Effect of environmental factors on the fecundity, hatchability and survival of snail *Lymnaea* (Radix) *acuminata* (Lamarck): vector of fascioliasis

Harsh Vardhan Jigyasu and Vinay Kumar Singh

ABSTRACT

Lymnaea acuminata breeds round the year. The effect of pH, temperature, dissolved oxygen, carbon dioxide, light/dark period and clean/polluted water on the fecundity, hatchability and survival of young snails of *L. acuminata* were studied. It was observed that these environmental variant abiotic factors caused a significant variation in fecundity, hatchability and survival of young snails. Maximum reproduction of this snail was observed in the months of March to May. A significant positive correlation ($p < 0.05$) between D.O. (3.1–7.7 ppm)/pH (7.01–8.96) of water with fecundity (6.0–196.33/20 snails), hatchability (54.69–96.91%) and survival (61.3–95.86%) of young snails was observed for each month and each interval of 24–72 h. In contrast, a significant negative correlation between dissolved CO_2 (4.6–16.6 ppm)/temperature (16–37°C) of water was noted with fecundity, hatchability and survival of snails. Percent hatchability in the eggs in different regimens of water was between 96.91–54.69%. The hatching period was prolonged (2–14 days) in snail exposed to different groups of water compared to the control group (2–9 d). This study conclusively shows that variant abiotic factors in different months of the year can significantly alter the reproductive ability and development process in the snail *Lymnaea acuminata*.

Key words | environmental factors, fecundity, hatchability, *Lymnaea acuminata*, polluted water

Harsh Vardhan Jigyasu
Vinay Kumar Singh (corresponding author)
Department of Zoology,
D.D.U. Gorakhpur University,
Gorakhpur 273009, U.P.,
India
Tel.: +91 0551 220 2187 (Office);
+91 941 585 5488 (Mobile)
E-mail: *vksinghgpu@yahoo.co.in*

INTRODUCTION

Fascioliasis is one of the most debilitating zoonotic diseases of domestic herbivores and human beings (Ashrafi *et al.* 2006; WHO 2006; Lewin 2007; Alatoom *et al.* 2008). Earlier, fascioliasis was limited to populations within well-defined watershed boundaries: however; recent environmental changes and modification in human behavior have increased the risk in other new populations (Savioli *et al.* 1999). Liver flukes *Fasciola hepatica* or *Fasciola gigantica* are the causative agent of fascioliasis (Ghanaei *et al.* 2006; Taheri *et al.* 2007). The freshwater snail *Lymnaea acuminata* is the intermediate host of *F. gigantica* (Singh & Agarwal 1981). An effective method to reduce the incidence of fascioliasis is to control the population of vector snails, thereby breaking the lifecycle of these flukes, or by reducing the reproductive capacity of snails (Godan 1983; Katz 1986; Agarwal & Singh 1988; Singh *et al.* 1996). Earlier studies have shown that the reproductive capacity of snails varies from one season to another (Maat *et al.* 1983; Wayne 2001). It has also been conclusively shown that oviposition in snails is induced by a neuroendocrine hormone of the Caudo-Dorsal Cells (CDCs) in the cerebral ganglion (Geraerts & Bohlken 1976; Takeda 1977; Maat & Lodder 1980; Maat *et al.* 1982; Singh *et al.* 2008). Several mechanisms are involved in the release of the ovipository hormone by environmental factors (Highnam & Hill 1977; De Jong-Brink *et al.* 1992). Environmental factors such as temperature, pH, dissolved oxygen, carbon dioxide and light/dark period are major seasonal variants that affect the morphological characteristics of CDCs (Joosse 1964; Maat *et al.* 1983; Wayne 2001).

doi: 10.2166/wh.2009.035

The objective of this study was to explore the possibility whether seasonal changes in the abiotic factors temperature, pH, dissolved oxygen and carbon dioxide, dark and light exposure can influence the fecundity, hatchability and survival of young snail *L. acuminata* in each month of the year 2006–2007. This will be helpful in deciding the most suitable time in the year for their control.

METHODOLOGY

Animals

Adult *L. acuminata* (2.60 ± 0.30 cm in length) were collected locally from ponds, pools, lakes and low-lying submerged areas located almost adjacent to our University campus. The collected snails were acclimatized in dechlorinated tap water for 72 h.

Experimentation

The following experiments were carried out in different regimens of water:

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

Six groups of twenty snails were kept in six glass aquaria separately for each regimen with 5 liters of different regimens of water. The aquaria were covered with wire netting to prevent the animals from escaping. *L. acuminata* laid their eggs in the form of elongated gelatinous capsules containing 2–180 eggs on the lower surface of leaves of aquatic vegetation. After every 24 h up to 96 h, the total number of eggs oviposited by the snails were counted in each aquarium. Dissolved O_2, CO_2, pH and temperature of different regimens of water were measured simultaneously. Temperature and pH were measured by a thermometer and digital pH meter, respectively. Dissolved O_2 and CO_2 were estimated according to methods prescribed by APHA (2005).

