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EFFECTS OF MEDIA CONDITIONED BY HETEROSPECIFICS AND CONSPECIFICS OF DIFFERENT AGE GROUPS ON OVIPOSITION IN A *BULINUS TROPICUS*

M. ASGHAR CHAUDHRY

Department of Zoology, King Saud University
Riyadh—11451, Saudi Arabia

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M. Asghar Chaudhry (1988) Effects of media conditioned by heterospecifics and conspecifics of different age groups on oviposition in a snail, *Bulinus tropicus*. *Bull. Inst. Zool., Academia Sinica* **27**(2): 217-224. Water conditioned by conspecific snail effectively inhibited oviposition in *B. tropicus*, whereas lettuce infusion or the faecal homogenates of conspecific snails has no such effect. Media conditioned by sexually mature snails or by the maturing juveniles were effective in inhibiting ovipositon but similar water conditioned by egg masses hatching into neonates or one week old neonates was without effect. Media conditioned by closely related species were also effective in inhibiting oviposition but taxonomically distant species had no such effect. The inhibitory properties of snail-conditioned water were impaired when it was bubbled and filtered through a column of activated charcoal.

Key words: *Bulinus tropicus*, Oviposition, Heterospecific, Inhibition.

In a previous communication (Chaudhry and Morgan, 1986) it was shown that, in common with other freshwater pulmonates, oviposition in *Bulinus tropicus* declined when kept in closed cultures. From the evidence presented this decline appears to be independent of changes in the inorganic composition of the medium, and is unaffected by changes in temperature or oxygen tension. Instead the results are more consistent with the progressive accumulation of some non-dialysable organic component in the water.

Similar inhibitory feedback mechanisms have been reported in the control of oviposition in insects, where chemical marking of oviposition sites with compounds of both plant and animal origin has been repoted (Prokopy, 1981). The literature regarding the control of oviposition in freshwater pulmonates is more controversial. Snail-conditioned water has been found to enhance and also to diminish egg laying (e. g. Wright, 1960; Thomas *et al.*, 1975 and Chaudhry, 1986) and inhibition has been demonstrated between heterospecific as well as conspecific snall populations (e. g. Levy *et al.*, 1973; Madsen, 1979, 1981). Wright (1960), working with *Bulinus forskalii*, suggested that such inhibition might result from a pheromone produced by the snails themselves and this hypothesis is favoured by a number of authors (Chernin and Michelson, 1957, a, b; Madsen, 1979 and Lazaridou-Dimitriadou and Daguzan, 1981). Moreover Berrie and Visser (1963) and Levy *et al.*, (1973) have been able to extract and concentrate the inhibitory factors(s) from media containing crowded cultures of *Biomphalaria sudanica* and *Fossaria cubensis* respectively. However as (Thomas,

1973; Thomas *et al.*, 1975) has pointed out, snail-conditioned water is a complex medium and plant food, micro-organisms and snail faeces must all be considered as potential sources of inhibitory compounds.

The present paper describes the results of some preliminary experiments on the source of the inhibitory compound(s) affecting oviposition in *B. tropicus*.

MATERIALS AND METHODS

The snails used in these experiments were cultured as indicated previously (Chaudhry and Morgan, 1986). Three series of experiments were carried out. In the first lettuce extract and snail faeces were investigated as a possible source of an inhibitor substance(s). Lettuce infusion was attained by incubating the leaves in fish-conditioned water for three weeks at 24°C. In an other experiment, actively laying snails were maintained, five per 400 ml fish-conditioned water for three weeks and fed dried scaled lettuce. The snails were then removed and their faeces separated by filtration before being homogenized in 10 ml of fish-conditioned water. The final volume of each faecal homogenate was made up to 400 ml with fish-conditioned water. The above experimental media were assayed on actively laying groups of snail (each group consisted of 5 animals in 400 ml water). In order to investigate the progressive accumulation of oviposition inhibitor(s) in the medium, five actively laying snails were kept in 400 ml fish-conditioned water without change for a period of three weeks. Each week the water was filtered through a column of activated charcoal after gently bubbling with an air pump for about five minutes and the oviposition rate was recorded.

