

Effect of Shell Damage on Mortality, Reproduction
and Regrowth in Helisoma trivolvis

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Abstract

In order to see the effect of non-lethal shell damage, imitating that of the crayfish Orconectes rusticus on Helisoma trivolvis populations, the apertures of H. trivolvis shells were artificially damaged at different levels (0, 2, 4 and 9 mm of shell removed) during their reproductive period. The number of egg sacs, eggs, deaths and the amount of shell regrowth were all recorded for a period of one month. The preliminary hypotheses were that the snails that were damaged would divert energy and nutrients to repair and thus decrease their egg output, or, the damaged snails would put all energy into reproduction and subsequently die.

Since reproduction and death did not differ significantly among treatments, and there was more regrowth in the higher damaged treatments, both hypotheses were incorrect. Since the non-living shell is a necessary component of defense that must continually be repaired, the snail is incapable of stopping shell growth. The damaged snails increased their shell growth rate and continued with reproduction without any mortality. Therefore, non-lethal shell damage of H. trivolvis, during the latter part of its reproductive season, by O. rusticus probably does not have any effect on the population of H. trivolvis.

Introduction

Snails live within shells that act as protection for them. This means that a predator must find a way of getting past the shell in order to get to the edible snail. This can be done in several different ways, which include drilling, peeling, crushing and pulling out the intact snail by reaching into the aperture (Vermeij 1979, 1982). Snails and their predators have coevolved in ways that enhance their ability to survive predation and to catch prey, respectively (Vermeij and Covich, 1978). Freshwater gastropods, however, have developed far fewer predatory defenses in their shells than marine gastropods (Vermeij and Covich, 1978). This may be due to the limiting physicochemical characters of the freshwater bodies, lack of time for coevolution (due to the relatively young age of most freshwater bodies) and a lower diversity of predators in freshwater (Vermeij and Covich, 1978). The fact that freshwater snails have developed fewer predatory defenses would seem to leave their populations open to strict control by any abundant or efficient freshwater predator. In lakes of northern Wisconsin, a predator that controls snail populations is the crayfish. In Trout Lake, crayfish feed readily on snails and limit their distributions to areas of low predation pressure, which are not optimal feeding areas for snails (Lodge et al., 1987). This control can also be seen in the limitation and reduction of snails in macrophytes by the crayfish (Lodge and Lorman, 1987).

However, predators may be unsuccessful in their attempts to remove a snail from its shell. This is evident in non-lethal damage of the snail shell by crayfish (Olsen, 1989) and marine predators (Raffaelli, 1978; Vermeij, 1982). In laboratory experiments in which freshwater Orconectes crayfish prey on snails, intermediate sized shells are most often non-lethally damaged (Olsen, 1989). This is because larger snails are not handled as easily and have stronger shells, and small snails are almost always successfully eaten due to their lower shell strength.

It seems that there are two possible outcomes of non-lethal shell damage of snails by crayfish. The first possibility is that an injured snail may divert energy and nutrients from

growth and reproduction to repair its damaged shell. Repair would be undertaken because if it is not, and the snail subsequently dies or is eaten before reproduction as a result of the injury, then the snail population may be limited severely as a result of the non-lethal shell damage. However, even if shell damage has no immediate effect on mortality in some snails (Zipser and Vermeij, 1980), indicating that repair may take place, repair of the shell may have its own effects on the reproduction of the snails. Since energy, nutrients and time are being diverted away from growth and reproduction and toward repair, there will be less growth and reproduction, especially if the non-lethal shell damage occurs during the reproductive (breeding) period of the snail. In this way non-lethal shell damage by a predator can limit snail populations even though the snail is not killed in the original attack.

