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The Relative Importance of Bacteria and Algae as a Food Source for Crustacean Zooplankton

by

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Abstract

Recently, with the realization that much of the primary production of aquatic systems may pass directly into bacterioplankton, attention has been focussed on the fate of bacterial secondary production. Although bacteria are generally too small to be eaten by large crustaceans, a "microbial loop" has been identified in which flagellates and ciliates ingest the bacterial production and are then subsequently grazed by crustaceans. It has been suggested that in this way much of the carbon requirements of crustaceans may actually pass through this loop rather than come directly from phytoplankton. Alternatively, because of the number of trophic levels involved in the microbial loop, most of the bacterial secondary production might be rapidly remineralized to inorganic nutrients with only small amounts of carbon being transferred to crustaceans. This study was designed to evaluate these postulated roles for the microbial loop. Using [^{14}C] labelled glucose and bicarbonate inoculated into *in situ* mesocosms, I was able to quantify the movement of carbon from bacterial and algal sources to crustaceans. I found that when copepods were the dominant members of the crustacean community, bacteria contributed 1-2% of the total carbon flow to crustaceans. This is due largely to the necessity for bacterial carbon to first

pass through at least one intermediate trophic level before becoming accessible to copepods. When cladocerans were present in large numbers, bacterial carbon may have contributed as much as 23% of the carbon used by crustaceans. In this case, although bacterial carbon is directly available to cladocerans through direct grazing, the six fold greater biomass of algal carbon resulted in most crustacean carbon originating from this source.

Resumé

Récemment, avec la découverte qu'une fraction importante de la production primaire pourrait passer directement dans le bactérioplankton, l'emphase est porté sur le sort de la production secondaire bactérienne. Même si les bactéries sont généralement trop petits pour être consommées par les crustacés, un cycle microbien a été identifié dans lequel les flagellés et les ciliés ingèrent la production bactérienne et sont ensuite broutés par les crustacés. Cela suggère qu'une partie du besoin en carbone des crustacés passe directement par ce route au lieu de travers le phytoplankton. Alternativement, en raison du nombre plus élevé de niveaux trophiques à l'intérieur du cycle microbien, la majorité de la production secondaire pourrait être reminéralisée en éléments nutritives inorganiques au lieu d'être transférée aux crustacés. Le but de cette recherche était de déterminer lequel des hypothèses domine. Les movements de carbone d'origine bactérienne et celui d'origine algale aux crustacés ont été quantifiés avec l'aide du glucose et du bicarbonate marques de [^{14}C]. Les résultats démontrent, lorsque les copépoïdes dominent la communauté de crustacés, que les bactéries ne sont responsable que de 1% ou 2% du flux de carbone aux crustacés. Lorsque les

cladocères dominent, cette valeur pourrait s'élèver à 23%. Dans ce cas, même si le carbone bactérien est disponsible aux cladocères par le broutage, la biomasse totale des algues, qui est de six fois supérieure à celui des bactéries, est la source principale de carbone.

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Introduction

Studies of nutrient flux in aquatic food webs have traditionally centered on the direct transfer of carbon from phytoplankton to zooplankton via grazing. With the development of techniques for estimating bacterial abundance (Paul 1982, Porter and Feig 1980, Hobbie et al. 1977, Zimmerman and Meyer-Reil 1973) and production (reviewed by Azam and Fuhrman 1984) recent investigations have centered on the role of microbes as producers and remineralizers of organic carbon and as a potentially important source of nutrients for higher trophic levels (Azam et al. 1983, Ducklow 1983, Williams 1984, Pomeroy 1974). Bacteria are now accepted as being numerically abundant (Azam and Fuhrman 1984) generally averaging 10-40% of algal biomass (Ducklow 1983) and at times equalling or exceeding algal standing crops (eg. Coffin and Sharp 1987, Coveney et al. 1977). Techniques for measuring bacterial production, although still requiring further refinement to determine specifically what is being measured (Carman et al. 1988, Coveney and Wetzel 1988, Hollibaugh 1988, Servais et al. 1987, Wicks and Robarts 1987), suggest that bacterioplankton are active, with measured doubling time varying from a few hours to several days. The carbon source used by these bacteria for growth appears to be derived from dissolved organic carbon (DOC), released directly from phytoplankton (Larsson and Hagstrom

1982, 1979), from zooplankton via excretion, release of faecal pellets and "sloppy feeding" (Azam and Fuhrman 1984) and from allochthonous inputs (external inputs from watershed of lake).

