FEEDING RELATIONSHIPS OF HARPACTICOID COPEPODS AND MICROBIAL FLORA IN A SALT MARSH

Alan W. Decho

INTRODUCTION

Benthic harpacticoid copepods represent a predonderate link in the estuarine food web. In the upper food web they serve as food for many invertebrates and fish fry. In the lower portion of the web, it has been suggested that they feed to a large extent on the microbial flora and are therefore important in the recycling of benthic energy. While their relationships with microbes have been the subject of much literature, information regarding the actual food resources of harpacticoids is limited. Most of these studies are strictly laboratory investigations, using food material which is either easily culturable or easily accessible but not necessarily representative of the animals' natural habitat.

In small drainage channels of Great Sippewisset Marsh there occurs definite zonation of photosynthetic microbial flora. The relationships of certain harpacticoid copepod species with these photosynthetic microbes (and their associated heterothrophic bacteria) are examined.

METHODS

Characterization of Organisms

The study site was in proximity to perennial microbial mats (Howarth and Marino, 1983). Across the surface sediment (0.05 cm depth) there is a distinct zonation of photosynthetic bacteria and diatoms, distinctly visible by the color patterns which they generate at high densities. The study site was divided into three areas, based on these zonations: 1) a "Diatom Area", located at the center of the channel, was composed of a dense mat of naviculoid diatoms; 2) a "Purple Area", located on either side of the diatom area, is composed of a photosynthetic purple-sulfur bacteria; 3) a "Clear Zone" dominated by neither the diatoms nor the purple-sulfur bacteria and located at the periphery of the channel.

Sampling was conducted during early July 1983 during low-water level. Two quadrats (20 x 15 cm) were placed in each area. Eight replicate cores (6 mm dia.) were randomly taken from each quadrat to a depth of 0.5 cm. Preliminary sampling indicated almost no animals below this depth. Cores were fixed on site in 10% formalin in seawater. Copepods were identified to species, densities recorded, then analyzed by ANOVA using BMDP computer programs.

For pigment analysis, frozen samples were thawed and the sediments filtered on Whatman GF/C glass fiber filters to remove excess water. Pigments were then extracted with 1 ml methanol (w $MgCo_3$) under darkness for 1 h and analyzed using a Gilford System 2600 Spectrophotometer from 660-800 nm. Within the diatom area, the photosynthetic organisms were composed almost exclusively of the naviculoid diatoms with lesser amounts of the cyanobacterium Oscillatoria sp. and underlain by purple sulfur bacteria. In the purple area the dominant photosynthetic organism was the purple-sulfur bacteria tentatively identified as Thiocapsa. Therefore pigment peaks of

chlorophyll-a and bacterialchlorophyll-a could be correlated with the presence of diatoms and Thiocapsa, respectively, and were used as semi-quantitative measure of their relative abundances in each area.

To enumerate the diatoms, purple-sulfur bacteria and the heterotrophic bacteria in each area, densities were estimated by the direct count method using acridine orange staining (Hobbie et al., 1977). The purple-sulfur bacteria could be distinguished from spherical heterotrophs by the presence of sulfur granules. Three replicate samples were taken from each area, fixed in 4% Formlin in Sw, sonicated then divided randomly into five subsamples. Since sonication destroys the diatoms, one fraction of each sample was not sonicated and separately used to estimate diatom densities. Standardization of densities according to the dry weight of sediment was used and densities were expressed as "no. bacterial/g sediment". Enumeration of samples was performed with a Zeiss WL Phase microscope fitted with a 4 FL Flourescence system, and a 51 Mercury AC burner, a 09 Edge filter, a 100/1.30 oil objective and M363 camera system.

LABEL EXPERIMENTS

To examine if 1) copepods are ingesting and incorporating the microbial flora, and 2) which types of microbes are being utilized, three types of labelling experiments were performed. Two copepod species were used: Pseudomesochra c.f. divaricata and Stenocaris pristina.

I. Photosynthetic Labelling Experiments:

Sediment slurries of the purple-sulfur bacteria Thiocapsa and the naviculoid diatoms, and their associated heterotropic flora, were colleted by pipette from freshly collected salt marsh sand near the study site. Slurries were concentrated using decantation then transferred to small labelling vials. Any copepods present in the slurries were removed.

