



A STUDY OF THE POPULATION DYNAMICS OF ACANTHOGYRUS PARASITES OF CATLA CATLA [CYPRINIFORMES: CYPRINIDAE] FROM GANGAPUR DAM, NASHIK, MAHARASHTRA

¹ Sushma V Singh, ² Varsha V. Andhare

¹ Research Scholar, ² Professor

¹ Department of Zoology, ² Department of Zoology

¹ The Institute of Science, Mumbai- 400032, India. ² The Institute of Science, Mumbai- 400032, India

ABSTRACT: Acanthocephalans are a family of endoparasitic helminth that parasitize fish worldwide. As an adult they use their thorny proboscis to clamp on to the host's gut tissues. Fish, which is a good source of high-quality animal protein for humans, loses some of its nutritional value as a result of the presence of these worms. 216 intestines of *Catla catla* fish were procured from the Godavari River in the Gangapur dam region and examined for infection on a monthly basis from April 2018 to March 2019. Most parasites recovered from fish were identified as being *Acanthogyryus spp.*, along with very few nematodes and cestodes parasites. As per the purpose of the present study, the rate of infection of *Acanthogyryus spp.* in *Catla catla* was highest in the summer season, low during the monsoon, and the same during the winter season as the overall prevalence.

Index Terms: *Catla catla*, Parasite, *Acanthogyryus*, Prevalence.

INTRODUCTION:

Acanthocephalans are a class of parasitic helminths that exhibit the presence of a retractable thorny proboscis, sometimes known as a spiny-headed or thorny-headed worm. Metazoan parasites of vertebrate hosts include nematodes, trematodes, and cestodes, among other helminths. However, Acanthocephalans are different, in the sense that they live in the small intestines of vertebrates only as adults and have an indirect life history with arthropods serving as intermediate hosts.

Acanthocephalans adhere to their host's intestine using their retractable and invaginable proboscis. The dietary value of the fish, which are an excellent source of animal protein for humans, is diminished by the presence of these worms. Acanthocephalan infection results in widespread fish mortality; damage to the inner wall of the intestine and, occasionally, obstruction of the gut lumen can result in the fish's death. Helminth parasites called Acanthocephalan employ intermediate hosts like arthropods and vertebrates to complete their life cycles.

These helminths are unique because they lack a digestive system and have a proboscis with attached hooks, a syncytial epidermis, and a lacuna system with circulatory channels that allow for direct nutrient absorption through the body wall. (Crompton, D. W. T. et al., 1985).

The genus *Acanthogyryus* was grouped under the class Eoacanthocephala, order Gyraacanthocephala, family Quadrigyridae, and subfamily Pallisentinae. Each acanthocephalan species develops from one or more intermediate hosts. Each and every adult Acanthocephalan is a gastrointestinal parasite.

Eggs are laid in the intestinal lumen and then released with faeces. Amphipods, isopods, copepods, and ostracods serve as the first intermediate hosts of the piscine acanthocephala. The first larva, the acanthor,

emerges from the eggs after being consumed by the proper invertebrate host. When their larvae in the invertebrate host are consumed by the definitive vertebrate host, some species will mature into adults.

(Schmidt, G. D., & Nickol, B. B. (1985). Non-predatory *catla catla* fish eat mostly phytoplankton and zooplankton in surface waters. According to a study on the impact of the environment on the severity of infection, seasonal variation affects host immunity and physiology by balancing energy requirements for essential life activities including fish reproduction and growth. Rohlenová, K. et al. (2011).

The Godavari River's hydrobiological parameters vary seasonally, and various researchers have periodically studied these variations. The resulting data is now available and shows a significant change in water parameters as a result of seasonal changes, with a focus on temperature, pH, dissolved oxygen, ammonia, and other factors. Bhutekar, D. D., et al. (2018).

Statistical parameters used to describe the epidemiology of parasites, like prevalence, mean intensity, mean abundance, etc., can be related to seasons. As for the aquatic animal environment, which is water, any change in water parameters might affect the distribution of parasites in fish and their epidemiology. The development of parasite eggs as well as zooplankton reproduction can be accelerated in the littoral shallows due to the water warming up more quickly there. (Dubinina, 1980).

