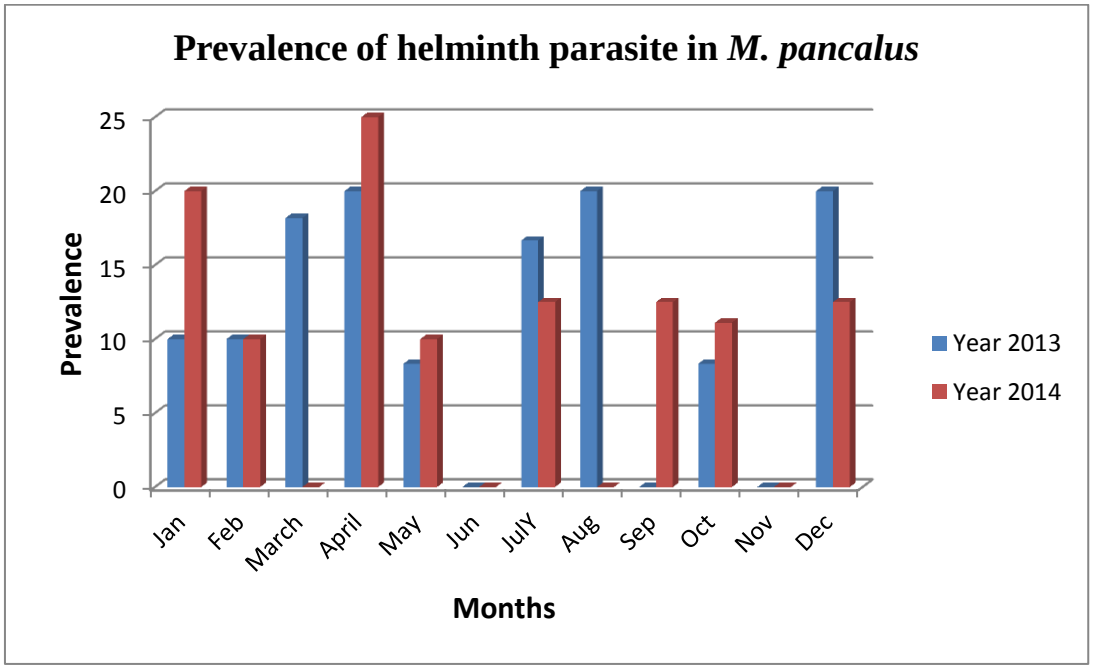


Figure 20: Prevalence (%) of Helminth Parasites of freshwater fish *M. pancalus* during, January 2013 to December 2014.

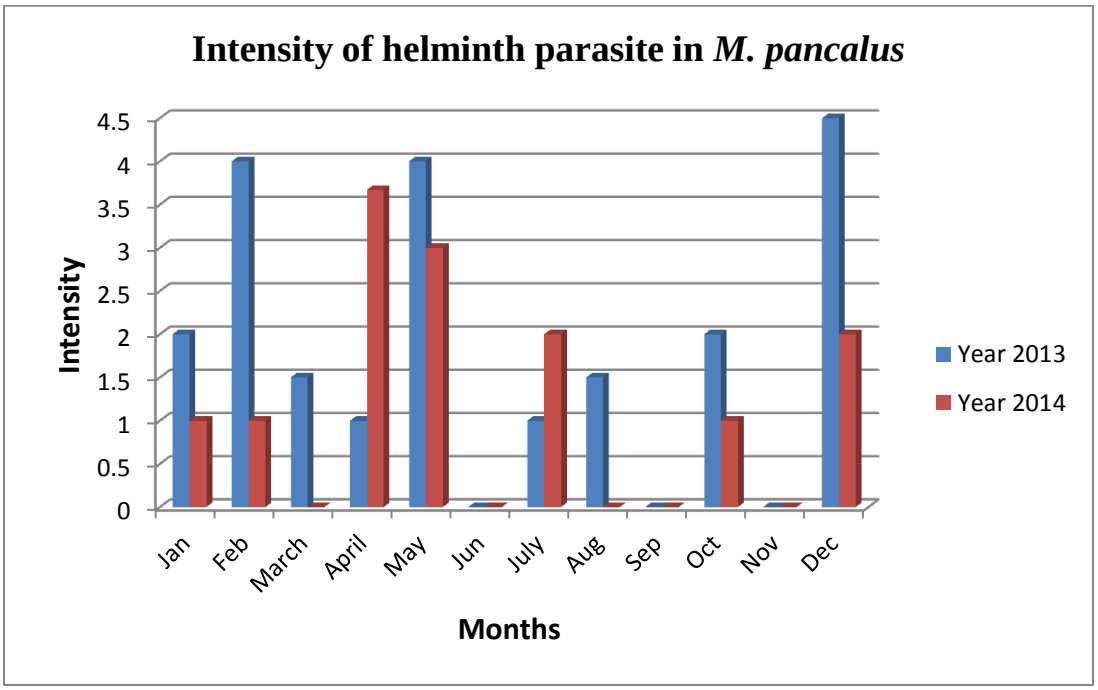


Figure 21: Intensity (%) of Helminth Parasites of freshwater fish *M. pancalus* during, January 2013 to December 2014.

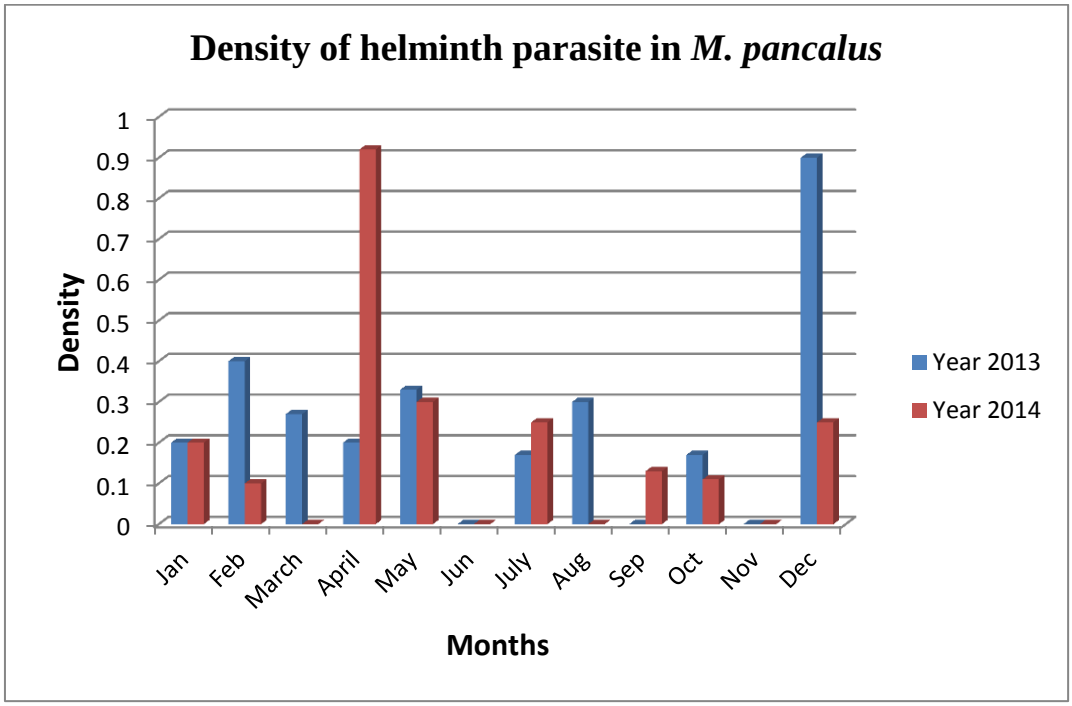


Figure 22: Density (%) of Helminth Parasites of freshwater fish *M. pancalus* during, January 2013 to December 2014.

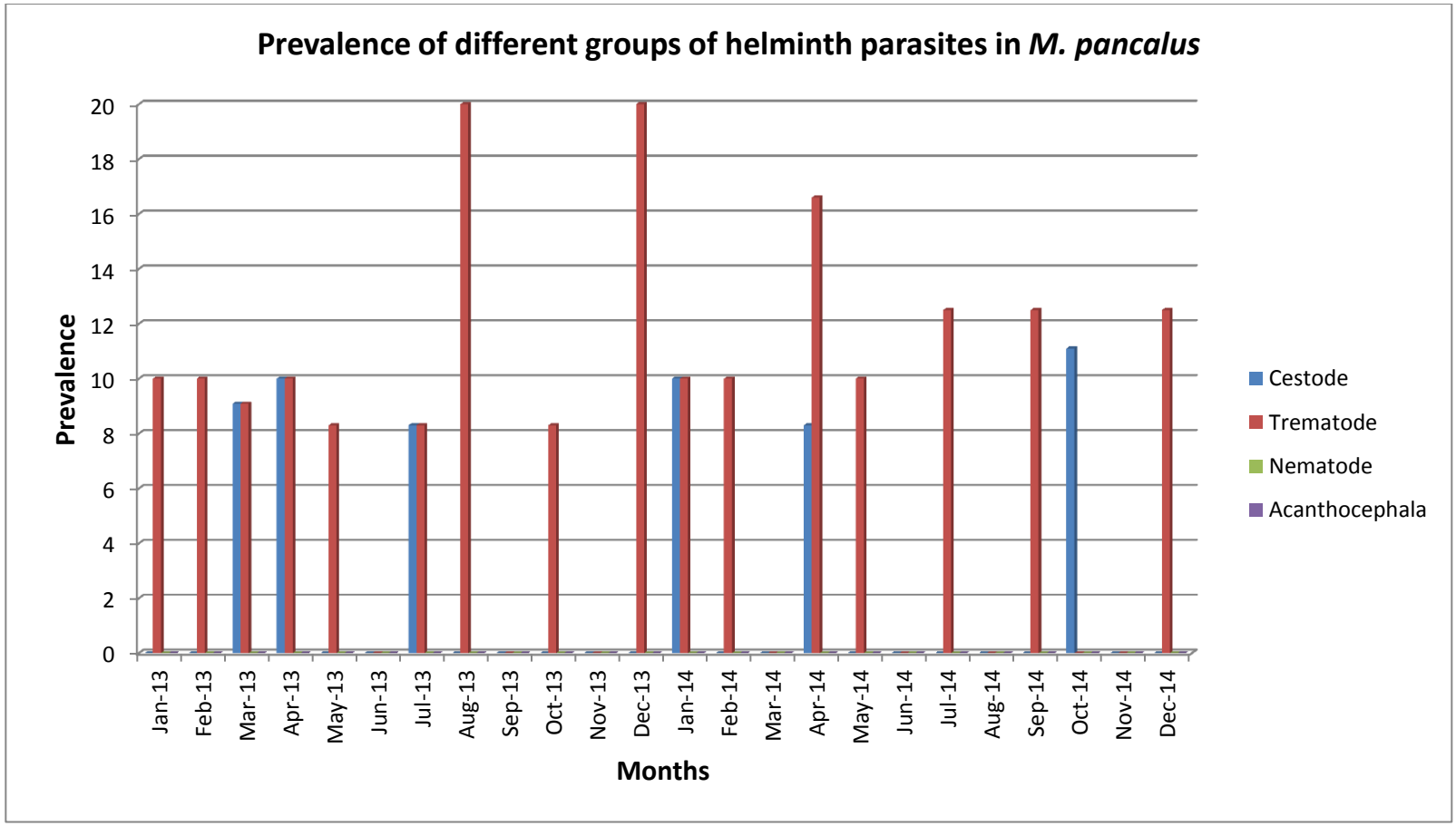


Figure 23: Prevalence (%) of different groups of helminth Parasites of freshwater fish *M. pancalus* during, January 2013 to December 2014.

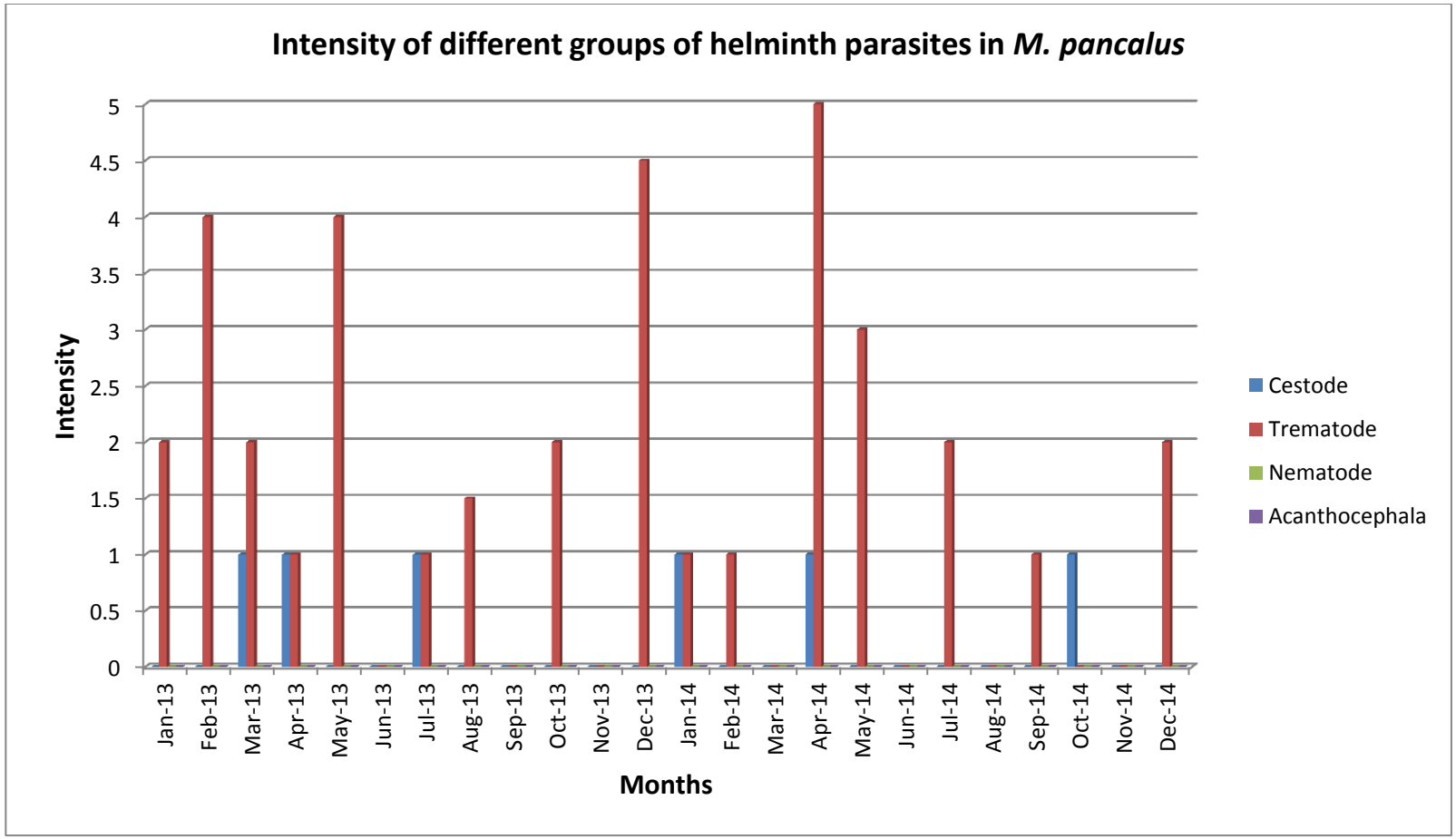


Figure 24: Intensity (%) of different groups of helminth Parasites of freshwater fish *M. pancalus* during, January 2013 to December 2014

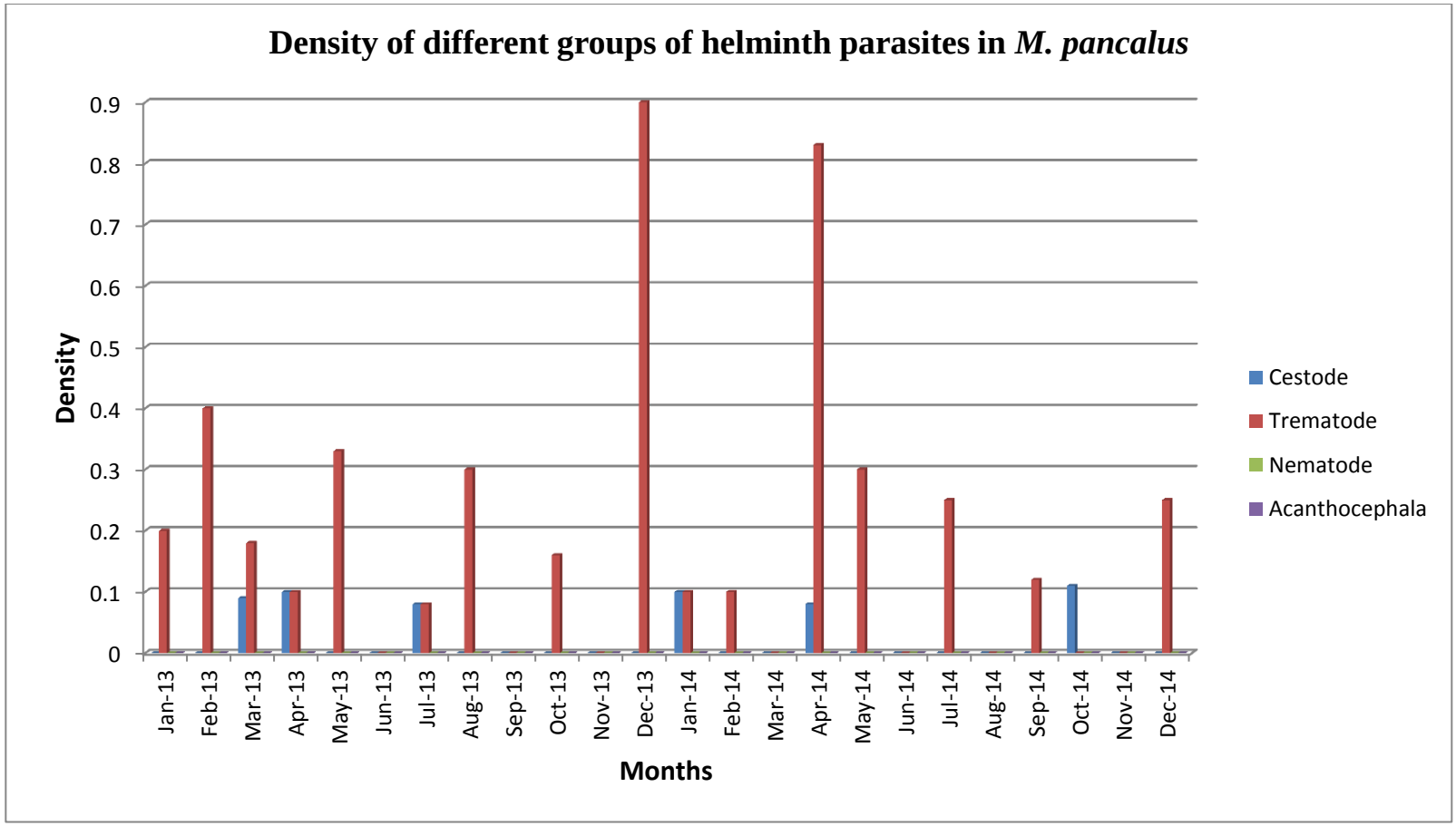


Figure 25: Density (%) of different groups of helminth Parasites of freshwater fish *M. pancalus* during, January 2013 to December 2014.

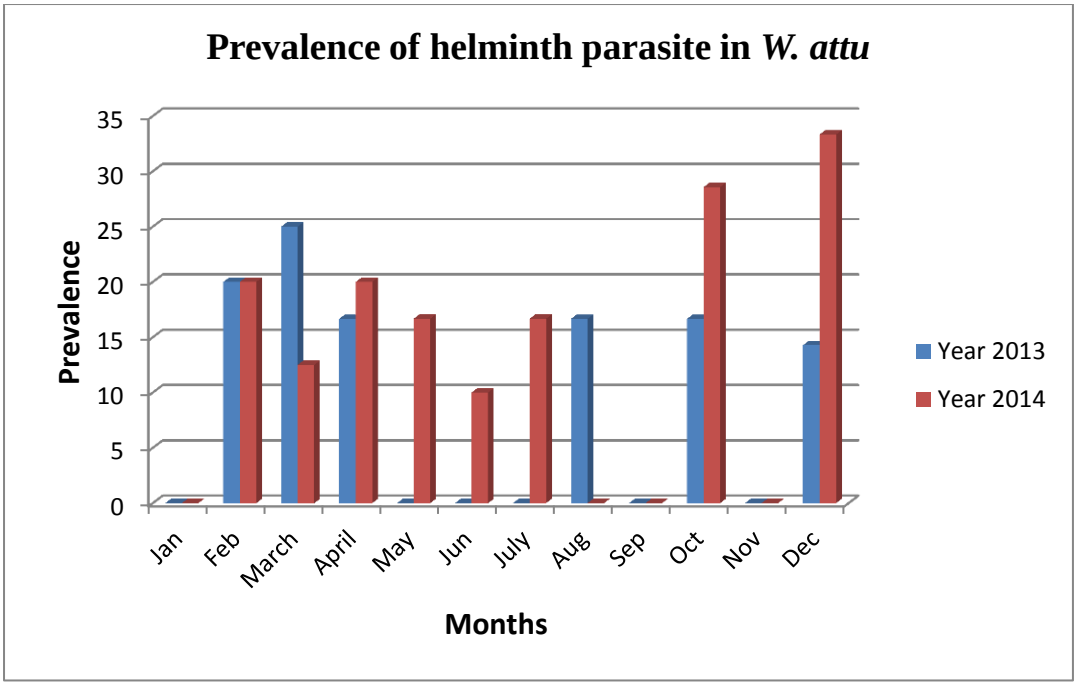


Figure 26: Prevalence (%) of Helminth Parasites of freshwater fish *W. attu* during, January 2013 to December 2014.

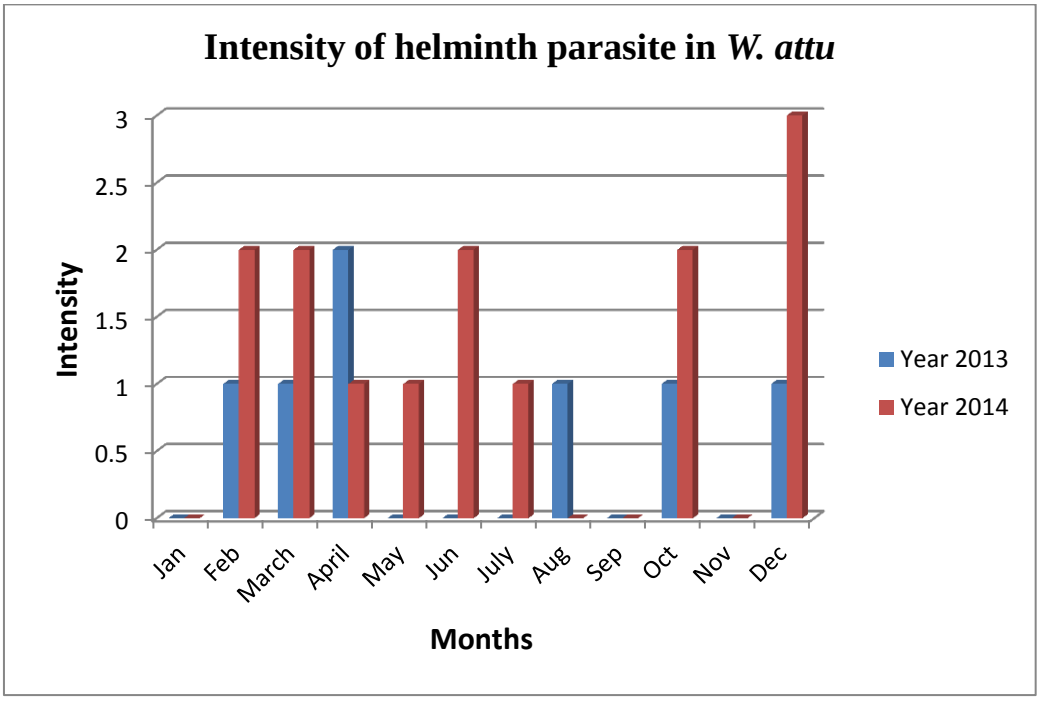


Figure 27: Intensity (%) of Helminth Parasites of freshwater fish *W.attu* during, January 2013 to December 2014

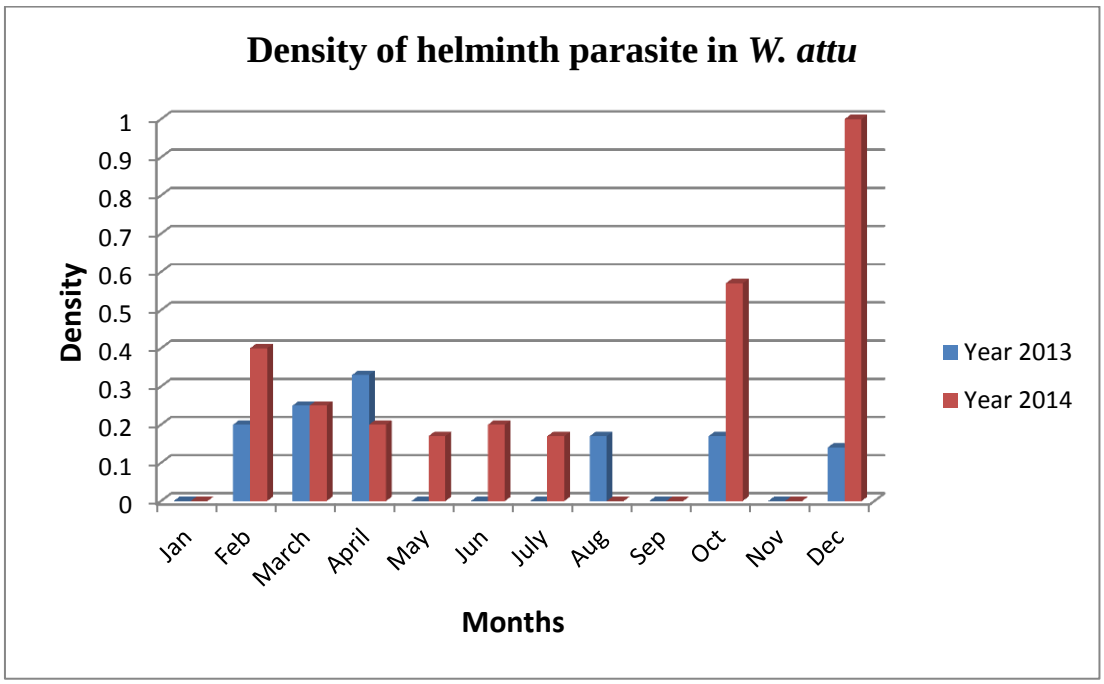


Figure 28: Density (%) of Helminth Parasites of freshwater fish *W. attu* during, January 2013 to December 2014.

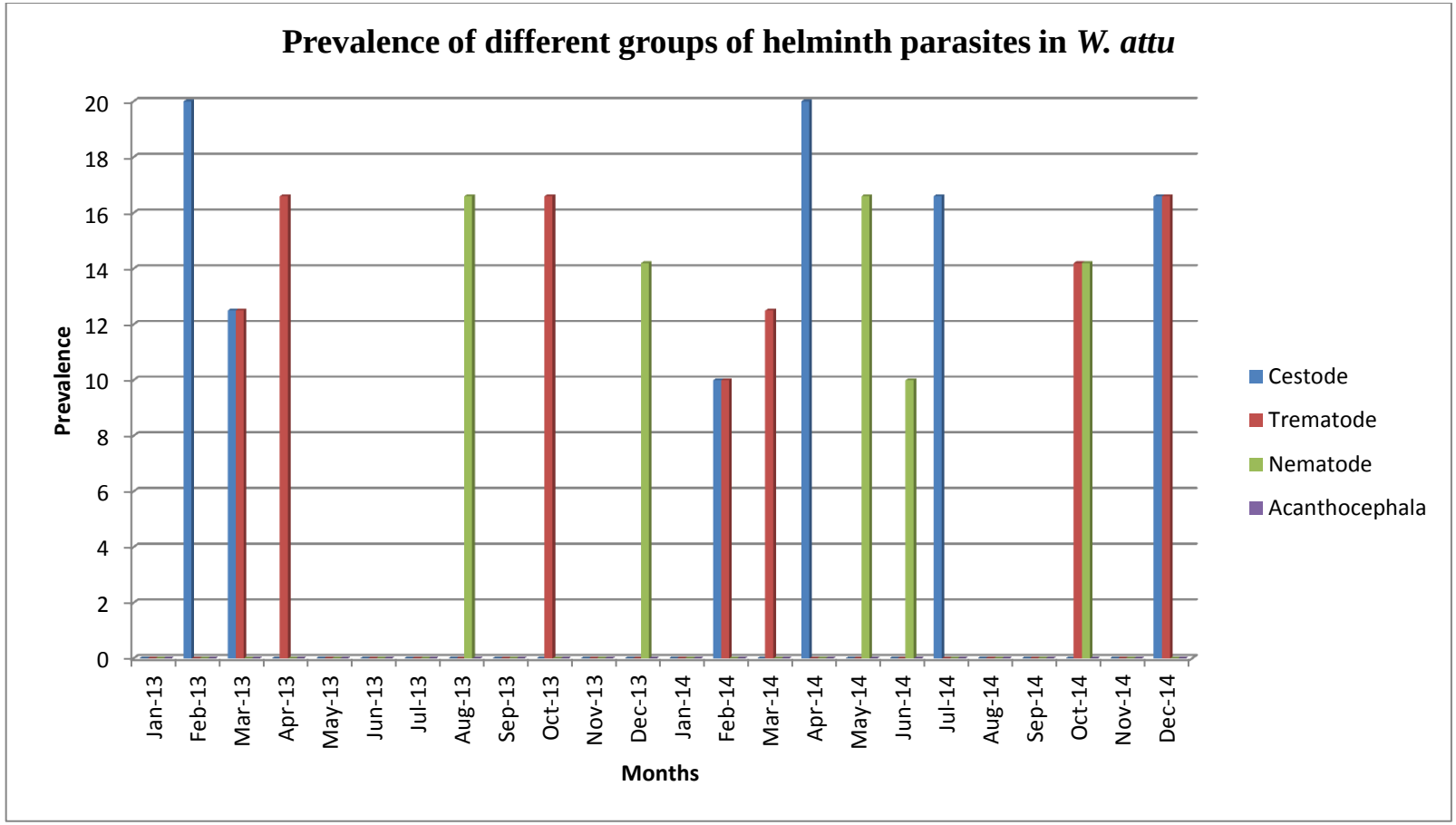


Figure 29: Prevalence (%) of different groups of helminth Parasites of freshwater fish *W. attu* during, January 2013 to December 2014.

