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Influence of Abiotic Factors and Infection of *Fasciola gigantica* on Oviposition of Vector Snail *Lymnaea acuminata*

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Authors' contributions

Authors may use the following wordings for this section: This work was carried out in collaboration between all authors. Author NS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PK and DKS managed the analyses of the study. Author DKS managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aim of the present study is establish a correlation between the abiotic factors and infection rate of *Fasciola gigantica* and their effect on the reproduction of vector snail *Lymnaea acuminata*.
Place and Duration of Study: Malacology Laboratory, Department of Zoology, DDU Gorakhpur University, Gorakhpur in between November 2009 - October 2010.
Methodology: Fecundity of infected/uninfected snail *Lymnaea acuminata* was noted in different months of year 2009-2010.Fecundity was dependent on variant abiotic environmental factors and infection rate of *Fasciola gigantica* larvae in snail body. In infected snail the highest fecundity was observed in winter and lowest in summer. In order to ascertain that such a relationship between fecundity and abiotic factors is not coincident, the nervous tissue of the snail was assayed for the activity of the acetylcholinesterase (AChE) in uninfected and infected snails in each months of the year. It was noted that abiotic factors and infection parameter in snail body influence the influence the fecundity of

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the snails in each month of year 2009-2010.
Results: There was a significant positive rank correlation between total fecundity of uninfected/infected snails and corresponding AChE activity in the nervous tissue of same snails.

Keywords: Fecundity; Fasciola gigantica; abiotic factors; infection; AChE

1. INTRODUCTION

Incidence of fasciolosis in the cattle population is very common in eastern region of the state of Uttar Pradesh in India [1,2]. Recently human fasciolosis is also reported in different parts of India [3]. The freshwater snail *Lymnaea acuminata* is the intermediate host of *F. gigantica* [4,5]. Carrier snail is one of the important factor in transmission of vector-borne diseases. Earlier, it has been reported that abiotic factors are involved in controlling fecundity of snail *L. acuminata* [6]. One of the major preventive method to reduce the incidence of fascioliosis is to control the population of vector snails and thereby, break the life cycle of *Fasciola* [6]. Vector snail *L. acuminata* is fast breeder and it lays eggs round the year [6]. It has also been conclusively observed by us that acetyl cholinesterase (AChE) in the nervous tissue of *L. acuminata* is very sensitive parameter influenced by abiotic environmental factors [7]. The objective of this study was to explore the possibility that whether seasonal changes in abiotic factors, namely temperature, pH, dissolved oxygen, carbon dioxide and electrical conductivity of the pond water can influence the reproductive capacity of uninfected/infected snails as well as AChE activity in the nervous tissue of snail in each month of year 2009-2010.

2. MATERIALS AND METHODS

2.1 Animals

Adult *Lymnaea acuminata* (length 2.0±0.30 cm) were collected from GIDA pond, located in the southern area of Gorakhpur, between latitude 26º 46' N and longitude 83º 22' E.

2.2 Fecundity and Measurement of Abiotic Factors

Naturally infected and uninfected (control) *L. acuminata* were collected along with their pond water. Six groups of twenty infected and uninfected snails were kept in six glass aquaria separately in 5 liters of pond water. The aquaria were covered with the wire netting to prevent the animals for escaping. *L. acuminata* laid their egg in form of elongated gelatinous capsules containing 2-180 eggs on the lower surface of leaves of aquatic vegetation. After every 24h up to 96h, total number of egg oviposited by the snails were counted in each aquarium. Temperature, dissolved oxygen, free CO_2, pH and conductivity of pond water were measured by thermometer, digital pH meter and conductivity meter, respectively. Dissolved oxygen and CO_2 were estimated according to method prescribed by APHA, [8]. Population density of snails per meter square in natural habitat were counted in six regions of GIDA pond in each month of year 2009-2010. Population has been expressed as mean ± SE.

2.3 Enzyme Assay

Acetylcholinesterase (AChE) activity was measured according to the method of Ellman et al. [9] as modified by Singh and Agarwal [10]: 50 mg of nervous tissue uninfected/infected snails was homogenized in 1.0 ml of 0.1 M phosphate buffer, pH 8.0, for 5 minute in an ice bath and centrifuged at 1000g for 30 minute at 4ºC.The supernatant was used as an enzyme source. The enzyme activity was measured in a 10 mm path length cuvette using incubation mixture consisting of 0.1 ml of enzyme source, 2.9 ml of 0.1 M phosphate buffer (pH 8.0); 0.1 ml of chromogenic agent DTNB (5,5-dithiobis 2 nitrobenzoate) reagent and 0.2 ml of freshly prepared acetylthiocholine iodide. The change in optical density at 412 nm was observed continuously on a spectrophotometer for 3 min at 25ºC. Protein estimation was carried out by the method of Lowry et al. [11]. Enzyme activity was expressed as µmoles 'SH' hydrolysed/min/mg protein. Each estimation was replicated six times and values were expressed as mean ±SE.

