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ANALYSIS

Occurrence and Seasonal dynamics of the digenean, *Tremiorchis ranarum* Mehra et Negi, 1926 (Digenea: Plagiorchiidae) in the Indian bull frog, *Hoplobatrachus tigerinus* Daudin, 1803 from Kadapa District, Andhra pradesh, India

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ABSTRACT

The Indian bull frog, *Hoplobatrachus tigerinus* Daudin, 1803 is frequently found to be infected with the digenean, *Tremiorchis ranarum* Mehra et Negi, 1926. The seasonal dynamics of the *T.ranarum* was studied in the frog during February, 2013 to February, 2014 from Kadapa District, Andhra Pradesh. Of the total of 130 frogs examined, only 18 frogs (13.84%) were infected with this digenean (n=530). Intensity of infection ranged from 1 to 229, with mean intensity (29.44±20.82), mean abundance (4.07±2.88) and index of infection (0.56). Monthly population dynamics of *T. ranarum* of *H. tigerinus* was analyzed in term of prevalence, mean intensity and mean abundance. Medium sized and larger frogs showed more infection and there is effect of sex on the parasitic infection of *T. ranarum*. Males are more infected when compared to females.

Keywords: *Hoplobatrachus tigerinus, Tremiorchis ranarum,* prevalence, mean intensity and meanabundance.

1. INTRODUCTION

Wild amphibian populations and their natural habitats are at stake due to global changes and severe pollution throughout the world which may also destroy their parasite biodiversity or may amplify the effect of pathogen and their diseases (Taylor et al., 2005; McKenzie, 2007; Tinsley et al., 2012). There are several areas in India, in particular in Southern India especially Kadapa where the biodiversity of metazoan parasites in vertebrate hosts is poorly known. Amphibians are one of the vertebrate hosts affected by the human activities. Amphibian hosts serve as the most preferred vertebrate hosts for a number of metazoan parasites like digeneans, monogeneans, cestodes, nematodes, acanthocephalans and protozoans. *Hoplobatrachus tigerinus* of the family Dicroglossidae Anderson, 1871 is the commonly occurring aquatic species of anurans inhabiting the natural and artificial (paddy fields) wetland areas of India (Alexander, 2011). *Tremiorchis ranarum* Mehra et Negi, 1925 is one of the most prevalent digenetic parasite infecting the Indian Bull frog, *Hoplobatrachus tigerinus.* In the present investigation, plagiorchiid digenean *T. ranarum* was collected from the intestine of *H. tigerinus* from various places of Kadapa district of Andhra Pradesh to analyze their seasonal dynamics. An appreciable amount of work has been contributed worldwide on metazoan parasites of Amphibians (Mehra et Negi,1926, 1928; Bhalerao,1926; Verma, 1930; Srivastava,1933; Fishchthal and Thomas, 1968, Fotedar, 1959a, 1959b; Agarwal,1966, 1968; Jahan, 1973; Khan and Mohiuddin,1968; Pandey and Chakravarti 1968; Mukerjee and Gosh 1970a, b, 1972; Pandey, 1973; Rao and Kameshwari, 1976; Simha, 1977; Hafeexullah and Dutta 1981, 1985; Diengdoh 1989; Diengdoh and Tandon,1991; Prudhoe and Bray 1982; Verma and Singh, 2000; Luque *et al*., 2005; Lemke *et al*., 2008; Schotthofer *et al*., 2009; Mohammad *et al*., 2010). However, only a negligible amount of work has been carried out on the seasonal dynamics, morphological intra-specific variations and life-cycles of *T.ranarum* from India (Mehra et Negi, 1926, 1928; Bhalerao, 1926; Verma, 1930; Bhardwaj, 1962; Ali and Karyakarte, 1970; Dwivedi and Chauhan, 1970; Sinha and Sahay, 1971, Pandey, 1973; Kalyanker and Palladwar, 1978; Karyakarte and Baheti, 1980; Pandey and Agarwal, 1981; Madhavi et al., 1987; Ratnakumari; 2006 a, 2006 b; Hafeexullah and Dutta 1981; Rajendran and Janardanan, 1993; Sah and Sahay, 1998; Pandey et al., 2013). Insufficient amount of work has been contributed from Southern peninsular India and these sites require an additional revision or new comprehensive approach, in particular Kadapa region of Andhra Pradesh. Hence, a serious attempt was made to study the occurrence and seasonal dynamics in *T. ranarum* in *H. batrachus*.