Total fish production in the state of Maharashtra was 523098 t in 2020–21, worth 7665 crores, of which 124587 t, worth 1659 crores, came from inland waters. A substantial portion of the nation's catch from fisheries and farmed fish are produced in the state of Maharashtra. The fishing sector is important in India because it not only produces high-quality fish meat, which is a substantial source of vitamins, food, and protein, but also gives millions of people jobs. People who reside near rivers and oceans usually include fish in their diet. Rohu, Calbasu, Catla, Singhara, and other notable freshwater fish that may be eaten are found in the state. Maharashtra State Government Report (2020-21)

MATERIAL & METHODS:

Fish intestines procured for the present study were mostly collected from the freshly caught fishes and sold in the market near Gangapur dam on Godavari River district Nashik. Intestines were brought to the laboratory in polythene bags in ice boxes for examination. Generally, 3-4 specimens were examined each day. A total of 216 *Catla* intestines were examined for the presence of acanthocephalan.

In order to help with the complete eversion of their proboscis, the worms from *Catla catla* gut were removed and then placed in the refrigerator for a few hours. The worms were also fixed in A.F.A. fixative for 24 hours at room temperature before being kept in 70% ethanol.

Parasites were processed in various alcohol grades, stained with acetoalum carmine, and mounted in DPX to create permanent slides. With the use of a light microscope, the prepared slides were inspected. The methods used by Margolis et al. in 1982 and Bush et al. in 1997 were used to calculate prevalence, mean intensity, and mean abundance.

$$\text{Prevalence} = (\text{number of hosts infected}) / (\text{total number of collected hosts})$$

$$\text{Mean intensity} = (\text{total number of parasites}) / (\text{total number of infected hosts})$$

$$\text{Mean abundance} = (\text{total number of parasites}) / (\text{total number of collected hosts})$$

OBSERVATION & RESULTS:

The taxonomic identification: The worms were identified as *Acanthogyryus* as per the taxonomical characters; the major taxonomic characters of the identified acanthocephalan are the proboscis hooks, spines of the collar and trunk region, Lemnisci size, proboscis sheath, tandem testes, and cement gland. These are fish parasites. The worm had the following characteristics of the genus *Acanthogyryus*: a sub globular proboscis with three transverse rows and eight hooks in each row. The spines are organised in two lateral longitudinal rows that reach all the way to the genital pore in the anterior portion of the trunk. There is a ganglion at the base of the proboscis sheath, and the lemnisci is long. The testes are tandem. Each cement gland has two or more nuclei. Eggs are rounded with elliptical embryos. They are fish parasites. Molecular taxonomy also confirmed the generic identification of *Acanthogyryus*.

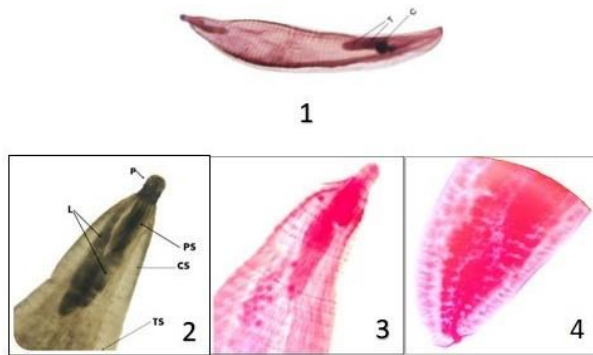


Fig.1: *Acanthogyryrus spp.* male whole worm T-Testes C-cement gland

Fig.2: *Acanthogyryrus spp.* male anterior view -TS-Trunk spines, L- Lemnisci long P-Proboscis, PH Proboscis hook

