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EFFECTS OF CALCIUM LEVELS ON DISTRIBUTION, FECUNDITY AND PREPONDERANCE OF LYMNAEA CAILLIAUDIA INFECTED WITH CLINOSTOMUM TILAPIAE, (UKOLI 1966).

R.A. M., RAMADAN AND O. A., SALEH

Fish health department-Central Laboratory of Aquaculture Research Abbassa, (CLAR).

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SUMMARY

Lymnaea cailliaudia were reared in stock culture and subjected to either 7-day or 60-day acclimatization periods in a complex CaCo3 media with calcium values ranging from 1.5mg/L to 75mg/L. Following 30-day acclimatization Snail from group1 were each exposed to 100-eggs of Clinostomum tilapiae. Snails of group II were each exposed to a 10-eggs. Snails of both experimental regimes were observed for mortality, growth, rate of infection and number of cercariae shed. Group I snails were also monitored for fecundity during acclimatization and following eggs exposure. Calcium levels 1.5 and 75mg/L resulted in snail mortality. Shell growth and rates of infection were positively correlated with calcium maintenance level. Snails with high fecundity prior to eggs exposure subsequently shed more cercariae. In contrast, post exposure fecundity of snails reared in media with up to 30 mg/L calcium were negatively correlated with calcium level, rate of infection and number of cercariae shed. Maximal cercarial shedding occurred at 30mg/L calcium. These results suggest that environmental calcium affect both the distribution patterns of snail hosts of Clinostomum tilapiae and the productivity of intramolluscan infection.

INTRODUCTION

Effect of environmental Calcium level on distribution and preponderance of freshwater gastropods has been investigated since Boycott, (1936) who reported that certain species of snail fauna were absent from calcium poor habitats in Great Britain. Studies on the effects of environmental calcium on snail growth, shell composition, fe-

cundity, mortality have established a physiological foundation for the effect of calcium on distribution (McMahon, 1983). Calcium also may be a requisite for the development of trematode stages within the gastropod intermediate host. Effective miracidial host-finding was reported to be correlated with calcium /magnesium ratio (Saladin, 1979). Maturation of intramolluscan larvae of Schistosoma mansoni has been shown to be temporary coincident with hypocalciemia in Biomphalaria glabrata (Sidner, 1982). The effect of calcium levels on Clinostomum tilapiae was determined by measuring molluscan infection rate cercarial shedding of Clinostomum tilapiae from Lymnaea cailliaudia reared in calcium concentration ratios ranging from 1.5 mg / L (very soft water) to 75 mg/L (hard water) according to Eric and Eileen,(1986). Exoskeletal calcification in the blue crabs is achieved with calcium levels absorbed from seawater at salinity of 12% , with calcium level reduced to 60-80% of normal, decreased the calcification rate without increasing shedding mortality, (Harriet, Perry et al, 2001). The aim of this work was to throw a light on the effect of calcium levels on growth, shell composition,fecundity,mortality of Lymnaea cailliaudia snail the intermediate host of Clinostomum tilapiae.

MATERIALS AND METHODS

Lymnaea cailliaudia were bred from 50 adults (self- fertilization) which were collected from Central Laboratory for Aquaculture Research Abbassa, (CLAR) ponds and streams . Snails were reared in aquaria in well-aerated water (Ulmer, 1970). Low-density condition of snail/300ml water maintained to avoid crowding effects. Snails were subjected to 12hrs light:12hrs darkness cycle and a controlled environment room temperature of about 26°C-28°C. Snails were feed Lattice, unfed Lattice was removed daily to avoid fouling of the water and allow for maximal oxygenation (Van der Steen, 1967). Aquaria were fitted with filters to remove debris. The pH of aquaria was maintained between 7 and 7.5 with phosphate buffer. Complete water changes were performed on a biweekly basis. When juvenile snails were observed in breeding tanks, adults were removed. For experiments on snail fecundity and cercarial shedding, 6 concentrations of calcium were selected (1.5, 15,30,45,60 and 75.0 mg / L calcium as CaCO3). The calcium concentrations were added to an aqueous medium consisting of 5mg/ LKno3, 5mg/L KH2po4, 35 mg/L NaCl and 35 mg/LMgSo4 in demineralizd distilled water (Thomas and Benjamin, 1974) in 3-liter plastic aquaria. 7-day old duckling was fed of about 10-20 encysted metacercaria of Clinostomum tilapiae and fecal examination daily till eggs appeared in feces.

