

Studies on Nematode Helmenth Parasitic Diversity in Freshwater Fishes order Perciformes at Lower Manair Dam Karimnagar Dt. Telangana State

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ABSTRACT: Two nematode parasitic species *Rhabdochona sp.* and *Philomitra abdominalis* was found in Perciformes fish species at Lower Manair Dam. The seasonal and monthly parasitic diversity was noticed from December 2014 to November 2015 in the presence of a pre-anal sucker, well developed caudal alae and gubernaculum, 3 lips and 3 teeth but differs in the undivided oesophagus, simple, equal spicules and the presence of distinct caudal pores, The main characteristics of this nematode are mouth with large simple triangular lateral lips armed with one tooth or more. Body movement is at anterior region sluggish and 1/3rd of the body was coiled, at posterior region active and penetrates host muscle from coelomic cavity to out of the body with small spicules. The oviparous species lodged with embryonated eggs. Shannon-Weiner diversity, species richness (S), $H_{max} = \ln(S)$ Maximum diversity possible were studied during this period.

KEYWORDS: Oviparous, Coelomic cavity, Spicules, parasite diversity, Shannon-Weiner diversity, species richness (S), $H_{max} = \ln(S)$ Maximum diversity possible.

I. INTRODUCTION

The Indian region has a rich diverse fauna and the parasites of the region are quite fascinating. Nearly 50 years ago, Baylis (1936-1939) published in the series of "Fauna of British India", a comprehensive account of the helmenth parasites of the region known at that time. Since then an enormous amount of literature has been published on this group and in the last 3 decades there has been an avalanche of new species and genera emanating from this region. Most of these descriptions have been published in a wide range of journals, some of which are not accessible.

Nematodes belong to the most frequent and the most important parasites of fishes in the freshwater, brackish-water and marine environments throughout the world. The present knowledge of these parasites remains still incomplete, especially as to their biology and ecology, but also taxonomy, phylogeny, zoogeography, and the like as well as in studies of their geographical distribution and diversity in different parts of the world and those of their biology. Opportunities for more detailed studies of fish nematodes have recently greatly improved with the use of some new methods, in particular SEM and DNA studies. There is a need to create a new classification system of these parasites reflecting phylogenetic relationships; a prerequisite for this is taxonomic revisions of different groups based on detailed studies of individual species, including mainly their morphology, biology and genetics. Further progress should concern studies on various aspects of biology, ecology and host-parasite relationships, because these data may have practical implications.

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Nematodes infect freshwater, marine and brackish water fish species, pathology normally occurs within the intestines but can affect all organs and substantial damage to the host can occur. Some nematodes cause high levels of fish mortality in wild fish populations. Nematodes can infect fish as adults but larval stages of nematodes infecting piscivorous birds, mammals or reptiles, or less frequently predatory fish, can also infect fish species. Ingestion of uncooked infected fish meat poses a zoonotic threat to humans. Following ingestion by humans the larvae are activated by high temperatures, acidic pH and pepsinogen within the stomach. Nematodes also cause an economic threat to the market value of fish, through consumer attitudes towards the presence of these parasites within food products. Infected fillets are rejected and can increase production costs. Disease is mainly found within wild fish populations as captive fish are fed non-infected feed.

Nematodes cause an array of different pathogenicity within fish and can cause damage to the Gastro Intestinal tract, swim bladder, gonads, internal organs (especially the liver), gills, eye and skin. Mechanical damage to the mucosa and submucosa by nematode migration and proteolytic damage from nematode enzymes are common. Larger parasites can cause deformation of organs and body shape, mesenteric and visceral adhesions, granulomas, haemorrhage, deep nodules within the stomach wall and general inflammation. Some nematodes can cause blockages to the Gastro Intestinal tract.