Fig. 3: *Acanthogyryrus spp.* female anterior view

Fig. 4: *Acanthogyryrus spp.* female posterior view

Population Analysis: Between April 2018 and March 2019, a total of 216 *C. catla* fish intestines were dissected, and 103 of these were found to be infected with *Acanthogyryrus spp.* Observations are made throughout the three succeeding seasons and the data is provided in below Table. The study was done during the summer, monsoon, and winter seasons of 2018 and the summer and winter seasons of 2019. Seasonal variations in *Acanthogyryrus spp.* infection prevalence, severity, and abundance were observed. Throughout the period of collection, *C. catla* from the Godavari River had a significant incidence of *Acanthogyryrus spp.* infection. According to one study, acanthocephalan actively reproduces throughout the year. Violante González et al. (2017). 103 fish out of the 216 evaluated in total (or 47.69%) had this acanthocephalan infection. At the end of February and March 2019, it was observed that the rate of infection started to increase, and in April and May 2018, data showed the highest prevalence, indicative of high infection in the summer. The prevalence starts to rise in November and December of 2018 and gradually increases in February and March of 2019. The rate of infection was relatively greater during the summer season. *Catla* fish breed in the months of May and June; this is the period when their immune systems must be compromised, which might be one of the reasons for the high prevalence percentage in these months. The infection rate began to decline in June 2018, during the monsoon, by 44%, and it reached its lowest point in September 2018, by 25%. Drop in temperature due to rains leads to a low metabolic rate and changes in other water parameters might not be supporting the transmission of acanthocephala. There are more zooplankton in the Godavari River's water body throughout the summer, or the pre-monsoon season, than there are during and after the monsoon season due to biological processes and human involvement. Zooplankton serves as both an intermediate host in the life cycle of Acanthocephalans and the primary food source for catla fish. Nalawade, P. M., Kadam, et al. Prevalence grew by 39% over the winter in October 2018 and reached a slight peak in December 2018 (55%), before declining once more in January 2019 (47%). The level of infection also displayed a distinct trend. By interfering with the life cycles of the parasite and its intermediate hosts as well as the metabolism of the ultimate host, our results show that environmental factors like temperature, relative humidity, and rainfall play a significant role in determining the density of parasitic infection. With a high in the summer, reaching (18) in May 2018, a medium low in the monsoon season, minimum (1.29) in August 2018, and low in the winter, January 2019. Highest abundance was reported (11.7) in May 2018, followed by the winters in 2018 and the monsoon in 2018, which had the lowest infections (0.44) in September 2018. In winter, the metabolic rate decreases, leading to a weak immune system of the host and therefore density is higher than monsoon but lower than summer.

PREVALENCE /MEAN INTENSITY/ABUNDANCE (Table)							
<i>Acanthogyris spp.</i>							
Season	Month of sample collection	Total number of hosts examined	Number of infested hosts	Prevalence	Total no. of parasites found	Mean intensity	Mean Abundance
				In %			
Summer	April 2018	18	11	61.11	180	16.36	10.00
	May 2018	20	13	65.00	234	18.00	11.70
Monsoon	June 2018	18	8	44.44	50	6.25	2.78
	July 2018	16	5	31.25	11	2.20	0.69
	August 2018	18	7	38.89	9	1.29	0.50
	September 2018	16	4	25.00	7	1.75	0.44
Winter	October 2018	18	7	38.89	41	5.86	2.28
	November 2018	21	10	47.62	75	7.50	3.57
	December 2018	18	10	55.56	100	10.00	5.56
	January 2019	19	9	47.37	107	11.89	5.63
Summer	February 2019	18	10	55.56	110	11.00	6.11
	March 2019	16	9	56.25	120	13.33	7.50
		216	103	47.69	1044	10.14	4.83



Fig.4: Graphs

4a: Prevalence, 4b: Mean Intensity, 4c: Mean Abundance respectively of *Acanthogyris spp.* in *Catla catla* fish from Gangapur dam during April 2018 till March 2019

DISCUSSION: Parasites are primarily host-specific. The study observed the presence of the sole endohelminth, *Acanthogyris* Thapar (1927), in the intestines of *C. catla*. Similar aspects were observed in the present investigation as well. In the current study, *Catla catla* fish were used to examine the seasonal prevalence and distribution of endoparasitic helminth. Acanthocephalan *Acanthogyris* sp. was abundant, along with very few sporadic occurrences of nematodes and cestodes. The parasite is found in the intestinal region. Extensive research on the seasonal fluctuations of the host's parasites has been conducted on various fish parasites.

As per Sanjeev kumar Verma et al (2018) Acanthocephalan parasites infection in *Channa punctatus* of river Gomti, Lucknow was studied during January 2015 to December 2015 and reported prevalence % and mean intensity of Acanthocephalan parasites as highest in summer (67.22%,0.67) and lowest in winter (38.75%,0.38). However, the highest relative density of Acanthocephalan parasites was in the summer season (0.72) and lowest in autumn season (0.50). The current study also shows that summer has the highest prevalence and mean intensity (65.00%; 18.00), as well as the highest mean abundance (11.7) and lowest mean abundance (0.44).

