Avoidance responses of some indigenous and exotic freshwater pulmonate snails to leech predation in South Africa

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The avoidance behaviour elicited from four species of freshwater pulmonate snails, *Physa acuta* and *Aplexa marmorata* (Physidae), *Bulinus tropicus* (Planorbidae) and *Lymnaea natalensis* (Lymnaeidae), following contact with a molluscivorous leech *Helobdella conifera* (Glossiphoniidae), was examined experimentally. Although *H. conifera* showed no species preference for any of those species it attacked, the physids, both exotics, were killed less often than would be expected by chance. The indigenous species, *B. tropicus* and *L. natalensis*, were more susceptible to leech attack than the introduced species. Size-preference trials using *P. acuta* showed decreased susceptibility to leech attack with an increase in snail size. These results are interpreted with reference to the mode of leech attack and to differences in snail morphology.

Vier spesies pulmonate varswaterslakke, *Physa acuta* en *Aplexa marmorata* (Physidae), *Bulinus tropicus* (Planorbidae) en *Lymnaea natalensis* (Lymnaeidae), se ontwykingsgedrag tydens eksperimentele blootstelling aan die slakvretende bloedsuier, *Helobdella conifera* (Glossiphoniidae), is ondersoek. Hoewel *H. conifera* geen voorkeur vir enige van hierdie slakspesies getoon het nie, is die uitheemse Physidae minder dikwels gedood as wat verwag sou word. Die inheemse slakspesies, *B. tropicus* en *L. natalensis*, is meer dikwels gedood. Eksperimente met *P. acuta* waarin die voorkeur van *H. conifera* ten opsigte van slakgrootte ondersoek is, het getoon dat 'n afname in suksesvolle aanvalle voorgekom het met 'n toename in slakgrootte. Hierdie waarnemings word na gelang van die aanvalwyse van *H. conifera*, asook die verskille in die morfologie van die slakke, vertolk.

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Many glossiphoniid leeches feed on the body fluids of benthic invertebrates, primarily small oligochaetes, chironomid larvae and molluscs. Predation on gastropods is well documented (Elliott 1917; Chernin, Michelson & Augustine 1956; Michelson 1957; Harry & Aldrich 1958; Mc Annaly & Moore 1966; Crewe & Cowper 1973; Young & Ironmonger 1980 *inter alia*). Some forms are known to feed primarily (e.g. *Glossiphonia complanata*) or exclusively (e.g. *Helobdella triserialis*) on molluscs and particularly on gastropods (Sawyer 1986). Several authors have examined snail / leech interactions, and have described leech avoidance responses by snails, ranging from simple operculum closing in prosobranchs (Kelly & Cory 1987) to elaborate and vigorous shell shaking and detachment from the substratum in pulmonates (Wrede 1927; Frieswijk 1957; Townsend & McCarthy 1980). *Physa acuta* and *P. fontinalis* are able to distinguish between leech and mechanical stimulation (Wrede 1927), and also between molluscivorous leeches and those species known not to prey on snails (Frieswijk 1957).

The aim of the present paper was to document the repertoire and efficacy of avoidance responses of four snail species against leech attack. More specifically, experiments were conducted with the following questions in mind; firstly whether *H. conifera* encounters and attacks its prey opportunistically, or whether any of the four species or size classes of snails are selected for or against; secondly, whether those species which display more complex avoidance behaviours are less susceptible to leech attack. The snails studied were two indigenous species, *Bulinus tropicus* (Krauss) (Planorbidae) and *Lymnaea natalensis* (Krauss) (Lymnaeidae), and two exotic species, *Physa acuta* (Draparnaud) (Physidae) and *Aplexa marmorata* (Germain) (Physidae). Of the exotic species, the former is a successful invasive, while the latter snail has only recently been recorded from southern Africa (Appleton, Brackenbury & Tonin 1989).

Materials and Methods

All experimental animals used were bred from specimens collected in the Durban / Pietermaritzburg area of Natal, and were housed in aerated aquaria (90 × 25 × 20 cm) and subjected to a 12 h light / 12 h dark light regime at 23 ± 10°C.

Avoidance responses

Six response categories, each more complex than the former, were created so as to encompass all observed responses to leech attack. These are:

Type 1: No response (but includes retraction of the body part stimulated).
Type 2: Retraction of the whole body into the shell.
Type 3: Floating.
Type 4: Shaking of the shell with the foot remaining in contact with the substratum.
Type 5: Shaking of the shell and detachment of the foot from the substratum.
Type 6: Shaking of the shell, detachment of the foot from the substratum and floating to the surface.