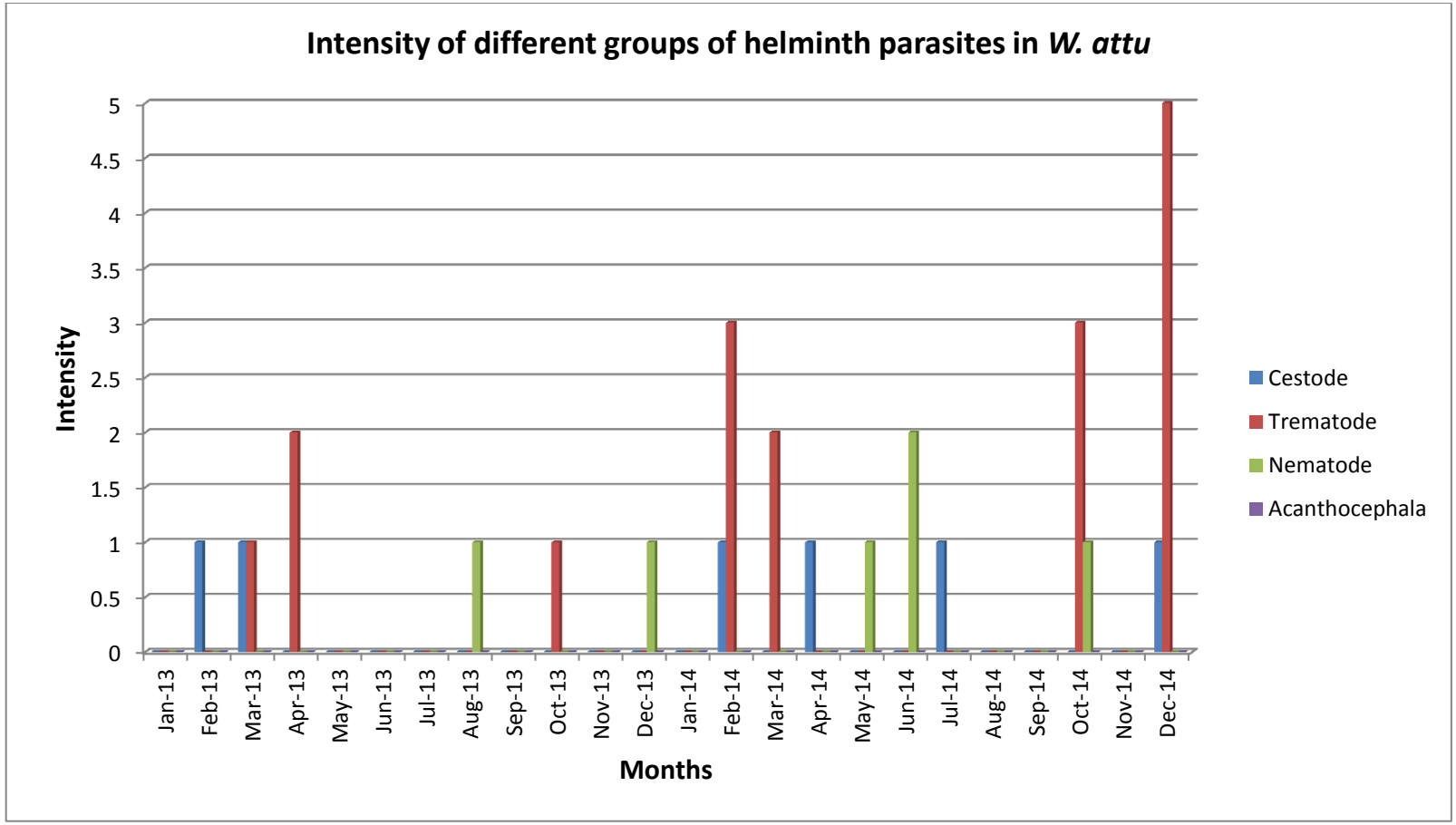


Figure 30: Intensity (%) of different groups of helminth Parasites of freshwater fish *W.attu* during, January 2013 to December 2014

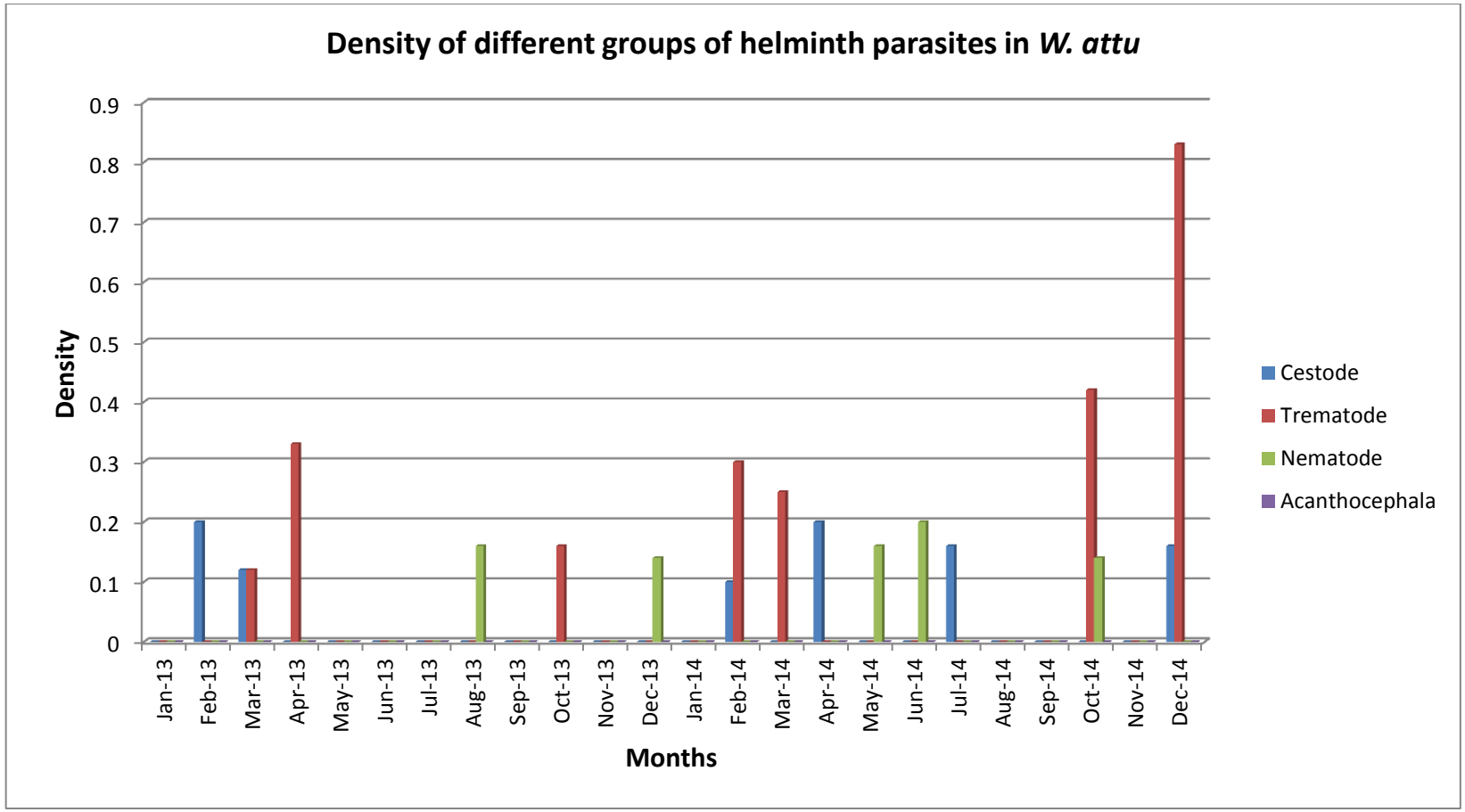


Figure 31: Density (%) of different groups of helminth Parasites of freshwater fish *W. attu* during, January 2013 to December 2014.

Discussion

The present study has been designed to investigate the population dynamics of helminth fauna of five freshwater fishes available in abundance all-round the year in the River Gomti, Lucknow of Uttar Pradesh. The study was carried out for two consecutive years 2013 and 2014.The fresh water fishes selected on the basis of their popularity and easy availability throughout the year in River Gomti, Lucknow were *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu*. Different parameters of population dynamics study viz. Prevalence, intensity of infection, density and of parasites in host fish species were evaluated and reported.

Overall prevalence of helminth parasites in selected fresh water fish

A total of 1491 fishes belonging to genus *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu* were dissected for parasitic examination, out of which 293 fishes were found to be infected with different helminth parasites. The overall prevalence of helminth parasites in selected fishes was observed to be 19.65 %. The highest prevalence (34.28%) was observed in *C. punctatus,* followed by *H. fossilis* (14.72%), *M. vittatus,* (12.28%), *W. attu* (11.84%)and *M. pancalus* (11.01%). The collected helminth parasites were closely observed and found to be belonging to four major classes viz., Trematoda, cestoda, acanthocephala and nematoda. All the four major groups of helminth parasites i.e. Acanthocephalan (16.04%), Trematode (12.08%), Nematode (4.39%) and Cestode (1.7%) were found in *C. punctatus.* These four classes of parasites were also observedin *M. vittatus*in the order of Trematode (6.84%), Nematode (2.66%), Acanthocephalan (2.28%) and Cestode (0.76%). In *H. fossilis* and *W. attu*Cestode, Trematode and Nematode parasites were found while Acanthocephalan parasite was not observed at all. While in *M. pancalus,* only Cestode (2.64%) and Trematode (8.37%) parasites were found while Nematode and Acanthocephalan were absent.The reason for the difference in the rate of helminth infection could beattributed to variations in salinity tolerance of fish hosts. Besides, it could also be related to the presence or absence of many freshwater intermediate hosts of the intestinal helminth parasites (Wogu and Osaka, 2012).

Population dynamics of helminth parasites infecting *Channa punctatus*:

In *Channa punctatus*, the most prevalent (16.04%) parasite was found to be Acanthocephalan, followed by Trematode (12.08%), Nematode (4.39%) and Cestode (1.7%). However, Trematode parasites were found to be most abundant (Abundance: 0.37) and least abundant parasitic group was found to be Cestode (Abundance: 0.01). The investigation on monthly variation of parasites revealed that the incidence of infection was maximum in month of April, 2013 and minimum in November, 2013. In 2014, the highest incidence of infection was recorded in the month of July and minimum in the month of November. *C. punctatus s*howed highest intensity of infection in the month of August during 2013and in the month of July during 2014. It was found lowest in March during 2013 and in December during 2014. The highest density was recorded in April in both year of study. In 2014, the highest density was also observed in March. The lowest Density was recorded in the month of November in both year of study.

Studies on population dynamics of helminth parasites in *Channa punctatus* have been carried out by several workers in India and have reported similar observations (Yadav *et al*., 2010; Gupta *et al*., 2012; Kaur *et al.*, 2012; Rahman *et al*., 2007; Chandra, 2006; Kanth and Srivastava, 1987; Jha *et al.*, 1992; Verma *et al.*, 2013; Dhole *et al.*, 2010). In their study, Yadav *et al*. (2010) examined a total of 359 *Channa punctatus* fish, out of which 223 were found infected with trematode parasites. They reported a minimum prevalence (10%) in September. According to their observations, the maximum Mean intensity was reported in the month of March (2.72) whereas minimum was in September and October (1.0). Similarly, maximum relative density was reported in the month of March (2.45) and minimum in September (0.1).

Dhole *et al*. (2010), summarized the on data of incidence, intensity and density of infection of helminth parasites in freshwater fishes in relation to environmental factors from Marathwada region (M. S.) India and reported the prevalence of seven genera of helminth parasites during their study: four cestodes, two trematodes and one nematode.

In another comparative study on the population dynamics of Pallisentis (Acanthocephala), in relation to host sex from freshwater fishes, in two species of

Channa from Rohilkhand region, Gupta *et al*., (2012) reported that out of a total of 517 fishes (*Channa punctatus*, n= 198 and *C. striatus*, n= 319) examined regularly from August 2006 to February 2010, the overall prevalence of *Pallisentis* in *C. striatus* was higher in females (67.78%) as compared to males (63.52%). Whereas, in case of *C. punctatus*, overall prevalence of Pallisentis was higher in males (53.77%) as compared to females (52.17%) whereas, relative density was higher in females (61.41%) than in males (52.72%). Intensity (2-3 parasite/host, in both sexes), density (1.36 in males and 1.69 in females) and infection index (0.73 in males and 0.88 in females) were recorded by these authors.

Kaur *et al.* (2012), investigated the prevalence of different helminth parasites of freshwater murrel, *Channa punctatus* (Bl.) and reported that *E. heterostomum* (prevalence=28.0%) was the dominant species followed by *Pallisentis* sp. (26.6%), while *Clinostomum complanatum* showed least prevalence (20.0%).

To find out the parasite infection in several edible fish including *Channa punctatus,* Verma and Capoor (2013) conducted extensive survey at different places in river Yamuna at Agra in the rainy season and concluded that the incidence, intensity and density of parasites varied with the fish and their parasite species and also with the different experimental year. In the present study also, there variation in the different parameters was observed in the separate years during the study period.

Kumar (2014) determined the prevalence of infection in sampled species of *Labeo* and *Channa* and investigated the variations in the density of infection and the relationship between the prevalence and mean intensity of *Labeo rohita* (Hamilton), and *Channa punctatus* (Bloch). In their study a total of 360 samples of fresh water fishes were collected from the different sites of Bareilly region, out of which, 246 trematode parasites were collected. The authors reported both Monogenetic and Digenetic trematodes were isolated from the selected fishes

Population dynamics of helminth parasites infecting *Heteropneustes fossilis*:

In *Heteropneustes fossilis*, Trematode parasites were found to be the most prevalent (9.89%) and abundant (0.66). The Trematode group was followed by Nematode (Prevalence: 2.79%; Abundance: 0.03) and Cestode (Prevalence: 2.03%;

Abundance: 0.02). Acanthocephalan was not found at all in *H. fossilis*. In the present investigation, regarding the monthly infestation, maximum prevalence was observed in month of June in 2013 and July in 2014. The minimum prevalence of was recorded in January, April and October in 2013 and in January and June during 2014. The lowest intensity was recorded in months of March, Oct. and December, 2013. In *H. fossilis*, the maximum intensity was recorded in July, 2013. The highest value of density was recorded in July, 2013 and the lowest value of density was calculated in month of October, 2013.

Other workers for example Khanum *et al*. (2008) reported similar findings in another freshwater fish *Rita rita* from the rivers and their tributaries of Dhaka. They reported 50 fishes to be infected by helminth parasites and collected a total of 148 parasites with 2.96 mean intensity. They found that the prevalence of collected cestode larvae was 13% while the intensity was 1.53.

Mofasshalin *et al*. (2012) in their studies on the parasitic infection of three Indian minor carps (*Labeo bata, Labeo gonius, and Cirrhinus reba*) from different fresh water bodies of Rajshahi district, Bangladesh reported that infection and infestation rate of parasites varied with fish size and season and were found to be high in the post-monsoon and winter period (November-March), when fish were found to be most susceptible to parasites.

Population dynamics of helminth parasites infecting *Mystus vittatus*:

In *Mystus vittatus*, Trematode parasites were found to be the most prevalent (6.84%) and abundant (0.17). It was followed by Nematode (Prevalence: 2.66%; Abundance: 0.03), Acanthocephalan (Prevalence: 2.28%; Abundance: 0.03) and Cestode (Prevalence: 0.76%; Abundance: 0.007).The observations revealed that the incidence of helminth parasites was observed to be maximum in month of May in 2013 and in December during 2014. While the incidence of helminth parasites was observed as minimum in month of August in 2013 and in months of March, May and October during 2014. The maximum percent intensity of helminth parasites was recorded in the month of August in 2013 and in March during 2014. The minimum percent intensity of helminth parasites was recorded in months of Feb, June, July, and November, 2013. The maximum percent density was recorded in the month of May,

2013 and in March and July during 2014. The minimum percent density was recorded in the month of May, 2014.

Rafique *et al*. (2002), studied freshwater fish *Mystus vittatus* from a pond at Roy walla, Kasur for the occurrence of intestinal helminthes and reported only one species of nematode from intestine of the fish. In their study they showed 100% prevalence of acanthocephalan infection in this fish species. In another study, Schmidt (1998), on the basis of their observations concluded that the factors like muddy bottom water and feeding habits of *M. vittatus* might also contribute for the incidence of helminth parasites in the fish. However, these authors did not find other helminth parasites such as trematodes, cestodes and nematodes were not found in this fish species.

Kaur *et al*., (2013) investigated the distribution of helminth parasites in catfishes belonging another species Mystus, among others fishes of family Bagridae. They reported five helminth species which included two digenetic trematodes, one species of cestode, and two species of acanthocephalan. The maximum prevalence (100%) of parasites was reported in *Mystus tengara* followed by *Mystus cavasius* (80%) and minimum (36.36%) in *Sperata seenghala*. Thus, the authors concluded that the variation in distribution or prevalence of parasites in a particular host fish may depend on their feeding habit or immunity of fish.

Population dynamics of helminth parasites infecting *Mastacembelus pancalus*:

In *Mastacembalus pancalus* Trematode parasites were found to be the most prevalent (8.37%) and abundant (0.21). It was followed by Cestode parasites (Prevalence: 2.64%; Abundance: 0.02). Nematode and acanthocephalan parasites were not found at all in the fish. The maximum and minimum prevalence of infection was recorded in the months of April, August and December, 2013 and in May and October respectively. In 2014, the maximum and minimum prevalence of infection was recorded in the months of April and in February and May respectively. The minimum percent intensity of infection was observed in the months of April and July. The investigation revealed that the minimum percent of density of infection was recorded in the months of July and October. In 2014, the maximum percent intensity was recorded in the month of April and minimum was observed in the months of January,

Feb and October. The investigation revealed that the minimum percent of density was recorded as in the month of February in 2014.

Population dynamics of helminth parasites infecting *Wallago attu*:

In *Wallago attu*, Trematode parasites were found to be the most prevalent (4.60%) and abundant (0.11). The Trematode group was followed by Cestode (Prevalence: 3.94%; Abundance: 0.03) and Nematode (Prevalence: 3.28%; Abundance: 0.03). Acanthocephalan parasites were not found at all in *W. attu.*

The observations during 2013 revealed that the maximum incidence of infection was recorded in the month of March while minimum in the month of December. The maximum percent of intensity and density observed in the month of April. The minimum intensity of infection was recorded in the months of Feb, March, August, October and December. The minimum density of infection was recorded in the month of December. The Maximum index of infection was observed in the month of March while minimum index of infection was recorded in the month of December. In year 2014, the maximum prevalence of infection was recorded in the month of December while the minimum in the month of June. The maximum intensity of infection was recorded in the month of December and the minimum in the months of April, May and July. The investigation revealed that the maximum percent of Density and index of infection was recorded in the month of December and the minimum in the months of May and July.

Conclusion

The findings accomplished that *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu* were common species of edible fishes in river Gomti at Lucknow. These were found to be attacked by helminth parasites externally and internally, and the organs infected were gills, stomach, intestine swim bladder and body cavity. The major classes of helminth parasites i.e Cestoda, Trematoda, Nematoda and Acanthocephala were observed in the fish species. The observationson population dynamics revealed that number of fish and the helminth infection fluctuated round the year. The dynamics of different parameters including percent incidence percent, percent density and index of infection was found to be highly variant in selected fish species in both years of the study period. Similar variations in the years of study period were also reported by Verma and Capoor (2013). The highest Cestode infection was observed in *W. attu*, highest Trematode, Nematode and Acanthocephalan infection in *C. punctatus*. The differences may be due to a difference in the intensity of pollution at different locations of study sites. Climate change in aquatic systems will affect most organisms and their functional roles in the ecosystem. Changes in these roles may be difficult to detect, but examination of parasite communities in fish may provide insight into any structural and functional alterations in the system. Thus, the knowledge provided by this study could be greatly useful as previous information could be utilized for intensive production of fishes where the fish health could be maintained by checking the parasitic infestation.

PLATE - A

Fig. 1: Lateral view of *Channa punctatus*

Systematic Position of *C. punctatus*

- Phylum : Chordata
- Class : Teleostomi
- Order : Ophiocephaliformes
- Family : Ophiocephalidae
- Genus : *Channa*
- Species : *punctatus*

PLATE - B

Fig. 2: Lateral view of *Heteropneustes fossilis*

Systematic Position of *Heteropneustes fossilis*

- Phylum : Chordata
- *Class* : Teleostomi
- Order : Cypriniformes
- Family : Heteropneustidae
- Genus : *Heteropneustes*
- Species : *fossilis*

PLATE - C

Fig. 3: Lateral view of *Mystus vitattus*

Systematic Position of *Mystus vitattus*

- Phylum : Chordata
- Class : Teleostomi
- Order : Cypriniformes
- Family : Bagridae
- Genus : *Mystus*
- Species : *vittatus*

PLATE - D

Fig. 4: Lateral view of *Mastacembalus pancalus*

Systematic Position of *Mastacembalus pancalus*

- Phylum : Chordata
- Class : Teleostomi
- Order : Mastacembeleformes
- Family : Mastacembelidae
- Genus : *Mastacembalus*
- Species : *pancalus*

PLATE - E

Fig.5: Lateral view of *Wallago attu*

Systematic Position of *Wallago attu*

- Phylum : Chordata
- Class : Teleostomi
- Order : Cypriniformes
- Family : Siluroidae
- Genus : *Wallago*
- Species : *attu*

PLATE – F

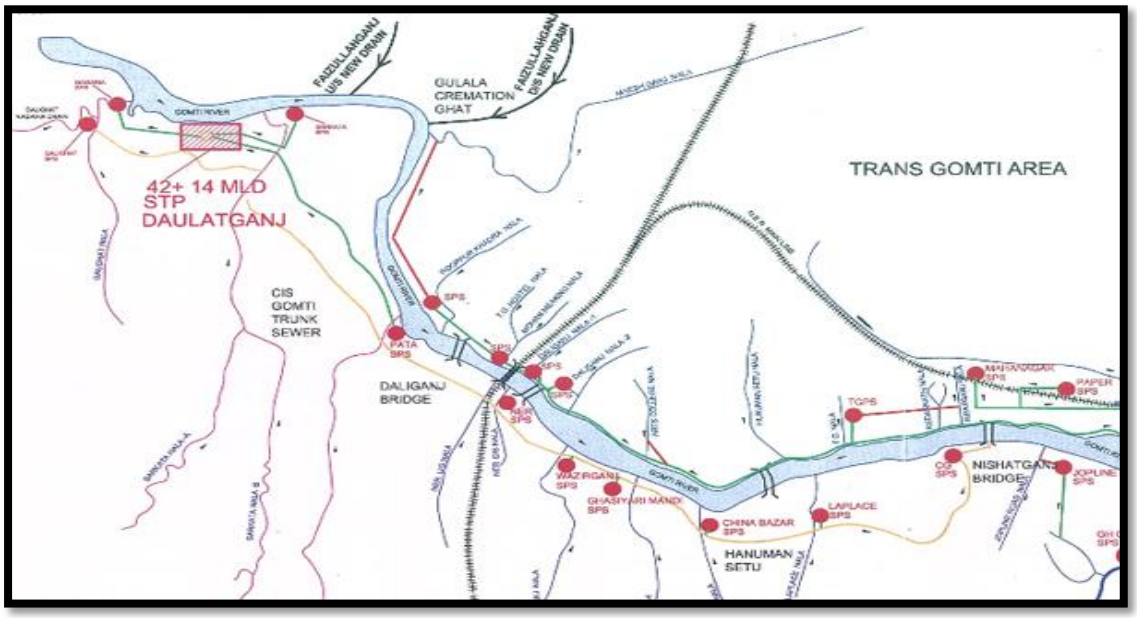


Fig. 1: Map showing Gomti River from where the fishes are collected.

Source: lucknowinfo.com

Fig. 2: One site of River Gomti from where the fishes are collected.

Source: https//en.wikipedia.org/wiki/Gomti_River

PLATE - 1

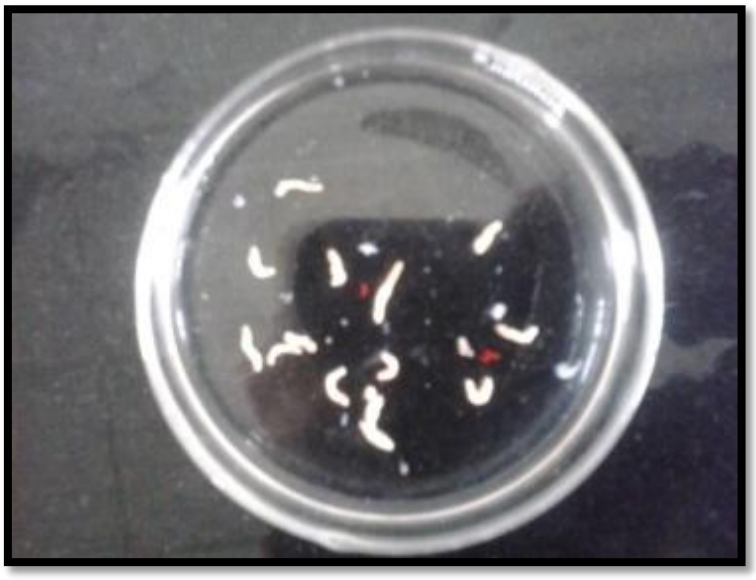


Fig. 1: Relaxation stage of helminth parasites in saline

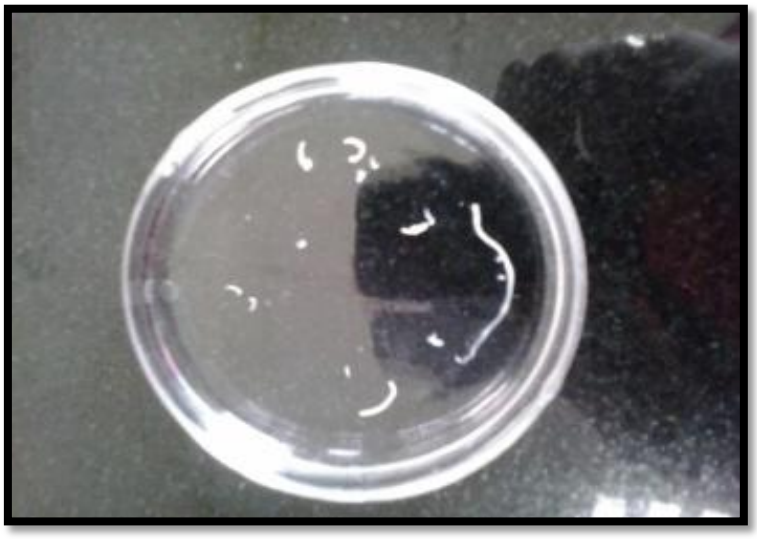


Fig. 2: Relaxation stage of helminth parasites in saline

PLATE – 2

Fig. 1: Trematode parasite

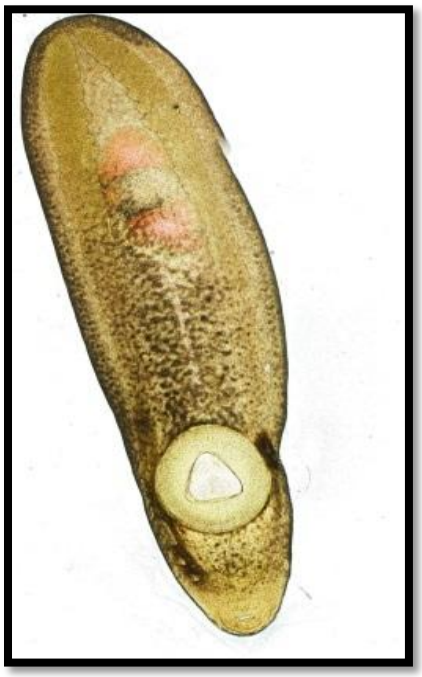


Fig. 2: Trematode parasite

Fig. 3: Trematode parasite

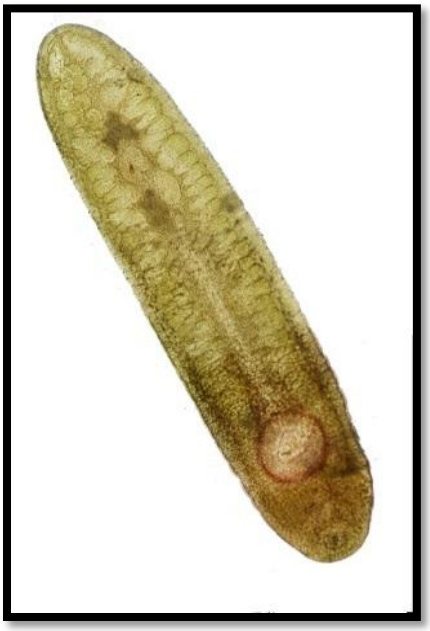


Fig. 4: Trematode parasite

PLATE – 3

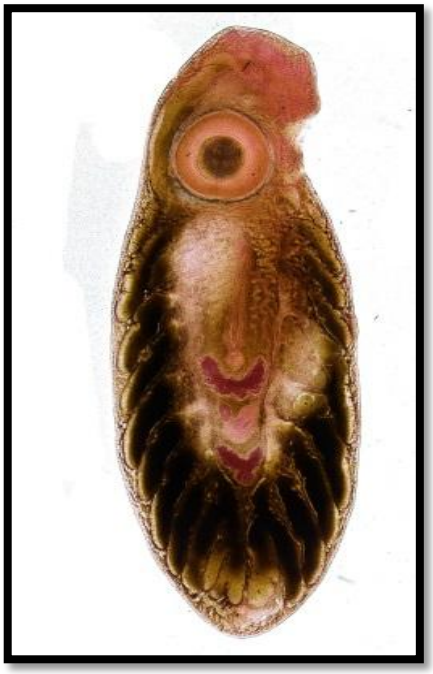


Fig. 1: Trematode parasite

Fig. 2: Trematode parasite

Fig. 3: Trematode parasite

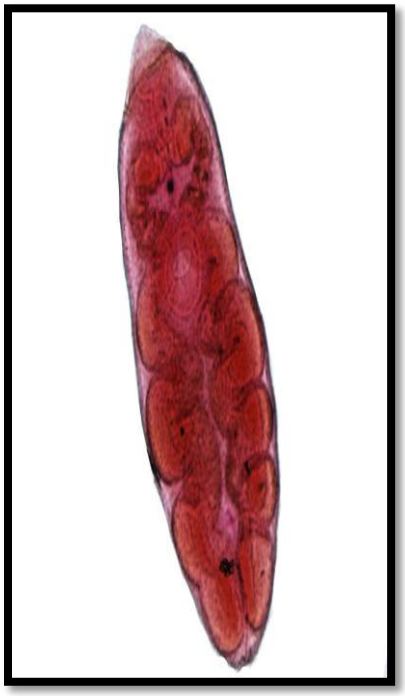


Fig. 4: Trematode parasite

PLATE – 4

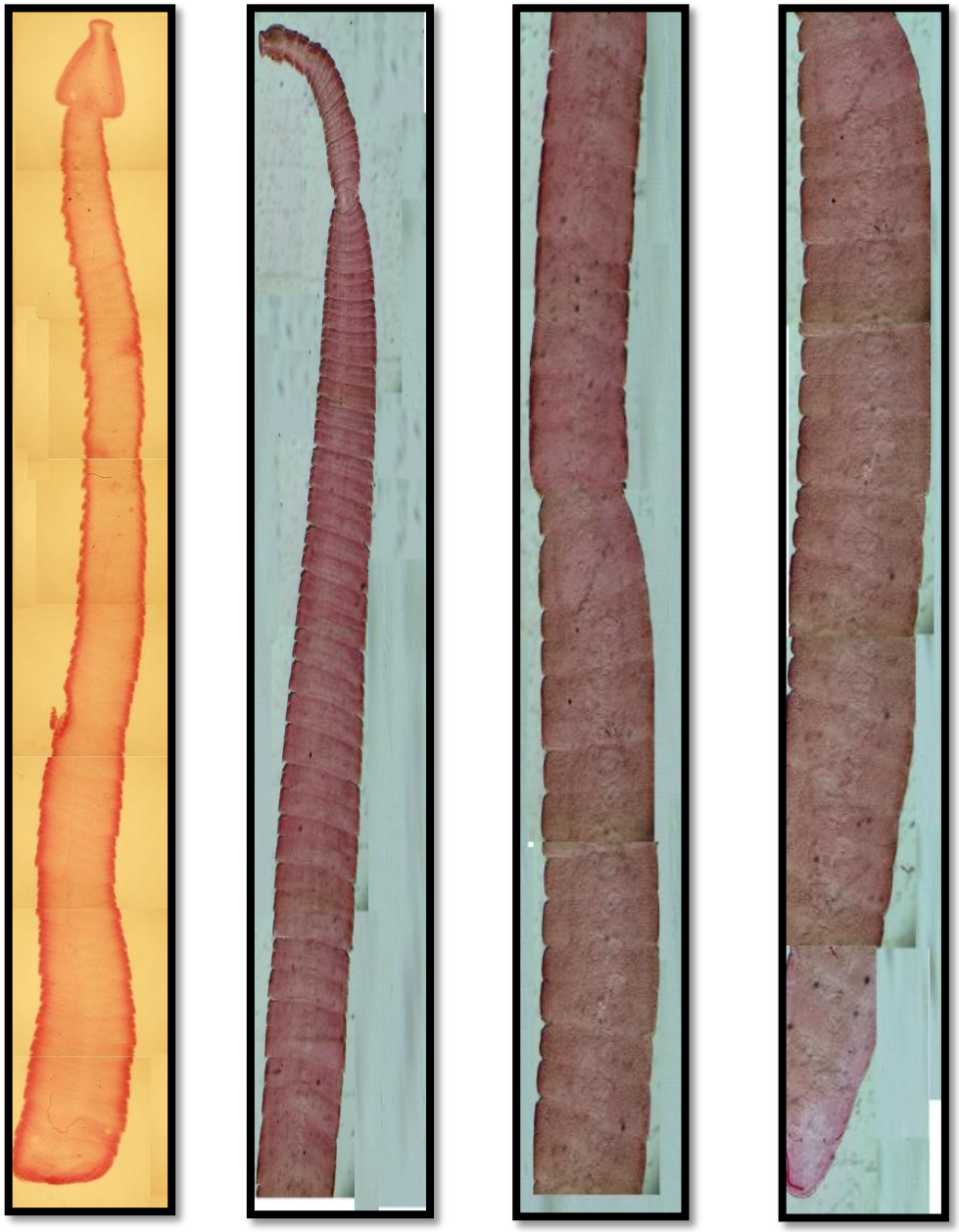


Fig. 1: Cestode parasites

PLATE – 5

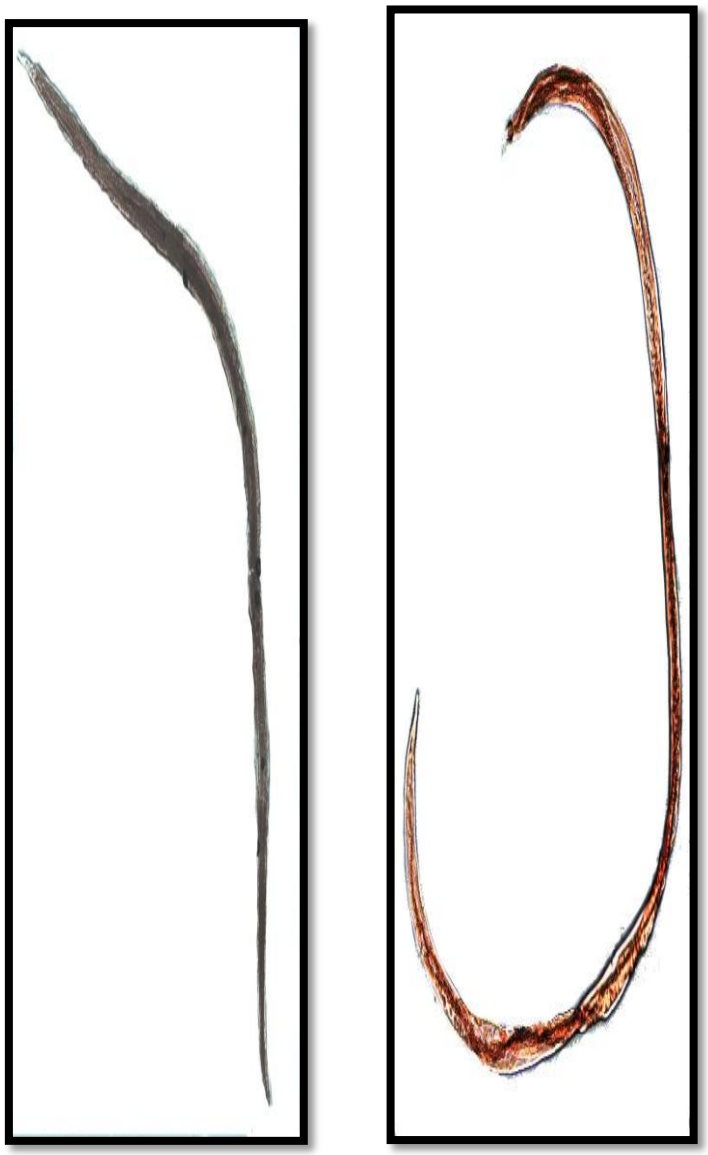


Fig. 1: Nematode parasites

PLATE – 6

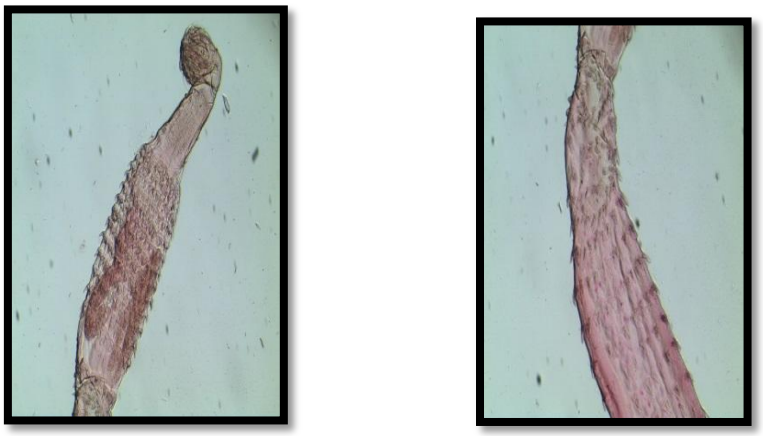


Fig. 1: Anterior region

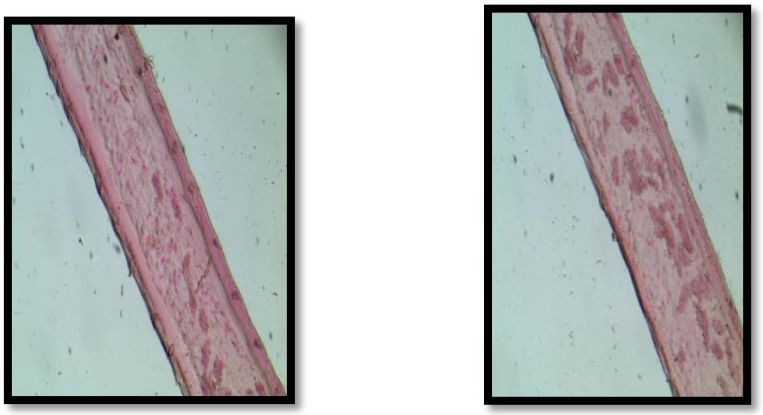


Fig. 2: Middle region

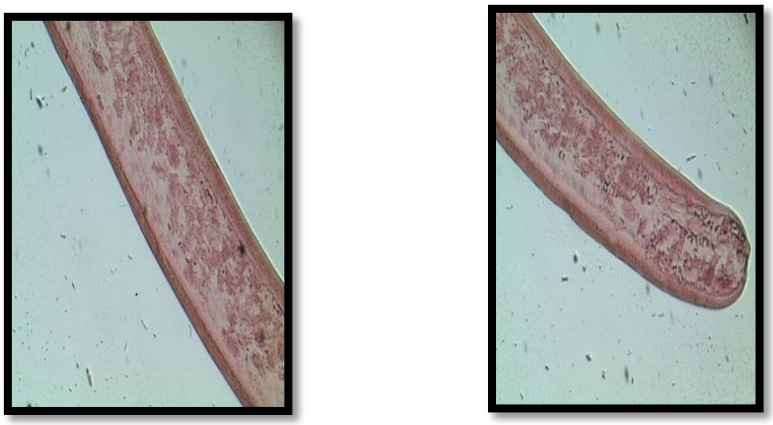


Fig. 3: Posterior region

Acanthocephala parasites

PLATE – 7

Fig. 1: Acanthocephala

Chapter 4

Study of the Relationship between Prevalence of Helminth Infection and Length, Weight and Sex of Host Fish

Introduction

As the world population grows, fish resources are being depleted at an increasing rate as a result of environmental degradation, over harvesting, and pollution and therefore fish production could no longer meet the demand of the growing population. This had led to an increase in the involvement of stakeholders in aquaculture. The methods of fish farming are in general, plagued by problems of overcrowding, poor environmental conditions and pollution which often results in reduced immunity of fish and higher susceptibility to parasites and diseases (Murray, 2005; Biu et al., 2014). Parasites have been shown to be responsible for delay in fish growth and gain of weight by affecting the food ingestion (Barber *et al*., 2000; Barker *et al*., 2005). The relationship between weight and length has been used as a tool to estimate body conditions of healthy fish (Tavaes-Dias *et al*., 2000a) and parasitized fish (Laidley *et al*., 1988; Tavaes-Dias *et al*., 1999a; Ranzani-Paiva *et al*., 1997; Tavaes-Dias *et al*., 2002; Lizama *et al*., 2006).