To one type-vial (Diatom-treatment) was added $NaH^{14}CO_3$ (New England Nuclear, spec. act; 10µg/µCi, final concentration of 0.5µCi; ml^{-1}). The suspension was incubated under light conditions (approx. 22°C) on a shaker (100 rpm) for 4 h to label the diatom flora from the sediment. The slurry was then washed three times using filter sterilized seawater on Whatman GF/F glass filters, resuspended in small petri dishes in 10 sterile SW and 0.1 ml subsamples removed for incorporated label, pigment concentration, and diatom densities. To each dish were then added 25 prestarved (1 h) gravid female copepods (using gravid females facilitated separation of species) of a similar size and of a given species. The copepods were allowed to feed on the labelled-slurry for 5 h. At the conclusion of the 4 h labelled-feeding the copepods were removed and washed in sterile SW. Label levels of the remaining slurry were measured. Ten copepods were immediately fixed in 6% filtered formalin in SW and the remaining animals were transferred to a "cold" unlabelled diatom slurry for 6 h. At the conclusion of this "cold feeding" the animals were cleaned, fixed in 6% formalin, bissected and placed in scintillation vials. The cold feeding allows labelled material which is ingested but is not utilized by the copepod as food material to pass through the gut. The animals were transferred to scintillation vials using 0.1 ml SW and were solubilized in Protosol (50°C) for 12 h then suspended in Packard Insta-Gel cocktail and counted on a Beckman 330 Scintillation Counter. Each vial contained 10 animals. All experiments were run in triplicate.

To label the purple sulfur bacteria, Thiocapsa, the same procedure as above was followed, however, D.C.M.U. (5×10^{-6}m final conc.) was added to inhibit oxygenic metabolism of any diatom or blue green contaminants.

II. Heterotroph Labelling Experiments:

To determine if the copepods were utilizing the heterotrophic bacteria associated with the diatoms or purple sulfur bacteria, approximately 4.0 nM ^{3}H-thymidine (final conc; specific act. of 6Ci/mmol) were used to label the heterotrophic portion of the slurry. At these low concentrations thymidine is not readily taken up actively by photosynthetic bacteria or diatoms. Therefore uptake of thymidine by the photosynthetic organisms should be negligible.

III. Control Experiments:

Experiments were designed to control for uptake of label via 1) passive diffusion, and active transport across the body surfaces of the copepods, and 2) epizoic bacteria attached to the copepod cuticle. Prestarved copepods were added filter-sterilized SW containing $NaH^{14}CO_3$ or ^{3}H-thymidine (same conc. as above) and incubated for 4 h. At the conclusion the animals were washed, then ten animals were fixed and ten animals transferred to sterile SW for 6 h .

Chase Experiments:

To distinquish 1) between the labelled material which is taken up but not utilized and material which is utilized by the copepod, and 2) the time needed to clear the gut of ingested food material, the following feeding-chase experiments were run:

100 copepods of a given species were added to a dish containing labelled bacteria. After a 2 h incubation, the animals were removed and washed in sterile SW. Ten animals were immediately fixed in 6% formalin, bissected and placed in scintillation vials (using 0.1 ml SW). The remaining animals were placed in a dish containing "cold" non-labelled bacteria from the same slurry and allowed to feed for varying amounts of time (0-9 h) in order to void their guts of non-incorporated labelled material. At the conclusion of a given time interval, 10 animals were fixed then placed in a scintillation vial. Before being placed in the vial each animal was bissected in order to facilitate solubilization of the tissues. Each experiment was run in triplicate. This experiment determined the incubation times of cold feeding for other labelling experiments.

RESULTS

DISTRIBUTIONAL DATA:

I. Harpacticoid Copepods:

A total of seven harpacticoid copepod species were found in the three areas sampled; Stebicarus pristina., Harpacticus nipponicus, Pseudomesochra c.f. divaricata, H. glaber, Scutellidium sp., Harpacticus sp., Diosacus sp. three of these species were present in high enough occurances to permit analysis of densities by ANOVA (Figure 1). The results of ANOVA on log (x+1) transformed density data are summarized in Table I. Significant differences in mean densities were noted for species and in the interaction of species and area ($p<0.001$). Using Bonferroni pairwise comparison tests with pre-adjusted a-levels to dissect the interaction term, the mean densities of Stenocaris were highest ($p<0.001$) in the diatom area when compared to the purple and clear area (Table II).

Harpacticus nipponicus showed highest densities ($p<0.05$) in the purple area. Both Pseudomesochra and H. glaber showed high densities in the clear area (these densities however did not differ from the purple area $p<0.05$) but did differ from the diatom area ($p<0.001$).

II. Pigment Analysis:

Analysis of photosynthetic pigments by absolute methanol extraction showed a large peak (Figure 2) at 665 nm indicating a high concentration of chlorophyll-a ($695 \pm 78 = \bar{x} \pm SD$; µg; cm^{-3} sediment) in the diatom area. Microscopical examination of these sediments showed this was primarily a result of the naviculoid diatom (Amphora sp.) and to a small extent the cyanobacterium Oscillatoria sp. A smaller peak at 770 nm (bacterial-chlorophyll-a) is caused by the purple sulfur bacteria Thiocapsa (127 ± 57 µg) which underlies the diatom mat. In the purple area there is a high concentration of Thiocapsa (525 ± 98 µg) and a smaller concentration of chl-a (206 ± 71 µg), a result of diatom contamination from a nearby mat. The clear area contains a substantial amount of both chl-a (341 ± 126 µg) and Bchl-a (234 ± 116 µg). The former however due to several cyanobacteria (Spirulina, Microcoleous, Lyngbya) and the latter once again a result of Thiocapsa.