Since it is difficult to detect the mother snails for a particular spawn, capsules containing eggs from each aquarium were incubated at 30°C in covered Petri dishes containing the same regimen of water as given to the adult snails. At regular intervals, the development of embryos was observed under a binocular microscope till their hatching. Dead eggs were removed, to avoid any contamination. Young snails were immediately transferred to the same regimen of water and their survival was observed up to 72 h after hatching.

Statistical analysis

Each experiment was replicated at least six times, and values of temperature, pH, dissolved O_2 and CO_2 are expressed as the mean of six replicates. Values of fecundity, hatchability and percent survival were expressed as mean ± SE. The 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/percent survival of snails in each months of the year 2006–2007 (Sokal & Rohlf 1973).

RESULTS

L. acuminata laid their eggs in gelatinous strings, each egg floating in albuminous fluids bounded externally by a membrane. The ovoid eggs were laid in two rows. There was a significant ($p < 0.05$) variation in the fecundity of *L. acuminata* kept in different regimens of water, viz. A, B, C, D and E (Tables 1 and 2); in the control group of snails the maximum fecundity was observed in the month of May (196.33 eggs/20 snails) and the minimum (20 eggs/20 snails) in July (Tables 1 and 2). A significant positive correlation between dissolved oxygen/pH of water and fecundity, hatchability and survival of young snails was noted for each month and each interval of 24 h up to 72 h. In contrast, a significant negative correlation between LC_{50} and dissolved CO_2 and with water temperature was noted. (Tables 1 and 2). The control group of animals hatched into young snails within 2–9 d (Table 1). Complete embryonic development was lacking in the eggs of snails exposed to different regimens of water. Percent hatchability in the eggs of different regimens of water was between 96.91–54.69

Table 1 | Effect of temperature, pH, dissolved oxygen and CO_2 in different water samples on the fecundity, hatchability and percent survival of the snail *Lymnaea acuminata* in the months November 2006 to October 2007

