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Habitat Preference and Seasonal Fluctuations in the Helminthofauna of Amphibian Hosts of Rohilkhand Zone, India

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Abstract: The helminthofauna of 350 amphibian hosts (250 *Bufo melanostictus* and 100 *Rana tigrina*) was investigated and the influence of ecological factors (host habitat and season) on parasitic incidence was observed in them. The highest frequency and concentration index of nematode parasites (*Oxysomatium* and *Oswaldocruzia*) occurred at Site A (Bareilly) while in case of the cestode parasite (*Proteocephalus*) the highest frequency index occurred at Site E (Aligarh) although the highest concentration index was again at Site A (Bareilly). All the parasites were most abundant during the rainy season followed by summer, spring and winter.

Key words: *Bufo melanostictus*, *Rana tigrina*, Habitat, Season, Frequency index, Concentration index.

Introduction :

Parasites can have a wide range of impact on the ecology of their hosts, in terms of health (Arme and Owen, 1967), and regulation of host populations (Freeland, 1979) and behavior (Milinski, 1984; Moore, 1984). This makes it interesting to examine the ecological factors that determine parasite loads. Potential factors determining the transmission of parasites include environmental conditions that affect the viability and behaviour of parasite propagules (Rogers and Sommerville, 1963) and feeding, movement and defecation patterns of the host, which indicate that the parasites have encountered (Price *et al*., 1988; Lozano, 1991).

Environmental conditions and host behaviour are influenced by habitat and season, while physical state reflects internal conditions, though this may be affected by external factors. In this study, we examine the effect of habitat (locality) and season on the intestinal parasite loads of the amphibians (*Bufo melanostictus* and *Rana tigrina*) of Rohilkhnad Zone, India.

Materials and Methods :

The experimental hosts (250 *Bufo melanostictus* and 100 *Rana tigrina*) were collected regularly for three years (June 2002- June 2005) from Rohilkhand and adjoining areas of Uttar Pradesh, India, in all four seasons (Summer, Rainy, Winter and Spring) of the year. The hosts were brought from wild conditions and maintained in the laboratory under normal conditions of food, water and aeration. The hosts were pithed, dissected and the (GI) gastrointertinal tract was scanned for

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helminth infection. The parasites were taken out in normal saline and live helminths were washed 2-3 times in normal saline. They were further processed and permanent slides were made according to Gibson (1984). "Frequency Index" is defined as the percentage of infected hosts per sample and "Concentration Index" as the number of worms per infected host (Margolis *et al*., 1982) and were calculated accordingly.

To observe the impact of ecological parameters on the frequency and concentration indices of the parasite populations, the data were analysed as follows :

(1) Infectivity of parasites in hosts collected from different localities.

(2) Infectivity of parasites during different seasons of the year.

In order to observe the effect of the locality of the host population on parasite incidence, the amphibians were collected from different sites as follows and infectivity recorded in each of them.

Site A : Bareilly
1. Company Garden
2. Cantonment Area
3. Civil lines
4. University Campus

Site B : Banbasa

Site C : Bisalpur

Site D : Pilibhit

Site E : Aligarh

In order to observe the effect of different seasons on the frequency index and concentration indices of different parasites in amphibian hosts, the whole year was divided into four seasons and the data was recorded and tabulated.

Summer	:	May – July
Rainy	:	August – October
Winter	:	November – January
Spring	:	February – April

Results and Discussion :

The nematode parasites, *Oxysomatium* (site: rectum) and *Oswaldocruzia* (site: stomach and small intestine) and the cestode parasite, *Proteocephalus* (site: small intestine) were recovered from the GI tract of *Bufo melanostictus* and *Rana tigrina*, the urinary bladder and lungs were devoid of helminthic infection.

(1) Infectivity of parasites in hosts collected from different localities : *Oxysomatium*, appeared to have a habitat preference for Site A. Frequency index of the parasite was highest at this site in *Bufo* (100%) as well as in *Rana* (84%). *Rana* from Site C were free from this parasite, while *Bufo* from Site E showed minimum infectivity of *Oxysomatium* (31%). Maximum values of its concentration index occurred from Site A, 4 (70 par/ host), while in case of *Rana* it was 20 par/ host from Site A, 1. The minimum value was 5 par/ host from Site B in both the hosts (Table 1 and 2).

