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Seasonal variation in helminth parasites of snakeheads *Channa punctatus* and *Channa striatus* (Perciformes: Channidae) in Uttar Pradesh, India

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Summary

Snakehead fishes are widely consumed throughout South East Asia, China and India because of their good taste of meat and high nutrient values such as presence of prostaglandins, thromboxane and Omega-6 fatty acid. Parasitic infection constitutes significant economic loss in fish production. The aim of this work was to study the seasonal variation of helminths in snakeheads. In the presented study, a three-year survey has been performed. A total of 1013 individuals of *Channa punctatus* and 247 individuals of *Channa striatus* were examined. A total of 3783 helminths were collected, with an average of 3.02 helminths/fish. 43.50 % individuals of *C. punctatus* and 59.10 % of *C. striatus* were found to be infected with acanthocephalans, trematodes, nematodes and cestodes per year. The prevalence and mean abundance of *Pallisentis* sp. was at its peak in summer. However the prevalence of trematodes, nematodes and cestodes was at peak during autumn. Mean abundance of nematodes was at peak in summer. Interestingly, the males were found more infected as compared to the females and the infection rate in males peaked in summer. In comparison to other weight groups, medium size hosts (21 – 40 g) were found more consistently infected. Thus the results indicate that there are seasonal variations in parasitic helminths infecting *C. punctatus* and *C. striatus* which also depend upon sex and weight. These variations may be attributed to various environmental and biological factors including parasite life cycle and immune level of host.
Keywords: nematode; fish; helminths; parasites; prevalence; snakeheads

Introduction

A large proportion of proteins obtained from animal sources come from fishes. Out of total worldwide protein obtained by animal sources, 25 % alone is contributed by fish and shellfish thus; fish is one of the most valuable sources of protein in food. About 80 % infection of warm water fishes is caused by parasites (Eissa, 2002). Helminths play a key role in internal parasitic infections of fishes which leads to low body weight gain and high mortality rate. Parasitic infection either alone or in conjunction with stress may reduce host weight and reproduction which leads to economic loss (Rohde, 1993). Parasites also upset the normal reproduction of the hosts (Faust, 1940).

The infection of parasites interferes with nutrition, metabolism and secretory function of the alimentary canal, damages nervous system (Markov, 1961) which may also lead to gastrointestinal abrasions and facilitate the invasion by opportunistic microorganisms. Unfavorable environmental conditions contribute to stress which also weakens the immunity and opens the pathway to pathogens (Eissa, 2002). The study of diversity and distribution of helminths

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started in the middle of the 19th century in India and numerous works has been done by Bhalerao, 1937; Gupta, 1984; Soota, 1981; Sood, 1989; Tondon *et al*., 2005; Bhure, 2008, Pandey & Agrawal, 2008; Deshmukh, 2015 in different parts of India.
The distribution of helminths is not only affected by seasons but also by host age, size, diet, abundance of fishes and an independent number of parasites within the fish. Change in climatic conditions is predicted to affect the prevalence of parasites in freshwater and marine ecosystems. A study of Chubb (1977 & 1979) showed the seasonal occurrence of helminths of freshwater fishes from different climatic zones.

Price and Clancy (1983) had also reported that larger bodied fish species harbor more parasitic diversity than small-bodied fish, but according to Sasal *et al*. (1997) there is no association between fish body size and parasite species richness. Available data on factors which potentially control the number of parasite species in freshwater and marine fishes (Sasal *et al*., 1997; Luque & Poulin, 2004) are very little in consistency. Moreover, available studies on the helminth parasitic fauna of snakeheads in relation to seasonal population dynamics, size and sex of the host are very little. The aim of this study was to investigate the seasonal variation of parasitic helminths of freshwater fishes from Uttar Pradesh, India with relation to host sizes and sex.