Swim bladder damage can be caused by species such as *Cystidicola* and *Anguillicola*, and epithelial hyperplasia, hyperaemia and necrotic areas around migrating larvae can be seen in the swim bladder wall, which can give rise to secondary bacterial infections. Continual damage by migrating larvae cause fibrosis and thickening of the swim bladder. Swelling of the scale sacs and inflammation and haemorrhaging under the scales can be seen with nematodes such as *Philometra* and *Philometroides* which are known to infect the skin, fins and gill epithelium.

II. RELATED WORK

The genus *Rhabdochona* Railliet 1916, is worldwide in distribution and is found mostly in fresh water fishes [1], [3], [4], [5], [10], [11], [13], [22], [23], [24], [25], [26], [27], [28], [29], [31], [33], [36], [39]. A large number of species, more than 32 are known from India (36). Recently, *Rhabdochona* sp. are reported from Thailand (9) and Arizona (19). A few species have been described from fishes of Pakistan [2], [15], [16], [17], [21], [32], [34], [40], including one species from a marine fish. Species of *Rhabdochona* have also been reported from Islamabad [20] and Jammu and Kashmir [30]. Here a new species, *Rhabdochona kharani* is described. Present description is based on both male and female specimens from a fish *Labeo gedrosicus* from Garruk, District Kharan, Balochistan. This is the first record of genus *Rhabdochona* from Balochistan.

František Moravec et al (František Moravec 2010) [14], Specimens of three little-known species of *Rhabdochona* (Nematoda: Rhabdochonidae) were collected during occasional examinations of some freshwater fishes in India: *R. (Rhabdochona) hellichi turkestanica* (Skryabin, 1917) [35] in *Schizothorax* sp. (Cyprinidae, Cypriniformes) from the Lodhomakhola and Rangit Rivers, West Bengal and Sikkim, respectively; *R. (R.) hospeti* Thapar, 1950 [38], in *Tor* sp. (Cyprinidae) from the Rangit River; and *R. (Globochona) mazeedi* Prasad et Sahay, 1965 in *Clupisoma garua* (Hamilton) (Schilbeidae, Siluriformes) from the Farakka Dam Lake, West Bengal. Their detailed light and electron microscopical studies revealed some taxonomically important, previously not observed features and made possible their redescription. Fourth-stage larvae of *R. hospeti* are described for the first time. *R. kashmirensis* Thapar, 1950, *R. schizothoracis* Siddiqi et Khattak, 1984 [34], is proposed as a subspecies, differing from the nominotypical subspecies *R. hellichi hellichi* (Šrámek, 1901) [37], mainly in the shape of the distal end of the left spicule, molecular data and geographical distribution. *Rhabdochona moraveci* Katoch et Kalia, 1991 (a homonym to *R. moraveci* Duggal et Kaur, 1987) is renamed *R. indica* nom. n. The following six species are considered new junior synonyms of *R. hospeti*: *Comephronema* [sic] *mackiewiczzi* Malhotra et Rautela, 1984, *Rhabdochona moraveci* Duggal et Kaur, 1987, *R. bifidum* Kakar et Bilqees, 2007, *R. uvaginus* Kakar et Bilqees, 2007, *R. bolani* Kakar, Bilqees et Ahmad, 2008 and *R. cephalodiverticula* Kakar, Bilqees et Ahmad, 2008.

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III. MATERIALS AND METHODS

On November 2014 -2015 January, 408 freshwater fishes of order perciformes are Gobiidae, Mastacembelidae, Anabantidae, Nandidae, Cichlidae and Ambassidae were collected from Lower Manair Dam. Fishes were caught with different craft and gear. The samples were taken to the laboratory of Zoology, Government Degree College, Jammikunta. Dissection and examination for parasites were performed under dissecting microscope, followed standard methods [18], [19]. The dissection procedure include, opening of the body cavity on the ventral midline of the fish from its anus to head and removing the entire digestive tract, stomach, viscera and swim-bladder. All the organs were placed in Petri-dishes separately, containing physiological saline. The internal organs were opened lengthwise and each parasite was isolated carefully. 193 nematodes (168 females and 25 males) were recovered from 408 fishes, seven is the maximum number of parasites were observed in a fish. Parasites were fixed in 70% alcohol and glycerol (1:1) (7). To expose key anatomical features and outer integument of nematodes were observed, lactophenol was used (66). Parasites were mounted between slide glass and cover glass using 2 to 3 drops of glycerol. Nail polish was pasted around the sides of cover slips to hold them firmly. Diagrams were made with the help of camera lucida and measurements are given length by width in millimeters. Only nematodes specimens were obtained, fixed in 4% formalin, examined under microscope after mounting in glycerin or water. The specimens then transferred to water adding 70% ethyl alcohol which was examined. Some live specimens and exoskeleton were observed with camera and video attached microscopes. Photographs and live video graph were taken using digital microscope (Digivision) (DN-300M).