III. Microbial Densities:

Heterotrophic bacterial densities ranged from 2.27×10^7 to 8.67×10^7 per g dry sediment. Heterotrophic counts were highest in the purple area. Within each sample the distribution of recovered microbes was homogeneous except for the diatom counts in the clear area. The results of epifluorescence enumeration are shown in Table IV.

IV. Labelling Experiments:

Preliminary feeding chase experiments to determine the gut-retention time of food material in the harpacticoids are summarized in Figure 4. Removing copepods from cold feeding at one hour intervals indicates that after 6 h post-feeding on labelled Spirulina, Pseudomesochra had lost $92 \pm 3.5\%$ of its originally present label. The 6 h post-feeding represented the time at which the mean no longer decreased sharply with time. Also the standard error of the mean was greatly reduced between replicates. Using Thiocapsa as another labelled food-source, retention was $75 \pm 9\%$ after 6 h and the slope of decrease was very low by this time. Therefore in subsequent experiment, 6 h was used as the amount of time necessary to clear the copepod gut of unused labelled food material.

Feeding Experiments:

Results of feeding experiments using Stenocaris pristina and Pseudomesochra divaricata vary depending on the type of photosynthetic microbe and/or heterotrophic bacteria used. Results for Stenocaris are summarized in Table III. Stenocaris consumed both labelled diatoms and heterotrophs associated with the diatoms, but only retained a significant portion of the heterotrophic bacteria after 6 h cold feeding. Most (66%) of the labelled diatoms were passed through the gut. Diatom densities of food material, estimated by direct counts of sub sample (0.1 ml of slurry) were 2.31×10^6 ml^{-1} while heterotrophs associated with the diatoms were 7.56×10^7 ml^{-1}. The copepods consumed over half of the total ^3H label used to label the heterotrophs but only about 2% of the total diatom bicarbonate label.

Pseudomesochra consumed Thiocapsa, the heterotrophs associated with the Thiocapsa, and the cyanobacterium Spirulina sp. However, this copepod only retained a large portion of the Thiocapsa label after 6 h cold feeding. Less than half of the total ^{3}H label (used to label the heterotrophs) and about 10% of the bicarbonate label (Thiocapsa & Spirulina) was taken up. Densities of microbes used in feeding experiments were 6.04×10^{7} ml^{-1} for heterotrophs.

DISCUSSION

The significantly high densities of certain harpacticoid species in areas of high densities of certain microbes provides an ideal opportunity to examine possible causal relationships for these copepod distributions. Stenocaris pristina exhibited extremely high densities for meiofauna in the diatom area (1032 ± 126 cm^{-3}). Pseudomesochra divaricata occurred in high densities in the purple and clear areas where there was an abundance of purple sulfur bacteria. The correlation of these species distributions with certain microbial flora allow the possibility to examine the nature of the food resources of these copepods.

Some studies in recent years regarding harpacticoid feeding have indicated preferential uptake of carbon foods. Using an unidentified alga and freeze-killed bacteria, Brown and Sibert (1977) showed that certain harpacticoids ingested heterotrophically derived carbon nine times faster than autotrophically derived carbon. Lee et al., (1977) using a food patch choice experiment involving 8 species of algae indicated that the harpacticoids Nitocra typica and Leptocaris brevicornis showed differential food preferences. Vanden Berghe and Bergmans (1981) showed a similar preferential uptake, with more quantitation. These studies suggest that some harpacticoids may preferentially ingest specific types of carbon sources in nature. These specificities most likely represent specific nutritional requirements (Coull, 1973; Lee et al., 1975). Most of these studies however equate ingestion of labelled food material with assimilation and utilization of the same resource. For a specialist feeder this is a sound assumption to make. However, not all meiofauna are specialist feeders, in the selective uptake sense. This means that some meiofauna may be 1) generalists--which uptake and utilize a variety of food sources, or 2) uptake a variety of food types but only utilize one of these as a nutritional source. This was first suggested to occur in some oligochaetes (Chua & Brinkhurst, 1975). Our data suggest that these harpacticoids take up a variety of microbes, then vary selectively retain and utilize a single food resource. Stenocaris was semi-selective in its ingestion of labelled microbes. It did not ingest Ocillatoria, a cyanobacterium which occurs in its natural habitat but did ingest naviculoid diatoms and the heterotrophs associated with these diatoms, both of which predominate in its natural habitat. It retained, however, on the heterotrophic portion after 6 h (the time necessary to empty the gut). This suggests that while both the diatoms and heterotrophs are ingested, only the heterotrophs--or portion of them--are digested and utilized by the Stenocaris as a food resource. The unused material is passed out in feces relatively intact. Indeed microscopical examination of Stenocaris and its feces by epifluoresence confirm the presence of diatoms in the gut and also in the fecal pellets. P. divaricata occurs in high densities in area which contain an abundance of purple sulfur bacteria. Using feeding experiments it was shown that this harpacticoid ingested the Thiocapsa--a purple bacteria, the heterotrophs associated with Thiocapsa, and Spirulina--a cyanobacterium, but only retained the purple sulfur bacteria as a food source. Examination of fecal pellets show a few