Khan, A. et al. (1991) found the presence of *P. ophiocephali* and *A. betwai* from *Channa striatus* and *Labeo rohita* from Kalri Lake, Pakistan. Between March and May, *P. ophiocephalis* showed a higher prevalence in *C. striatus*. In June and July, *L. rohita* displayed greater rates of *A. betwai* infection. The results of the current study differs in that *L. rohita* is associated with higher rates of *A. betwai* infection in June and July, while the infection rate begins to decline in the preceding months. Our study also demonstrates a higher prevalence of

Acanthogyryus in the period of March and May, which is similar to the high prevalence of *P. ophiocephalis* infection in *C. striatus* during the same period, i.e., between March and May.

Gupta, N. et al. (2012) According to this study on the population dynamics of *Pallisentis* in *C. straita* and *C. punctatus*, it was found that all parameters, including frequency, intensity, density, relative density, and infestation index, were highest at low temperatures, i.e., from November to March, in both males and females. The lowest prevalence was reported in summer (May 11.11%; June 7.14%), in contrast to the absolute prevalence reported in winter (February and March, 100%). The current study differs from their findings in that *Pallisentis* infection rates peaked in the winter, whereas *Acanthogyryus* infection rates peaked in the summer. Such unfavourable variations in prevalence rates could be caused by the host, parasite, and study location being highly diverse.

As per Kanth and Srivastava (1987), *Pallisentis ophiocephali* experienced two peak periods in May and August. Following that, the incidence of infection decreased steadily from September to February, then gradually increased through March to reach peaks in May and August. Current findings are consistent with the above work in that the rate of infection is extremely low during the monsoon season, which is September. The highest prevalence was observed in May in the studies conducted by Kanth and Srivastava, which are similar to the current research.

According to research done by Boping and Wenbing (2007) on the seasonal population of *Pallisentis (Neosentis) celatus* in the intestine of the rice field eel *Monopterus albus*, the parasite's prevalence exhibits a clear seasonal trend, peaking in the spring and declining in response to a drop in temperature. Additionally, no discernible seasonal changes in mean intensities were discovered. Similar findings were seen in the current investigation, when increased prevalence was noted in the summer months of February and March (the beginning of the summer season). And in terms of low prevalence throughout the winter, their findings matched ours.

Raju, P., described the seasonal population dynamics of *Pallisentis ophiocephali* (Acanthocephala) in *Channa striatus*. An investigation (from February 2019 to January 2021) shows prevalence of 37.42% and 43.48%, respectively. For the 2020–2021 year, the prevalence ranged from 22.91% (January 2012) to as high as 81.81%, with an average monthly prevalence of 22.64% (January 2019) to 63.33% (October 2019). (June, 2021). Rainy weather (53.8%) had the highest prevalence percentage, followed by summer (44.93%) and winter (31.72%). Our study results are dissimilar to theirs with respect to the rate of infection being highest at the beginning of the monsoon, whereas ours was low. However, the fact that the winter season had a lower prevalence than the summer season is similar to the current result.

CONCLUSION: It may be inferred from the current study that the acanthogyryus parasite infestations in *Catla catla* varied monthly. The change in seasons brings about the change in physicochemical characteristics of water bodies. The study also supports the importance of temperature in influencing the epidemiology of parasitic fauna either directly or indirectly. The prevalence and intensity depend on several parameters, such as the type of parasite and the host's feeding patterns. There are other important factors that may be emphasized which might affect a parasite's ability to transmit disease depending on the season and sex of the host. Due to the importance of temperature on their metabolic processes, aquatic organisms react immediately to changes in the environment.

Environment quality and fish illness state have a significant and inverse link in aquatic environments. One of the most important constraints for aquaculture is the presence of parasites. The seasonal fluctuations of Acanthocephalan parasites in *C. catla* are examined in this study. The research aims for understanding host-parasite interactions, which will benefit the timely management of helminthic diseases more effectively to increase quality fish farms. Many applications and implications in aquaculture and fisheries can emerge from studies on acanthocephalans found in freshwater food fish. The management and conservation of these host fish species can be benefited from awareness of the presence and effects of Acanthocephala parasites on freshwater food fish populations. The food safety division can be informed about potential health hazards associated with these parasites by studying them. Future research can be done to determine how *Acanthogyryus* affects the health and behaviour of their host fish because such a change could affect the ecological balance of freshwater ecosystems as a whole. Understanding how these parasites evolved and adapted to various host species and habitats can be gained from studying them.