These categories incorporate the avoidance responses documented by Frieswijk (1957) and Bronmark & Malmqvist (1986), but are expanded in order to facilitate the inclusion of floating behaviour, either in isolation (Type 3) or in combination with additional reactions (Type 6). While it may be argued that the above rankings are subjective, we believe that the six responses types do represent manoeuvres of an increasingly elaborate and / or composite nature.

Trials to examine the responses exhibited by the different snail species under experimental conditions were conducted in 9 cm diameter petri dishes containing 50 ml of filtered aquarium water. Five dishes, each containing five snails of the same species were used in each and were left for 20 min before commencement of each experiment. The response types shown by individual snails were recorded when either a live leech (0,07–0,1 g), or the anterior portion, severed just posterior to and including the oral sucker, was placed in contact with particular body parts. Since the detection of leeches was expected to have some chemosensory basis, the possible effects of diluted body fluids, or the build up such fluids in the experimental chamber, were minimized by using each severed body part to stimulate only five snails before being discarded and the water in the petri dishes replaced. Where the site of stimulation was easily accessible, whole, live leeches held in forceps were used in preference to severed head regions. The latter were used to test body regions such as the mantle digitations (fringe) of *P. acuta* and the foot (= tail region) of those species whose tails did not project beyond the shell sufficiently to allow the point of contact with the live leech to be determined with certainty. Stimulation was applied until the snail responded, or the arbitrary cut-off period of 5 s expired, after which the result of the test was recorded as negative. This cut-off time is much shorter than the 30 s limit imposed by Townsend & McCarthy (1980) in similar experiments. However, since it is often the attachment of a leech to the snail that determines both the success of the attack and the effect of any avoidance responses, the shorter period provided a better indication of the survival benefit that a response might afford.

Stimulation of snails was performed sequentially in such a way that only one snail in each petri dish was touched before moving on to the next container. This gave the snails time to rest and re-settle, and also permitted the exclusion of the most recently tested snails from the next round of tests. One hundred responses were recorded for each form of stimulation. As a control, mechanical stimulation was achieved by touching the snails with a blunted dissecting needle. This served to contrast responses to leech attack to those elicited by contact with an innocuous foreign body.

Snail species / size susceptibility

Non-specificity of leech attack

In order to test whether *H. conifera* is an opportunistic or selective feeder on any of these snail species, one member of each species (shell heights 7,5–10,5 mm) was placed in a petri dish together with a leech (0,07–0,1 g). One hundred leech-initiated contacts were observed and their frequency and the species of snail involved were recorded. In the event of a leech gaining attachment to the snail, the two were gently separated. This procedure was repeated using different size classes (viz. 4,0–7,5; > 7.5–10,5 and > 10,5–13 mm) of one species, *P. acuta*, in order to test the randomness of leech / snail contacts with snails of varying size.

Size susceptibility

Trials to determine whether size influenced the efficacy of leech attack, were conducted in 350 ml transparent plastic cylinders enclosed at either end by fine gauge plankton netting and vertically suspended in aerated aquaria, with the greater part of the container submerged. This allowed a through-flow of oxygenated water, thereby standardizing the conditions inside the containers, affording a three-dimensional environment in which those avoidance responses that involved floating or detachment from the substratum could be expressed to their full extent.

One starved leech (0,07–0,1 g) and three snails of the size classes 4,0–7,5; > 7,5–10,5 and > 10,5–13 mm, were introduced into each container. The trials, performed only on *B. tropicus* and *P. acuta,* were run and observed continuously for 1 h, and repeated until the size classes of the victims of 100 leech attacks were recorded. Snails that had failed to evade attack, but on which feeding had yet to commence, were removed and replaced, and the leech was allowed to continue foraging. Such interruption of feeding did not seem to adversely affect the activity of the leech and new victims were often attacked within 5 min of the previous one.

Snail species susceptibility

The apparatus described above was also used to test for differential species susceptibility to leech attack. One similarly sized specimen (7,5–10,5 mm) of each snail species was introduced into the containers together with an unfed leech (0,07–0,1 g). As in the size preference trials, snails that had been subdued were removed and replaced with an individual of the same species and size class, and the trial continued. Avoidance response data were analysed by 2×2 (Yates corrected) chi-squared contingency tables (1 degree of freedom).

Results

Avoidance responses

Figure 1a–h depicts the frequency of both leech-stimulated and mechanically stimulated responses elicited by the four species of snail examined. *P. acuta* and *A. marmorata* were stimulated on the head, posterior protrusion (= tail) of the body and the mantle fringe, whereas *B. tropicus* and *L. natalensis* were stimulated only on the head region as their mantle fringe does not extend over the shell and the tail does not protrude sufficiently beyond the shell.

