

Stream Periphyton, Insect Herbivores, and Pesticides:  
An Experiment on Periphyton Response to Invertebrate  
Consumption

BIOS 569 - Practicum in Aquatic Biology

Eric J. Willman

30 Pangborn Hall

Dr. Gary A. Lamberti

1991

## Abstract

The effects of limiting nutrients such as nitrogen and phosphorous on periphyton growth have been well documented. However, in conjunction with light and nutrients, invertebrate consumption may play a role in periphyton growth and accumulation. The goal of this experiment was to determine if an increase in stream periphyton would result from exclusion of invertebrate grazers by means of insecticides or by physically removing them. Sealed clay pots, some containing insecticides, served as the substrates for periphyton attachment. The pots were placed in planktonic and benthic regions of Tenderfoot Creek, a slow-moving, lake-outflow stream in Gogebic County, Michigan. Periphyton growth was sampled three times during a 39-day period. Biomass and chlorophyll a contents, as well as insect numbers, were determined for each sample. No significant differences were detected between substrates with or without invertebrate grazers, suggesting that herbivores did not play a major role in periphyton accumulation in Tenderfoot Creek.

## Introduction

Aquatic insects vary greatly in the methods they use to acquire food. In any single section of a stream, one might find a variety of insects taking advantage of a variety of food sources. Shredders, such as some Plecoptera and Trichoptera, can be found on decomposing macrophytes. Many Trichoptera and Diptera can be found on fixed surfaces, filter-feeding for the fine particulate organic matter that drifts in the water column. Predators, such as some Coleoptera, must be mobile so that they can capture and then ingest the contents of other invertebrates. The scrapers also are opportunistic and should be expected to be found in various regions of the stream including the benthos in lotic regions and the planktonic region in lentic areas of the stream.

The stream herbivores most responsible for periphyton consumption are the scrapers, which are invertebrates that graze on benthic algae and other material attached to submerged surfaces (Lamberti and Moore 1984). By reducing scraper abundance, it would seem likely that an increase in periphyton would follow. Indeed, by excluding the larvae of the caddisfly *Helicopsyche borealis* via elevated substrates, Lamberti and Resh (1983) observed significant increases in benthic biomass and chlorophyll a. Further, Eichenberger and Schlatter (1978) observed increased standing crop of periphyton by excluding chironomid larvae from artificial outdoor channels by using periodic insecticide treatments. Both of these methods, in addition to physically picking off the invertebrates, were attempted in this experiment.

## Materials and Methods

The substrate for periphyton growth consisted of porous clay pots (surface area = 89 cm<sup>2</sup>; radius from 3 to 4 cm) sealed with plexiglass squares and silicone sealant. Inside the pots was an agar medium in which two insecticides were dissolved. Insecticide solutions consisted of the following: 1) 12 ml of Liquid Sevin (27% carbaryl) added to 1.5 L of distilled water and agar and 2) 12 ml of Malathion 50 Plus (50% 0,0-dimethyl phosphorodithioate of diethyl mercaptosuccinate) added to 1.5 L of distilled water and agar. Initially, sixteen pots contained agar with Malathion, sixteen contained agar with Liquid Sevin, and thirty-two pots contained agar alone. Sixteen of the thirty-two agar-containing pots were set aside for controls, the other sixteen were used as substrates from which the insects were physically inhibited.

## Peri. Response to Inv. Consumption

The pots were placed in a reach of Tenderfoot Creek, Gogebic Co., MI, where a riffle area spilled into a slow-moving pool with a deep, fine detrital bottom. There were few reaches fitting this description on the stream. For the most part, the water was slow moving and shallow, generally no deeper than 1.5 meters. During the sampling dates, the stream showed drastic increase in aquatic vegetation; some reaches were choked with macrophytes. This may be due to the relatively high amounts of inorganic nutrients and increasing temperatures (Table 1). The riparian zone of Tenderfoot Creek is dense, consisting mostly of tangled alders (*Alnus* spp.). A dense mat of mosses and shrubs extended to the wetted channel. The active channel and the wetted channel are often coincident along the stream.

Place Table 1 here

The planktonic pots were suspended in the pool. Four pots were fastened to one piece of lumber which was raised by anchoring each end to vertical rebars (Fig. 1).