Month	Parameter	*T*	pH	D.O. (mg/l)	CO_2 (mg/l)	Fecundity after 24 h (egg/20 snails)	Hatchability percentage (hatching period in days)	Percent survival 24 h	Percent survival 48 h	Percent survival 72 h
Nov., 06	Control	24°C	8.61^{*}	5.1^{+}	12.0^{*}	119.00 ± 0.81	100 (2–6)	100	100	100
	A	24°C	8.64^{*}	5.2^{+}	13.0	83.66 ± 0.33	90.47 ± 0.40 (2–9)	90.80 ± 0.87	81.36 ± 0.73	71.71 ± 0.33
	B	25°C	8.70^{*}	4.2^{+}	16.6^{*}	21.50 ± 0.22	76.98 ± 0.79 (3–10)	81.25 ± 0.30	62.87 ± 0.32	48.36 ± 0.54
	C	26°C	7.84^{*}	7.7^{+}	8.1	42.50 ± 0.34	73.45 ± 0.74 (3–13)	64.92 ± 0.79	44.01 ± 1.18	28.34 ± 0.34
Dec., 06	Control	21°C	8.67^{*}	5.7	12.6^{*}	92.00 ± 0.77	100 (2–5)	100	100	100
	A	21°C	8.56^{*}	5.5^{+}	13.3^{*}	94.33 ± 0.95	92.99 ± 0.47 (3–7)	90.95 ± 0.92	74.50 ± 1.44	64.20 ± 0.87
	B	21°C	8.81^{*}	5.5^{+}	14.0^{*}	17.50 ± 0.56	78.12 ± 0.49 (3–9)	64.61 ± 0.78	28.75 ± 0.11	18.52 ± 0.33
	C	21°C	8.73^{*}	6.9	8.0	57.83 ± 0.54	56.67 ± 0.77 (3–10)	61.52 ± 0.67	32.47 ± 0.86	15.78 ± 0.50
Jan., 07	Control	16°C	8.68^{*}	6.1^{+}	9.0^{*}	62.33 ± 0.84	100 (3–7)	100	100	100
	A	16°C	8.69^{*}	6.0^{+}	9.0^{*}	59.00 ± 0.51	89.82 ± 0.09 (4–9)	94.33 ± 0.05	77.07 ± 0.71	58.19 ± 0.52
	B	16°C	8.39^{*}	5.8^{+}	10.0^{*}	20.00 ± 0.93	82.55 ± 0.40 (4–11)	72.74 ± 0.47	60.51 ± 0.60	51.71 ± 1.02
	C	16°C	8.83^{*}	7.5^{+}	8.0^{*}	31.00 ± 0.44	67.35 ± 0.44 (4–14)	61.21 ± 0.76	41.81 ± 1.15	29.84 ± 0.59
Feb., 07	Control	20°C	8.17^{*}	5.7^{+}	13.0	112.00 ± 0.57	100 (2–7)	100	100	100
	A	20°C	8.19	5.5^{+}	14.0^{*}	113.00 ± 1.12	96.91 ± 0.61 (3–11)	93.30 ± 0.75	86.76 ± 0.39	71.84 ± 0.47
	B	20°C	8.53^{*}	5.2^{+}	16.0^{*}	23.50 ± 1.20	81.53 ± 1.09 (3–13)	75.59 ± 0.26	62.61 ± 0.49	52.87 ± 1.15
	C	20°C	8.38^{*}	6.4^{+}	11.0^{*}	44.16 ± 0.91	59.97 ± 0.32 (4–14)	73.67 ± 1.21	62.15 ± 0.91	43.99 ± 1.04
Mar., 07	Control	23°C	8.75^{*}	5.8^{+}	12.0^{*}	97.16 ± 0.54	100 (2–9)	100	100	100
	A	23°C	8.73^{*}	5.2^{+}	13.0^{*}	74.50 ± 0.72	95.54 ± 0.45 (2–9)	94.90 ± 0.43	81.18 ± 0.53	58.60 ± 0.12
	B	23°C	8.96	4.0^{+}	15.0^{*}	19.16 ± 0.65	84.26 ± 0.50 (3–10)	87.53 ± 0.46	61.72 ± 0.82	34.93 ± 0.77
	C	23°C	8.91^{*}	6.6	9.0	64.00 ± 0.96	68.16 ± 0.82 (4–12)	77.01 ± 0.64	47.96 ± 0.96	29.43 ± 0.47
Apr., 07	Control	33°C	8.54^{*}	3.9^{+}	10.1^{*}	115.33 ± 0.42	100 (2–5)	100	100	100
	A	33°C	8.66^{*}	3.8^{+}	11.5^{*}	114.50 ± 0.72	92.13 ± 0.04 (3–6)	93.84 ± 0.28	80.58 ± 0.54	75.82 ± 0.57
	B	33°C	8.86^{*}	3.6^{+}	14.8^{*}	28.33 ± 0.55	77.66 ± 0.35 (3–8)	80.34 ± 0.67	66.68 ± 0.54	54.48 ± 0.75
	C	33°C	8.43	4.5^{+}	9.0^{*}	96.83 ± 0.54	71.60 ± 0.53 (3–12)	66.84 ± 0.71	54.81 ± 0.33	44.95 ± 0.44
May., 07	Control	35°C	8.22^{*}	4.1	9.0^{*}	196.33 ± 0.61	100 (2–8)	100	100	100
	A	35°C	8.20^{*}	3.5^{+}	10.8^{*}	103.33 ± 0.76	93.72 ± 0.60 (4–9)	95.86 ± 0.01	80.37 ± 0.03	73.14 ± 0.04
	B	36°C	7.15^{*}	2.8^{+}	10.5	76.50 ± 0.50	70.38 ± 0.55 (4–11)	81.41 ± 0.53	60.98 ± 0.12	57.26 ± 0.13
	C	36°C	7.82	4.2	5.8	90.50 ± 0.50	59.48 ± 0.26 (3–12)	75.84 ± 0.07	52.93 ± 0.60	46.73 ± 0.47
Jun., 07	Control	37°C	7.18^{*}	3.1	10.1^{*}	84.66 ± 0.61	100 (3–6)	100	100	100
	A	37°C	7.22^{*}	3.2^{+}	11.0^{*}	77.00 ± 0.77	86.35 ± 0.29 (4–7)	91.71 ± 0.35	81.70 ± 0.76	65.15 ± 0.19
	B	37°C	7.94^{*}	2.7^{+}	13.3^{*}	17.50 ± 0.50	77.04 ± 0.67 (4–9)	77.61 ± 0.86	65.47 ± 0.53	43.08 ± 0.75
	C	37°C	7.42	4.6^{+}	6.8^{*}	53.33 ± 0.76	59.99 ± 0.16 (5–11)	74.97 ± 0.36	53.62 ± 0.29	37.99 ± 0.46
Jul., 07	Control	36°C	7.16^{*}	4.6	11.0^{*}	20.0 ± 0.68	100 (3–4)	100	100	100
	A	35°C	7.20^{*}	4.5^{+}	11.0^{*}	13.66 ± 0.49	91.59 ± 0.82 (5–6)	91.96 ± 0.20	83.94 ± 0.41	66.69 ± 0.64
	B	35°C	7.41^{*}	3.2^{+}	12.0	6.00 ± 0.44	63.74 ± 1.35 (6–8)	63.47 ± 1.08	36.50 ± 1.08	0
	C	35°C	7.54^{*}	5.4^{+}	8.0^{*}	12.83 ± 0.40	57.18 ± 0.71 (6–9)	72.61 ± 0.75	54.76 ± 1.50	27.38 ± 0.75
Aug., 07	Control	35°C	7.01^{*}	5.9^{+}	6.1^{*}	96.66 ± 0.76	100 (4–6)	100	100	100
	A	35°C	7.08^{*}	5.8^{+}	7.8	84.83 ± 0.65	92.72 ± 0.20 (7–9)	92.79 ± 0.25	79.87 ± 0.28	62.48 ± 0.34
	B	35°C	7.25^{*}	5.5^{+}	7.5^{*}	13.33 ± 0.33	77.42 ± 0.58 (5–8)	80.53 ± 0.66	62.92 ± 1.04	43.46 ± 1.11
	C	35°C	7.29^{*}	6.8^{+}	6.3	25.00 ± 0.63	61.30 ± 0.14 (6–11)	67.26 ± 0.94	56.54 ± 0.18	33.77 ± 1.16