Snails were randomized into 6 groups at different calcium concentrations. Ten adults and 15 pre-adult snails (less than 8mm in shell diameter.) were used / aquarium. Experiments were per-

formed in 5-replicate and involved 30-day acclimatization periods during which snails were monitored daily for mortality and weekly for growth and fecundity (Eric and Eileen, 1986). At day 31st, snails were washed, measured for shell diameter, and placed in vials containing 1ml of dechlorinated tap water buffered to pH 7.3. In group I snails were exposed to100-eggs for 5hr under low light and then were returned to aquaria. Group II was conducted similarly, except that experiments were performed in duplicate and finished acclimatization of pre-adults snails for a week prior to exposure to a 10-eggs. In both groups, assessment of fecundity was accomplished by gently scraping egg masses from the sides of the aquaria, shells of snails, and lattice, and counting the number of eggs microscopically (Eric and Eileen, 1986). From day 28 post-exposure, snails were observed for cercarial shedding, observation were daily for 7 days, followed by at least 6 weekly determinations. Snails were removed from their aquaria, rinsed, measured, and isolated in vials containing 1ml of dechlorinated tap water buffered to (pH 7.3 and 26°C). Vials were left under overhead fluorescent lighting for 4hr at the time of day shown to be synchronous with peak cercarial shedding (Glaudel and Etges, 1973) and were observed microscopically for cercariae. Cercariae were fixed in absolute 2-propanol and stained with 0.005% phloxine-b. Cercarie were stirred to dissociate clumps, and 2.0 ml of the suspension was pipettid and suction-filtered through gridded (1cm2) filter papers (5.5cm in diameter). Cercariae in all squares were counted, and the total number per sample was calculated as followed: Cercariae counted x 10 = total cercariae according to Eric and Eileen, (1986). Mortality and infection rates were analyzed by Chi-square testing. Analyses of variance (ANOVA) were used to analyze data on reproduction and on cercarial shedding and Linear regression methods were employed to determine relationships between calcium level and infection rate, growth, fecundity and cercarial shedding (Sokal and Rohlf, 1981).

RESULTS

Biological and morphological aspects:

Adult *Lymnaea cailliaudia*, (Fig, 12) are 25x20 mm in size, oval with a thin or transparent shell and light yellowish-brown in color . They attached to water plants in fish ponds and swamps where egg lying occure. *Lymnaea cailliaudia,* feed on different microenvironmental nutrients including eggs of the parasites (Clinostomum tilapiae), Fig. (1), which are coming from the droplets of the birds landing on water plants. At this time the intramolluscan cycle begin. Hatching of the eggs take place inside the hemoceal of the snail producing Meracdia, (Fig. 2&3). The Meracidia burrowing through the snail hepatopancreas where they metamorphosed into Redia stage, (Fig. 13). The Redia developed into doughter sporocysts, (Fig. 14), no mother sporocysts seen, which containing different stages of ill developed, (Fig.