Place Figure 1 here

The physical inhibition pots and the control pots were located several meters upstream so that they would not be contaminated by the pots containing Malathion and Liquid Sevin. The benthic pots were placed in the riffle. The physical pots were raised several inches off of the bottom with rebar and lumber to inhibit direct access by benthic invertebrates. Similar to the planktonic site, the physical pots were located upstream from the insecticide treatments. All the pots which were to be physically inhibited from invertebrates had the invertebrates picked off approximately once per week. The malathion, sevin, and control pots were anchored to the bottom with rebars; six pots were randomly chosen and tied to three rebars. Consequently, there were a total of four groups of pots containing random pots for the four treatments.

The pots were placed in the stream on May 31 and sampled on June 11, June 27, and July 8. During each sampling date, two pots for each treatment from both sites were randomly sampled. Sampling consisted of removing and preserving all insects in 90% ethanol. Periphyton was then scraped into 35 mm film canisters, which were then placed into a cooler and brought back to the lab where they were refrigerated until further analysis. The extra

## Peri. Response to Inv. Consumption

two pots for each treatment were put in the stream in the event that any of the pots were lost or damaged.

To determine biomass and chlorophyll a abundance, the periphyton from each sample was placed in distilled water, mixed, and volumetrically divided into two equal portions. The portions were filtered onto preweighed, glass-fiber filters and placed in the freezer until further analysis. To determine chlorophyll a, the periphyton on one filter was extracted in 25 ml of methanol. Analysis was done on a fluorometer and the results were converted to areal chlorophyll a for the pot surface. Biomass was calculated by drying the other preweighed filter in a drying oven and then subtracting the weight of the filter from the weight of the filter with periphyton. For both biomass and chlorophyll a, the results were multiplied by two since only one-half of the sample was analyzed by each method.

## Results

### Benthic Biomass

Periphyton biomass was very low on day 12 but then exponentially grew, followed by a decline as periphyton showed senescence (Fig. 2). Periphyton biomass on the physical pots remained lower than all other treatments throughout the experiment. At the end of the experiment (day 39), an ANOVA test indicates that the benthic biomass was not significantly different among treatments ( $F_{3,4} = 2.321$ ;  $p = .217$ ).

Place Figure 2 here

### Benthic Chlorophyll a

As with the biomass, the chlorophyll a content indicates an exponential growth and decline of algae for most treatments between days 12 and 28 (Fig. 3). The physical treatment had moderate chlorophyll a, whereas the malathion samples had the lowest chlorophyll a content. An ANOVA test on day 39 indicates that benthic chlorophyll a was not significantly different among treatments ( $F_{3,4} = 2.116$ ;  $p = .241$ ).

Place Figure 3 here

## Peri. Response to Inv. Consumption

### Planktonic Biomass

In contrast to the benthic samples, the planktonic algae exponentially increased between 0 and 12 days, then declined after day 12 (Fig. 4). On day 28, there was a small additional increase in biomass on the malathion, sevin, and control treatments. The physical treatment was lower than the other treatments for all but the second sampling date. An ANOVA test at day 39 indicates that planktonic biomass was not significantly different among treatments ( $F_{3,4} = .799$ ;  $p = .556$ ).

Place Figure 4 here

### Planktonic Chlorophyll a

Similar to benthic biomass, an exponential increase in chlorophyll a occurred between the beginning of the experiment and the second sampling date, followed by a decline (Figure 5). However, chlorophyll a did not display a final surge as the biomass did, but rather continues to drop. On the first sampling date, the physical and control treatments appeared to contain more chlorophyll a than the insecticide treatments. An ANOVA test on day 39 indicates that the planktonic chlorophyll a was not significantly different among treatments ( $F_{3,4} = .376$ ;  $p = .776$ ).

Place Figure 5 here

### Invertebrates

#### Benthic

The total number of invertebrates collected from the pots for each treatment for all dates were summed and categorized as snails (all were *Ammicola* sp.) or insects (Fig. 6). Most of the insects on the physical treatments were blackfly larvae (Diptera: Simuliidae). Another big contributor was *Hydropsyche* (Trichoptera: Hydropsychidae), which totaled 48 on the physical pots. Small *Baetis* (Ephemeroptera: Baetidae) also were fairly common, with a total of 14 collected from the physical pots. The only other type of aquatic insect collected was *Ceratopsyche* (Trichoptera: Hydropsychidae), which may be grouped with the *Hydropsyche*. One specimen was collected on the physical pots during sampling periods. The physical pots in the benthic area were raised slightly off the bottom to inhibit benthic insects from crawling on them. Invertebrate densities indicate that the opposite probably occurred. For the remainder of the treatments, very few insects were collected; no black flies were found and Trichoptera and Ephemeroptera were sporadic. Snails, in general, were present

## Peri. Response to Inv. Consumption

in numbers similar to all the species of insects combined.

