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The Effect of Abiotic Factors on Certain Biochemical Changes in Ovotestis of Snail *Lymnaea acuminata*: Intermediate Host of Trematode Diseases

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Abstract: Every month during the year 2006-2007, the fecundity, hatchability, survival and simultaneous measurement of total protein, free amino acid, nucleic acid levels were made in the ovotestis of *L. acuminata,* with concomitant estimations of certain abiotic factors, viz. temperature, pH, dissolved oxygen and carbon dioxide both in control and test water. On the basis of these observations, it was noted that abiotic factors caused significant variation in the protein, amino acid and nucleic acid levels in control as well as tested water through out the year. A significant positive correlation ($p<0.05$) was observed between fecundity and dissolved oxygen / pH of water. On the contrary, a negative correlation was observed with dissolved CO_2 / temperature of water. There was a significant ($P<0.01$) positive correlation between total protein / free amino acid / nucleic acids and fecundity of snails. A maximum level of DNA (63.43µg/mg) in the ovotestis of snail was noted in the month of May, when fecundity was maximum at 35°C temperature, 8.22 pH, 4.1 mg/l dissolved oxygen, 9.0 mg/l carbon dioxide. Maximum level of RNA (30.76 µg/mg) was in the month of October, when fecundity (158.83 eggs/20 snail) and temperature, pH, dissolved oxygen and CO_2 were 158.83 egg/20 snail, 34°C, 8.45, 5.3 mg/l, 7.5 mg/l, respectively. The protein (84.48 µg/mg) and amino acid (46.11 µg/mg) level were also maximum in the month of May and October, respectively. This study conclusively showed that abiotic factor significantly alter the reproductive capacity of snails with simultaneous biochemical changes in ovotestis of snail in different test water in each month of year.

Key words: Environmental factors · Fascioliasis · Biochemical parameters · Fecundity · *Lymnaea acuminata*

INTRODUCTION

Fascioliasis is a world wide zoonotic disease [1-3], caused by the liver flukes *Fasciola hepatica* and *F. gigantica* [4]. Incidence of fascioliasis in the cattle population is very common in eastern region of the state of Uttar Pradesh in India [5]. Fresh water snail *Lymnaea acuminata* is the intermediate host of *F. gigantica* [6]. Fascioliasis cause high economic losses in the animal husbandry industry [7]. Human fascioliasis has been reported in 51 different countries from five continents [8]. Climate change influences the characteristics of the trematodiases in concrete areas whereas these diseases are emerging in recent years [7]. An effective method to reduce the incidence of fascioliasis is to control the population of vector snails, thereby, breaks the life cycle of these flukes or by reducing the reproductive capacity of snails [6, 9, 10]. Earlier studies have shown that the reproductive capacity of snails vary from one season to other [10-12]. Conclusively, it has been shown that oviposition in snails are induced by neuroendocrine hormone of Caudo-Dorsal Cells (CDCs) in the cerebral ganglion [13-17]. Several mechanisms are involved in the release of ovipository hormone by environmental factors [18-19]. The metabolic activities of invertebrates are influenced to a large extent by changes in both biotic and aboitic factors, which depends on the season [20]. The aquatic environment has numerous physical and chemical parameters that may influence the physiology of fresh water organisms [21]. Environmental factors such as temperature, pH, dissolved oxygen, carbon dioxide and light/dark period are major seasonal variant that affect the morphological characteristics of CDCs [11-12, 22-23].

Earlier, we have observed that various abiotic factors significantly reduced the reproduction of snail *L. acuminata* [10]. The present study was designed simultaneously to observe its effect on different biochemical parameters in the ovotestis of *L. acuminata* in each month of the year 2006-2007.

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MATERIALS AND METHODS

Treatment of Animals: Fresh water snail *L. acuminata* (2.60±0.30 cm in length) were collected in each month of the year 2006-2007 from the ponds, pools, lakes and low lying submerged area located in almost adjacent to our university campus. In each month snails were acclimatized in dechlorinated tap water for 72 h.

The following experiments were made in different regimen of water,

- Dechlorinated tap water (control).
- Dechlorinated tap water changed at 24h (Group A).
- Ramgarh lake water (dirty / polluted water) (Group B).
- Well aerated dechlorinated tap water changed at 24h (Group C).
- Dechlorinated tap water kept in dark (aquaria covered with black cloth) for 24h (Group D).
- Snails exposed for 6h light in 24h (Group E).