A second series of experiments was designed to ascertain whether the efficacy of inhibition was related to the age of the anails conditioning the medium. Water conditioned by newly laid egg masses, and by four different age groups of snails was assayed. To investigate the effect of eggs alone actively laying anails were allowed to oviposit in 400 ml of fish conditioned water, producing atoal of 75 egg masses with a mean of 15 ± 2.05 eggs in each. The snails were then removed and the water replaced with fresh fish-conditioned water, which was left undisturbed for a period of three weeks. During this time most of eggs hatched. Similarly, groups of neonates about one week old at the start of the experiment, and of three week old juveniles, were maintained separately in different dishes in 400 ml fish-conditioned water for a period of three weeks, after which the medium was assayed, the total biomass of the neonate and juvenile groups at the start of the experiment being approximately equivalent to that of five mature specimens. Further groups of five mature, actively laying snails and of five eight month old specimens of *B. tropicus* which produced only a few egg masses were also assayed. Six groups of test snails, each of five animals, were selected randomly from the laboratory stock and transfered to 400 ml of fish-conditioned water. In all groups the culture water was replaced weekly with fish-conditioned water for the first three weeks and at the beginning of week four, different groups were transferred to three week old culture media conditioned by conspecifics of different age groups as described above.

In the third series of experiments the specificity of the inhibitor was investigated. Here water was conditioned by sexually mature specimens of three different freshwater pulmonate species, *Bulinus globosus*, *Biomphalaria glabrata*, *Physa* sp., and by a prosobranch *Melanoides tuberculata* each species being maintained at a density of five snails in 400 ml for a period of three weeks. Aliquotes from each medium were removed and stored at −20°C after filtering through a Whatman filter paper No. 1 prior to analysing the ionic composition as indicated ealier (Chaudhry and Morgan, 1983). Four groups of *B. tropicus*, selected randomly from laboratory stock were transferred, five to a dish,

to 400 ml fish-conditioned water and their media replaced weekly for the first three weeks. At the beginning of the fourth week each groups was transferred to a medium previously conditioned by a heterospecific snail monoculture for a similar period. In all experiments the snails were fed dried scaled lettuce *ad libitum* during the conditioning period. Unless otherwise mentioned, three replicates of each experiment were set up.

RESULTS

Possible source of oviposition inhibition

The source of oviposition inhibitory compound(s) was investigated in a preliminary experiment in which water from the snail rearing dishes was weekly filtered over activated charcoal and the oviposition rate was recorded (Fig. 1d). There was a slight decrease in weekly oviposition but this decrease was not significant when compared with that shown by snails transferred to conspecific conditioned water (Fig. 1b). The results are consistent with the accumulation of some organic compound in the medium and if conditioning does indeed occur in this way, the possibility that the compound(s) may be of plant food origin or leaks from the accumulated snail faeces cannot be overlooked. Thus, in snails transferred to dishes containing filterted lettuce infusion the oviposition rate remained comparable to those in which the medium was replaced weekly with fish-conditioned water (Fig. 1c). Similarly, in conspecific faecal homogeneate the oviposition rate of snail was not significantly lower than in the first three weeks of the experiment when culture media were being replaced with fish-conditioned water alone (Fig. 1a).

In contrast, the oviposition of a similar group of actively laying snails transferred at the end of three weeks to three week old conspecific conditioned water from which the faecal material had been removed was significantly reduced (see Fig. 1b).

Inhibition by conspecific snails of different age groups

The effect of three week old culture media conditioned by conspecific of different age groups were tested on actively laying snails and their oviposition rate was monitored for two weeks without changing the experimental media (for details see methods). Snails transferred to water conditioned by egg masses hatching into neonates continued to lay at the same rate as before (Fig. 2a). Although, the oviposition rate of snails transferred to three week old medium conditioned by neonates (one week old) at the beginning of conditioning period declined during the fifth week of the experiment (Fig. 2b), the immediate reduction in egg laying which characteristically follows transfer to media containing inhibitor(s) was not shown by this group. In contrast the oviposition was considerably decreased or inhibited altogether in media conditioned by mature specimens of *B. tropicus* or by three week old juveniles, which started laying during the last week of the conditioning period (Fig. 2c and 2d). Water conditioned by old months specimens of *B. tropicus* also lowered the reproduction actively of the assaying conspecifics but to a lesser degree (Fig. 2e). In a control group where the culture water was replaced weekly with fish-conditioned water oviposition continued at approximately the same rate throughout five weeks of the experiment (Fig. 2f).

