

Aspects of the Ecology of Proteocephalid Cestode Parasites of *Hoplobatrachus tigerinus* (Daudin, 1803) and *Duttaphrynus melanostictus* (Schneider, 1799) from YSR (Kadapa) District, Andhra Pradesh, India

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MH and CSK collected the host samples and parasites and prepared the initial manuscript. Author AV framed and formatted the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/29053

Editor(s):

(1) J. Rodolfo Rendón Villalobos, Department of Technological Development, National Polytechnic Institute, México.

Reviewers:

(1) Mohamed Moussa Ibrahim, Suez Canal University, Egypt.

(2) Serdar Dusen, Pamukkale University, Turkey.

(3) Rewaida Abdel-Gaber, Cairo University, Egypt.

Complete Peer review History: <http://www.sciencedomain.org/review-history/16724>

Short Research Article

Received 20th August 2016
Accepted 18th October 2016
Published 28th October 2016

ABSTRACT

The Indian bull frog, *Hoplobatrachus tigerinus* Daudin, 1803 and Asian common toad, *Duttaphrynus melanostictus* Schneider, 1799 are frequently found to be infected with the proteocephalid cestodes. The seasonal dynamics of the *Proteocephalus* sp. was studied in both amphibians during February, 2013 to January, 2015 from YSR (Kadapa) District, Andhra Pradesh. Of the total 300 *H. tigerinus* examined, only 18 frogs (6%) were infected with the cestode, *Proteocephalus tigrinus* and of the 46 *D. melanostictus* examined, only 15 frogs (32.6%) were found to be infected with *Proteocephalus* sp. Intensity of infection ranged from 1 to 11 (n=31) in *H. tigerinus* and 1 to 2 (n= 19) in *D. melanostictus*. Monthly population dynamics of cestodes of *H. tigerinus* and *D. melanostictus* were analysed in terms of prevalence, mean intensity, mean abundance and index of infection. The effect of habitat predilection and the impact of season on the parasitic load were analysed. The impact of host size and sex on the intensity of infection was also studied.

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Keywords: *Hoplobatrachus tigerinus*; *Duttaphrynus melanostictus*; *proteocephalus*; prevalence; mean intensity; mean abundance; index of infection.

1. INTRODUCTION

Parasitism is the one of the most common ecological relationships representing a complex web of interactions among hosts. Host represents a resource and a habitat where the parasite can survive. Amphibians serve as definitive, intermediate, or paratenic hosts for many macroparasites such as trematodes, cestodes, nematodes, acanthocephalans and leeches in aquatic and terrestrial food webs. Individual frogs and toads harbours a dozen of macroparasite species in thousands of number [1,2]. The class Cestoda is one of the major and widespread parasitic classes of the phylum Platyhelminthes infecting all classes of vertebrates around the world. The cestodes of the genus *Proteocephalus* are reported to occur in freshwater fishes, amphibians and reptiles. The work on proteocephalid cestodes was contributed from all over the world by [3-26] from various vertebrate hosts. Proteocephalid cestodes were the only cestode parasites obtained from two species of amphibians, *Hoplobatrachus tigerinus* and *Duttaphrynus melanostictus* during the present parasitological survey. In the present study, *Proteocephalus tigrinus* was reported from *H. tigerinus* where as *D. melanostictus* showed infection with *Proteocephalus* sp. whose species name is not identified in the present study. This study was mainly focused on the various aspects of ecology of the proteocephalid cestodes from these two hosts which will provide a comprehensive knowledge about their seasonal occurrence and community structure in these hosts.

2. MATERIALS AND METHODS

2.1 Sampling Sites

Four different localities where natural vegetation's are disturbed by anthropogenic activities were selected:

- Site 1:** Industrial Estate area (Lat. 14°47'N 78°76'E, 138 meters Altitude), YSR (Kadapa) district.
- Site 2:** Ramapuram village (14.05°N 78.75°E, 143 meters), Raychoti and
- Site 3:** Campus area of Yogi Vemana University (Lat.14°28'N 78°49'E, 137 m Altitude), located in YSR Kadapa District of Andhra Pradesh.