Each regimen of 5 liter water was kept in six aquaria separately, containing 20 snails in each aquarium. The aquaria were covered with wire netting to prevent the animals from escaping. *L. acuminata* laid their eggs in the form of elongated gelatinous capsule containing 2-180 eggs on the lower surface of leaves of aquatic vegetation. After every 24 hours up to 96 hours, the total number of eggs oviposited by the snails were counted in each aquarium. Temperature, pH, dissolved oxygen and dissolved free carbon dioxide of different regimen of water were measured simultaneously. Temperature, pH were measured by thermometer and digital pH meter, respectively. Dissolved O_2 and CO_2 were estimated according to methods prescribed by APHA [24].

After 96 hours, the snails exposed to different regimen of water were removed from aquaria and washed with fresh water. The ovotestis were dissected out and placed on filter paper for removal of adherent water and weighed. Protein, free amino acids, DNA and RNA activities in ovotestis were then measured.

Estimation of Total Protein and Free Amino Acid: Total protein (μg/mg) was estimated according to Lowry *et al.* [25] using bovine serum albumin as a standard. Homogenates of ovotestis were prepared in 10 % (w/v) trichloro acetic acid (TCA). Total free amino acids (μg/mg) were determined according to the method of Spies [26].

Estimation of Nucleic Acids: DNA and RNA in ovotestis of *L. acuminata* were estimated according to Schneider [27], using diphenylamine and orcinol reagents, respectively. Homogenates (1mg / ml; w / v) were prepared in 10 % TCA at 90°C and centrifuged at 5000 g for 20 minutes. The supernatants were used for DNA and RNA estimations.

Statistical Analysis: Each experiment was replicated at least six times, values of temperature, pH, dissolved O_2 and CO_2 are expressed as mean of six replicates. Values of fecundity and biochemical parameter (protein, amino acid, DNA and RNA) were expressed as Mean±SE. Product moment correlation coefficient was applied to determine significant ($P<0.05$) differences between environmental factors such as temperature, pH, dissolved O_2, CO_2, exposure of dark / light and fecundity of snails. Whereas, rank correlation coefficient was applied in between fecundity and biochemical parameters (protein, amino acid, DNA and RNA) [28].

RESULTS AND DISCUSSION

There was a significant ($p<0.01$) change in the endogenous level of protein, amino acid, DNA and RNA in the ovotestis of snail *L. acuminata* exposed to different regimen of water (Tables 1-4). In control group of snails, significant ($p<0.01$) variation in the protein, amino acid, DNA and RNA levels in the ovotestis was observed in different months. (Tables- 1, 3). The maximum level of DNA (63.43 μg/mg) and RNA (30.76 μg/mg) in the control group of snails was observed in the month of May and October, respectively. The protein and amino acid level were maximum in May (84.48 μg/mg) and October (46.11 μg/mg), whereas minimum in July (29.84 μg/mg and (8.30 μg/mg, respectively) (Table 1, 3). Fecundity was maximum (196.33 eggs / 20 snails) in the month of May. Maximum reduction in protein, amino acid, DNA, RNA in the ovotestis of snails and lowest fecundity (20 eggs/ 20 snails) were observed in dirty/polluted water exposed group B (Table 2, 4). There was a significant ($p<0.05$) negative product moment correlation between the temperature of water and dissolved oxygen in different months of the year and positive correlation between the temperature and fecundity of snails. Carbon dioxide concentration in different months of water shows a significant ($p<0.05$) negative and dissolved oxygen / pH of water shows positive correlation with fecundity of snails (Tables 1- 4). There was a significant ($p<0.01$)

Table 1: Effect of temperature, pH, dissolve oxygen and CO_2 in the different water sample on the levels of DNA (µg/mg) and RNA (µg/mg) in the ovotestis of the snail *Lymnaea acuminata* in the month of November 2006 to October 2007.