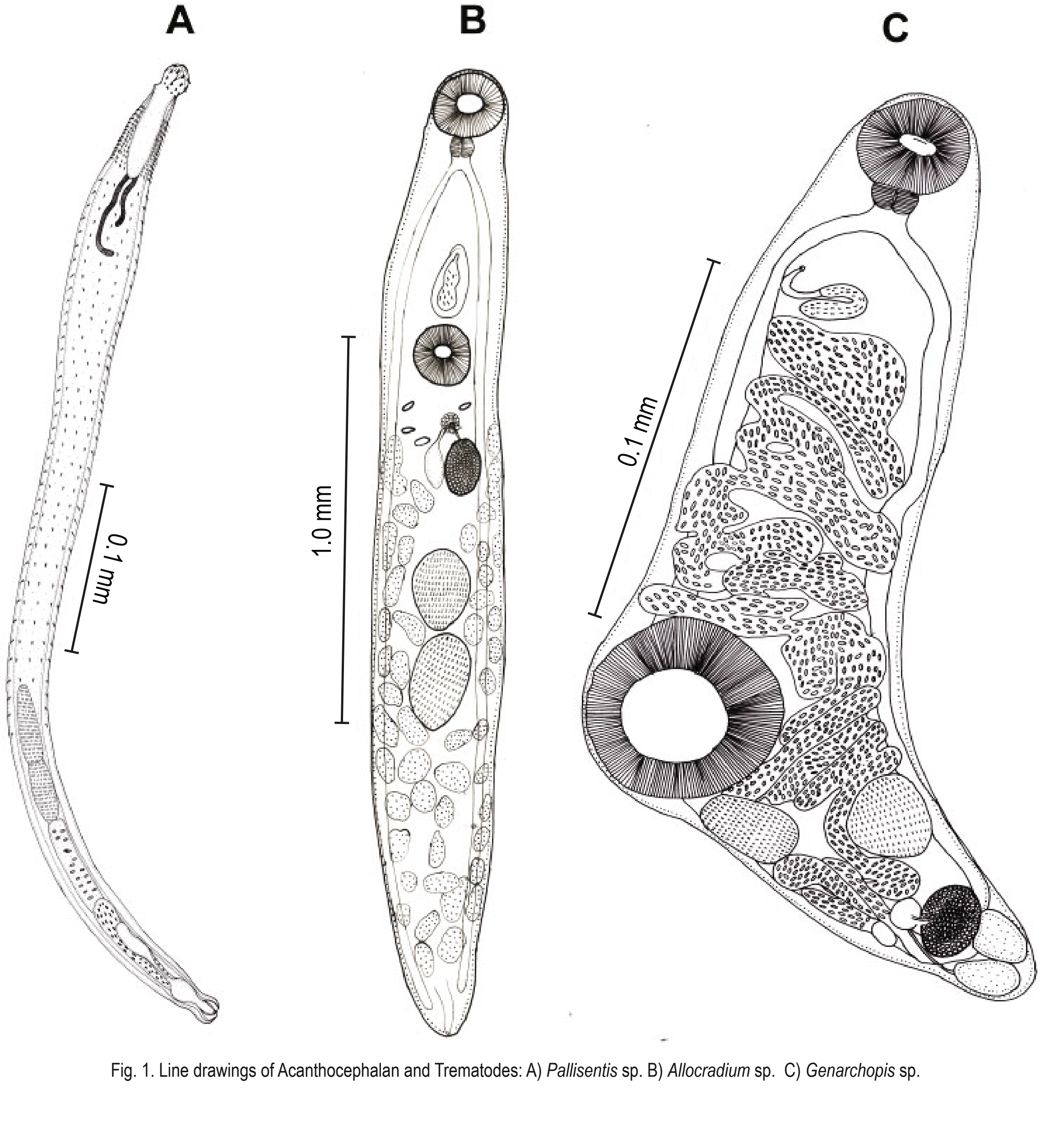

Fig. 1. Line drawings of Acanthocephalan and Trematodes: A) *Pallisentis* sp. B) *Allocradium* sp. C) *Genarchopis* sp.