The second possible outcome of non-lethal shell damage on a snail would be that a snail of reproductive age could forego repair and put all of its energy and nutrients into reproduction (Kirkwood, 1981). This could occur especially for semelparous snails, which reproduce only once in their lifetime (Boerger, 1975). If the snails, being semelparous, are not going to contribute to reproduction in future years, why should they decrease their reproduction in order to repair themselves? In other words, snails that are damaged during their time of reproduction should not repair themselves because it would decrease their reproductive value (Kirkwood, 1981). Their reproductive value is all that is important because natural selection stops working on individuals which are past their reproductive potential. This is why organisms past their reproductive time senesce quicker and die. If the snail did opt to put all of its energy into reproduction one would expect to see high mortality in damaged snails since they have not repaired themselves (Kirkwood, 1981). This means that the snail population would not be affected by non-lethal shell damage because reproductive value was not decreased even though the snail was damaged.

Prior experiments have revealed that Orconectes rusticus removes Helisoma trivolvis from its shell by peeling away its planospiral ("ram's horn") shell, starting at the aperture

(Olsen, 1989). Since H. trivolvis lacks a nacreous strengthening near its aperture, the crayfish can readily peel the shell and submit the snail to non-lethal shell damage (Olsen, 1989). This is in contrast to H. companulata which has a nacreous strengthening at the aperture and therefore must be broken at its dorsum or ventrum by the crayfish in order to be removed from the shell so it can be eaten. This means that the crayfish may have a significant effect on H. trivolvis abundance through the mechanism of reduced growth and reproduction due to non-lethal damage, or it may have no effect on the abundance but cause high mortality in the parents because repair is excluded in favor of reproduction. The second option is only feasible for semelparous organisms in their reproductive period (Kirkwood, 1981). This is therefore feasible for H. trivolvis since it is semelparous with a life cycle of two years, at the end of which occurs the breeding period (Boerger, 1975).

This experiment will compare the effects on snail reproduction, death and repair of different levels of non-lethal shell damage (simulating damage produced by O. rusticus) on H. trivolvis.

Materials and Methods

The experiment was conducted at the University of Notre Dame Environmental Research Center (UNDERC) in the Upper Peninsula of Michigan from 1 June - 1 July, 1989. Upon arrival at the research area in late May the experiment was set up as soon as possible in order to have it take place during the reproductive period of *H. trivolvis*, which is from early spring to late summer (Boerger, 1975). Potential sites for *H. trivolvis* collection were taken from a survey of snail abundance in area lakes (Weinsheimer, unpublished). Adult snails (mean shell diameter of 24.6 mm, range= 21-29 mm) were collected from Mullahy and Ward lakes.

Shells were artificially damaged by peeling the shell away from the aperture with forceps. There were four different treatments (0,2,4 and 9 mm of shell removed) with seven replicates of 10 snails each. Each of the 28 experimental units was a shallow fiberglass tray (30 cm by 35 cm by 8.5 cm deep), filled to a depth of 5 cm with Tenderfoot Lake water. Snails were randomly assigned to treatments. The trays were placed on makeshift benches exposed to uneven natural lighting. Replicates were randomly placed on the benches by drawing numbers out of a pool of all replicates.

Each tray contained 10 snails (all with the same degree of damage) and 2-3 periphyton covered rocks (6-10 cm diameter) to provide snail food. The natural amount of food was not known, but food level was kept as constant as possible throughout the experiment by replacing water and rocks about once per week (three times during the experiment).

Each tray was checked every other day for snail mortality and egg sacs. Dead snails were removed. Egg sacs were collected with a scalpel from tray sides, rocks and shells of snails. Eggs in each egg sac were then counted under a dissecting microscope.

Every snail was measured for new growth near the end of the experiment (24 June). In the three treatments that had actual artificial damage, the original (damaged) edge of the shell was visible in most cases. The new growth measurements were all done on the same

part of the snail shell in order to eliminate differences due to uneven cracking at the beginning of the experiment.

Results

When snail shells were cracked with the forceps, the crack was occasionally jagged. Therefore, the damage was not always exactly 2, 4 and 9 mm. However, the damage done to each shell in the damage levels did not overlap into the other damage levels.

During the experiment, Helisoma trivolvis often "shed" an outer layer of their shell (apparently the periostracum) and also a narrow ring of the shell occurring near the aperture. I believe this has never been documented before; its significance is not known.