Many studies have reported the highest prevalence of parasites in adult fish, which is an indicator that size of the fish is important in determining the parasitic burden compared to juveniles (Dar *et al*., 2012). Geets and Ollevier (1996), and Oniye and Aken'Ova (1999) observed an increase in the abundance of parasites with host size. Anosike *et al*. (1992) also observed that number and diversity of parasites increased with the age of fish. Mohammed *et al*. (2009) reported an increase in incidence of infection as the fish grows, and attributed it to the longer time of exposure to the environment by the larger body size.

Esch *et al*. (1990) observed that the relationship between parasitism and host size may indicate how parasite infra-communities structure changes during the life cycle of the host. Community organization may be influenced by host age and size through changes in diet or the volume of ingested food, ontogenetic changes in immune-competency, and changes in the possibility of contact with intermediate hosts.

According to Bauer (1959), Kennedy (1969) and Collard (1970), the variations in infections of three parasitic helminth species (*Diplectanum aequens*, *Acanthostomum absconditum*, *Hysterothylacium* sp.) according to seasons, host

length, size, age and sex of host fish might be influenced by many factors such as the hydro biological conditions of water, changes in host diets and feeding habits and physiological state of fish.

Helmintho-fauna infections in fishes have been noticed to have marked relationship with sex and size (Ezenwaji and llozumba 1992; Machado *et al*., 1994; Saliu, 1998; Guidelli *et al*., 2003; Araoye, 2005; Lizama *et al*., 2005, 2006; Hassan *et al*., 2010; Omeji, 2012). However, extensive details in literature as regards to the relationship between sex and prevalence are scarce. To its a few example, Emere (2000) attested differences in infestation between males and females to differential feeding pattern, which could be in terms of value and quantity. They concluded that it could also be attributed to differences in the degree of resistance to infection. Emere and Egbe (2006) stated that the physiological state of the female fishes could have reduced resistance to infection by parasites and they also found that there was no relationship between parasite burden and size of fish.

There is an increasing awareness of the importance of parasitic diseases as one of the major detrimental factors in fish farming (Paperna, 1996; Keremah and Inko-Tariah, 2013) and that the sizeand sex of the fish bears a relation to the parasitic burden carried by the host. Further, the increased demand of fish has made the need for a continuous study on fish and their parasites as important for determining the health of the fish. Therefore, the present study was aimed at investigating the relationship between the prevalence of helminth parasites and the length, weight and sex of selected host fishes from River Gomti, Lucknow.

Materials and Methods

Study area: - Daliganj, Hanuman Setu, Pakka Pul, Nishatganj Pul, Gaughat, and Gomti barrage regions of River Gomti, Lucknow**.**

Study period: - January, 2013 to December, 2014.

Test organisms: - *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus, and Wallago attu***.**

Collection of host fishes: - During the collection period, a total of 1491 live specimens of freshwater fishes viz., *Channa punctatus* (455), *Heteropneustes fossilis* (394), *Mystus vittatus* (263), *Mastacembalus pancalus* (227), *and Wallago attu* (152) were collected from different places on the Gomti river, Lucknow, viz., Daliganj, Hanuman Setu, Pakka Pul, Nishatganj Pul, Gaughat, and Gomti barrage for the study of various indices.

Methodology

Collection and determination of parameters of fish

The fishes were anaesthetized by using clove oil. Before dissection, the Total Length (TL) and Standard Length (SL) of the fishes were recorded using stainless steel scale, and weight (W) of individual fish was measured using analogue weighing balance. Each fish was dissected by slitting on ventral side near the genital pore/anal region and it was cut open towards the head up to the opercular region for observation of the parasitic infection. Sex was determined by observing the gonads.

Total and Standard Length measurement

The total and standard length of each fish was measured in centimeter (cm) using stainless steel scale. The total length was measured from the tip of the snout to the extreme end of the caudal fin, while standard length was measured from the tip of the snout to the end of caudal peduncle.

Weight measurement

Fish sample was weighed to the nearest gram (g) using weighing balance (Paperna 1996).

Sex determination

The sex of the fish was initially determined by examination of genital pore and later by exposing the gonads after dissection (Martinez-Aquino *et al*., 2004).

Determination of parasitic infection load/prevalence and identification of parasites

The protocols for the fixation, identification and prevalence of parasites accordingly followed the standard methods and have already been described in detail in Chapter 1.

Determination of prevalence of Helminth infection in relation to length, weight and sex of fishes

The fishes were grouped into different classes corresponding to the parameters studied *i.e.*, length, weight and sex. The prevalence of helminth parasitic infection in relation to length, weight and sex of the host fishes was recorded and calculated according to Margolis *et al.* (1982).

$$\text{Prevalence} = \frac{No.of\ Host\ infected}{Total\ Host\ examined} \times 100$$

Statistical analysis

Microsoft excel 2010 was used in the analysis where in descriptive analyses such as sum, average and standard deviation were computed. Chi-squared Goodness of fit was employed in order to statistically determine if there was any significant difference between prevalence of infection and sex of the host between prevalence of infection and standard length of the host and likewise between prevalence of infection and weight of host fish.

Results

A total of 1491 specimens of selected freshwater fishes belonging to 5 species namely: *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus, Wallago attu* were examined over the study period from January, 2013 to December, 2014 for the presence of endoparasitic helminthes infection. Results of this examination revealed that these host fishes were parasitized by helminthes belonging to all the classes viz., Trematoda, Cestoda, Nematoda, and Acanthocephala. The acanthocephalan parasites were not detected in *Heteropneustes fossilis, Mastacembalus pancalus* and *Wallago attu,* while the nematode parasites were not detected in the *Mastacembalus pancalus* only.

Relation between Parasitic load and length, weight, and sex in host fish

1. *Channa punctatus*

The helminth infection in relation to size (length), weight and sex of host was analyzed. Out of 455 *Channa punctatus* examined, 156 were found to be infected by the helminthic parasites. Overall 34.28% of the fish were found to be infected with the helminth parasites which belonged to the classes Trematoda, Cestoda, Nematoda and Acanthocephala.

The fishes were grouped according to their length into two classes viz., class I (7 – 11.9 cm) and class II (12 – 16.9 cm) and examined for their parasitic load. The results are shown in Table 1, and Figure 1. In class I (7 - 11.9 cm) a total of 254 fishes were examined out of which 82 fishes were found to be infected with the helminthic parasites. The overall prevalence was observed to be 32.28% in the fishes with this range of length. In class II (12 – 16.9 cm) a total of 201 fishes were examined, out of which 74 fishes were found to be infected, showing an overall prevalence of 36.81%. Although a higher prevalence of infection was observed in fishes of Class II, the variation in the parasitic loads of the two groups of fishes was not statistically significant ($\chi^2 = 0.5995$; $P>0.05$).

The fishes were also grouped into different classes according to their weight viz., class I (15 – 29.9g), class II (30 – 44.9g), class III (45 – 59.9 g), class IV (60 –

74.9g), class V (75 – 89.9 g) and class VI (90 – 104.9 g) and subsequently examined for parasitic load. The results are shown in Table 2, figure 2. In class I (15 - 29.9 g) a total of 164 fishes were examined out of which, 39 fishes were found to be infected (Prevalence=23.78%). In class II (30 - 44.9 g) a total of 133 fishes were examined out of which 49 fishes were found to be infected (Prevalence=36.84%). In class III (45 – 59.9 g) a total of 87 fishes were examined, out of which 24 fishes were found to be infected (Prevalence= 27.58%). In class IV (60 – 74.9 g) a total of 39 fishes were examined, out of which 26 fishes were found to be infected (Prevalence =66.66%). In class V (75 – 89.9 g) only 27 fishes were examined, out of which 15 fishes were found to be infected with an overall prevalence of 55.55%. In class VI (90 – 104.9 g) there were only 5 fishes, out of which 3 fishes were found to be infected. The overall prevalence of infection in this Class VI was observed as 60.00%. Chi square test revealed that the variations in the parasitic load in relation to weight of the fish was not statistically significant ($\chi^2 = 1.378$; $P>0.05$)**.**

The relation between prevalence of infection in *Channa punctatus* in relation to the sex of the host was also determined and the results are shown in Table 3, Figure 3. A total of 266 male fishes were examined, out of which 71 fishes were found to be infected (overall prevalence was (26.69 %). A total of 189 female fishes were examined out of which 85 fishes were found to be infected, depicting an overall prevalence of infection of 44.97%. The higher prevalence of parasitic infection observed in female host fish as compared to the male fish was statistically significant ($\chi^2 = 5.153$; $P<0.05$).

2. *Heteropneustes fossilis*

The helminthic infection load in relation to size (length), weight and sex of host fish *Heteropneustes fossilis* was analyzed. A total of 394 fishes were examined, out of which, only 58 were found to be infected by the helminthic parasites. The prevalence of infection was observed to be 14.72%. The helminth parasites isolated from this fish species belonged to classes Trematoda, Cestoda and Nematoda. Acanthocephalan parasites were not observed in any member of this species during the period of study.

The fishes were grouped in two classes according to their lengths viz., class I (10 – 14.9 cm) and class II (15 – 19.9 cm) and examined for parasitic infection. The results are shown in Table 4, figure 4. In class I, a total of 293 fishes were examined, out of which 42 fishes were found to be infected with the helminthes parasites. The overall prevalence was observed as14.33%. In class II (15 – 19.9 cm) a total of 101 fishes were examined, out of which 16 fishes were found to be infected giving an overall prevalence of 15.84%. A little higher prevalence of helminthes infection was found in the fishes of Class II, however, chi square test revealed that this was not significant ($\chi^2 = 0.1358$; $P>0.05$).

The *H. fossilis* fishes were grouped into different classes according to their weight viz., class I (15 – 34.9g), class II (35 – 54.9g), class III (55 – 74.9 g), class IV (75 – 94.9g), class V (95 – 114.9 g) and class VI (115 – 134.9 g) and examined for their parasitic load. The results are shown in Table 5, Figure 5. In class I (15 - 34.9 g) a total of 222 fishes were examined, out of which 30 fishes were found to be infected (Prevalence=13.51%). In class II (35 - 54.9 g) a total of 125 fishes were examined out of which 19 fishes were found to be infected (Prevalence=15.20 %). In class III (55 – 74.9 g) a total of 11 fishes were examined out of which 3 fishes were found to be infected (Prevalence= 27.27 %). In class IV (75 – 94.9 g) a total of 8 fishes were examined and were not found to be infected with any helminthic parasite. In class V (95 – 114.9 g) only 18 fishes were examined, out of which 4 fishes were found to be infected, with an overall prevalence of 22.22%. In class VI (115 – 134.9 g) only 10 fishes were examined out of which, only 2 fishes were found to be infected (overall prevalence of infection was observed as 20.00%. Chi square test revealed a significant difference ($\chi^2 = 1471.885$; $P<0.05$) in the prevalence of helminthes infection in relation to the weights of the examined host fishes in all the six classes.

The relation between prevalence of infection and sex of *H. fossilis* was also studied in which, out of a total of 233 male fishes examined, 30 were found to be infected by helminthes parasites. The overall prevalence was found to be 12.87 %. In the same study, a total of 161 female fishes were examined out of which 28 fishes were found to be infected, with an overall prevalence of infection of 17.39 % (Table 6, Figure 6). However, although a higher prevalence of infection was observed in the female fish, it was not statistically found to be significant ($\chi^2 =1.546$; $P>0.05$).

3. *Mystus vittatus*

Studies on the relationship between the prevalence of helminthic infection and size (length), weight and sex of host fish *Mystus vittatus* was analyzed, and out of a total of 263 fish examined, 36 were found to be infected by the helminth parasites with an overall prevalence of 13.68%. The helminth parasites isolated belonged to the classes Trematoda, Cestoda, Nematoda and Acanthocephala.

The fishes were grouped into two classes in accordance to their length viz., class I (5 – 9.9 cm) and class II (10 – 14.9 cm) and examined for their parasitic load. The results are shown in Table 7, Figure 7. In class I (5- 9.9 cm) a total of 251 fishes were examined out of which 34 fishes were found to be infected with the helminthes parasites. The overall prevalence observed was 13.54% in the fishes with this range of length. In class II (10 – 14.9 cm) a total of 12 fishes were examined, out of which 02 fishes were found to be infected with an overall prevalence of 16.66%. Higher prevalence of infection was found in Class II, however, chi square test revealed that the difference in the occurrence of helminthes parasites in the two classes of the examined host fishes was not significant (χ^2 =0.094; P>0.05).

The fishes were also grouped into different classes according to their weight viz., class I (15 – 24.9g), class II (25 – 34.9g), class III (35 – 44.9 g) and examined for the status of infection by the helminth parasites. The results are shown in Table 8, Figure 8. In class I (15 - 24.9 g), a total of 114 fishes were examined, out of which, 18 fishes were found to be infected (Prevalence=15.78%). In class II (25 - 34.9 g), a total of 137 fishes were examined and out of these 16 fishes were found to be infected (Prevalence= 12.40 %). In class III (35 –44 .9 g) a total of 12 fishes were examined out of which 02 fishes were found to be infected (Prevalence= 16.66 %). Chi square test revealed that there is no significant difference (χ^2 = 0.984; P>0.05) in the prevalence of helminth infection among the six classes of the examined hosts, grouped according to their weights**.**

Prevalence of infection in *M. vittatus* was also studied according to the sex of the host and the results are shown in Table 9, Figure 9. Out of a total of 152 male fishes examined, 17 fishes were found to be infected, with the overall prevalence of 11.18 %. Whereas, out of a total of 111 female fishes examined, 19 were found to be

infected with the helminth parasites (prevalence of infection of 17.11%). Higher prevalence of infection was observed in female hosts but it was not statistically significant (χ^2 = 1.911; P>0.05).

4. *Mastacembalus pancalus*

The helminth infection load in relation to size (length), weight and sex of host fish *Mastacembalus pancalus* was studied and a total of 227 fishes were examined, out of which, 25 were found to be infected by the helminth parasites. An overall prevalence of 11.01% was observed, and the isolated helminth parasites belonged to classes Trematoda, and Cestoda only. No nematode or Acanthocephalan parasites were observed.

The fishes were grouped into two classes according to their length viz., class I (10-14.9 cm) and class II (15- 19.9 cm) and examined for parasitic infection. The results are shown in Table 10, Figure 10. In class I (10-14.9 cm) a total of 205 fishes were examined out of which 23 fishes were found to be infected. The overall prevalence was observed as 11.21% in the fishes with this range of length. In class II (15-19.9 cm) a total of 22 fishes were examined, out of which 02 fishes were found to be infected with an overall prevalence of 9.09%. Higher prevalence of helminth infection was found in Class I, however, chi square test did not reveal it to be significant (χ^2 =0.0915; P>0.05).

The fishes were grouped into different classes according to their weight viz., class I (15 – 29.9gm), class II (30 – 44.9gm), class III (45 – 59.9 gm) and examined for infection by helminth parasites**.** The results are shown in Table 11 and Figure 11. In class I (15 - 29.9 gm), a total of 147 fishes were examined out of which 19 fishes were found to be infected (Prevalence=12.92%); in class II (30 - 44.9 gm) a total of 65 fishes were examined out of which 4 fishes were found to be infected (Prevalence=6.15%).; and in class III (45 – 59.9 gm) a total of 15 fishes were examined out of which 02 fishes were found to be infected (Prevalence= 13.33%). However the differences in the prevalence of helminth infection among the six groups of the examined hosts classified according to their weights was not found to be significant (χ^2 = 2.197; P>0.05).

The prevalence of infection in *M. pancalus* was also studied in accordance to the sex of the host fish and the results are shown in Table 12, Figure 12. A total of 129 male fishes were examined, out of which 13 fishes were found to be infected by the helminth parasites. The overall prevalence of infection was found to be 10.07%. In case of the total of 98 female fishes examined, 12 were found to be infected with an overall prevalence of infection of 12.24%. The higher prevalence of infection observed in female hosts as compared to the male host fishes was however not significant statistically (χ^2 =0.267; P>0.05).

5. *Wallago attu*

The helminth infection in relation to size (length), weight and sex of host fish *Wallago attu* was studied and out of a total of 152 fishes examined, 18 were found to be infected by helminth parasites. The overall prevalence of infection observed was 1.84%. Three helminth parasitic classes were identified: the Trematoda, Cestoda and Nematoda.

The fishes were grouped into two classes according to their lengths viz., class I (15 – 24.9 cm) and class II (25 – 34.9 cm) and examined for infection by helminth parasites. The results are shown in Table 13, Figure 13. In the length class I (15 - 24.9cm), a total of 79 fishes were examined, out of which 07 fishes were found to be infected. The overall prevalence was 8.86%. In the length class II (25 – 34.9cm), a total of 73 fishes were examined out of which 11 fishes were found to be infected. The overall prevalence was 15.06%. Higher prevalence of infection was observed in Class II as compared to class 1, but the difference was statistically non-significant (χ^2 = 1.400; P>0.05).

The fishes were grouped into different classes according to their weights viz., class I (38 – 97.9g), class II (98 – 157.9g), class III (158 – 217.9g) and examined for helminth infection. The results are shown in Table 14 and Figure 14. In class I (38 - 97.9 g), a total of 118 fishes were examined out of which 13 fishes were found to be infected. The overall prevalence was 11.01%. In class II (98 - 157.9 g) a total of 18 fishes were examined out of which only one fish was found to be infected. The overall prevalence was 5.55% and in class III (158 – 217.9 g) a total of 16 fishes was examined out of which 04 fishes were found to be infected by the helminth. The

overall prevalence was found to be 25%. Chi square test revealed that differences in prevalence of helminthes infection in the three classes of host fishes are statistically significant ($\chi^2 = 923.07$; $P<0.05$).

The fishes (non-infected and infected) were grouped according to their sex and examined for the infection by helminth parasites. The results are shown in Table 15, Figure 15. Out of a total of 89 male fishes examined, 11 were found to be infected giving an overall prevalence of 12.35%. A total of 63 female fishes were examined in which 07 fishes were found to be bearing the helminth infection. The overall prevalence of infection was 11.11%. However, the prevalence of infection in male and female host varied non- significantly ($\chi^2 = 0.344$; $P>0.05$).

Table 1: Relation between the host length and the prevalence of infection in *Channa punctatus*

Classes	Standard length (cm)	No. of fish examined	No. of fish infected	Prevalence
I	7 - 11.9	254	82	32.28
II	12 - 16.9	201	74	36.81
	Total	455	156	34.28

Table 2:-Relation between the host Weight and the prevalence of infection in *Channa punctatus*

Classes	Body weight (g)	No. of fish examined	No. of fish infected	Prevalence
I	15 – 29.9	164	39	23.78
II	30 – 44.9	133	49	36.84
III	45 – 59.9	87	24	27.58
IV	60 – 74.9	39	26	66.66
V	75 – 89.9	27	15	55.55
VI	90 – 104.9	5	3	60.00
	Total	455	156	34.28

Table 3:- Relation between the host sex and prevalence of infection in *Channa punctatus*

Sex of fish (Host)	No. of fish examined	No. of fish infected	Prevalence
Male	266	71	26.69
Female	189	85	44.97
Total	455	156	34.28

Table 4:-Relation between the host length and the prevalence of infection in *Heteropneustes fossilis*

Class	Standard length (cm)	No. of fish examined	No. of fish infected	Prevalence
I	10 – 14.9	293	42	14.33
II	15 – 19.9	101	16	15.84
	Total	394	58	14.72

Table 5:-Relation between the host Weight and the prevalence of infection in *Heteropneustes fossilis*

Class	Body weight (gm)	No. of fish examined	No. of fish infected	Prevalence
I	15 – 34.9	222	30	13.51
II	35 – 54.9	125	19	15.20
III	55 – 74.9	11	03	27.27
IV	75 – 94.9	08	00	00
V	95 – 114.9	18	04	22.22
VI	115 – 134.9	10	02	20
	Total	394	58	14.72

Table 6:- Relation between the host sex and prevalence of infection in *Heteropneustes fossilis*

Sex of fish (Host)	No. of fish examined	No. of fish infected	Prevalence
Male	233	30	12.87
Female	161	28	17.39
Total	394	58	14.72

Table 7:-Relation between the host length and the prevalence of infection in *Mystus vittatus*

Class	Standard length (cm)	No. of fish examined	No. of fish infected	Prevalence
I	5 - 9.9	251	34	13.54
II	10 – 14.9	12	02	16.66
	Total	263	36	13.68

Table 8:-Relation between the host Weight and the prevalence of infection in *Mystus vittatus*

Class	Body weight (g)	No. of fish examined	No. of fish infected	Prevalence
I	15 – 24.9	114	18	15.78
II	25 – 34.9	137	16	12.40
III	35 – 44.9	12	02	16.66
	Total	263	36	13.68

Table 9:- Relation between the host sex and prevalence of infection in *Mystus vittatus*

Sex of fish (Host)	No. of fish examined	No. of fish infected	Prevalence
Male	152	17	11.18
Female	111	19	17.11
Total	263	36	13.68

Table 10:-Relation between the host length and the prevalence of infection in *Mastacembalus pancalus*

Class	Standard length (cm)	No. of fish examined	No. of fish infected	Prevalence
I	10 – 14.9	205	23	11.21
II	15 – 19.9	22	02	9.09
	Total	227	25	11.01

Table 11:- Relation between the host Weight and the prevalence of infection in *Mastacembalus pancalus*

Class	Body weight (g)	No. of fish examined	No. of fish infected	Prevalence
I	15 – 29.9	147	19	12.92
II	30 – 44.9	65	04	6.15
III	45 – 59.9	15	02	13.33
	Total	227	25	11.01

Table 12:- Relation between the host sex and prevalence of infection in *Mastacembalus pancalus*

Sex of fish (Host)	No. of fish examined	No. of fish infected	Prevalence
Male	129	13	10.07
Female	98	12	12.24
Total	227	25	11.01

Table 13:-Relation between the host length and the prevalence of infection in *Wallago attu*

Class	Standard length (cm)	No. of fish examined	No. of fish infected	Prevalence
I	15 – 24.9	79	07	8.86
II	25 – 34.9	73	11	15.06
	Total	152	18	11.84

Table 14:-Relation between the host Weight and the prevalence of infection in *Wallago attu*

Class	Body weight (g)	No. of fish examined	No. of fish infected	Prevalence
I	38 – 97.9	118	13	11.01
II	98 – 157.9	18	01	5.55
III	158 – 217.9	16	04	25
	Total	152	18	11.84

Table 15:- Relation between the host sex and prevalence of infection in *Wallago attu*

Sex of fish (Host)	No. of fish examined	No. of fish infected	Prevalence
Male	89	11	12.35
Female	63	07	11.11
Total	152	18	11.84

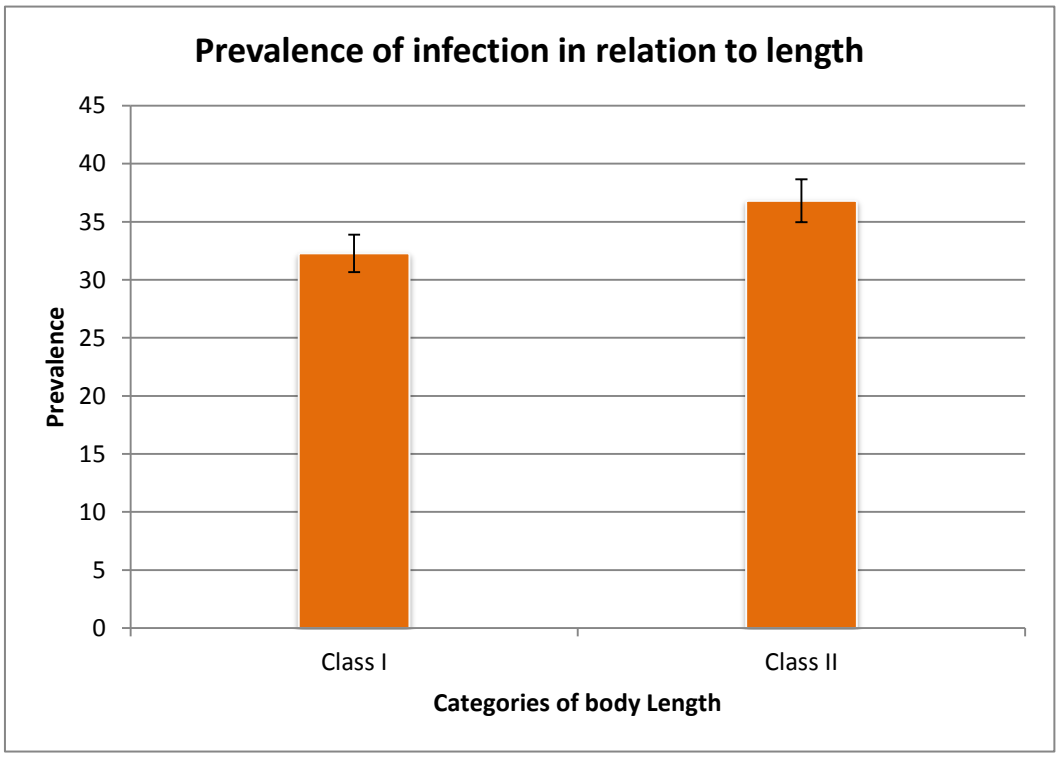


Figure 1:-Relation between the host length and the prevalence of infection in *Channa punctatus*.

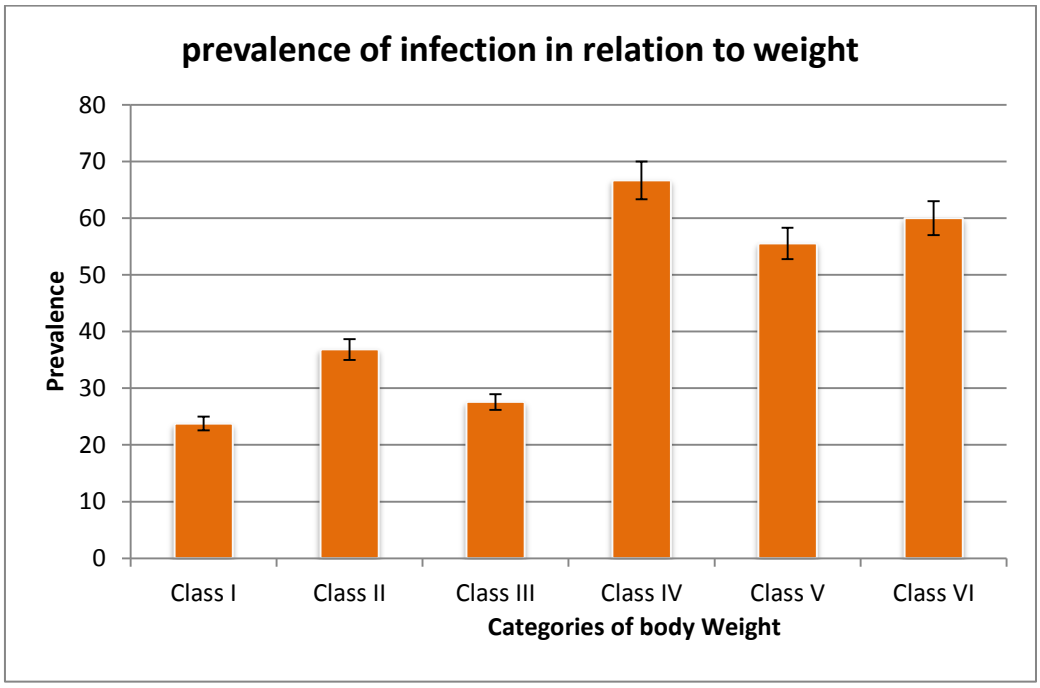


Figure 2:- Relation between the host body weight and the prevalence of infection in *Channa punctatus*.