Site-4: Bouinpalli village, Kadapa.(Lat.14°28'N 78°52'E, 379 meters).

All four sites were sampled frequently for two years, February, 2013 to January, 2015 for the experimental hosts, *H. tigerinus* (n=300) and from September, 2013 to September, 2014 for *D. melanostictus* (n=46) from YSR (Kadapa) district, Andhra Pradesh, India.

Various parameters such as sex, snout-vent length and weight of the each amphibian were recorded for ecological studies according to standard procedure. Amphibians were collected and brought to the laboratory, dissected and examined for the parasites. The animals were dissected and various organs such as oesophagus, stomach, intestine and rectum were expansively examined for the cestode parasites. The tissues of the amphibians were dissected out separately in petri dish containing 0.7 percent saline solution. A number of adult cestodes were obtained from both the hosts which were easily identified due to their ribbon like body and its small, round scolex with four suckers. Parasite was carefully segregated from the intestinal walls without causing any damage to the parasite [8,27]. (Anatomical and morphological characters of the cestode parasites were observed under the Lynx trinocular microscope (N-800M) and figures were drawn with the aid of attached drawing tube. Ocular micrometer measurements of the parasite were in Micrometers unless otherwise stated. Pearson's coefficient of correlation 'r' was applied to study the relationship between host's snout-vent length and parasitization. The influence of host sex on the parasitic abundance and prevalence of parasites was analysed by applying Mann-whitney (Vassarstat.net/utest.html). The quantitative and qualitative analysis of the data and various statistical calculations such as prevalence, mean intensity, mean abundance, standard deviation, correlations, t-tests, Chi-square tests were carried out with Microsoft Excel (2007) and SPSS IBM 21 Version and standard statistical books [28-31]. The ecological terminology in parasitology was adopted from [32].

3. RESULTS AND DISCUSSION

3.1 Monthly Population Dynamics

The seasonal occurrence of the cestode species in *H. tigerinus* was analysed from February, 2013

to January, 2015 and the seasonal occurrence of *Proteocephalus* sp. was analysed in *D. melanostictus* from September, 2013 to September, 2014 because of their less obtainability. Monthly population dynamics of Protocephalid cestode of *H. tigrinus* and *D. melanostictus* was investigated in terms of prevalence, mean intensity and mean abundance. For *H. tigrinus*, the two annual cycles are slightly different with highest overall prevalence in the month of October, moderate in November, and January for 2013-14 cycle while the rest of the months showed no infection (Fig. 1a). However, 2014-2015 cycle showed a different track with highest prevalence being in the month of April, moderate in March, September, November and December. Mean intensity was highest in January, moderate in October and November for first annual cycle while rest of the months showed no infection. 2014-2015 cycle showed variation from the previous cycle with highest mean intensity being in the months of March, April, September and November and slightly moderate values in May and December and no infection in the rest of the months (Fig. 1b). Mean abundance and index of infection was high in October for 2013-14 cycle while it was high in April for 2014-2015 cycle with rest of the months showing zero to negligible infection for both cycles (Figs.1c and 1d). For *D. melanostictus*, the prevalence of *Proteocephalus* sp. infection was highest in September, 2014 while it is moderate to low in September and 2013, March, April, June and July 2014 (Fig. 2a). Mean intensity was high in September 2013 and August 2014, moderate in November 2014 (Fig. 2b) whereas mean abundance and index of infection was high in the months of September, 2013 and August, moderate in February and May 2014 and low in December, January 2013 and June, July and August 2014 (Figs. 2c and 2d). These variations are due to various characteristics and inconsistency of collection of the host species in sufficient numbers.

3.2 Seasonal Dynamics

H. tigrinus showed highest prevalence of infection during rainy and lowest during winter for 2013-14 cycle whereas it was highest during winter and lowest during summer for 2014-15 cycle (Fig. 3a). These inconsistent results show that seasons doesn't show any impact on the rate of parasitization. However, *D. melanostictus* showed highest

prevalence of infection during rainy and lowest during summer which might be due to the low metabolic activities of the host during the aestivation period during summer season (Fig. 3b).