Table 1 | *(continued)*

Month	Parameter	*T*	pH	D.O. (mg/l)	CO_2 (mg/l)	Fecundity after 24 h (egg/20 snails)	Hatchability percentage (hatching period in days)	Percent survival 24 h	Percent survival 48 h	Percent survival 72 h
Sep., 07	Control	35°C	7.82^*	5.7^+	6.8^*	45.16 ± 0.94	100 (2–4)	100	100	100
	A	35°C	7.73^*	5.5^+	7.8^*	36.00 ± 1.00	94.41 ± 0.16 (3–7)	94.64 ± 0.41	80.94 ± 0.51	74.11 ± 0.69
	B	35°C	7.42^*	3.7^+	12.0	22.66 ± 1.02	69.91 ± 0.63 (3–10)	83.26 ± 0.83	64.15 ± 0.63	55.87 ± 0.63
	C	35°C	7.50^*	5.7^+	6.0^*	15.83 ± 1.08	54.69 ± 0.61 (4–11)	61.37 ± 0.98	51.94 ± 1.27	38.61 ± 0.98
Oct., 07	Control	34°C	8.45^*	5.3^+	7.5^*	158.83 ± 0.98	100 (3–5)	100	100	100
	A	34°C	8.44^*	5.2^+	7.8^*	155.00 ± 0.98	93.69 ± 0.24 (5–8)	95.51 ± 0.49	83.74 ± 0.38	70.08 ± 0.38
	B	34°C	8.61^*	4.4^+	8.8^*	63.00 ± 1.09	72.71 ± 0.40 (7–12)	86.87 ± 0.29	61.87 ± 0.70	53.44 ± 0.31
	C	34°C	8.68^*	5.9^+	4.6^*	83.66 ± 2.29	63.06 ± 0.68 (7–10)	72.25 ± 0.37	50.48 ± 0.21	38.20 ± 0.24

Each experiment was replicated 6 times and the value of pH, dissolved oxygen, dissolved free carbon dioxide is the mean of six replicates and fecundity is the mean ± SE measured after 24 h period. Product moment correlation coefficient in between the fecundity (egg/20 snail), hatchability, percent survival and different parameters indicate significant ($p < 0.05$) (+) positive/(*) negative correlation. A- Tap water change at every 24 h up to 96 h. B-Ramgarh lake water. C-Aerated water.

(Tables 1 and 2). The hatching period was prolonged (2–14 d) in the snails exposed to groups A, B, C, D and E than in the control group (2–9 d). Maximum prolongation (7–12 d) of hatchability was observed in eggs of group B in the month of October whereas the minimum (2–9 d) was in group A in the month of November (Table 1). The newly hatched snails exposed to different regimens of water were mostly found attached to the walls of the container. They had very thin shells in comparison with the control group. The movement of newly hatched snails of groups A, B, C, D and E was slow and they had smaller tentacles than the control. There was a significant negative correlation between the survival time and the survival of young snails hatched from eggs laid by snails exposed to different regimens of water. The fecundity of group C was more than group B but the maximum reduction in survival was observed in the young ones of group C.

DISCUSSION

It is evident from the results that temperature, pH, dissolved oxygen and carbon dioxide alter the fecundity, hatchability and survival of snails. In the summer season (June–August) the temperature of the water is high (35–37°C). The fecundity of the snails was usually high when the temperature of water increased up to 35°C, i.e. in the month of May. In contrast, when the temperature of the water increased above 35°C, i.e. in the months of June and July, there was a marked decrease in fecundity. An earlier study has shown that the decrease in temperature from 20°C to 8°C stopped the oviposition of the snail *Lymnaea stagnalis* because of a reduction in the activities of neurosecretory cells (CDCs) (Dogterom *et al.* 1984; Wayne 2001). It seems that for normal fecundity, hatchability and survival of young snails *L. acuminata* the average temperature of water should be between 23°C and 35°C, as evident in the control group of snails.

