

Two Invertebrate  
Potential Grazers on Epipellic Algae

BIOS 569 - Practicum in Aquatic Biology

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## Abstract

Two invertebrate potential grazers (chironomids and trichopterans) were studied on epipellic algae in separate laboratory tests to see the effects of larval insects and sediment type on epipellic algal chlorophyll a. Densities of 0, 1290, and 1911 chironomids/m<sup>2</sup> were placed on epipellic algae on two sediment types in laboratory containers (10cm diameter X 5cm deep). Chlorophyll a was measured weekly for 4 weeks as an indicator of changes in algal biomass. In a separate experiment, trichopterans (*Limnephilus sp.*) were added directly to sediment-filled containers and chlorophyll a was again used to measure changes in algal biomass. Results of the chironomid experiment show chlorophyll a accumulation was significantly ( $p < .05$ ) affected by sediment type, but not by larvae concentration, while the trichopteran experiments showed no significant changes in chlorophyll a, yet profound sediment morphology changes were observed. Results of both experiments suggest factors besides larval insect grazers may effect algal chlorophyll a accumulation in lakes, and that larvae may contribute to changes in algal community morphology.

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### Introduction

Many aquatic grazers, such as fish, adult snails, and larval insects effect algal growth, biomass, distribution, and species composition. With larval insects, Winterbourn (1990) concluded from interactions between Chironomidae larvae and periphyton that algal standing crop was decreased by the feeding of the larvae. In another experiment, Lamberti and Resh (1983) found higher levels of algae when trichopteran larvae (*Helicopsyche borealis*) were excluded. Lamberti and Moore (1984) concluded that "grazer assemblages, which included chironomid larvae, could significantly reduce the biomass of lentic epiphytic algae."

In lakes, larval insect grazing on algae growing on the soft sediments of the bottom has not been studied to the extent that grazers affect on other types of attached algal growth has (Cattaneo 1983; Botts, 1993). Also, although many data exist on attached algae (Winterbourn, 1990; Lamberti and Resh, 1983), this data usually deals with streams. Nevertheless, Hansson (1992) stated that periphytic algae may contribute up to 80% to primary production of the whole lake and also have high biomass, with invertebrate grazers being a potential avenue for this fixed carbon to become available to consumers and higher trophic levels. Therefore epipelon and its associated larval insect grazers impact lake productivity.

According to Pinder (1986), in terms of benthic insects which are in contact with epipelon, the Chironomidae family is usually the most abundant. Lodge unpublished data for the lakes at the University of Notre Dame Environmental Research Center where some of the sediments for this experiment were collected show Chironomini's mean density to be 745.6, Tanypodinae's to be 280.2, and Tanytarsini's mean density to be 2762.7 chironomids/m<sup>2</sup>. Also, chironomid densities in excess of 100,000/m<sup>2</sup> (Welton, et. al., 1991) have been reported in streams. With regards to grazing, Mason and Bryant (1975) found periphyton losses attributed to chironomid larvae to be 75 mg dry wt\*m<sup>-2</sup>\*day<sup>-1</sup> (Kelsner 1981), with this rate being 44% of the periphyton net accumulation rate. Also, chironomids have been shown to be specific grazers on algae selected from their surrounding mud (Armitage 1968). These exemplify how chironomids are abundant, decrease algal standing crop, and that previous assumptions of their insignificance are unfounded. I dealt with multiple taxa of Chironomidae and their effects on epipellic algae incubated on Nitex mesh placed on two sediment types in the laboratory. The two sediment types, Paul and Trout Lakes, were used due to the presumably different nutrient regimes between the organic

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Paul sediments and the sandy Trout sediments.

Trichopterans, while not understudied, are usually dealt with only in streams and/or with their effects on algae other than epipelon (Lamberti and Resh, 1983). Given these facts, my experiments dealt with *Limnephilus* sp. placed directly on sediments in the laboratory. In the trichopteran experiment, only one sediment type, Paul Lake, was used. Trichopterans are particularly interesting in that the effects of small, larval grazers such as these, could differ in their effects on algae compared to those of larger grazers such as snails and fish because of their preference for attached substrates where larva are able to find fine particulate matter for tube building (Winterbourn 1990) and small size and higher population densities.

I added larval insect potential grazers, trichopterans and chironomids, to algal incubations in the lab and measured chlorophyll a as an indicator of algal biomass changes. In the chironomid experiment, I also used two different substrate treatments, flocculent, organic Paul Lake and sandy Trout Lake sediments, to see if this would change algal chlorophyll accumulation. I hypothesized a substantially slower increase in chlorophyll a would occur in treatments containing the higher levels of chironomids and trichopterans due to larval grazing. I also believed the treatments using Paul sediments would show higher levels of chlorophyll a accumulation because of an assumption that additional nutrients are available to the algae from these organic, flocculent sediments. I believed the algae with the fewest larvae and greatest nutrient availability (Paul sediments) would show the greatest increase in chlorophyll a, with the opposite occurring with higher insect densities on the Trout sediments.