Oviposition inhibition and species specificity

Media conditioned by *Bulinus globosus* and *Physa* sp. significantly reduced the oviposition rate of *B. tropicus*, the latter being rather less effective (Fig. 3a, 3b). Media conditioned by *Biomphalaria glabrata* also lowered the oviposition rate of *B. tropicus* but not significantly so (Fig. 3c). In contrast the egg laying activity of *B. tropicus* was relatively unaffected by *Melanoides tuberculata* conditioned water (Fig. 3d).

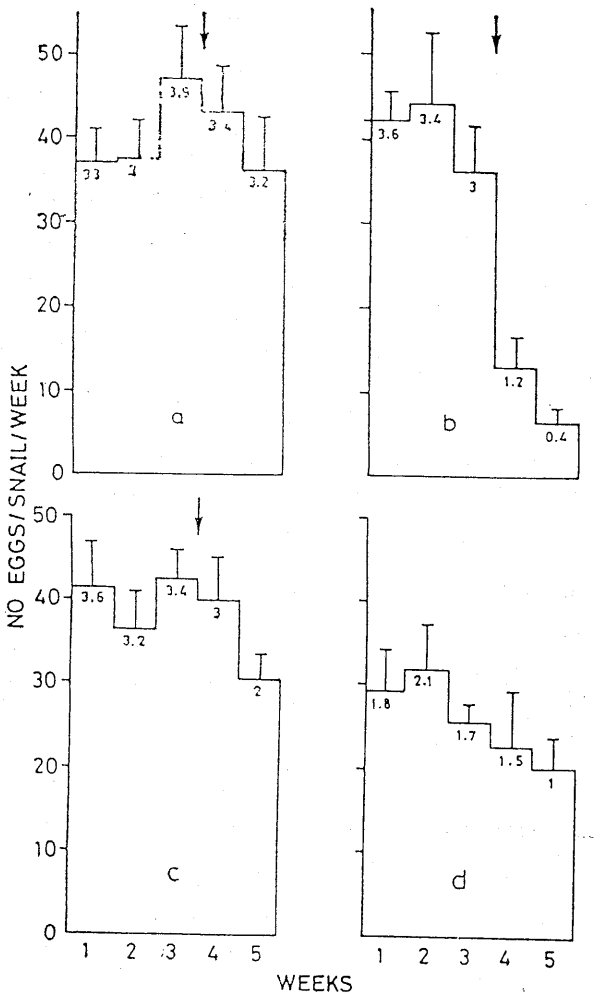

Fig. 1. The oviposition rate of *B. tropicus* following transfer of snails to different experimental media containing: faecal homogenates of conspecific snails, three week conspecific conditioned water and three week old lettuce infusion (a to c respectively). The water was replaced with fish conditioned water at the end of each week for the first two weeks of each experiment with the experimental medium at the end of week three, (indicated with the arrows). In fig. 1d the water remained unchanged but was filtered each week through a column of activated charcoal after bubbling with compressed air. Figures at the column heads indicate the number of egg masses per snails.

The ionic composition of media conditioned by different snail species is represented in Table 1. It is evident that the various snail species did not change the ionic composition of their media more drastically than does *B. tropicus*.

DISCUSSION

The results described above are essentially similar to those reported by other workers in that media conditioned by conspecific and heterospecific snails inhibit oviposition (e. g.