(1) Statistical analysis

The mathematical expression of Shannon - Wiener Diversity Index is

Shannon-Wiener Index denoted by $H = -\text{SUM} [(p_i) \times \ln(p_i)]$

SUM = summation

p_i = proportion of total sample represented by species i

Divide no. of individuals of species i by total number of samples

S = number of species, = species richness

$H_{\max} = \ln(S)$ Maximum diversity possible

E = Evenness = H/H_{\max}

IV. EXPERIMENTAL RESULTS AND DISCUSSIONS

(a) Genus *Rhabdochona* Railliet, 1916

Description

Rhabdochona (*Rhabdochona*) sp. n. (Figs. 1-3)

Host: Gobid fishes

Location: Stomach, Intestine, Liver and Gonads

Locality: Lower Manair Dam waters

Number of host examined: 408

Number of host infected: 125

Number of specimens recovered: 12 male & 168 female

Date of collection: 2014-2015

Generic Diagnosis [36]

Rhabdochonidae : Head and body bear, Mouth with two lips, Prostome funnel-shaped, supported by short longitudinal ridges projecting anteriorly as sharp teeth, mesostome long, narrow and of uniform diameter. Oesophagus of moderate

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length, composed of two distinct parts. Male: Tail conical, pointed, curved ventrad; caudal alae narrow, numerous, simple preanal papillae and three to six pairs of postanal papillae; spicules unequal and dissimilar; gubernaculum absent. Female: Tail straight and elongated; Vulva in middle region of body; uterine branches opposed. Oviparous, eggs with or without filaments. Parasite of the body cavity or intestine of fishes [36]

Type of species and observations to the species

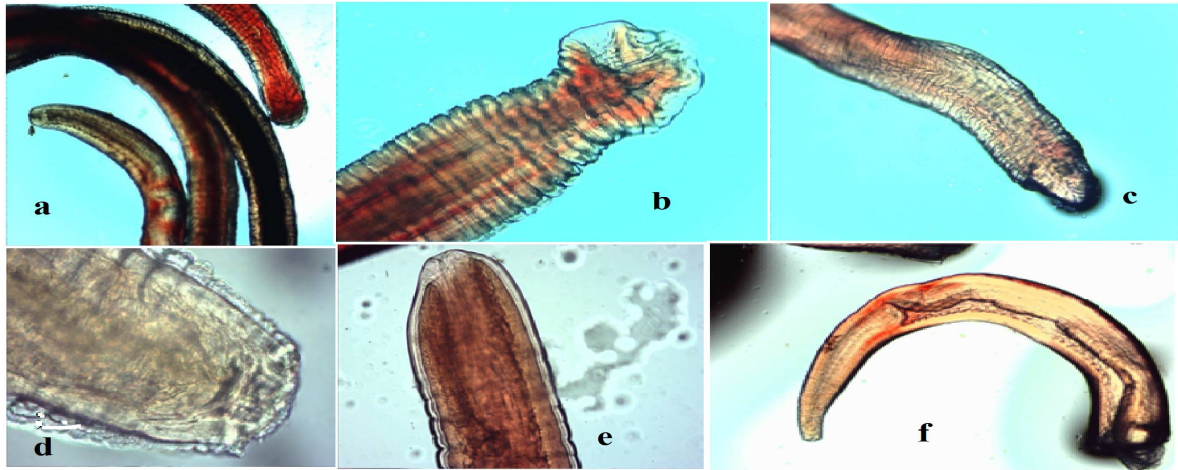
1. Eggs non-filamented
2. Female tail round, with a stumpy projection having four pointed spines
3. Vulva preequatorial
4. Tail pointed
5. Spicules spiny or spine-like projections
6. Four pairs of caudal papillae, caudal alae narrow