A 100% infectivity of *Oswaldocruzia* occurred in *Bufo* from Site A, 1 (Table 1). *Rana* also showed a maximum frequency index (60%) from Site A, 1(Table 2). On the other hand, in *Bufo*, Site B showed a minimum frequency index (15%), while in Site C, *Rana* were free from the above parasite infection (0% frequency index). The highest concentration index of *Oswaldocruzia* in *Bufo* occurred in Site A,

4 (12 par/ host), while in *Rana*, it occurred in Site A, 1 and 3 (3 par/ host) (Table 2). Least concentration index was recorded from Site E (2 par/ host) in *Bufo*, while in *Rana* from Site B (1 par/ host). *Rana* from Site C was free from the above parasitic infection.

The cestode parasite *Proteocephalus* was not recovered from *Rana tigrina*. In case of *Bufo*, *Proteocephalus* showed maximum frequency index (79%) from Site E and a minimum (8%) from Site D (Table 1). The highest concentration index occurred in Site A, 4 (7 par/ host) and the lowest (1 par/ host) from Sites C and D.

Amphibians, particularly anurans have a rich parasite fauna (Prudhoe and Bray, 1982) perhaps due to the typical life style of amphibians favouring vulnaribility to microbial and parasitic infection. The damp habitats preferred by amphibians favour survival of infective stages of parasites. Moreover, most amphibians are associated with both aquatic and terrestrial habitats and, hence with two different ranges of parasitic species. The diversity of parasites is, thus increased with different transmission characteristics which may combine together.

Locality is one of the important ecological factors which plays a substantial role in the occurrence of parasitic species. Literally, the term locality refers to a geographic locale of the external environment from where the parasite is isolated. It refers to the geographic position from where the individual population of community is obtained. Bush *et al*. (1997) also referred to locality as the spatial region from where a host is collected or it might refer to the spatial region from where a substrate is examined for parasites.

In the present piece of work, the above terminology is endorsed and the term is said to refer to the place from where the host is collected. Out of the five major sites taken under study, maximum infectivity was found in the amphibian population (both species *Bufo* and *Rana*) collected from Company Garden, Bareilly. From this locality 100% infectivity of *Oxysomatium* and *Oswaldocruzia* was recorded in *Bufo* and 57% of *Nyctotherus*. The infectivity in *Rana* for *Oxysomatium* was 90% and *Oswaldocruzia* 60%. *Proteocephalus* was recorded at the highest frequency index from Site E (79%) in *Bufo*, although it was completely absent in *Rana* collected from all localities.

An investigation on the concentration index of parasites collected from different localities indicates that *Oxysomatium* occurred at highest density at Site A4 (70 par/host) in *Bufo* whereas they were the maximum (20 par/host) at site A1 in *Rana*. Site A1 was again favourable for *Oswaldocruzia* in both *Bufo* and *Rana* and *Proteocephalus* occurred at highest density at Site A4 in *Bufo*. Site A1 appears to be the most favoured site for parasite infectivity in both species of amphibians sampled, whereas Site D for *Bufo* and Site C for *Rana* are the least favoured sites for parasite invasion.

The above findings indicate the preference of the habitat in the incidence of different species of helminth fauna in *Bufo* and *Rana*. The findings suggest that the locality from where the host is collected plays an influential role in the occurrence of parasites. When a parasite is

Table-1 : Infectivity of helminth parasites in *Bufo melanostictus* from different localities

Locality	No. of Hosts Examined	Parasites Recovered								
		Oxysomatium			*Oswaldocruzia*			*Proteocephalus*		
		No. of hosts found infected	Freq. index (%)	Conc. index (Par/host)	No. of Hosts found infected	Freq. Index (%)	Conc. Index (Par/host)	No. of hosts found Infected	Freq. index (%)	Conc. index (Par/host)
SITE-A										
1. Company Garden	70	70	100	55	70	100	12	15	21	6
2. Cantonment Area	40	40	100	60	21	52	10	14	35	5
3. Civil Lines	34	34	100	57	10	29	9	12	35	5
4. University Campus	34	34	100	70	13	38	11	10	30	7
Average	44.5	44.5	100	60.5	28.5	54.7	10.5	12.7	30.2	5.7
SITE-B Banbasa	20	17	85	5	3	15	6	3	15	2
SITE-C Bisalpur	21	16	76	27	4	19	4	5	24	1
SITE-D Pilibhit	12	9	75	22	2	17	3	1	8	1
SITE-E Aligarh	19	6	31	7	4	21	2	15	79	3

studied by itself apart from the locality from where it is collected only a part and often a small part of its total biology can be understood. The community principle in ecological theory emphasizes the orderly manner in which diverse organisms usually live together and how different parasitic species may affect them. This co-relation between type of habitat and species of parasite occurrence has been observed by earlier workers. (Ingles, 1936; Rankin, 1937, 1945). The latter author conducted an ecological study of helminth parasites of amphibian and reptiles of Western Massachusetts and its vicinity making valuable observations on the ecology of parasite fauna. They observed the physical conditions of localities from which hosts were collected, position of worm in the host, condition of the host, season, and life cycle of parasite in addition to the morphology and taxonomy of the parasites.

Muzzall *et al.* (2001) examined green frogs *Rana clamitans* from six localities

Table-2 : Infectivity of helminth parasites in *Rana tigrina* from different localities

Locality	No. of hosts examined	Parasites Recovered					
		Oxysomatium			*Oswaldocruzia*		
		No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host	No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host
SITE-A Bareilly							
1. Company Garden	20	18	90	20	12	60	3
2. Cantonment Area	20	16	80	19	10	50	2
3. Civil Lines	20	16	80	16	9	45	3
4. University Campus	15	13	86	13	8	53.3	2
Average	18.7	15.7	84	17	9.7	52	2.5
SITE-B Banbasa	15	4	26.6	5	3	20	1
SITE-C Bisalpur	10	-	-	-	-	-	-

in South Western Michigan and observed that adult frogs had significantly higher values than did juveniles at each location and stressed the importance of local ecological conditions on helminth community structure.

Our studies when compared to the observations of earlier workers suggest that the locality from where the host is collected may account as one of the important factors accounting for variance in frequency index and concentration index of the parasite fauna in various amphibian populations. This may be due to the fact that an ecological complex is formed at each locus composed of the parasite, the vector, the host and various features of the host's environment which

is not something absolute and static, but is ever changing and these factors may account for variance in infectivity as indicated in the present findings.

(**2) Infectivity of parasites during different seasons of the year :** 100% frequency index was recorded for *Oxysomatium* from *Bufo* in rainy season clubbed with the highest concentration index (80 par/ host). A similar observation was again recorded in *Rana* where 80% frequency index and 19 par/ host concentration index was observed during the rainy season. Minimum infectivity occurred in winters when the frequency index declined to 50 % and concentration to 15 par/host in *Bufo* and in *Rana*, *Oxysomatium* infection reduced to nil in winter (Table 3 and 4).

Oswaldocruzia also followed a similar pattern and showed highest infectivity in rainy season in both the host species. In *Bufo* maximum frequency index and concentration was 60 % and 12 par/host, respectively. In *Rana* it was 51% and 6 par/host, respectively. On the other hand, a drastic fall in infectivity occurred in winters, the frequency index and concentration index fell down to 24% and 1 par/host, respectively in *Bufo*. In *Rana,* infection of *Oswaldocruzia* was totally absent in winter season (Table 4).

The cestode, *Proteocephalus* also showed maximum infectivity in rainy season and minimum in winter in *Bufo melanostictus*. The maximum frequency and concentration indices being 46% and 6 par/host, respectively, in spring season, the frequency index was 20% and concentration index 3 par/host This parasite was totally absent in the winter season. On the other hand, this parasite was not found in *Rana tigrina*.

Investigations on seasonal dynamics of amphibian parasites with respect to the fluctuation of the entire parasite fauna of any host species is still meager. Climatic factors are regarded to be of utmost importance, while considering seasonal fluctuations and incidence of parasite infectivity. Of these, humidity and thermal changes of the environment may play the decisive roles. In fact, temperature plays an important environmental influence on the biology of all animals. The responses to temperature may be complex, but at its simplest, temperature affects the rate at which life processes (growth, metabolism, reproduction) proceed. Every animal sustains to the maximum at optimum temperature and at low temperature, the metabolism of the organism slows down due to the decrease in kinetic energy imparted to reactions. This is reflected in changes in the level of activity displayed by the animal.

The influence of rainfall and temperature on host parasite relationship is of dual nature: they can directly influence the final host (the frog) changing its living conditions (and thus also its possibilities of infection) or indirectly through the change of the trophic conditions of the biocoenosis causing a successive appearance of animals being the food of frogs and at the same time the intermediate hosts of their parasites.

The parasites of amphibians are still more affected due to seasonal and climatic factors due to the reason that firstly they are cold-blooded and secondly they are

Table-3 : Infectivity of helminth parasites in *Bufo melanostictus* during different seasons

Season	No. of hosts examined	Parasites Recovered								
		Oxysomatium			*Oswaldocruzia*			*Proteocephalus*		
		No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host	No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host	No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host
Summer	96	91	95	60	50	52	5	28	29	5
Rainy	80	80	100	80	48	60	12	37	46	6
Winter	24	12	50	15	6	24	1	-	0	-
Spring	50	43	86	20	23	46	3	10	20	3

Table-4 : Infectivity of helminth parasites in *Rana tigrina* during different seasons

Seasons	No. of hosts examined	Parasites Recovered					
		Oxysomatium			*Oswaldocruzia*		
		No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host	No. of Hosts found Infected	Freq. Index (%)	Conc. Index Par/host
Summer	30	22	73.3	15	12	40	2-3
Rainy	45	36	80	19	23	51	6
Winter	5	-	-	-	-	-	-
Spring	20	9	45	2	7	35	1

amphibious in nature and, thus the climatic/ seasonal changes directly affect the parasite population and the amphibious mode of life provides a wider exposure to both terrestrial and aquatic conditions, thus favouring parasitism.

Our investigations have indicated that both the species of amphibians examined (*Bufo melanostictus* and *Rana tigrina*) are affected by parasite invasion to the maximum during the rainy season. *Oxysomatium* occurred at the highest frequency index (100%) and peak concentration index (80 par/host) in the rainy season itself in *Bufo melanostictus*. Similar results were obtained for *Oswaldocruzia*, whereas *Proteocephalus*, the cestode parasite was totally absent in *Rana tigrina*, but was prevalent to the highest degree again in the rainy season in *Bufo*. Parasitic infectivity of the rainy season was followed by summer, then by spring and was minimum during the winter season.

Parasite Infection :

Rainy season > Summer season > Spring season > Winter season

During winters, the frogs hibernate and during this period there is a reduction in metabolism resulting in hypobiosis. The effects of hypobiosis are poorly understood especially in poikilotherm hosts. All variations are recorded: in some hosts, parasites may be killed; in others they may become quiescent and occasionally the parasites may continue to develop. The influence of hibernation can be more properly considered as being the sum of the influence of several factors involving cessation of feeding. The drop in temperature retards reproduction and may lead to a reduction in the number of parasites. The sum total of the influence of hibernation may lead to its effect on parasite infectivity.

The low infectivity during winters in the present work finds support from those of earlier workers when Markov and Rogoza (1953) observed the same phenomenon in frogs from the Leningrad area and by Lees (1962) who observed a fall in helminth infestation during December and January. Plasota (1969) stressed the influence of rainfall and temperature on parasite infectivity and added that the hosts living conditions may affect the penetration of parasites like *Oswaldocruzia*. Baker (1979) observed the seasonal population changes in *Rhabdias ranae* parasitizing *Rana sylvatica* and recorded lowest infection in summers and highest in spring and early fall in winters. Vanderburgh and Anderson (1987) made some preliminary observations on the prevalence and intensity of the nematode parasite *Cosmocercoides* in *Bufo americanus* and recorded 4th stage larvae in the lungs of the host in late April to early May.

Griffin (1988) compared the duration of development of egg and larval stages of *Oswaldocruzia filiformis* under changing temperatures with the values predicted from constant temperature experiments and recorded an acceleration in the rate of development of eggs to hatching under regimes of both sudden and gradual temperature changes. Wharton (1999) stressed the role of low temperature on the growth rate, development and metabolism of parasites and suggested that the parasites are at a potentially lethal

risk of freezing at sub zero level.

The above studies on parasitic infectivity in relation to seasonal variations, climatic changes and temperature variables indicate that various authors from all over the world have recorded variance in infectivity of the amphibian parasite fauna. As observed from the above findings, maximum infection was recorded during the rainy season when the amphibian population was abundant, the climate was moist and humid. These conditions favoured parasite occurrence and multiplication where not only the frequency index of the parasites was higher but also the concentration index reached their peak values. On the other hand, the infectivity was lowest during the winter season when the frogs were hibernating and their metabolic activities were the lowest. As parasites are characterized by metabolic dependence on their hosts, the conditions during the winters did not promote parasite occurrence perhaps because they could not metabolically depend on their host. During winters, the frogs hibernate and their feeding is minimum. It has also been suggested that from the end of November till the commencement of the breeding season, the blood sugar levels of frogs is low (Smith, 1950). Moreover the lack of food specially for intestinal parasites during this period may also account for their low sustenance as has also been opined by Mazurmovich (1951) that lack of adequate food for helminths during hibernation may be a sound reason for their decline in number.

This low value of infection started to increase during the spring when the hosts regained their metabolic activities and resumed feeding by the end of winter with consequent opportunities of acquiring new infections. This spring stimulation of parasites was also recorded by Markov and Rogoza (1955). The parasite infection increased further during the summer when the frogs were quite active and metabolically sound. During the rainy season, the hosts were most abundant and the higher metabolic activity of the host coupled with suitable climatic and humid conditions promoted parasitic existence which may account for the highest infectivity during the rainy season.

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