3.3 Community Structure

Of the total 300 *H. tigrinus* examined, only 18 frogs (6%) were infected with *Proteocephalus tigrinus* and of the 46 *D. melanostictus* examined, only 15 frogs (32.6%) were found to be infected with *Proteocephalus* sp. The prevalence of *Proteocephalus tigrinus* in *H. tigrinus* was 6%, with a mean intensity of 1.72 ± 1.21 , mean abundance 0.10 ± 0.07 and index of infection 0.006 whereas the prevalence of *Proteocephalus* sp. infection was 32.6%, with mean intensity (1.26 ± 0.89), mean abundance (0.41 ± 0.28) and index of infection (0.134) for *D. melanostictus* (Table 1). Intensity of infection ranged from 1 to 11 (n=31) in *H. tigrinus* and 1 to 2 (n= 19) in *D. melanostictus*.

The term locality refers to a geographic milieu of the external environment from where the parasite is segregated. It refers to the geographic spot from where the individual population of community is attained. Locality serves as one of the vital ecological factors playing a momentous role in the occurrence of parasitic species [33-45,14,22,23].

Out of the four major sites taken under study, incidence of *P. tigrinus* infection was high for the *H. tigrinus* examined from Industrial estate (Site-1). Of the 195 hosts examined from site-1, only 14 hosts (7.17%) were parasitized (n=24), with mean intensity of 1.71 and the parasitic load was almost nil for *D. melanostictus* from this site. Site-2: Ramapuram village, Raychoti and Site-3: University campus area, Kadapa showed least parasitic incidence. However, site-4: Bouinpalli village, Kadapa showed highest parasitic incidence in *D. melanostictus*. Of the 13 hosts examined from site-4, 7(53.8%) were infected (n=10) with mean intensity 1.42 and the parasitic load was nil for *H. tigrinus* from this site (Table-2). Only site-1 and site-4 seems to be the preferred habitats for *Proteocephalus* sp. infestation during the present survey. The above outcomes signify the preference of the habitat in the incidence of the *Proteocephalus* sp. in both the hosts. Hence, locality from where the host was collected plays an influential role in the occurrence of the parasite.