Month	Parameter/ water regimen	Temp.	pH	D.O.(mg/L)	CO_2(mg/L)	Fecundity after 24 hr(egg/20 snails)	DNA(µg/mg)	RNA(µg/mg)
Nov.,06	Control	24°C	8.61*	5.1^+	12.0*	119.00±0.81	60.07±0.29	26.63±0.20
	A	24°C	8.64*	5.2^+	13.0	83.66±0.33	44.68±0.33	16.31±0.21
	B	25°C	8.70*	4.2^+	16.6*	21.50±0.22	18.47±0.62	8.85±0.52
	C	26°C	7.84*	7.7^+	8.1	42.50±0.34	34.94±0.42	9.16±0.92
Dec.,06	Control	21°C	8.67*	5.7	12.6*	92.00±0.77	41.66±0.85	14.44±0.08
	A	21°C	8.56*	5.5^+	13.3*	94.33±0.95	44.68±0.96	17.00±0.21
	B	21°C	8.81*	5.5^+	14.0*	17.50±0.56	7.38±0.42	6.76±0.23
	C	21°C	8.73*	6.9	8.0	57.83±0.54	33.26±0.45	11.97±0.23
Jan.,07	Control	16°C	8.68*	6.1^+	9.0*	62.33±0.84	16.80±0.85	6.85±0.28
	A	16°C	8.69*	6.0^+	9.0*	59.00±0.51	17.63±0.22	14.75±1.09
	B	16°C	8.39*	5.8^+	10.0*	20.00±0.93	21.83±0.62	6.94±0.29
	C	16°C	8.83*	7.5^+	8.0*	31.00±0.44	15.11±0.45	6.17±0.04
Feb.,07	Control	20°C	8.17*	5.7^+	13.0	112.00±0.57	55.10±0.42	24.47±0.23
	A	20°C	8.19	5.5^+	14.0*	113.00±1.12	57.72±0.33	21.52±0.04
	B	20°C	8.53*	5.2^+	16.0*	23.50±1.20	24.05±0.08	9.71±0.21
	C	20°C	8.38*	6.4^+	11.0*	44.16±0.91	25.19±0.45	12.84±0.22
Mar.,07	Control	23°C	8.75*	5.8^+	12.0*	97.16±0.54	45.69±0.42	16.97±0.09
	A	23°C	8.73*	5.2^+	13.0*	74.50±0.72	17.13±0.36	6.25±0.26
	B	23°C	8.96	4.0^+	15.0*	19.16±0.65	18.47±0.33	9.02±0.34
	C	23°C	8.91*	6.6	9.0	64.00±0.96	34.40±0.45	14.06±0.23
Apr.,07	Control	33°C	8.54*	3.9^+	10.1*	115.33±0.42	49.72±0.42	22.21±0.43
	A	33°C	8.66*	3.8^+	11.5*	114.50±0.72	49.72±0.43	25.27±0.41
	B	33°C	8.86*	3.6^+	14.8*	28.33±0.55	53.69±0.44	25.79±0.17
	C	33°C	8.43	4.5^+	9.0*	96.83±0.54	55.70±0.26	23.53±0.84
May.,07	Control	35°C	8.22*	4.1	9.0*	196.33±.61	63.43±0.16	25.35±0.23
	A	35°C	8.20*	3.5^+	10.8*	103.33±0.76	47.37±0.45	22.21±0.43
	B	36°C	7.15*	2.8^+	10.5	76.50±0.50	47.70±0.42	21.11±0.44
	C	36°C	7.82	4.2	5.8	90.50±0.50	49.65±0.70	27.73±0.25
Jun.,07	Control	37°C	7.18*	3.1	10.1*	84.66±0.61	21.66±0.50	17.00±0.21
	A	37°C	7.22*	3.2^+	11.0*	77.00±0.77	30.70±0.16	12.35±0.08
	B	37°C	7.94*	2.7^+	13.3*	17.50±0.50	15.28±0.16	3.03±0.24
	C	37°C	7.42	4.6^+	6.8*	53.33±0.76	25.13±0.59	10.58±0.17
Jul.,07	Control	36°C	7.16*	4.6	11.0*	20.0±0.68	12.42±0.76	6.15±0.08
	A	35°C	7.20*	4.5^+	11.0*	13.66±0.49	9.40±0.50	4.85±0.10
	B	35°C	7.41*	3.2^+	12.0	6.00±0.44	4.70±0.21	4.50±0.55
	C	35°C	7.54*	5.4^+	8.0*	12.83±0.40	8.06±0.45	5.25±0.29
Aug.,07	Control	35°C	7.01*	5.9^+	6.1*	96.66±0.76	49.05±0.42	19.26±0.23
	A	35°C	7.08*	5.8^+	7.8	84.83±0.65	35.61±0.67	14.92±0.22
	B	35°C	7.25*	5.5^+	7.5*	13.33±0.33	9.46±0.81	4.15±0.16
	C	35°C	7.29*	6.8^+	6.3	25.00±0.63	16.62±0.50	7.11±0.32
Sep.,07	Control	35°C	7.82*	5.7^+	6.8*	45.16±0.94	19.65±0.34	9.71±0.44
	A	35°C	7.73*	5.5^+	7.8*	36.00±1.00	18.64±0.93	8.00±0.31
	B	35°C	7.42*	3.7^+	12.0	22.66±1.02	25.53±0.42	8.55±0.09
	C	35°C	7.50*	5.7^+	6.0*	15.83±1.08	16.96±0.16	3.98±0.39
Oct.,07	Control	34°C	8.45*	5.3^+	7.5*	158.83±0.98	59.13±0.67	30.76±0.27
	A	34°C	8.44*	5.2^+	7.8*	155.00±0.98	58.46±0.40	29.99±0.28
	B	34°C	8.61*	4.4^+	8.8*	63.00±1.09	28.89±0.42	12.35±0.08
	C	34°C	8.68*	5.9^+	4.6*	83.66±2.29	38.97±0.43	14.71±0.08

Each experiment was replicated 6 times and the value of temperature, pH, dissolved oxygen and dissolved free carbon dioxide is the mean of six replicate. Fecundity and DNA, RNA is the mean ± SE were measured after every 24h period up to 96h and 96h respectively.

Product moment correlation coefficient in between the fecundity and abiotic factors indicates significant ($p<0.05$) (+) positive / (*) negative correlation.

Rank correlation coefficient between fecundity and DNA, RNA indicates a significant positive ($p<0.01$) correlation.

A- Tap water change at every 24h upto 96hB- Ramgarh lake water (dirty/polluted) C- Aerated water

Table 2: Effect of temperature, pH, dissolve oxygen and CO_2 in different water samples on the levels of DNA (µg/mg) and RNA (µg/mg) in the ovotestis of the snail *Lymnaea acuminata* in the month of November 2006 to October 2007.

Month	Parameter/ water regimen	Temp.	pH	D.O.(mg/L)	CO_2(mg/L)	Fecundity after 24 h (egg/20 snails)	DNA(µg/mg)	RNA(µg/mg)
Nov.,06	Control	24°C	8.61*	5.1^+	12.0*	119.00±0.81	60.07±0.29	26.63±0.20
	D	26°C	7.61*	5.1^+	13.3	36.33±0.55	23.31±0.06	11.80±0.34
	E	26°C	7.64*	5.8^+	12.3*	74.33±0.76	17.47±0.76	15.10±0.35
Dec.,06	Control	21°C	8.67*	5.7	12.6*	92.00±0.77	41.66±0.85	14.44±0.08
	D	21°C	8.23*	4.5^+	14.0*	28.83±0.54	10.03±0.69	9.71±0.21
	E	21°C	8.57*	5.5^+	13.0	64.66±0.49	39.35±0.89	17.28±0.28
Jan.,07	Control	16°C	8.68*	6.1^+	9.0*	62.33±0.84	16.80±0.85	6.85±0.28
	D	16°C	8.49*	5.3^+	10.0*	25.50±0.34	20.83±0.43	8.15±0.17
	E	16°C	8.69	6.1	9.0*	55.66±0.49	27.61±0.09	10.55±0.08
Feb.,07	Control	20°C	8.17*	5.7^+	13.0*	112.00±0.57	55.10±0.42	24.47±0.23
	D	20°C	8.54*	4.2^+	18.0*	33.33±1.14	25.19±0.45	9.16±0.26
	E	20°C	8.28	5.4	15.0	85.50±0.67	41.66±0.42	14.23±0.21
Mar.,07	Control	23°C	8.75*	5.8^+	12.0*	97.16±0.54	45.69±0.42	16.97±0.09
	D	23°C	8.53*	2.3^+	18.0*	41.33±0.61	29.23±0.45	12.14±0.21
	E	23°C	8.71*	5.2^+	13.0*	72.66±0.92	39.64±0.67	12.14±1.10
Apr.,07	Control	33°C	8.54*	3.9^+	10.1*	115.33±0.42	49.72±0.42	22.21±0.43
	D	33°C	8.33*	3.1^+	16.6*	55.16±0.94	54.29±0.42	14.26±0.04
	E	33°C	8.46*	3.6^+	13.8	99.00±0.81	56.84±0.25	23.62±0.18
May.,07	Control	35°C	8.22*	4.1	9.0*	196.33±.61	63.43±0.16	25.35±0.23
	D	36°C	7.50	3.4	12.5	80.16±0.30	33.26±0.45	22.35±0.89
	E	36°C	7.80*	3.8	8.1*	102.66±0.76	45.02±0.42	17.70±0.26
Jun.,07	Control	37°C	7.18*	3.1	10.1*	84.66±0.61	21.66±0.50	17.00±0.21
	D	37°C	7.20	3.4	13.0	40.50±0.50	28.69±0.33	9.02±0.21
	E	37°C	7.33*	3.7^+	10.3*	62.16±0.75	18.31±0.84	11.97±0.23
Jul.,07	Control	36°C	7.16*	4.6	11.0*	20.00±0.68	12.42±0.76	6.15±0.08
	D	35°C	7.54*	3.7^+	10.0*	8.66±0.21	6.54±0.51	6.24±0.57
	E	35°C	7.30*	4.0	9.0*	14.66±0.55	8.74±0.21	5.71±0.42
Aug.,07	Control	35°C	7.01*	5.9^+	6.1*	96.66±0.76	49.05±0.42	19.26±0.23
	D	35°C	7.55	6.2^+	12.3	16.83±0.40	16.29±0.40	5.03±0.22
	E	35°C	7.17*	6.3^+	7.3*	37.16±0.40	28.89±0.42	7.63±0.24
Sep.,07	Control	35°C	7.82*	5.7^+	6.8*	45.16±0.94	19.65±0.34	9.71±0.44
	D	35°C	7.28*	3.9^+	9.0*	13.50±0.72	13.43±0.21	3.20±0.20
	E	35°C	7.43*	4.5^+	6.8*	28.16±1.45	16.29±0.47	4.24±0.24
Oct.,07	Control	34°C	8.45*	5.3^+	7.5*	158.83±0.98	59.13±0.67	6.85±0.28
	D	34°C	8.44*	4.5^+	6.8*	106.83±1.35	49.52±0.72	30.76±0.27
	E	34°C	8.53*	4.9^+	7.3	141.16±1.42	52.29±0.69	29.29±0.27

Each experiment was replicated 6 times and the value of temperature, pH, dissolved oxygen and dissolved free carbon dioxide is the mean of six replicate. Fecundity and DNA, RNA is the mean±SE were measured after every 24h period up to 96h and 96h respectively.

Product moment correlation coefficient in between the fecundity and abiotic factors indicates significant ($p<0.05$) (+) positive / (*) negative correlation.

Rank correlation coefficient between fecundity and DNA, RNA indicates a significant positive ($p<0.01$) correlation.

D- Dark E-Light

Table 3: Effect of temperature, pH, dissolve oxygen and CO_2 in different water samples on the levels of protein (µg/mg) and amino acid (µg/mg) in the ovotestis of the snail *Lymnaea acuminata* in the month of November 2006 to October 2007.

Month	Parameter/ water regimen	Temp.	pH	D.O.(mg/L)	CO_2(mg/L)	Fecundity after 24 hr (egg/20 snails)	Protein(µg/mg)	Aminoacid(µg/mg)
Nov.,06	Control	24°C	8.61*	5.1^+	12.0*	119.00±0.81	82.05±0.32	38.09±0.18
	A	24°C	8.64*	5.2^+	13.0	83.66±0.33	61.29±0.43	19.18±0.53
	B	25°C	8.70*	4.2^+	16.6*	21.50±0.22	53.83±0.41	18.92±0.42
	C	26°C	7.84*	7.7^+	8.1	42.50±0.34	52.86±0.32	18.61±0.69
Dec.,06	Control	21°C	8.67*	5.7	12.6*	92.00±0.77	47.83±0.68	14.60±0.58
	A	21°C	8.56*	5.5^+	13.3*	94.33±0.95	76.54±0.45	27.75±0.19
	B	21°C	8.81*	5.5^+	14.0*	17.50±0.56	45.07±0.32	10.16±0.14
	C	21°C	8.73*	6.9	8.0	57.83±0.54	62.91±0.41	26.20±0.19
Jan.,07	Control	16°C	8.68*	6.1^+	9.0*	62.33±0.84	54.17±0.32	23.20±0.58
	A	16°C	8.69*	6.0^+	9.0*	59.00±0.51	28.86±0.41	11.45±0.28
	B	16°C	8.39*	5.8^+	10.0*	20.00±0.93	46.05±0.41	20.90±0.18
	C	16°C	8.83*	7.5^+	8.0*	31.00±0.44	46.54±0.33	10.74±0.61

Table 3: Continued

Feb.,07	Control	20°C	8.17*	5.7+	13.0	112.00±0.57	81.08±0.41	36.95±0.86
	A	20°C	8.19	5.5+	14.0*	113.00±1.12	83.84±0.39	37.23±0.18
	B	20°C	8.53*	5.2+	16.0*	23.50±1.20	63.24±0.43	18.32±0.57
	C	20°C	8.38*	6.4+	11.0*	44.16±0.91	69.08±0.79	22.34±0.62
Mar.,07	Control	23°C	8.75*	5.8+	12.0*	97.16±0.54	67.66±0.28	31.64±0.14
	A	23°C	8.73*	5.2+	13.0*	74.50±0.72	34.83±0.85	22.91±0.57
	B	23°C	8.96	4.0+	15.0*	19.16±0.65	21.89±0.86	16.32±0.22
	C	23°C	8.91*	6.6	9.0	64.00±0.96	70.37±0.41	23.48±0.18
Apr.,07	Control	33°C	8.54*	3.9+	10.1*	115.33±0.42	82.05±0.32	26.92±0.36
	A	33°C	8.66*	3.8+	11.5*	114.50±0.72	78.16±0.32	32.22±0.19
	B	33°C	8.86*	3.6+	14.8*	28.33±0.55	59.03±0.32	21.05±0.19
	C	33°C	8.43	4.5+	9.0*	96.83±0.54	74.59±0.41	31.07±0.14
May.,07	Control	35°C	8.22*	4.1	9.0*	196.33±.61	84.48±0.58	40.67±0.36
	A	35°C	8.20*	3.5+	10.8*	103.33±0.76	78.16±0.32	33.79±0.18
	B	36°C	7.15*	2.8+	10.5	76.50±0.50	69.07±0.62	26.34±0.17
	C	36°C	7.82	4.2	5.8	90.50±0.50	71.02±0.43	34.68±0.18
Jun.,07	Control	37°C	7.18*	3.1	10.1*	84.66±0.61	78.65±0.39	14.88±0.18
	A	37°C	7.22*	3.2+	11.0*	77.00±0.77	44.11±0.20	15.03±0.76
	B	37°C	7.94*	2.7+	13.3*	17.50±0.50	27.25±0.67	10.45±0.34
	C	37°C	7.42	4.6+	6.8*	53.33±0.76	63.56±0.64	24.28±0.13
Jul.,07	Control	36°C	7.16*	4.6	11.0*	20.0±0.68	29.84±0.41	12.03±0.22
	A	35°C	7.20*	4.5+	11.0*	13.66±0.49	71.52±0.78	12.46±0.19
	B	35°C	7.41*	3.2+	12.0	6.00±0.44	17.83±0.41	8.30±0.18
	C	35°C	7.54*	5.4+	8.0*	12.83±0.40	35.35±0.86	12.88±0.38
Aug.,07	Control	35°C	7.01*	5.9+	6.1*	96.66±0.76	79.13±0.41	34.65±0.18
	A	35°C	7.08*	5.8+	7.8	84.83±0.65	75.24±0.41	27.77±0.18
	B	35°C	7.25*	5.5+	7.5*	13.33±0.33	40.32±0.60	11.17±0.31
	C	35°C	7.29*	6.8+	6.3	25.00±0.63	32.92±0.77	9.74±0.43
Sep.,07	Control	35°C	7.82*	5.7+	6.8*	45.16±0.94	35.85±0.63	20.90±0.53
	A	35°C	7.73*	5.5+	7.8*	36.00±1.00	50.27±1.25	14.10±0.22
	B	35°C	7.42*	3.7+	12.0	22.66±1.02	50.75±0.46	21.05±0.19
	C	35°C	7.50*	5.7+	6.0*	15.83±1.08	24.81±0.40	13.17±0.57
Oct.,07	Control	34°C	8.45*	5.3+	7.5*	158.83±0.98	84.32±0.59	46.11±0.18
	A	34°C	8.44*	5.2+	7.8*	155.00±0.98	78.48±0.42	43.96±0.34
	B	34°C	8.61*	4.4+	8.8*	63.00±1.09	55.45±0.43	24.20±0.14
	C	34°C	8.68*	5.9+	4.6*	83.66±2.29	79.62±0.88	26.77±0.26

Each experiment was replicated 6 times and the value of temperature, pH, dissolved oxygen and dissolved free carbon dioxide is the mean of six replicate. Fecundity and protein is the mean±SE were measured after every 24h period up to 96h and 96h respectively.

Product moment correlation coefficient in between the fecundity and abiotic factors indicates significant ($p<0.05$) (+) positive / (*) negative correlation.

Rank correlation coefficient between fecundity and protein, amino acid indicates a significant positive ($p<0.01$) correlation.

A- Tap water change at every 24h upto 96h B- Ramgarh lake water (dirty/polluted) C- Aerated water

Table 4: Effect of temperature, pH, dissolve oxygen and CO_2 in different water samples on the levels of protein (µg/mg) and amino acid (µg/mg) in the ovotestis of the snail *Lymnaea acuminata* in the month of November 2006 to October 2007.

Month	Parameter/ water regimen	Temp.	pH	D.O.(mg/L)	CO_2(mg/L)	Fecundity after 24 h (egg/20 snails)	Protein(µg/mg)	Amino acid(µg/mg)
Nov.,06	Control	24°C	8.61*	5.1+	12.0*	119.00±0.81	82.05±0.32	38.09±0.18
	D	26°C	7.61*	5.1+	13.3	36.33±0.55	49.62±0.43	22.19±0.14
	E	26°C	7.64*	5.8+	12.3*	74.33±0.76	69.41±0.33	26.34±0.28
Dec.,06	Control	21°C	8.67*	5.7	12.6*	92.00±0.77	47.83±0.68	14.60±0.58
	D	21°C	8.23*	4.5+	14.0*	28.83±0.54	48.97±0.32	10.71±0.29
	E	21°C	8.57*	5.5+	13.0	64.66±0.49	48.32±0.54	22.71±0.28
Jan.,07	Control	16°C	8.68*	6.1+	9.0*	62.33±0.84	54.17±0.32	23.20±0.58
	D	16°C	8.49*	5.3+	10.0*	25.50±0.34	29.19±0.25	12.88±6.22
	E	16°C	8.69	6.1	9.0*	55.66±0.49	33.08±1.25	18.61±0.69
Feb.,07	Control	20°C	8.17*	5.7+	13.0*	112.00±0.57	81.08±0.41	36.95±0.86
	D	20°C	8.54*	4.2+	18.0*	33.33±1.14	55.78±0.41	19.48±0.57
	E	20°C	8.28	5.4	15.0	85.50±0.67	74.59±0.41	24.25±0.06

Table 4: Continued

Mar.,07	Control	23°C	8.75*	5.8+	12.0*	97.16±0.54	67.66±0.28	31.64±0.14
	D	23°C	8.53*	2.3+	18.0*	41.33±0.61	61.95±0.82	21.05±0.19
	E	23°C	8.71*	5.2+	13.0*	72.66±0.92	56.10±0.32	28.35±0.31
Apr.,07	Control	33°C	8.54*	3.9+	10.1*	115.33±0.42	82.05±0.32	26.92±0.36
	D	33°C	8.33*	3.1+	16.6*	55.16±0.94	69.08±0.43	24.34±0.18
	E	33°C	8.46*	3.6+	13.8	99.00±0.81	76.21±0.32	39.10±0.19
May.,07	Control	35°C	8.22*	4.1	9.0*	196.33±.61	84.48±0.58	40.67±0.36
	D	36°C	7.50	3.4	12.5	80.16±0.30	68.19±0.19	30.50±0.19
	E	36°C	7.80*	3.8	8.1*	102.66±0.76	80.43±0.41	33.79±0.18
Jun.,07	Control	37°C	7.18*	3.1	10.1*	84.66±0.61	78.65±0.39	14.88±0.18
	D	37°C	7.20	3.4	13.0	40.50±0.50	66.81±0.41	23.48±0.18
	E	37°C	7.33*	3.7+	10.3*	62.16±0.75	68.69±0.74	14.02±0.52
Jul.,07	Control	36°C	7.16*	4.6	11.0*	20.00±0.68	29.84±0.41	12.03±0.22
	D	35°C	7.54*	3.7+	10.0*	8.66±0.21	44.92±0.39	9.16±0.18
	E	35°C	7.30*	4.0	9.0*	14.66±0.55	26.75±0.48	10.45±0.14
Aug.,07	Control	35°C	7.01*	5.9+	6.1*	96.66±0.76	79.13±0.41	34.65±0.18
	D	35°C	7.55	6.2+	12.3	16.83±0.40	23.35±0.52	11.45±0.36
	E	35°C	7.17*	6.3+	7.3*	37.16±0.40	67.46±0.41	14.02±0.18
Sep.,07	Control	35°C	7.82*	5.7+	6.8*	45.16±0.94	35.85±0.63	20.90±0.53
	D	35°C	7.28*	3.9+	9.0*	13.50±0.72	34.70±0.33	17.46±0.28
	E	35°C	7.43*	4.5+	6.8*	28.16±1.45	39.81±0.44	12.05±0.57
Oct.,07	Control	34°C	8.45*	5.3+	7.5*	158.83±0.98	84.32±0.59	46.11±0.18
	D	34°C	8.44*	4.5+	6.8*	106.83±1.35	70.69±0.59	24.63±0.65
	E	34°C	8.53*	4.9+	7.3	141.16±1.42	78.49±0.41	29.78±0.18

Each experiment was replicated 6 times and the value of temperature, pH, dissolved oxygen anddissolved free carbon dioxide is the mean of six replicate. Fecundity, protein and amino acid is the mean ± SE were measured after every 24h period up to 96h and 96h respectively.