intact sulfur bacteria and many isolated sulfur granules (intact Thiocapsa contain sulfur granules when sufficient sulfide is present in the environment) indicating the rest of the cell has been removed in some way.

Table I. Summary table for ANOVA using log (x-1)n transformed densities of harpacticoid species from Great Sippewissett Marsh.
(significance: *=0.05, **=0.01, ***=0.001)

Source	df	SS	MS	F
Species	3	4.6805	1.5602	24.28***
Area	2	0.1490	0.0745	1.16
S x A Interaction	6	16.6499	2.7750	43.19***
Error	168	10.7932	0.0642	
Total	179			

Table II. Densities of harpacticoid species in three study area of Great Sippewissett Marsh (no. animals x ± SE, cm^{-3} sediment).

SPECIES	AREA		
	Diatom	Purple	Clear
Stenocaris pristina	1032±125	18.8± 8.3	89.6± 32.7
P. divaricata	127± 68.7	367.8±78	631.8±118
H. nipponicus	28± 13.4	117 ±21.4	14 ± 10.25
H. glaber	28± 15	226 ±39	363 ± 63

Table III. Summary of feeding experiments using Stenocaris pristina and various labelled food sources.*

TYPE OF MICROBE	LABEL	CPM/COPEPOD TIME 0*	CPM/COPEPOD TIME + 6 HR FEEDING	% OF TOTAL FOOD LABEL CONSUMED BY 10 COPEPODS	% LABEL CONSUMED BUT LOST AFTER 6 HR.
Stenocaris					
Diatom	$NaH^{14}CO_3$	80 ± 11.9	27.4± 4.3	2.2± 0.32	66%
Diatom-- heterotrophs	^3H-thymidine	6347.4±684	4986.9±313	51.3± 3.7	21
Oscillatoria	$NaH^{14}CO_3$	0	0	--	--
Pseudomesochra					
Thiocapsa	$NaH^{14}CO_3$	145 ± 7.2	103 ± 15	10.4± 1.2	29
Thiocapsa heterotrophs	^3H-thymidine	2534.9±291.5	508 ±159	44.9±11.4	80
Spirulina	$NaH^{14}CO_3$	1337.3±984	125.6± 24.9	--	82

Table IV. Cell counts obtained per g sediment determined by epifluorescence from samples collected at Great Sippewissett Marsh.

SITE	RANGE	$\bar{x}$±SD g dry sed.
Diatom Area		
Diatoms	1.89 - 3.71 x 10^6	2.59 ± .47 x 10^6
Purple-S Bacteria	1.77 - 2.40 x 10^8	1.99 ± .16 x 10^8
Heterotrophs	3.60 - 5.18 x 10^7	4.43 ± .50 x 10^7
Purple Area		
Diatoms	0.41 - 1.53 x 10^6	0.95 ± .34 x 10^6
Purple-S Bacteria	2.12 - 3.09 x 10^8	2.59 ± .31 x 10^8
Heterotrophs	4.44 - 8.67 x 10^7	5.77 ± .86 x 10^7
Clear Area		
Diatoms	---- 0.41 x 10^5	0.14 ± .12 x 10^6
Purple-S Bacteria	1.54 - 2.09 x 10^8	1.82 ± .16 x 10^8
Heterotrophs	2.27 - 4.30 x 10^7	3.42 ± .58 x 10^7