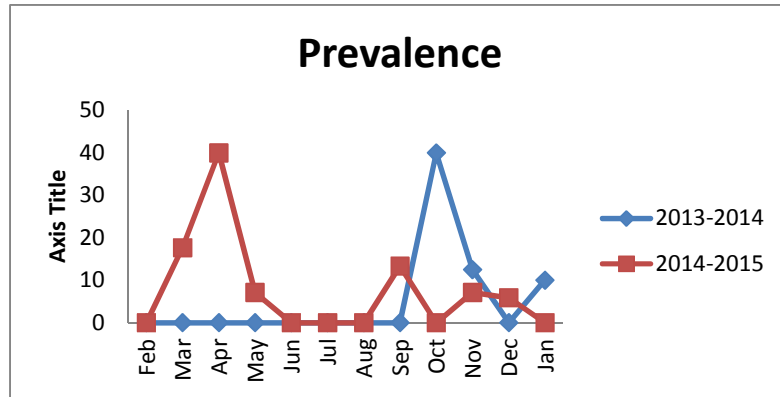


Fig. 1a. Prevalence of cestode in *H. tigrinus*

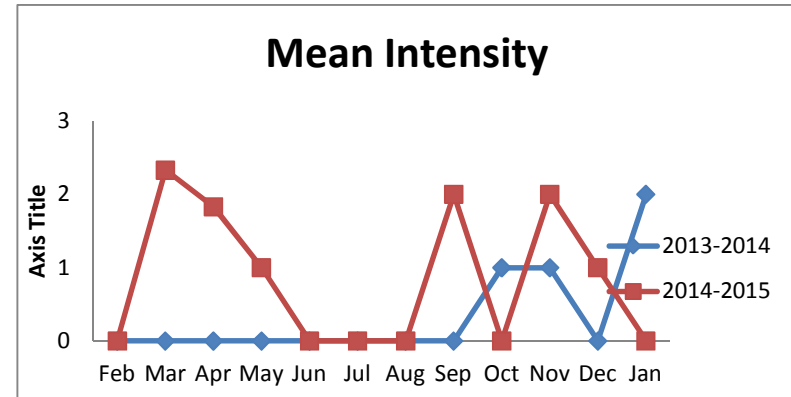


Fig. 1b. Mean intensity of cestode in *H. tigrinus*

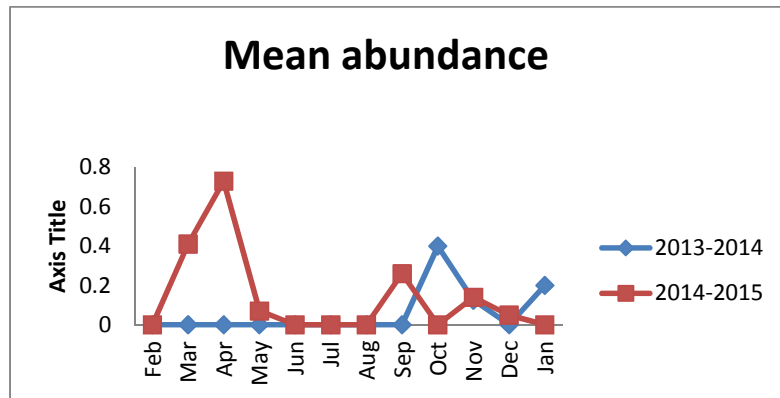


Fig. 1c. Mean abundance of cestode in *H. tigrinus*

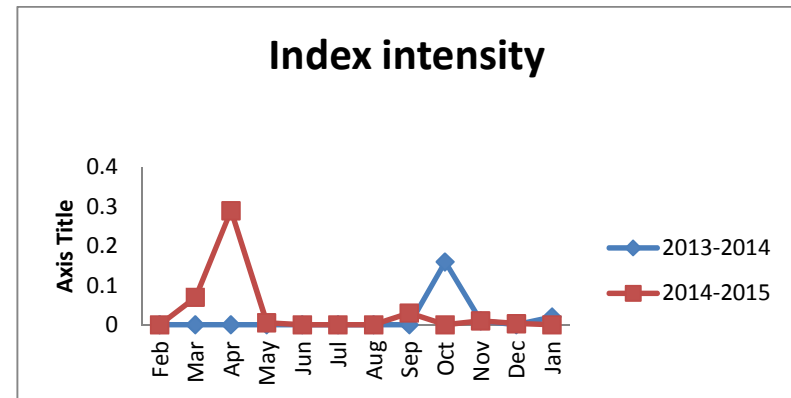


Fig. 1d. Index of intensity of cestode in *H. tigrinus*

Fig. 1. Monthly population dynamics of cestode parasites in *H. tigrinus*
 a) prevalence; b) Mean intensity; c) Mean abundance; d) index of infection