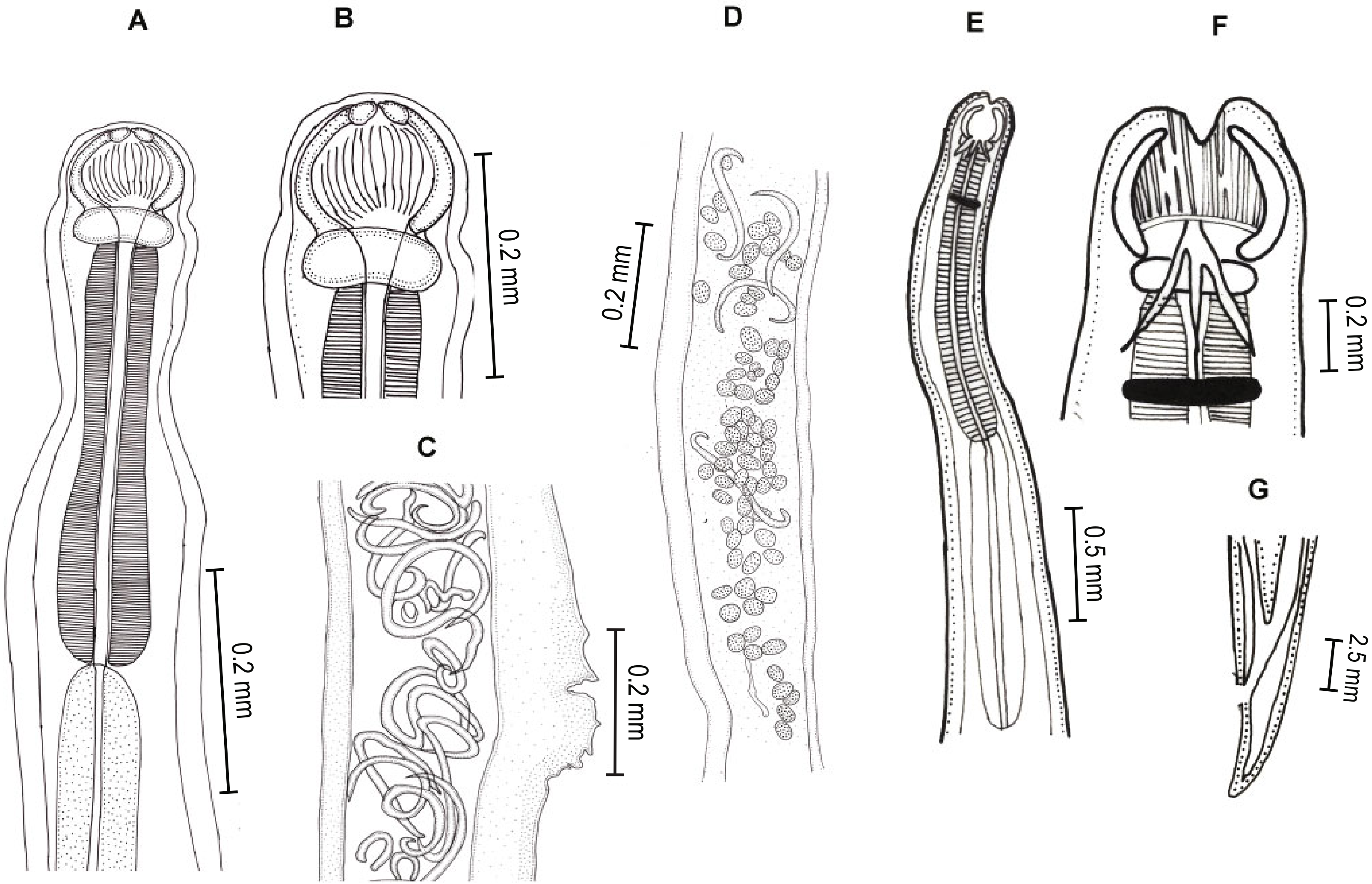

Fig. 2. Line drawings of Nematodes: A) anterior extremity of *Neocamallanus* sp. B) Buccal capsule of *Neocamallanus* sp. C) Vulvar region of female *Neocamallanus* sp. D) Gravid of female *Neocamallanus*. E) Anterior extremity of *Procamallanus* sp. F) Buccal capsule of *Procamallanus* sp. G) Vulvar region of female *Procamallanus* sp.