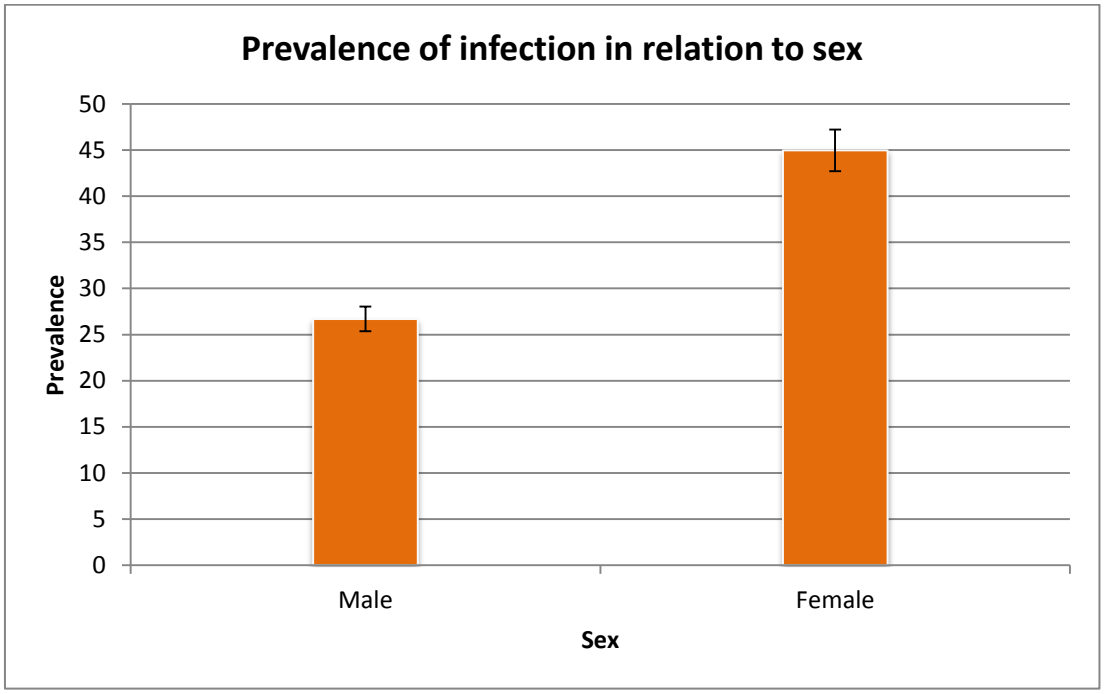


Figure 3:- Relation between sex of the host and the prevalence of infection in *Channa punctatus*.

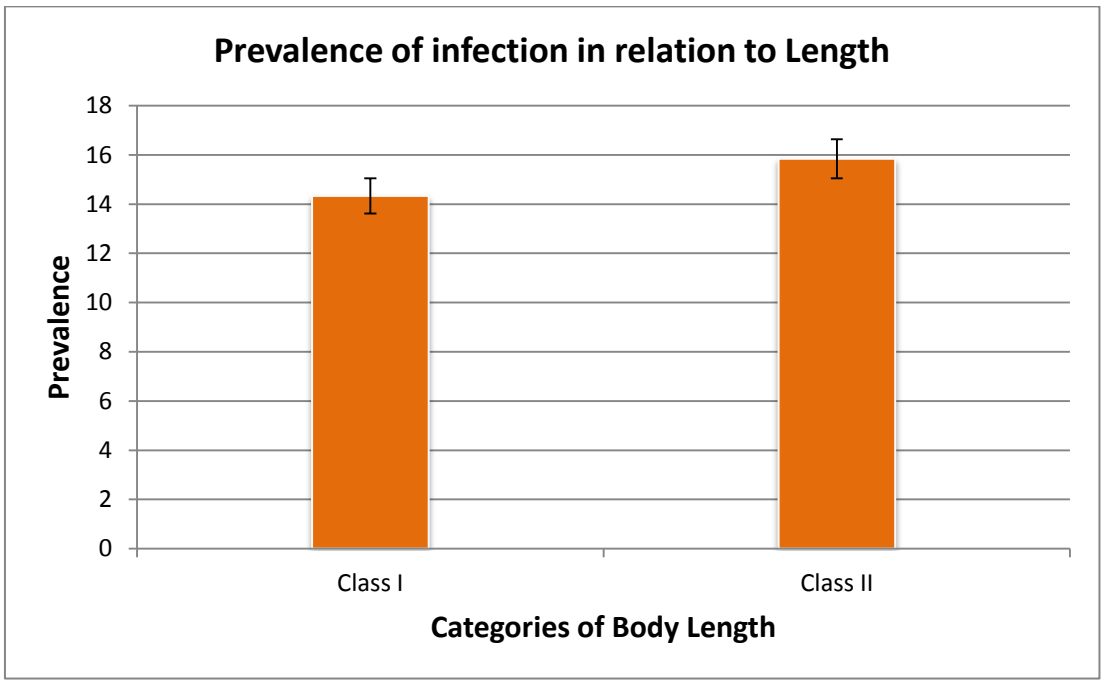


Figure 4:- Relation between the host length and the prevalence of infection in *Heteropneustes fossilis*.

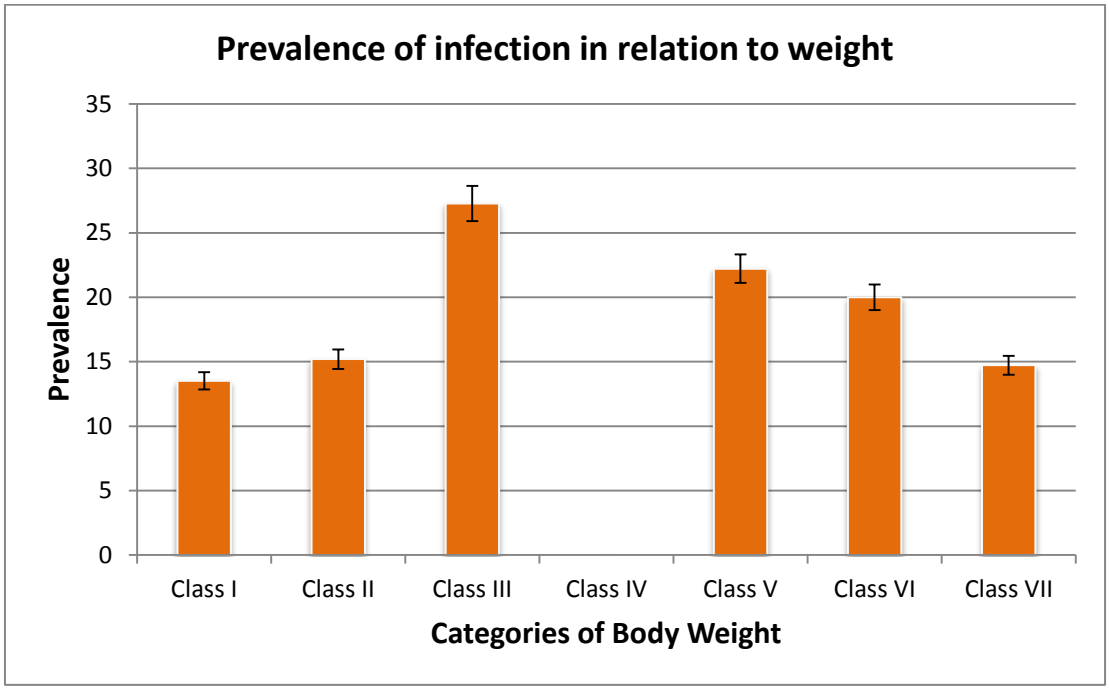


Figure 5:- Relation between the host body weight and the prevalence of infection in *Heteropneustes fossilis*.

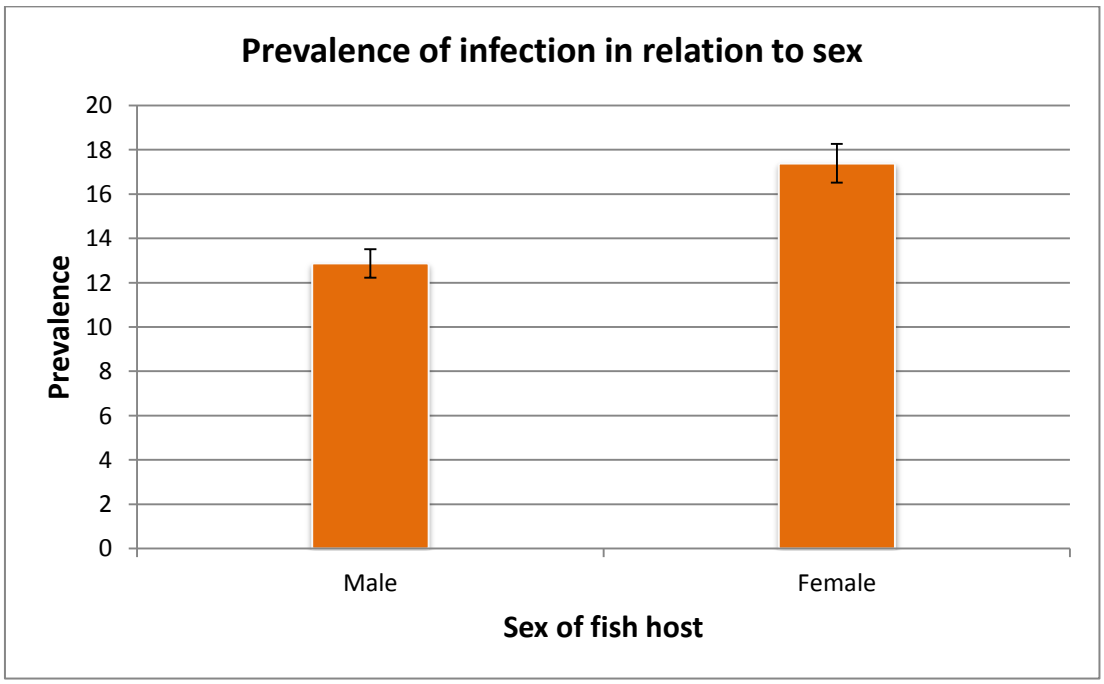


Figure 6:- Relation between the sex of the host and the prevalence of infection in *Heteropneustes fossilis*.

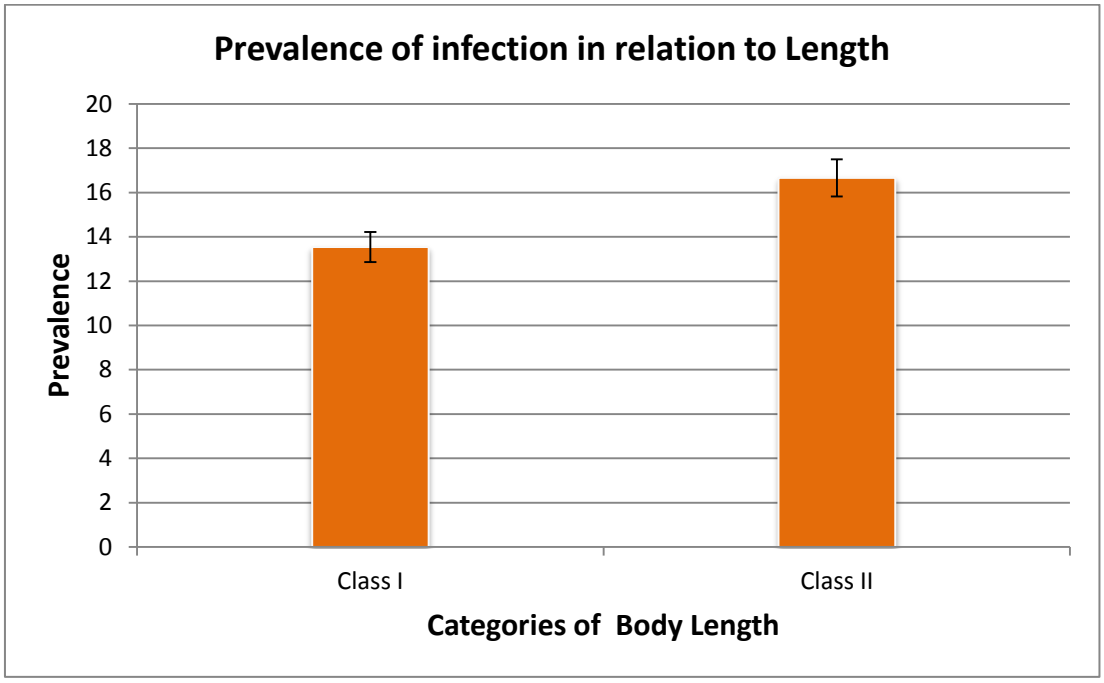


Figure 7:- Relation between the host length and the prevalence of infection in *Mystus vittatus*.

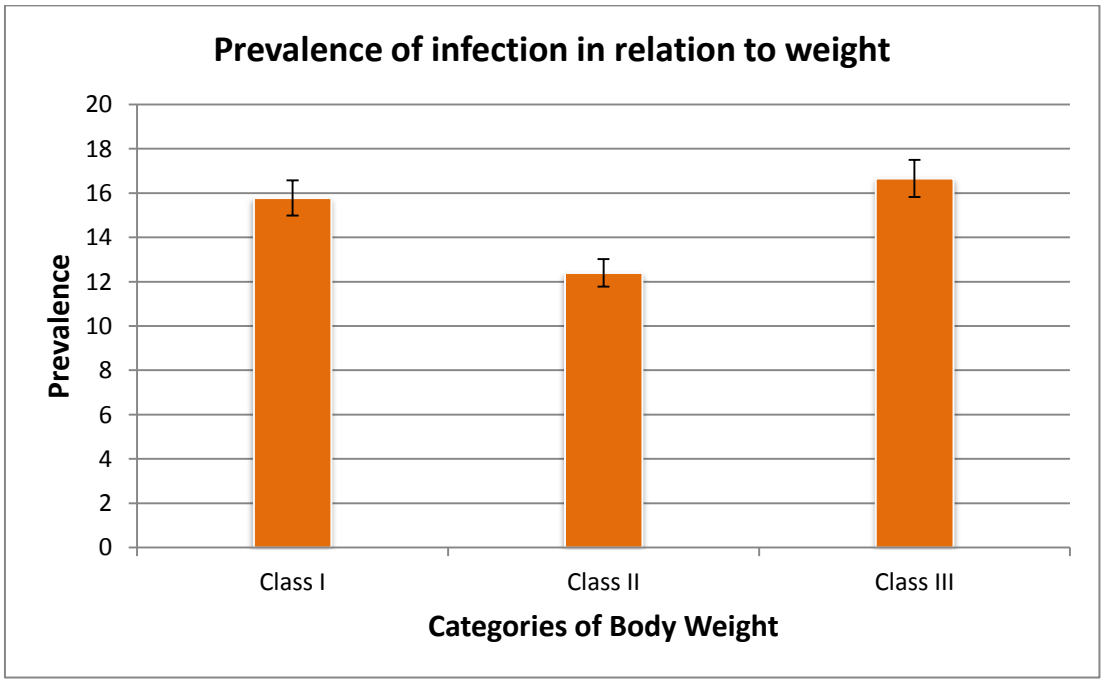


Figure 8:- Relation between the host body weight and the prevalence of infection in *Mystus vittatus*.

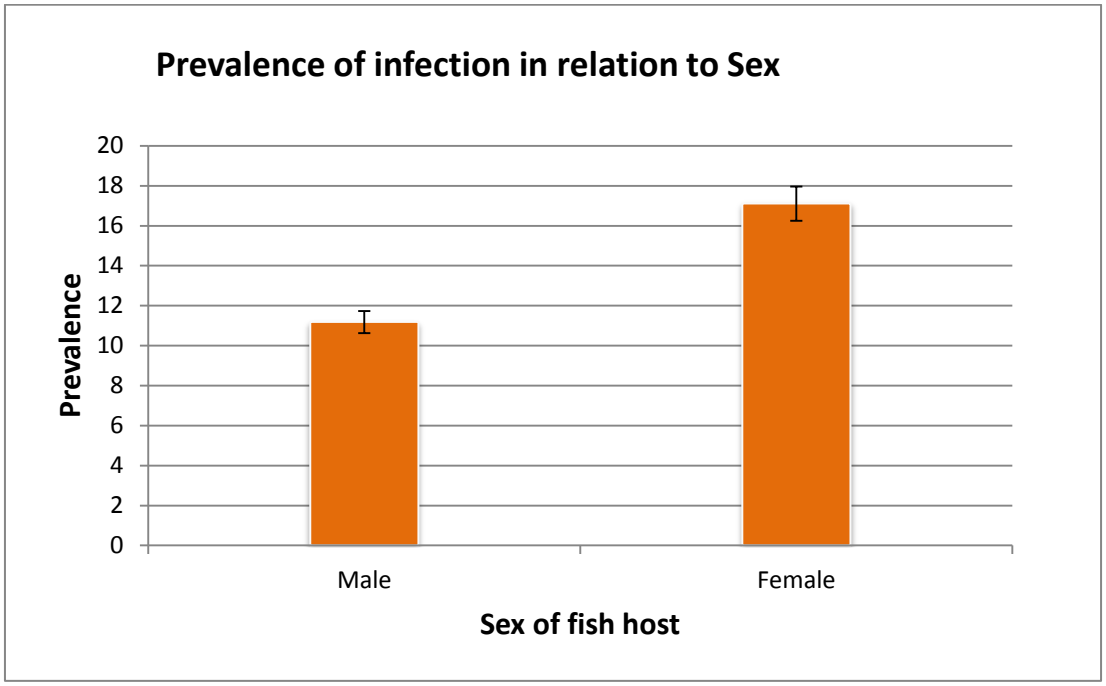


Figure 9:- Relation between the sex of host and the prevalence of infection in *Mystus vittatus.*

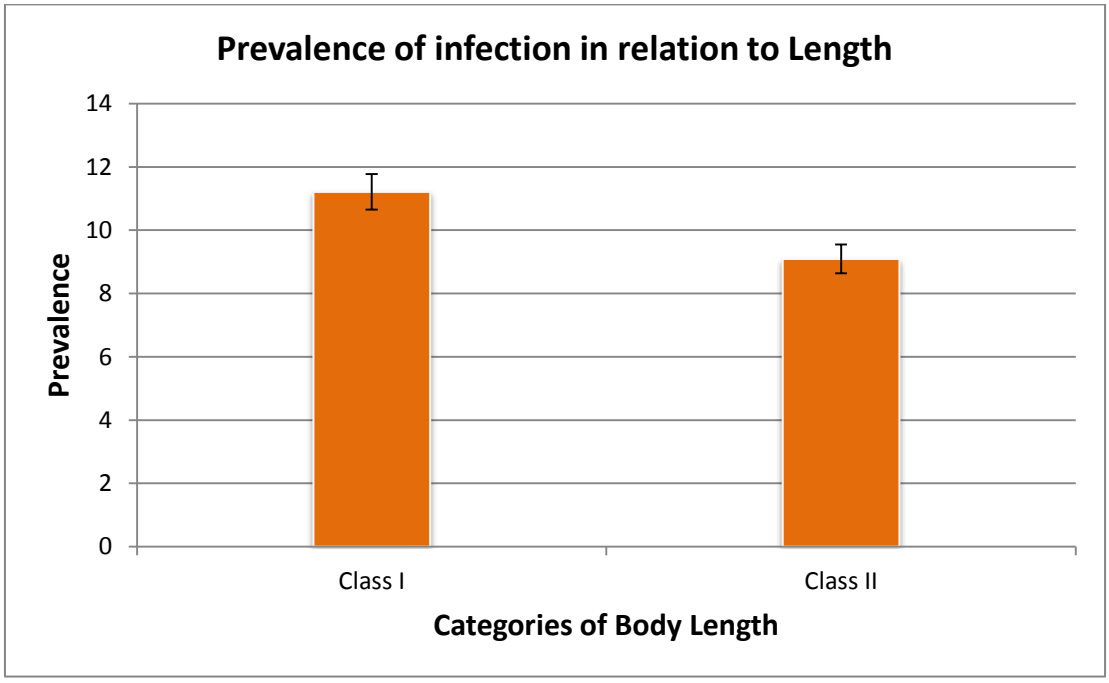


Figure 10:- Relation between the host length and the Prevalence of infection in *Mastacembalus pancalus*.

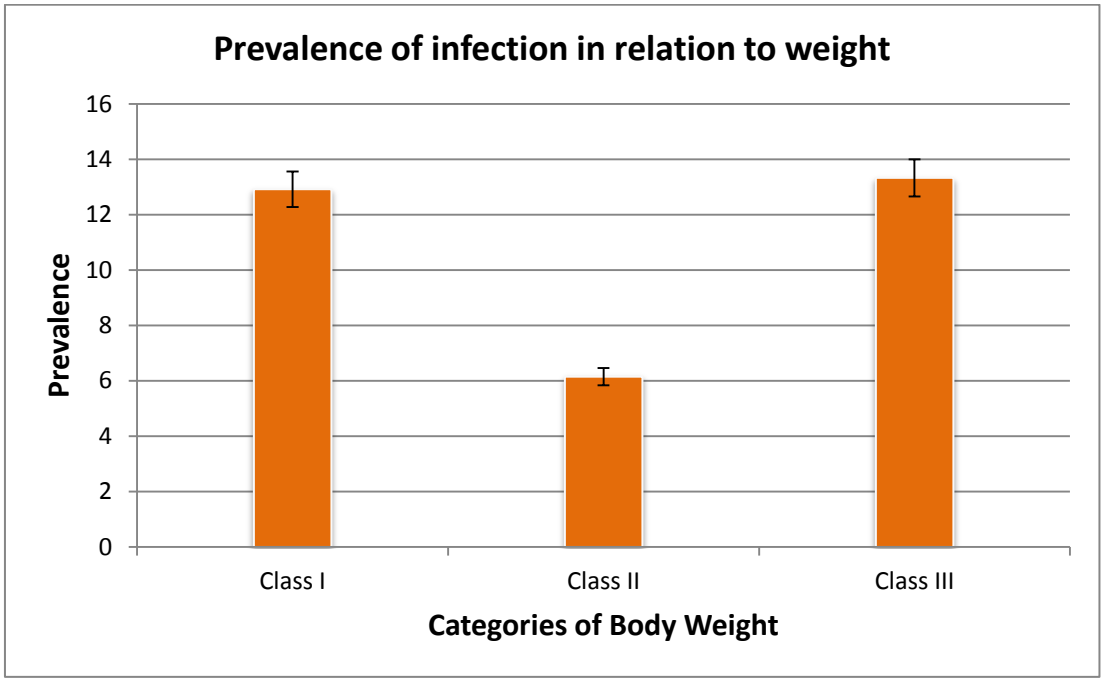


Figure 11:- Relation between the host body weight and the prevalence of infection in *Mastacembalus pancalus*.

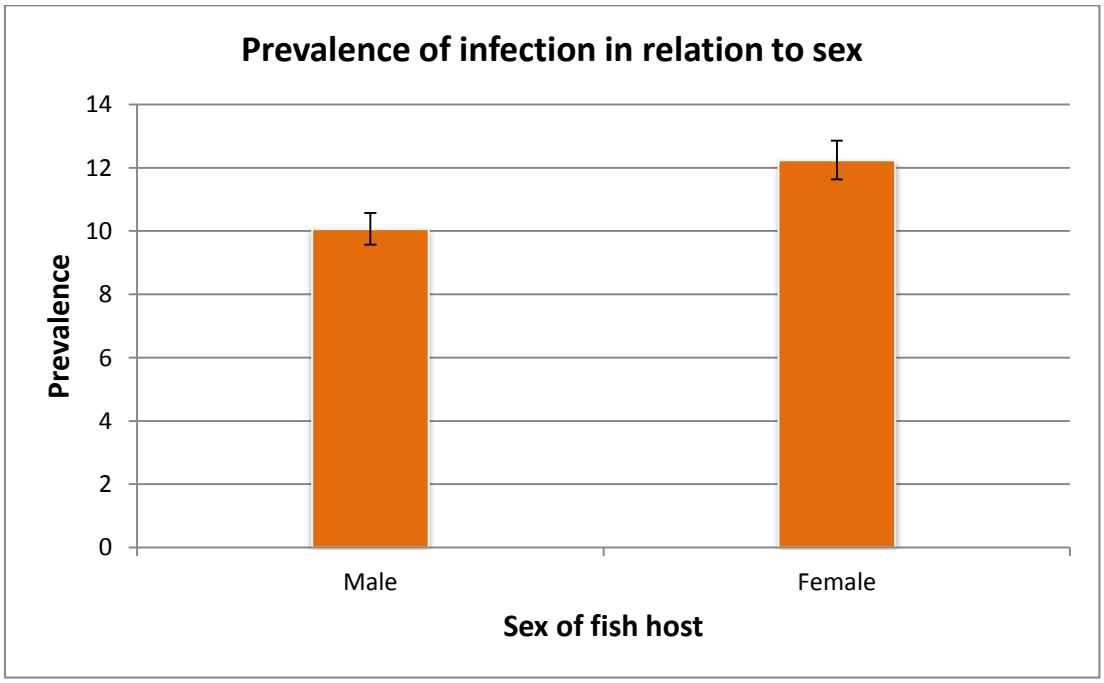


Figure 12:- Relation between the sex of host and the prevalence of infection in *Mastacembalus pancalus*.

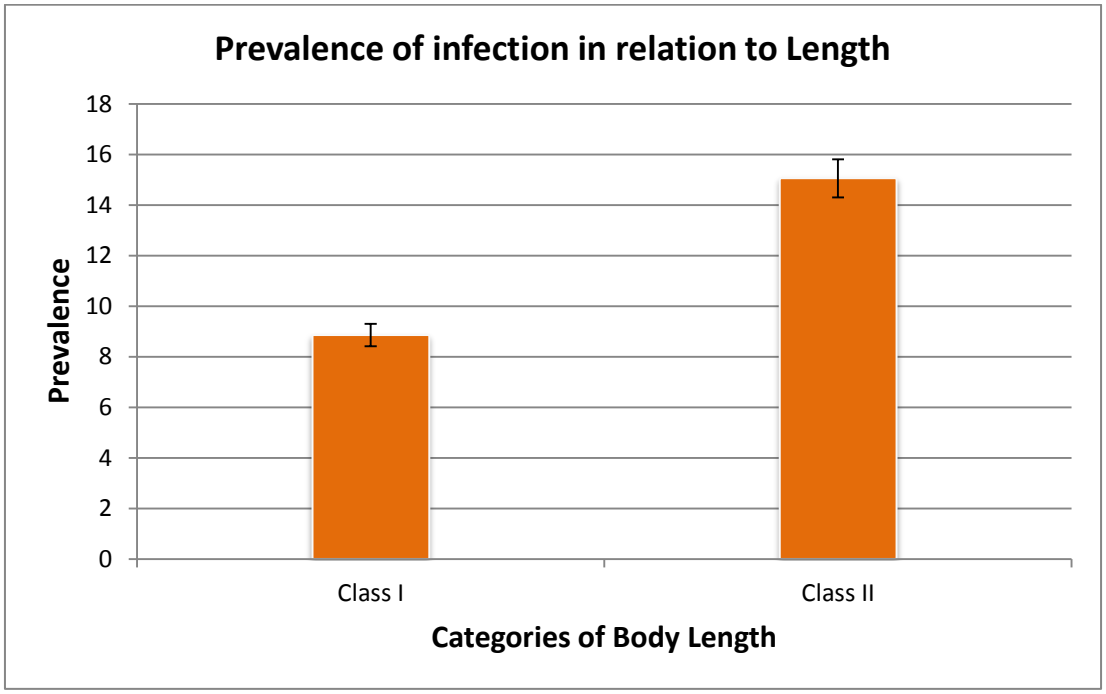


Figure 13:- Relation between the host length and the prevalence of infection in *Wallago attu*.

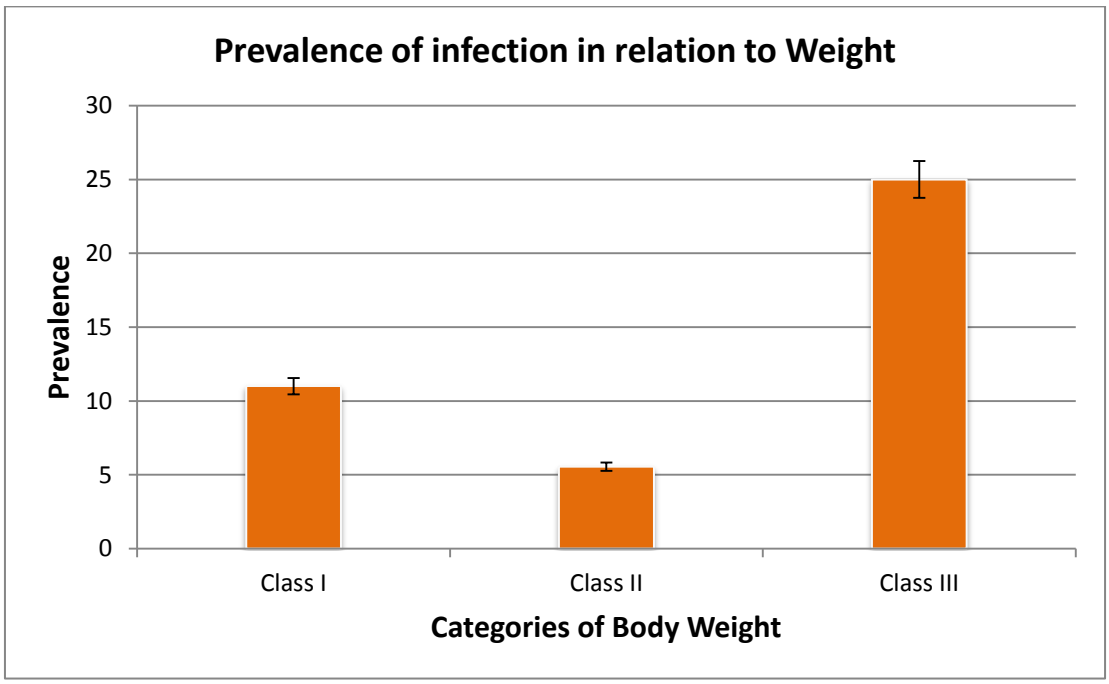


Figure 14:- Relation between the host body weight and the prevalence of infection in *Wallago attu*.

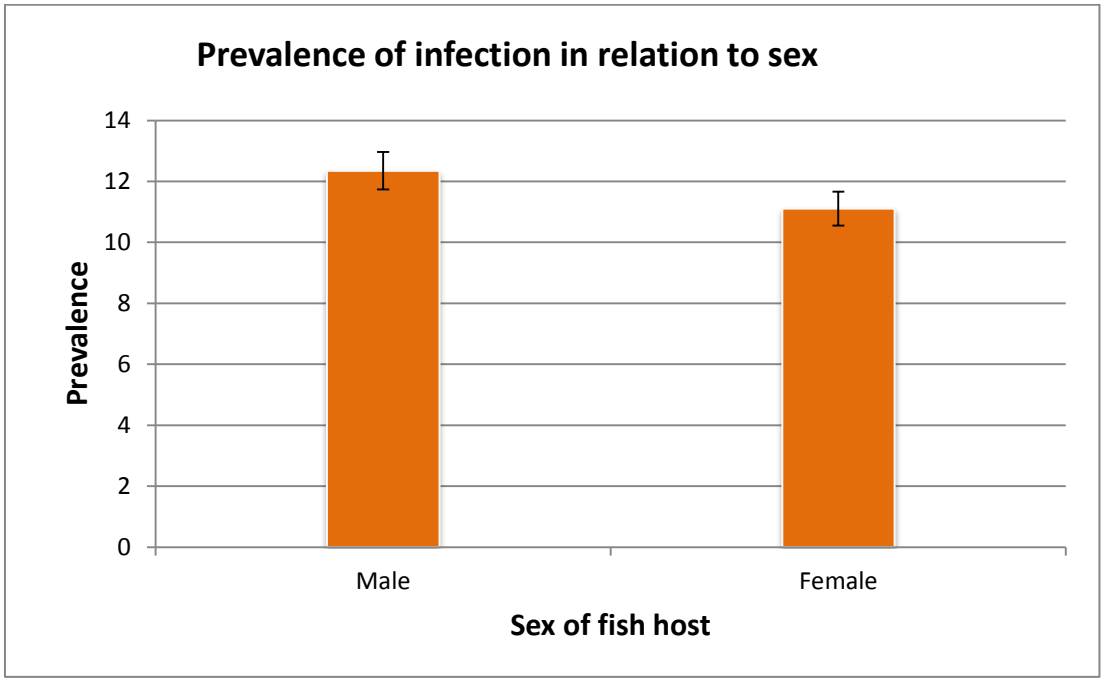


Figure 15:- Relation between the sex of the host and the prevalence of infection in *Wallago attu*.

Discussion

Helmintho-fauna infections in fishes have been reported to have a marked relationship with sex and size of the fish (Ezenwaji and llozumba 1992; Machado *et al.,* 1994; Saliu, 1998; Guidelli *et al.,* 2003; Araoye, 2005; Lizama *et al.,* 2005, 2006; Hassan *et al.,* 2010; Omeji, 2012).

The present study was designed to study the prevalence of helminth infection in relation to length, weight and sex of the selected host fishes. The study was conducted on food fishes of Gomti river, Lucknow for the two years 2013 and 2014. During the collection period, a total of 1491 live freshwater fishes viz., *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus, Wallago attu* were collected from different places in the Gomti river, Lucknow. The highest prevalence of infection was observed in *Channa punctatus* and lowest in *Mastacembalus pancalus*. The fishes were grouped into different classes according to the parameters and corresponding studies on the prevalence of helminth infection was carried out to determine the relationships, if any.

Prevalence of helminth parasites in relation to length of host fishes

In the present study, the smaller fishes *Channa punctatus, Heteropneustes fossilis, Mystus vittatus* and *Wallago attu* were found to be relatively less infected than the longer fishes, although the results were not statistically significant. Bashirullah, 1973 also made similar observations and suggested that higher prevalence in longer length fishes may be due to more ingestion of food by the fishes as they grow. Maximum parasitic infection was also due to accumulation of plerocercoids in fishes with age as it survives in the body for long term (Arme, 2002). Kaur *et al.* (2012), Kumar (2014), Mohammed *et al*. (2009) have also reported the similar findings in *Channa punctatus* fish species. Gaber *et al.* (2015) pointed out that the high incidence of infestation obtained in bigger fish is an indicator of importance of fish size in determining the parasitic load. Oniye and Aken'Ova (1999) stated that high prevalence in lengthy fishes could be attributed to the longer time of exposure to the environment by body size. The high prevalence in lengthy and bigger fishes may be due to the bigger internal organs of the hosts leading to the increase in the surface areas of infection (El-Naggar and Khidr, 1986; Khidr, 1990; Hagras *et al.,* 1995;

Muzzall *et al.,* 1990). Bashirullah (1973) and Dogiel (1961) reported that the degree of parasitism was obviously related to the food habit and age of the fishes. Imam and Dewu (2010) have reported similar findings in *Clarias* sp. and Masarat (2012) has also reported the same results in freshwater fish species *Cyprinus carpio*.