2. MATERIALS AND METHODS

Sampling Sites

Kadapa District (Lat. 14°28´N 78°49´E, 137 m Altitude), located in Andhra Pradesh state was sampled during February, 2013 to February, 2014. The fieldwork was based in an Industrial estate areas, Ramapuram village and University campus areas where natural vegetations are disturbed by anthropogenic activities. *Hoplobatrachus tigerinus* (n=130), procured from these areas were transported alive to the laboratory and were anaesthesized before dissecting to examine the parasites under the stereozoom microscope (LM-52-3621 Elegant) from February, 2013 to February, 2014. Snout-vent length and sex of each frog was note down carefully and all organs were examined discretely for collection and counting. *H. batrachus* measured 4-18.5 cm (mean= 10.97±3.78) in total length. The average total snout-vent length of female (11.15±3.84 cm, n=46) and male (10.88±3.77 cm, n=84) frog in the sample were not significantly different (t= 0.69). Pearson's coefficient of correlation 'r' was applied to study the relationship between host's snout-vent length and parasitisation. The influence of host sex on the abundance and prevalence of parasites was analysed by applying Chi-square test. During the present study, only 530 parasites were collected which were cleansed thoroughly byordinary tap water, killed and fixed quickly under pressure of the cover glass by using a fixative,A.F.A (85:10:5) for 24 hours and were washed thoroughly in running tap water for few minutes and stained with alum caramine. The specimens after being stained with Alum caramine were briefly washed in tap water and dehydrated through series of graded alcohols (70%, 90%, 95% and 100%) cleared in xylol and finally mounted in Canada balsam or DPX Mountant and due care is taken to avoid the formation ofair bubbles during and after mounting (Hiware et al., 2003; Madhavi et al., 2007). Specific characterizations were carefully observed under the Lynx Trinocular microscope (N-800M) and figures are drawn with the aid of attached drawing tube. Conventional terms are used to describe the species. All measurements will be taken with the aid of an ocular micrometer in millimeters.Quantitative and qualitative analysis of the data were carried out by following the standard statistical books (Snedecor and Cochran, 1967; Sundara Rao and Richards, 1996; Daniel, 1998) and the statistical computations like prevalence, mean intensity, mean abundance, standard deviation were carried out using Microsoft Excel (Office 2007). Ecological terminology is as suggested by Margolis et al., 1982 and Bush et al., 1997.

3. RESULTS AND DISCUSSION

Taxonomic summary

***Host**: Hoplobatrachus tigerinus* Daudin, 1926
Infection site: Intestine
Collection site: Kadapa

Body 2.2-4.10× 0.47-1.07, small backwardly directed spines extend from anterior end to little posterior to hind end of the posterior testis. Cuticle thick and smooth.Oral sucker 0.12- 0.35 × 0.14-0.35. Prepharynx small, thin walled; pharynx 0.1-0.15× 0.1-0.15. Fore body, 1.1-2.5 × 0.7 -1.20; hind body, 1.1-2.5 × 0.55-0.95.Intestinal caecasimple extending up to anterior or hind end of anterior testis. Acetabulum, 0.15-0.25 × 0.14-0.22.Testis two, anterior testis, 0.3-0.37 × 0.2-0.42; posterior testis, 0.3-0.32 × 0.2-0.45. Cirrus sac, 0.54-0.55 × 0.14-0.15. Ovary, 0.20-0.28

× 0.16-0.26. Vitelline follicles, 0.05-0.11× 0.025-0.10. Uterus small on posterior region. Eggs small and non operculated, 0.0125-0.0275 × 0.010 – 0.015.

Seasonal dynamics

Hoplobatrachus tigerinus is the extremely aquatic species of anurans inhabiting the natural and artificial (paddy fields) wetland areas of India (Alexander, 2011). The aquatic environment of frog permits the metacercariae larvae of plagiorchiid trematodes to enter their body when they feed on metacerceriae infected tadpoles, resulting in infection (Rajendran and Janardhanan, 1993). Of the 130 hosts examined, only 18 frogs (13.84%) were found to be infected with 530 (range = 1-229) *T. ranarum*. It is a recurrently occurring plagiorchiid digenean infecting the intestines of the hosts. *T. ranarum* represented a prevalence of 13.84%, with mean intensity (29.44±20.82), mean abundance (4.07±2.88) and index of infection (0.56) (Table-1). Monthly changes in the prevalence, mean intensity, mean abundance and index of infection of *T. ranarum* were depicted graphically (Fig-1). Prevalence was high in the months of May, September, December to February, 2014 and almost nil in the months of July, October and November. Mean intensity, mean abundance and index of infection were high in the month of December as the maximum number of *T. ranarum* (429) was recovered from the 20 frogs examined. Prevalence of parasitization, mean intensity, abundance and index of infection was relatively higher in winter followed by rainy and summer (Fig.2). High population levels of *T.ranarum* in winter can be attributed to high levels of occurrence of hosts in winter months. Fall in temperature might be one of the causes for reduced immunological response in hosts. Coefficient of Correlation 'r' was designated to study the possible relationship between host size and parasitization. The computed value 'r' 0.019 illustrated a very insignificant positive correlation between host size and *T. ranarum* infection. Parasitization was relatively high in medium sized frogs (Group-II) and slightly less in smaller (Group-I) and larger (Group-III) frogs (Table-2). The present study supports the views of Aho (1990), Sluys et al., (1994), Koyun (2012) and Comas et al., (2014) that there is a positive relationship between body size and parasite intensity. Impact of host sex on the parasitic loads of amphibians is unpredictable among different host and parasite species and is seems to be more complex cause than the size of the host (Hollis, 1972; Kehr *et al*., 2000; Verma and Singh, 2000 and Sluys et al., 2006, Begum and Bhanu, 2012). Of the total sample of 130 *H. batrachus* screened, 84 (64.6%) were males and only 46 (35.3%) were females. Only 12 (14%) males and 06 (13%) were infected by these parasites (Table-3). Though females showed marginally high prevalence than males, however, there is significant sex-related difference in the parasitic abundance of *T. ranarum* between male and female *H. batrachus* which is evidenced by the calculated χ^2 -value (46.37) at 5% level of significance and 1° degree of freedom being greater than the tabulated χ^2 –value (3.84).