## Materials and Methods

### Chironomid Experiment

#### Incubation of Algae

1mm Nitex mesh (2.25m<sup>2</sup> total) was incubated on the sediments of West Long Lake on the University of Notre Dame Environmental Research Center (UNDERC) property. The Nitex was secured in a plastic frame on 1/2 inch PVC tubing, tied at the edges, and held down with brick weights at the corners so to keep the apparatus in contact with the sediments. This was incubated at a depth of 1.5m

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from May 25, 1993 until June 25, 1993. The growth of algae and structural integrity of the frame were visually monitored on May 31 and June 7 with as little disturbance as possible.

### Preparation of sediments and containers

On June 15, 1993, the sediment containers to be used in the lab were prepared with twenty-five round plastic containers (10cm diameter x 5cm deep) being filled approximately 4.0cm high (leaving 1cm standing water) with sandy, coarse sediments from Trout Lake at a depth of 1.5m at the University of Wisconsin Trout Lake Field Station. Sediments were also taken from Paul Lake at UNDERC, but due to the organic, flocculent nature of these sediments a PVC corer was used by SCUBA divers to fill each container, with care given to try and preserve sediment integrity. These containers provided the 40 experimental containers with two different sediment types needed for the experiment.

The 10 extra containers were used to measure the interstitial water's nutrient concentrations. After centrifuging the sediments for 10 minutes at high speed (4,500-5,000 rev./min) in order to isolate the interstitial water from the sediments, this water was sampled using a needle and syringe. The initial nutrient concentrations were found per Wetzel and Likens (1991) and averaged for each sediment type (See Table 1). At the end of the experiment, one block was analyzed to find the final values with the same procedure being followed.

The other 40 sediment containers were then microwaved until their internal temperatures reached 50-65C for 10 minutes. This procedure allowed sediment integrity and composition to remain controlled while potentially eliminating any larval insects (or other potential grazers) which could have affected grazing measurements. Finally, sediments were arranged in the lab in a randomized block pattern as shown in Figure 1.

Each container was aerated with aquarium tubing. The flow of air was adjusted with air flowing at approximately 1 bubble per second in all containers throughout the experiment. Finally, "no-see-um" mesh was placed around the tops of the containers, with rubber bands, to capture any emerging insects. On June 25, the Nitex mesh was removed from the floor of West Long lake by SCUBA divers with care taken to preserve the algal mat that had developed over the month incubation period. The Nitex and associated mat was cut into 40 circles (approximately 9cm diameter) and placed directly