ACKNOWLEDGEMENTS: The authors are thankful to the Director, Head of the Department of Zoology, and the Institute of Science in Mumbai for providing the lab facility for this work.

REFERENCES:

- 1) Crompton, D. W. T., Crompton, D. W. T., & Nickol, B. B. (Eds.). (1985). *Biology of the Acanthocephala*. Cambridge University Press.
- 2) Schmidt, G. D., & Nickol, B. B. (1985). *Development and Life Cycles*: [Chapter 8 in *Biology of the Acanthocephala*].
- 3) Bhutekar, D. D., Aher, S. B., & Babare, M. G. (2018). SPATIAL AND SEASONAL VARIATION IN PHYSICO-CHEMICAL PROPERTIES OF GODAVARI RIVER WATER AT AMBAD REGION, MAHARASHTRA. *Journal of Environment and Biosciences*, 32, 15-23.
- 4) Rohlenová, K., Morand, S., Hyršl, P., Tolarová, S., Flajšhans, M., & Šimková, A. (2011). Are fish immune systems really affected by parasites? An immunoeological study of common carp (*Cyprinus carpio*). *Parasites & vectors*, 4(1), 1-18.
- 5) Dubinina, M. N. (1980). Tapeworms (Cestoda, Ligulidae) of the fauna of the USSR. Tapeworms (Cestoda, Ligulidae) of the fauna of the USSR.
- 6) <https://fisheries.maharashtra.gov.in/sites/default/files/Fish%20production%20report%202020-21.pdf>
- 7) Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., & Schad, G. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *The Journal of parasitology*, 68(1), 131-133.
- 8) Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *The Journal of parasitology*, 575-583.
- 9) Violante-González, J., Villalba-Vásquez, P. J., Monks, S., García-Ibáñez, S., Rojas-Herrera, A. A., & Flores-Garza, R. (2017). Reproductive traits of the acanthocephalan *Neoechinorhynchus brentnickoli* in the definitive host. *Invertebrate Biology*, 136(1), 5-14.
- 10) Nalawade, P. M., Kadam, V. B., & Bagul, A. B. Study of Impact of Seasonal Variation in Water Quality on Planktons Population Found in River Godavari.
- 11) Sanjeev Kumar Verma, Sita Yadav, AM Saxena (2018) An ecological analysis of Acanthocephalan parasites of *Channa punctatus* of river Gomti, Lucknow, Uttar Pradesh, India. *International Journal of Zoology Studies* ISSN: 2455-7269.
- 12) Khan, A., Bilquees, F. M., & Shaukat, S. S. (1991). Seasonal variation in the occurrence of *Pallisentis ophiocephali* and *Acanthosentis betwai* (Acanthocephala) in relation to their fish hosts. *Angewandte Parasitologie*, 32(3), 165-171.
- 13) Gupta, N., Singhal, P., & Gupta, D. K. (2012). Population dynamics of a parasite *Pallisentis* in two species of fish *Channa punctatus* and *Channa striatus*. *Journal of environmental Biology*, 33(2), 195.
- 14) Kanth, L. K., & Srivastava, L. P. (1987). The occurrence of *Metaclinostomum srivastavai* (Pandey and Baugh, 1969) (Digenea), *Genarchopsis goppo* (Tubangui) Ozaki, 1925 (Digenea) and *Pallisentis ophiocephali* (Rai, 1967) (Acanthocephala) in the fresh water fish, *Channa punctatus* (Block). *Indian journal of parasitology*, 11(2), 155-157.
- 15) Boping, Z., & Wenbin, W. (2007). Seasonal population dynamics of *Pallisentis* (*Neosentis*) *celatus* (Acanthocephala: Quadrigyridae) in the intestine of the rice-field eel *Monopterus albus* in China. *Journal of helminthology*, 81(4), 415-420.
- 16) Raju, P. Population dynamics of *Pallisentis ophiocephali* in *Channa striata* from Karimnagar, Telangana, India.
- 17) Bhattacharya, S. B. (2007). *Handbook on Indian Acanthocephala*. Zoological Survey of India.
- 18) Rekha, G. B. Morphology and Morphometry of Acanthocephalan, *Acanthogyrus* *Acanthogyrus* found in Fish Host *Catla catla* of Hyderabad and Rangareddy Dist. 2016
- 19) Marcogliese, D. J. (1995). The role of zooplankton in the transmission of helminth parasites to fish. *Reviews in fish Biology and fisheries*, 5(3), 336-371.
- 20) Didier, B. B. E., Armel, M. J., & Félix, B. B. C. (2020). Epidemiological study of *Acanthogyrus tilapiae*, gut parasitic helminth of *Hemichromis elongatus* in the Mefou hydrographic system (South-Cameroon): Effect of the environment. *Statistics*, 2(119.7), 1-5E.
- 21) Eure, H. (1976). Seasonal abundance of *Neoechinorhynchus cylindratus* taken from largemouth bass (*Micropterus salmoides*) in a heated reservoir. *Parasitology*, 73(3), 355-370.
- 22) Radwan, N. A., El Gamal, M. M., Fayed, R. H., & Khalil, A. I. Ultrastructure and occurrence of *Acanthogyrus* (*Acanthosentis*) *tilapiae* (Acanthocephala: Quadrigyridae) from *Oreochromis niloticus*, Nile River, Egypt.
- 23) Amin OM. 1998. Marine Flora and Fauna of the Eastern United States (Acanthocephala). NOAA Technical Report NMFS 135. The Scientific Publications Office, National Marine Fisheries Service, NOAA, pp. 1-28. (Also methods given for staining).

- 24) Gautam, N. K., Misra, P. K., & Saxena, A. M. (2018). Seasonal variation in helminth parasites of snakeheads *Channa punctatus* and *Channa striatus* (Perciformes: Channidae) in Uttar Pradesh, India. *Helminthologia*, 55(3), 230.
- 25) Sakthivel, A., & Gopalakrishnan, A. (2020). Prevalence and pathology manifestation of *Acanthocephalus ranae* infestation in finfishes of Tamil Nadu, southeast coast of India. *World News of Natural Sciences*, 33, 1-19.
- 26) Chakraborty, R. D., & Murty, D. S. (1972). Life history of Indian major carps *Cirrhinus mrigala* (Ham.), *Catla catla* (Ham.) and *Labeo rohita* (Ham.). *J. Inland Fish. Soc. India*, 4, 132-161.
- 27) Thapar, G.S. (1927). On *Acanthogyrus* n.g. from the intestine of the Indian fish *Labeo rohita* with a note on the classification of the *Acanthocephala*. *J. Helminthol.*, 5 : 109-120.
- 28) George, P. V., & Nadakal, A. M. (1973). Studies on the life cycle of *Pallisentis nagpurensis* Bhalerao, 1931 (*Pallisentidae*; *Acanthocephala*) parasitic in the fish *Ophiocephalus striatus* (Bloch). *Hydrobiologia*, 42(1), 31-43.
- 29) White, K. A. J., Grenfell, B. T., Hendry, R. J., Lejeune, O., & Murray, J. D. (1996). Effect of seasonal host reproduction on host-macroparasite dynamics. *Mathematical biosciences*, 137(2), 79-99.
- 30) Lizama, M. D. L. A. P., Takemoto, R. M., & Pavanelli, G. C. (2006). Influence of the seasonal and environmental patterns and host reproduction on the metazoan parasites of *Prochilodus lineatus*. *Brazilian Archives of Biology and Technology*, 49, 611-622.
- 31) Rauque, C. A., Semenas, L. G., & Viozzi, G. P. (2006). Seasonality of recruitment and reproduction of *Acanthocephalus tumescens* (*Acanthocephala*) in fishes from Lake Moreno (Patagonia, Argentina). *Journal of Parasitology*, 92(6), 1265-1269.
- 32) Shaikh Abdullah, S., & Lohar, P. S. (2011). Biochemical composition and gonadosomatic index of three major carps in Hatnoor reservoir, Maharashtra, India. *Journal of Ecobiotechnology*, 3(6), 01-04.
- 33) White, K. A. J., Grenfell, B. T., Hendry, R. J., Lejeune, O., & Murray, J. D. (1996). Effect of seasonal host reproduction on host-macroparasite dynamics. *Mathematical biosciences*, 137(2), 79-99.
- 34) Lizama, M. D. L. A. P., Takemoto, R. M., & Pavanelli, G. C. (2006). Influence of the seasonal and environmental patterns and host reproduction on the metazoan parasites of *Prochilodus lineatus*. *Brazilian Archives of Biology and Technology*, 49, 611-622.