Materials and Methods

A three-year survey (September 2013 to August 2016) was performed. Fish samples were collected four times in a year during winter (December – February), autumn (September – November), summer (March – May) and rainy season (June – August). Fishes were collected from water bodies near Lucknow (26°84'67"N; 80°94'62"E). Fishes were weighed, measured and identified according to Vazzoler (1996).

Bodies were opened along the midventral line from the anal region to the mouth. The surface of the visceral organs, intestine and the body cavity were examined carefully. The alimentary canal was separated and kept in Petri dishes containing normal saline (0.9 %). The stomach and intestine were opened to dislodge parasites. A few drops of the methanol were added to the normal saline, containing the parasites adhered to the intestinal wall for immobilization and loosening of the grip on the intestinal wall.

Live specimens of parasites collected from the stomach and intestine of host and were kept in normal saline. Parasites were flattened with the help of a glass cover slip and fixed in AFA {Alcohol (50 %): formalin: acetic acid (100: 6: 2.5)}. Specimens were stained with acetoalum carmine, dehydrated in ascending grades of ethanol (30 %, 50 % 70 %, 90 % and absolute ethanol), cleared in xylene and mounted in DPX. Figures were drawn with

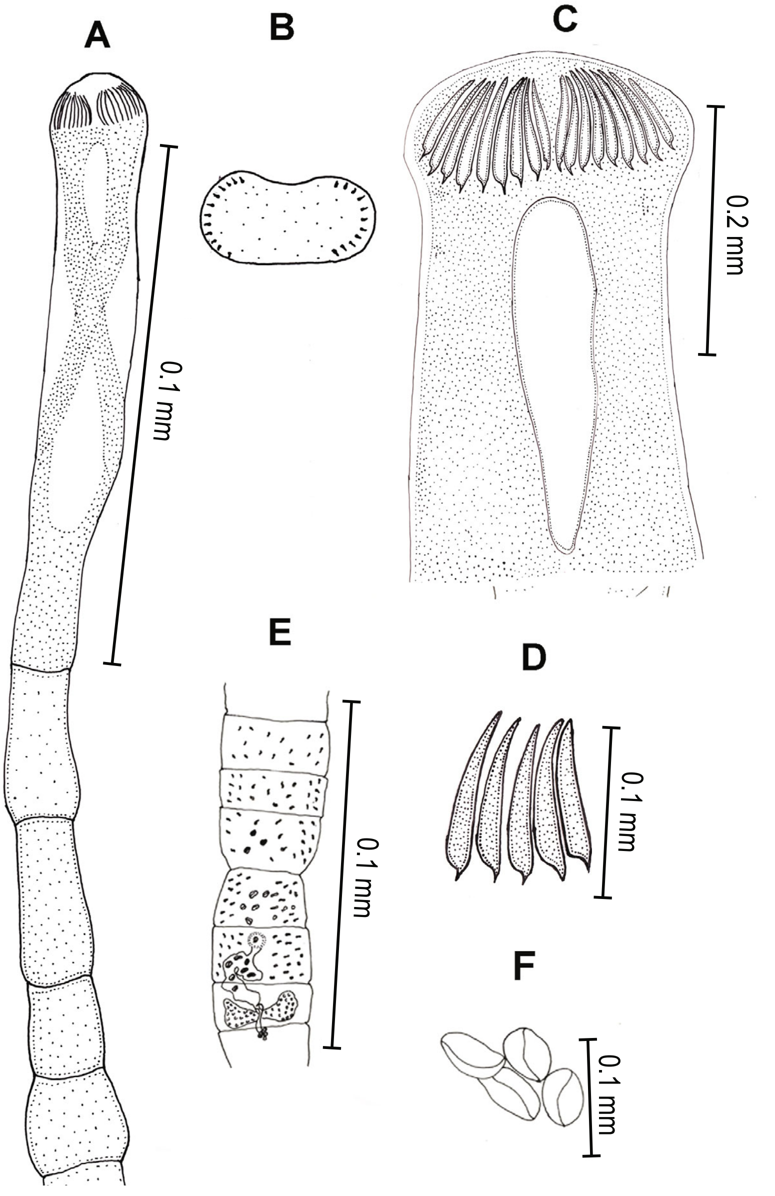

Fig. 3. Line drawings of *Senga* sp. A) Scolex and neck. B) Apical disc of scolex with hooks. C) Enlarge view of scolex. D) Enlarge view of scolex hooks. E) Mature proglottid. F) Eggs.