Description is based on mature male and female worms. A total of 3 males and 8 females were recovered from stomach. Microscopic study was shown that the body smooth, long, delicate, blunder anteriorly and narrow posteriorly. Anterior extremity rounded, posterior pointed, widest at the post-esophageal region. Prostome with three teeth, mesostome almost triangular shape. Excretory pore is pre-equatorial. Males are smaller than females. Spicules are equal and pointed, wider at the distal end and pointed at the proximal end. Caudal papillae four pairs including a pairs postanal. In female there is a small intestinal cecum, vulva is pre-equatorial with large prominent, almost triangular anterior lip, vagina muscular, long, enclosed in a large glandular structure forming a voluminous, bulbous, vaginal complex. This structure is prominent even in the immature female specimens. Uterus is long, extending anteriorly to the base of glandular esophagus and posteriorly terminates far anterior to the anal opening. Tail is relatively short, pointed. Eggs are numerous, large, oval, smooth and non filamented.

Male: Body length 2.5 – 4.2cm, width 0.02 – 0.03, prostome 0.021 – 0.022 wide, mesostom 0.022 – 0.025 in length, 0.012 – 0.013 wide, anteriorly, muscular esophagus 0.020– 0.061 x 0.013 – 0.015, glandular esophagus 0.172 – 0.175 x 0.017 – 0.019, excretory pore at a distance of 2.9 to 3.2 from the anterior extremity, spicules 0.1 – 0.12 mm. Tail 0.30 – 0.32 in length.

Female: Body length 4.4 – 4.8 cm, width 0.025 – 0.037, prostome 0.022 – 0.024 wide, mesostome 0.023 – 0.025 in length, 0.022– 0.023 wide anteriorly, muscular esophagus 0.095 – 0.098 in length, 0.017 – 0.020 in width, glandular esophagus 0.26 – 0.29 in length, 0.028 – 0.027 in width, intestinal cecum approximately 0.026 in length, vulva at a distance of 5.8 – 7.1 from the anterior end. Anterior lip of vulva 0.09 – 0.072 x 0.039 – 0.039 in mature female. Vaginal complex 0.21 – 0.24 x 0.065 – 0.068, vagina 0.21 – 0.19 x 0.032 - 0.035. Eggs 0.042 – 0.057 x 0.020 – 0.021. Tail 0.28 – 0.31 in length.