However, in case of *Mastacembalus pancalus*, the longer fishes were found to bear relatively less infection of helminthes parasites in comparison to the smaller sized fishes. Onyedineke (2010) also made similar observations and suggested that it may be due to low level of immunity in smaller sized fish. Minimum parasitic infection recorded in the higher length group of this fish may also be attributed to the possible random selection of the specimens and the possible high level of immunity in the larger sized fishes (Akinsanya *et al*., 2007). Dar *et al*. *(*2012) has also reported similar findings in the coldwater fish species *Schizothorax plagiostomus* and *Diptychus maculatus*.

Khurshid and Ahmad (2013) in their study on survey of helminthes in cyprinoid fish of Shallabugh wetland have also revealed that the length of the host was an important factor affecting the prevalence and mean number of parasites per host.

Prevalence of helminth parasites in relation to weight of host fishes

In all the fishes examined in the present study, a high infection was observed in the intermediate weight range of the fishes and the results were found to be significant in *Channa punctatus*, *Heteropneustes fossilis* and *Wallago attu*. This increase in infection in moderately sized fishes may be due to random selection of fishes. Kennedy (1978) and Lawrance (1970) have suggested it may be due to availability of food, feeding, distribution and environment of host. Khanum and Parveen (1997), in their study made similar observations in *Macrognathus acculeatus* and *Mastacembelus armatus* and described that parasite infestation follows a direct relationship with size of the fishes. Nahar (1988) reported that the intermediate size group was more infected by the parasites than the other size groups. Sultana and Salam (2015) in their study, similarly conducted in *Channa punctatus*, described that the host age and habitat play a vital role and influence the prevalence and intensity of the parasites. Paling (1965), Meshego (1989) and Davey and Gee (1976) have also

observed the similar results in *Heteropneustes fossilis*. Rahman and Parween (2001) reported maximum prevalence and intensity of parasites in intermediate and smaller size group respectively in *Heteropneustes fossilis*, *Channa punctatus* and *Colisa fasciatus.* Kumar (2014) has also reported similar findings in *Labeo rohita*, *Channa striatus* and *Channa punctatus* fish species. Gaber *et al*. (2015), Ayanda (2009), and Omeji *et al.* (2010, 1999) observed that the increase in infection in larger and heavy fishes might be due to the fact that bigger fishes cover wider areas in search of food than the smaller ones, and as a result, intake more food and this could expose them to infestation by more parasites.

According to Omeji, 2013 the higher number of parasitic infestation recorded in bigger fish could be attributed to their quest for survival (Ayanda 2009).

According to Esiest (2013) parasitic worms can cause swollen abdomen in fishes thereby contributing to both pseudo-weight and length of fishes and can also lead to stunted growth thereby reducing the length and weight of the fish. An increase in size is a reflection of increase in length and weight, which is hereby considered as a measured of age. Therefore, in the study the juvenile fish had no parasite while the sub-adults and adults had higher prevalence which implies that infestation increased with age of fish (Dan-Kishiya *et al.*, 2013).

Imam and Dewu (2010) explained that bigger fishes feeding on the smaller ones coupled with their greater body size and omnivorous food habit, could possibly be the reason for high rate of parasitic infestation. However, Tasawar *et al.* (2007) reported the higher parasite load in smaller fish than their bigger counterparts to be due to lower immunity of the smaller sized fishes.

Prevalence of helminth parasites in relation to sex of host fishes

It has been confirmed by several authors that the sex of the host has relevant effect on the regulation and periodicity of the parasites (Smith, 1969; Sanwal and Agarwal, 1974; Sinha, 1984; Rajaiah, 1977). In the present study a significantly higher prevalence of infection was observed in female fishes of *Channa punctatus, and Heteropneustes fossilis.* Thomas (1964), Chandra (1985), Khanum and Parveen (1997), Khanum *et al.* (2008), Rahman and Saidin (2011), Holden and Reed (1972)

also reported similar results and suggested that this may be due to lower physiological resistance of the female fishes. Maximum parasitic infection in the female fish has been observed during the breeding season (Dobson, 1961; Kaur *et al.*, 2012). Siddiqui (2014) suggested that the differences in the infection levels of the parasites in the male and female hosts may be due to the physiological changes in the steroid hormones like androgens of male and estrogens of the female hosts. Kisielewska (1970) observed that during summer the rate of infection is more in females than in males.

The differences in parasite fauna between animals of different sexes are less common and less well understood. The difference of distribution of parasites between the different sexes must be not due to one single factor, but due to combination of several factors including host's diet and their physiology. Omeji *et al.* (2011), Emere and Egbe (2006) and Ayanda (2009) reported higher parasitic prevalence in female *C. gariepinus* and suggested that during breeding season increased rate of food intake by female fishes to meet their requirements for the development of their egg might have exposed them to more contact with the parasites, which subsequently increased their chance of being infected.

In the present study in *Wallago attu,* the female fishes were found to be relatively less infected than the male fishes. These results are in accordance with the reported works of Anosikel *et al.* (1992) and Oniye *et al.* (2004) who have also reported more parasitic prevalence of infestation in male fish than the female. Omeji *et al. (*2013) and Sultana and Salam (2015) have also reported similar findings in *Channa punctatus*. Akther (1995) also showed the same result in *Anabas testudineus.* According to Aloo *et al. (*2004) the main reason for the differences in parasitic load with sex is physiological.

Conclusion

The present study was designed to examine the helminth parasites in important food fishes (*Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus, Wallago attu*) of River Gomti in Lucknow and to evaluate the relationship of helminth infection with respect to length, weight and sex of the selected host fishes. The results of the present study revealed that these host fishes were parasitized by helminth belonging to four classes Trematoda, Cestoda, Nematode and Acanthocephala. The present study on the relationship of length, weight and sex of host fishes with the prevalence of helminthes parasites gave variable results and has shown significant differences in some cases. In *Channa punctatus* the prevalence of helminth infection varied significantly in sex of host fishes and it was observed to be higher in the female hosts. In *Heteropneustes fossilis* and *Wallago attu* the prevalence of helminth infection was found significantly higher in the intermediate weight group. In the present study the length of the host fishes was not found to be a remarkable feature for the prevalence of helminth infection, however, higher prevalence was observed in the lengthy fishes. These results can prove to be an effective tool for determination of fish health for fisheries management and to initiate management strategies for conservation of fish. In addition, the increased demand on fish as a source of protein should trigger further studies on fish parasites. In conclusion, though heavy fish parasitism has been recorded in this study, it is worth mentioning that fish farmers should adopt good management practices like avoidance of overcrowding and taking care to avoid introduction of already infected broodstock. Good culinary practices should be ensured as this will reduce greatly, any risk of infection.

PLATE – 8

Fig. 1: Length measurements of fish

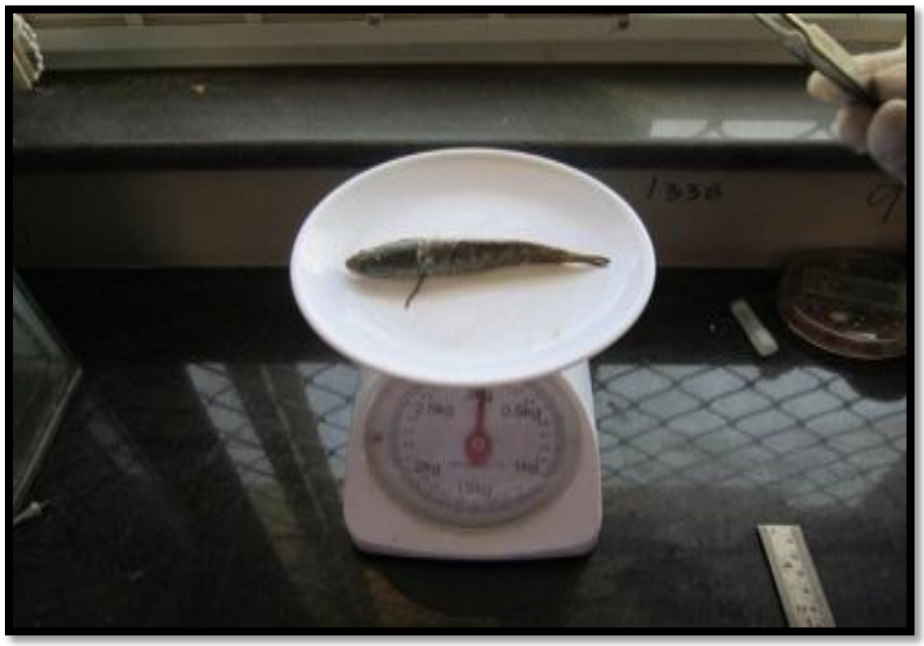


Fig. 2: Weight measurements of fish

Chapter 5

Study of Haematological Parameters of Helminth Infected Freshwater Fishes

Introduction

Fishes are important food product as they are a source of high amount of good protein, phosphorus and vitamin. In order to get better nutrition from fishes, they must be free from diseases. Fish diseases may be due to parasitic or nonparasitic causes. Parasitic diseases of fishes are very common all over the world. The effects of helminth parasites on freshwater fish consist of nutrient decline (Hassan, 2010), changes of biology and behavior (Lafferty, 2008), decreases of immune capability, induction of blindness, mortality growth and fecundity reduction (Nmor, 2004) and mechanical damages depending on the parasite species and burden (Echi, 2009).

Haematological parameter is one of the most reliable tools for diagnosis of disease of the fishes (Beneriee, 1964) and act as physiological indicators to changing external environments as a result of their relationship with energetic (metabolic levels), respiration (haemoglobin) and defence mechanisms (leukocyte levels) (Caruso, 2005; Oriakpono, 2012). It is well known that blood comprises 1.3-7% of the total body weight of fish and it is one of the most active components that contributes to metabolic processes by ensuring gas exchange between the organism and environment. For this reason, haematological parameters are increasingly used as indicators of the physiological condition in fish to endogenous or exogenous changes.

Changes in heamatological parameters depend upon the aquatic biotope, fish species, age, sexual maturity and health status (parasitic infection, bacterial infection), food habit, chemical and environmental stress (Blaxhall, 1972; Patriche, 2011; Radu, 2009). The fish haematological parameters such as RBC, WBC, Hb, PCV, MCV and MCHC values etc., are shown to be influenced by many factors including environmental factors, seasonal conditions and different period of reproductive cycle, chemical stress and parasitic or bacterial infection.

RBC mainly helps in transport of oxygen and carbon dioxide and is nucleated in all vertebrates except mammals. RBC parameters can be used for the diagnosis of anemia (Tavares-Dias and Moraes, 2004, 2007; Affonso *et al*., 2007; Pavlidis *et al*., 2007). In case of parasitic infection, fishes become anemic resulting in low production and destruction of red blood cells.

WBC or leucocytes are blood cells containing no respiratory pigments. There are various types of WBC's which are usually classified as granular and nongranular and as basophil or acidophil according to their staining reaction. The WBC (leukocytes), are the primary line of immunological defense and provide an important representation of defense cells throughout the body (Tavares-Dias and Moraes, 2004; Tavares-Dias and Moraes, 2007; Affonso *et al*., 2007; Pavlidis *et al*., 2007). Thus, one of the most elementary ways to assess the immune system is to explore changes in the number or appearance of fish circulating white blood cells (WBC_s).

Haemoglobin is a conjugated protein, important as a carrier of oxygen necessary for cell survival. Hb estimation as an assessment tool is employed because this blood component is a part of the sophisticated oxygen delivery system that provides the desired amount of oxygen to the tissues under an extensive variety of environments (Voet and Voet, 1990). Hb production and destruction are balanced under physiological conditions and any deviation in either one is associated with a significant haematological disorder and a rapid decline in Hb concentration. The determination of haemoglobin concentration can be a good indicator of anaemic conditions in fish (Blaxhall and Daisley, 1973).

Haematocrit is a main tool for determining the amount of plasma and corpuscles in the blood and used to determine the oxygen carrying capacity of the blood (Larsson *et al*., 1985). It is also defined as the volume employed by erythrocytes in a given volume of blood. Haematocrit are secondary indicators of chronic stress. If parasites act as stress inducers, haematocrit values could be increased by splenic release of stored blood cells, loss of plasma, or by swelling of red blood cells (Bowers *et al*., 2000; Wagner and Mckinley, 2004; Jones and Grutter, 2005). Depending on their number, parasites can also cause a reduction in the host's haematocrit by blood ingestion (Wagner and Mckinley, 2004; Jones and Grutter, 2005) or by osmoregulatory failure, caused by exposed lesions (Jones and Grutter, 2005).

The calculated haematological indices, MCHC, MCH and MCV; are the other essential indicators in the analysis of anaemia in most animals (Coles, 1986). MCH is an index of the concentration of Hb in an average erythrocyte of a population of cells.

MCH value increases due to the infection of parasites, not only indicating macrocytosis but confirming that more erythroblasts, immature red cells are present in the circulation (Tondan and Joshi, 1973). MCHC values are also decreased during the infection of parasites.

The studies on blood components in the fishes are therefore important from the diagnostic point of view. It was therefore considered desirable to evaluate the hematological changes in the blood parameters of the three selected food fishes bearing high parasitic infection viz., *Channa punctatus, Heteropneustes fossilis* and *Mystus vittatus*. *Mastacembalus pancalus* and *Wallago attu* showed very poor infection levels therefore they were not considered for this study. The present work was aimed at investigating the impact of helminthes parasites on the various hematological characteristics of the selected fishes.

Materials and Methods

Collection of Blood samples

Blood samples for haematological investigations was collected from fish hosts (*Channa punctatus*, *Heteropneustes fossilis*, *Mystus vittatus*) in glass tubes containing EDTA and properly labeled. The blood was diluted with appropriate diluting fluids for red blood corpuscle (RBC) and white blood corpuscle (WBC) counts and the individual cells were counted in the counting chamber (haemocytometer). Routine Sahli's haemometer was used to estimate haemoglobin content (Hb%). Mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) was calculated from the average values of Hb%, while the packed cell volume (PCV) was calculated according to the standard method.

Haematological Investigations in Fish

Analysis of Red Blood Corpuscle (RBC)

Enumeration of formed elements (blood cells) is a quantitative measure of the population of blood cells in circulation. The counting of cells was done manually with the help of a microscope after diluting blood and making a special type of wet mount as per method given by Rusia and Sood (1992). The technique is popularly known as haemocytometry and aided by Neubaur grid, on the haemocytometer which show cell counting areas for the estimation.

The Haemocytometer (Neubaur counting chamber) has a ruled area of 9 sq.mm. consisting of a central heavy ruled area of 1 sq.mm and four ruled areas of the same size in the four corners. The central area is divided into 25 squares which are subdivided into 16 small squares. For counting total RBC, the ruled areas at 5 centres were counted and total RBC measured as number/cubic millimetre. For counting white blood corpuscles (WBC) corner small squares i.e. 16 x 4 = 64 sq.mm were counted and number of cells reported as number/cubic millimetre.

The blood was drawn into the RBC pipette up to 0.5 mark and immediately the diluting fluid containing, Hayem solution was drawn up to 101 mark. This gives a

dilution of 1:200. The solution is added by shaking gently and allowed to be settled for 2 to 3 minutes. The counting chamber and cover glass were properly cleaned and the cover glass was placed over the ruled area. Again the solution was mixed gently and the stemfull of solution was expelled and a drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 45^0. It was allowed to settle for 2 to 3 minutes and the RBCs after settling without air bubble under the coverslip, were counted. Then the ruled counting area was focused under the microscope and the number of RBC's were counted in fine small squares of the counting area under high power lens and the total number of RBCs per cubic millimeter were calculated by using the following formula:

$$\text{No. of RBC} = \frac{No.of\ RBC \times Dilution\ counted}{Area\ counted \times Depth\ of\ fluid} \quad (million\ \ cu.mm)$$

Analysis of White Blood Corpuscle (WBC)

Blood collection and processing procedure was same as described in the above except for the dilution factor which is 1: 20. As far as the counting (Neubaur counting chamber) procedure of WBC is concerned, each of these 4 square millimeter area is subdivided into 16 squares. By using low power objective and a maximum occular care, counting of cells was carried out in the Neubaur chamber. The following formula was taken for the estimation of the total number of WBCs per cubic millimeter.

$$\text{No. of WBC} = \frac{\text{No.of WBC} \times \text{Dilution counted}}{\text{Area counted} \times \text{Depth of fluid}} \quad (\text{million}\ \ \text{cu.mm})$$

Analysis of Hemoglobin (Hb)

Hemoglobin was determined by the more commonly used Sahli's method. In this method, hemoglobin in the blood sample is converted to acid hematin which gives brown colour. Briefly the procedure is described below.

The hemoglobinometer tube and pipette were cleaned and dried. 5 drops of the 0.1 N HCL was placed at in the bottom of the graduated tube. The blood was taken up to the mark 20 in the pipette and added to HCL in graduated tube and allowed to stand

for 5 minutes until it changed to dark brown colour. The solution was diluted by adding distilled water drop by drop (each time mixing the solution with a stirring rod) until it matched with the standard colour. Then reading was taken from the scale on the graduated tube and the Hb concentration was expressed as gram percent.

Analysis of Packed Cell Volume (PCV)

PCV is the volume of erythrocytes expressed as a percentage of the volume of whole blood in a sample. Packed cell volume was calculated according to microhematocrit method, which requires only a small volume of blood and since it is done with a high- speed centrifuge, it takes less time. Briefly the procedure is described below.

A capillary tube is filled two – third to three – quarters as with blood and one end of the tube is sealed with clay. The filled hematocrit tubes are in the radial grooves of the microhematocrit centrifuge, with their heads opposite each other. Centrifugation is carried out for 5 minutes at 12000 rpm, there after the tubes are placed in the microhematocrit reader and the Packed cell volume is read.

Analysis of Mean Corpuscular Volume (MCV)

Mean Corpuscular Volume refers to the average volume of red cells. Because the size of the cell is very small, volume is expressed in cubic microns (μm3). It is calculated by using the following formula:

$$\text{MCV (fl)} = \frac{\text{PCV (\%)}}{\text{RBC count in million/m3}} \times 10$$

Analysis of Mean Corpuscular Haemoglobin (MCH)

Mean Corpuscular Haemoglobin (MCH) is the average haemoglobin content of the red blood cell. MCH is influenced by the size of the cell and concentration of haemoglobin. It is derived by the following formula:

$$\text{MCH (pg)} = \frac{\text{Hb (g/dl)}}{\text{RBC count in million/m3}} \times 10$$

Analysis of Mean Cell Haemoglobin Concentration (MCHC)

The MCHC is an expression of the average haemoglobin concentration per unit volume (100) of packed cells (W/V). Hence it is expressed in g/dl which is the same as percent (%).

$$\text{MCHC (g/dl)} = \frac{\text{Hb (g/dl)}}{\text{PCV (\%)}} \times 100$$

Statistical Analysis

Student's t-test was employed to calculate the significance in the differences between non-infected and infected host (Fisher, 1950).

Results

Haematological estimation of common blood parameters of naturally infected *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* from river Gomti from Lucknow region was carried out to gather information on the effects of helminth parasites on the fish haematology. The haematological parameters studied were Hb, PCV, TEC, TLC, MCV, MCH and MCHC. The results of the haematological studies on the infected/ non-infected fishes viz., *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* have been shown in Tables1-3 respectively.

Haematological estimation of blood parameters of naturally infected *Channa punctatus* :

The range of different haematological parameters (RBC count, WBC count, Hb, PCV, MCV, MCH, MCHC) of non-infected and helminth infected *Channa punctatus* are presented in Table 1. The total red blood corpuscle (RBC) count observed in the normal *Channa punctatus* was 3.51 ± 0.44× 10^6/ mm^3 and in the infected fish was 2.31 ± 0.46 × $10^6/mm^3$. Results show that RBC value was decreased significantly ($P < 0.001$) in helminths infected fishes as compared to the non-infected fishes. The total white blood corpuscle (WBC) count observed in the non-infected fish was 38.84 ± 4.95 × 10^6/ mm^3 and in the infected fish it was 43.55 ± 5.00 × 10^6/ mm^3. The value was found to be significantly increased ($P < 0.01$) in the infected fishes. The haemoglobin content observed in the non-infected fish was 8.55 ± 1.49 gm/dl and in the infected fish was 6.28 ± 2.26 gm/dl. The Hb concentration was found to be significantly decreased ($P< 0.001$) in the infected fish in comparison to the non-infected fish. The PCV value observed in the non-infected fish was 30.93 ± 3.32 % and in the infected fish was 28.93 ± 3.31%. The average value showed that this concentration was decreased in the infected fish in comparison to the non-infected fishes, however, the difference was found to be statistically insignificant ($P > 0.05$). The MCV value observed in the non-infected fish was 88.84 ± 10.11 and 127.31 ± 12.90 in the infected fish. The difference in the MCV was found to be highly significant ($P < 0.001$).The mean value of MCH was recorded 43.43 ± 6.59 in infected fishes which was also found to be increased significantly ($P < 0.001$) when compared to non-infected fishes. The MCHC observed in the non-infected fish was 35.90 ± 2.52 and in the infected fish it was 33.97 ± 2.33. The observed values

represent that MCHC value was decreased significantly (P <0.05) in helminth infected fishes as compared to non-infected fishes.

Haematological estimation of blood parameters of naturally infected *Heteropneustes fossilis*:

The range of different haematological parameters of non-infected and infected *Heteropneustes fossilis* are presented in Table 2. The total red blood corpuscle (RBC) count observed in the non-infected fish was 3.89 ± 0.28× 10^6/ mm^3 and in the infected fish it was 2.77 ± 0.24 × $10^6/mm^3$. The value was found to be decreased significantly (P < 0.001) in infected fishes as compared to non-infected *H. fossilis.* The total white blood corpuscle (WBC) count observed in the non-infected fish was 14.93 ± 2.35 × 10^6/ mm^3 and in the infected fish it was found to be increased to 19.89 ± 2.30 × $10^6/mm^3$ which was statistically significant (P < 0.001). The haemoglobin content observed in the non-infected fish was 10.64± 1.73 gm/dl and in the infected fish was 8.47± 1.28 gm/dl. The mean value shows that the haemoglobin content in infected fish was significantly decreased (P < 0.001) when compared to the non-infected fish. The PCV value observed in the non-infected fish was 36.42 ±1.73 % and in the infected fish, it was 32.84 ± 1.85 %. The average value showed that the PCV was significantly decreased (P < 0.001) in infected fish in comparison to non-infected fishes. The MCV values observed in the non-infected fish was 93.62 ± 2.67 and 118.72 ± 6.67 in the infected fish. The higher MCV in the infected fish was found to be statistically significant (P < 0.001). The mean value of MCH was recorded 42.83 ± 2.46 in infected fishes which was found to be significantly highly increased (P < 0.001).The mean MCHC value observed in the non-infected fish was 35.99 ± 1.06 while in the infected fish it was 36.07 ± 0.59. However, the difference was statistically non- significant (P >0.05).

Haematological estimation of blood parameters of naturally infected *Mystus vittatus*:

The range of different haematological indices in non-infected and infected *Mystus vittatus* is given in Table 3.The total red blood corpuscle (RBC) count observed in the non-infected fishes was 2.01 ± 0.20 × 10^6/ mm^3 and in the infected fish it was 1.26 ± 0.27 × $10^6/mm^3$. The difference in the mean value was found to be

statistically highly significant (P < 0.001) when comparison was made between infected and non-infected *M. vittatus.* The total white blood corpuscle (WBC) count observed in the non-infected fish was 22.07 ± 1.76 × 10^6/ mm^3which was significantly highly increased (27.55 ± 1.81 × $10^6/mm^3$) than in the infected fish (P < 0.001). The haemoglobin content observed in the non-infected fish was 9.67±0.85 gm/dl and in the infected fish were 8.25± 1.38 gm/dl. The mean value shows that Hb concentration was decreased significantly (P<0.001) in the infected fish. The PCV value observed in the non-infected and infected fish was 17.31 ± 1.57 % and 12.52± 1.45 % respectively. The difference in the mean value was found to be highly significant (P < 0.001) statistically. The MCV value observed in the non-infected fish was 95.10 ± 8.66 and 101.40 ± 11.45 in the infected fish. However the difference in the values was not statistically significant (P >0.05). The mean value of MCH was recorded 72.90 ± 11.90 in infected fishes. It was found that the mean value of MCH was highly increased significantly (P < 0.001) in the infected fish as compared to the non-infected fish. The MCHC value observed in the non-infected fish was 57.13 ± 2.56 and in the infected fish, 71.55 ± 4.55 which was statistically very significant (P <0.001).

Table:1 Haematological parameters of non-infected and infected *Channa punctatus*.

Parameter of blood	Noninfected (n=20)		Infected (n=20)	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
RBC × $10^6/mm^3$	2.86 – 4.10	3.51 ± 0.44	1.75 – 3.01	2.31 ± 0.46***
WBC × $10^3/mm^3$	30.25 – 45.05	38.84 ± 4.95	35.10 – 50.00	43.55 ± 5.00**
Hb (gm/100ml)	6.2 – 10.2	8.55 ± 1.49	3.04 – 8.8	6.28 ± 2.26***
PCV (%)	28.0 - 38.0	30.93 ± 3.32	25.0 – 35.0	28.93 ± 3.31^{ns}
MCV (fl)	75.63 – 110.78	88.84 ± 10.11	101.76 – 143.25	127.31 ± 12.90***
MCH (pg)	27.27 – 37.76	31.79 ± 3.39	33.88 – 51.68	43.43 ± 6.59***
MCHC (%)	30.34 – 38.28	35.90 ± 2.52	29.14 – 36.29	33.97 ± 2.33*

Each value is mean ±SD of 20 observations, Values are significant at *P<0.05; **P<0.01; ***P<0.001, ns = Value are non-significant at P > 0.05.

Table:2 Haematological parameters of non-infected and infected *Heteropneustes fossilis*.

Parameter of blood	Non-infected (n=20)		Infected (n=20)	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
RBC × $10^6/mm^3$	3.62 – 4.52	3.89 ± 0.28	2.50 – 3.46	2.77 ± 0.24***
WBC × $10^3/mm^3$	12.0 – 19.6	14.93 ± 2.35	17.0 – 25.1	19.89 ± 2.30***
Hb (gm/100ml)	8.0 – 12.4	10.64 ± 1.73	6.8 – 10.1	8.47 ± 1.28***
PCV (%)	34.0 – 40.0	36.42 ± 1.73	30.0 – 36.1	32.84 ± 1.85***
MCV (fl)	87.83 – 98.07	93.62 ± 2.67	104.3 – 123.6	118.72 ± 6.67***
MCH (pg)	32.0 – 35.7	33.68 ± 1.03	37.5 – 50.9	42.83 ± 2.46***
MCHC (%)	34.17 – 37.53	35.99 ± 1.06	34.9 – 37.7	36.07 ± 0.59^{ns}

Each value is mean ±SD of 20 observations, Values are significant at ***P<0.001, ns = Value are non-significant at P > 0.05.

Table:3 Haematological parameters of non-infected and infected *Mystus vittatus*.

Parameter of blood	Noninfected (n=20)		Infected (n=20)	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
RBC × $10^6/mm^3$	1.80 – 2.53	2.01 ± 0.20	0.86 – 1.81	1.26 ± 0.27***
WBC × $10^3/mm^3$	19.0 – 25.1	22.07 ± 1.76	25.0 – 30.2	27.55 ± 1.81***
Hb (gm/100ml)	7.8 – 10.8	9.67 ± 0.85	5.8 – 9.6	8.25 ± 1.38***
PCV (%)	15.1 – 20.1	17.31 ± 1.57	10.2 – 15.5	12.52 ± 1.45***
MCV (fl)	82.96 – 110.43	95.10 ± 8.66	85.63 – 123.40	101.40 ± 11.45^{ns}
MCH (pg)	42.68 – 52.15	49.05 ± 2.79	53.03 – 93.02	72.90 ± 11.90***
MCHC (%)	53.64 – 60.89	57.13 ± 2.56	61.93 – 80.39	71.55 ± 4.55***

Each value is mean ±SD of 20 observations, Values are significant at ****P*<0.001, ns = Value are non-significant at $P > 0.05$.

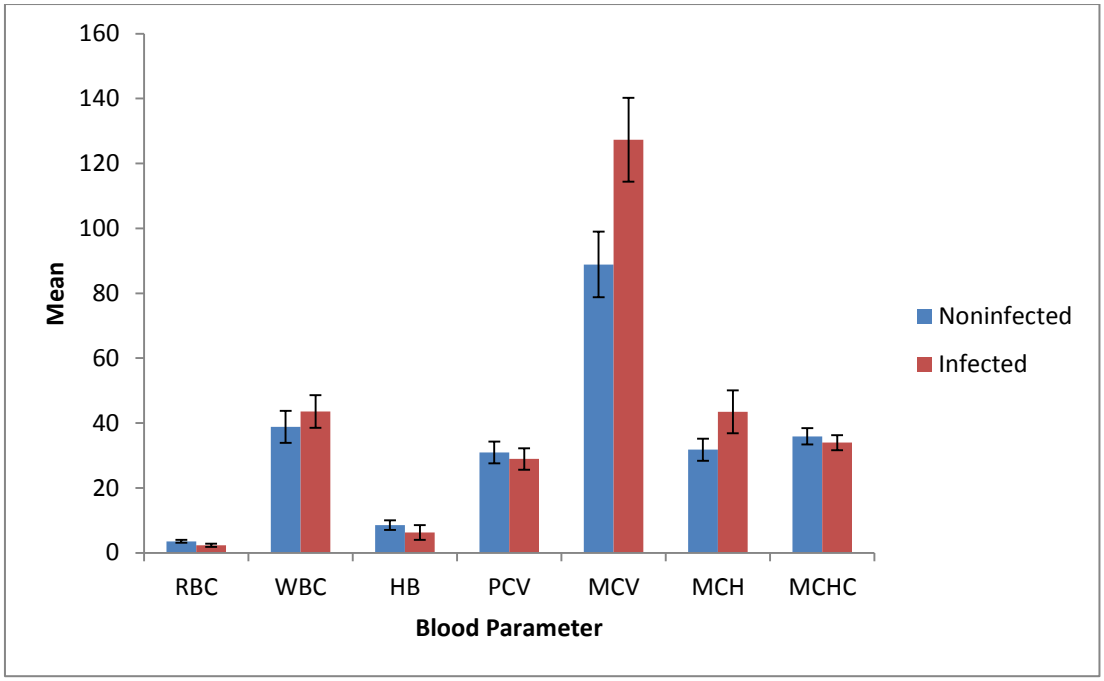


Figure 1: Means of blood parameters of non-infected and helminth infected *Channa punctatus*.

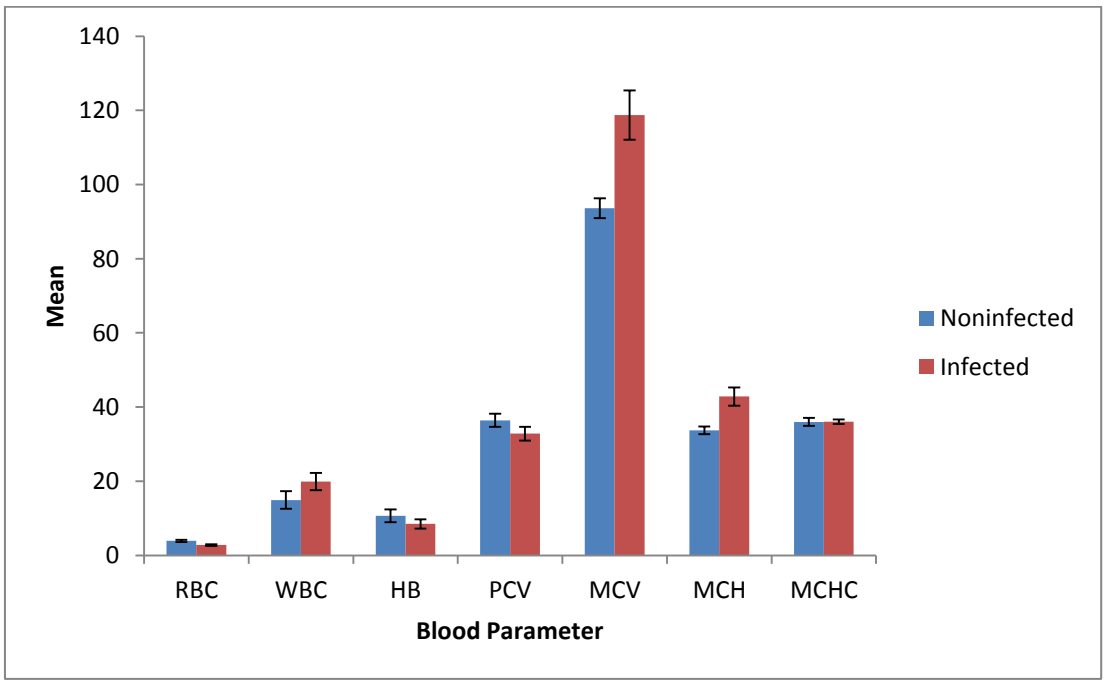


Figure 2: Means of blood parameters of non-infected and helminth infected *Heteropneustes fossilis*

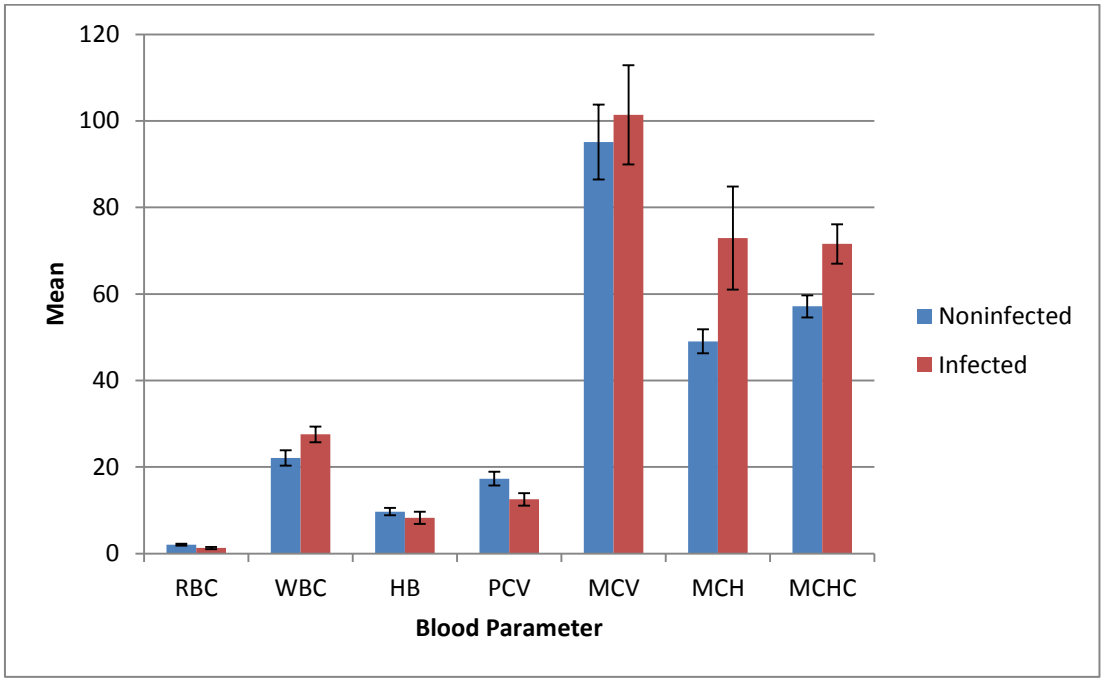


Figure 3: Means of blood parameters of non-infected and helminth infected *Mystus vittatus*

Discussion

Blood is a good bio-indicator of the health of an organism and acts as a pathological reflector of the whole body. Hence, haematological parameters are important in diagnosing the functional, nutritional and physiological status of the fish (host) infected by parasites (Joshi *et al.*, 2002; Chagas and Val, 2003). In the present study, haematological parameters Hb, PCV, TEC, TLC, MCV, MCH, MCHC were observed in infected and non-infected fish and the results revealed that helminth parasites caused severe haematological changes in all the studied fish species viz., *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*. Some of the haematological parameters studied were significantly varied ($P<0.001$) between the infected and the non- infected fishes. The observations made in the present study are supported by the findings of other researchers, as described below.