Camera Lucida, attached to Phase Contrast Microscope (Olympus CX-41). Parasites were identified up to genus level by using the keys of Yamaguti, 1958; Rego *et al*., 1999; Amin *et al*., 2000; Bhattacharya, 2007 and Gibbons, 2010. The slides of collected specimens were deposited in the "Helminthology lab, Department of Zoology, University of Lucknow, U.P., India. Collection numbers: LU/Z/2017/13, LU/Z/2017/14, LU/Z/2017/15, LU/Z/2017/16, LU/Z/2017/17 and LU/Z/2017/18 were assigned for *Pallisentis* sp., *Allocredium* sp., *Genarchopsis* sp., *Neocamallanus* sp., *Procamallanus* sp. and *Senga* sp. respectively.

Only the species that had prevalence equal or greater than 10 % in at least one of the collections were further included in the analysis. Prevalence, intensity range, mean intensity and mean abundance were calculated following Rohde *et al*. (1995). Possible differences between the prevalence in different seasons in relation to total samples were evaluated with Kruskal-Wallis H test .The Wilcoxon Sign Rank test was used for the analysis of infection in male and female. We set the null hypothesis, H_o: infection in male and female were equal. i.e. $p_1 = p_2$. We also set alternate hypothesis, H_1: Infection in male and female were not the same. i.e. $p_1 \neq p_2$. From the given data we got interested in testing whether the infection in male and female *C. punctatus* and *C. striatus* are same or different.

The ecological terminology used was recommended by Bush *et al*. (1997). The level of statistical significance was $p < 0.05$.

Ethical Approval and/or Informed Consent

Animal care and handling were carried out in accordance with national guidelines.

Results

A total of 1013 individuals of *C. punctatus* and 247 individuals of *C. striatus* were examined. Overall 3783 helminths were found with an average of 3.02 helminths/fish collected. On an average 43.50 % *C. punctatus* and 59.10 % *C. striatus* were found to be infected with Acanthocephalans (*Pallisentis* sp., Fig.1A), trematodes (*Allocredium* sp. Fig.1B and *Genarchopsis* sp. Fig.1C), nematode (*Neocamallanus* sp. Fig.2A–2D and *Procamallanus* sp. Fig.2E–2G) and cestodes (*Senga* sp. Fig.3A–3F) per year.

The prevalence, intensity range, mean intensity and mean abundance of various helminths in *C. punctatus* and *C. striatus* were observed (Table 1). The digestive tract was found to be harbouring one acanthocephalan, two trematodes, two nematodes and one cestode genus. Acanthocephalan *Pallisentis* spp. was the most prevalent and abundant parasite (46.50 % infected specimen in *C. punctatus* and 59.11 % in *C. striatus*). We could find only one cestode *Senga* sp. in both *C. punctatus* and *C. striatus*

The highest mean intensity and abundances were found in *Pallis-*

Table 1. Prevalence, intensity range, mean intensity and mean abundance of helminths of snake headed fishes in Uttar Pradesh, India.

	Parasites	Prevalence (%)	Intensity range	Mean Intensity ± SD	Mean Abundance ± SD	Site of infection
C. punctatus	**Acanthocephala**					
	Pallisentis sp.	46.50	12.33 – 0.64	3.79 ± 3.31	1.70 ± 1.52	Intestine
	Trematode					
	Allocredium sp.	21.74	2.78 – 0.00	0.97 ± 0.79	0.23 ± 0.25	Intestine
	Genarchopsis sp.	19.66	3.68 – 0.17	1.31 ± 0.98	0.30 ± 0.28	Stomach
	Nematode					
	Procamallanus sp.	14.53	3.83 – 0.00	1.38 ± 0.95	0.25 ± 0.25	Intestine
	Cestode					
	Senga sp.	13.04	2.25 – 0.00	1.21 ± 0.68	0.20 ± 0.21	Stomach and Intestine
C. striatus	**Acanthocephala**					
	Pallisentis sp.	59.11	20.21 – 0.13	5.11 ± 6.92	3.42 ± 5.98	Intestine
	Trematode					
	Allocredium sp.	26.32	7.78 – 0.00	2.00 ± 2.21	0.56 ± 0.78	Intestine
	Genarchopsis sp.	18.62	3.22 – 0.00	1.15 ± 1.01	0.28 ± 0.43	Stomach
	Nematode					
	Neocamallanus sp.	17.81	7.60 – 0.00	1.70 ± 2.11	0.36 ± 0.51	Intestine
	Cestode					
	Senga sp.	14.57	7.50 – 0.33	2.15 ± 2.37	0.27 ± 0.31	Stomach and Intestine

Table 2. Seasonal differences in the prevalence (%) (Mean ± SD) of helminths in snakehead fishes U. P. India.

Host	Parasites	Acanthocephala	Trematode		Nematode	Cestode
	Seasons	*Pallisentis* sp.	*Allocredium* sp.	*Genarchopsis* sp.	*Procamallanus* sp.	*Senga* sp.
C. punctatus	**Autumn**	68.85 ± 3.83	18.19 ± 4.12	22.35 ± 5.96	22.09 ± 18.62	29.93 ± 21.97
	Winter	34.79 ± 9.68	25.15 ± 13.96	15.67 ± 4.15	13.94 ± 7.98	11.66 ± 6.58
	Summer	65.21 ± 5.90	27.71 ± 5.93	22.19 ± 6.17	18.38 ± 2.94	12.03 ± 1.10
	Rainy	36.47 ± 11.64	26.26 ± 10.00	24.87 ± 9.68	20.30 ± 7.41	13.28 ± 3.64
	Chi-square	8.641	2.487	1.974	1.564	1.051
	p-value	0.034	0.478	0.578	0.668	0.789
		Pallisentis sp.	*Allocredium* sp.	*Genarchopsis* sp.	*Neocamallanus* sp.	*Senga* sp.
C. striatus	**Autumn**	66.72 ± 5.23	37.02 ± 15.00	34.48 ± 12.36	25.48 ± 9.97	18.87 ± 6.62
	Winter	22.22 ± 11.11	11.11 ± 11.11	25.93 ± 16.97	7.41 ± 6.42	18.52 ± 6.42
	Summer	79.97 ± 9.50	28.79 ± 4.67	2.66 ± 2.43	19.46 ± 6.49	10.14 ± 2.51
	Rainy	40.15 ± 6.44	16.53 ± 6.93	14.69 ± 9.28	11.60 ± 2.34	13.35 ± 5.78
	Chi-square	9.974	7.667	7.821	7.539	5.539
	p-value	0.019	0.053	0.050	0.057	0.136

entis sp. while the trematode *Allocredium* sp. and cestode *Senga* sp. were least abundant in *C. punctatus* and *C. striatus* respectively. There were statistically significant differences of prevalence between different seasons of *Pallisentis* spp. in *C. punctatus* (p = 0.034) and *C. striatus* (p = 0.019). But there were no significant differences in other helminths in either *C. punctatus* or *C. striatus* (Table 2). The prevalence of *Pallisentis* spp. was maximum in autumn and summer in *C. punctatus* (68.85 ± 3.83 and 65.21 ± 5.90 respectively) while in *C. striatus*, prevalence of *Pallisentis* spp. was maximum during summer (79.97 ± 9.50).

Mean abundance of most of the helminths species was higher during summer and never reached maximum observed during winter season (Table 3). Acanthocephalans and nematodes in both the host attained peak mean abundance during summer while trematode *Genarchopis* sp. in *C. punctatus* and *Allocradium* sp. in *C. striatus* showed maximum during rainy season. Only cestode *Senga* sp. in *C. punctatus* and trematode *Genarchopis* sp. in *C. striatus* showed maximum mean abundance during autumn.

Infection rates of parasitic helminths were observed for different sexes in changing seasonal conditions to study the effect of sex on infection. There was a statistically significant difference between the infection rates of male and female *C. punctatus* and C. *striatus* (Table 4). During all the seasons except autumn in *C. punctatus* and throughout all the seasons in *C. striatus* there were significantly higher infected males as compared to females.

Comparing different weight groups in hosts, we found that there was a tendency in medium weight group (21 – 40 g) to become more infected. In C. *punctatus* weight group 60 – 200 was infected most while as regards to the *C. striatus* in 21 – 40 weight group it was autumn season (Table 5). In winter season the infection rates were higher in the weight group of 21 – 40 for both C. *punctatus* and *C. striatus*. The infection rates in summer season were highest in weight group 41 – 60 in C. *punctatus* and in weight group 00 – 20 in *C. striatus*. In the rainy season, the highest prevalence of the infection was in the weight group 21 – 40 and 41 – 60 in C. *punctatus* and *C. striatus* respectively.

Discussion

The present study on two snakehead fishes was aimed to survey the occurrence and distribution of endoparasitic helminths and to explore the seasonal prevalence of these parasites. Six genera of helminths were found from trematode, cestode, nematode and acanthocephalans. Most of the parasites were found in the intestine region and the acanthocephalan *Pallisantis* spp. were most prevalent ones.

Seasonal variations of parasites in the host are already well studied. Distinct seasonal variation was reported by Boping and Wang (2007) and they found that the prevalence of *Pallisentis caelatus* (Neosentis) was at the highest in spring and decreased with fall in temperature. Earlier, Kanth and Srivastava (1987) had also reported that infestation rate of *Pallisentis ophiocephali* gradually increases and achieves two peaks in May and August. In our study, the infection rate differed seasonally and the maximum parasites were generally found during autumn and summer seasons. Among the nematode and cestode, peak prevalence was in autumn in both hosts, following the end of the peak breeding period of the host fishes. The Prevalence of trematode was not similarly regular

Table 3. Seasonal differences in mean abundance of helminths of snakeheaded fishes in U. P. India.

Host	Parasites	Total	Autumn	Winter	Summer	Rainy
C. punctatus	**Acanthocephala**					
	Pallisentis sp.	0.96	0.72	0.66	0.89	0.85
	Trematode					
	Allocredium sp.	0.54	0.40	0.33	0.72	0.58
	Genarchopsis sp.	0.50	0.46	0.40	0.55	0.60
	Nematode					
	Procamallanus sp.	0.54	0.53	0.42	0.58	0.54
	Cestode					
	Senga sp.	0.46	0.62	0.44	0.45	0.33
C. striatus	**Acanthocephala**					
	Pallisentis sp.	0.82	0.75	0.66	0.85	0.85
	Trematode					
	Allocredium sp.	0.46	0.31	0.40	0.66	0.80
	Genarchopsis sp.	0.65	0.88	0.33	0.60	0.66
	Nematode					
	Neocamallanus sp.	0.30	0.23	0.22	0.50	0.33
	Cestode					
	Senga sp.	0.33	0.35	0.40	0.42	0.18

and differed in both hosts. For *C. striatus* the peak was in autumn but *C. punctatus* attained its peak in both summer and rainy seasons. Many authors have also reported that high prevalence of cestode (Bhure *et al*., 2014) in summer whereas low in the monsoon season. Similarly Vincent and Font (2003) reported that the prevalence, mean abundance and mean intensity of nematodes were higher in summer than in winter. According to Genc *et al*., (2005) the parasitic infection showed seasonal variations with the highest prevalence in the summer season.

Two main categories of factors may be held responsible for the seasonal variations in host infectivity, those linked to the host and other linked to the parasites. Ibiwoye *et al*., (2004) observed that susceptibility to infections in fishes are generally due to weakened body after hibernation. According to Bhuiyan *et al*., (2007) decrease in water volume during dry seasons results in imbalanced nutritional conditions also make fishes vulnerable to the infections. The authors also concluded that decreased water temperature also made the hosts susceptible to infections by weakening immune systems. So many other parasite associated factors are also held responsible for the development of parasites such as high temperature and

Table 4. Seasonal differences of helminths of *C. punctatus* and *C. striatus* during different seasons.

Host	Seasons	Male	Female	Z	p*
C. punctatus	Autumn	6.22 ± 6.01	2.33 ± 1.73	1.90	0.057
	Winter	6.11 ± 6.60	0.44 ± 0.72	2.03	0.042
	Summer	20.33 ± 18.01	4.55 ± 2.35	2.36	0.018
	Rainy	5.22 ± 7.67	1.88 ± 3.14	2.13	0.033
C. striatus	Autumn	4.33 ± 2.23	1.66 ± 1.22	2.55	0.011
	Winter	2.11 ± 1.26	1.11 ± 1.05	2.04	0.041
	Summer	6.66 ± 7.36	0.88 ± 1.36	2.37	0.018
	Rainy	2.66 ± 2.00	0.88 ± 1.36	2.45	0.014

Z value of Kruskal-Wallis H test, *significance level $p \leq 0.05$

Table 5. Helminths infecting *Channa punctatus* and *Channa striatus* of different weight groups as 00 – 20 g, 21 – 40 g, 41 – 60 g and 61 – 200/300 g.

		Weight (g)			
Host	**Seasons**	**00 – 20 (p_1)**	**21 – 40 (p_2)**	**41 – 60 (p_3)**	**60 – 200/300 (p_4)***
C. punctatus	Autumn	26.3	48.75	7.69	70.0
	Winter	18.30	51.28	31.25	13.33
	Summer	50.0	52.90	59.23	51.28
	Rainy	36.20	78.76	39.47	0.00
C. striatus	Autumn	64.15	76.08	50.0	0
	Winter	46.66	75.55	71.42	50.0
	Summer	90.0	73.33	34.48	11.53
	Rainy	0	26.66	32.14	11.76

**Channa punctatus* maximum weight was 200 g and *Channa striatus* 300 g.

low rainfall (Jadhav & Bhure, 2006). Kennedy (1970 & 1977) had explained that feeding habits of the host, availability of infective host and parasite maturation are also responsible for influencing the parasitic infections. Recently, Sheema *et al*., (2015) and Ritika *et al*., (2012) have suggested that abundance of helminths increase with the rising temperature in summer and slow down during winter. The development of intermediate hosts of helminths during summer season also leads to better availability of infective stages resulting in higher helminths prevalence in summer (Khurshid & Ahmad, 2012).

During our study we also found significant differences in infestation rates on the basis of sex and weight groups. In overall four different seasons, the infestation rate in male C. *punctatus* was higher in the summer. The association between reproduction and increase in prevalence and abundance of parasites has been attributed the fact to the physiological stress of the host during the breeding period, as a higher investment in reproduction may decrease the energy allocated to the immune system and thereby facilitate parasitic infestation (White *et al*., 1996; Lizama *et al*., 2006). There are contrasting reports in this regard as some authors have observed more infection in male hosts as compared to females (Zelmer & Arai, 1998) and this view is supported by our study. Other observers have reported a greater susceptibility of females (Ibiwoye *et al*., 2004; Singhal & Gupta, 2009). These differences may be because of various factors including host species, infective species and geographical conditions.

Variations in infection rates in different weight group were less profound in summer where in the rest seasons, there were more variations. Overall, the mid-weighed snakeheads were more prone to infection as compared to lower and higher weight groups. Our results are in accordance with some earlier observations. Nahar (1988) have also reported similar finding that the hosts with intermediate size were more infected by the parasites than the smaller and larger individuals. Similar reports by Polyanski (1961) had supported the view that intermediate length and weight group host had a higher prevalence and intensity than those of smaller and larger length and weight group. Major factors such as food, lifespan, variety of habitats, population density and size attained by host were suggested to affect the parasites prevalence and intensity in fish.

Our study explores the diversity and seasonal variations of helminthic parasites in snakeheads. Our study explores the diversity and seasonal variations of helminthic parasites in snakeheads. These types of studies will lead to the better understandings of host-parasite interactions what will be beneficial for the improvement of infectious diseases management and also contribute to the increase in fish production.

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Conflict of Interest

Authors state no conflict of interest.

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