Fig: 1. Microscopic photographs of *Rhabdochona sp.n.*



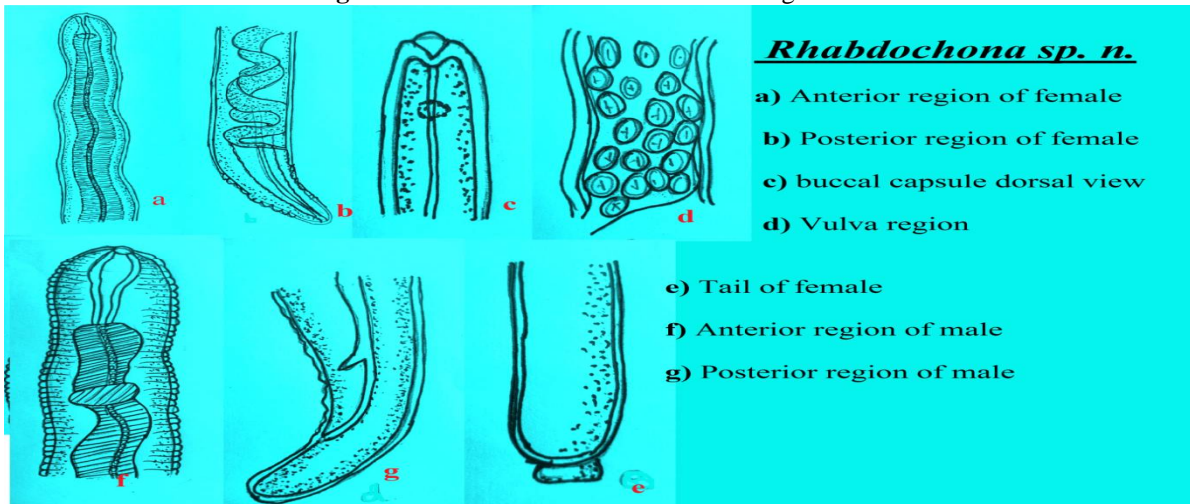
a) *Rhabdochona sp. n.* **b)** *Rhabdochona* female head region
c & d) *Rhabdochona* female tail region. **e)** *Rhabdochona* male anterior region
f) *Rhabdochona* posterior end of lateral view

Fig: 2. Microscopic photographs of Uterus and Embryo sac with fertilized eggs



Uterus with numerous non-filamented eggs and embryonic developmental stages

Fig: 3. *Rhabdochona* male and female enlarged areas



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Deepak et al 2013 reported to facilitate the identification of the species of *Rhabdochona* (*Filochona*) *Saidov*, 1953, parasitizing in carp fishes, a bipartite key for 12 species of the subgenus is provided. A large number of worms, *Rhabdochona* (*Filochona*) *hellichi turkestanica* (*Skrjabin*, 1917) *Moravec et al.* 2010, were recovered from *Schizothorax plagiostomus* *Heckel* in river Ravi at Chamba, a district of Himachal Pradesh (India). They observed during the study on the morphology and taxonomy of three spp. of *Rhabdochona* parasitizing fishes in India, that the European and Asian representatives of *Rhabdochona* (*Filochona*) *hellichi* (*Sramek*, 1901) *Chitwood*, 1933 (*Chitwood*, 1933), exhibit some small morphological differences, especially in the shape of distal end of the left spicule and considered it to be within the intraspecific variability of the species. Therefore, *R. (F.) hellichi turkestanica* (*Skrjabin*, 1917) was differentiated from the nominotypical subspecies *R. (F.) hellichi hellichi* (*Sramek*, 1901) mainly in having the ventral process of the distal tip of the left spicule distinctly longer than the dorsal process (versus both processes approximately equally long). This categorization has also been supported by the molecular studies (*Cernotikova*, 2010) of *R. hellichi* specimens from *Barbus barbus* (*Linnaeus*) in Czech Republic and also those from *Schizothorax* in West Bengal and Sikkim (India). Needless to add that *R. (F.) hellichi hellichi* parasitizes barbels (*Barbus* spp.) in Europe and *R. (F.) hellichi turkestanica* found as parasitic mainly in species of *Schizothorax* and some related fish genera in Central and South Asia (*Moravec et al.*, 2012). In this species Body length, width, prostome is wide, mesostome is wide anteriorly, muscular esophagus, glandular esophagus are present. Testinal cecum approximately 0.026 in length, vulva at a distance of 5.8 – 7.1 from the anterior end. Anterior lip of vulva is present in mature female. Vaginal complex and vagina is distinguishable. Embryonated eggs are present.

The classification of the genus *Rhabdochona* *Railliet* 1916, and its subgenera based on morphology of eggs, have been discussed by several workers [7], [8], [9]. Character of eggs has been emphasized for diagnosis of subgenera. *Moravec* divided the genus into three subgenera based on the type of eggs which was accepted by some workers [10]. But, *Moravec* considered that only egg character is not sufficient to divide the genus into three subgenera and other characters must also be included, such as number and arrangements of teeth in the prostome, presence of cervical alae, shape of the female tail tip and shape of deirids. Based on these characters, he raised the number of subgenera into four including *Rhabdochona*, *Globochona*, *Globochonaides* and *Sinonema*. This was accepted by *Bilqees*; *Soota* and *Sarkar* and *Soota*.