Place Figure 6 here

### *Planktonic*

In the slow-moving water, *Ammicola* snails were extremely common (Fig. 7). There was a total of 51 on the control pots, 62 on the physical, 57 on the Malathion 50 Plus, and 80 on the Liquid Sevin. One *Baetis* mayfly was found on the controls; one *Baetis* on the physical; one *Baetis* and one caddisfly on the malathion; and six *Baetis* on the Sevin treatment.

Place Figure 7 here

## Discussion

Although the statistical analysis indicated that the treatments resulted in no significant differences in biomass or chlorophyll a in either the benthic or planktonic regions, this does not necessarily mean that insect grazing has no effect on periphyton growth. There may have been problems with the experimental design; this portion of Tenderfoot Creek may not have been the ideal place to run this experiment; or the results may have been significant although not statistically significant. All of these possibilities will be expanded upon in the forthcoming discussion.

### Consideration of Experimental Design

In designing the layout of the benthic pots, a choice had to be made. The four treatments could have been isolated from each other by grouping them, and then isolating them in their own area of the riffle. Alternatively, the pots could be randomly placed in the same area of the riffle. By choosing the first option, the insecticides would probably not influence the control and physical pots. However, by separating the pots, the stream conditions are changed. If periphyton on the insecticide pots flourished, one could never really say that reduction of grazing was the cause since the pots were exposed to different stream conditions. For this reason, a random design was used with the exception of the physical pots, which were placed slightly upstream from the rest. Since they were exposed to different conditions anyway (i. e., they were raised), this action was justified.

The planktonic pots posed a different problem. Since this pool was relatively homogenous in its bottom structure (loose detritus) and the water moved very slowly, diffusion of chemicals

### Peri. Response to Inv. Consumption

was the larger concern. For this reason, the pots were grouped according to treatment and spaced several meters apart, which is much farther than the benthic pots. But because of the fairly homogenous conditions, any differences in results could not be attributed to different stream conditions.

In summary, a compromise had to be made in pot placement. Perhaps placing the pots near each other allowed the insecticides to influence the insects on all pots and therefore caused insignificant differences in biomass and chlorophyll a content. Since this possibility was not examined in this experiment, it was accepted in favor of spreading the pots out in different areas of the stream, which would lead to more uncertainty in the significance of the results. In contrast to influencing all pots, perhaps the insecticides in this experiment were not leaching out in lethal doses, thereby not killing the insects on any pots. A study of malathion and sevin diffusion from clay pots and the resulting potency would need to be conducted to resolve the issue.

### Insect Exclusion Approaches

It appears that the physical inhibition treatment produced consistently low periphyton standing crop. Perhaps this was due to disturbance of algae when the invertebrates are picked off. A better method of deterrence is needed. Such a method might be to ignore picking off the insects and prevent the insects from getting on the pots in the first place. The rebar on the physical pots could have been replaced with a suspension mechanism that first came out of the water and then bent back down into it, with the idea being that the aquatic insects will not crawl out of the water. This method was used by Lamberti and Resh (1983) in their exclusion of *Helicopsyche borealis* caddisflies via elevated substrates. However, this still does not prevent invertebrates that are drifting in the water column from colonizing the pots. In addition to bent rebars, some other means of inhibition would be required.

Another systematic error could have arisen with the insecticide treatments. Throughout the experiment it was assumed that periphyton was not affected by the insecticides. Examination of benthic chlorophyll a (Fig. 4) suggests that the malathion treatment was much lower than the other treatments. It is possible that the malathion inhibited algal growth. Conversely, any rise in periphyton during the experiment could be attributed to nutrients provided by the insecticides. For example, malathion

## Peri. Response to Inv. Consumption

contains organic phosphorous and sevin contains nitrogen. However, because the insecticides are large molecules, the nitrogen and phosphorous may not be available in a form that could be assimilated by the algae.

Before the insecticides can eliminate the periphyton grazers, the grazers must be present. The insects that were collected from the pots are not strictly classified as scrapers according to the functional feeding group classification (Cummins 1973, Merritt & Cummins 1984). *Baetis* (Ephemeroptera: Baetidae) are collectors and gatherers as well as scrapers, with their main food source being detritus and not algae. *Ceratopsyche* and *Hydropsyche* (Trichoptera: Hydropsychidae) are collectors and filterers with their food sources being diatoms, detritus, and small animals. Their horn-shaped nets were observed wherever they were picked off the pots. The blackflies are filter feeders; they comb the water with the large, cephalic brushes. Even if all these insects were strictly algal scrapers it is not clear that they consumed significant amounts of algae since they were so few in number. However, the fact remains that there could have been transient grazers that simply were not present when the pots were sampled.

One grazer was present in significant numbers. The snail *Ammnicola* apparently was not affected by the insecticides. If there was any significant increases in the amount of periphyton due to insect exclusion, grazing by the snails could have compensated for that difference on the insecticide and control treatments. The study area of Tenderfoot Creek, and perhaps this creek in general, might be dominated by molluscan grazers and thus may not have been the best site for this experiment. A site where abundant insect scrapers are known to exist would be ideal.

Yet another reason for insignificant differences in periphyton exists. Grazing may not always decrease the standing crop of algae, but rather may enhance its growth in some instances. Lamberti and Moore (1984) proposed some ways in which this might occur. Grazers may remove dead or senescent cells and thereby increase the turnover rates of algal cells. They may increase the available nutrients by their wastes or by breaking open cells. Grazers may selectively remove slower growing algae and allow faster growing species to survive. If any of these possibilities occurred, deterrence of grazers by insecticides may have a smaller effect on periphyton when compared to the controls.

At the final sampling date, malathion and sevin treatments had more chlorophyll *a* and biomass than the physical and control treatments in both the benthic and planktonic sites. The benthic algae exponentially grew and then displayed senescence for all

### Peri. Response to Inv. Consumption

treatments. Perhaps, when the algae grows exponentially, grazers cannot significantly affect the rapidly growing food source. Perhaps, only when the algae slows its growth, and has exhausted its space and nutrients, will the grazers significantly change the algal content. If this is true, then the third sampling date may show the beginning of the trend. It may not be solely chance that both insecticide treatments are higher in both chlorophyll a and biomass in both the planktonic and benthic regions. The ANOVA p-values for benthic biomass and chlorophyll a are insignificant but relatively low (.217 and .241, respectively). Thus, there is about a 75% to 80% chance that the insecticide treatments had some effect on periphyton growth, presumably by reducing grazing.

It is apparent that there was considerable variation in this experiment. In order to control the variation, several things must be done. The diffusion and potency range of malathion and sevin from porous clay pots must be determined to resolve the issues of pot placement and optimal concentration. A better approach to physical inhibition of grazing must be designed. An area that contains abundant insect scrapers would be more suitable for this experiment. Finally, more replicate pots must be used and sampled to increase the amount of data for each treatment, and therefore increase the certainty of the results. When all of these conditions have been met, the relationships between insect scrapers, insecticides, and periphyton could be more clearly resolved.

### Acknowledgements

I would like to thank The Bernard J. Hank Family Endowment for allowing me the opportunity to conduct this experiment. I would also like to thank Dr. Lamberti for all of his assistance.

Peri. Response to Inv. Consumption

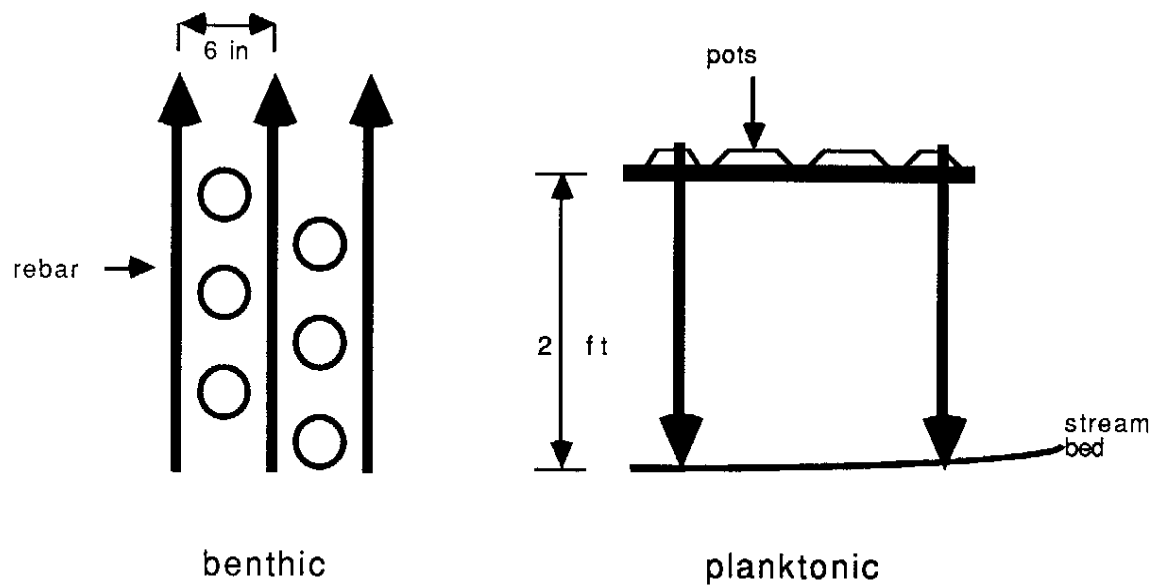
References Cited

- Cummins, K. W. 1973. Trophic relations of aquatic insects. *Annu. Rev. Entomol.* 18: 183-206.
- Eichenberger, E. and A. Schlatter. 1978. The effect of herbivorous insects on the production of benthic algal vegetation in outdoor channels. *Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen.* 20: 1806-10.
- Lamberti, G. A., and J. W. Moore. 1984. Aquatic Insects as Primary Consumers. p. 164-195 *in:* (V. H. Resh and D. M. Rosenberg, eds.): *The Ecology of Aquatic Insects.*
- Lamberti, G. A., and V. H. Resh. 1983. Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. *Ecology* 64: 1124-35.
- Merritt, R. W. & K. W. Cummins [eds.]. 1984. *An introduction to the aquatic insects of North America*, 2nd ed. Kendall Hunt, Dubuque, Iowa.

Table 1: Description of Tenderfoot Creek on July 20, 1991

	<u>Benthic</u>	<u>Planktonic</u>
PO <sub>4</sub> <sup>3-</sup> (mg/L)	.11	.13
NO <sub>3</sub> <sup>-</sup> (mg/L)	.02	.02
NH <sub>3</sub> (mg/L)	.16	.18
pH	7.36	7.16
O <sub>2</sub> (mg/L)	5.22	5.4
water temp °C	23	23

Figure 1 : The experimental design and the layout of the pots.



**Pot Placement**

p = physical  
 c = control  
 m = malathion  
 s = sevin

○ = pot

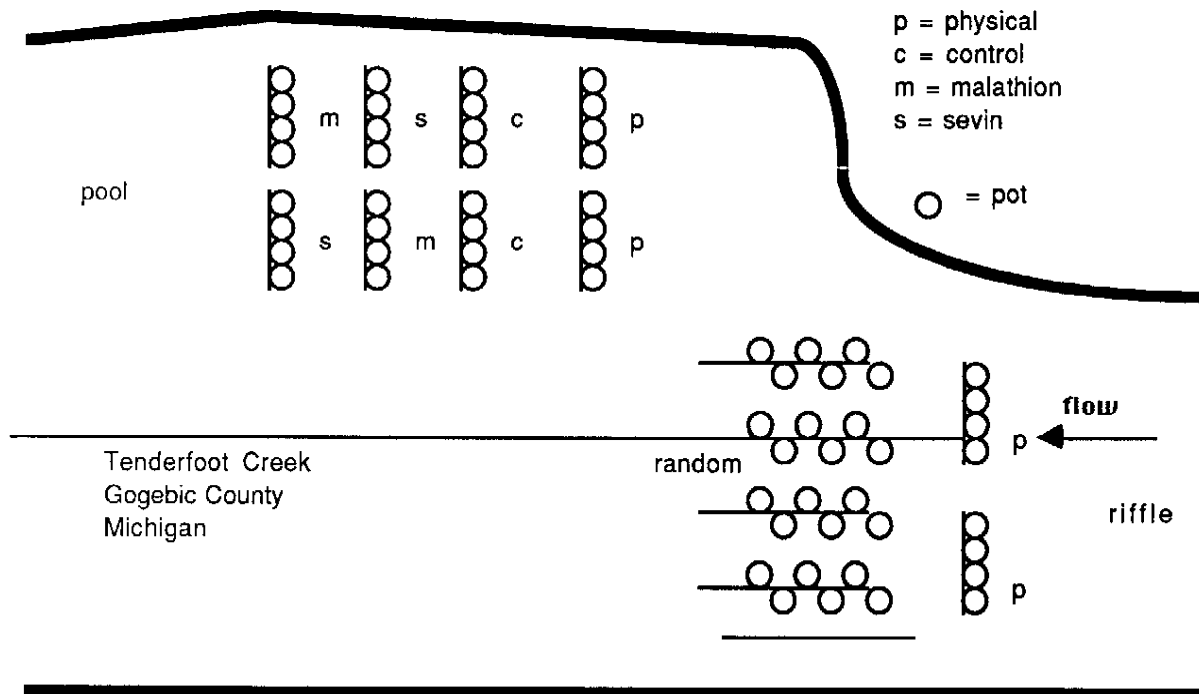


Fig.2: Benthic Biomass

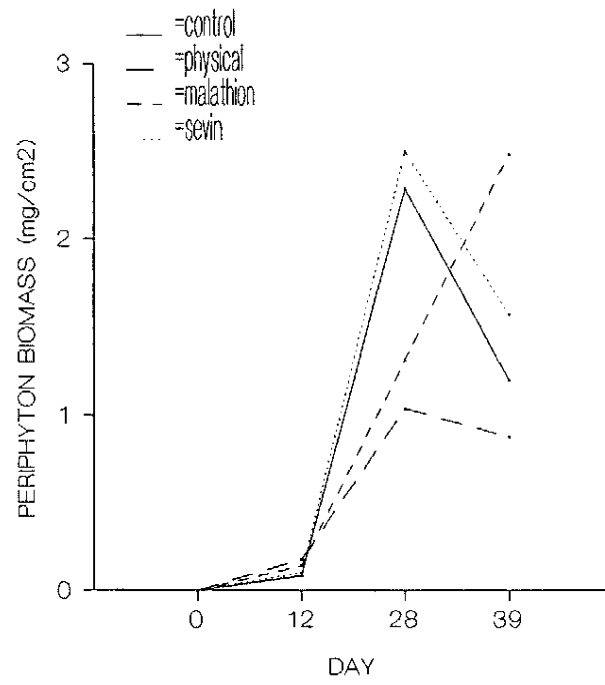


Fig.3: Benthic Chlorophyll a

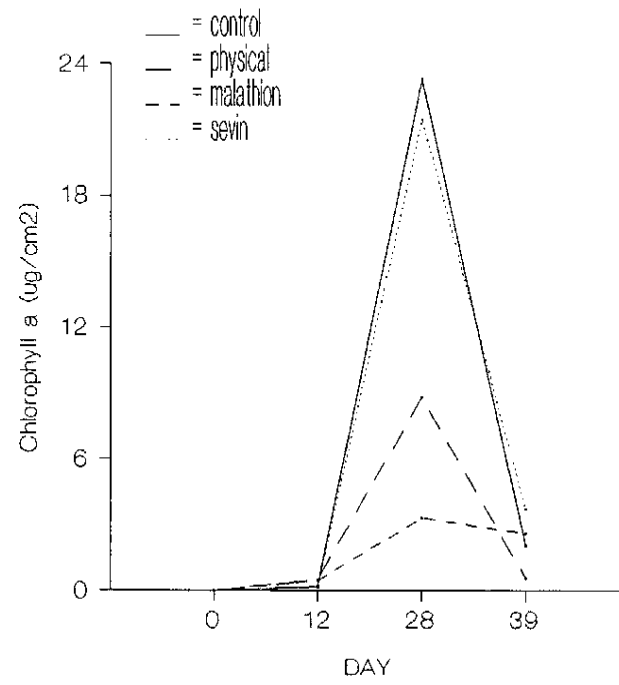


Fig.4: Planktonic Biomass

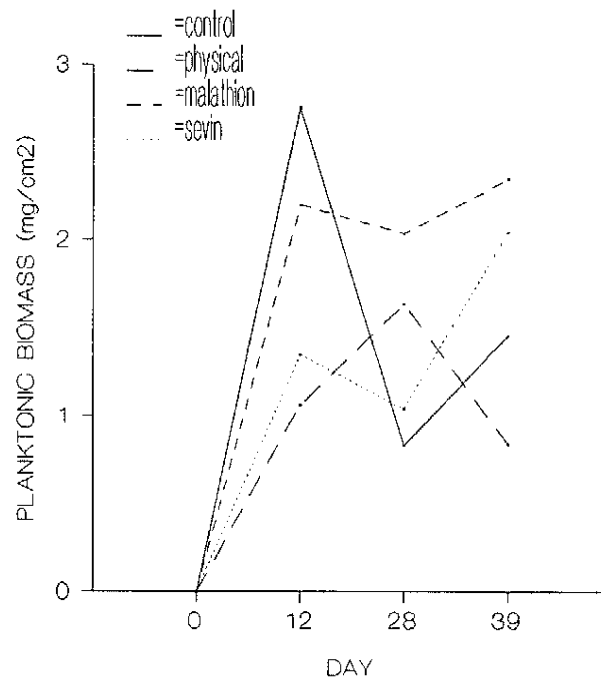


Fig.5: Planktonic Chlorophyll a

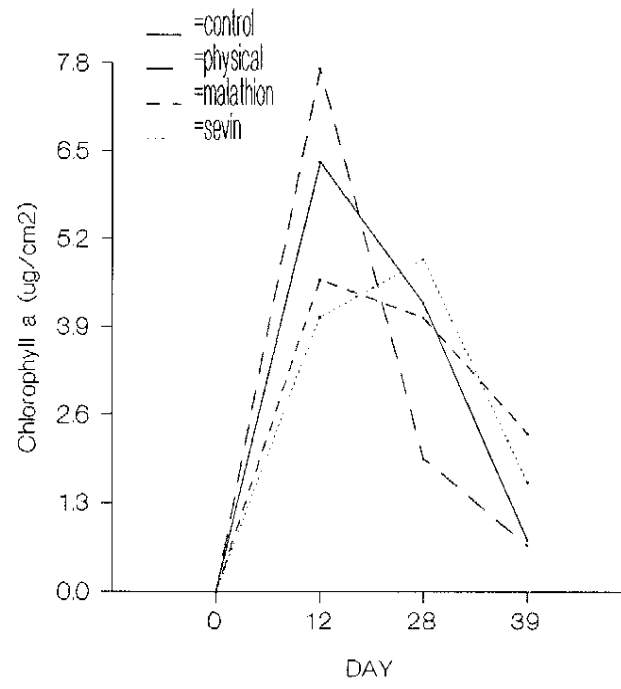


Fig.6: Benthic Invertebrates

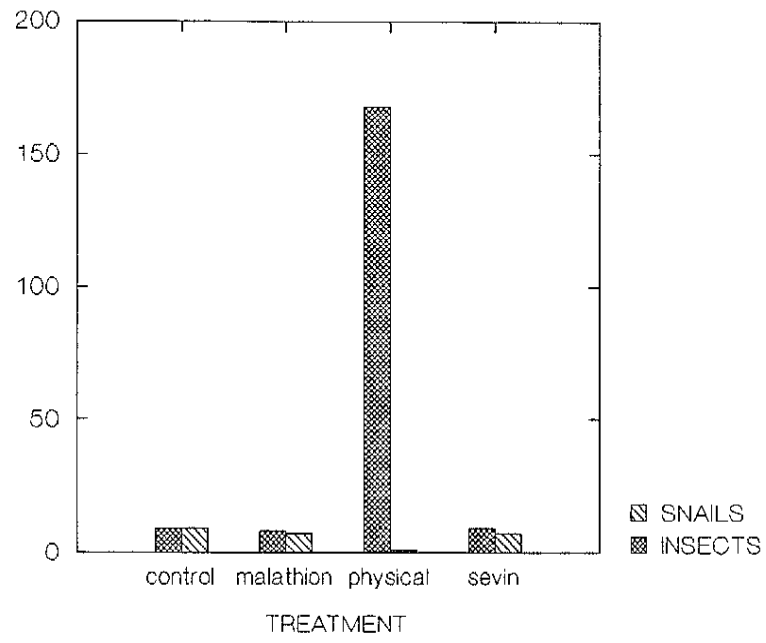


Fig.7: Planktonic Invertebrates