Effect on Red blood cell (RBC) counts

The RBC counts were found to be significantly reduced ($P<0.001$) in infected fish of all the three genera studied i.e. *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* as compared to the non-infected fishes. Kundu *et al*. (2016) pointed out that the RBC count of fish *C. punctatus* infected with parasites was reduced significantly ($P<0.05$) in comparison to non-infected fish. Bag *et al.* (2007) have also reported the RBC count to be decreased by 55.19% in the fishes infected with parasites and decreased even further on the increase in infection which could be an indicator of anemia due to infection. However, Eldeen *et al*. (2010), in their study on reported that RBC count is a highly variable blood parameter among the different fishes.

Effect on White blood cell (WBC) counts

It is known that WBCs are normally lower in healthy fishes and can be used as a significant indicator for infectious diseases and to predict the health status of fish (Gabriel, *et al*., 2004). In the present study, a significant increase in WBC was observed in infected fishes which indicated the poor health of the infected fish. Shah *et al*. (2009) observed a positive correlation between WBC and prevalence of helminth infection i.e. WBC increased according to intensity of infection. Kaur *et al.*

(2012) also reported similar results in *Nandus nandus* parasitized by *Clinostomum complanatum.* Ali *et al*. (2012) reported an increase in WBC count in *Cyprinus carpio* infected with monogenean parasites. According to Hassen (2002) and Lebola *et al.* (2001) the increase in WBC counts occurred as a pathological response since WBCs play a major role during infestation by stimulating the haemopoietic tissues and immune system to produce antibodies and chemical substances. These antibodies work as defense agents against infection. Differential Leucocyte Count (DLC) was also studied by several workers in fishes due to parasitic invasion (Shah *et al*., 2009; Denisove, 1979; Ali *et al.,* 2012; Malgorzata *et al*., 2010). An increase in the lymphocyte count in *Heteroponeutus fossilis* infected with *Lucknowia indica* has been reported by Saxena and Chauhan (1993). A higher degree of eosinophilia has been observed in *Clarias batrachus* carrying helminth infections by Sinha (2000).

Effect on Haemoglobin (Hb)

Haemoglobin values are one of the most thoroughly documented haematological parameters for evaluating fish health (Kawatsu *et al*., 1969; Smith *et al.,* 1952). In the present study, the average concentration of haemoglobin was decreased in infected fish as compared to non-infected fish of the selected genera viz., *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*. It has been reported by other workers that parasitization by pathogens like cestode, trematode, nematode and acanthocephalan (parasites) was metabolically dependent on their host and alter the host physiology (Moore, 1987). Therefore, the decrease in Hb concentration in fishes may be due to helminth infection. But many workers have attempted to correlate relative haemoglobin concentration with fish activity i.e. higher fish activity means the higher haemoglobin concentration (Eisler, 1965). Khalil *et al*. (2014) observed the decrease in Hb by 0.59% in infected *Heteropneustes fossilis.* Bag *et al.* (2011) observed the decrease in Hb by 37.80% in trypanosome infected *Clarias batrachus*. Klinger *et al*. (2002) have also reported the decrease in Hb concentration along with anemia, emaciation and reduced vitality due to the effect of helminth parasites.

Effect on Packed cell volume (PCV)

Korzhuez, (1964) stated that fish PCV values usually varies from 20-35%, or sometimes can attain more than 50% under different conditions In the present study the PCV was observed to be decreased in parasitized fishes which was statistically significant. The parasites act as stressors and during the primary stages of stress, the PCV are altered due to the release of catecholamine (Wells *et al*., 1990). Parasitic infection also induced RBC swelling as a result of fluid shift into the intracellular compartment was reported by Chiocchia and Motais (1989) and that thus can mobilize RBCs from the spleen was documented by Wells *et al*. (1990).

Effects on Mean corpuscular volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC)

The MCV and MCH values observed in infected fishes are generally enhanced, which confirms the pathological occurrence of pernicious anemia (Kundu *et al*., 2016). An increase in MCV indicating erythrocyte swelling, probably due to stress-related catecholamine secretion has been reported by Nikinmaa *et al*. (1984). Swelling is a low-cost mechanism of increasing oxygen transport capacity, as diluted haemoglobin exhibits higher oxygen binding affinity (Nikinmaa *et al*., 2001 and Kind *et al*., 2002). In the present study also, an increase in MCH was observed, which may suggest haemoglobin synthesis via circulating erythrocytes; this possibility was earlier reported by Speckner *et al*. (1989) and the MCV and MCH levels remained elevated until the end of their experiment. Similar results were obtained by Kurovskaya and Osadchaya (1993), who did not report anaemia in common carp infested with *I. multifiliis*, however said that MCHC values showed a non-significant decline in infected fish in comparison to the non-infected fish. On the other hand, Tavares-Dias *et al.* (2002) reported a significant decrease in MCHC accompanied by an increase in MCV in *Oreochromis niloticus* with gill infection with *ichthyophthiriasis* and *saprolegniosis.*

Conclusion

The present study was designed to study the haematological parameter of helminth infected fish species *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*. Haematological parameters are regarded as valuable tools for monitoring fish health. The range of normal values of the key haematological parameters is still undefined for different species in different aquaculture conditions. Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture. The results of the present study showed that there are significant changes in important blood parameters of the selected infected fish species in comparison to the non-infected (control) fishes. Therefore, the study may be effective in providing information for using blood parameters as an effective tool for monitoring the effects of parasitic infestation in fish which would be helpful in fishery management programs.

Chapter 6
Study of Biochemical Parameters of Helminth Infected Freshwater Fishes

Introduction

On a global scale, fish is a major source of food for human nutrition providing an important amount of dietary protein, carbohydrate and lipid (Vivekanandan and Jayasankar 2008; Bouriga *et al*., 2010; Nguyen Thi *et al*., 2010; Ravichandran *et al*., 2011). The flavour is often due to the presence of specific amino acids and oil characteristic of each fish species (Kirk and Sawyer, 1991). Edirisinghe (1998) stated that fishes are rich source of omega-3 polyunsaturated fatty acid which is recognized as an important nutritive supplement for preventing a number of coronary heart diseases. Lipids are structural materials, reserve supply of fuels, vitamins, emulsifiers, flavours, aromatic compounds and barriers to the environment (Harris, 1989; Nestel, 1990). Glycogen is the most abundant organic molecules in cells constituting 50 percent or more of their dry body weight. It is found in every cell since it is fundamental in all aspects of cell structure and function.

The nutritive value of fish may be degraded due to the presence of helminth parasites and their cysts (Hassan *et al.,* 2015). Parasites cause damage to the hosts by depriving them of digested food and by feeding on host tissues, sera, or blood (Kundu *et al.,* 2016).The pathogenicity of parasitism has been reported to cause extensive damage to the host leading to the lower production of the fish (Rai, 1986). Parasitic diseases, either by causing mortality or retarding growth rate of fish, lead to heavy economic losses in the farm. In fish, helminth parasites are conspicuous and located in the body cavity, intestine or embedded in the muscle (Mitchum, 1995). Unencysted larvae of these parasites migrate under the skin and in the muscles, causing extensive inflammation and necrosis. Encystation occurring in the viscera, namely liver, spleen, or gonads causes severe pathologic changes in the adjacent tissue (Paperna, 1974). Cestodes completely lack alimentation in all stages of life history and utilize the food directly from the intestine of host.

The regulation of helminth population in the host's gastrointestinal tract is a complex process, influenced by host immunological and nutritional status, age and breed of the animal (Von Brand, 1979). The impact of helminth infection on the hosts physiology and nutrition has been the subject of numerous investigations over the past decade (Stephenson, 1993; Solomons, 1993; Solomons and Scott, 1994; Eduisinghe

and Tomkins, 1995; Coop and Holmes, 1996; Knox, 2000). The parasite's metabolism depends on the feeding habits and the rich nourishment available in the intestine of the host, and uses this nourishment for their normal development and growth (Sonune, 2014).

Nutritional deficiencies as a result of intestinal helminth infection have also been studied by several workers (Hadju *et al.*, 1996; Lunn and Nothrop-Clewer, 1996). Intestinal helminthes may affect the nutritional status by causing increased nutrient loss, in addition to decreased food intake and nutrient absorption (Edirishinghe and Tomkins, 1995). The influence of host nutrition on helminth population has received relatively little attention and limited information is available as only a few studies have examined the effects of nutrition on the parasitic response in the infected host.

Parasitic worms compete for energy reserves with their fish host (Meakin, 1974; Tierney, 1991; Walkey and Meakins, 1970). There is a clear variation among hosts and parasites in the extent of such effects. This may be due to difference in the extent to which the parasites comprises nutrient reserves. Bundy and Golden (1987) described mechanisms by which host nutrition might influence helminth infection: nutritionally mediated changes in host defence and malnutrition of the parasites. Gastrointestinal helminthes have very specific physico-chemical requirements of their host gut environment, and nutritionally mediated changes might have a direct influence on the parasite population (Crompton and Nesheim, 1976). Hence, to maximize productivity and to reduce fish mortality due to diseases and parasites, continuous evaluation of the physiological status of the fish is essential in overall growth of fishery sector.

Haemato-biochemical indices have been employed in effectively monitoring the responses of organisms to stressors and thus its health status under such adverse conditions. The changes associated with biochemical parameters due to various parasites establish a database, which could be used in diseases diagnosis and in guiding the implementation of the treatment or preventive measures (Yaji and Auta, 2007).

Keeping the above facts in mind, the present study has been undertaken to examine the changes in nutritive value of fishes after parasitic infection. In the present study, investigators were made to assess the level of major biopolymers *i.e*. proteins, glycogen and lipids in muscle tissue, and the level of proteins and glucose in the serum of helminth infected (bearing natural heavy infection) *Channa punctatus, Heteropneustes fossilis* and *Mystus vittatus.*

Materials and Methods

A. Tissue Biochemical Estimations in host Fish

Tissue Biochemical Estimation

The tissue of the infected fish was collected, weighed and processed for biochemical estimations using standard methods. The estimation of protein content in the host tissues was carried out by Lowry's method (1951), the glycogen estimation by the method of Kemp *et al.* (1954) and lipid estimation as per method of Folch *et al.* (1957). The tissue of non-infected fish was taken as control.

1. Estimation of Protein

Reagents required:

1. BSA stock solution (1mg/ml).
2. Analytical reagents:
    a. 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solutions (0.4 gm in 100 ml distilled water.)
    b. 10 ml of 0.5% copper sulphate solution mixed with 10 ml of 1% sodium potassium tartarate solution. Prepare analytical reagents by mixing 2 ml of (b) with 100 ml of (a)
3. Folin – Ciocalteau reagent solution (1N) Diluted commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water).

Procedure

Total protein content was estimated by the method of Lowry *et al.* (1951). 5% homogenates of muscle were prepared in 5% Phosphate buffer saline and centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded and the suspended protein residue was dissolved in 1 ml of 1N NaOH. From this 0.2 ml of the extract was taken into the test tube and 5 ml of alkaline copper solution (50 ml of 2% Na_2CO_3 and 1ml of 0.5% $CuSO_4$. $5H_2O$ in 1% sodium potassium tartrate) was added. The contents

were mixed well and allowed to stand for 10 minutes. To this 0.5 ml of 50% Folin - Ciocalteau reagent (diluted with distilled water in 1:1 ratio) was added. After 30 minutes, the optical density was measured at 620 nm in a spectrophotometer against a blank. The standard graph was plotted by the method of Lowry *et al.* (1951) with bovine serum albumin (Sigma chemical Company, U.S.A). The values were expressed as mg/g wet weight of the tissue. All the samples were taken in triplicates.

2. Estimation of Glycogen

Reagents required

1. Deproteinizing solution. Trichloroacetic acid (5g. A.R.) and Ag_2SO_4 (100 mg . A.R.) are dissolved in water and made up to 100 ml.
2. Sulphuric acid, 98%
3. Methanol, 80%

Procedure

The glycogen was estimated by the method of Kemp *et al.* (1954). 50 mg muscle tissues of the fish were homogenized with 5 ml of deproteinizing solution with the help of mortar- pestle. 5% homogenates of muscle tissues were prepared in 80% methanol and centrifuged at 3000 rpm for 10 minutes. The tissue residue was suspended in 5 ml of deproteinizing solution and boiled for 15 minutes at 100^0C and then cooled in running water. The solution was made up to 5 ml with deproteinizing solution to compensate for evaporation and then centrifuged. From this, 2 ml of supernatant was taken into the test tube and 6 ml of concentrated H_2SO_4 was added and the mixture was boiled for 10-15 minutes. The mixture was cooled and the optical density was measured at 520 nm in a spectrophotometer against a blank. The standard graph was plotted by the method of Kemp *et al.* (1954) with bovine glycogen and expressed as mg of glycogen/g wet weight of the tissue.

3. Estimation of total lipids

Extraction of tissue

Tissues were extracted according to the procedure of Folch *et al.* (1957)

Reagents required for extraction

Alcohol-ether mixture (3: 1, v/v)

Chloroform-methanol mixture (1: 1v/v)

Procedure

250 mg of the tissue was extracted with 3: 1 alcohol ether mixture into 15ml test tubes and kept at 65^0C in a water bath for 2 hours, and cooled and centrifuged for half an hour Another 6 ml of 3: l alcohol-ether mixture was added to the residue and heated for 2 hours at 65^0C. Centrifuged and decanted supernatant in to the same tube containing previously decanted filtrate. Then 6 ml of 1: 1 chloroform methanol mixture was added to the residue and heated at 65^0 C for 1 hour Centrifuged and decanted the supernatant to the same tube containing the filtrate previously decanted. The total volume was made to 25ml with chloroform methanol mixture. This was used for the estimation of total lipids.

Estimation of total lipids

The lipid was estimated by the method of Frings and Dunn (1970).

Reagents required

Conc. H_2SO_4

Vanillin 0.6%.

Phospho-vanillin reagent: 200 ml 0.6 vanillin in 800 ml conc. o-phosphoric acid.

Standard - Olive oil. Stock 1 g % in ethanol. Working standard - 400mg % in ethanol.

Procedure for estimation

Three test tubes were labelled as test, blank and standard. 0.1 ml of lipid extract of tissues 0.1 ml distilled water, and 0.1 ml working standard were taken respectively in the 3 test tubes. 2.0 ml concentrated H_2SO_4 was added to each tube and they were heated in a boiling water bath for 10 minutes. Cooled and pipetted 0.1 ml of the digested mixture from each tube and transferred them into another 3 test tubes. 0.1 ml

concentrated H_2SO_4 and 5 ml phospho-vanillin reagent were added to all tubes and incubated at $37^0\ C$ for 15 minutes. The optical densities were measured at 540 nm in a spectrophotometer.

B. Blood Biochemical Estimations in host Fish

Blood Biochemical Estimation

1. Estimation of blood glucose

Blood glucose was estimated using the method of Folin and Wu (1919).

Reagents

1. 10% Sodium tungstate
2. 2/3 N Sulphuric acid
3. Phosphomolybdic acid
4. 0.1% Glucose stock
5. 0.01% Glucose working standard
6. Alkaline copper reagent

Procedure

Deproteinisation of blood:

Fresh blood without clot was collected. From this, 1 ml was pipetted out into 7 ml distilled water in a test tube so as to form a layer at the bottom. The pipette was washed with the top layer of water in the test tube till it was cleared off the blood. 1 ml of 10% sodium tungstate was added followed by 1 ml of 2/3 N sulphuric acid to make the total volume to 10 ml. The contents were mixed well by vigorous shaking and allowed to stand for a few minutes till the colour of the precipitate changed to chocolate brown, and then filtered through Whatman No. 41 filter paper. The protein free filtrate was collected in a dry test tube.

2. Estimation of blood glucose

Three Folin Wu tubes were marked A, B and C for test, standard and blank. 2 ml of protein free filtrate was taken in tube A, 2 ml of standard glucose solution in tube B and 2 ml distilled water in tube C. To each tube 2 ml alkaline copper reagent

was added. The content of each tube was mixed well by tapping gently between palms. The tubes were kept in boiling water bath for 8 minutes. After 8 minutes the tubes were removed from the water bath and immediately cooled under running tap water for 1minute. 2 ml of phosphomolybdic acid was added without delay to each tube. The contents were tapped between palms till the effervescence stopped. The tubes were heated in boiling water bath for 1 minute to stabilize the colour. It was cooled and made up to the mark with water. The contents were mixed well by inverting the tubes. Read test and standard against the blank at 680nm.

3. Estimation of total protein

Total Protein was estimated by using the Biuret method (Gornall *et al.*, 1949).

Reagents

1. Biuret reagent
2. 0.85% Sodium chloride
3. 2.5 N Sodium hydroxide
4. Standard protein (BSA)

Procedure

In a test tube 0.5 ml plasma was mixed with1.5 ml of 0.85% sodium chloride solution. 8 ml Biuret reagent was added to it. To prepare the standard, 1 ml of standard protein was mixed with 1 ml of 0.85% sodium chloride solution. Then 8 ml of Biuret reagent was added to this. To prepare the blank 2 ml of 0.85% sodium chloride solution was mixed with 8 ml of Biuret reagent. All the three test tubes were shaken well and allowed to stand for 30 minutes. Read test and standard against the blank at 520 nm.

Statistical analysis

Student's t-test was applied to test significant difference between levels of biomolecules in the infected and non-infected fishes.

Results

A total of 20 non-infected and 20 naturally infected fishes were subjected to blood and tissue biochemical studies in order to investigate the effects of helminthic infections on the levels of certain biomolecules in the fish body. The fishes selected for the study were *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* only as they were found to show high parasitic burdens during the study.

Tissue Biochemical Estimations in host Fish

The tissues from 20 infected host fishes each belonging to *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* were individually subjected to biochemical studies in order to evaluate the levels of protein, glycogen and lipid during natural infection by helminth parasites.

Tissue protein in host fishes

The total protein content in muscle tissue samples of host fishes was estimated by the method of Lowry (1951). The range of total protein level and mean value of total protein level in naturally infected and non-infected host fishes are given in Table 1, Figure 1**.** In *Channa punctatus* the total protein level observed in the infected fish was 105.0 ± 1.81 mg/g and in the non-infected fish, it was 115.4 ± 0.60 mg/g. The total protein level was found to be decreased significantly ($P<0.01$) in the helminth infected fishes as compared to the non-infected fishes.

In *Heteropneustes fossilis*, the total protein level observed in the infected fish was 90.1 ± 5.4 mg/g and in the non-infected fish it was 96.5 ± 1.6 mg/g. The difference in total protein content in infected and non-infected *H. fossilis* was found to be statistically significant ($P<0.01$).

In *Mystus vittatus* the total protein observed in the infected fish was 90.1 ± 1.2 mg/gm and in the non-infected fish was 100.8 ± 5.4 mg/g. The difference in total protein content in infected and non-infected *M. vittatus* was found to be highly significant ($P < 0.001$).

Tissue glycogen in host fishes

The estimation of total glycogen level in muscle tissue samples of host fishes was done by the method of Kemp *et al.* (1954). The range of glycogen levels and its mean value in infected and non-infected host fishes are given in Table 2, Figure 2. In *Channa punctatus* the glycogen level observed in the non-infected fish was 2.56 ± 0.01mg/gm and in the infected fish, it was 1.29 ± 0.39 mg/g. The average values showed that the glycogen level was very significantly decreased in the infected fish ($P < 0.001$).

In *Heteropneustes fossilis* the glycogen level observed in the infected fish was much lower (2.1 ± 1.5 mg/g) as compared to the levels in the non-infected fish (3.5 ± 1.6 mg/g). The difference in glycogen levels in the two groups was highly significant ($P < 0.001$).

In *Mystus vittatus* the mean value of glycogen was recorded 2.8 ± 0.12 mg/g in infected fishes and 4.7 ± 0.12 mg/gm in non-infected fish. Student's t-test revealed that the mean value of glycogen was found to be decreased significantly ($P < 0.001$) in infected fish when compared to the non-infected fish.

Tissue total lipids in host fishes

The range of total lipid content and its mean value in infected and non-infected host fishes are given in Table 3, Figure 3. In *Channa punctatus* the level of total lipids observed in the non-infected fish was 12.6 ± 0.09 mg/g and in the infected fish it was 9.8 ± 0.13 mg/g. Data shows that lipid value was decreased significantly ($P <0.001$) in the helminths infected fishes as compared to non-infected fishes.

In *Heteropneustes fossilis* the total lipids observed in the non-infected fish was 11.0 ± 0.18 mg/g and in the infected fish it was 9.8 ± 0.73 mg/g. The results show that total lipid content in tissue sample was reduced significantly ($P<0.01$) in helminths infected fishes as compared to non-infected fishes.

In *Mystus vittatus* the total lipid value observed in the non-infected fish was 29.5 ± 1.0mg/g and in the infected fish was 27.3 ± 0.78 mg/g. The average value showed that this level was decreased significantly ($P < 0.001$) in the infected fish.

Blood Biochemical Estimations in host Fish

The blood of 20 naturally infected host fishes was collected and stored by using standard procedures respectively. The serum was subjected to biochemical studies in order to evaluate the protein and glucose levels in the naturally infected *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* only as these fishes showed higher burden of helminthes parasites during the study period.

Serum total proteins in host fishes

The effect on total protein level in the serum of host fishes was observed and estimated by the Biuret method (Gornall *et al.*, 1949). The total protein content in infected and non-infected fishes was evaluated and the results are given in Table 4 and Figure 5.

In *Channa punctatus* the serum total protein was estimated as 4.25 ± 0.38mg/dl and 3.07 ± 0.36mg/dl for non-infected fishes and infected fishes respectively.

In *Heteropneustes fossilis*, the serum total protein content in non-infected fishes was 4.26 ± 0.80mg/dl, while in the infected fishes it was 3.15 ± 1.01mg/dl.

In *Mystus vittatus*, the serum total protein content in non-infected fishes was 4.64 ± 0.98mg/dl and in infected fish it was estimated at 3.10 ± 0.67mg/dl.

In all the fishes examined, the total protein content in serum was found to be significantly decreased ($P<0.001$) in the infected fishes as compared to the non-infected ones.

Serum glucose in host fishes

The effect on glucose level in the serum of host fishes was observed and estimated by Folin and Wu (1919) method. In *Channa punctatus* the serum glucose level observed in the non-infected fishes was 57.92 ± 4.48mg/dl and in the infected fishes it was 72.99 ± 7.91mg/dl. The results show that glucose level was increased significantly ($P < 0.001$) in helminths infected fishes when compared to non-infected fishes **(**Table 5, Figure 5).

In *Heteropneustes fossilis*, the blood glucose level observed in the infected fishes (126.34 ± 16.71mg/dl) was found to be significantly increased (P<0.01) when compared to non-infected fishes (105.78 ± 31.27mg/dl)

Similarly, in *Mystus vittatus* the blood glucose level observed in the infected fishes (120.58 ± 16.33mg/dl) was found to be significantly increased (P<0.001) when compared to non-infected fishes (85.72 ± 14.33mg/dl).

Table:1. Total Protein content in tissue (muscles) of non-infected and infected host fishes

Hosts	Total Protein(mg/g)			
	Non-infected		Infected	
	Range	Mean ± SD	Range	Mean ± SD
C. punctatus	104.4 - 131.5	115.4 ± 0.60	94.4 - 121.0	105.0 ± 1.81**
H. fossilis	88.7 – 108.7	96.5 ± 1.6	83.0 – 108.7	90.1 ± 5.4**
M. vittatus	95.0 -106.7	100.8 ± 5.4	85.0 – 96.2	90.1 ± 1.2***

Each value is mean ±SD of 20 observations, Values are significant at ***P*<0.01; ****P*<0.001.

Table: 2. Glycogen content in tissue (muscles) of non-infected and infected host fishes

Hosts	Glycogen (mg/g)			
	Non-infected		Infected	
	Range	Mean ± SD	Range	Mean ± SD
C. punctatus	1.74 - 3.83	2.56 ±0.01	0.69 – 2.0	1.29 ± 0.39***
H. fossilis	2.4 – 5.9	3.5 ± 1.6	0.87 – 3.82	2.1 ± 1.5***
M. vittatus	4.1 – 5.3	4.7 ± 0.12	2.0 – 3.8	2.8 ± 0.12***

Each value is mean ±SD of 20 observations, Values are significant at ****P*<0.001.

Table: 3.Total Lipid content in the tissue (muscles) of non-infected and infected host fishes

Hosts	Total Lipid (mg/g)			
	Non-infected		Infected	
	Range	Mean ± SD	Range	Mean ± SD
C. punctatus	11.4 - 14.0	12.6 ± 0.09	8.2 – 12.0	9.8 ± 0.13***
H. fossilis	10.0 – 12.7	11.0 ± 0.18	8.2 – 12.7	9.8 ± 0.73**
M. vittatus	27.6 - 32.0	29.5 ± 1.0	25.0 – 29.5	27.3 ± 0.78***

Each value is mean ±SD of 20 observations, Values are significant at $^{**}P<0.01$; $^{***}P<0.001$.

Table: 4. Total Protein content in the serum of non-infected and infected host fishes

Hosts	Total Protein (g/dl)			
	Non-infected		Infected	
	Range	Mean ± SD	Range	Mean ± SD
C. punctatus	3.90-4.98	4.25 ± 0.38	2.3-3.8	3.07 ± 0.36***
H. fossilis	3.4-5.9	4.26 ± 0.80	2.0-5.0	3.15 ± 1.01***
M. vittatus	4.0-7.0	4.64 ± 0.98	2.7-5.0	3.10 ± 0.67***

Each value is mean ±SD of 20 observations, Values are significant at $^{***}P<0.001$.

Table: 5. Glucose content in the serum of non-infected and infected host fishes

Hosts	Glucose (mg/dl)			
	Non-infected		Infected	
	Range	Mean ± SD	Range	Mean ± SD
C. punctatus	56-70	57.92 ± 4.48	61-88	72.99 ± 7.91***
H. fossilis	81-177	105.78 ± 31.27	97-185	126.34 ± 16.71**
M. vittatus	63-102	85.72 ± 14.33	105-153	120.58 ± 6.33***

Each value is mean ±SD of 20 observations, Values are significant at $^{**}P<0.01$; $^{***}P<0.001$.