Tail region

Stimulation of the tail region of *P. acuta* and *A. marmorata* revealed no difference in response between the two species to leech or mechanical stimulation (Figure 1a, b), although *P. acuta* was seen to demonstrate significantly more complex escape responses (21% Type 4 and 1% Type 5) in response to leech stimulation (χ^2 = 21,28; p < 0,001).

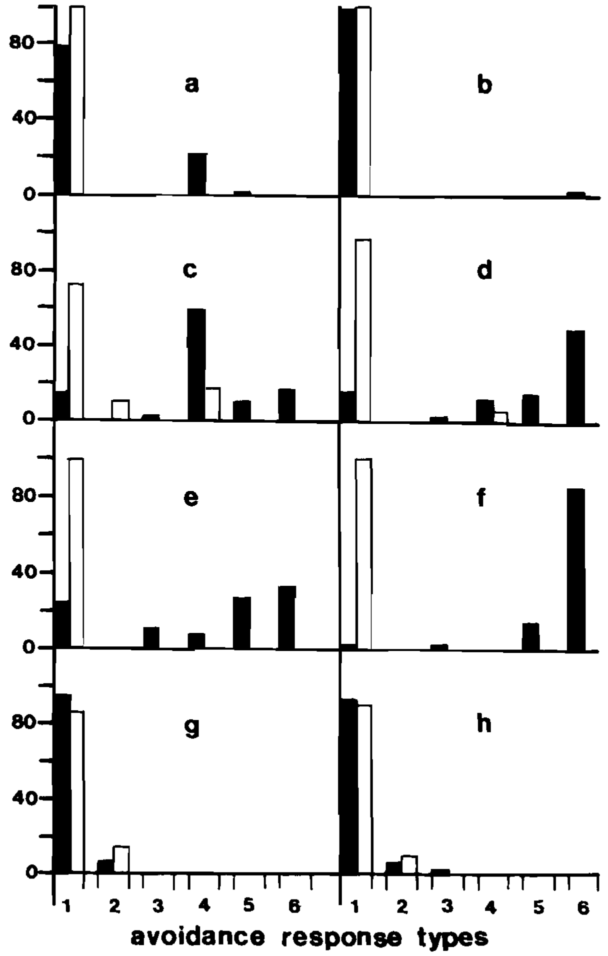

Figure 1 Frequency (%) of the six types of avoidance responses elicited from four species of snail by leech (shaded) and mechanical (unshaded) stimulation on the tail region (a — *P. acuta*, b — *A. marmorata*), mantle fringe (c — *P. acuta*, d — *A. marmorata*) and head region (e — *P. acuta*, f — *A. marmorata*, g — *L. natalensis*, h — *B. tropicus*).

Mantle fringe

Comparison between the responses to stimulation of the mantle fringe revealed a marked difference between leech and mechanically stimulated results for *P. acuta* and *A. marmorata* (Figure 1c,d). Of the two snails, *A. marmorata* appeared to be better able to distinguish between leech and mechanical stimulation of this tissue (97% Type 1 responses as opposed to 73% Type 1 response in *P. acuta*, $\chi^2 = 20,75; p < 0,001$).

Leech-stimulated experiments revealed similar response types for both species, with the only marked difference lying in the frequency of each elicited response. *A. marmorata* showed a greater complexity of escape responses, (59% Type 6) compared with *P. acuta* with 17% for this category ($\chi^2 = 35,67; p < 0,001$).

Head region

A. marmorata and *P. acuta* (Figures 1e,f), showed similar responses, with 100% of mechanically stimulated snails of both species showing Type 1 responses. The leech-mediated responses for these two species are similar to the results of the mantle fringe stimulation trials, with *A. marmorata* demonstrating a higher percentage of more complex responses (13% Type 5 and 84% Type 6) compared with the 26% Type 5 and 31% Type 6 responses elicited from *P. acuta* ($\chi^2 = 18,03; p < 0,001$). The latter snail species failed to respond in 26% of the leech-stimulated trials; while *A. marmorata* responded to 99% of leech contacts with the head region. Of the two snails, *P. acuta* displayed a marginally broader repertoire of response types.

The head-stimulation experiments with the two indigenous species (*L. natalensis* and *B. tropicus*) revealed no significant difference between their responses to either leech ($\chi^2 = 4,5; p < 0,0339$) or mechanically stimulated trials ($\chi^2 = 0,55; p < 0,459$) (Figure 1g,h). Both *L. natalensis* and *B. tropicus* were similarly unresponsive ($\chi^2 = 0,55; p < 0,459$) to leech stimulation (90% and 92% Type 1 responses respectively). Type 3 responses (2% *B. tropicus*) represented the most complex avoidance response elicited from these two snail species.