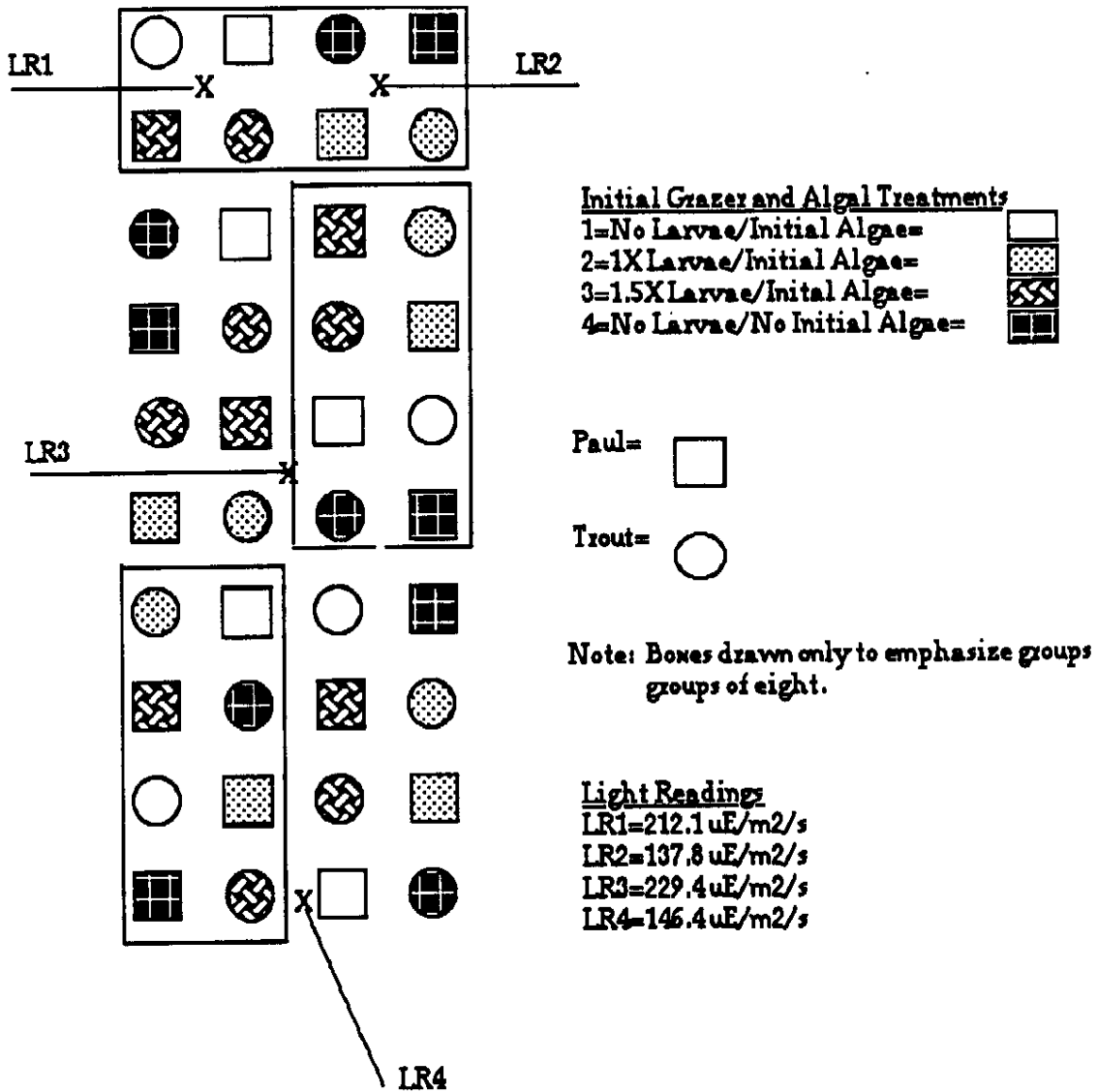
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Soluble Reactive Phosphorus (SRP) in ug/L		
	Day 179	198 Variance in Parentheses
Paul Seds	98.331	175.42 (85.039-408.118)
Trout Seds	62.839	189.12(109.552-254.552)

Table 1: Soluble Reactive Phosphorus (SRP) Concentrations

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**Figure 1: Chironomid Experiment Set-up**



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on the sediments in the 40 containers, with small rocks used as weights. The containers were filled with water and the first chlorophyll samples were taken (see Replicated measurements of algal growth section for method). The containers were placed on a lab bench under timed lights of a natural photoperiod. Light intensities are shown of Figure 1. Note: Established algal light saturation is 200-300  $\mu\text{E}/\text{m}^2/\text{s}$  (Jasper and Bothwell, 1986).

### Addition of grazers to containers

A goal of approximately 3,500 chironomid insect larvae/ $\text{m}^2$  was targeted as the ambient density for grazing chironomids (Lodge unpublished data). This density was based on Ekman grabs taken in the summer 1992 in Paul Lake (also on the UNDERC property). To collect chironomids for this experiment, Ekman grabs were taken on June 17 and 18 at 1.5m depth, sieved with 250 $\mu\text{m}$  mesh, and picked, with the same methods being used as in the previous years. Densities of 1290 individuals/ $\text{m}^2$  as the "ambient" density (1X) and 1911/ $\text{m}^2$  as the higher density (1.5X) were used simply due to chironomid scarcity evident during collection, and these densities of 10 and 15 per container respectively were added in a randomized pattern. Other treatments included a "no chironomid, with the algal mat present" treatment to show algal accumulation over time without chironomids present and a "no chironomid, initially clean Nitex" treatment to show how much algae would have grown without the initial algal mat present (See Figure 1).

### Replicated measurements of the effect of Chironomidae on epipelton

Four measurements of chlorophyll *a* were used to measure algal accumulation over time. To do this, 1 $\text{cm}^2$  squares (approximately) of mesh were cut from random points from all 40 mesh circles for each measurement on four days (Julian calendar: 179, 183, 189, 198). The chlorophyll was then extracted from the samples with 25 ml of methanol (Optima) after the squares had been frozen for at least 24 hours. Chlorophyll was measured with a fluorometer and micrograms of chlorophyll *a* calculated using standard curve 93A in the manner specified in the Cascading Trophic Interactions Manual (Marker, et. al., 1980; Strickland and Parsons, 1968; Holm-Hansen, 1978). The micrograms of chlorophyll were then divided by square areas from which they were extracted. Areas were calculated by digitizing the square size.

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### Analysis

Statistical analyses performed by SYSTAT statistics program. ANOVA repeated measures analyses performed for all data.