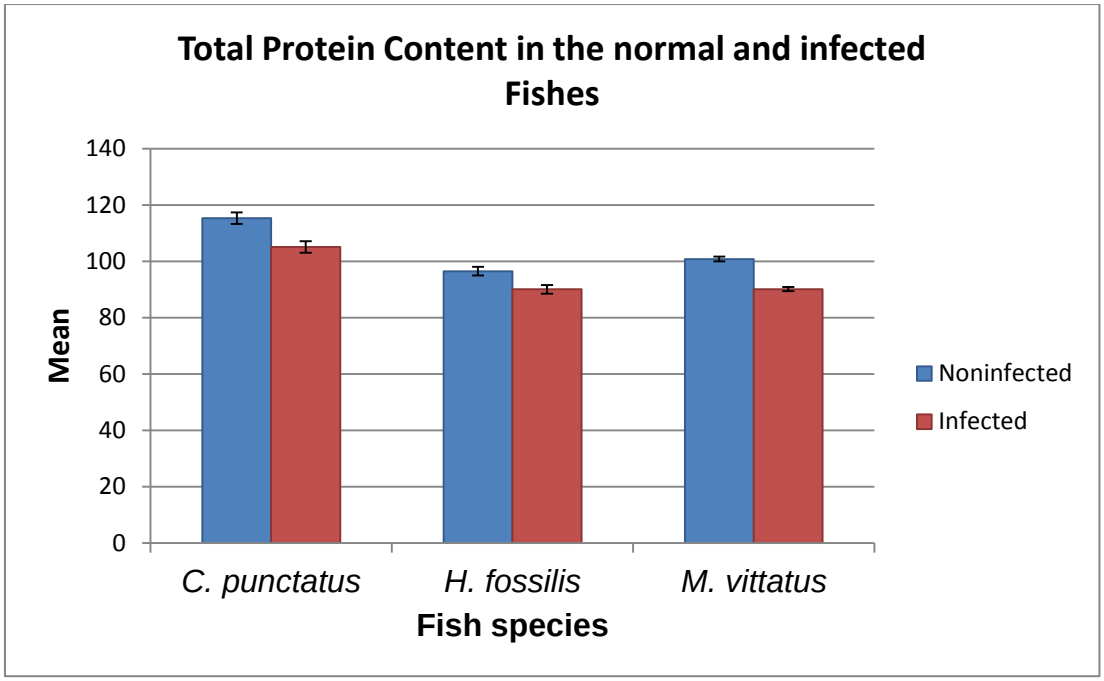


Figure 1: Total Protein content in tissue (muscles) of non-infected and infected host fishes

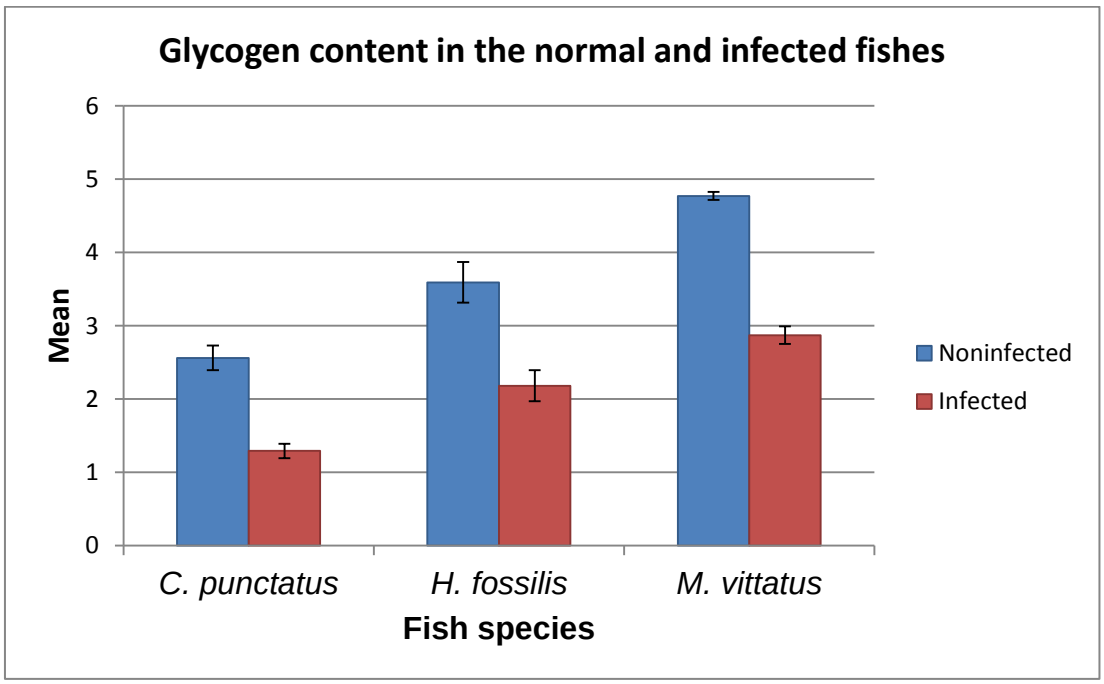


Figure 2: Glycogen content in tissue (muscles) of non-infected and infected host fishes

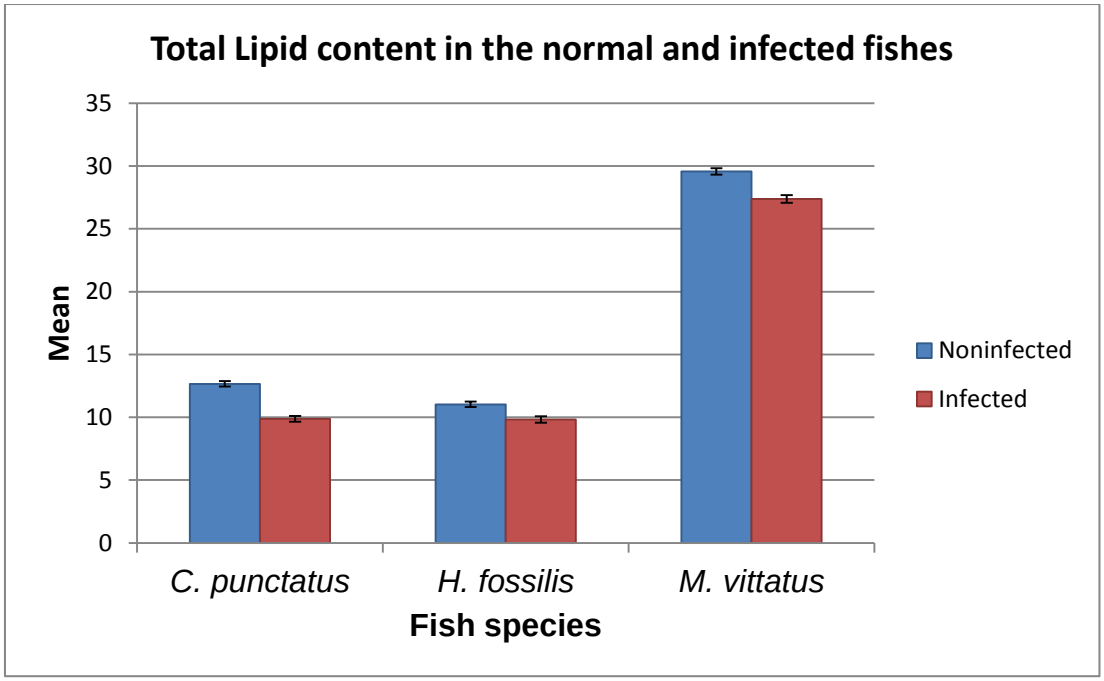


Figure 3: Lipid content in the tissue (muscles) of non-infected and infected host fishes

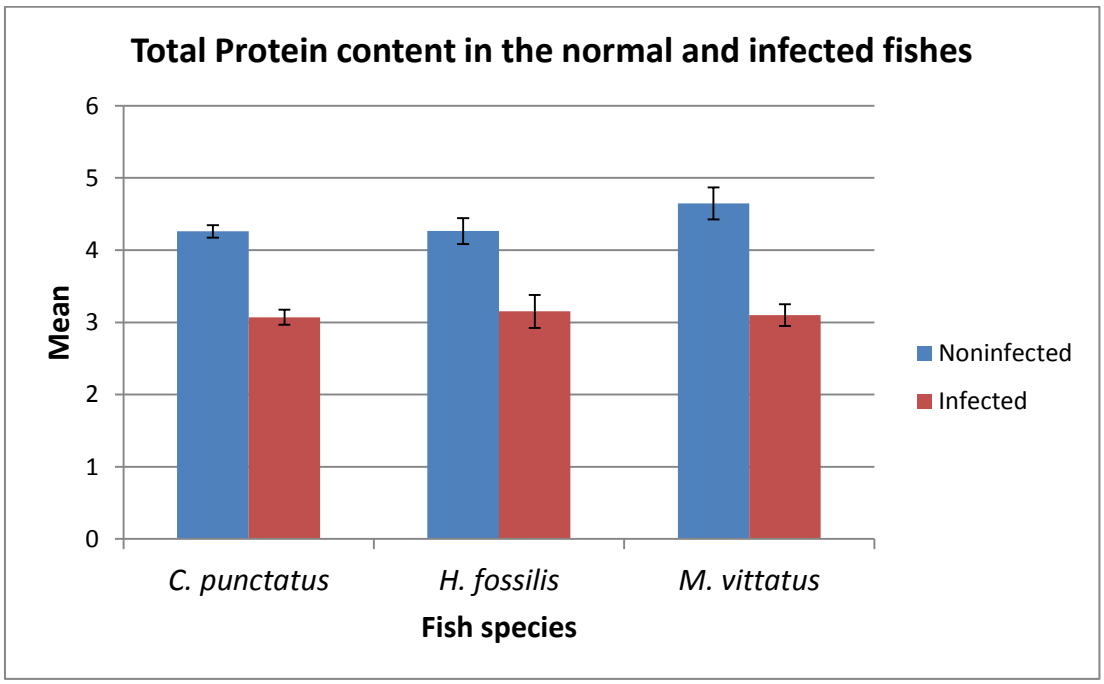


Figure 4: Total Protein content in the serum of non-infected and infected host fishes

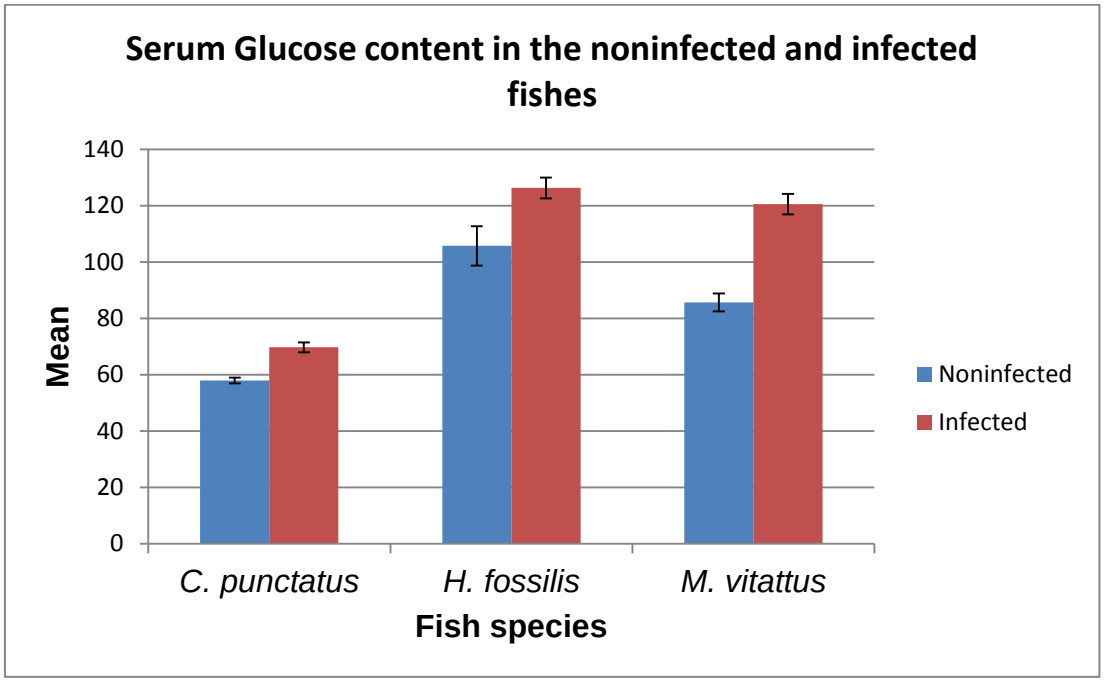


Figure 5: Glucose content in the serum of non-infected and infected host fishes

Discussion

The present study showed the effect of helminth parasites on the level of protein, glycogen and lipid in muscle tissue of host fishes. The study also estimated the level of protein and glucose in the blood (serum) of host fishes. The infected fishes used in the present study were *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus* bearing high burden of helminth parasites.

Effect of helminth parasites on protein level of host tissue

The value of protein content is considered as an important tool for evaluation of physiological standards (Chezhian *et al.*, 2010). In *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*, the protein level was found to be significantly reduced in the naturally infected fishes as compared to the non-infected fishes. A drop in the protein level in fishes during the helminth infection was also reported by Patwardhan (1953), Lomukhin (1971). According to Joshi (1979) the reason behind the decrease in protein level in the infected fish is the obstruction of the bile duct as a result of the presence of a large number of parasites which leads to improper digestion and absorption of nutrients.

However, some workers have reported increased protein content in helminth infected fishes (Kameshwari, 1978; Bhonsle, 1980; Thabitha, 1982). According to Kumar (2014) the increase could be attributed to the tissue repair mechanism operating in the host system in order to cope with the parasitic invasion. Repair of the connective tissue is a mandatory procedure in the infected hosts and this process is supported and brought about by the increased shunt of proteins.

Effect of helminth parasites on glycogen level of host tissue

The present study showed that glycogen level was significantly reduced in the naturally infected fishes (*Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*) as compared to the non-infected fishes. Similar results were reported by Linnik *et al.* (1980) and Ali (2001). They concluded that the decrease in glycogen may be due to the stress since the parasite obtains nourishment through cyst wall from the host. The reason may be the improper digestion and absorption of nutrients due to

obstruction of bile duct with the heavy parasitic load (Joshi, 1979). Hassan *et al*. (2015) have also reported similar findings in *E. summana* fish species.

Effect of helminth parasites on lipid level of host tissue

The present study showed that lipid level was significantly reduced in infected fishes (*Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*) as compared to the non-infected fishes. According to Harper (1983) the decrease in tissue lipid might be due to their utilization in cell repair and tissue organization with the formation of lipoproteins, which are important cellular constituents of cell membranes and cell organelles present in the cytoplasm. Tazeen *et al*. (1996) mentioned the possibility of reduction of total lipids is due to the excessive lipolysis subsequently used for synthesis of glucose. Amudha *et al*. (1993) suggested that lipid content of fish may also be reduced with increasing water pollution.

Effect of helminth parasites on serum protein level in host fishes

The present study showed that protein level was significantly reduced in the serum of all the studied naturally infected fishes viz., *Channa punctatus*, *Heteropneustes fossilis* and *Mystus vittatus*. Kundu *et al*. (2015) and Aly *et al.* (2005) also reported similar findings in *C. punctatus* and *C. gareipinus* fish species.

Effect of helminth parasites on serum glucose level in host fishes

The present study showed that glucose level was significantly increased in the naturally infected fishes. Similar results were also given by several workers (Oruc and Uner, 1998 and 1999; Al-Akel and shamsi; 2000; Al-Kahem, 1996; Aly *et al.*, 2005; Ali *et al*., 2012). Elnemaki (2003) suggested that the stress caused by the infestation of the parasites in all fish organs may lead to hypoxic or anoxic conditions which therefore enhance glycogenolysis and gluconeogenesis and these metabolic activities result in the rise of serum glucose level in the naturally infected fish.

Conclusion

The results obtained in the present study showed that the protein, lipid, and glycogen levels were significantly decreased while serum glucose level was significantly increased in the helminth infected fishes. Thus, the parasites seem to have altered the crucial physiological aspects of the host system and the host appears trying to face the infection by altering its physiology. The results of this study provide information regarding the characteristic features of tissue and serum biochemical changes in *C. punctatus, H. fossilis* and *M. vittatus* due to parasitic helminth infection. The study suggests that tissue and serum biochemical studies may be effective in monitoring the effects of parasitic infestation in fish. This knowledge would be effective in fishery management programs.

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Appendix

APPENDIX

PAPERS PUBLISHED IN JOURNALS

S.No	Title of the paper	Authors	Journal Name/ Volume (issue)/ Page Number	Year
1	Population dynamics of Helminths parasites in freshwater fishes of River Gomti in Lucknow, Uttar Pradesh.	**Anita singh** and Suman Mishra	Journal of Environmental science, Computer science and Engineering & Technology (JECET) Volume 5, Issue 3, 640-647 ISSN: 2278- 179X. Impact factor= 5.048	2016
2	A Comparative study of Helminths parasites in catfishes.	**Anita singh** and Suman Mishra	International journal of science and research (IJSR) Volume 5 Issue 7, ISSN (Online): 2319-7064 Impact factor= 6.391	2016

ARTICLES/CHAPTERS PUBLISHED IN BOOKS

S.No.	Title of the Chapter	Book Title , Editor & Publisher	ISSN/ISBN No.
1	**Singh, A.** and Mishra, S. **,** Population dynamics of trematode parasites in freshwater fish *Channa punctatus* from Lucknow region, Uttar Pradesh	**Trends in Biodiversity: floral, faunal and ecological aspects** Published by Narendra Publishing House, Delhi.	ISBN: 978-93-84337-65-0, pp, 67-74 (2016)

PAPERS PRESENTED IN CONFERENCES/ SYMPOSIA/ SEMINARS

S.No	Title of the Paper	Conference/Symposium/ Workshop	Date
1.	**Singh, A.** and Mishra, S. Infestation of Helminth parasites in *Heteropneustes fossilis* (Bloch) fishes of river Gomti, Lucknow (U.P.)	26th All India Congress of Zoology and International conferences on Innovation in Animal Sciences For food security, Health security and Livelihood-2015	29th -31st October, 2015
2.	**Singh, A.** and Mishra, S. Survey of Helminth Parasites of two freshwater catfishes from Lucknow Region, Uttar Pradesh, India.	25th National Congress of Zoology and National Seminar on "Threats to Biodiversity and Ecosystems: Impact of Developmental Projects and Climate Change", Gurukula Kangri University, Haridwar	17th-19th November, 2014
3.	**Singh, A.** and Mishra, S. Population Dynamics of Trematode Parasite in Freshwater Fish *Channa punctatus* from Lucknow Region, Uttar Pradesh, India	International symposium on "Biodiversity: Status, Utilization and Impact of Challenging Climatic Conditions, B.B.A. University, Lucknow	30th-31th October, 2014.
4.	**Singh, A.** and Mishra, S. Population Dynamics of Cestode Parasite in *Wallago attu* from Lucknow Region, Uttar Pradesh, India	2nd Lucknow Science Congress, Babasaheb Bhimrao Ambedkar University, Lucknow	27-28th March 2014.
5.	**Singh, A.** and Mishra, S., Prevalence of Helminth parasites in freshwater fish *Channa punctatus* (Bloch) from Lucknow region, Uttar pradesh	National workshop on Biomolecular Parasitology and Resource Sustainability, held at University of Allahabad, Allahabad	3-5th Dec 2013
6.	**Singh, A**., Climate change, its implication and management strategies in aquatic environment	World Environment Day, Babasaheb Bhimrao Ambedkar University, Lucknow	5th June 2013
7.	**Singh, A.** and Mishra, S. Studies on prevalence of acanthocephalan parasites in freshwater fish *Channa*	1st Lucknow Science Congress, Babasaheb Bhimrao Ambedkar University, Lucknow	20th -21st March 2013

	punctatus from Lucknow region, Uttar Pradesh.		
8.	**Singh, A.** and Mishra, S. Survey of the Helminth parasites of freshwater fish from Lucknow region, Uttar Pradesh.	National Workshop on Capacity Building in Disease Control & Sustenance & Mini Symposium on Parasitic Diseases, Dept. of Zoology, University of Allahabad, Allahabad, U.P.	5^{th}-7^{th} November, 2012
9.	Singh, A. and Mishra, S., Present status and biodiversity of ornamental fishes used in trade in Lucknow City, Uttar Pradesh.	National Seminar on Status of Environment and Biodiversity Rio + 20 and Role of Space Technology 2012 Department of Zoology and Environmental Science, Gurukula Kangri University, Haridwar in collaboration with Indian Academy of Environmental Sciences..	2^{nd}-3^{rd} November. 2012
10	**Singh A.** and Mishra, S. Studies on the Helminth parasites of fish in Uttar Pradesh: A Review.	23^{rd}All India Congress of Zoology & National Seminar of Conservation and Management of Faunal Resources of Sustainability, Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai in association with Zoological Society of India, Bodh Gaya.	3^{rd}-5^{th} October, 2012

PARTICIPATION IN TRAINING PROGRAMS/ WORKSHOPS

S.No	Name of the Workshop, etc.	Name of the Sponsoring Agency	Place and Date
1	Hands-on-Training on SEM, FTIR, FPLC and Ion Chromatography	University Science Instrumentation Centre, Babasaheb Bhimrao Ambedkr University, Lucknow	Babasaheb Bhimrao Ambedkar University, Lucknow 18^{th} Feb, 2015 to 20^{th} Feb. 2015
2.	National workshop on Biomolecular Parasitology and Resource Sustainability (BPRS-2013)	CSIR, DAE-BRNS, DBT, DRDO, ICMR, Min. Earth Sci., UGC and ACU, Parasitology laboratory, Department of Zoology, University of Allahabad,	University of Allahabad, Allahabad 3-5^{th} December, 2013
3.	National Workshop on Capacity Building in Disease Control & Sustenance & Mini Symposium on Parasitic Diseases (CBDCS-2012)	DBT, DRDO, UGC, CSIR, Min. Earth Sci. and ACU, Parasitology laboratory, Department of Zoology, University of Allahabad,	University of Allahabad, Allahabad 5-7^{th} November, 2012

CONFERENCES/ SYMPOSIA/ SEMINARS ATTENDED

S.No	Name of the Seminar/Conference / Symposia / Workshop, etc.	Name of the Sponsoring Agency	Place and Date
1.	One Day Seminar on "ICT In Higher Education: Need of the Hour"	Information & Guidance Bureau, Placement Cell & DLIS, BBAU, Lucknow	20th January, 2014
2.	Symposium on " Building an Ecologically Sustainable Society"	Indian Academy of Social sciences, Prof. H.S. Srivastava Foundation for Science & technology, Association of Socio-Economic Development Studies, Lucknow, Society of Earth Scientists	B.B. Ambedkar University, Lucknow 16th August, 2013
3.	Patent workshop-2013	University placement cell and Information & Guidance Bureau, BBAU, Lko	University placement cell and Information & Guidance Bureau, BBAU, Lko 18th march, 2013
4.	National Workshop on "Crime Against Women: Legal Issues"	SHS, BBAU, Lko	SHS, BBAU, Lko, 7th march, 2013
5.	Programme on "Wetlands In Biodiversity Conservation"	U.P state Biodiversity Board and DAAS, BBAU, Lko	U.P state Biodiversity Board and DAAS, BBAU, Lko. 2nd Feb, 2013
6.	International Conference on "Recent Trends in Climate Change Researches vis-à-vis Biodiversity"	Department of Animal Science, MJP Rohilkhand University, Bareilly	Department of Animal Science, MJP Rohilkhand University, Bareilly 3rd-4th December 2012

MEMBERSHIP OF PROFESSIONAL BODIES/ORGANIZATIONS

- Life member**, Indian Science Congress Association**
- Life Member, **Zoological Society of India**
- Life Member, **Indian Society for Parasitology**

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Section A: Environmental Science

Research Article

Population dynamics of helminths parasites in freshwater fishes of River Gomti in Lucknow, Uttar Pradesh

Anita Singh and Suman Mishra*

Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, VidyaVihar, Raebareli Road, Lucknow-226025 (U.P.)

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Abstract: Five freshwater fish species viz. *Channa punctatus*, *Heteropneustes fossilis* (Bloch), *Mystus vittatus*, *Mastacembalus pancalus*, and *Wallago attu* were examined for the presence of helminth parasitic infection. A total of 793 fishes examined, 163 (20.5%) were found infected with helminth parasites. These parasites were isolated from body cavity, muscles, Intestine, stomach of the infected fishes. In general species wise prevalence of the parasitic infection was found in *Channa punctatus* (37.5), *Heteropneustes fossilis* (Bloch) (14.7), *Mystus vittatus* (13.5), *M. pancalus (11.6)*, and *Wallago attu* (10%). In present study, the highest infection (37.5) was found in *Channa punctatus* and lowest infection (10%) in *Wallago attu* species

Key-word: Helminth parasites, Freshwater fishes, Fish species, Prevalence, Infection.

INTRODUCTION

Fishes are aquatic vertebrates that are naturally cold blooded. They are abundantly found in the sea water and in freshwater. Fishes are a best source of protein and vitamins like A, D, E, K, and B12 and

are easily digested. They are also a rich source of calcium, iron, phosphorous, iodine and omega-3-fatty acid. Fish oil contains omega–3–essential fatty acids which are necessary for the proper functioning of the brain, heart and immune system[1]. Millions of human being suffers due to hunger and malnutrition. In order to meet the demand of food partly, fishes can be used as alternative source of food. *Channa punctatus* (Bloch), *Mystus tengra, Mystus vittatus* (Sykes), *Mystus bleekeri, Mastacembalus armatus, M. pancalus, Wallago attu* (Schn), *Heteropneustes fossilis* (Bloch), *C. striatus, Labio bata, Labio rohita, Clarias batrachus* (Linn) *and Anabas testudineus* are one of the common freshwater fish species of Uttar Pradesh abundantly found in River Gomti. In the present studies five freshwater fishes ie, *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu,* found in River Gomti Lucknow.

EXPERIMENTAL

1. **Study area:** River Gomti, Lucknow.
2. **Study period:** January 2013 to December 2013.
3. **Study organism:** Freshwater fishes (*Channa punctatus, Heteropneustes fossilis* (Bloch), *Mystus vittatus, M. pancalus,* and *Wallago attu*).
4. **Collection of host fishes:** Live fresh specimens of *Channa punctatus, Heteropneustes fossilis* (Bloch), *Mystus vittatus, M. pancalus,* and *Wallago attu* obtained from River Gomti Lucknow city. The specimens were brought in the laboratory alive in a small container with water and maintained in glass aquaria.
5. **Methodology**

I. **Examination for helminth parasites:** In the laboratory, all samples were examined and processed as per standard protocol. Fishes were opened up dorso-ventrally and all the internal organs were examined separately. The entire digestive system and bladder was removed and placed in a petri dish with saline for further examination.

II. **Processing of parasites for identification:** The trematodes were fixed in hot 10% formalin, cestodes and acanthocephala were fixed in Alcohol - Formaline – Acetic acid (AFA) solution for 24 hours. The parasites were dehydrated using different grades of alcohol: 30%, 50%, 70%, 90%, 100% alcohol for a period of 10 to 15 minutes each was depending on the thickness of specimen. After dehydration, the parasites were stained by borax carmine. After staining, parasites were washed with distilled water, dehydrated in ascending grades of alcohol, cleared in xylene, mounted in D.P.X. The slides were observed under a light microscope and the parasites identified with the help of Yamaguti[2-5].

III. Formula for Prevalence study: Prevalence, Mean Intensity and Abundance were determined by following the formula proposed by Margolis *et al*.[6]

$$\textbf{Prevalence} = \frac{\text{Total no. of infected fishes}}{\text{Total no. of fishes host examined}} \times 100$$

$$\textbf{Mean Intensity} = \frac{\text{Total no. of parasites recovered}}{\text{Total no. of infected host examined}}$$

$$\textbf{Abundance} = \frac{\text{Total no. of parasites recovered}}{\text{Total no. of fish hosts examined}}$$

RESULTS AND DISCUSSION

To find out the parasitic infection in edible fishes *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu* survey a have been made at different location in river Gomti at Lucknow in the year 2013. The fishes collected from river Gomti were found to be infected with four classes of helminth parasites. They were attacked in the intestine (Cestode), liver, stomach, intestine and body cavity (Trematode), intestine, body cavity, airbladder (Nematode), and intestine (Acanthocephala) of freshwater fishes. A total of 793 fishes examined comprising *Channa punctatus, Heteropneustes fossilis, Mystus vittatus, Mastacembalus pancalus and Wallago attu*, 163 were found infected with helminth parasites. The overall prevalence of infection with helminth parasites (20.5%) was recorded. The maximum parasitic infection (37.5%) was showed by *Channa punctatus* and minimum (10%) by *Wallago attu*. **(Table 1).**

Table 1: Overall prevalence of the Helminth parasites

Species	No. of fish examined	No. of fish infected	Prevalence
Channa punctatus	240	90	37.5
Heteropneustes fossilis	223	33	14.7
Mystus vitattus	140	19	13.5
Mastacembalus pancalus	120	14	11.6
Wallago attu	70	7	10
Total	793	163	20.5

Population dynamics of helminth parasites infecting *Channa punctatus* in River Gomti at Lucknow: Out of 240 specimens of *Channa punctatus* examined, 90 were found infected with helminth parasites. The overall prevalence of the infection with helminth parasites (37.50%) was recorded **(Table 1, figure 1)**. The maximum prevalence (75%) was recorded in month of December while the minimum prevalence (20%) was recorded in August and September (**figure 2**). In *C. punctatus*, the maximum intensity (10) was recorded in Aug while the lowest intensity (1.54) was recorded in March **(figure 3**). The recorded value of abundance ranged from 0.45 – 2.0. The highest abundance (2.0) was recorded in Aug and the lowest abundance (0.45) in November **(Figure 4)**. The present investigation revealed that the incidence of infection was maximum in month of December *i.e.*, during winter month. Kaur *et al.* also recorded the maximum prevalence in month of December in *Channa punctatus*. The

minimum incidence of infection was recorded in the month of September. Kanth and Srivastava also reported the similar finding. *C.punctatus* showed highest intensity of infection in the month of August, 2013. Jha *et al.* also reported the similar finding. The percent abundance of helminth parasites was recorded highest in the *Channa punctatus* during month of August, 2013. Present study were also reported prior by Verma *et al.*[7] The lowest abundance was observed in month of November, 2013. Akhter *et al.* also reported the similar finding in the different fish species *H. molitrix*.

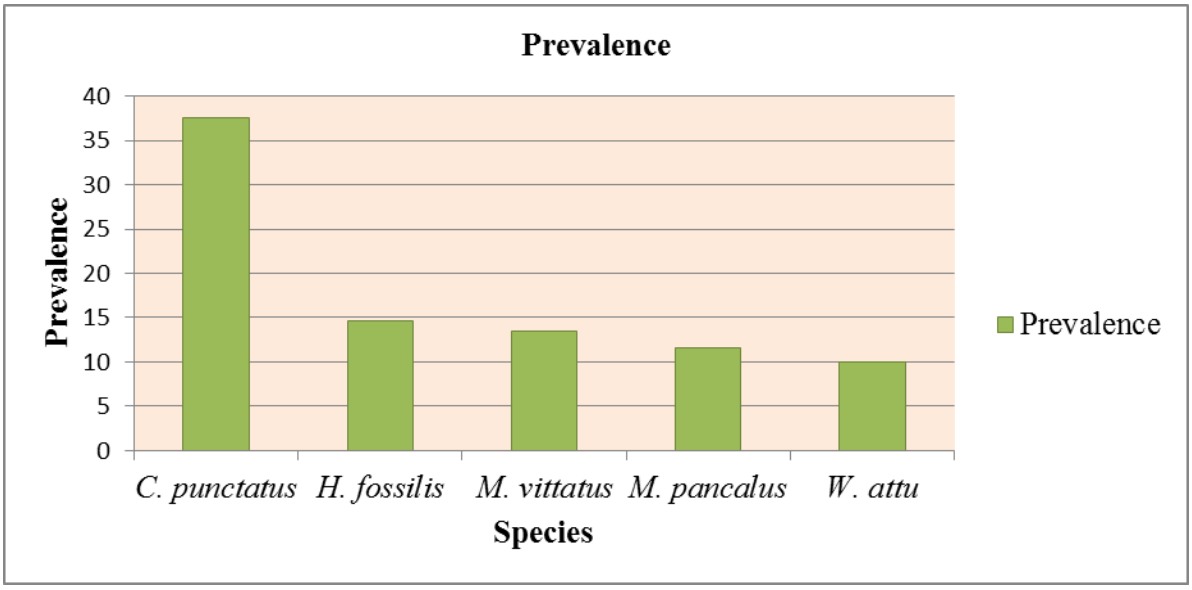

Figure 1: Overall prevalence of helminth parasites

Out of 223 specimens of *Heteropneustes fossilis* examined, 33 were found infected with helminth parasites. The parasites were recovered from intestine, muscles and body cavity and showed an overall prevalence of 14.79% **(Table 1, figure 1).** The maximum prevalence of 25% was recorded on month of June while the minimum prevalence of 10% was recorded in January, April and October **(Figure 2)**. In *H. fossilis*, the maximum intensity (6.75) was recorded in July, 2013. The lowest intensity (1.0) was recorded in months of March, Oct. and December, 2013 **(Figure 3)**. The recorded value of abundance ranged from 0.10 – 1.35. The highest value of abundance (1.35) was recorded in July and the lowest value of abundance (0.10) was calculated in month of October. **(Figure 4)**. During the study period, helminth parasites, belonging to three classes Cestode, Trematode, and Nematode were observed and collected. In the present investigation, regarding the monthly infestation, maximum prevalence was observed in month of June. Khanum *et al.* also reported the similar finding in fish species *Rita rita*. The minimum prevalence was recorded in January, April and October. Similarly, the minimum prevalence is also found in January by Pinky Kaur et al.[8] in fish species *Channa punctatus*. In *Heteropneustes fossilis*, the maximum intensity was recorded in July. Khanum *et al.* also reported the similar finding in fish species *Rita rita*. The lowest intensity was recorded in months of March, Oct. and December. Similarly, the lowest intensity was also found in October by Pinky Kaur *et al*.[8] in fish species *Channa punctatus*, Bhopal, M.P. The highest value of abundance was recorded in July and the lowest value of abundance

was calculated in month of October. Similarly, the lowest abundance was also found in October by Pinky Kaur *et al.* [8] in fish species *Channa punctatus*, Bhopal, M.P.

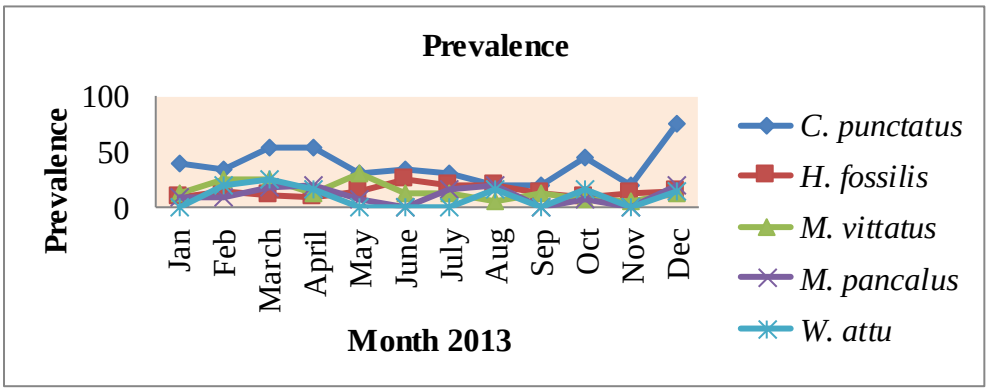

Figure 2: Monthly variation in the prevalence of helminth parasites

The results showed that parasites collected from *Mystus vittatus* were Cestode, Nematode and Acanthocephala parasites were found in intestine and Trematode parasites were found in Airbladder. Out of 140 specimens of *M. vittatus* examined, 19 were found infected with helminth parasites. The overall incidence of helminth parasites was observed 13.57% **(Table 1, Figure 1)**. The observations revealed that the incidence of helminth parasites was observed to be maximum 30% in month of May while the incidence of helminth parasites was observed as minimum 5.88% in month of August **(Figure 2)**. However, the maximum percent intensity of helminth parasites was recorded as 3.0% in the month of August and the maximum percent abundance was recorded as 0.6% in the month of May.

The minimum percent intensity of helminth parasites was recorded as 1.0% in months of Feb, June, July, November and the minimum percent abundance was recorded as 0.06% in month of November **(Figure 3, Figure 4).** The observations revealed that the prevalence of helminth parasites was observed to maximum in month of May. Shilpi Yadav *et al*. are also reported the similar finding in fish species *Channa punctatus* were obtained from River Gomti, Lucknow and Pinky Kaur *et al*. are reported the similar finding in fish species *Channa punctatus* were obtained from Bhopal, M.P. while the incidence of helminth parasites was observed as minimum in month of August. Similarly, the minimum prevalence is also found in August by M.S. Mofasshalin *et al*. [9] in fish species *C reba* in Bangladesh. However, the maximum percent intensity of helminth parasites was recorded in the month of August. The maximum percent density was recorded in the month of May. Md. Redwanur Rahman *et al*. is also reported the similar finding in fish species *C. idellus* in Bangladesh.

The minimum percent intensity of helminth parasites was recorded in months of Feb, June, July, and November. Similarly, the minimum intensity is also found in November by M.S. Mofasshalin *et al.* [9] in fish species *C reba* in Bangladesh. The minimum percent abundance was recorded in month of November. Similarly, the minimum abundance is also found in November by M.S. Mofasshalin *et al.* [9] in fish species *C reba* in Bangladesh.