As current estimates of bacterial secondary production indicate that bacterioplankton could potentially consume a significant amount of primary production, it is of interest to determine the fate of bacterial carbon. Bacterial numbers are generally in a steady state over a period of days or weeks (Pomeroy and Wiebe 1988, Ducklow 1983) which suggests that bacteria are efficiently removed as quickly as they are produced. Four loss processes are possible 1) lysis, 2) bacteriophages, 3) sedimentation, and 4) grazing. Lysis of aquatic bacteria has not been quantified but it is not generally believed that healthy actively growing cells would lyse to any great extent (Ducklow 1983, Fuhrman and Azam 1982). Bacteriophages have not been extensively studied in aquatic environments, however, as with lysis it is not thought that this is a major loss process for aquatic bacteria (Wiggins and Alexander 1985). Sedimentation may be important for attached bacteria (Ducklow et al. 1982) but the majority of bacteria are free living (Hodson et al. 1981, Azam and Hodson 1977,) and do not sediment to any great extent due to their small size (Ducklow et al. 1982).

It is now thought that grazing is the major process for the removal of secondary production of bacteria (Pace 1988,

Ducklow 1983). Aquatic bacteria are generally too small to be grazed effectively by crustaceans (Rieper 1978, Nival and Nival 1976, Jorgenson 1975, Kibby 1971) (daphnids may be an exception and will be discussed in greater detail in the following sections), however, protozoan grazers, mainly flagellates and ciliates, can effectively graze bacterioplankton (Sherr and Sherr 1987, Sherr et al. 1987a, Anderson et al. 1986, Lessard and Swift 1985, Fenchel 1982) and are present in numbers which could potentially remove the large numbers of bacteria produced in aquatic systems (Sherr and Sherr 1987, Sherr et al. 1987a, Lessard and Swift 1987). Since these protozoans are in a size range which can be effectively ingested by crustaceans, Azam et al. (1983) postulated that organic carbon lost from the phytoplankton may pass back to the crustaceans via the bacteria, flagellates and ciliates, a pathway that they termed the "microbial loop". Alternatively, the transfer of nutrients through several microbial trophic levels may simply result in the rapid remineralization of organic nutrients to an inorganic form available for algal uptake.

Recently, Ducklow et al. (1986) attempted to quantify the flux of nutrients through the microbial loop to determine which of the postulated roles dominates. Using [^{14}C] glucose as a tracer of bacterial carbon they found that less than 1% of the total added label was passed to the crustacean fraction (>100 um) over a period of 13 days. Most of the

label was remineralized to inorganic or refractory organic compounds. From this they concluded that bacteria and protozoans were a sink. Nutrients are rapidly remineralized with only an insignificant amount of carbon being transported back to crustaceans.

Subsequently, this study was severely criticized by Sherr et al. (1987b). In particular Sherr et al. cited an investigation by Davies (1984) designed to follow the movement of algal carbon (labelled with [^{14}C] bicarbonate) to crustaceans in which less than 1.2% of the [^{14}C] label was transferred to crustaceans. This suggests that the transfer of carbon in aquatic food webs may be inefficient and the small amount of label that Ducklow et al. observed being transferred to crustaceans from bacteria may actually represent a significant part of the total carbon input to crustaceans. Although the study of Davies casts some doubt on the conclusions of Ducklow et al. it does not in itself provide the means to simultaneously determine the relative importance of bacteria and algae as a food source since it deals only with algal carbon flow.