While collecting egg sacs in the beginning of the experiment, some of the sacs were mangled because the collection technique was not yet mastered. This only affected the count of the number of eggs per sac for a few of the sacs in the first collection time. The mangled egg sacs' number of eggs was determined as accurately as possible by putting the egg sac back together under a dissecting microscope.

The regrowth on many of the H. trivolvis' shells was observed to be very thin as compared to the rest of the shell. This, along with the original fracture line, made it easy to measure the regrowth on all of the snails where these observances occurred, but where they did not occur and the regrowth blended in with the old shell, measurements were taken from areas where the regrowth seemed to begin. This problem was especially found in the control snails (no damage) because they had no fracture line to begin with.

While egg sac and egg counts seemed to show that the greatest number of egg sacs and total number of eggs were being laid by treatment 4 with the most damage (9mm), cumulative number of eggs laid, as tested at the conclusion of the experiment, did not differ among treatments (Fig. 1, ANOVA $p=0.40$).

Some mortality was observed during the course of the experiment. Until after day 16 there was only one death per treatment (Fig. 2). Thereafter, there was a greater number of deaths. The 4 mm treatment had the most deaths, followed by 0 mm, 9mm and 2 mm. An ANOVA on the last day's data indicated these differences were borderline, but not significant ($p= 0.0517$).

Regrowth differed significantly among treatments (Fig. 3, ANOVA $p < 0.00$). The 9 mm treatment had the most growth, followed by the 4 mm, 0 mm and 2mm treatments. All treatments, except the 0 mm and 2 mm treatments, differed significantly (Student-Newman-Keuls, $p < 0.00$).

Discussion

The problems of uneven, jagged cracking of the shells, a few "mangled" egg sacs at the first collection day and the shedding of the shells probably had little effect on the outcome of the experiment.

The difference in deaths among treatments was borderline significant ($p=0.0517$), but probably not biologically meaningful. Apparently, snails with 9 mm and 2 mm of shell damage survived when snails with 0 and 4 mm of damage died, but no plausible biological explanation can explain this lack of increasing or decreasing relationship between damage and mortality. This agrees with the conclusions of Zipser and Vermeij (1980) that shell damage does not immediately cause mortality in snails.

The only response that differed significantly among treatments was amount of repair which increased with increasing damage. Since it was harder to measure growth on the shells where no damage was done (treatment 1), these data may be inaccurate. Even if it was, it shows that minor damage (treatment 2) is similar to the natural growth of the snail shell (treatment 1), and may be a common part of shell growth. Even though none of the treatments (2,3,4) repaired close to the amount removed, the shells with more damage had more regrowth because more shell needed to be replaced after the damage. The shell is important for the survival of the snail, so it is regrown.

An important distinction should be made at this point which may help to understand the results of this experiment: the snail's shell is an inanimate object and not part of the living snail. This is why the snails did not die even in the treatments with the most damage, although they may have if they were in the natural environment with its stress and predators. Even though the snails are semelparous and breed once at the end of a two year life cycle (Boerger, 1975), they are not going to put all of their energy into reproduction when their shells are damaged during their reproductive period, as is possible for damage to living tissue according to Kirkwood (1981). The snails did not put all of their energy into reproduction because no living part of them had been damaged. However, the shell

must be repaired since it is necessary for the snail's defense. The snail shell is analogous to the immune system and epithelial cells described in Kirkwood (1981). All of these systems are involved in both defense and repair. The snail shell is constantly growing and being repaired under normal circumstances. Shell growth is a natural and necessary part of the snail's life and it may be a "hardwired" (unable to be stopped) part of the snail's genome. This is evident in the fact that shells were repaired without any decrease in reproduction, and even the controls grew in a time when all energy was supposed to be going toward reproduction. This is why the snail was able to continue its normal reproductive rate and avoid mortality while still repairing its necessary defense system.