### Trichoptera Experiment

Ten plastic containers (20cm x 10cm x 10cm) were filled with sediments from 1.5m depth in West Long lake, with sediments 6cm deep and overlying water approximately 3cm deep. Five containers had no trichopterans added and 8 trichopterans were added to the other 5 for densities of 0 and 200/m<sup>2</sup> (See Figure 2 for pattern). Containers were arranged randomly under the lights in the lab, and sampled on July 8 (before larvae added) and on July 19 by coring the sediments to a depth of 1cm (to get epipelagic algae with a surface area of 5.47cm<sup>2</sup>) and extracting this sample with 24.6ml of methanol. Again, a fluorometer was used to measure the chlorophyll a of the samples. The trichopterans used for this experiment were collected from the bottom West Long Lake and were identified as *Limnephilus sp.*

### Analysis

Statistical analyses performed by SYSTAT statistics program. ANOVA repeated measures analyses performed for all data.

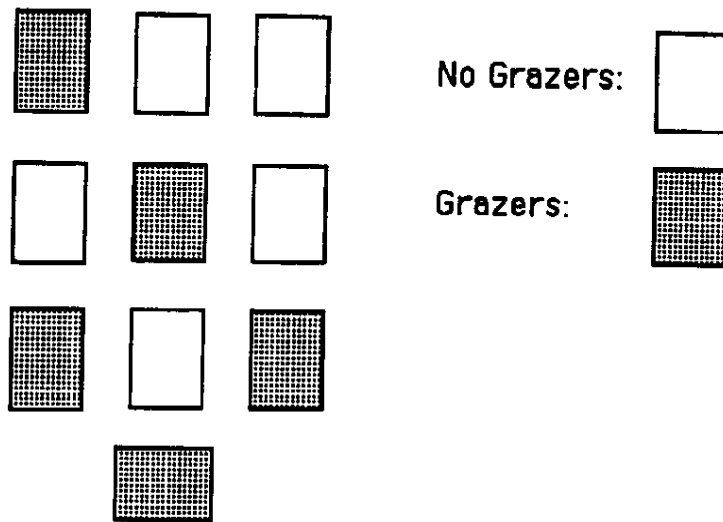
## Results

### Chironomidae results

Statistically significant results ( $p < .05$ ) illustrate the importance of sediment type (See Table 2A). The only  $p < .05$  are the sediment, time, and sedimentXtime statistical variables. This showed some feature(s) differing between the two sediment types was/were significant in influencing algal growth. This effect also was significant over time, with the greatest and quickest chlorophyll a accumulation occurring in Paul sediments. Neither larval treatments, 1X or 1.5X, were significant at influencing algal accumulation over time. Looking at mean values of chlorophyll a averaged for each treatment over time in Figures 3a and 3b, no grazing effect on either the Paul or Trout sediments is

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**Figure 2:** Trichoptera experimental set-up



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<b>Chironomid Statistics</b>				
Variable	Deg. Freedom	Mean-Square	F-Ratio	P
Sediment	1	317.258	100.709	0
Larvae	2	4.815	10528	0.237
Sediment X Larvae	2	0.344	0.109	0.897
Time	3	178.084	44.212	0
Time X Sediment	3	46.623	11.575	0
Time X Larvae	6	1.254	0.311	0.857
TimeXSedimentXLarvae	6	0.724	0.18	0.94