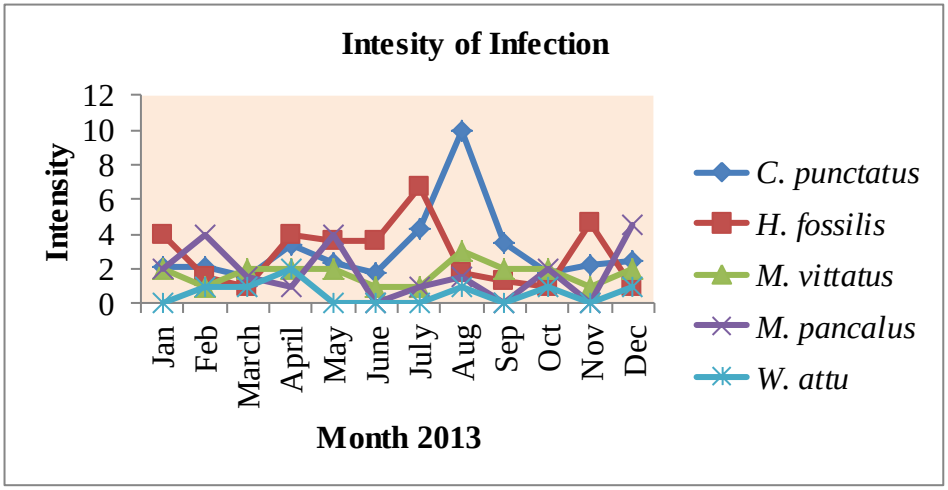

Figure 3: Monthly variation in the Intensity of helminth parasites

Out of 120 specimens of *Mastacembalus pancalus* examined, 14 were found infected with parasites **(Table 1, Figure 1)**. The maximum prevalence of infection was recorded 20.0% in the months of April, August and December while minimum prevalence of infection was recorded 8.33% in the months of May and October and no any parasitic infection in the months of June, September and November (**Figure 2)**. The maximum percent intensity and abundance was recorded 4.5% and 0.90% in the month of December and the minimum percent intensity of infection was observed 0.1% in the months of April and July (**Figure 3, Figure 4)**. The investigation revealed that the minimum percent of abundance of infection was recorded as 0.17% in the months of July and October. The maximum prevalence of infection was recorded in the months of April, August and December. Similar reports were also observed by Nadia Maika *et al.*[10] in August and December month in fish species of family Bagridae and similarly, the maximum prevalence of infection was recorded in month of April by H.puinyabati *at el.*[11]. The minimum prevalence of infection was recorded in the months of May and October. Similarly, the minimum prevalence of infection was recorded in month of May by M.S. Mofasshalin *et al.* [10] in fish species *L. bata* in Bangladesh and similarly, the minimum prevalence of infection was recorded in month of October by H.puinyabati *at el.*[11] in fish species *A. testudineus*. The maximum percent intensity and density were recorded in month of December. Similarly, the maximum percent intensity of infection was recorded in month of December by Md. Redwanur Rahman *et al*. in fish species *C.carpio var.specularis* in Bangladesh and similarly, the maximum percent of abundance was recorded in month of December by Nadia Maika *et al.*[10] and same result observed Rumeet Kaur *et al.*[12] The minimum percent intensity of infection was observed in the months of April and July. The miniimum percent of intensity was recorded in month of April by Rumeet Kaur *et al*. and the minimum intensity was recorded in July month by M.S. Mofasshalin *et al.*[10] in fish species *L. bata* in Bangladesh. The investigation revealed that the minimum percent of abundance of infection was recorded in the months of July and October. Similarly, the minimum density was recorded in July month by M.S. Mofasshalin *et al.*[10] in fish species *L. bata* in Bangladesh and similarly, the minimum percent abundance of infection was recorded in month of October by Md. Redwanur Rahman *et al*. in fish species *C.carpio var.communis* in Bangladesh.

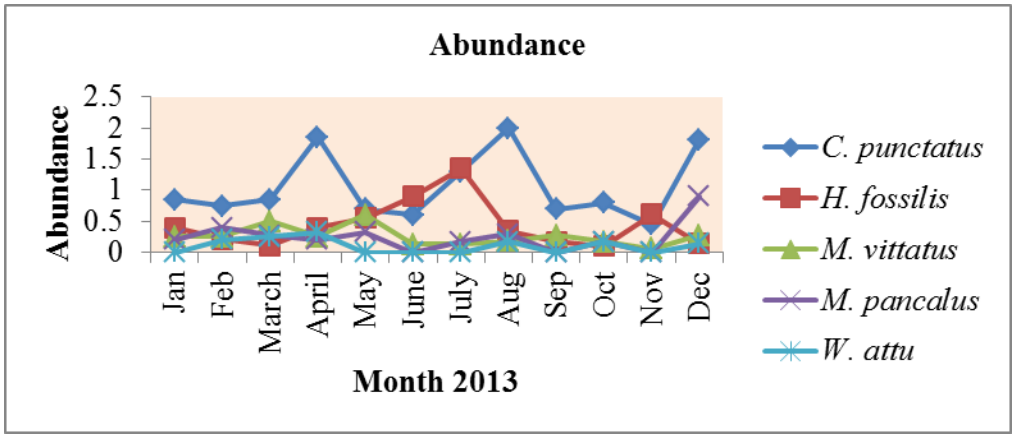

Figure 4: Monthly variation in the Abundance of helminth parasites

Out of 70 specimens of *Wallago attu* examined, 7 were found infected with helminth parasites **(Table 1)**. The observations revealed that the maximum incidence of infection was recorded 25% in the month of March while minimum incidence of infection was recorded 14.28% in the month of December and no found any parasitic infection in the months of January, May, June, July, September and November **(Figure 2)**. The maximum percent of intensity and abundance 2.0% and 0.33% in the month of April and the minimum intensity of infection was recorded 1.0% in the months of Feb, March, August, October and December **(Figure 3, Figure 4)**. The minimum abundance of infection was recorded 0.14% in the month of December **(Figure 4)**. The observations revealed that the maximum incidence of infection was recorded in the month of March. Similarly, the maximum percent incidence of infection was recorded in month of March by Rumeet Kaur *et al*.[12] in fish species *C. punctatus* in Bhopal The minimum incidence of infection was recorded in the month of December. The maximum percent of intensity and abundance in the month of April and the minimum intensity of infection was recorded in the months of Feb, March, August, October and December. The minimum abundance of infection was recorded in the month of December.

CONCLUSION

The one year survey has shown that freshwater fishes from the River Gomti in Lucknow harbor a wide range of parasites especially the helminth parasites. The study has established that the *Channa punctatus* fish is one of the most heavily infected fish species as compare to *Heteropneustes fossilis*, *Mystus vittatus*, *Mastacembalus pancalus* and *Wallago attu*. This study thus highlights on the parasitic infection of freshwater fishes in monthly wise. We concluded that the freshwater fishes harbour a wide range of helminth parasites especially trematode and acanthocephala. Out of a total of 793 fish examined 163 were found to be infected with helminth parasites. The overall prevalence of helminth parasites was found to be 20.5%. We concluded that the maximum infection of helminth parasites was found in the month of December in *Channa punctatus* and in the *Heteropneustes fossilis*, *Mystus vittatus*, *Mastacembalus pancalus, Wallago attu*, the maximum infection of helminth parasites was found in the month of June, May, April, March respectively. Due to the occurrence of the parasites, the fish suffering from parasitic infection or disease result into fish growth is retarded and physiological activities of the fishes are hindered which cause severe damage to fisheries industry. For successful inhibition and removal of such infections, it is very important to achieve early and correct diagnosis of the parasites.

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Corresponding Author: **Suman Mishra**
Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, VidyaVihar, Raebareli Road, Lucknow-226025 (U.P.)

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A Comparative Study of Helminths Parasites in Catfishes

Anita Singh[1], Suman Mishra[2]

Department of Applied Animal Sciences, BabasahebBhimraoAmbedkar University, VidyaVihar, Raebareli Road, Lucknow-226025 (U.P.)

Abstract: ***Fish is one of the best sources of proteins for human beings, so it is important that they should be healthy and free from infections. Fishes are often found infected with a parasites; Trematoda, Nematoda, Cestoda, and Acanthocephala; causing infectious diseases. Among different fish diseases, Gyrodactylussalaris, Ichthyophthiriusmultifiliis, flukes, are most prevalent in fish population that causes great mortality. In case of severe infection by parasites, fish development is hindered causing emaciation of affected fish. The major parasitic groups found in freshwater fishes are trematodes, cestodes, acanthocephalans and nematodes. In the present study, the prevalence of helminth parasites has been observed two freshwater fishesChanna punctatus (Bloch) and Wallago attu. Live fresh specimens of Channa punctatus (Bloch) and in Wallago attu of all sizes, weight and sex were collected from river gomti, Lucknow and transported from the sampling area to the laboratory in aerated container in dechlorinated water. A total of 180 fishes were examined for the presence of helminth parasites in which a total no. of parasites found in Channa punctatus was 100 and in Wallago attu was 80. In case of Channa punctatus (Bloch) 31% of total collected fishes and in Wallago attu 13.7% of total collected fishes were found to be infected with helminth parasites.***

Keywords: Survey, Helminth parasites, Freshwater fishes, Channa punctatus, Wallago attu.

1. Introduction

Fishes are good source of protein that can bedigested easily. These are important components of ecosystem from ecological, medicinal, nutritional and economical point of view. A majority of freshwater fishes carry heavy infection of parasites (Trematodes, Nematodes, Cestodes and Acanthocephalans) which cause deterioration in the food value of fish and even result in their mortality. Besides these, there are a number of helminth parasites which are transmitted to human beings only through fish (Gupta, 1959). These parasites use the fish for their shelter and food, and destruct every organ resulting in pathogenic effects (Dogiel, 1958). Parasites interfere with the nutrition; metabolism and secretary function of alimentary canal, damage nervous system (Markov, 1961), and even upset the normal reproduction of the host (Faust, 1949). Verma*et al*. (2013) studied the parasitic infection index in edible fish *ie*.,*Catlacatla, Channa punctatus* and *Cirrhinusmrigala*, in extensive survey made at different places in river Yamuna at Agra in the rainy seasons during year 2009 and 2010. Recently, Qadri*et al*. (2013) have made some attempts to explore the fauna of helminth parasites in three commercially important catfishes, *Wallago attu, O. pabda* and *A. seenghala* in regions of Bhopal.*Wallago attu*and *Channa punctatus* both, are common food catfishes. The *Channa punctatus* is very economical with high nutritional value and market demand because of its relatively low cost and high availability; and *Wallago attu* are the easily available in the fish market. Due to their feeding habit, fishes can act as an intermediate or a final host for many helminth parasites. To obtain healthy and quality meat fish, it is essential that the fish should be free from all types of infections like viral, bacterial, and parasitic. The major parasitic groups found in freshwater fishes are trematodes, cestodes, acanthocephalans and nematodes. Since human preferring white meat source is utilizing these fishes, so it is important to study the prevalence of parasites in these fishes.The present study is aimed to investigate the burden and effect of helminth parasites in freshwater catfishes *Wallago attu* and *Channa punctatus* (Bloch) obtained from River Gomti in Lucknow, U.P.

2. Materials and MMethods

1) **Study area:** GomtiRiver, Lucknow.
2) **Study periods:** July, 2012 –December, 2012.
3) **Study organism:** Freshwater fishes (*Channa punctatus and Wallago attu*).
4) **Collection of host fishes:** Live fresh specimens of *Channa punctatus*and *Wallagoattu*of all sizes, weight and sex werecollected from River Gomti in Lucknow. The specimens were brought in the laboratory alive in a small container with water and maintained in glass aquaria.
5) **Methodology:**

a) Total and Standard Length and Weight measurement:

The total and standard length of the fish were measured in centimeter (cm) using a measuring board. Fish were weighed to the nearest gram (g) using weighing balance (Paperna, 1996).

b) Examination for Helminth Parasites:

In the laboratory all samples were examined and processed as per standard protocol. External examination of each fish for the helminths ectoparasites was conducted using hand lens. Each gill was examined individually for the presence of parasite. Fishes were opened up dorso-ventrally and all the internal organs were examined separately. The entire digestive system was removed and placed in a petri dish with saline for further examination.

c) Processing of parasites for identification:

The Trematodes were fixed in hot 10% formalin, cestodesand acanthocephala were fixed in AFA solution following staining by borax carmine. After staining,

parasites were washed with distilled water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in D.P.X. Helminth parasites were identified up to class level on the basis of available taxonomical characters as described by Yamaguti, 1958; 1961; 1963.

d) Formula and statistical Analysis

Prevalence, Abundance and Mean density, Index of infection were determined by following the formula proposed by Margolis et al.(1982).

$$\text{Prevalence} = \frac{\text{Total no. of infected fishes}}{\text{Total no. of fishes host examined}} \times 100$$

$$\text{Abundance} = \frac{\text{Total no. of parasites recovered}}{\text{Total no. of fish hosts examined}}$$

$$\text{Mean density} = \frac{\text{Total no.of parasites recovered}}{\text{Total no.of infected host examined}}$$

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

Statistical analysis (Chi-squared test) was done by SPSS 16.0 version software.

3. Results and Discussion

The results obtained in the present study are depicted in table 1 to 7 and figure 1to 7. A total no. of 180 specimens of the two species of freshwater catfishes*Channa punctatus*and *Wallago attu*were examined, for the presence of helminths parasites. Out of a total of 180 fishes, 31(31%)*Channa punctatus* and 11 (13.75%)*Wallago attu* were found to be infected with different helminth parasites. **(Figure 1)**

Table 1: Species wise prevalence of helminth parasites

Fish species	No. of fish examined	No. of fish infected	No. of parasites	Prevalence
Channa punctatus	100	31	60	31
Wallago attu	80	11	18	13.75
Total	180	42	78	

1: Prevalence of parasites in Catfishes, *Wallago attu and Channa punctatus*

To observe parasitic infection in two edible fish species viz., *C. punctatus*and *Wallago attu* were collected from River Gomti, Lucknow and examined critically. Out of 100 specimens of *C. punctatus* were examined, 31 were found infected with helminths parasites. Whereas 11 out of 80 specimens of *Wallago attu* werefound to be infected. The maximum prevalence (31%) showed by *C. punctatus*while (13.75%) shown by *Wallago attu*.**(Table 1, Fig. 1).** Recently,Verma*et al.*, 2013, also recorded prevalence of helminth parasites in same fish *C. punctatus*they were found to be infected with three species of helminth parasites, the parasites were associated with gill, stomach and intestine of fishes respectively and *Qadri et al*2013, also reported prevalence of helminth parasites and compare with the other fish.

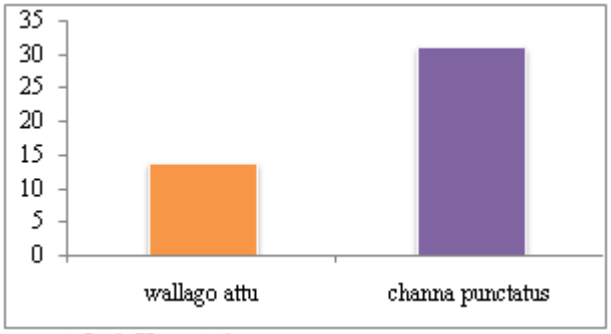

Figure 1: Prevalence of parasite in two catfishes, Wallago attu and Channa punctatus

Relation between host (*Channa punctatus*) length and parasitic infection- It was observed that an increase in the size of fish host was accompanied with an increase inparasitic infection. The larger fishes (11-14.9cm) were more heavily infected than the smaller fishes (7-10.9cm). Figs.2.shows the increase in the Index and mean intensity with the increase in size (length) of the host. Kaur *et al.* observed that the similarly, the large fishes (<15 cm) were more heavily infected than the smaller fishes (>10cm). Arme(2002) further explained the reason for gradual increase in intensity of infection with increase in size (length) and according to the author it may be due to the accumulation of plerocercoids in fish as they grow and it is accepted that the plerocercoids may survive in fish for several years.

Chi square test revealed that there was significant difference in prevalence of helminthes parasites among the three length classes of the examined host.

Table 2: Relation between standard length and different indices of parasitic infection in Channa punctatus.

Length group	No. of fish examined	No. of fish infected	No. of parasites	Prevalence	Abundance	Mean density	Index of infection
7-10.9	29	8	10	27.5	0.34	1.25	2.7
11-14.9	55	18	39	32.7	0.70	2.1	12.7
15-18.9	16	5	11	31.2	0.68	2.2	3.4
Total	100	31	60	91.4	1.72	5.55	18.8

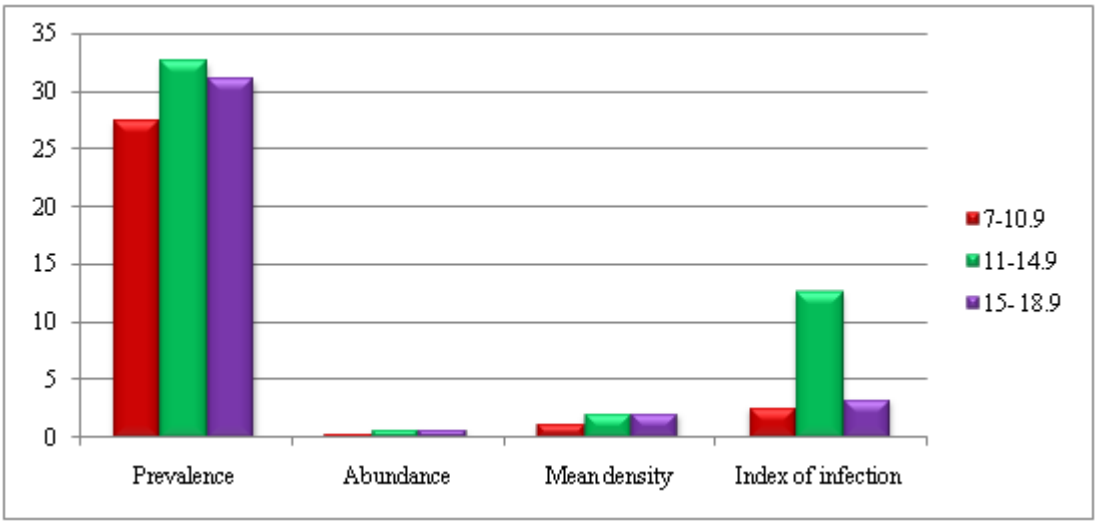

Figure 2: Relation between standard length and different indices of parasitic infection in Channa punctatus.

Relation of host Body weight and parasitic infection: - In relation to studies on host body weight and parasitic infection,it was observed that the percentage of infection increased with increasing weight. The bigger fishes (71 to 90 gm) were more heavily infected than the smaller fishes (20 to 50 gm) **(Table 3, Figure 3**). Parallel observations were stated by Ayanda (2009) and Olurin and Samorin, (2006), According to these workers, the heavier the fish, the greaterthe vulnerability to parasitic infection. This observation could berecognized to the fact that larger fish provides larger surface area for the infection to multiply in numbers than the smaller ones. Another plausible reason could be that the relation may be the result of changes in diet from phytoplankton and zooplankton to insects, larvae, snails, worms and crustaceans forfood as smaller fishes grow into larger ones (Obano*et al*., 2010b). Chi square test revealed that there were significant differences in prevalence of helminthes among the three weight classes of the examined host.

Table 3*:* Relation between body weight and different indices of parasitic infection in Channa punctatus

Body weight	No. of fish examined	No. of fish infected	No. of parasites	Prevalence	Abundance	Mean density	Index of infection
20-50	60	21	42	35	0.7	2	14.7
51-70	35	8	15	22.8	0.42	1.8	3.4
71-90	5	2	3	40	0.6	1.5	1.2
Total	100	31	60	97.8	1.72	5.3	19.3

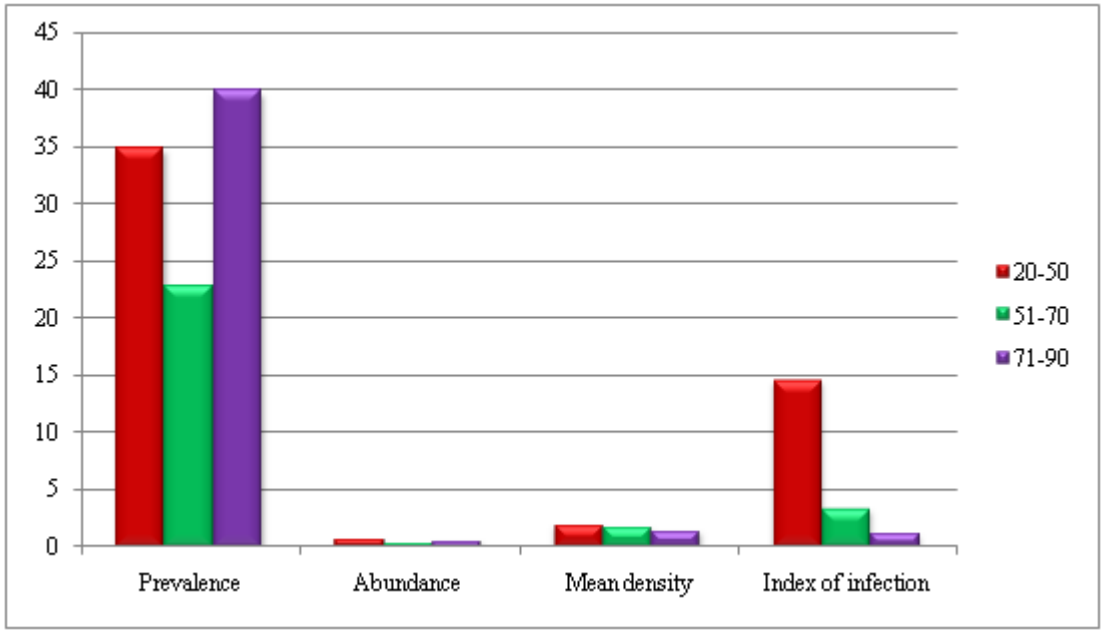

Figure 3: Relation between body weight and different indices of parasitic infection in Channa punctatus

Relation between host Sex and Parasitic infection–The total fishes were (*Channa punctatus*) examined, 18 out of 55 females (32.72%) were found infected with parasites as comparison to 13 out of 45 (28.88 %) males **(Table 4, Figs. 4).** Present study was revealed that female host was observed to be more infected than males. Similar reports were also observed by Thomas (1964) Chandra (1985)Khanum and Parveen (1997)and Rahman and Saidin (2011).These workers concluded that this relation may be due to lower physiological resistance of female fishes as compared to the males. According to Dobson (1961) female are more susceptible to parasite infection during breeding season.

Chi square test revealed that there was non- significant the differences in prevalence of the infection in males and female of the examined host fishes.

Table 4: Relation between body sex and different indices of parasitic infection in Channa punctatus

Sex	No. of fish examined	No. of infected fish	No. of parasites	Prevalence	Abundance	Mean density	Index of infection
Male	45	13	21	28.88	0.46	1.61	6.06
Female	55	18	29	32.72	0.52	1.61	9.49
Total	100	31	50	61.6	0.98	3.22	15.55

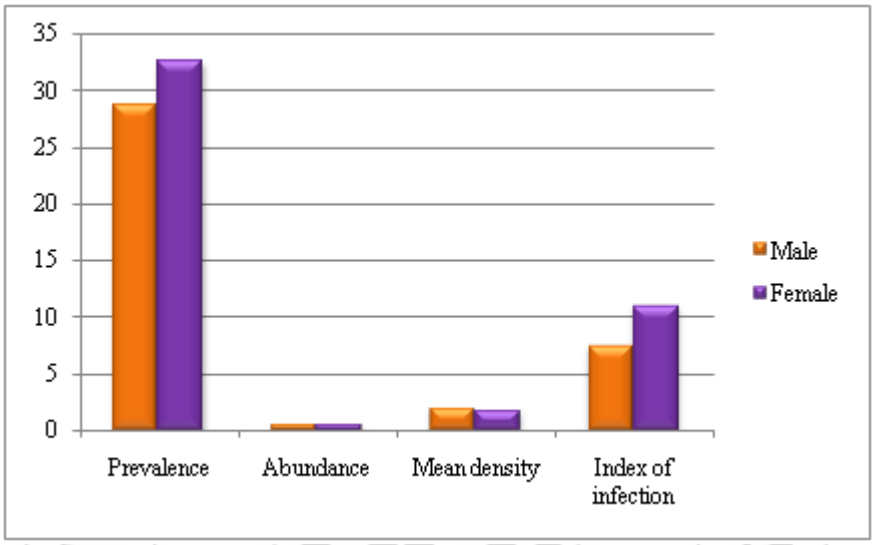

Figure 4: Relation between body sex and different indices of parasitic infection in Channa punctatus

Relation betweenhost (*Wallago attu*) size (length) and parasitic infection:In this study, we observedthe relationship between length of the fishand the percentage of infected fishes. It is also investigated which length of *Wallago attu*more infectedwith parasites than the others. The size of normal and infected fishes was grouped in length classes (17 to 21.9, 22 to 26.9 and 27 to 31.9).(**Table 5,Fig. 5)**showed that the percentage of infection of both the normal and infected fishes in each length group. Itcan be concluded from the given data that the smallest fishes in length group from 17cm to 21.9cm) were relatively less infected than the other length groups and the percentage of infectionincreases with increasing fish length. It was concluded that, larger fishes were heavily parasitized thansmaller ones. Bashirullah(1973)and Dogiel (1961)also reported that the degree of parasitism was obviously related to the food habit and age ofthe fishes.

Chi square test revealed that there was no significant difference in prevalence of helminths among the three length classes of the examined host.

Table 5: Relation between standard length and different indices of parasitic infection in Wallago attu.

Length group	No. of fish examined	No. of fish infected	No. of parasites	Prevalence	Abundance	Mean density	Index of infection
17-21.9	24	2	5	8.3	0.20	2.5	.41
22-26.9	40	7	9	17.5	0.22	1.2	1.5
27-31.9	16	2	4	12.5	0.25	2	0.5
Total	80	11	18	38.3	0.67	5.7	2.41

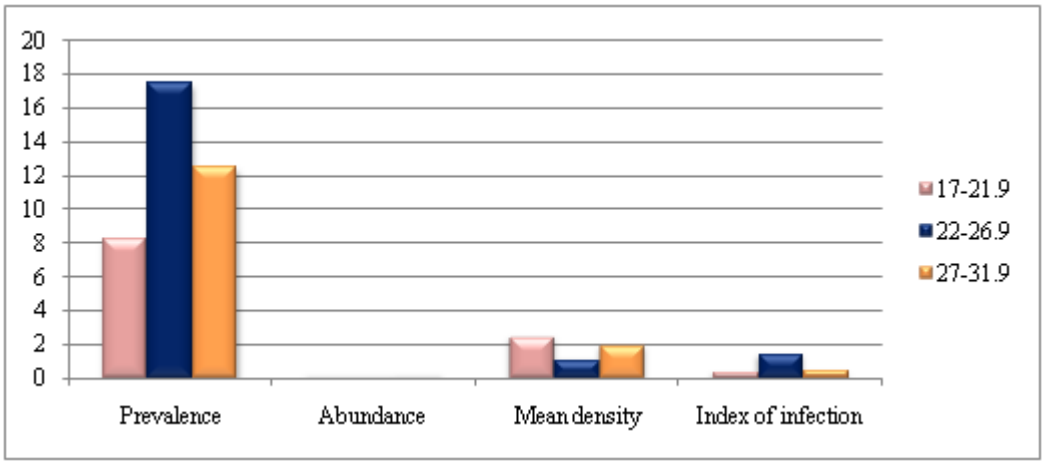

Figure 5: Relation between standard length and different indices of parasitic infection in Wallago attu.

Relation between host (*Wallago attu*) Body weight and parasites–We observed that the bigger fishes (151 to 210gm) were more heavily infected than the smaller fishes (30 to 90gm). Table1 and Figure 6: shows prevalence, abundance, mean density of parasitic infection and index of infection according to body weight of *Wallago attu.* Highest Prevalence (30%) was observed in the body weight range of 151 to - 210gm with Abundance of 0.7, Density of infection of 2.3 and Index of infection of 2.1. There was an observed increase in Incidence of infection with increase in body weight.Chi square test revealed that there were significant($P<0.05$) differences in prevalence of helminthes among the three weight classes of the examined host.

Table 6: Relation between body weight and different indices of parasitic infection in Wallago attu .

Body weight	No. of fish examined	No. of fish infected	No. of parasites	Prevalence	Abundance	Mean density	Index of infection
30-90	55	4	6	7.2	0.1	1.5	0.43
101-160	15	4	5	26.6	0.3	1.2	1.3
161-220	10	3	7	30	0.7	2.3	2.1
Total	80	11	18	63.8	1.1	5	3.83

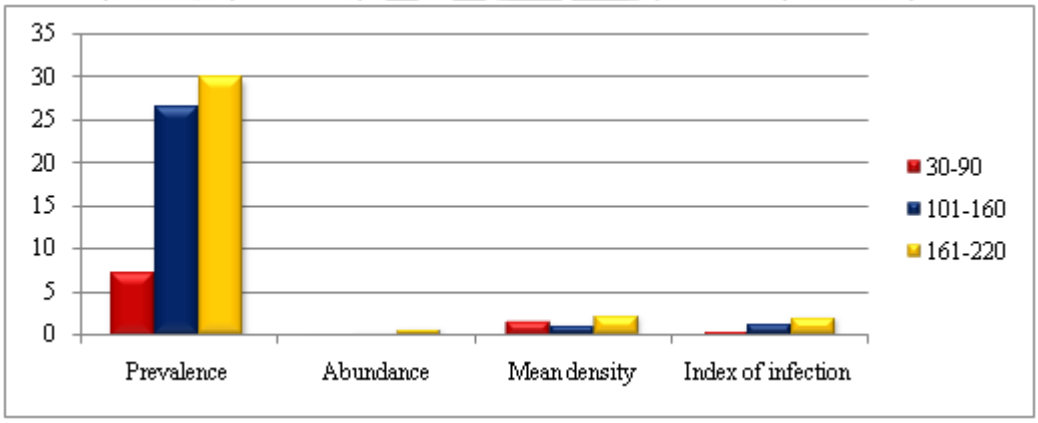

Figure 6: Relation between body weight and different indices of parasitic infection in Wallago attu

Relation to Sex of host and parasitic infection:-Out of the total fishes examined, 5 were infected out of 35 female and 6 were infected out of 45 males. The Prevalence of the parasitic infection (%) in the female and male was 14.2 and 13.3 respectively during this period. The abundance, density and index of infection in female was 0.2, 1.4 and 1 and in male .24, 1.8 and1.46 respectively (**Table 7, Fig, 7**).Chi square test revealed that there was no significant difference in prevalence of the infected males and female of the examined host fishes.

Table 8: Relation between sex and different indices of parasitic infection in Wallago attu

Sex	No. of fish examined	No. of infected fish	No. of parasites	Prevalence	Abundance	Mean density	Index of infection
Male	45	6	11	13.3	.24	1.8	1.46
Female	35	5	7	14.2	0.2	1.4	1
Total	80	11	18	27.5	0.44	3.2	2.46

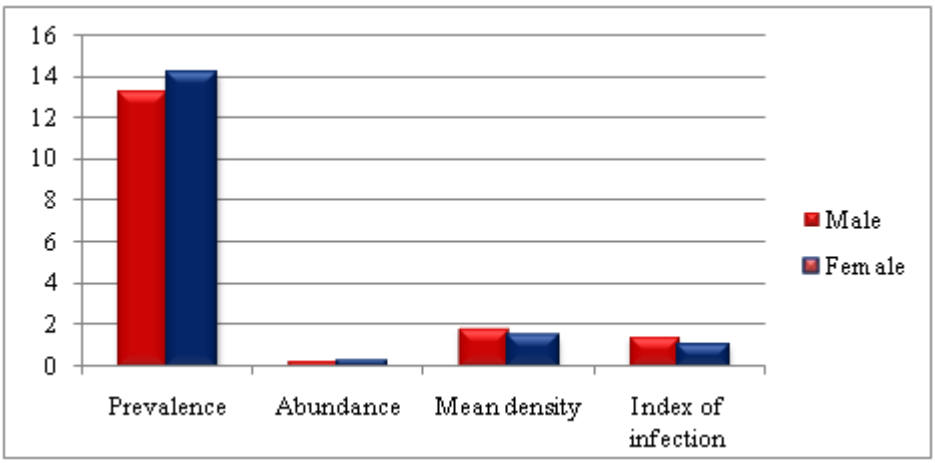

Figure 7: Relation between sex and different indices of parasitic infection in Wallago attu

4. Conclusion

The present study has shown that two catfishes from the River gomti harbor a wide range of parasites especiallythe helminth parasites. The study has established that the *Channa punctatus* fish is one of the most heavily infected fish species as compare to *Wallago attu*. This study thus highlights on the parasitic infection according to the length, weight andsex. We concluded thatthe freshwater fishes harbour a wide range of helminth parasites especially trematodes and acanthocephala. Out of a total of 180 fish examined 42 were found to be infected with helminth parasites. The overall prevalence of parasites was found to be 23.3. The maximum infection of helminth parasites was found in *Channa punctatus* in comparison *Wallago attu*.The maximum infection of helminth parasites was found in the fishes weighing 71-90g and in the fish length ranging from 11-14.9cm in case of *channa punctatus*. The maximum infection of helminth parasites was found in the fishes weighing 161-220g and in the fish length ranging from 22-26.9cm in case of *Wallago attu.*

5. Future Scope

Fishes arean important source of protein that consumed by human being. Butmajority of freshwater fishes carry heavy infection of parasites which cause deterioration in the food value of fish and even result in their mortality.The fishery industry also plays an important role in the Indianeconomy as they provide employment opportunities. However, due to the occurrence of these parasites, the physiological activities of the infected fishes are hindered and their growth is retarded. Beside this, there is always the possibility of their transfer to the human beings by consumption of infected fishes. Therefore, it is important to carry out the prevalence of helminth parasites in fishes.

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Author Profile

Anita Singh is pursuing PhD from Department of Applied Animal Sciences at Babasaheb Bhimrao Ambedkar University (A central University) Lucknow under the supervision of Dr. Suman Mishra. She has participated and presented her work in several International and National Conferences. Her area of Research Interest includes Fishries and Parasitology

Dr. Suman Mishra is working as Assistant Professor in Department of Applied Animal Sciences at Babasaheb Bhimrao Ambedkar University (A central University) Lucknow. She has a decade long and bright teaching and research experience. She is having one International and one national patent to her credit. She has published several Research paper in reputed International and national journals and 6 Books. Dr. Mishra has been honoured with several prestigious awards. She is also member of editorial team of the Indian journal of Helminthology. She has participated the InspiredTeacher In - Residence Programme of the President of India at Rashtrapatibhawan in New Delhi. Her area of research interest includes Parasitology