2.4 Statistical Analysis

Each experiment was replicated at least six times, and values were expressed as mean ± SE of six replicates .Two way ANOVA was applied between the temperature, pH, CO_2, O_2 conductivity and fecundity of uninfected and infected snail in different months of year 2009-2010.The product moment correlation coefficient was applied between different abiotic environmental factors and fecundity of snails in each month of year 2009-2010. Rank correlations were applied between acetylcholinesterase activity of control (uninfected/ infected) snail group and corresponding fecundity of snails in different months [12].

3. RESULTS

The temperature of water in GIDA pond in different months of year 2009-2010 was in between 16 (January) to 36ºC (June). The water of GIDA pond was alkaline and its pH varies in between 7.55 to 8.53. Highest dissolved oxygen in GIDA pond water was recorded in month of February (3.21 ppm) and minimum in April (1.3 ppm). Maximum CO_2 was noted in month of June (25.20 ppm) and minimum in December (13.25 ppm). Maximum conductivity was recorded in month of October (42.68 µ mhos cm^{-1}) and minimum in May (21.60 µ mhos cm^{-1}) (Table 1).

There was a significant ($p<0.05$) positive correlation in between the fecundity of uninfected (control) snail group in each month and temperature of water and pH ($p<0.05$). There was no marked correlation was noted in between fecundity of uninfected snail group with conductivity, dissolved oxygen and free carbon dioxide. In control group highest fecundity/20 snail in 24 h was observed in August (217.00 eggs/20snails), whereas lowest in February (100.10 eggs/20snails) (Table 1).

There was a significant ($p<0.05$) negative correlation between fecundity of infected snail group and temperature, free carbon dioxide. Significant positive ($p<0.05$) correlation was noted with dissolved oxygen. There was no marked correlation in between fecundity of infected snail group with pH and conductivity. A highest fecundity/20 snail in infected snail group was observed in month of January (184.00±0.33), whereas lowest in month of June (78.18±0.90). Significant higher fecundity was noted in uninfected group of snails than infected group of snails (Table 1**).**

Table 1. Fecundity of infected and uninfected *Lymnaea acuminata* in different months of year 2009-2010

Parameter	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct
+Temp°#(°C)	25±0.33	17±0.42	16±0.75	23±0.36	27±0.33	33±0.48	34±0.36	36±0.49	35±0.49	34±0.31	33±0.45	31±0.43
+pH#	8.02± 0.003	7.94± 0.004	7.67± 0.005	7.75± 0.006	7.55± 0.003	7.91± 0.013	8.08± 0.007	8.06± 0.49	8.22± 0.05	8.26± 0.009	8.53± 0.008	8.34± 0.004
+DO_2*(ppm)	2.18± 0.06	2.07± 0.07	2.72± 0.06	3.21± 0.09	2.71± 0.04	1.30± 0.04	1.62± 0.05	1.42± 0.06	1.65± 0.04	2.28± 0.09	2.38± 0.08	2.47± 0.06
+°DCO_2°(ppm)	19.25± 0.38	13.25± 0.45	15.63± 0.17	13.73± 0.15	25.08± 0.14	23.01± 0.28	24.70± 0.13	25.20± 0.09	21.69± 0.23	22.53± 0.14	13.64± 0.18	15.78± 0.08
+Conductivity (μ mhos cm^{-1})	39.58± 0.06	38.45± 0.04	39.98± 0.05	29.35± 0.08	28.20± 0.06	26.55± 0.07	21.60± 0.06	23.45± 0.08	29.67± 0.05	27.50± 0.09	38.7± 0.08	42.68± 0.03
+24h USF	170.50± 0.76 (100)	150.83± 0.30 (100)	142.32± 0.59 (100)	100.10± 0.78 (100)	165.00± 0.37 (100)	155.00± 0.49 (100)	185.67± 0.37 (100)	188.32± 0.70 (100)	193.50± 0.55 (100)	217.00± 0.78 (100)	174.33± 0.59 (100)	215.00± 1.02 (100)
48h USF	60.00±0.33	30.50±0.20	78.85±0.48	97.47±1.83	95.70±1.72	102.23±0.73	101.03±0.23	94.00±0.34	50.40±0.56	51.33±0.36	67.42±0.49	39.50±0.33
72h USF	24.40±0.88	30.21±0.65	30.12±0.47	90.00±0.49	86.50±0.36	85.44±0.30	66.23±0.67	49.33±0.75	28.19±0.33	31.51±0.75	62.00±0.33	10.40±0.67
96h USF	13.30±0.48	19.16±0.43	13.33±0.36	52.83±0.33	50.80±0.51	74.50±0.49	51.00±0.59	8.40±0.36	11.56±0.62	16.50±0.43	14.65±0.47	15.40±0.33
+24 ISF	137.66± 0.76 (80.74)	147.83± 0.36 (98.01)	184.00± 0.33 (129..30)	180.16± 0.90 (179.87)	153.00± 0.21 (92.72)	150.50± 0.54 (96.77)	95.44± 1.05 (51.40)	78.18± 0.90 (41.51)	86.00± 0.54 (44.44)	104.50± 0.33 (48.17)	150.16± 0.40 (86.14)	145.59± 0.21 (67.72)
48h ISF	67.00±0.36	29.96±0.47	21.50±0.47	53.21±0.47	45.38±0.36	22.20±0.59	60.38±0.36	76.50±0.43	53.16±0.47	89.20±0.91	70.30±0.49	37.34±0.48
72h ISF	25.19±0.43	26.22±0.57	15.82±0.47	33.40±0.48	14.90±0.36	17.44±0.43	24.00±0.43	46.00±0.49	34.30±0.47	67.30±0.59	56.19±0.36	31.20±0.43
96h ISF	14.00±0.36	28.19±0.48	2.50±0.36	14.32±0.49	13.29±0.59	12.00±0.36	18.27±0.31	13.23±0.47	24.56±0.48	6.00±0.42	13.20±0.67	28.55±0.23