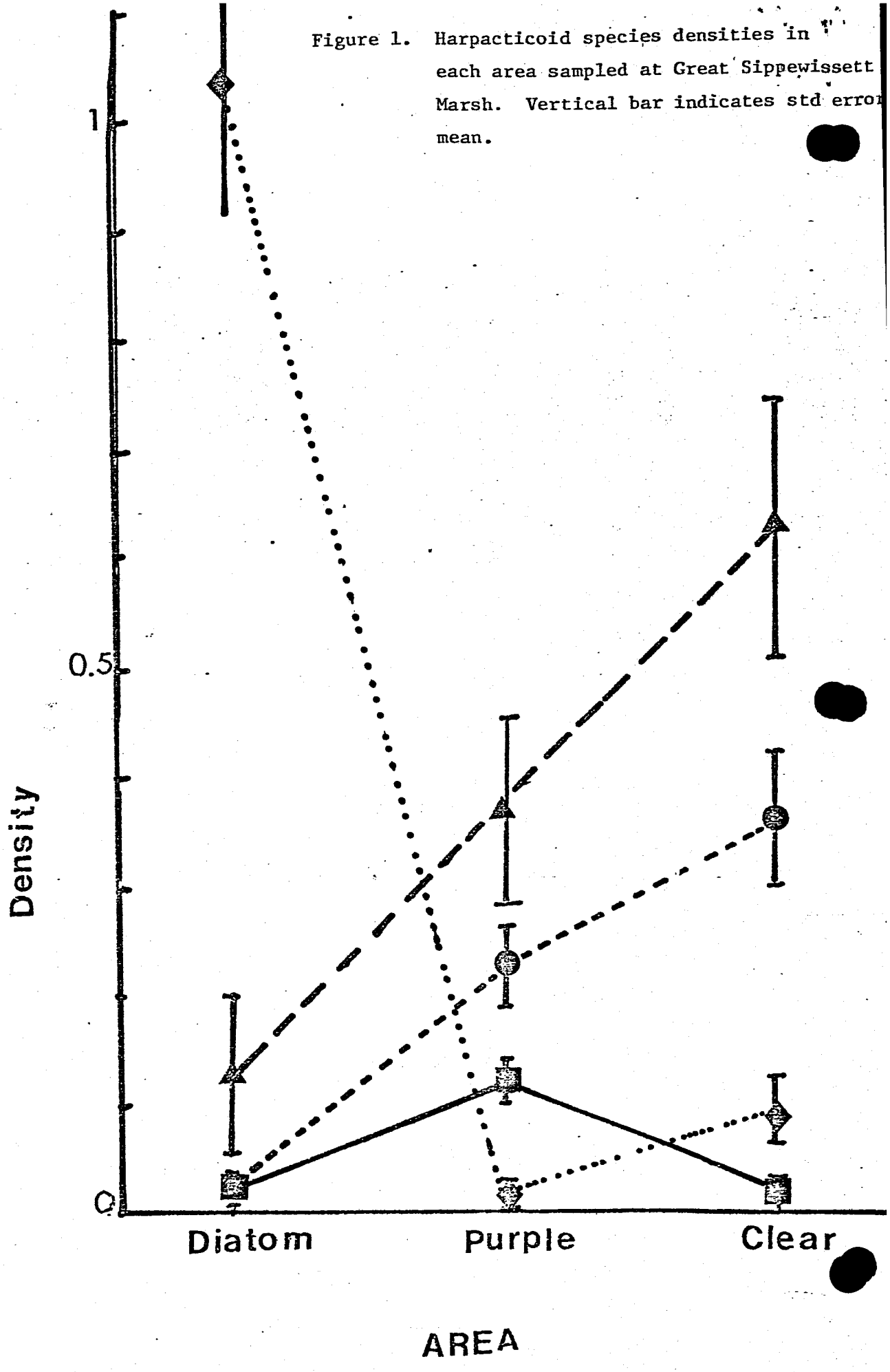
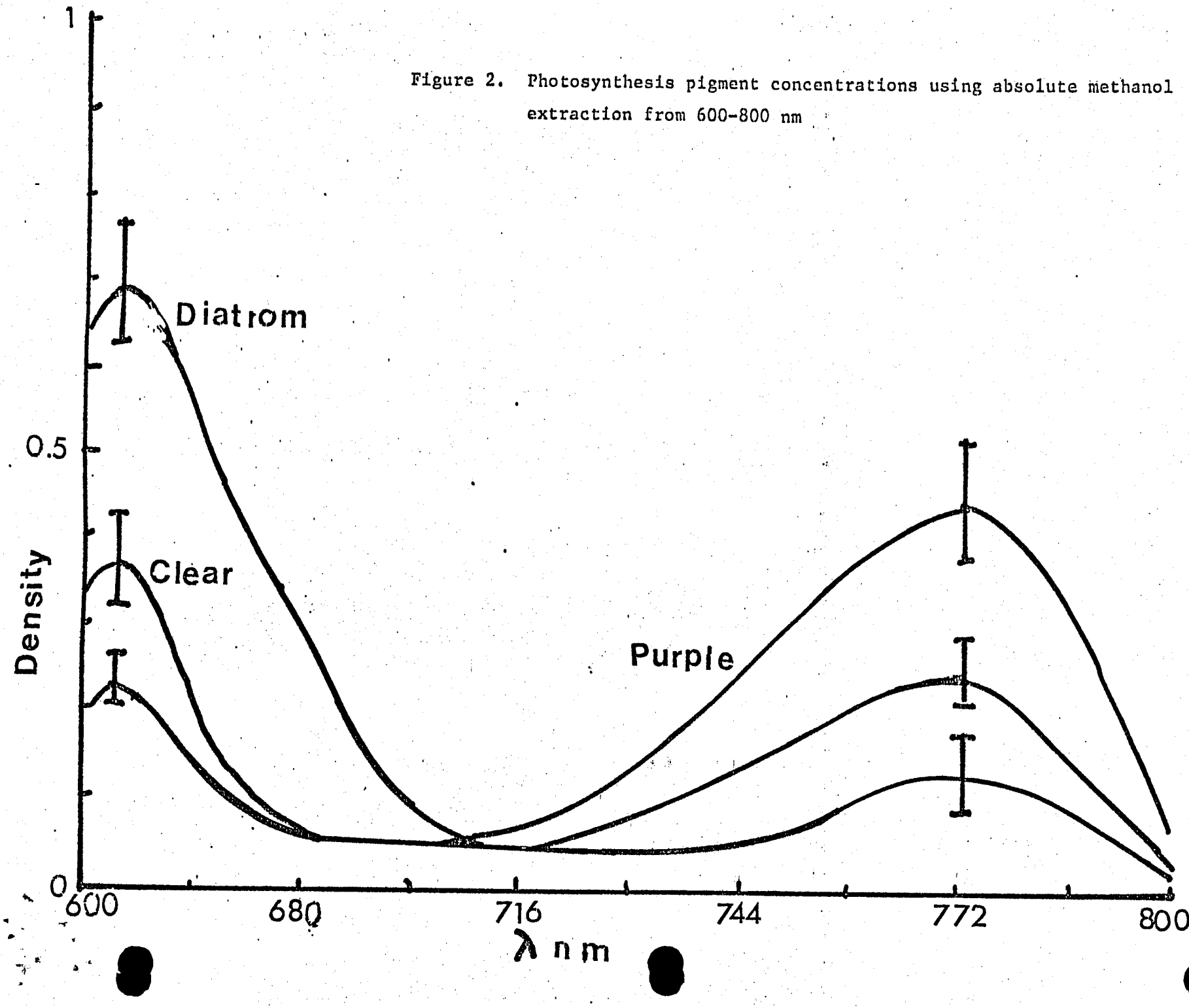