The results indicate that the shell is repaired at a faster rate if there is more damage done to the shell. This may be misleading because it was observed that some of the regrown shells were very thin, and therefore the shell may not have been completely repaired. Thus, it is conceivable that all treatments put the same amount of energy into growth. This would seem to go along with modern scientific theories on trade-offs, since no trade-offs were evident in the treatments that had greater regrowth. However, it still seems that this observation is outweighed by the large difference in growth between the treatments. Therefore, this leads one to conclude that the trade-offs were not able to be seen in the scope of this study. A more detailed and long-term study would have to be done to elicit what is being damped in order for the shell to grow more quickly.

Neither of the initial predictions of this experiment turned out to be true. The snails did not decrease their reproduction in order to repair themselves so they could survive. This was evident in the fact that there were no significant differences between the treatments in reproductive egg totals. The damaged snails did not put all of their energy into reproduction and then die afterwards either. All damaged and undamaged reproductive outputs were the same and the death totals were decided to be insignificant. Both of these rejections mean that the non-lethal shell damage inflicted on *H. trivolvis* during the latter stages of its reproductive season (Boerger, 1975) by the crayfish *Q. rusticus* does not

have any effect on the population of H. trivolvis. The snails continue to reproduce while going about the usual business of repairing their shells (although a little quicker when there is more damage). Even though the size of the H. trivolvis used in the experiment (mean of 24.6 mm) was not the same as the 10 mm H. trivolvis that is most often non-lethally damaged by O. rusticus (Olsen, unpublished), the experiment showed that non-lethal shell damage does not affect H. trivolvis populations because the greatest effect of shell damage would be seen in the reproductively active group of snails. H. trivolvis are not reproductively active until they are 18 mm long (Boerger, 1975). The effect of non-lethal shell damage occurring at the end of the main reproductive season (Boerger, 1975) is seen in this experiment to be insignificant to the snail populations.

There are a few things stemming from this study which could be addressed in further research. First is egg viability. It is possible that some of the eggs produced by the damaged snails might not have been as viable as eggs made by undamaged snails. The fact that the food amount was not strictly controlled is also a problem. The rocks with periphyton may have given the snails a superabundance of food which in turn might have allowed the damaged snails to do as well as the undamaged ones. The question of whether size or age determines whether or not an H. trivolvis is reproductively active came up several times in this study as well as in Boerger (1975). The answer to this could be quite useful. This experiment explored the effects of damage during the reproductive period on the snails, but it was done during the very end of H. trivolvis' main reproductive time of the year according to Boerger (1975), and, also, the snail physiology may be such that it produced all of its eggs prior to the non-lethal shell damage. If this experiment were done a few months earlier, during early spring, the results would show the effect of non-lethal shell damage over the whole reproductive season of the snails. Another experiment could be done during the winter, or with a known iteroparous snail species, in order to compare the above results with an organism that has much future reproductive time left. Earlier seasonal experiments lead into the fact that the general trends of reproduction and death,

although insignificant, were intriguing. If the experiment were started earlier, the trend of increased production until day 12 and increased mortality after day 16 (which looks a lot like putting all energy into reproduction and then dying) could probably be better clarified.

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Figures

Figure 1. (a) Mean cumulative number of eggs \pm 2SE (standard errors) throughout the experimental period (ANOVA $p=0.40$). (b) Mean cumulative number of egg sacs \pm 2SE throughout the experimental period. \circ 0 mm damage, \bullet 2 mm damage, \square 4 mm damage and \blacksquare 9 mm damage.

Figure 2. Mean cumulative deaths \pm 2SE throughout the experimental period (ANOVA $p=0.0517$). \circ 0 mm damage, \bullet 2 mm damage, \square 4 mm damage and \blacksquare 9 mm damage.

Figure 3. Histogram of mean shell growth \pm 2SE for the four damage levels (ANOVA $p<0.00$). The horizontal bar indicates the results of the Student-Newman-Keuls Test.

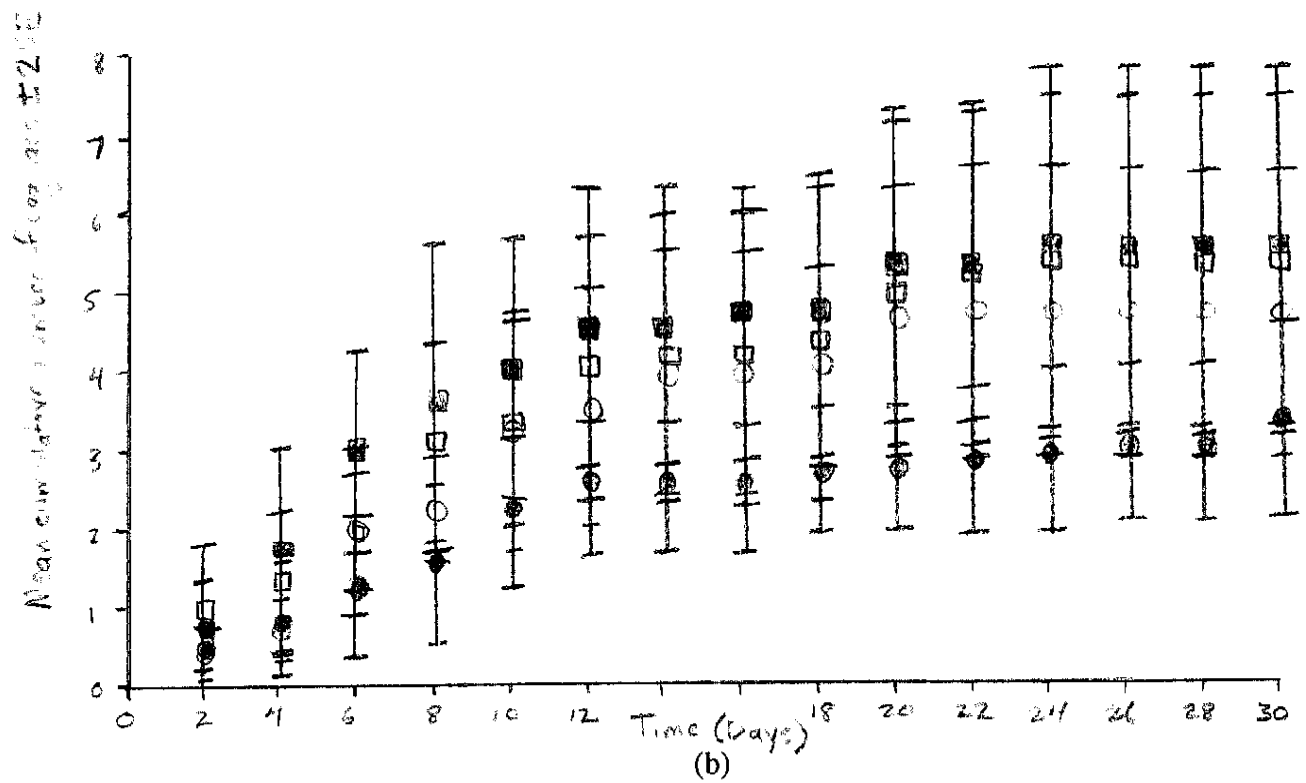
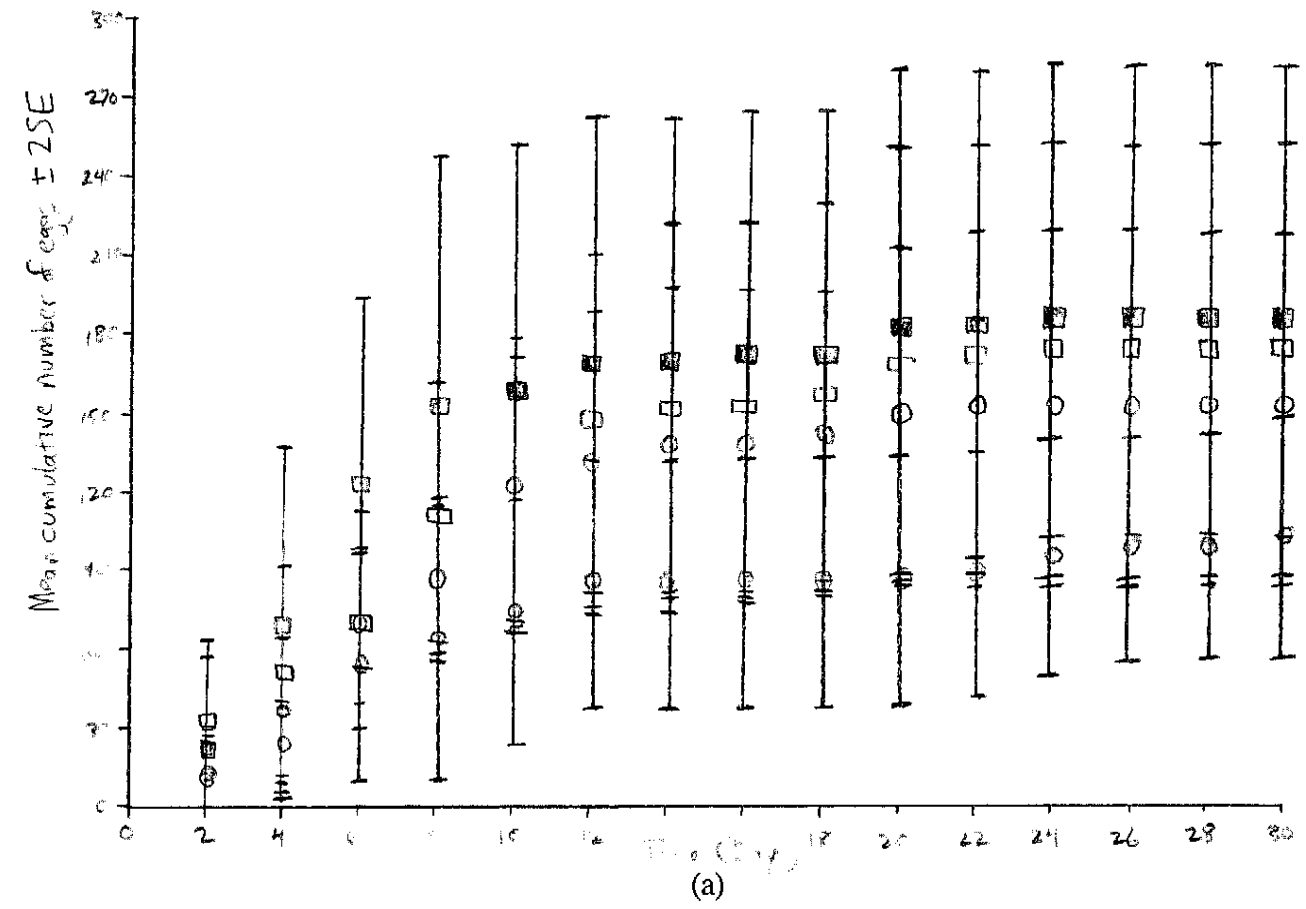
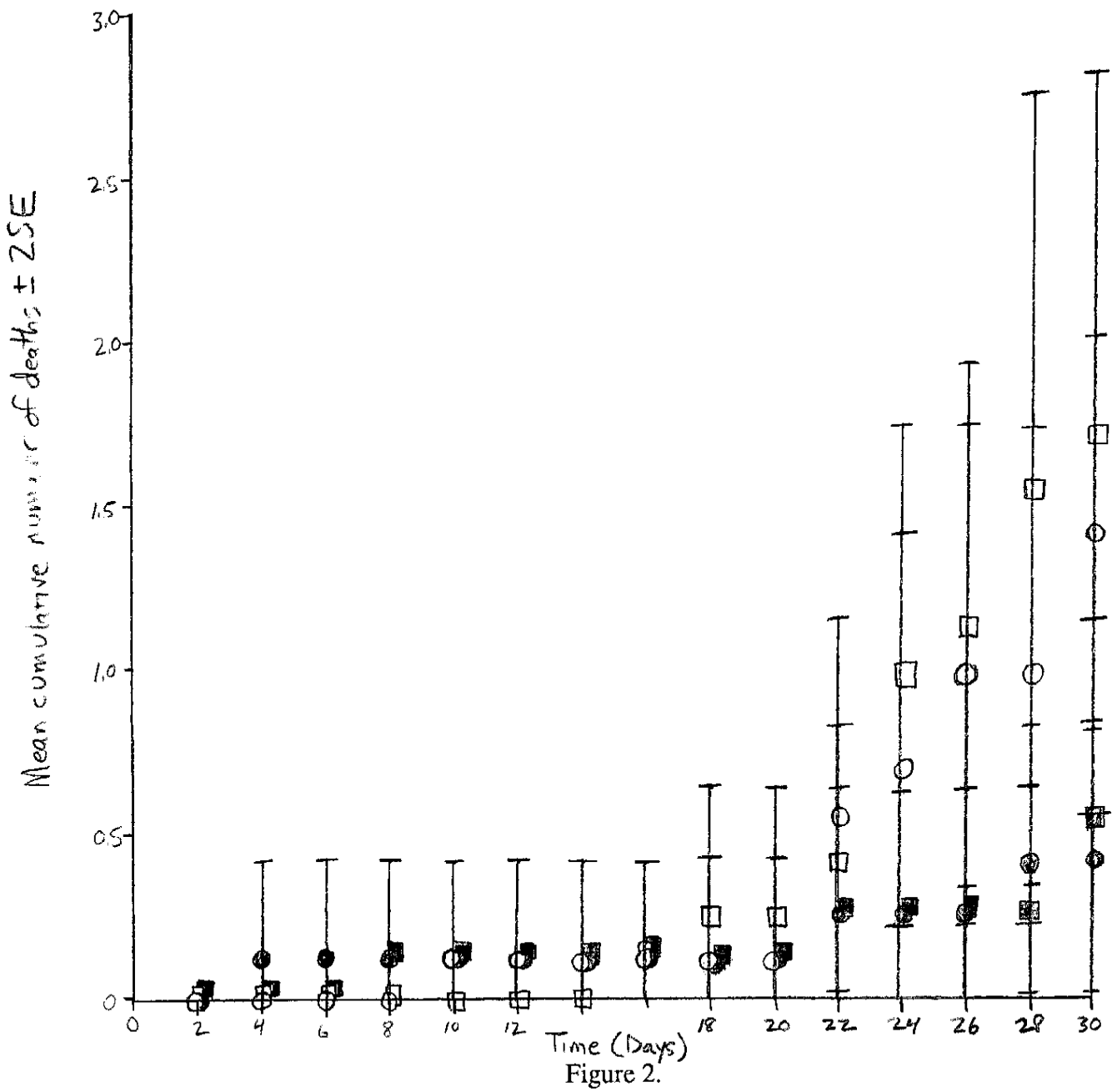


Figure 1.



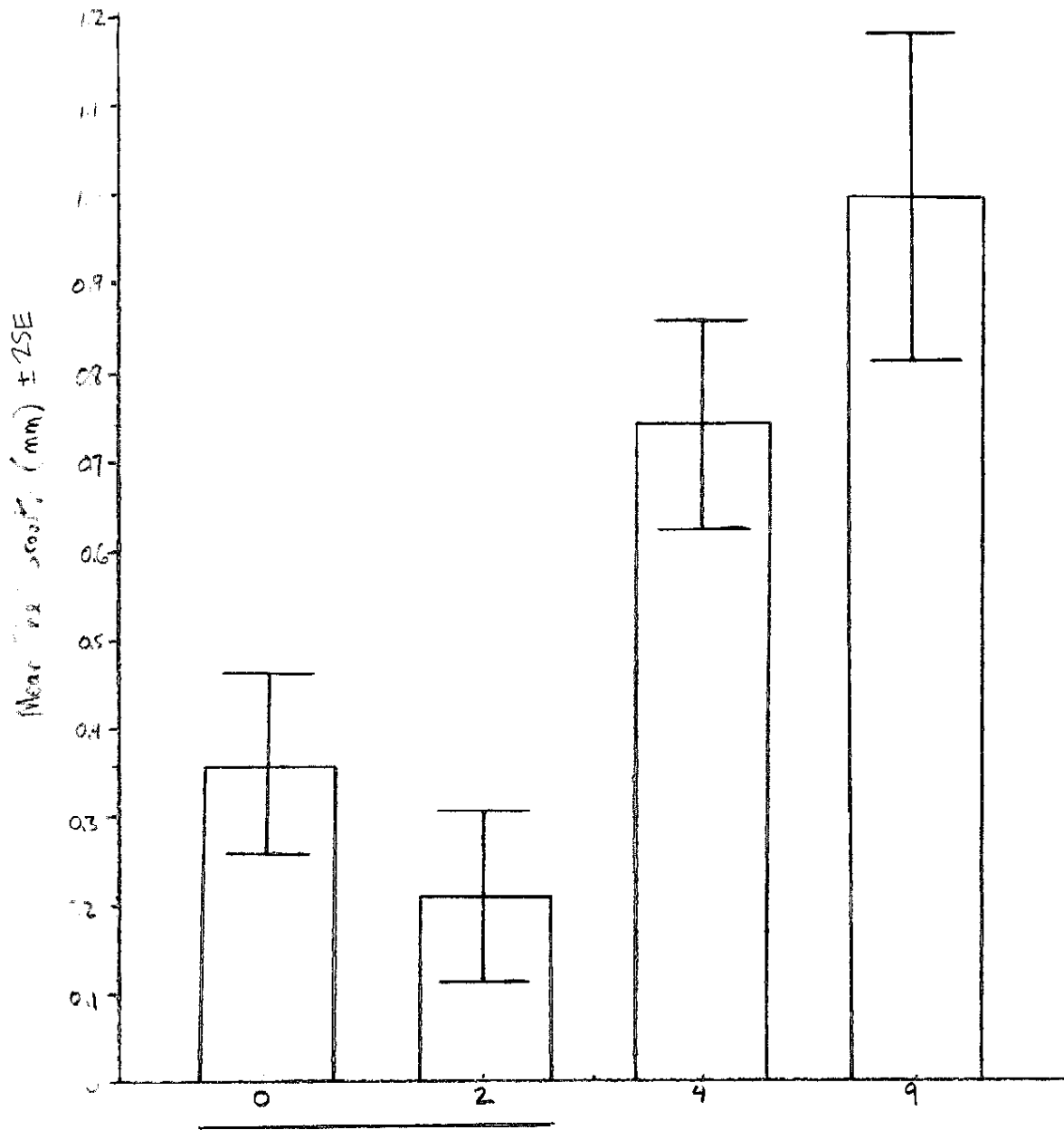


Figure 3.

Appendix 1. Data on total eggs laid per replicate.

Rep	Treatment (Damage)			
	1 (0mm)	2 (2mm)	3 (4mm)	4 (7mm)
1	198	122	118	164
2	115	33	149	72
3	330	98	214	231
4	137	212	269	123
5	120	116	335	457
6	120	71	94	103
7	42	46	28	150
Σ	1067	698	1207	1302
\bar{X}	152.4	99.7	172.4	186.0

Appendix 2. Data on total deaths per replicate.

Rep	Treatment (Damage,			
	1 (0 mm),	2 (2 mm),	3 (4 mm),	4 (9 mm)
1	0	1	4	2
2	2	0	2	0
3	1	0	2	1
4	1	0	1	1
5	2	1	0	0
6	2	0	0	0
7	2	1	3	0
Σ	10	3	12	4
\bar{X}	1.43	0.43	1.71	0.57

Appendix 3. Data on mean growth of snails per replicate.

Rep	Treatment (Lavage)			
	1 (0mm)	2 (2mm)	3 (4mm)	4 (9mm)
1	0.40	0.36	0.56	0.66
2	0.20	0.27	0.69	1.09
3	0.29	0.7	0.58	1.04
4	0.52	0.13	0.97	0.99
5	0.52	0.18	0.81	1.36
6	0.35	0.22	0.59	0.73
7	0.20	0.04	0.70	1.12
\bar{x}	0.35	0.21	0.74	1.00