8&9) and well developed Cercariae, (Fig.10). Under the environmental chemo-phototactic effect Cercariae shedding occurs and the Cercariae invade the skin, (Fig. 6) and subcutaneous tissues of fish, also gills and bronchial cavity, (Fig.7) where they encapsulated as encysted Metacercariae, (Fig. 4). Metacercaria were yellow to orange grup- like pea sized cysts. The excysted larvae are stout, longuform, convex dorsally and concave ventrally. The oral sucker is subterminal in the center of the oral field, the ventral sucker was large spherical in shape. In the oral field the mouth cavity opened into a short prepharynx which lead to prepharngeal bulb. The esophagus was lacking and the intestinal caeca were very long extended to the posterior end and provided with lateral pouches behind the ventral sucker. The two testes were strongly digitized, subdivided into small lobes and situated in the posterior part of the middle third of the body, the anterior testis. The cirrus pouch was oval in shape situated between the two testes. The ovary was small, located beside the cirrus pouch. Ootype was large complicated structure, situated between the two testes. The uterus runs around the left margin of the anterior testes to open into the uterine sac was long and tubular .The uterine sac was constricted into a narrow tube which rune in the space between the right edge of the anterior testis and right caecum, opening into the genital atrium. The biometrics comparison of Clinostomum tilapia, Metacercaria and adults were clarified in Table (1).The first adult egg, small ovoid brownish-yellow, (Fig. 5) apear after 30 days from feeding 7-day old duckling on the encysted Metacercari-

Table (1) : Biometrics Comparison of Clinostomum tilapia, Metacercaria and Adults

Points of comparison	Metacercaria	Adult
Body measures	8-17mm long and 4-7mm wide	11-12mm long and 5-7mm wide
Oral sucker	0.20-0.30mm	0.29-0.32mm
Pharynx	0.20-0.4 Length 22 - 0.51mm wide.	0.31-0.5 Length 34 - 0.54mm wide.
Acetabulum	1.10X1.16mm	1.10X2.1mm
Ovary	0.19 - 0.31mm 0.13-0.23mm	0.40 - 0.981mm 0.20-0.43mm
Anterior testis	0.41-0.45mm 0.43-0.56mm	0.61-0.85mm 0.33-0.76mm
Posterior testis	0.29-0.42mm 0.51-0.53mm	0.39-0.82mm 0.81-0.93mm
Circus pouch	0.2-0.6mm 0.14mm	0.9 - 1.02mm 0.70m
Ootype	0.43-0.73mm 0.21-0.51mm	0.5 3- 0.93mm 0.40 -0.89mm
Uterus	1.5- 2.1mm.	2.15 - 3.1mm.

ac.

Table (2) a and b experiments showed group (1) mortality, infection rate and shell diameter for snails. Two periods of snails growth and mortality were observed: the day 30 pre-exposure period which end by exposure of snails to eggs and the day 30 post-exposure period (Prepatent period end by cercarial shedding). By the end of the day 30 pre-exposure significant mortality had occurred in snails maintained in 1.5mg/L Ca++ ($P<0.01$). While, significantly smaller shell diameters were observed at 1.5mg/L9.96+0.16 ($P<0.05$), relative to all other calcium levels ranged from (10.96 + 0.36- 13.11+0.24 at 45 mg/L). Prepatent mortality was also maximized at 1.5mg/L, where 76.6% of the snails had died by the end of the period. In contrast, the snail population maintained at 75 mg/L,(65.3%) which had high mortality during the pre-exposure period, had no additional moralities following cercarial exposure. 15-day post-exposure shell diameter was positively correlated with calcium level from (11.60 + 0.10 to 13.21 + 0.76) where r=0.84. Although heavy mortality encountered at 1.5mg/ml, 46 (76.6%) resulted in a significantly depressed incidence of infection (8) snail by 25.8% preexposure and 57.14% postexposure ($P<0.05$), snails maintained in 15mg/L also exhibited a significantly lower rate of infection (39.13 % and 37.5% in prexposure and postexposure respec-

Table (2): Mortality and infection rate, shell diameter of Lymnaea cailliaudia exposed to 100- eggs of Clinostomum tilapiae. (Snails measured in mm)

(a)

Ca level mg/l	day 1	day 30		day 60		No snail Infected	% infected	
	No.of snail exposed	No.of snail survived	%	No.of snail survived	%		A	B
1.5	60	31	51.6*	14	23.3*	8	25.8	57.14
15	60	46	76.6	48	80.0	18	39.13	37.5
30	60	43	71.6	45	75.0	28	56.1	62.1
45	60	46	76.6	46	76.6	25	54.34	54.34
60	60	41	86.3	43	71.6	22	53.65	51.16
75	60	35	58.3*	39	65.3	21	60.0	53.84