Snail species / size susceptibility

Non-specificity of leech attack

There was no significant selection of any of the snail species tested ($\chi^2 = 20,47$; 3 *d.f.*) or of size classes of *P. acuta* ($\chi^2 = 0,16$; 2 *d.f.*) in leech-initiated leech / snail encounters.

Size susceptibility to leech attack

Differences in the susceptibility to leech attack of the three size classes of both snail species tested, were greater than could be expected by chance (Figure 2; $\chi^2 = 19,9$ and 21,78 respectively; 2 *d.f.*; $p < 0,001$). The smaller size classes were clearly more vulnerable than the larger snails.

Species susceptibility to leech attack

Susceptibility of all four snail species to leech attack differed significantly from that expected by chance ($\chi^2 = 34,71$; 3 *d.f.*; $p < 0,001$). Subsequent Bonferroni intervals (Randal Byers & Steinhorst 1984) indicated that *A. marmorata* and *P. acuta* were subdued significantly less often, while *B. tropicus* and *L. natalensis* were subdued signifi-

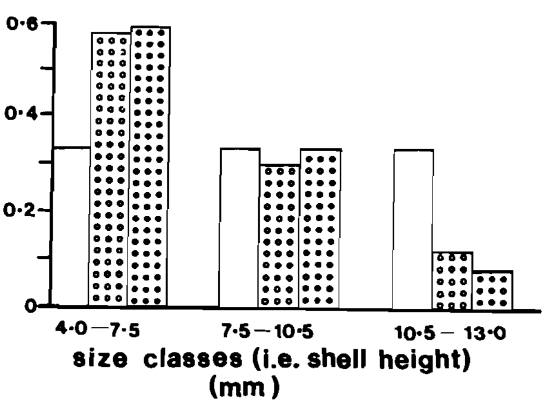

Figure 2 Proportions of three size classes (i.e. shell heights) of *P. acuta* (closed circles) and *B. tropicus* (open circles) subdued by *H. conifera*. Expected results are shown in the unshaded columns.

cantly more often than expected by chance ($\alpha = 0,05$ and $k = 4$ categories).

Leech attack patterns

H. conifera displays foraging and attack patterns that share many features with those of another glossiphoniid leech, *Glossiphonia complanata*, described in detail by Sawyer (1986). The following brief account is based on observations performed during the size and species susceptibility trials. Upon encountering a snail, the leech usually commences its attack by attaching the anterior sucker to the shell of the snail. With the caudal sucker attached to the substrate the leech contracts and attempts to dislodge the snail. Subsequent behaviour, though variable, seems to follow one of two strategies which appear to depend on the size and / or strength of attachment of the snail and the leech to the substratum. In the event of an attack on a small, easily dislodged snail, the leech contracts, lifts the snail and wraps the anterior portion of its body around it. Having thus immobilized the snail, the leech attempts to gain access to the soft parts with its anterior sucker and proboscis.

It appears that the above behaviour is attempted as a matter of course, and that it is only when attempts to dislodge the victim fail that the second approach is adopted. In this event, the leech releases the grip it has on the substratum and places the caudal sucker on the snail's shell. From this position the leech waits for the snail to emerge and then attempts to enter the shell through the gap between the shell and the substratum. This invariably elicits a Type 2 response from the snail, forcing the leech to withdraw. This happens until the leech either gains access to the snail or aborts the attempt.

Discussion

All avoidance responses are of some benefit in deterring leech attack, and even simple behaviours such as repeated retraction of the body into the shell can be successful in this regard. It must, however, be stressed that it is those responses which pre-empt adherence of the leech to the shell that provide the greatest survival to the snail.

The results indicate that *H. conifera* forages opportunistically and that the resulting leech / snail encounters occur on an essentially random basis. However, the actual susceptibility of the snails to leech attack is greater for the less responsive forms as well as for the smaller size classes of all the snail species tested. How well do these findings correlate with the types and frequencies of leech initiated avoidance responses elicited for each species? It is clear from the experimental findings that both *P. acuta* and *A. marmorata* respond in a more elaborate manner to leech stimulation than either *B. tropicus* or *L. natalensis*. In addition, both the former snails have the benefit of three sensitive areas available to them for leech detection, namely the head and tentacles, the mantle fringe and the tail which projects posteriorly from beneath the shell for some distance. It may therefore be expected that the ability to escape or deter leech attack would be greater in these snails than in *B. tropicus* and *L. natalensis*, which in effect have to rely on only the head and tentacles to inform them of the presence of a leech. To a great extent the results meet these expectations; the species susceptibility trials show that the two indigenous forms are eaten to a greater extent than expected by chance, while their more reactive, exotic counterparts fall prey to leech attack less often than could be expected by chance.