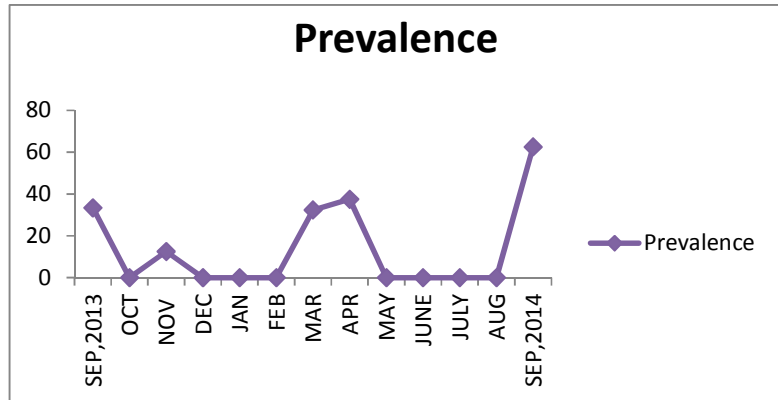


Fig. 2a. Prevalence of cestode in *D. melanostictus*

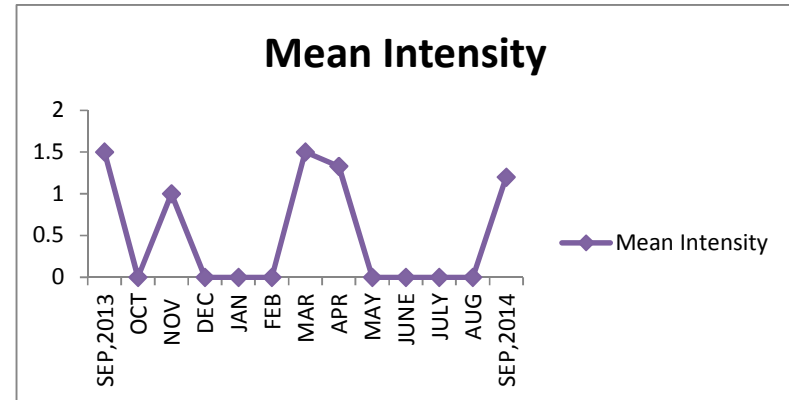


Fig. 2b. Mean intensity of cestode in *D. melanostictus*

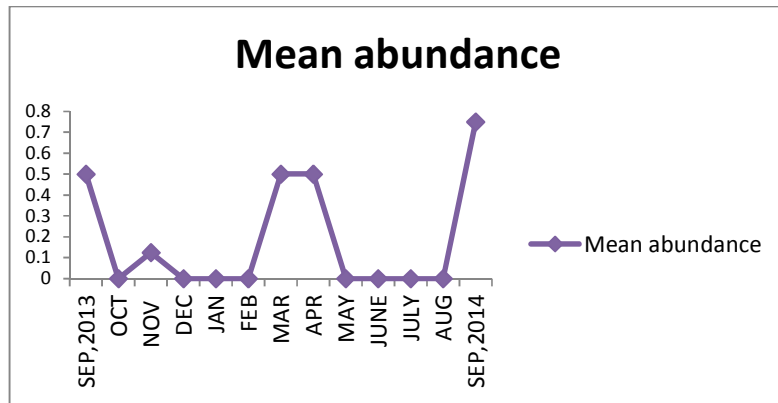


Fig. 2c. Mean abundance of cestode in *D. melanostictus*

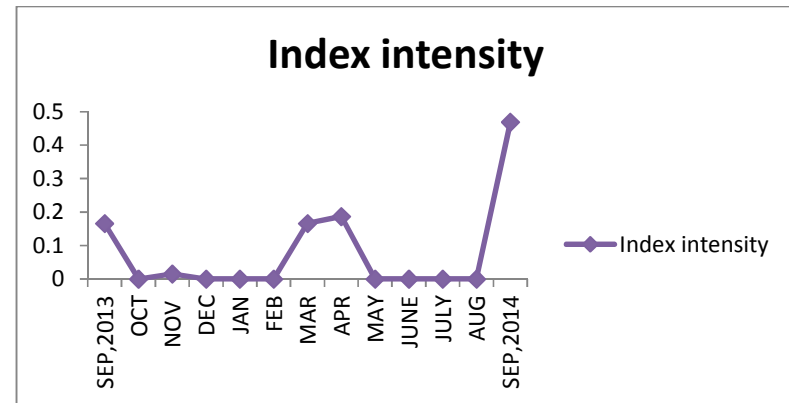


Fig. 2d. Index of intensity of cestode in *D. melanostictus*

Fig. 2. Monthly population dynamics of cestode parasites in *D. melanostictus* prevalence; b) Mean intensity; c) Mean abundance d) Index of infection