(b)

Ca level mg/l	day 1 MSD ± SE	day 30 MSD ± SE	day 60 MSD ± SE	infected MSD ± SE
1.5	6.51 ± 0.53	9.96 ± 0.16**	11.66 ± 0.16	11.91± 0.51
15	6.59 ± 0.53	11.75 ± 0.76	12.96 ± 0.92	12.26 ± 0.46
30	6.21 ± 0.54	12.27 ± 0.66	12.26 ± 0.26	14.06 ± 0.31
45	6.50 ± 0.61	13.11 ± 0.24	12.36 ± 0.38	15.19 ± 0.23
60	6.76 ± 0.54	10.96 ± 0.37	12.16 ± 0.46	16.06 ± 0.36
75	7.06 ± 0.55	10.96 ± 0.36	13.21 ± 0.76	16.96 ± 0.36

a= infection rate of snails exposed to 100-eggs.
b= infection rate of snails surviving pre-patency. MSD= mean shell diameter
**=Significantly smaller shell diameter in relative to all Ca ++ concentration

tively (P<0.05).

Table (3), Clarify the reproductive parametres of group (1) experiments. Fecundity expressed as ®=egg masses/snail/day and ©=eggs/snail/day for 30-day pre-exposure period, 15-day pre-patent period, and cercarial-shed period are presented. Significant difference occurred in either reproductive parameter during the pre-exposure period with the maximum and minimum observed at 75mg/L and 1.5mg/L Ca++ respectively. During the pre-patent period. Increased reproductive activity was observed at 1.5mg/L and 15mg/L Ca++ accompanied by a trend of decreased fecundity with increased calcium level (egg masses /snail/day r=0.993 and egg /snail/ day r=0.881). With the onset of cercarial shedding, fecundity of all groups decreased as infected snailsí rarely laid eggs.

Table (3): Daily fecundity of Lymnaea cailliaudia exposed to100-eggs of Clinostomum tilapiae.

Ca level mg/l	# X ± SE	day 30 pre-exposure X ± SE	day 30 post-exposure X ± SE	Fecundity
1.5	0.83 ± 0.67	0.25 ± 0.52	0.68 ± 0.27	®
	7.73 ± 1.93*	8.16 ± 1.58	12.03 ± 1.41*	©
15	0.39 ± 0.23	0.34 ± 0.27	0.28 ± 0.62	®
	8.09 ± 0.94	7.79 ± 0.11	11.09 ± 0.53*	©
30	0.46 ± 0.67	0.17 ± 0.61	0.36 ± 0.53	®
	8.16± 0.54	7.0 ± 0.18	10.86 ± 0.66	©
45	0.40 ± 0.66	0.24 ± 0.28	0.24 ± 0.28	®
	9.28 ± 0.21	5.17 ± 1.22	8.88 ± 0.21	©
60	0.16 ± 0.54	0.15 ± 0.71	0.19± 0.71	®
	9.86 ± 0.22	4.62 ± 0.25	8.02 ± 0.22	©
75	0.56 ± 0.25	0.20 ± 0.72	0.26 ± 0.25	®
	10.46 ± 0.05*	3.65 ± 0.05	7.45 ± 0.45	©

60-day Acclimatization period ® = egg mass/snail/ day
© = egg /snail/ day

Table (4), showing the cercarial emergence increased in a linear fashion between 1.5mg/L and 30mg/L Ca++group emergence r=0.993 individual emergence r=0.998 with significantly lower group emergence observed at 1.5mg/L when compared with all other groups. At concentration greater than 30mg/L a general pattern of declining emergence was noted with restively sharp reductions observed at 45mg/L. Rates of infection and number cercaria shed from individual snail were correlated ,with the exception of snail maintained at 15 mg/L, where a low infection rate and subsequent low group emergence was coupled with high individual cercarial output. In contrast, snail maintained at 45mg/L, had a high infection rate with relatively low number of cercaria shed from individual snails.