Dissolved oxygen is one of the most important ecological parameters. Water holds more oxygen in the winter season than in the summer season (Ingram *et al.* 1997). *Lymnaea* is very sensitive to the dissolved oxygen content of water (Janse 1981; Maat *et al.* 1983). It has been reported that dissolved oxygen below 20% saturation causes stress to freshwater mussels (Ellis 1937; Ingram 1957;

Table 2 | Effect of temperature, pH, dissolved oxygen and CO_2 in different water samples on the fecundity, hatchability and percent survival of the snail *Lymnaea acuminata* in the months November 2006 to October 2007

Month	Parameter	Temp.	pH	D.O. (mg/l)	CO_2 (mg/l)	Fecundity after 24 h (egg/20 snails)	Hatchability percentage (hatching period in days)	Percent survival 24 h	Percent survival 48 h	Percent survival 72 h
Nov., 06	Control	24°C	8.61*	5.1+	12.0*	119.00 ± 0.81	100 (2–6)	100	100	100
	D	26°C	7.61*	5.1+	13.3	36.33 ± 0.55	78.47 ± 0.85 (3–10)	77.92 ± 1.16	57.80 ± 0.48	36.79 ± 0.78
	E	26°C	7.64*	5.8+	12.3*	74.33 ± 0.76	85.65 ± 0.14 (3–9)	89.02 ± 0.95	79.68 ± 0.95	63.36 ± 0.77
Dec., 06	Control	21°C	8.67*	5.7	12.6*	92.00 ± 0.77	100 (2–5)	100	100	100
	D	21°C	8.23*	4.5+	14.0*	28.83 ± 0.54	74.91 ± 1.12 (3–8)	77.5 3 ± 0.85	65.97 ± 0.35	49.03 ± 0.36
	E	21°C	8.57*	5.5+	13.0	64.66 ± 0.49	85.60 ± 0.91 (3–7)	76.51 ± 1.01	69.00 ± 0.31	67.16 ± 0.46
Jan., 07	Control	16°C	8.68*	6.1+	9.0*	62.33 ± 0.84	100 (3–7)	100	100	100
	D	16°C	8.49*	5.3+	10.0*	25.50 ± 0.34	72.51 ± 0.38 (3–10)	83.57 ± 0.31	53.16 ± 0.48	41.40 ± 0.52
	E	16°C	8.69	6.1	9.0*	55.66 ± 0.49	85.33 ± 0.17 (3–9)	89.46 ± 0.07	76.83 ± 0.16	66.30 ± 0.22
Feb., 07	Control	20°C	8.17*	5.7+	13.0*	112.00 ± 0.57	100 (2–7)	100	100	100
	D	20°C	8.54*	4.2+	18.0*	33.33 ± 1.14	77.43 ± 0.60 (3–11)	83.38 ± 1.17	72.34 ± 0.50	62.75 ± 0.93
	E	20°C	8.28	5.4	15.0	85.50 ± 0.67	86.23 ± 0.63 (3–8)	89.59 ± 0.51	80.80 ± 0.48	66.59 ± 0.21
Mar., 07	Control	23°C	8.75*	5.8+	12.0*	97.16 ± 0.54	100 (2–9)	100	100	100
	D	23°C	8.53*	2.3+	18.0*	41.33 ± 0.61	79.46 ± 0.52 (4–9)	81.22 ± 0.38	69.03 ± 0.33	47.70 ± 0.34
	E	23°C	8.71*	5.2+	13.0*	72.66 ± 0.92	88.77 ± 0.31 (3–8)	90.68 ± 0.09	75.70 ± 0.12	50.14 ± 0.37
Apr., 07	Control	33°C	8.54*	3.9+	10.1*	115.33 ± 0.42	100 (2–5)	100	100	100
	D	33°C	8.33*	3.1+	16.6*	55.16 ± 0.94	82.40 ± 1.10 (3–7)	84.98 ± 0.52	60.13 ± 0.68	49.84 ± 0.32
	E	33°C	8.46*	3.6+	13.8	99.00 ± 0.81	87.54 ± 0.32 (3–6)	91.16 ± 0.44	75.78 ± 0.60	66.92 ± 0.32
May., 07	Control	35°C	8.22*	4.1	9.0*	196.33 ± 0.61	100 (2–8)	100	100	100
	D	36°C	7.50	3.4	12.5	80.16 ± 0.30	76.29 ± 0.09 (4–10)	79.28 ± 0.34	70.13 ± 0.06	61.95 ± 0.21
	E	36°C	7.80*	3.8	8.1*	102.66 ± 0.76	83.43 ± 0.12 (4–9)	85.98 ± 0.12	73.92 ± 0.06	68.46 ± 0.27
Jun., 07	Control	37°C	7.18*	3.1	10.1*	84.66 ± 0.61	100 (3–9)	100	100	100
	D	37°C	7.20	3.4	13.0	40.50 ± 0.50	69.94 ± 0.35 (4–10)	84.13 ± 0.63	69.43 ± 0.29	58.55 ± 0.67
	E	37°C	7.33*	3.7+	10.3*	62.16 ± 0.75	80.44 ± 0.60 (3–10)	89.01 ± 0.58	79.01 ± 0.58	63.34 ± 0.66
Jul., 07	Control	36°C	7.16*	4.6	11.0*	20.00 ± 0.68	100 (3–4)	100	100	100
	D	35°C	7.54*	3.7+	10.0*	8.66 ± 0.21	76.84 ± 0.58 (6–7)	69.83 ± 1.00	53.57 ± 1.60	42.45 ± 0.25
	E	35°C	7.30*	4.0	9.0*	14.66 ± 0.55	84.17 ± 1.00 (5–6)	83.67 ± 0.59	75.52 ± 0.88	54.07 ± 0.14
Aug., 07	Control	35°C	7.01*	5.9+	6.1*	96.66 ± 0.76	100 (4–6)	100	100	100
	D	35°C	7.55	6.2+	12.3	16.83 ± 0.40	71.31 ± 0.48 (4–9)	74.94 ± 0.54	59.74 ± 0.93	43.04 ± 0.87
	E	35°C	7.17*	6.3+	7.3*	37.16 ± 0.40	86.53 ± 0.14 (4–7)	91.19 ± 0.47	71.50 ± 0.23	53.83 ± 0.24
Sep., 07	Control	35°C	7.82*	5.7+	6.8*	45.16 ± 0.94	100 (2–4)	100	100	100
	D	35°C	7.28*	3.9+	9.0*	13.50 ± 0.72	74.10 ± 0.64 (4–9)	79.70 ± 1.14	53.19 ± 1.02	43.04 ± 1.40
	E	35°C	7.43*	4.5+	6.8*	28.16 ± 1.45	86.46 ± 0.57 (3–8)	91.13 ± 0.25	78.68 ± 0.39	64.28 ± 0.68
Oct., 07	Control	34°C	8.45*	5.3+	7.5*	158.83 ± 0.98	100 (3–5)	100	100	100
	D	34°C	8.44*	4.5+	6.8*	106.83 ± 1.35	79.71 ± 0.16 (4–9)	80.43 ± 0.21	52.64 ± 0.74	46.14 ± 0.54
	E	34°C	8.53*	4.9+	7.3	141.16 ± 1.42	85.15 ± 0.80 (4–8)	90.43 ± 0.48	76.83 ± 0.20	68.31 ± 0.18

Each experiment was replicated 6 times and the value of pH, dissolved oxygen, dissolved free carbon dioxide is the mean of six replicates and fecundity is the mean ± SE measured after 24 h period. Product moment correlation coefficient in between the fecundity (egg/20 snail), hatchability, percent survival and different parameters indicate significant ($p < 0.05$) (+) positive/(*) negative correlation. D-Dark exposed water. E-Light exposed water.

Holliday 2005). It may be possible that high oxygen concentration changes the chemical composition of water as well as morphological characterstics of CDCs and the physiology of animals. The effects of high CO_2 exposure cause a reduction of metabolism, protein synthesis, growth rate and reproduction in marine animals (Portner *et al.* 2004). When the CO_2 combines with water it forms carbonic acids and releases hydrogen ions (Royal Society 2005). The hydrogen ions determine the acidity of the water (Ruttimann 2006). Reduction in fecundity, hatchability and survival of well-aerated water may be due to excess O_2 and CO_2 concentrations. Day length is a crucial factor in egg laying in *Lymnaea* (Bohlken & Joosse 1982; Maat *et al.* 1983). Due to the hypothalamus–hypophysis–gonadal system in snails light and day length may influence the release of tentacular hormone, which alters the activity of CDCs (Takeda 1977). In the present study the snails are exposed in light for only 6 h in 24 h dark period which initiates higher egg laying in comparison to snails kept in the dark for 24 h. Wayne & Block (1992) have reported that eyes play a role in mediating photoperiodic information in the reproductive system in *Aplysia*. Singh (1987) has reported there is no significant change in the egg laying pattern of *L. acuminata* kept in the dark for 24 h with respect to control snails (normal daylight). In *Helix aspersa*, the absence of photoperiod inhibited egg laying (Jess & Marks 1998). However, in the present study we have observed that the egg laying was higher in the control group with respect to eggs kept for 24 h in the dark. If the snails were exposed to 6 h light and kept for the rest of the period in darkness it indicates that light stimulation plays some role in the fecundity of snails. Low level of fecundity, hatchability and survival of the snail *L. acuminata* in dark group D in comparison to light-exposed snails, group E, may be due to the influence of light on CDCs.

Reduction in survival of newly hatched snails in groups A, B, C, D and E in comparison to the control indicate that it may be due to low intake of oxygen due to low surface tension between water and the respiratory surface, increase in CO_2 concentration and low pH (Shirayama 2005). Variation in pH, dissolved oxygen and carbonate hardness levels in water caused detrimental effects on the growth and reproduction of *Bithynia gracea* (Eleutheriadis & Lazaridou-dimitriadou 2001). Formation of the shells of newly hatched snails is sensitive to environmental factors, but dissolved CO_2 (hypercapnia), which alters the rate of shell calcification by changing pH, is one the most important factor (Shirayama 2005). It may be possible that variations in different environmental factors in the surrounding water affect the reproduction and development processes of the snails.

CONCLUSIONS

This study conclusively shows that variant abiotic factors can significantly alter the reproductive ability as well their development process. It has been observed that molluscicides used in the control of the snail *L. acuminata* are very effective in the summer season, i.e. March to May (Singh & Singh 2008). The present study clearly demonstrates that between March to May maximum reproduction of snail was observed in the control group of snails. It is obvious that the most suitable period for the control of the snail *L. acuminata* in India is during the months of March to May.

ACKNOWLEDGEMENTS

One of the authors (HVJ) is grateful to the University Grants Commission (UGC), New Delhi (Rajiv Gandhi National Fellowship, no.F-14-2 (SC)/2007 SA-III) for financial assistance.

REFERENCES

Agarwal, R. A. & Singh, D. K. 1988 Harmful gastropods and their control. *Acta Hydrochim. Hydrobiol.* **16**, 113–138.

Alatoom, A., Sheffield, J., Gander, R. M., Shaw, J. & Cavuoti, D. 2008 Fascioliasis in pregnancy. *Obstet. Gynecol.* **112**, 483–485.

American Public Health Association (APHA) 2005 *Standard Methods for the Examination of Water, Sewage and Industrial Waste*, 21st edition. APHA, Washington, DC.

Ashrafi, K., Valero, M. A., Massoud, J., Sobhani, A., Solaymani-Mohammadi, S., Conde, P., Khoubbane, M., Bargues, M. D. & Mas-Coma, S. 2006 Plant-borne human contamination by fascioliasis. *Am. J. Trop. Med. Hyg.* **75**, 295–302.

Bohlken, S. & Joosse, J. 1982 The effect of photoperiod on female reproductive activity and growth of the fresh water pulmonate snail *Lymnaea stagnalis* kept under laboratory breeding condition. *Int. J. Invertebr. Reprod.* **4**, 213–222.

De Jong-Brink, M., Hordi, K. P. L., Vergeest, D. P., Schallig, H. D., Kits, K. S. & ter Maat, A. 1992 The ant-gonadotropic neuropeptide schistosomin interferes with peripheral and central neuroendocrine mechanisms involved in the regulation of reproduction and growth in the schistosome-infected snail *Lymnaea stagnalis*. *Prog. Brain Res.* **92**, 385–396.

Dogterom, G. E., Hofs, H. P., Wapenaar, P., Roubos, E. W. & Geraerts, W. P. 1984 The effect of temperature on spontaneous, and ovulation hormone-induced female reproduction in *Lymnaea stagnalis*. *Gen. Comp. Endocrinol.* **56**, 204–209.

Eleutheriadis, N. & Lazaridou-dimitriadou, M. 2001 The life cycle, population dynamics, growth and secondary production of *Bithynia graeca* (Westerlund, 1879) (Gastropoda) in Lake Kerkini, Northern Greece. *J. Mollusc. Stud.* **67**, 319–328.

Ellis, M. M. 1937 Detection and measurement of stream pollution. *Bull. US Bur. Fish.* **48**, 365–437.

Geraerts, W. P. M. & Bohlken, S. 1976 The control of ovulation in the hermaphroditic fresh water snail *Lymnaea stagnalis* by neurohormone of the Caudo-Dorsal Cells. *Gen. Comp. Endocrinol.* **18**, 61–71.

Ghanaei, F. M., Alizadeh, A., Pourrasouli, Z. & Naghipour, V. M. R. 2006 Sonographic findings of human fascioliasis. *Iran. J. Radiol.* **4**, 11–15.

Godan, D. 1983 *Pest Slugs and Snails, Biology and Control* (D. Godan (ed.) (translated by S. Gruber). Springer-Verlag, Heidelberg, Berlin.

Highnam, K. C. & Hill, L. 1977 *The Comparative Endocrinology of Invertebrates*. University Park Press, Baltimore, MD.

Holliday, T. R. 2005 What effect does dissolved oxygen level have on *Viviparis malleatus* (Trapdoor snail) behavior? *California State Science Fair* project number J: 1909.

Ingram, W. M. 1957 Use and value of biological indicators of pollution: fresh water clams and snails. In: C. M. Tarzwell (ed). *Biological Problems in Water Pollution,* pp. 94–135, U.S. Public Health Service.

Ingram, B. A., Hawking, J. H. & Shiel, R. J. 1997 *Aquatic Life in Fresh Water Ponds; A Guide to Identification and Ecology of Life in Aquaculture Ponds and Farm Dams in South-Eastern Australia*. Co-operative Research Center for Fresh Water Ecology, Albury, NSW, Australia.

Janse, C. 1981 The effect of oxygen on gravity orientation in the pulmonate snail *Lymnaea stagnalis*. *J. Comp. Physiol.* **142**, 51–59.

Jess, S. & Marks, R. J. 1998 Effect of temperature and photoperiod on growth and reproduction of *Helix aspersa* var. *maxima*. *J. Agric. Sci.* **130**, 367–372.

Joosse, J. 1964 Dorsal bodies and dorsal neurosecretory cells of the cerebral ganglia of *Lymnaea stagnalis*. *L. Arch. Neerl. Zool.* **15**, 1–103.

Katz, N. 1986 Possibilidade de controle da equistosomose. *J. Bras. Med.* **50**, 85–88.

Lewin, M. R. 2007 A case study. *Johns Hopkins Microbiol. News L.* **26**(3), 3–4.

Maat, A. T. & Lodder, J. C. 1980 A biphasic cholinergic input on the ovulation hormone producing Caudo-Dorsal Cells of the fresh water snail *Lymnaea stagnalis*. *Comp. Biochem. Physiol.* **66**, 115–119.

Maat, A. T., Lodder, J. C., Veenstra, J. & Goldschmeding, J. T. 1982 Suppression of egg-laying during starvation in the snail *Lymnaea stagnalis* by inhibition of the ovulation hormone producing Caudo-Dorsal Cells. *Brain Res.* **239**, 535–542.

Maat, A. T., Lodder, J. C. & Wilbrink, M. 1983 Induction of egg laying in the pond snail *Lymnaea stagnalis* by environmental stimulation of the release of ovulation hormone from the Caudo-Dorsal Cells. *Int. J. Invert. Reprod.* **6**, 239–247.

Portner, H. O., Langenbuch, M. & Reipschlager, A. 2004 Biological impact of elevated ocean CO_2 concentration. *J. Oceanogr.* **60**, 705–718.

Royal Society 2005 *Ocean Acidification Due to Increasing Atmospheric Carbon Dioxide*. Policy document 12/05. Clyredon Press, Cardiff.

Ruttimann, J. 2006 Sick seas. *Nat. News Feature* **442**, 978–980.

Savioli, L., Chitsulo, L. & Montresor, A. 1999 New opportunities for the control of fascioliasis. *Bull. WHO* **77**, 330–331.

Shirayama, Y. 2005 Effect of increased atmospheric CO on shallow water marine benthos. *J. Geophys. Res.* **9**, 110.

Singh, S. K. 1987 *Studies on Chemosterilization and Neurosecretory Control of Reproduction in the Gastropod Lymnaea acuminata*. PhD Thesis, University of Gorakhpur, Gorakhpur, U.P., India.

Singh, O. & Agarwal, R. A. 1981 Toxicity of certain pesticides to two economic species of snails in northern India. *J. Econ. Entomol.* **14**, 568–571.

Singh, V. & Singh, D. K. 2008 The effect of abiotic factors on the toxicity of cypermethrin against the snail *Lymnaea acuminata* in the control of fascioliasis. *J. Helminthol.* **83**, 39–45.

Singh, A., Singh, D. K., Misra, T. N. & Agarwal, R. A. 1996 Molluscicides of plant origin. *Bio. Agric. Horticult.* **13**, 205–252.

Singh, R. N., Kumar, P., Singh, V. K. & Singh, D. K. 2008 Effect of binary combination of deltamethrin + MGK-264 on the levels of phospholipid and lipid peroxidation in the snail *Lymnaea acuminata*. *Chemosphere* **73**, 1032–1035.

Sokal, R. R. & Rohlf, F. J. 1973 *Introduction to Biostatistics*. W. H. Freeman, San Franscico.

Taheri, M. S., Aminzade, Z., Shokohi, B. & Aghazade, K. 2007 Hepatobiliary fascioliasis: clinical and radiological features. *Iran. J. Parasitol.* **2**, 48–55.

Takeda, N. 1977 Stimulation of egg laying by nerve extracts in slugs. *Nature* **267**, 513–514.

Wayne, N. L. 2001 Regulation of seasonal reproduction in mollusks. *J. Biol. Rhythms* **16**, 391–402.

Wayne, N. L. & Block, G. D. 1992 Effects of photoperiod and temperature on egg-laying behaviour in a marine mollusk, *Aplysia californica*. *Biol. Bull.* **182**, 8–14.

WHO 2006 *Report of the WHO Informal Meeting on Use of Triclabendazole in Fascioliasis Control, WHO Headquarters, Geneva, Switzerland, 17–18 October*. WHO/CDS/NTD/PCT/2007.1. WHO, Geneva.

First received 26 February 2009; accepted in revised form 31 May 2009. Available online 9 November 2009