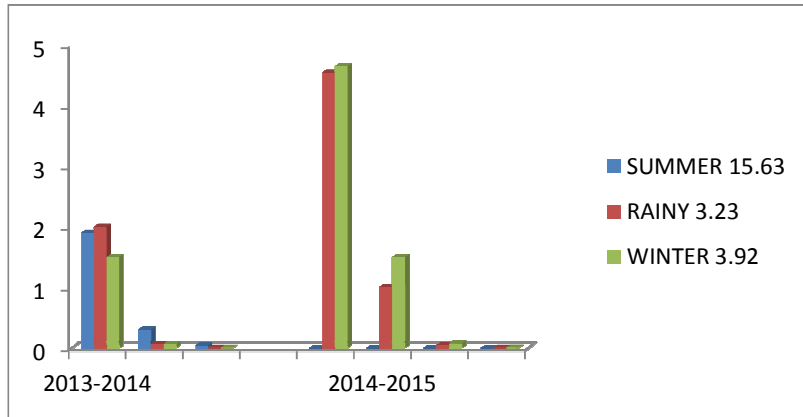


Fig. 3a. Seasonal dynamics of cestode in *H. tigerinus*

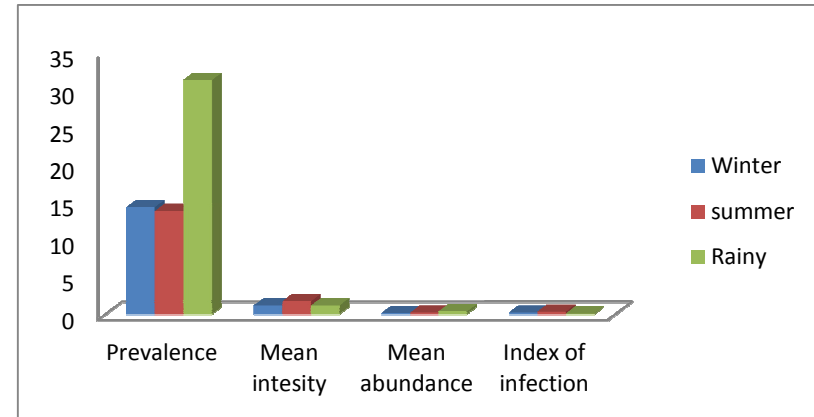


Fig. 3b. Seasonal dynamics of cestode in *D. melanostictus*

Table 1. Diversity parameters and distribution pattern of *Proteocephalus* sp. in *H. tigerinus* and *D. melanostictus*

Name of host	Infected frogs	Total no. of parasites	Prevalence (%)	Mean Intensity	Mean abundance	Index of infection	Range	Location
<i>H. tigerinus</i>	18	31	6	1.72±1.21	0.10±0.07	0.006	1-11	Intestine
<i>D. melanostictus</i>	15	19	32.6	1.26±0.89	0.41± 0.28	0.134	1-2	Intestine

Table 2. Infectivity of cestode parasites in *H. tigerinus* and *D. melanostictus* from different localities

Locality	No. of hosts examined (a)	Parasites recovered								
		<i>Hoplobatrachus tigerinus</i>				<i>Duttaphyrnus melanostictus</i>				
		No. of infected hosts (b)	No. of parasites (c)	Prevalence (%)	Mean intensity	No. of hosts examined (a)	No. of infected hosts	No. of parasites	Prevalence (%)	Mean intensity
Site-1: Industrial Estate, Kadapa	195	14	24	7.17	1.71	-	-	-	-	-
Site-2: YVU campus	45	2	4	4.44	2	15	03	4	20	1.3
Site-3: Ramapuram	60	2	3	3.33	1.5	18	5	5	27.7	1
Site-4: Bouinpalli	-	-	-	-	-	13	7	10	53.8	1.42

Table 3. Correlation coefficient (r) between size and parasitic number of *Proteocephalus tigrinus* in *H. tigrinus*

Sl. no.	Size groups	Class intervals	No. of parasites	Correlation coefficient (r)
1	Group-I	4-9 cm	23	r = -0.1283
2	Group-II	9-14 cm	8	
3	Group-III	14-19 cm	0	