Table (4): Cercarial shedding after 100-eggs exposures.

A) Group cercarial shedding group (1).

Ca level mg/lCa	No.of infected snails	Cercarial ± SE	Multiple range analysis
1.5	10	5,852 ± 1,977.2	
15	20	9,595.0 ± 1,328.8	
30	31	12,881.2 ± 1,121.3	***>1.5
45	28	7,831.9 ± 741.0**	
60	26	11.544.4 ± 1,319.0	
75	25	8,803.7 ± 1,253.7	

B) Individual cercarial shedding (group 1)

Ca level mg/l	No.of infected snails	Cercarial ± SE	Multiple range analysis
1.5	24	2,543.3 ± 504.52	
15	31	6,190.4 ± 937.8	***>1.5
30	32	12,479 ± 1,816.5	***>1.5,15,45,75
45	32	6,852.9 ± 1,074.7	***>1.5
60	32	10.101.4 ± 1,425.0	****>1.5
75	32	6,877.7 ± 8758.5	***>1.5

Table (5): Cercarial shedding after 10-eggs exposures.

A) Group cercarial shedding (group 11):

Ca level mg/l	No.of infected snails	Cercarial ± SE	Multiple range analysis
1.5	6	1921.7 ± 640.5	
15	16	2,733.6 ± 1.068.8	
30	16	6,668.9 ± 1,068.8	***>1.5,15,45
45	16	4,073.1 ± 1,018.3	
60	16	8,209.4 ± 1.273.4	****>1.5,***>45
75	16	10.295.0 ± 1,489.7	***>1.5,15,45

B) Individual cercarial shedding (group1).

Ca level mg/ml	No.of infected snails	Cercarial ± SE	Multiple range analysis
1.5	3	1,882.8 ± 1,875.2	
15	4	9,695,0 ± 1,328.8	
30	10	10,881.9 ± 1.13	***>1.5
45	7	7,831.9 ± 741.0	
60	12	11.940.4 ± 1,319.0	
75	13	10,891.3 ± 2,321.7	

*** P<0.05 **** P<0.01

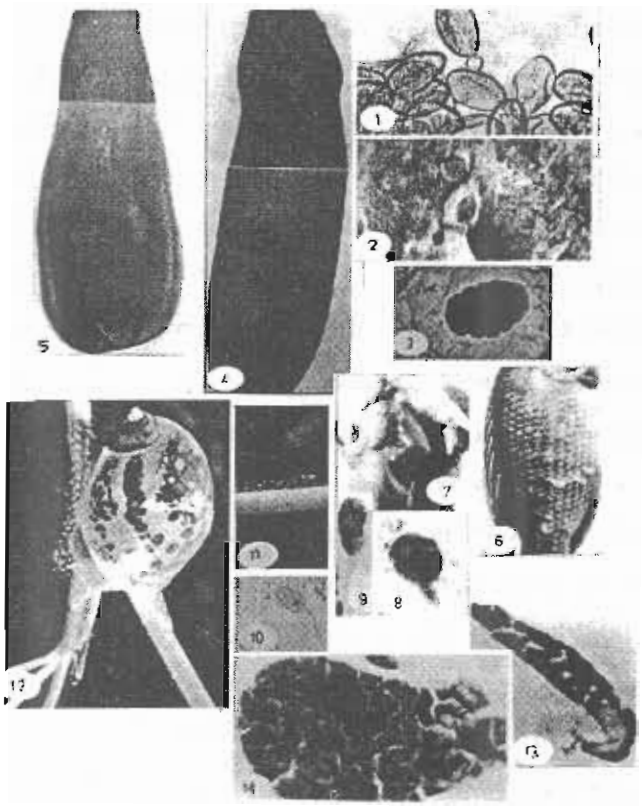
Table (5), Clarify that Cercarial shedding experiments for group 11 exposed to 10-eggs of parasites and showed that group shedding was positively correlated with increased calcium concentration (r=0.91). Significant difference in the number of cercariae shed by the groups occurred (P<0.01) with statistically high emergence at 75mg/L(10.29+1,4897, 60mg/L (8.209+1,273.4 , and 30mg/L(6.668,9+ 1.06) than at 1.5mg/L,15mg/Land 45mg/ Ca++ were 1,921.7+640.5, 2.733+1.08.8 and 4,073.1+1,018.3 respectively. No significant difference were observed of individual snails, although snails maintained at 1.5mg/L shed fewer cercariae than snails of the other groups As group (I)(100-eggs/snail), snails maintained at 15mg/L had low rate of infection, and group cercariae output, coupled with high individual cercarial emergence. Similarly at 45mg/L a comparable high rate of infection was a companied by relatively low cercariae output.

Table (6) summarizes data on mortality, shell diameter, and rate of infection of snails each exposed to a ten eggs of Clinostomum tilapiae (groupII) experiments. Post-exposure survival was affected only at 1.5 mg/L Ca++ where 27.3% of the snails had died by the 30-day post-eggs exposure. Significant difference occurred in the rate of infection of snails between groups with respect to the number of snails exposed P<0.01). The number of snails surviving the prepatent period (P<0.05) to subsequently shed cercariae. differential infection rates were primarily due to low frequencies of infection at 1.5 mg/L (16% of exposed snails and 27% of surviving snails infected) and 15% of exposed snails and surviving snails infected). Shell diameters significant difference (P<0.05) with the main effects due to the smaller individuals of the 1.5 mg/L Ca++group.

Table (6): Mortality and infection rate of Lymnaea cailliaudia snails each exposed to ten eggs of Clinostomum tilapiae .

Ca level mg/l	day 1			day 15	% infected	
	No.of snail exposed	No. snail survived	% survival	No.of snail exposed	% Precxposurec	% Prepatency
1.5	33	24	72.7	3	9.09	12.5
15	34	33	97.05	4	11.76	12.12
30	38	35	92.10	10	26.31	28.57
45	33	35	93.87	7	21.21	22.58
60	32	31	96.87	12	37.5	38.70
75	33	29	87.87	14	42.25	48.27

Preadult snails were acclimated for one week prior to exposure to 10 eggs of parasites for each



1) Clinostomum eggs: measured 0.10 X 0.05 mm X750
2) Eggs inside hepatopancreas of Lymnea cailludae snail
3) Miracdium inside snail inside hepatopancreas of Lymnea cailludae snail
4) Excysted Metacercariae X 250
5) Clinistimum adult X 250.
6) Encysted metacercariae in O.niloticus skin (hypodermis)
7) Encysted Metacercaria in O.niloticus Branchial cavity (gills)
8) Non differentiated cercariae X 500
9) III developed cercariae X 500
10) Developed cercariae X 500
11) Eggs of Lymnea cailludae on lattice parts.
12) Lymnea cailludae on luttice parts after oviposition.
13) Redia X 500.
14) Doughtier sporocyst X 250.