Figure 1. Harpacticoid species densities in each area sampled at Great Sippewissett Marsh. Vertical bar indicates std error mean.

Figure 2. Photosynthesis pigment concentrations using absolute methanol extraction from 600-800 nm



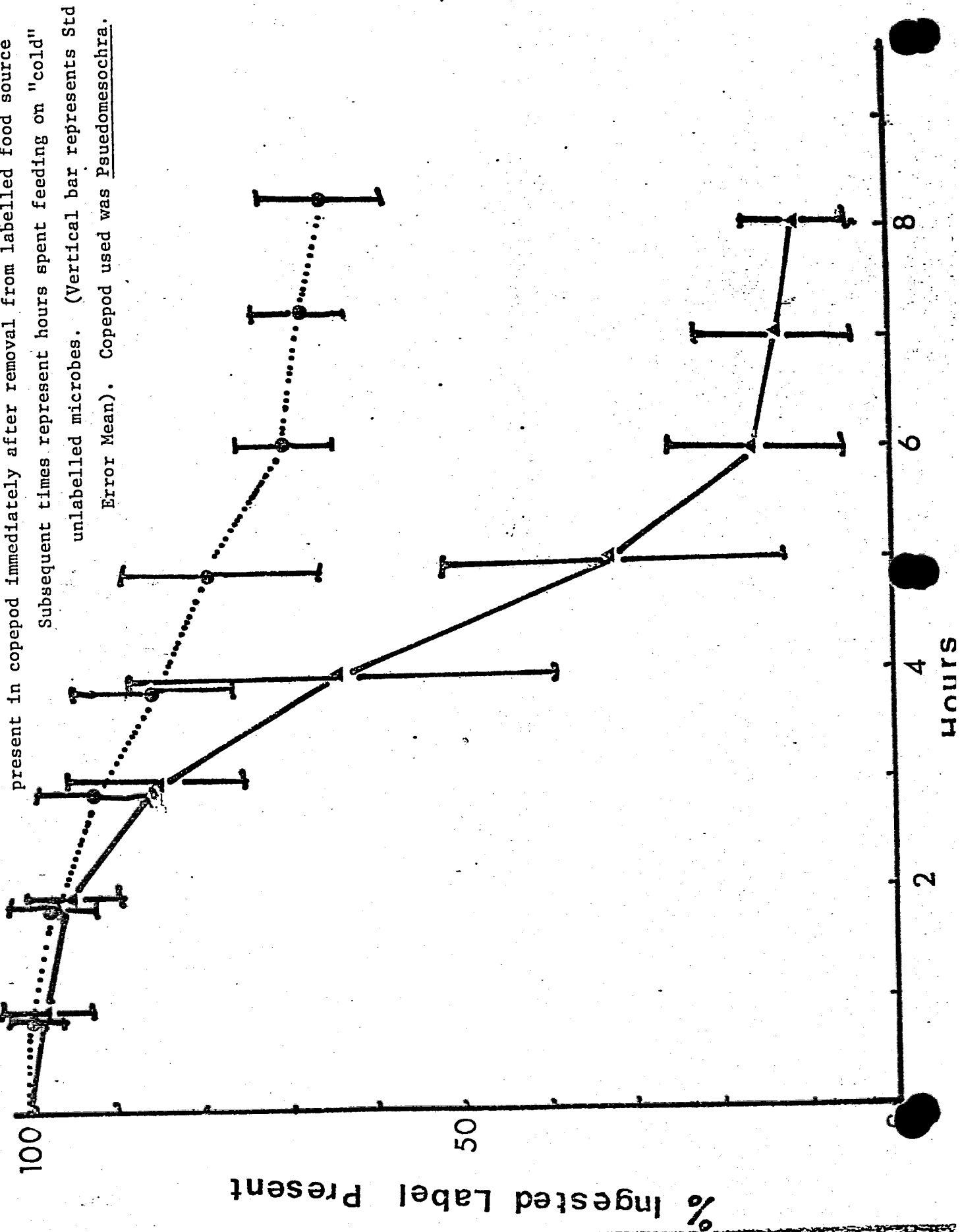
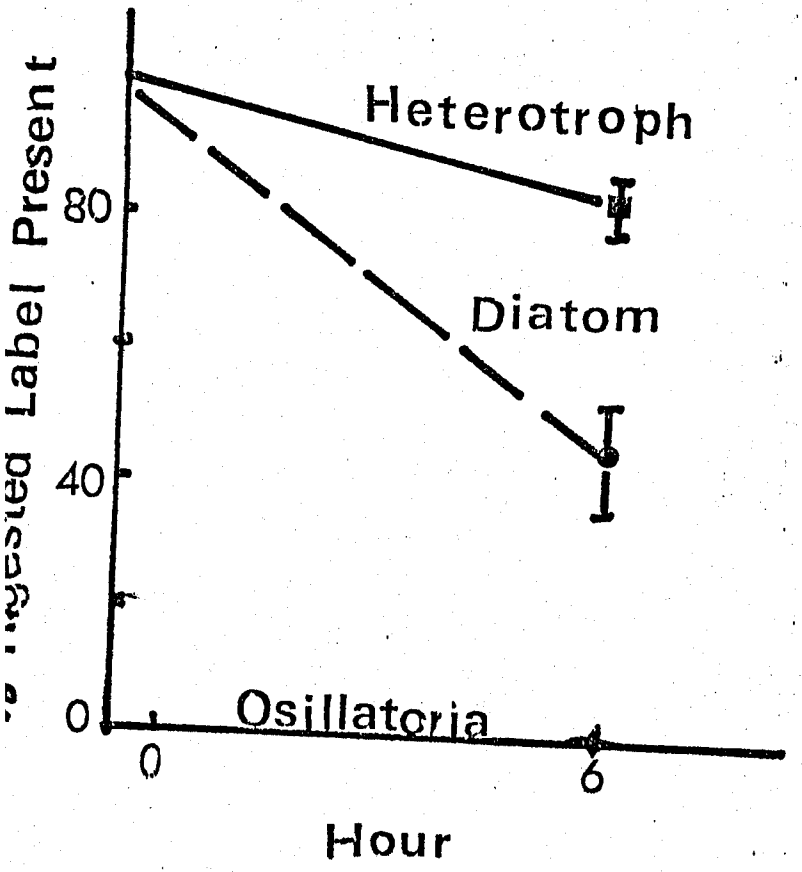
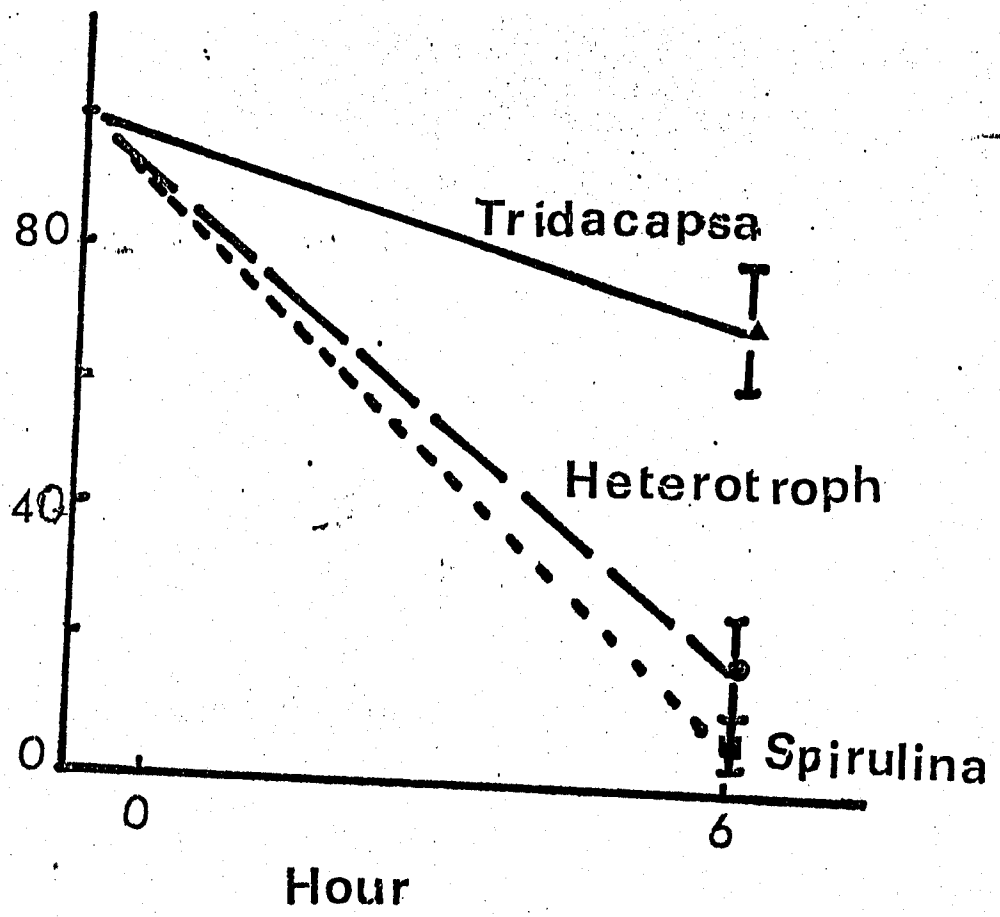