Table 2A: Chironomid Statistical Analyses

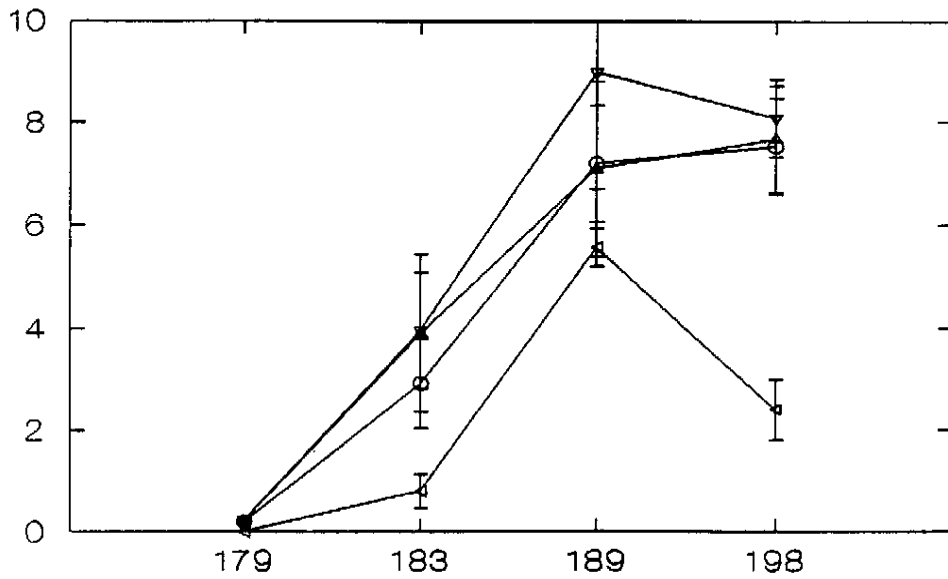
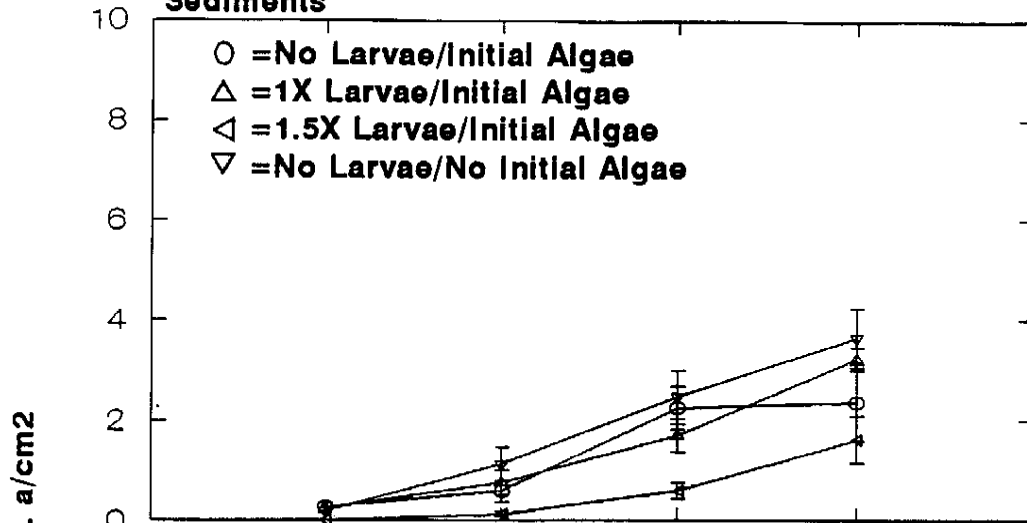
<b>Trichoptera Statistics</b>				
Variable	Deg. Freedom	Mean-Square	F-Ratio	P
Larvae (each day)	1	0.219	0.875	0.377
Larvae (across time)	1	1613.696	2.341	0.165

Table 2B: Trichoptera Statistical Analyses

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**CHLOROPHYLL *a* ACCUMULATION**

**Figure 3a: Trout Sediments**



**Figure 3b: Paul Sediments**

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apparent, and this was confirmed statistically. However, while not significant, the mean values of chlorophyll are ordered (from highest to lowest) by treatment in the same way in both graphs. Notice the No Larvae/No Initial Algae treatment had the greatest accumulation in both graphs, with the 1X, No Larvae/Initial Algae, and 1.5X treatments always ordered second, third, and fourth respectively at the final measurement. Note the small effect the initial algal mat had on starting levels of chlorophyll. The No Initial Algae treatments did not effect starting levels of chlorophyll, but seemed to accumulate chlorophyll in greater quantities.

Between the sediment types, it is easy to see the almost two-fold increase in ug CHL a/cm<sup>2</sup> which occurred in the Paul treatments compared to the Trout treatments by day 198 which was statistically significant. Table 1 shows a change in Soluble Reactive Phosphorus (SRP) concentrations occurred over the course of the experiment. For example, the Paul SRP went up by 2X, while the Trout SRP increased 3X, yet there was substantial variability in the measurements.

Table 3 shows the emergence of the larval insects into adults which were captured in the "no-see-um" mesh covering the containers. Treatments 1-4 correspond to the treatments shown on Figure 1. Interesting to note are the larvae which emerged from Trout treatments 1 and 4 which were microwaved and had no larvae added to them and the 20% emergence (12/60 total insects) which occurred in the 1.5X Trout treatments.

### Trichopteran results

The *Limnephilus sp.* did not significantly (all P's > .05) change algal chlorophyll a over time (See Table 2B). However, looking at Figure 4, showing mean chlorophyll a values for each treatment over time, there appears to be a positive relationship between the addition of sediment grazers and increase in epipellic chlorophyll a. For example, the No Trichopteran treatments' chlorophyll a increased by 31.04% with the Trichopteran treatments' increasing by only 7.86%. Also, profound differences in sediment morphology were noted between the two sediment types. In the treatments with trichopterans added to them, the sediments appeared gray and lumpy, with no apparent algae on the surface. However, in treatments with no trichopterans, a green algal mat grew.