DISCUSSION

Ca++ regulation in freshwater pulmonate snails is controlled by some limiting factors and the regulation performed in three modes . In optimal Ca++concentration, the snail epithelium (gut and cutaneous) act as a calcium electrode and calcium is absorbed passively into the body (Greenaway,1971).While calcium stored in the midgut gland allows calcification to begain shorly after blue crab ecdysis, these store comprise a small portion of the total calcium requirment (Greenaway, 1985). Exoskeletal calcification is achieved predominantly with calcium absorbed from external medium, with a rate of energy transport (Harriet Perry et al, 2001).Optimal Ca++ level differs between and within species, according to (McMahon, 1983) i.e Lymnaea stagnalis L. needs at least 20mg/L Ca++ (Greenaway, 1971).While Bimophlaria glabrata passivly absorbs Ca++ above 10mg/L (Thomas and Lough, 1974) and that of Pureto Reco have at least 16 mg/ Ca++ (Pimentel and White, 1959) well above needed for passively absorption . if calcium level below optimum the snail survive, but calcium uptake is by an active, energy-requiring transport system, (Thomas and Lough, 1974), (McMahon, 1983) and (Greenaway, 1985). The need for additional metabolic energy may cause a decrease in energy available for growth and reproduction. At very low calcium levels, the snails can no longer regulate osmoticaly (Pimentel and White, 1959). In the present study low level calcium less than 2mg/L Ca++ snails had smaller shell sizes, extreme shell fragility and pitting of periostracal and prismatic layers. These observation agreed with that of Thomas et al. (1974) who found increasing growth rate and higher relative shell weight of Bimophlaria glabrata with increases of calcium levels up to 80mg/L Ca++ and Eric and Eileen (1986) whom recorded that low level calcium less than 2mg/L Ca++ snails become smaller in size and retarded growth rates. Snail fecundity affected directly and indirectly by environmental calcium levels, this agreed with that reported by Thomas et al. (1974) who found that steadily increasing fecundity up to 80mg/L Ca++ and proposed that the association between fecundity and calcium level was indirectly (the larger snail is produced by the higher calcium levels being more found and Eric and Eileen (1986) who found that fecundity was greatest at 30 mg/L. In the present study, fecundity was greatest at 30-45 mg/L while Nduku and Harrison (1980a&b) found that the maximum from 5 to 30 mg/L.

Pattern of snail production were altered after eggs-exposure. Egg laying of snails each exposed to 100-eggs of parasite decreased linearly with increased Calcium levels. The relatively high reproductive levels of exposed snails at 1.5 and 15 mg/L Ca++ were similar to those observed by Eric and Eileen (1986) .They ascribed

the accelerated oviposition to imminent high mortality and decreased fecundity of infected snails. However, the difference in the reproduction output may reflect the reduce infection rates of 1.5 and 15 mg/L snails, suggesting an increased suppression of snail reproduction with increased eggs exposure. Cercarial shedding in snails exposed to 100-eggs followed a linear pattern with steep increase from 1.5 and 30mg/ Ca++. Cercarial emergence was significantly elevated at 30mg/L relative to all groups except at 60mg/L and there was a close correlation between pre-exposure snail fecundity and the number of cercarial shed. Ten eggs exposures resulted Ca++ related in increased cercarial emergence The difference in emergence pattern between 10 and 100-eggs exposure suggest that short term exposure to high calcium level may be potentate infection, where as long term maintenance may be moderately deleterious, also the number of eggs exposure may have influence the pattern of cercarial shed. Successful transmission and productivity of intramoluscan. Clinostomum infection is contingent upon egg/host finding, successful eggs penetration and host biochemistry conductive to cercarial development. Snails maintained at 15 mg/L Ca++and exposed to 10 and 100-eggs, exhibited a reduced group emergence this result correlated with low infection level rather than low cercarial emergence/ individual snail. The differential transmission of infection observed in this study agreed with that given by MacInnis, (1976) and Eric and Eileen, (1986), whom reported that the efficient of uptake of Ca++by snails in a high Ca++environment result in a negative calcium gradient between snail and environment which, with emission of Magnesium, may serve to establish an ionic ratio conductive to eggs attraction. Eric and Eileen, (1986) suggest that there may be a complex interaction between habitats and snail survival, and Bimophlaria glabrata infection. Trematode parasite may be totally absent from low Ca++environment because the snails requirements for calcium not met. This study agreed with that given by Curtis and Rau, (1980) who observed that calcium related restriction of Diplostomiasis to water with calcium levels greater than 5 mg/L Ca++in Quebec. while in more optimal, calcium levels where the maximal fecundity and infection rate are obtained, individual snail may be at increased risk of decreased fecundity and mortality associated with Clinostomum tilapiae infection.

Finally, this study, suggests that Ca++ environment levels is a limiting factor in biology ,snail survival, and Clinostomum tilapiae infection.

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