Figure 4. Plot showing retention of microbial label. vs. time. (Time 0 = label present in copepod immediately after removal from labelled food source Subsequent times represent hours spent feeding on "cold" unlabelled microbes. (Vertical bar represents Std Error Mean). Copepod used was Psuedomesochra.

Figure 5. Graph showing retention of various food-labels after 6 hr cold feeding in two harpacticoid species. (Vertical bars = std error mean)





DEGRADATION OF ORTHO PHENYL PHENOL BY STREPTOMYCETES IN CONTINUOUS CULTURES

Andreas Escher

INTRODUCTION

Phenolic compounds are quite commonly used as pesticides. Its degradation in soils is in absence of sun light relatively slow, since it is mediated by microbial activity alone. Investigations showed that mainly some fungi and streptomycetes are responsible for the degradation of these xenobiotic compounds. The mechanisms, however, are not well understood and it appears that traditional microbial assays, i.e. batch cultures and enrichment plates, are not very helpful. The intention of this summer work was to develop some different techniques for a better understanding of the mechanisms and the kinetics of biodegradation of phenolic compounds by streptomycetes. Ortho phenyl phenol has been selected since it is the most toxic compound of the different phenols and its possible products would be less toxic (no secondary inhibition by the product).

Xenobiotic compounds, i.e. phenols, can be detoxified by microorganisms either by metabolism or cometabolism. Studies at DuPont (J. Romesser, 1983) showed biodegradation of phenols with some fungi and actinomycetes. These experiments have been made with high amounts of biomass grown in batch cultures, decanted and resuspended in a solutions containing high amounts of substrate, nutrients, and xeonbiotic compounds. The focus of these studies was not to study growth in presence of toxic compounds, but metabolism, i.e. mineralization, and cometabolism of these compounds

The goal of the work was: (a) to measure the toxicity in terms of growth inhibition; (b) to determine whether streptomycetes metabolize or cometabolize phenolic compounds under conditions of continuous culturs; and (c) to measure whether energy is required or gained by degradation of toxic phenolic compounds.

Continuous Culture Dynamics

The use of chemostats with a steady state allows the application of simple mass balances to determine the rates of bioreactions. In the absence of xenobiotic compounds, i.e. carbon limitation, the system can be defined as follows:

1. $\underbrace{\frac{Vdc}{dt}}_{\text{accumulation}} = \underbrace{F(c_0 - c)}_{\text{flow in minus flow out}} - \underbrace{VK}_{\text{reaction rate}}$

By dividing this equation by volume, one obtains:

2. $\frac{dc}{dt} = D(c_0 - c) - K$

where D is the dilution rate. Under steady state conditions (d/dt = 0) it becomes: