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Prevalence and incidence of helminth infection in some freshwater fishes of Sukhnag stream in Kashmir

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Helminth infection, with high mortality rate, causes significant damage to fish industry worldwide In Kashmir, about 31 species of helminths are known to affect local fish population. In this study, we investigated seasonal variation of helminth parasites in snow trout (*Schizothorax plagiostomus* Heckel,1838) and scale carp (*Cyprinus carpio communis* Linnaeus, 1758) collected from Sukhnag stream for a period of two years (February 2017 to January 2019). Helminths *Adenoscolex oreini* (cestode), *Diplozoon kashmirensis* (trematode), and *Pomphorhynchus kashmirensis* (acanthocephalan) were recovered from *S. plagiostomus* while *Bothriocephalus acheilognathi* (cestode) and *P. kashmirensis* have been recovered from *C. carpio communis*. Prevalence and mean intensity of helminth infection was highest in *S. plagiostomus* (25.26% and 13.79) and lowest in *C. carpio communis* (10.82% and 8.47), respectively. Prevalence of helminths was at its peak in summer (June/July) and lowest in winter (December/January) in both native (*S. plagiostomus*) and exotic (*C. carpio communis*) fish species. This study revealed significant seasonal and monthwise variation in parasitic load of fishes which could be due to the difference in temperature, feeding habits of fish, fish species, immunity levels and other abiotic conditions of the water body.

Keywords: *Adenoscolex oreini*, *Bothriocephalus acheilognathi Cyprinus carpio communis*, *Diplozoon kashmirensis*, *Pomphorhynchus kashmirensis*, Scale carp, *Schizothorax plagiostomus*, Snow trout

Fish is taken as a high quality food throughout the world and has a beneficial role in diminishing cardiovascular disorders and cholesterol level in blood due to presence of polyunsaturated fatty acids[1]. As the world population grows, fish resources are being depleted at an increasing rate due to anthropological activities and presence of parasites. Different parasites regularly reported in fishes are protozoans, monogeneans, digeneans, cestodes, nematodes, acanthocephalans and crustaceans[2]. Parasites cause several degrees of damage in fish by altering their biology and behaviour[3]. Parasitic diseases cause slow growth rates, large-scale mortalities, and decrease marketability of fish[4,5]. Among different parasites, helminthic infections constitute a major factor in declining the production of fish in India. Helminth parasites not only cause severe pathological conditions in fish but also pose a serious threat to the well-being of people especially when raw fish is eaten[6].

Evaluation of parasitic load in fish is important as it provides information about health status of fish and also useful in depicting changes in the diversity, prevalence and abundance of helminths related to different ecological stressors. Among different fish species, *Schizothorax* (snow trout) and *Cyprinus carpio* (scale carp) are considered as the most predominant fish species in the water bodies of Kashmir. Parasitism and other ecological stressors have been found to cause a considerable decline in the population of *Schizothorax* (Kashmiri) over the last few decades[7]. Helminth parasites of fish species in various water bodies of Kashmir have been studied extensively but no work has been carried out on helminth parasites in the fishes of Sukhnag stream. Hence, in the present study, we investigated the helminthes infestation in *Schizothorax plagiostomus* and *Cyprinus carpio communis* which are principal fish species found in Sukhnag stream.

Materials and Methods

Study area and fish collection

This study has been approved by the Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences, University of Kashmir under Approval no. F(IAEC-Approval/KU/2018/118.

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The area selected for carrying out the current research was Sukhnag stream. Sukhnag, is a torrential stream and one of the important tributary of River Jhelum. It is among the five major inflows of the Wular Lake (Ramsar site). This stream flows through Budgam district and has its origin both from spring 'Sukhnag spring' (Sokha Nag; the spring of solace) and high altitude glacier called Damdam in Damsar near Tossa Maidan. Besides providing water for domestic and agricultural purposes, this water body harbours a wide variety of fish species, particularly *Schizothorax plagiostomus* and *Cyprinus carpio communis* and is considered an important source of economy in the areas through which it flows.

The fish hosts were collected monthly from four sites at Sukhnag stream for the period of two years (February 2017-January 2019). The annual study period was divided into four seasons; i.e., Summer (June-September), Autumn (September-December), Winter (December-March) and Spring (March-June). Site I: Beerwah located at 74°35′ 0″ East and 34º1′0″ North; Site II: Makhama located at 74°35′ 0″ East and 34º4′0″ North; Site III: Kanihama located at 74°36′ 0″East and 34º6′0″ North and Site IV: Narbal located at 74°39′ 0″East and 34º7′0″ North (Fig. 1). The fish hosts were collected with the help of local fisherman and brought to the Parasitological Research Laboratory. The fish hosts were identified as *Cyprinus carpio communis* Linnaeus, 1758 (Fig. 2A) and *Schizothorax plagiostomus* Heckel, 1838 (Fig. 2B) using the keys given by Skeleton[8] and registered in Zoological Museum with acquisition numbers as ZoKU-Pis/11043 and ZoKU-Pis/11110, respectively. The sample size was calculated using the formula given by Thrustfield[9].

$$n = \frac{1.962^2 . P\text{exp}\,(1 - P\text{exp})}{d^2}$$

where n = required sample size, Pexp = expected prevalence= 50%, d = desired absolute precision=5%. Hence, d = 0.05 and p= 0.5 (50%). Thus, the minimum desired annual sample size was calculated to 384.

Parasitological examination of fish

The fish hosts were first killed by giving strong blow on their heads and then examined externally for monogenetic and encysted digenetic parasites employing the methods of Cable[10]. The fishes were dissected for internal examination and scanned by stretching and opening their alimentary canal in normal saline. Other visceral organs like gall bladder, kidneys, liver, and spleen were also examined for helminth parasites in separate petridishes. Recovered parasites like trematodes, cestodes and acanthocephalans were fixed in carnoys fixative, stained with acetoalum carmine, dehydrated in ascending grades of ethanol, cleared in xylene, mounted in DPX and observed under microscope. Photomicrographs were taken with Olympus microscope (Digital camera attached to Research Microscope). Taxonomical identification of helminths parasites was then carried out by the keys given by

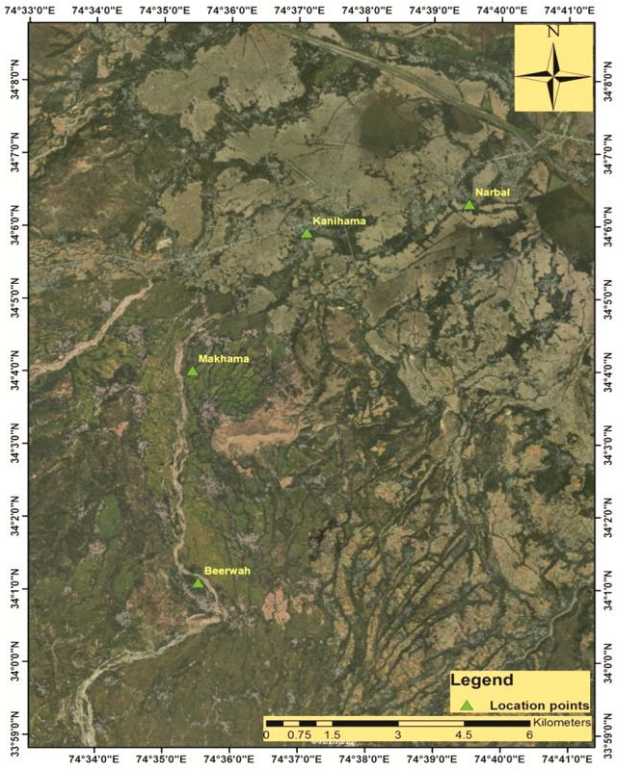

Fig. 1 — Fish collection sites at Sukhnag stream

Fig. 2 — (A) *Cyprinus carpio communis*; and (B) *Schizothorax plagiostomus* caught from Sukhnag stream, Budgam, Kashmir

Yamaguti[11]. The permanent slides of collected specimens of helminths were deposited in the "Zoological Museum", Department of Zoology, University of Kashmir, India. Acquisition numbers: ZoKU-Hel/04003, ZoKU-Hel/04201, ZoKU-Hel/04202 and ZoKU-Hel/04602 were assigned for *Diplozoon kashmirensis* Kaw,1950, *Adenoscolex oreini* Fotedar, 1958, *Bothriocephalus acheilognathi* Yamaguti, 1934 and *Pomphorhynchus kashmirensis* Kaw,1950, respectively.

Then level of parasite infection was calculated monthly as well as seasonally by using prevalence and mean intensity formulas as per Margolis *et al.*[12].

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

$$\text{Mean intensity} = \frac{\text{Total number of parasites}}{\text{Total number of infected hosts}}$$

Data analysis

Data was recorded and analyzed statistically by using Minitab Version 13. The prevalence of helminths in different seasons and months was analyzed by employing Chi square test. P-value <0.05 (at 5% level of significance) was considered as significant.

Results

Helminth parasites reported were trematode: (*Diplozoon kashmirensis* Kaw, 1950), cestode (*Adenoscolex oreini* Fotedar, 1958) and acanthocephalan (*Pomphorhynchus kashmirensis* Kaw, 1950) in *S. plagiostomus*. From *Cyprinus carpio communis*, cestode: (*Bothriocephalus acheilognathi* Yamaguti, 1934) and acanthocephalan (*Pomphorhynchus kashmirensis* Kaw, 1950) were recovered (Fig. 3).

Overall prevalence of helminth infection in fishes

During the entire study period, 768 fish specimens were examined, of which 166 were found infected with different helminth parasites giving a prevalence of 21.61%. While 74 fishes were found infected out of 384 examined during Feb. 2017-Jan. 2018, 92 fish were found infected out of 384 examined during Feb. 2018-Jan. 2019. Hence, from Feb. 2018-Jan.2019, the percentage prevalence was higher (23.95%) than from Feb.2017-Jan.2018 (19.27%). But difference in prevalence rate was not statistically significant as $P > 0.05$ (Table 1).

Prevalence and mean intensity of helminth parasites in *Schizothorax plagiostomus* and *Cyprinus carpio communis*

Out of 768 fishes examined, 574 were *S. plagiostomus* of which 145 were found infected

Table 1 — Year wise prevalence of helminth infection

Year	No. examined	No. infected	Prevalence	$\chi 2$ (p- value)
February 2017-January 2018	384	74	19.27%	1.606 (0.205)
February 2018-January 2019	384	92	23.95%	
Total	768	166	21.61%	

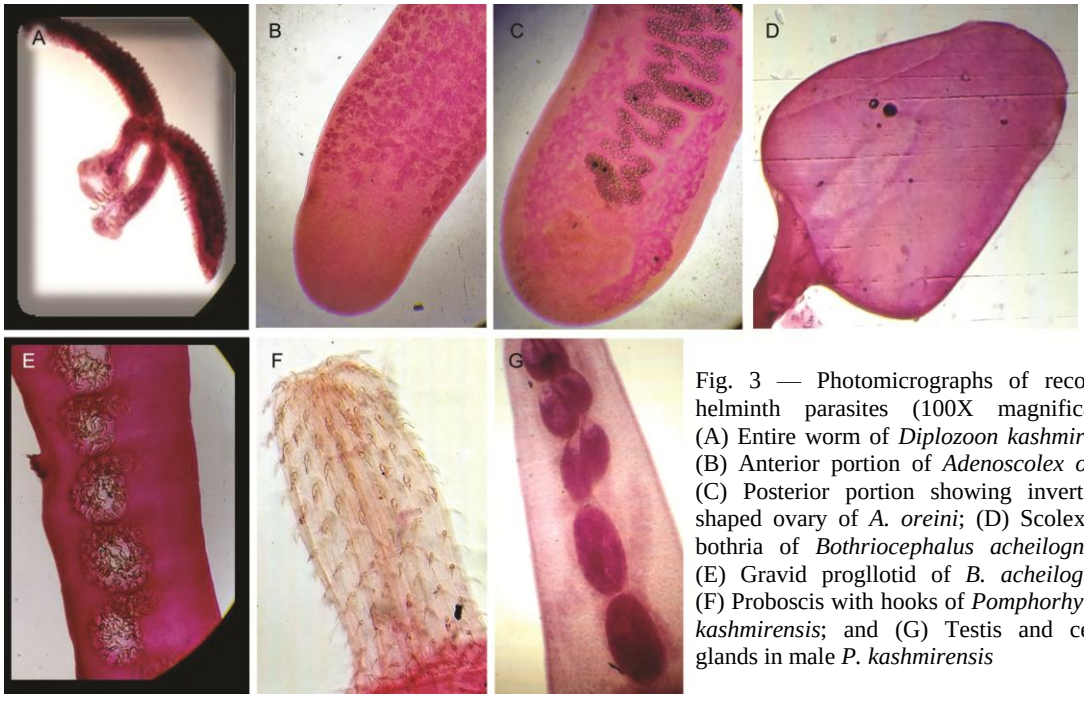

Fig. 3 — Photomicrographs of recovered helminth parasites (100X magnification) (A) Entire worm of *Diplozoon kashmirensis*; (B) Anterior portion of *Adenoscolex oreini*; (C) Posterior portion showing inverted A shaped ovary of *A. oreini*; (D) Scolex with bothria of *Bothriocephalus acheilognathei*; (E) Gravid progllotid of *B. acheilognathi*; (F) Proboscis with hooks of *Pomphorhynchus kashmirensis*; and (G) Testis and cement glands in male *P. kashmirensis*

showing a prevalence of 25.26%. Total number of parasites recovered from *S. plagiostomus* was 2000 and the mean intensity was 13.79. 194 specimens of *Cyprinus carpio communis* were examined, of which infection was reported in 21 specimens with a prevalence of 10.82%. In 21 specimens, 178 parasites were recovered giving a mean intensity of 8.47. The data is statistically significant as P <0.05 (Table 2).

Seasonal prevalence of helminth infection in fishes

From Feb. 2017-Jan. 2018, maximum seasonal prevalence of helminth parasites was reported in summer (29.7%) followed by autumn (21.5%), spring (15.05%) and lowest in winter (10.3%). From Feb. 2018-Jan. 2019, same trend of infection was reported that is maximum seasonal prevalence in summer (35.71%) followed by autumn (25%) then spring (21.64%) and lowest in winter (12.9%). The prevalence of infection in different seasons is statistically significant in both the years as P <0.05 (Fig. 4).

Cumulative season-wise prevalence of helminth infection

On cumulating the data of both years, there was significant difference (P <0.05) in helminth infection among the seasons. Maximum prevalence was reported in summer season i.e., 32.66% followed by autumn (23.28%), spring (18.42%) and lowest in winter (11.57%) (Fig. 5).

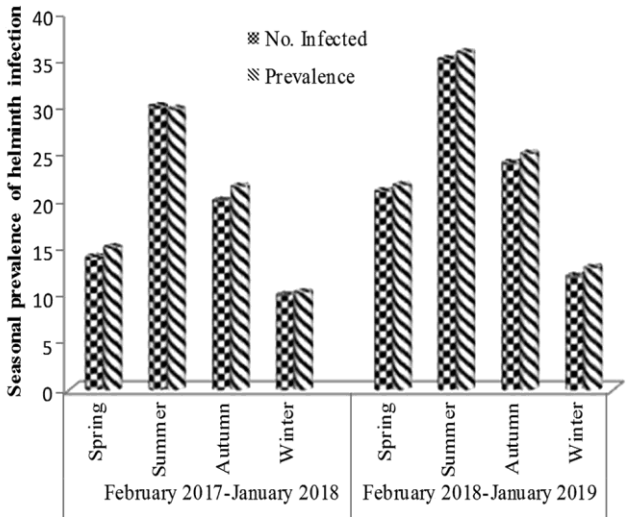

Fig. 4 — Seasonal prevalence of helminth infection in fishes

Month-wise prevalence of helminth parasites

The month-wise data revealed the highest prevalence of helminth infection in July (45.45%) in 2017-2018 and (48.48%) in 2018-2019, respectively. From 2017–2018, lowest prevalence of infection was reported in January (3.12%) and from 2018-2019, lowest infection was found in December (3.33%). Data regarding month-wise prevalence of helminth infection in two years is significant (P <0.05) (Fig. 6).

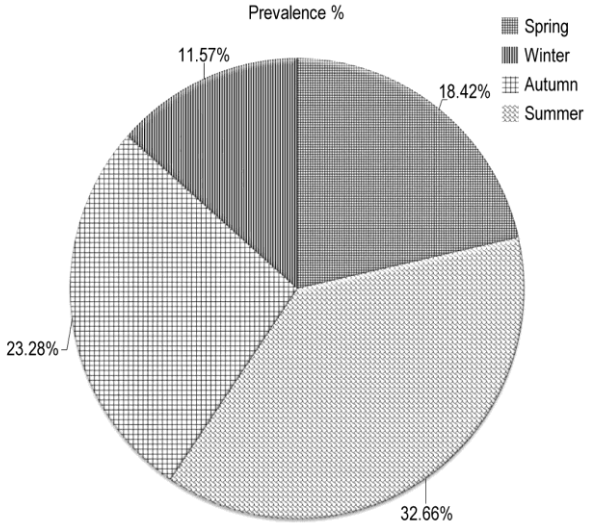

Fig. 5 — Cumulative season wise prevalence of helminth infection

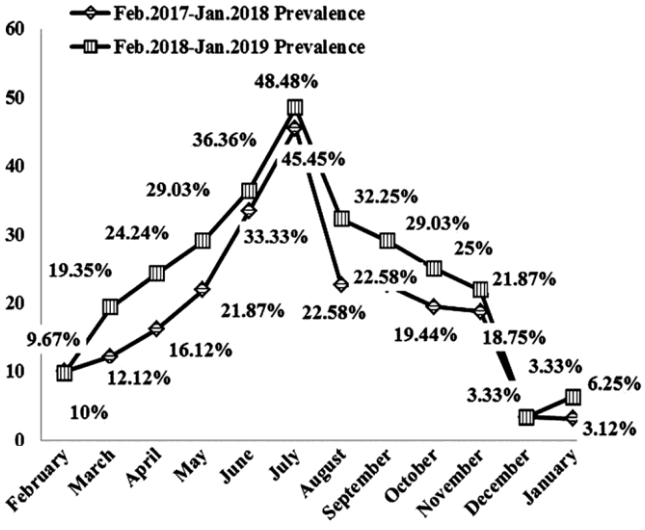

Fig. 6 — Month wise prevalence of helminth infection

Table 2 — Prevalence and mean intensity of helminth parasites in *Schizothorax plagiostomus* and *Cyprinus carpio communis*

Fish species	No. examined	No. infected	No. of parasites	Mean intensity	Prevalence	χ^2 (p-value)
Schizothorax. plagiostomus	574	145	2000	13.79	25.26%	12.248
Cyprinus carpio communis	194	21	178	8.47	10.82%	(0.000)

Discussion

During the entire span of the study, variations observed in the overall prevalence of helminths between two years can be attributed to density and maturity of the host population (Table 1). Highest infection in terms of prevalence and mean intensity of helminths was noticed in case of *Schizothorax plagiostomus* (native fish) when compared to *Cyprinus carpio communis* (exotic fish) (Table 2) that is in accordance to Choudhury *et al.*[13] who also observed highest prevalence of parasites in native fishes in comparison to non-native fishes of Little Colorado River Grand Canyon Arizona. These differences in parasitism can be attributed to different preferences of fish for food at different stages[14,15]. According to Sasal *et al.*[16] fish with a more diversified habits of feeding can encounter more intermediate host and thus harbor more parasitic species. Not only diet, but differences in the parasitic resistance and metabolic state of the fish host are also responsible for variations in prevalence and intensity of parasites in different fish species[17].

In our study, prevalence percentage differed seasonally and the maximum percentage prevalence of helminth infection was reported during summer season followed by autumn then spring and lowest during winter in both years and also in cumulative season-wise prevalence (Figs 4 and 5). Our results are in close proximity with Genc *et al.*[18], Wali *et al.*[19], Gautam *et al.*[1] and Khalid *et al.*[20] who reported the highest prevalence of helminth parasites during summer and lowest during winter. Variation in helminth infection in different seasons can be related with large variations in environmental conditions and in feeding habits of fish[21]. Temperature is considered a significant factor for increasing the development of intermediate hosts of helminths that may increase the chances of parasitic larvae development[22,23]. The low prevalence of helminth parasites in winter season may be attributed to low temperature that is not feasible for the development of preparasitic stages of parasites and decrease the feeding tendency of fish. The results of this study revealed the high prevalence of infection in month of July (Fig. 6) that is supported by Khalid *et al.*[20] who also reported high prevalence in the month of July in *S. plagiostomus* of river Swat, Khyber PakhtunKhwa, Pakistan. Lowest prevalence of helminths was found in month of January in first year of the study that is supported by Khan *et al.*[24] who also reported low infection of helminths in *Johniuss dussumieri* in January. The fluctuation in prevalence of helminths in different months is attributed to temperate climate of valley marked with wide variations in temperature[25] and other ecological aspects like pH and O_2[26].

Conclusion

This study makes it evident that *Schizothorax plagiostomus* (snow trout) and *Cyprinus carpio communis* (scale carp) of Sukhnag stream are infected with trematodes, cestodes and acanthocephalans. Helminths observed in *S. plagiostomus* were *Diplozoon kashmirensis*, *Adenoscolex oreini* and *Pomphorhynchus kashmirensis*. From *Cyprinus carpio communis*, parasites like *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* were recovered. The results clearly indicate that prevalence of helminth parasites show seasonal variation and also choice of host species. These variations in helminth parasitic load are attributed to different environmental factors, feeding habits and immunity levels in fishes. Parasitic infection cause economic loss to fishery industry and also severely affects humans on the top of the food chain.

Conflict of interest

The authors declare no conflict of interests.

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