Product moment correlation coefficient in between the fecundity and abiotic factors indicates significant ($p<0.05$) (+) positive / (*) negative correlation.

Rank correlation coefficient between fecundity and protein, amino acid indicates a significant positive ($p<0.01$) correlation.

D- Dark -E-Light

positive rank correlation between the fecundity of snail and corresponding biochemical parameters in the ovotestis of snails exposed to different regimen of water in different months of the year 2006-2007.

The data given above clearly demonstrate that abiotic factors (temperature, pH, dissolved oxygen, free carbon dioxide, dark / light period) of the aquatic environment vary seasonally. These abiotic factors significantly alter the biochemical parameters viz., protein, total free amino acids and nucleic acids (DNA, RNA) in the ovotestis of the snail *L. acuminata*. Changes in biochemical parameters may be the cause of the simultaneous variations in the fecundity, hatchability and survival of snail *L. acuminata* [10].

Earlier, it has been observed that photoperiod, food consumption, temperature, water quality and parasites regulate the reproduction of the snail *L. stagnalis* [19, 29]. In summer season (June-August) the temperature of water is usually high (35-37°C). Consequently, higher temperature alters the pH (low), dissolved oxygen concentration (low) and dissolved free carbon dioxide concentration (high) in water available to snails [9]. Contrarily, in winter season the temperature of water is low and it holds more oxygen than summer season [30-31]. Dissolved oxygen is one of the major components required by snail metabolic activity [11, 32]. Temperature has pervasive effects on oxygen consumption. In normal condition metabolic demand for oxygen increases substantially with temperature [33]. At higher temperature, the increasing rate of snail metabolism may release more CO_2, which affects the pH of water [34, 35]. This was evident from the elevated concentration of CO_2 which causes decrease in the pH of water during the summer season. Possibly cumulative effect of these abiotic factors on the protein, amino acids and nucleic acids in ovotestis may be direct or indirectly through caudo dorsal cells (CDCs), which release ovulation hormone and ultimately affect the reproduction of snails in different months.

The reduction in protein levels may be due to indirect interference of the environmental abiotic factors with protein synthesis. Protein synthesis is affected by pH, as it has crucial role on the activity of a number of enzymes involved in protein synthesis [36]. Amino acids are the building blocks for structural protein and enzymes. Purine and pyrimidine, which form essential components of DNA and RNA, are synthesized from amino acids.

Amino acids are of critical importance in energy metabolism of molluscs [37]. Certain amino acids (glutamic, aspartic, tyrosine, lysine, arginine and praline) have stimulatory effects on the development of parasite helminthes [38], because flukes have usual nutritional requirement for essential amino acid [37].

DNA and RNA were significantly influenced by the water temperature [39] as evident in result section. Small change in DNA/RNA levels can have a tremendous impact on biological process [40]. An elevated temperature from 5-25°C induce spermatogenic DNA synthesis and formation of spermatid and spermatozoa in ovotestis of snails [41]. RNA translation efficiency is lower at higher temperature [42]. Therefore, animals need higher concentration of RNA to maintain their metabolic function at colder temperature [39]. The synthesis of DNA and RNA also influence by the intracellular pH within physiological range. The activity increases with increasing pH from 7.0 - 8.0 [43]. The process of cellular growth and divisions requires the synthesis of nucleic acids and protein. The fact that RNA is obligate precursor to protein synthesis [44], therefore, it suggests a seasonal variation in RNA mainly governed by seasonal abiotic factors, abundance of food and associated with growth and breeding cycles. The seasonal trend in RNA varied, but common for most species was an increase from late winter towards spring and summer [45]. As a result, changes in RNA content often reflected by a change in protein synthesis rate [46-47].

Conclusively, this study showed that abiotic factors in different regimen of water can significantly alter the reproductive behavior of snails, which is due to the biochemical changes in ovotestis. The maximum reproductive capacity and development process of snail was in between the month of March to May, which is due to higher level of protein, amino acids and nucleic acids in ovotestis of snail. Nevertheless, water quality has also profound effect of the reproduction of snails. It is obvious, that most suitable period for the control of the snail *L. acuminata* and ultimately the incidence of fascioliasis in India is during the month of March to May.

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