Abbreviation: USF – Uninfected snail fecundity; ISF – Infected snail fecundity. Temp – Temperature, pH – pH, DO_2 – Dissolve Oxygen, DCO_2 – Dissolve Carbon dioxides.
Each experiment was replicated six times and values are the means ± SE of six replicates. Temperature, pH, conductivity, CO_2, O_2 were measured
+, significant ($p<0.05$) when two way ANOVA was applied between temperature, pH, conductivity, Dissolved oxygen and free carbon dioxide and fecundity in different months of year 2009-2010.
Significant (*) positive/(°) negative correlation was observed between infected snail fecundity and abiotic factors. Significant (#) positive/(Δ) negative correlation was observed between uninfected snail fecundity and abiotic factors

Acetylcholinesterase (AChE) activity in nervous tissue of uninfected snails was observed in between 0.630-0.730 µ mole SH hydrolysed/min/mg protein. Highest acetylcholinesterase (AChE) activity in uninfected snail was noted in April, while minimum in December (Table-2). AChE activity in infected snail was lower than uninfected snails. There was significant ($p<0.05$) negative correlation between the AChE activity in the nervous tissue of infected snail and uninfected snail in GIDA pond. Maximum acetylcholinesterase (AChE) activity of infected snail was observed in October/November (0.647 µ mole SH hydrolysed/min/mg protein), while minimum in June (0.510 µ mole SH hydrolysed/min/mg protein) (Table-2). There was significant positive rank correlation ($p<0.05$ $r = 0.85$) between % infection rate and AChE activity in nervous tissue/ (Table-2) fecundity of infected snails.

Table 2. Fecundity (eggs/20 snails) of uninfected and infected snail *Lymnaea acuminata* in 96 h in different months of year 2009-2010 and corresponding changes in acetylcholinesterase activity in the nervous tissue

Months	+Total fecundity Uninfected)	+Total fecundity (Infected)	+ %Infection in snail	AChE (µ mole SH hydrolysed /min/mg protein)	
				+Uninfected *	+Infected#
November	268.23	243.85	33.33±0.47	0.649±0.013 (100)	0.647±0.003 (99.69)
December	230.70	231.24	23.33±0.45	0.630±0.005 (100)	0.535±0.004 (84.92)
January	264.59	233.82	13.33±0.29	0.645±0.010 (100)	0.523±0.008 (81.08)
February	340.40	281.09	25.00±0.26	0.650±0.013 (100)	0.556±0.008 (85.53)
March	398.00	226.57	25.00±0.45	0.675±0.012 (100)	0.570±0.002 (84.44)
April	417.17	202.14	15.00±0.36	0.730±0.003 (100)	0.534±0.002. (73.15)
May	403.93	198.09	15.00±0.48	0.698±0.003 (100)	0.523±0.017 (74.93)
June	340.09	212.97	5.00±0.59	0.654±0.005 (100)	0.510±0.003 (77.98)
July	283.65	198.02	3.33±0.11	0.642±0.004 (100)	0.543±0.002 (84.58)
August	316.34	267.28	5.00±0.61	0.698±0.014 (100)	0.554±0.007 (79.36)
September	318.40	289.85	13.33±0.46	0.698±0.019 (100)	0.645±0.005 (92.48)
October	280.34	242.68	23.33±0.67	0.657±0.013 (100)	0.647±0.006 (98.48)

Values are the mean± SE of six replicates. Value in parenthesis indicate percent enzyme activity of uninfected/infected snails. AChE of control snail was taken as 100%.

Rank correlation in between total fecundity of infected snail in 96 h and AChE activity in infected snail/ total fecundity of uninfected snail in 96 h and AChE activity in uninfected snail in both group indicate significant (#/) positive correlation*

(+) a significant two way ANOVA was applied in between enzyme activity in control/infected group with fecundity in 96h eggs/20 snails uninfected/infected snails as given as in table 1.

4. DISCUSSION

A biotic factors of aquatic system play significant role in altering the snail population/infection rate as well as fecundity of snail *L. acuminata*. Studies have shown that there are five major external signals that regulate reproduction in snail: photoperiod, food consumption, temperature, water quality, and parasites [13]. Temperature is an important abiotic factor which controls the metabolism of snail. High temperature increases the fecundity of the snails [6]. It is evident from the present study that increase in temperature in summer months act as enhancer of fecundity in uninfected snails. Contrarily, temperature shows negative correlation with fecundity of infected snail. In infected snail higher temperature caused significant reduction in fecundity. It seems that energy in infected snail is utilized by the parasitic larvae in its over reproduction and ultimately snail fecundity is decreased. Infection of avian *schistosome Trichobilharzia* in snail *L. acuminata* caused cessation of egg laying [14,15]. The decline in host egg laying, benefits the parasite by diverting the energy that the host would have invested in its own reproduction in to the parasite reproduction [16]. *F. gigantica* intra-molluscan larval stages were mostly noted in ovotestis of infected snail *L. acuminata.* When parasite burden increases they depend on the beneficial tissue for their food. It may cause double stress on the host body in summer. First stress is due to the infection of *F. gigantica*, while second was due to high temperature. Both these characters show negative correlation with fecundity of infected group of snails. It clearly demonstrate that in summer temperature is suitable for uninfected snails but not for the infected snail. Too low and too high temperatures affect the metabolic processes of both host snail and parasite, thus interfering with parasite reproduction within the snail, growth and snail survival [17]. Some of the inhibitory effects on reproduction are caused by release of the peptide schistosomin from the *Lymnaea* central nervous system in response to parasitic infection of *Trichobilharzia* [18], which antagonizes the bioactivity of caudodorsal cell hormone at its target sites, blocking ovulation and many of the behaviours associated with egg laying [19]. In present study it was clearly noted that fecundity of uninfected snail was higher than infected snail. It may be possible that, larvae of *F. gigantica* interfere with the reproduction of vector snail.

Activity of AChE in the snail nervous tissue may be used as the infection indicator. AChE activity in the infected snail was lower than the uninfected snail in each month of the year 2009-2010. Ovulation and egg-laying behaviour are dependent on secretion of the neuropeptide caudodorsal cell hormone [20]. Studies done in *Lymnaea* have identified a number of environmental factors that regulate reproductive function. There is an impressive literature on the details of the cell physiology underlying function of the caudodorsal cells that regulate ovulation and behaviours associated with egg laying [21]. There are six main types of cells/organs known to be involved in regulating egg laying in *Lymnaea*: the endocrine dorsal bodies, the neuroendocrine caudodorsal cells, the lateral lobes, the hermaphroditic gonad, the hermaphroditic duct, and the accessory sex organs (including the albumen gland) [13].

In general, oxygenated water stimulates reproductive functions, while starvation, cold temperatures, and parasites inhibit reproduction. There were complex interactions between these signals that ultimately decide whether or not the snail will reproduce or not.

Dissolved oxygen is one of the major component, which is required for the metabolic activity of snails [22]. Dissolved oxygen concentration in water decreases with increase in temperature [7]. When the CO_2 combines with water it forms carbonic acids and release hydrogen ions. pH of water is one of the important factor that directly or indirectly influence

the metabolic activities and thereby the growth and abundance of freshwater molluscs [6,7] Aquatic organisms are affected by pH because most of their metabolic activities are pH dependent. The surface water of GIDA pond was always towards the alkaline side (Table 1) and significantly altered in different months [23]. Low conductivity range coincides with low snail abundance [24].

5. CONCLUSION

This study conclusively, indicate that abiotic factors such as temperature, pH, dissolved oxygen, carbon dioxide and electrical conductivity of pond water and infection of *F. gigantica* in vector snail. *L. acuminata* are crucial factors, which significantly alter the fecundity of uninfected /infected snail *L. acuminata*. Meteorological factors and *Fasciola gigantica* infection greatly influenced the ethology and fecundity of intermediate host molluscs. Use of these parameters will be beneficial in snail control programme. It will provide knowledge, when effective snail control method can be applied in the year to control fasciolosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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