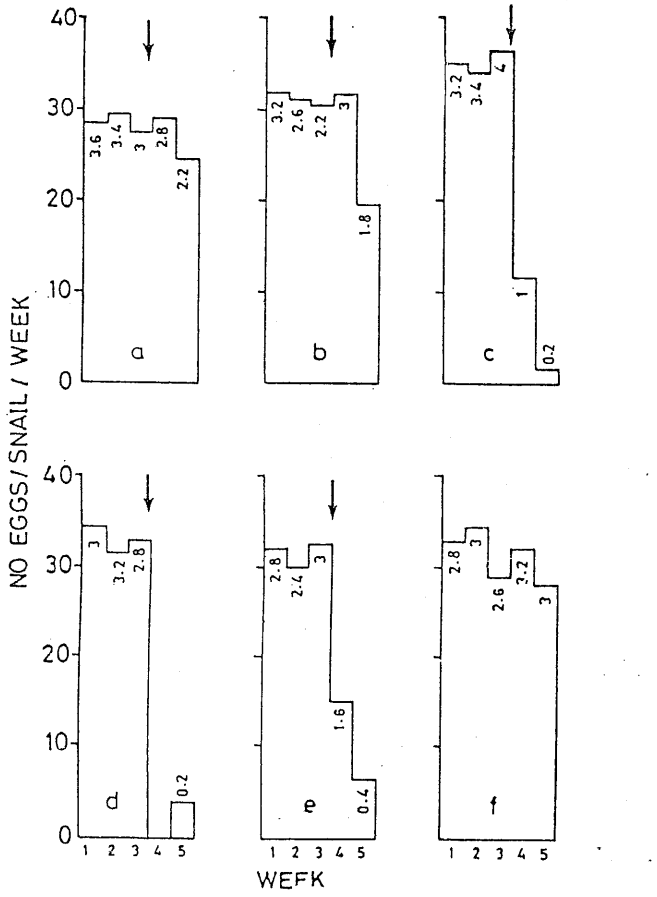

Fig. 2. The results of a single experiment in which the water was replaced by media conditioned by conspecific snails of different age groups. (a) Egg masses hatching into neonates, (b) one week old juveniles, (c) three week old juveniles, (d) actively laying specimens, (e) eight month old specimens, and (f) water changed weekly with fish conditioned water. Otherwise legend as for Fig. 1.

Chernin and Michelson, 1957a, b; Wright, 1960; Berrie and Visser, 1963; Levy *et al.*, 1973; Madsen, 1979, 1982; Lazaridou-Dimitriadou and Daguzan, 1981; Chaudhry and Morgan, 1986; Chaudhry, 1986) and are consistent with the suggestion that the inhibition is produced by the snails themselves.

Madsen (1979a, b), investigating the competition between *Helisoma duryi* and *Biomphalaria* species in the laboratory, found many unhatched egg masses in older aquaria and, as the effect showed no species specificity, he concluded that the factors responsible may have originated from the food or metabolic wastes. In the present study three week old lettuce infusion did not inhibit oviposition and although the possibility that the inhibitor may be a dietary by-product or of microbial origin cannot be precluded (e. g. see Thomas *et al.*, 1975) it is difficult to explain the absence of inhibition on transfer to certain heterospecifically conditioned media, or to media conditioned by neo-natal or juvenile snails in these terms, all groups of snails being fed on the same lettuce. Faecal material has been shown to influence the growth and development of a number of organisms (Rose, 1960; Akin, 1966). In particular, Thomas *et*