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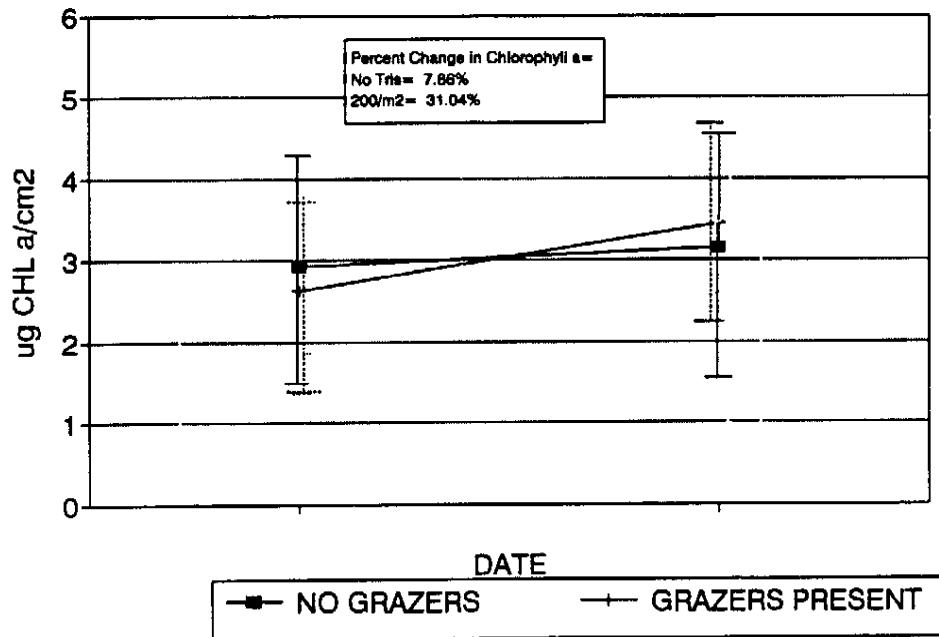
<b>Chironomid Emergence</b>						
<b>Paul Sediments</b>						
	<b>Day 179</b>	<b>183</b>	<b>189</b>	<b>198</b>	<b>Total Emerged</b>	<b>% of Total #</b>
<b>Treatment 1</b>	0	0	0	0	0	0
<b>2</b>	0	0	2	3	5	13
<b>3</b>	0	0	5	0	5	8.3
<b>4</b>	0	0	0	0	0	0
<b>Trout Sediments</b>						
	<b>Day 179</b>	<b>183</b>	<b>189</b>	<b>198</b>	<b>Total Emerged</b>	<b>% of Total #</b>
<b>Treatment 1</b>	0	0	1	0	1	N/A
<b>2</b>	0	1	0	0	1	2.5
<b>3</b>	0	1	6	5	12	20
<b>4</b>	0	0	1	0	1	N/A
<b>Note: See Figure 1 for treatment definitions.</b>						

**Table 3: Emergence of chironomid larvae during experiment**

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Figure 4:

**CHLOROPHYLL  $a$  ACCUMULATION  
TRICHOPTERANS**



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### Discussion

#### Chironomidae

My statistically significant results ( $p < .05$ ; Table 2A) show the importance of sediment type in determining epipellic algal chlorophyll a accumulation. By looking at Figures 3A and 3B and the chlorophyll a/m<sup>2</sup> raw data in Appendix A, the greater accumulation associated with the Paul Lake sediments is easily visible. Two differences in the sediments which could account for the accumulatory differences between the two sediment treatments during the laboratory experiment were nutrient concentration and sediment morphology.

Table 1 shows how SRP increased over time in both sediment types and the differences between the Paul and Trout values on either day. However, these measurements had great variability and were not replicated. Possible explanations for the increase include nutrient release by decaying larvae or algae and/or water evaporation (thereby concentrating nutrients). Since the differences between the sediments' nutrient concentrations were small, I believe the physical make-up of the sediments may have led to differences in nutrient distribution and, therefore, algal accumulation. The flocculent and muddy Paul sediments had more water associated with them than did the sandy, hard-packed Trout sediments. Therefore, not only would the Paul sediments provide greater surface area for the algae, but would also allow for easier water movement, enabling nutrients at the bottom of the container to reach the top. This effect could account for differences in chlorophyll a accumulation in Paul treatments, even though the water nutrient concentrations were not much higher at the end.