Now, *Chabauds'* classification is widely accepted and followed here. As mentioned above, species of the genus *Rhabdochona* reported from Pakistan are *R. (R) magna* *Khan and Yaseen*, 1969, *R. (R) cavasius* *Rehana and Bilqees*, 1973; *R. (R) Chanawensis* *R. (F) charsaddiensis* *Siddiqi and Khattak*, 1984; *R. (F) shizothoracis* *Siddiqi and Khattak*, 1984; *R. (R) megasacculata* *Ghazi and Rahim*, 1999; and *R. (R) rahimi* *Ghazi et al.*, 2003. All these species are from fresh water fishes except *R. (R) parastromaei* described from *Parastromateus niger* of Karachi coast (*Bilqees*, 1979). Description of *R. (R) megasacculata* is based on a single female specimen only.

(b) Genus *Philometra* *Costa*, 1845

Description (Fig: 4-5)

Host: Gobid fishes

Location: stomach, Intestine, Liver and Gonads

Locality: Lower Manair Dam waters

Number of host examined: 408

Number of host infected: 59

Numbers of specimens recovered: Females numerous, Males unknown

Date of collection: 2014-2015

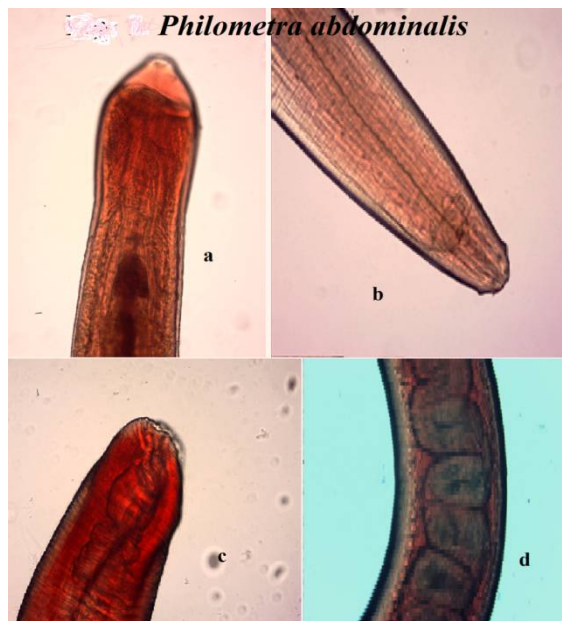
Generic Diagnosis: *Philometrinae*- Female enormously larger than male; body filiform; anterior and posterior extremities round; mouth with or without lips. Head and tail papillae present or absent. Oesophagus cylindrical, short, bulbous at the anterior end; oesophageal gland confined to walls of oesophagus; rudimentary ventriculus present. Male; posterior extremity rounded; cloaca terminal, border by two lips, spicules equal, needle-like; gubernaculum present.

Female: Anus and vulva atrophied; vulva seen at junction of middle and posterior third of body in young worms, Uterus occupying almost entire body, ovaries small, one at each end of body. Parasitic in the body cavity or tissues of the body [36], [41]

***Philometra abdominalis* Nybelin, (1928)**

Description: (after Pandey, Laal, Shah and Dubey, 1982): Body filiform 40-120 x 0.8-1.4, Mouth simple, without lips, oesophagus short, cylindrical. Uterus filled with embryos occupying entire body. Anus and vulva atrophied. *P. abdominalis* produce exudative peritonitis. The heavily infected fish had prolapsed abdomen with several worms attached to upper part of intestine filling abdominal cavity and some protruding out of body.