Table 1

Diversity parameters and distribution pattern of *Tremiorchis ranarum* in *H. batrachus*

Name of the parasite	Infected frogs	Total no. of parasites	Prevalence (%)	Mean Intensity	Mean abundance	Index of infection	Range	Location
Tremiorchis ranarum	18	530	13.84	29.44±20.8	4.07±2.88	0.56	1-229	Intestine

Table 2

Correlation coefficient (r) between size and parasitic number of *T. ranarum* in *H. batrachus*

Sl.No.	Size groups	Class intervals	No. of parasites	Correlation coefficient (r)
1	Group-I	4-10	144	
2	Group-II	10-16	241	r = 0.019
3	Group-III	16-22	145	

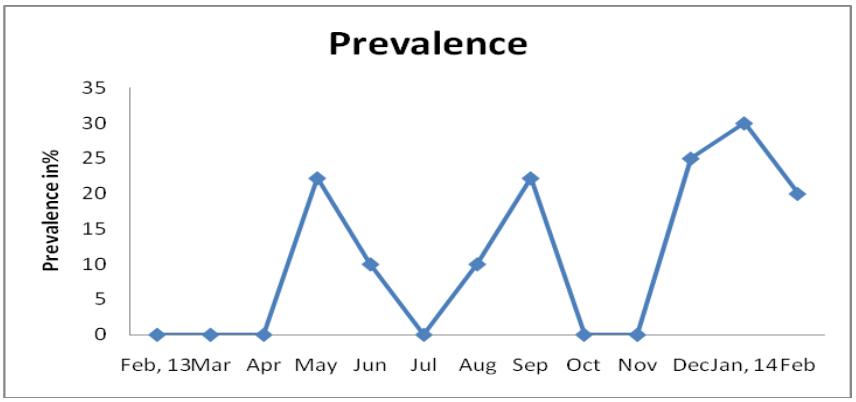
Table 3

Parasitization of *T. ranarum* in males and females of *H. batrachus* (N_m= 84; N_f= 46)

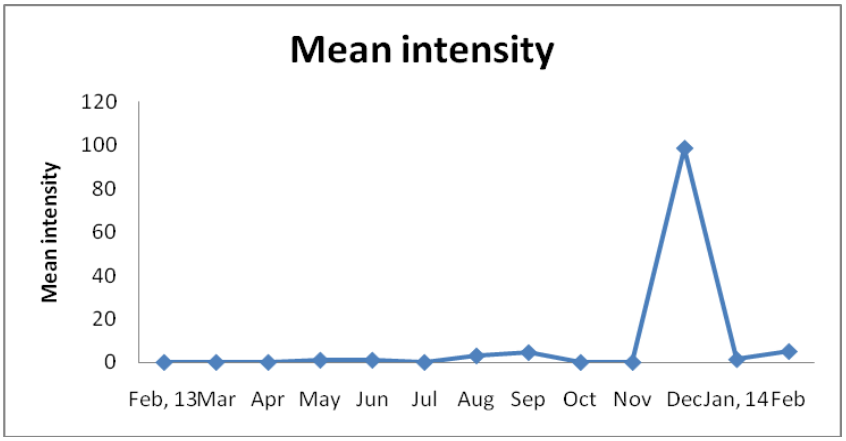
Name of the species	N_{mi}	N_{fi}	P_m	P_f	M_{im}	M_{if}	MA_m	MA_f
T. ranarum	12	6	14.28	13.04	43	2.33	6.14	0.30

N_m number of males examined, N_f number of females examined, N_m number of infected males, N_{fi} number of infected females, P_m and 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.

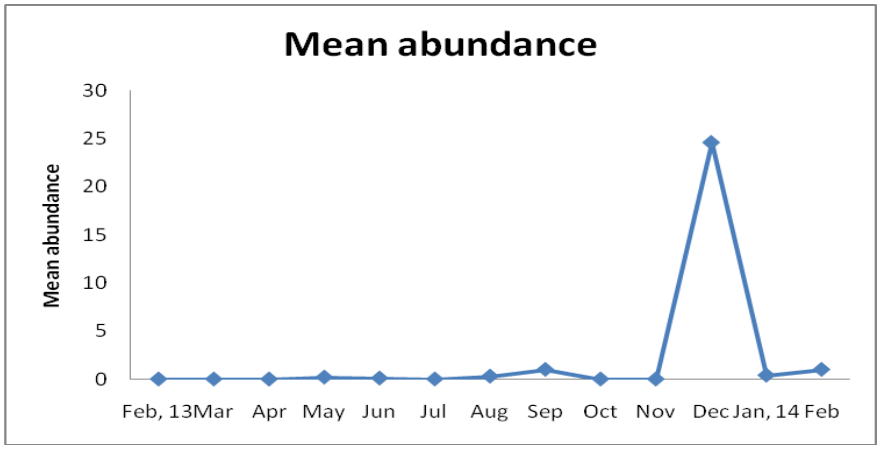
a.



b.



c.



d.

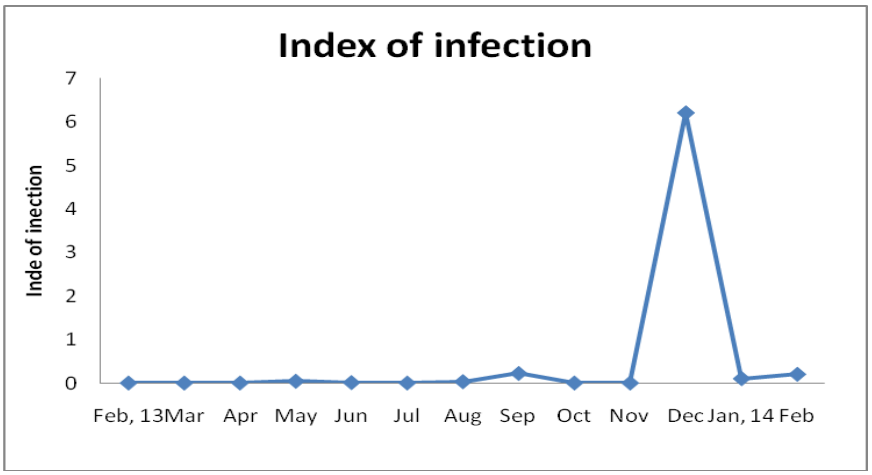


Figure 1

Monthly population changes of *Tremiorchis ranarum* in *H. batrachus;* **a.** Prevalence, **b**. Mean intensity, **c.** Mean abundance, **d.** Index of infection

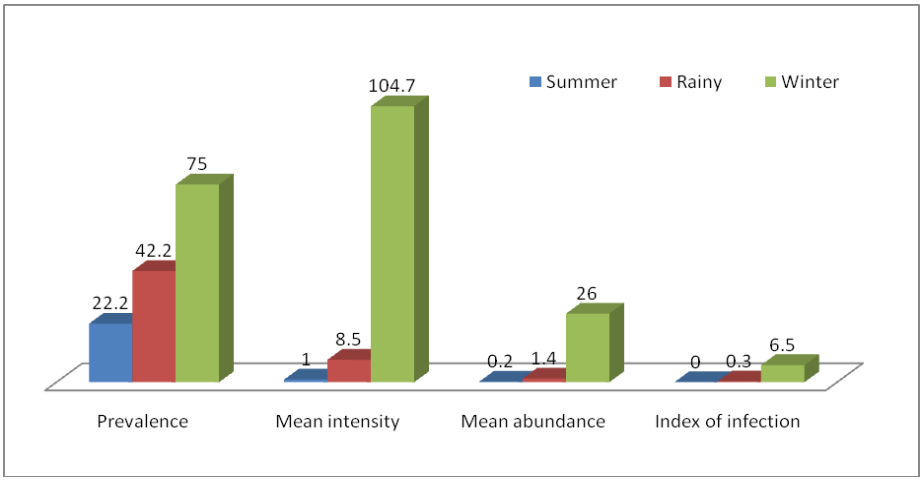


Figure 2

Seasonal variations in prevalence, mean intensity, mean abundance & index of infection of *T. ranarum* in *H. batrachus*

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