While only the sediment (and time) treatments were statistically significant, other areas of the experiment deserve interpretation. The identical vertical ordering of the mean chlorophyll values by treatment in both Figure 3A and Figure 3B is one of these areas. The No Larvae/No Initial Algae accumulated the most chlorophyll in both graphs. This is surprising since the other three treatments had an initial algal mat present. This leads to the assumption that the initial algal mat exerted a negative effect on algal chlorophyll a accumulation. This could be accounted for either by grazers which entered the experiment via the unsterilized mats which exerted grazing pressure or by algal species composition differences between the Initial Algae treatments and the No Initial Algae treatment which would change chlorophyll a accumulation rates.

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However, since an insect emerged from Trout treatment 4 (No Larvae/No Initial Algae) while none emerged from either Paul sediment No Larvae treatments this points to algal species differences between the colonies with an Initial mat and those without. This is because, if larvae were consistently entering the experiment via the unsterilized mat, more emergence in the No Larvae treatments would have occurred, while no emergence would have occurred from Trout treatment 4. Species composition differences between the two could verify this hypothesis.

Also deserving comment is the emergence of larval insects which occurred. Table 3 shows the variation of emergence between treatments. In Trout treatment 3 (1.5X larvae), 20 percent of the insects added eventually emerged and this could account for the lack of a grazing effect. Also, Trout treatments 1 and 4 (No Larvae treatments) had insects emerge. Since no larvae were added to these treatments, and because all sediments were microwaved as a sterilization technique, microwaving might not kill all larvae (and possibly other grazers) in the hard-packed Trout sediments. Along with emergence, larval mortality could also account for a lack of grazing effect (and nutrient release) and should be quantified in future studies.

Given the results of Winterboun (1990) and Pinder (1986), a chironomid grazing effect was expected. However, some explanations are possible for the lack of this effect being measured. One of these explanations deals with the larvae themselves. With established densities approaching 3,000 individuals/m<sup>2</sup> (Lodge unpublished) lakes and 100,000/m<sup>2</sup> (Welton, et. al., 1991) in streams, this experiment used only 1290 and 1911 /m<sup>2</sup> for the 1X and 1.5X treatments respectively. Therefore, a lower grazing effect would not be surprising. Secondly, I assumed that all larvae I used were grazers on epipellic algae. Evidence of tube building in the containers did lead me to believe that some of the chironomids I used were using the algae in one way or another. Finally, I am assuming that the larvae I used stayed alive throughout the experimental run. While some mortality probably occurred, substantial emergence also occurred (See Table 3), immediately dropping larvae densities because of the small container size.

Measurement techniques could also account for the lack of a measured grazing effect. Chlorophyll a analysis, while providing an indicator of biomass accumulation when species composition remains constant, fails when species composition of algae changes between measurements or treatments due to differences in chlorophyll fixing rates (Berg, personal communication) between algal species,

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leading to inaccuracies in measurements of grazing. Also, the method I used for sampling the chironomid treatments (cutting squares) could possibly have disturbed the replicates unnecessarily. Pre-cut squares are an alternative.

Nevertheless, conclusions can be drawn from this experiment. First, sediment differences, whether in nutrient concentration or availability, significantly effect chlorophyll a accumulation in epipelagic algae. Second, the presence of an initial algal mat tends to exert a negative effect (compared to control) on algal chlorophyll a accumulation. Finally, emergence of larval insects from experimental treatments can effect grazing measurements.

### Trichoptera

The chlorophyll a measurements did not yield statistically significant (See Table 2B) results indicating larval grazing. However, the percent change differences (No Trichopterans=7.86%; Trichopterans=31.04%) between the two treatments indicate a positive relationship between trichopteran addition and algal chlorophyll accumulation. Also, an effect was very noticeable in the grazer treatments simply from sediment morphology. In the grazer treatments, the sediments turned from dark brown to almost gray in color, with substantial granulation of sediments occurring in the grazer treatment. This makes me believe that the *Limnephilus sp.* either were grazing on something not containing chlorophyll, or more likely that species composition of algae changed substantially in the grazer treatments. Therefore, *Limnephilus sp.* tend to exert a positive influence on algal chlorophyll a accumulation, yet decrease the visible algal mat present on untreated sediments.