Fig: 4. Microscopic photographs of *Philometra abdominalis*



a) Anterior end of female b) Posterior end with spicules
c) Anterior nerve ring d) Ovaries

Fig: 5. *Philometra abdominalis* enlarged views

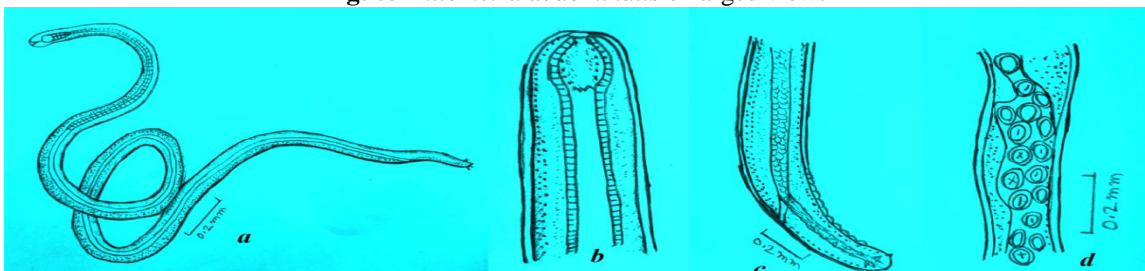


Fig: 2. *Philometra abdominalis*

a) Female entire view
b) Anterior end of female, dorsal view
c) Posterior end of female
d) Vulva region

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Shannon-Wiener Index diversity indices of helmenth parasites is represented in Table-2. The richness of fish species was highest in February 2015 and lowest in October 2015 (Table. 1), the parasite species diversity (H) ranged from 0.17 (August and September-2015) to 0.49 (Jan. 2014) (Fig: 6). the results indicated that minimal diversity index having in the Lower Manair Dam. The results indicated that the maximum diversity possible ln(S) range was 1.79 in all seasons. The parasitic species diversity evenness (E) is 0.10 to 0.27 (Fig: 7). It is clearly indicated that there is an even distribution of the helmenth parasitic diversity in the Lower Manair Dam.

Table: 1. Number of fish samples analyzed and recovered the Nematode Helmenth parasites

Month	No. of samples	Parasitic Males	Parasitic Females	Size of Fish	Weight of Fish
December-14	25	02	12	16.5 ± 0.42	75 ± 0.46
January-2015	28	01	09	17.8 ± 0.22	87 ± 0.39
February-2015	32	04	22	14.0 ± 0.34	69 ± 0.25
March-2015	42	02	19	12.5 ± 0.12	65 ± 0.36
April-2015	34	02	14	15.8 ± 0.21	72 ± 0.24
May-2015	28	03	16	19.6 ± 0.16	96 ± 0.42
June-2015	41	02	12	18.4 ± 0.11	87 ± 0.22
July-2015	29	02	09	18.2 ± 0.31	85 ± 0.25
August-2015	45	01	13	16.2 ± 0.53	75 ± 0.38
September-2015	43	02	15	14.4 ± 0.34	71 ± 0.49
October-2015	38	03	18	15.8 ± 0.28	79 ± 0.34
November-2015	22	01	08	12.7 ± 0.09	68 ± 0.28
	408	25	168		

Table: 2. Fish Population Diversity Index

Population	Dec-14	Jan-15	Feb-15	Mar-15	Apr-15	May-15	Jun-15	Jul-15	Aug-15	Sep-15	Oct-15	Nov-15
H	0.49	0.37	0.28	0.26	0.22	0.17	0.32	0.35	0.17	0.17	0.40	0.35
ln(S)	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79	1.79
Evenness E	0.27	0.21	0.16	0.15	0.12	0.10	0.18	0.20	0.10	0.10	0.22	0.20

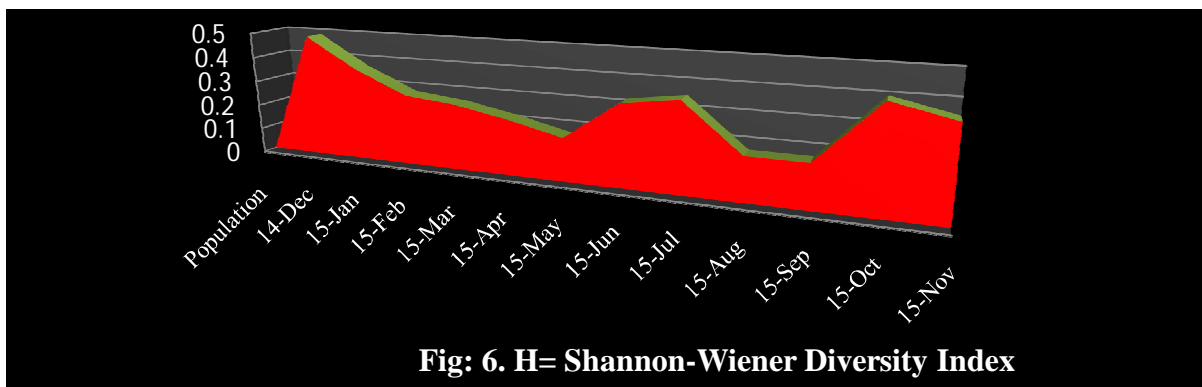
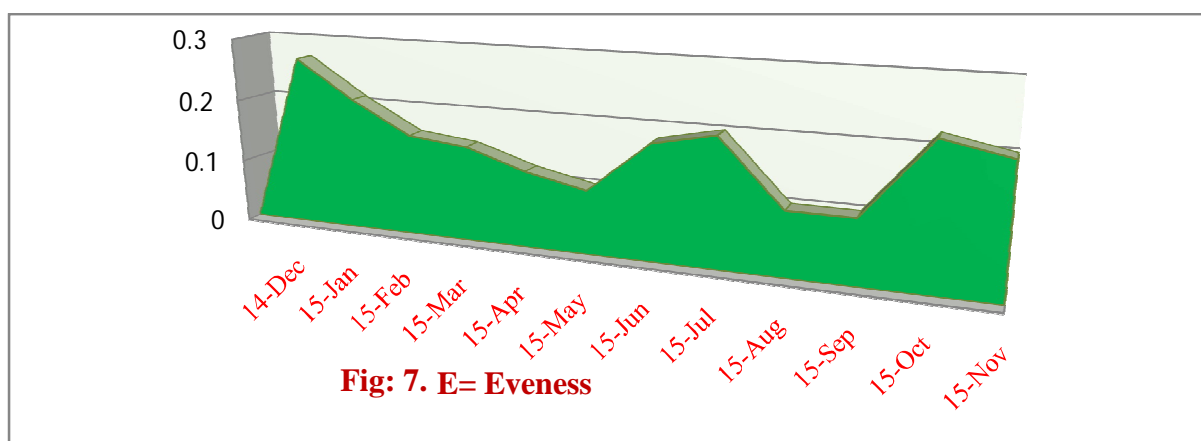


Fig: 6. H= Shannon-Wiener Diversity Index



V. CONCLUSION

The present study describes the intensity of parasitic infestation in different organs of perciformis collected from Lower manair Dam, Karimnagar district, Telangana. The nematode parasites were found in the stomach, liver and gonads of the fish. This is the first report of the genus *Rhabdochona* sp. (n) from Lower manair Dam. The highest peak of parasite abundance and prevalence was recorded in February, while the lowest was recorded in November during the study period. The present specimens are different in having prominent vulva with a large triangular anterior lips and posterior round sensible tail. The cephalic papillae are present and there is a small intestinal cecum of female specimen. One approach to the study of the function, development and nervous systems is to use an organism that is both amenable to genetic analysis and has a simple nervous system that may be completely characterized anatomically. The live parasites are penetrate the peritoneal cavity to out at the time of the fish is dead, because the required oxygen is not sufficient to the live parasites. Live video can play in google network, uploaded by Leela Bommakanti on 17th June 2015. <https://www.youtube.com/watch?v=QqYEqfaFUqU>

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