Table 4. Correlation coefficient (r) between size and parasitic number of *Proteocephalus* sp. in *D. melanostictus*

Sl. no.	Size groups	Class intervals	No. of parasites	Correlation coefficient (r)
1	Group-I	3-5 cm	6	r = -0.1521
2	Group-II	5-7 cm	11	
3	Group-III	7-9 cm	2	

Table 5. Diversity parameters of parasitic species in males and females and values of Mann-Whitney U-test to evaluate rate of host sex and parasitic abundance in *H. tigrinus* and *D. melanostictus*

Host name	<i>Proteocephalus</i> sp.								Mann –Whitney U test (Z)		
	N _{mi}	N _{fi}	P _m	P _f	MI _m	MI _f	MA _m	MA _f	Z (U)	P ₁ (significance level)	P ₂ (significance level)
<i>H. tigrinus</i> (N _m = 167, N _f = 133)	10	6	5.98	4.51	2.16	1.8	0.09	0.10	0.38	0.352	0.703
<i>D. melanostictus</i> (N _m =24, N _f =22)	9	6	37.5	27.2	1.22	1.33	0.45	0.36	0.52	0.301	0.603

*N_m = Number of males examined; N_f = Number of females examined; N_{mi} = Number of males infected;

N_{fi} = Number of females infected; P_m & P_f = Prevalence of males and females respectively;

MI_m & MI_f = Mean intensity of males and females; MA_m & MA_f = mean abundance of males and females respectively

3.4 Effect of Host Size on the Cestode Parasitization

Length of the hosts is considered to be one of the key factors in parasite infra population variation [46,47,13]. *H. tigerinus* measured 4-18.5 cm (mean= 10.15±7.17) in total length with average total snout-vent length of male (10.19±7.20 cm, n=167) and female (10.1±7.20 cm, n=133) in *H. tigerinus* where as *D. melanostictus* measured 3-9 cm (mean = 5.89±4.16) in total length. The average total snout-vent length of female (4.79±3.39 cm, n=22) and males (0.45±0.32 cm, n=24) frog in the sample were not significantly different. Pearson's Correlation coefficient 'r' was chosen to study the possible relationship between host size and cestode parasitization. The negative computed value 'r' -0.1283 in *H. tigerinus* and 'r'-0.152 in *D. melanostictus* proves that there is no influence of host size on the cestode parasitization (Tables-3 & 4). Small sized frogs (Group-1) shows infection rate compared to (Group-2 and Group-3) in *H. tigerinus* (Table-3). Medium sized toads (Group-2) showed high parasitization compared to (Group-1 and Group-3) in *D. melanostictus* (Table-4). Younger amphibians are more susceptible to parasite infection than the older ones. The present study is in accordance with the views of [48,49] who reported that the penetration of parasite larvae is easier in younger ones than older ones.

3.5 Effect of Host Sex on the Cestode Parasitization

Prevalence of parasites with respect to host sex is unpredictable as few reports imply that males show more infection than females while some report that females are parasitized than males. Also some studies suggest that there is no influence of host sex on parasitization. The present study is in total agreement with the views of [50-54] who opined that males show more infection due to high levels of testosterone which cause immune suppression making them more susceptible for infection than females. According to Mann-Whitney Z(U) test, there is little significant relation between host sex and parasite abundance in *H. tigerinus* (Z=0.38, P1=0.352, P2=0.703) and in *D. melanostictus* (Z=0.52, P1=0.301, P2=0.603) (Table-5). Males are slightly more infected than the females for both the hosts. The significant values with respect to sex from Z (U)-test might be due to the behavioral and physiological dissimilarity between the male and female hosts.

4. CONCLUSION

The present study showed a perceptible distinction in terms of seasonal occurrence and the parasitization of cestodes which might be due to the host biology and behavior, their feeding habits and habitat and immense diversity in the environmental conditions.

ACKNOWLEDGEMENTS

First author is grateful to University Grants Commission for providing the financial assistance as JRF and SRF under UGC-RGNF scheme (F1-17.1/2011-12/RGNF-SC-AND-5015).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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