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### Acknowledgements

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### Appendix A

Replicate	Grazers	Nutrient	Algae(i)	179	183	189	198
1	0	high	N	0	0.468548	6.713013	2.612575
2	0	high	N	0	0.785831	5.856662	0.609273
3	0	high	N	0	0.637933	5.679474	1.526509
4	0	high	N	0	0.062316	4.644557	3.383638
5	0	high	N	0	2.031366	4.928965	3.877551
TREATMENT		AVERAGE		0	0.797199	5.564534	2.401909
1	0	low	N	0	0.08478	0.461061	3.455376
2	0	low	N	0	0.016458	0.383795	0.81121
3	0	low	N	0	0.311684	1.278896	1.226562
4	0	low	N	0	0.095551	0.486766	1.533316
5	0	low	N	0	0.189672	0.409455	1.146423
TREATMENT		AVERAGE		0	0.139629	0.603995	1.634577
1	0	high	Y	0.201728	1.619811	4.867192	6.976869
2	0	high	Y	0.040921	3.423836	5.24855	5.554566
3	0	high	Y	0.16328	3.44745	6.12212	9.398401
4	0	high	Y	0.158901	0.482805	10.59276	10.07904
5	0	high	Y	0.402119	5.591855	9.1627	5.630396
TREATMENT		AVERAGE		0.19339	2.913151	7.198665	7.527855
1	0	low	Y	0.054179	0.55264	3.477495	0.386213
2	0	low	Y	0.14979	0.718867	1.928766	1.321359
3	0	low	Y	0.310003	1.370577	3.056445	1.849481
4	0	low	Y	0.385826	0.041528	1.626862	3.93283
5	0	low	Y	0.475698	0.31059	1.220002	4.359179
TREATMENT		AVERAGE		0.275099	0.59884	2.261914	2.369812
1	1	high	Y	0.111496	0.648116	6.013574	8.483504
2	1	high	Y	0.120427	3.125916	5.507419	4.877773
3	1	high	Y	0.237254	7.368333	9.67384	9.699662
4	1	high	Y	0.281875	0.706139	12.03545	5.557705
5	1	high	Y	0.331248	7.600613	2.237134	9.771379
TREATMENT		AVERAGE		0.21646	3.889823	7.093484	7.678004
1	1	low	Y	0.119474	1.12389	1.180871	3.510384
2	1	low	Y	0.209531	0.816767	2.409672	3.549917
3	1	low	Y	0.307787	1.446343	1.396932	2.737945
4	1	low	Y	0.643596	0.08481	0.981596	2.651058
5	1	low	Y	0.041473	0.400046	2.578734	3.712254
TREATMENT		AVERAGE		0.264372	0.774371	1.709561	3.232312
1	1.5	high	Y	0.115014	2.932397	2.569448	8.460321
2	1.5	high	Y	0.197467	5.921611	13.38321	6.496293
3	1.5	high	Y	0.259987	2.928823	4.83773	9.867755
4	1.5	high	Y	0.278846	0.815323	14.22657	6.135787
5	1.5	high	Y	0.345744	7.134755	9.954645	9.441786
TREATMENT		AVERAGE		0.239412	3.946582	8.994319	8.080388
1	1.5	low	Y	0.084131	1.180808	1.161578	1.677447
2	1.5	low	Y	0.219993	0.756297	1.432354	4.792706
3	1.5	low	Y	0.367172	2.203975	2.42497	3.226946
4	1.5	low	Y	0.221988	0.126643	3.541019	4.886678
5	1.5	low	Y	0.110619	1.447329	3.803199	3.642628
TREATMENT		AVERAGE		0.20078	1.143011	2.472624	3.645281
Means for All Treatments							
paul				179	183	189	198
				0.162315	2.886689	7.212751	6.422039
Trout							
				0.185063	0.663963	1.762023	2.720496

**POTENTIAL GRAZERS AND EPIPELIC ALGAE**

**Appendix A (Contd.)**

**Chlorophyll a Data--Trichopteran Experiment**

Date	TRICHOPS	Fb	Fa	Gain	ugChgl	ugChl/cm2	
189	0	376	110	5	16.4388	3.005265	
189	0	277	95	5	11.2476	2.056234	
189	0	382	106	5	17.0568	3.118245	SD=
189	0	421	114	5	18.9726	3.468483	1.324634
189	0	370	107	5	16.2534	2.971371	AV (200)=
AVERAGE						2.92392	3.153854
189	1	401	111	5	17.922	3.276417	
189	1	338	104	5	14.4612	2.643729	
189	1	296	106	5	11.742	2.146618	SD=
189	1	358	103	5	15.759	2.880987	1.263968
189	1	297	103	5	11.98920	2.191810	AV (200)=
AVERAGE						2.627912	3.443627
200	0	701	203	10	15.50772	2.835049	
200	0	674	204	10	14.6358	2.675649	
200	0	503	117	5	23.8548	4.361024	
200	0	380	105	5	16.995	3.106947	
200	0	360	113	5	15.2646	2.790603	SD=
AVERAGE						3.153854	1.397229
200	1	409	112	5	18.3546	3.355503	
200	1	380	106	5	16.9332	3.095649	
200	1	426	110	5	19.5288	3.570165	
200	1	433	114	5	19.7142	3.604059	
200	1	426	108	5	19.6524	3.592761	SD=
AVERAGE					0	3.443627	1.321383

% Change (No Tri)=7.863928

% Change (200/m2)=31.04041

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