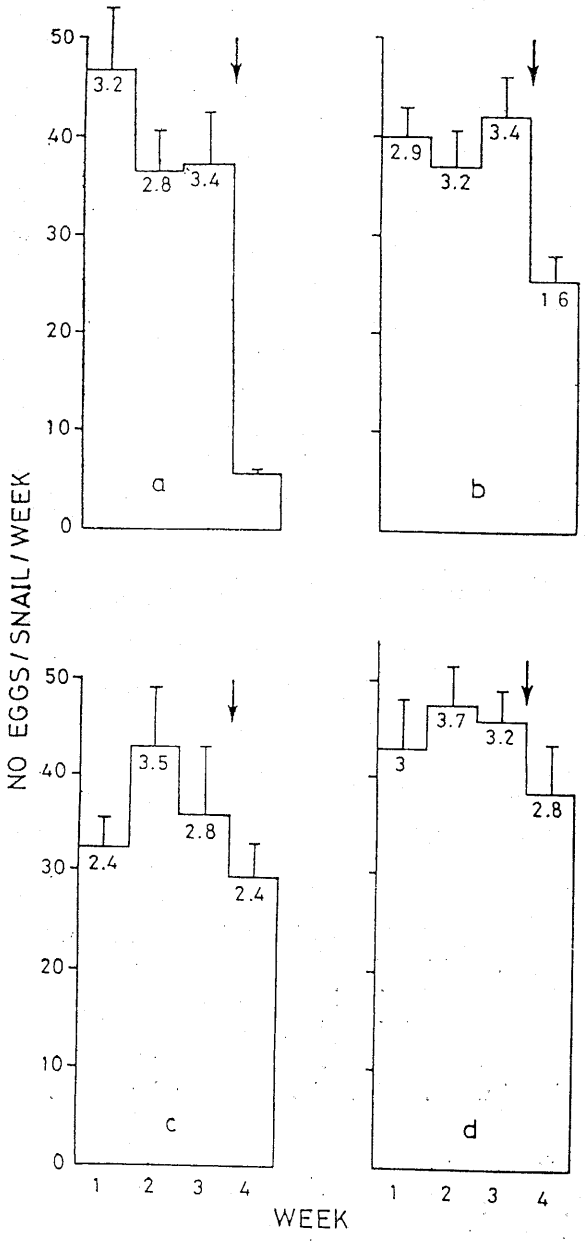

Fig. 3. The oviposition rate of *B. tropicus* after transfer to media conditioned by *B. globosus*, *Physa* sp., *B. glabrata* and *M. tuberculata* (a to d respectively). Otherwise legend as for Fig. 1.

al., (1975) have shown that faecal homogenates may both accelerate and retard the growth of *Biomphalaria glabrata*, the nature of the response being determined by the diet of the donor snail. Gazinelli *et al.*, (1970) have also reported the presence of an active component in faeces from a crowded culture of *B. glabrata* which inhibited the uptake of ^{59}Fe, therefore inhibit the growth of this species. But Thomas *et al.*, (1975) found that the oviposition of *B. glabrata* was unaffected by faecal material. The faecal homogenates are also found without effect on the oviposition of *Bulinus tropicus* (Fig. 1). It seems

TABLE 1
Ionic composition of snail media conditioned by different species, analysed after a period of three weeks (for details regarding analytical procedures, see Chaudhry, 1986). In each case five mature specimens were maitained in 400 ml fish conditioned water

Snail speoies	Na^+ (m. mol/ℓ)	K^+ (m. mol/ℓ)	Ca^{++} (m. mol/ℓ)	Mg^{++} (m. mol/ℓ)	Ammonia (ppm)
B. glabrata	1.66±0.208	1.29±0.332	0.083±0.015	0.221±0.018	4.69 ±0.363
B. globosus	1.06±0.158	0.70±0.120	0.11 ±0.06	0.17 ±0.011	3.46 ±0.202
Physa sp.	1.20±0.162	1.43±0.153	0.096±0.02	0.125±0.018	3.25 ±1.172
M. tuberculata	0.72±0.175	0.74±0.135	0.16 ±0.03	0.152±0.021	0.175±0.066

more likely therefore that the inhibitory effects observed in the present study result from some factor produced by the snails themselves, and this factor is removed from the medium by passing through activated charcoal.

Similar results have been obtained by Wright (1960) who reported the absence of inhibition of oviposition in *Bulinus forskalii* after such treatment. In the present study some inhibition was still observed after such treatment (Fig. 1) and probably reflects the difference in the duration of the deactivation, i. e. 5 minutes versus 24 hours in Wright's (1960) experiments. Inhibitory secretions have been postulated to account for the success of *Helisoma duryi* in competition with other helminthologically important snails (see Madsen, 1982, for references) and for other freshwater pulmonates (Levy *et al.*, 1973; Lazaridou-Dimitriadou and Daguzan, 1981) but in a recent study Madsen (1982) could find no evidence to support this hypothesis. He attributed the success of *Helisoma* to direct interaction, but did observe inhibitory effects in newly established aquaria with low densities of adult snails, conditions which have approximated to those of the present experiments.

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水質受異種及同種不同齡羣螺類影響對 *Bulinus tropicus* 之產卵效應

M. ASGHAR CHAUDHRY

B. tropicus 會因同種螺影響水質導致產卵被抑制。但水質若混有蒿苣汁或其糞便混合液則沒有此反應。水中有性成熟或老熟幼螺，皆會有效地抑制 *B. tropicus* 產卵，但若水中只有正孵化出新生螺的卵塊或孵化一週的新生螺，則不具此抑制效應。分類上相近種所生活過的水具此效應，但關係較遠者則無。卵此抑制效應可由水中通氣和活性炭過濾而減弱。