However, it remains to be explained why *P. acuta* and *A. marmorata*, which display a similar range of responses that vary mainly in the frequency of expression and both of which have three sensitized body regions available to them for leech detection, differ so markedly in their susceptibility to leech attack.

There are three possible explanations for this apparent anomaly:

(i) Differences in complexity responses.
The results indicate that the responses elicited from *A. marmorata* are, as a rule, more composite than those of *P. acuta*. However, whether this factor can account for the disparity in the results of the susceptibility trials for these two snails is doubtful.

(ii) Morphological differences.
Where the mantle fringe is concerned, differences in complexity of response become more significant when the tissue fringes of these two species are compared. In *P. acuta* the mantle fringe consists of small flap-like digitations that protrude over a very small area of the shell whereas the corresponding structure in *A. marmorata* is greatly enlarged and at times covers most of the shell.

Since it is usually the shell to which the leech initially attaches, the absence of an extensive mantle fringe constitutes a sizeable 'blind spot' and makes the snail more vulnerable to leech attack. Thus the extent of the mantle fringe in *A. marmorata* not only reduces the amount of insensitive area available to the leech for attachment, but allows the snail to react before the leech adheres to the snail. In addition, attachment to the mucus-covered mantle fringe itself may be more difficult to achieve.

Higher predation rates on the smaller size classes of snail tested are in agreement with the findings of other workers (Chernin, Michelson & Augustine 1956; Young & Ironmonger 1980). It was expected that the larger size classes of the more reactive *P. acuta* would be less susceptible to leech attack than similar sized unreactive *B. tropicus*. However, the similarity in the numbers of the larger size classes of each of the above species (Figure 2), do not indicate that this is the case. In addition, only a moderate difference in vulnerability of these two snails to leech attack was recorded (Figure 3). This in itself is unexpected since, as we have seen, *P. acuta* is seemingly much better equipped to survive attacks launched against it than is *B. tropicus*. The findings of the present paper indicate that in the case of *P. acuta*, elaborate avoidance responses are of surprisingly little use in escaping *H. conifera*.

Since the mantle fringe of *P. acuta* is only slightly reflected over the shell, the latter is largely insensitive and so precludes the expression of any avoidance behaviour before leech attachment. The response, when it is elicited, often coincides with, and is a result of, contraction of the leech in its attempt to dislodge the snail. Thus, as a result of

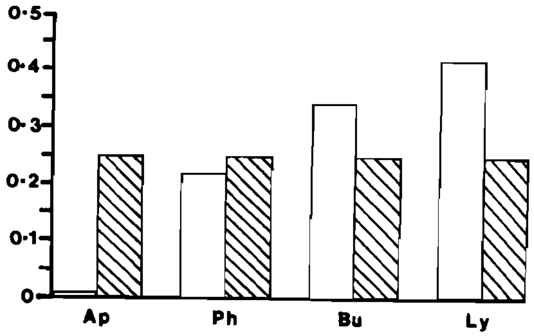

Figure 3 Proportions of the four snail species subdued by *H. conifera*, unshaded and hatched columns show observed and expected results respectively.

the mode of attack of *H. conifera*, the efficacy of the avoidance responses of *P. acuta* is minimal. Response Types 5 and 6 are, as a consequence of the extensive mantle fringes of *A. marmorata*, of greater benefit against *H. conifera* than to *P. acuta*.

It is reasonable to assume that, in the case of those snails having little or no mantle fringe, leech attack would counter selection pressure for avoidance responses that involve detachment. This indicates that predation pressure from leeches employing similar attack strategies to *H. conifera* comprise only a small fraction of the forces selecting for these responses, and that the overriding pressure stems from predators that do not rely on adhering to and dislodging their gastropod prey. The similarity of attack patterns between *H. conifera* and other common glossiphoniid leeches, and the relative ineffectiveness of *P. acuta* in avoiding leech predation, seems to support the contention of Bronmark & Malmqvist (1986) that anti-predatory responses elicited from snails are unlikely to be a result of specific coevolution between snail and leech, but rather a case of diffuse coevolution between snails and a number of species of invertebrate predators. Without first quantifying snail predators, their mode of attack, and the extent to which predation influences the distribution and abundance of gastropods, the extent to which avoidance responses enhance survival remains questionable. However, the occurrence of elaborate avoidance responses in the two exotic physids begs speculation as to the contribution of these responses to their invasive success.

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