The purpose of the present research is to address this debate by simultaneously measuring the transfer of carbon from bacteria and algae to crustaceans. Compartmental modelling will be used to determine if the small amount of carbon passed through the microbial loop may actually represent a significant source of carbon for crustaceans. To

my knowledge this will be the first experiment of its kind to quantify the role of the microbial loop in this way and, additionally, will be the first of its kind in freshwater, as Ducklow et al. (1986) and Davies (1984) both dealt with marine systems. In performing this experiment I will also be able to determine if the results of Ducklow et al. are typical of other environments. Their experiment was essentially the only one of its kind performed to date, and Sherr et al. (1987b) felt that it was questionable to draw general conclusions from a single experiment performed in only one area.

Like Ducklow et al. (1986), this paper will focus largely on heterotrophic bacteria and their role as a carbon source. However, at times much of the primary production in aquatic systems is by small autotrophs in the same size range as heterotrophic bacteria. As a result of their small size and the amount of carbon fixed, these autotrophs (hereafter referred to as autotrophic picoplankton) could also be responsible for a large portion of the carbon passing through the microbial loop (Stockner and Antia 1986). Therefore, when appropriate, the importance of autotrophic picoplankton as a carbon source for crustaceans will also be considered.

Methods and Materials

Experiments were conducted at Lake Maskinonge, an

oligotrophic freshwater lake (total phosphorus, June, 7.5 ug/l; orthophosphate, 2 ug/l; epilimnion temperature, June and August, respectively, 17°-19°, 25°-27°; epilimnion depth at site of experiments, 6 m) located on the property of Atomic Energy of Canada Ltd., Chalk River, Ontario. Two separate experiments were performed in June and August 1988. The following is a general description of the experimental protocol with differences between the two experiments to follow.

Briefly, by adding either labelled [^{14}C] glucose or bicarbonate to limnocorrals I was able to follow separately the movement of carbon to crustaceans from either heterotrophic or autotrophic sources. I used size fractionation to separate crustaceans from the various sizes of autotrophs or heterotrophs. By repeated sampling over a two to three week period I was able to measure the transfer of label to crustaceans from both sources.

Nine limnocorrals were deployed in the lake at the commencement of each experiment. The enclosures were 1 metre in diameter and either 6 metres (June) or 4 metres (August) in length. They were constructed of polyethylene closed to the sediments and held open at the top by an aluminum ring. The enclosures were launched by lowering the assembly to a point just above the sediments and raising the enclosure through the water column. This was repeated twice to insure

complete filling of the enclosures. Nalgene jugs were used as flotation devices.

Isotopes were used in the experiments to label bacteria ([^{3}H]-methyl-thymidine and [^{14}C]-D-glucose) or algae ([^{14}C]-sodium-bicarbonate). Isotopes were initially dispensed into 200 ml of lake water. This inoculated lake water was immediately pumped into the enclosures thru a 6 mm tygon tubing raised and lowered throughout the water column. Mixing of the enclosures was done with a secchi disk. Of the nine enclosures, six received [^{3}H] thymidine. Of these six, three received [^{14}C] bicarbonate while [^{14}C] glucose was inoculated into the remaining three. Three of the nine enclosures were not inoculated with isotopes and were used to follow the dynamics of the plankton community (outlined below). Plankton dynamics were followed in separate enclosures to avoid handling of radioactive samples.

Size-specific filtration of the plankton was used to separate various trophic levels (Ducklow et al. 1986, Currie and Kalff 1984, Berman and Stiller 1977). Polycarbonate filters with pores 0.2, 1.0, 3.0, and 10.0 um in diameter (Poretics corporation, Livermore, California) and 35.0 and 120.0 um nitex screen were used. These filters gave an approximate separation of the following heterotrophic fractions: 1) 0.2-1.0 um, free living bacteria, 2) 1.0-3.0 um, attached bacteria, 3) 3.0-10.0 um, flagellates, 4) 10.0-35.0 um and 35.0-120.0 um, ciliates and rotifers, (different

sizes of these heterotrophs overlap to some extent in these fractions), 5) >120.0 um, crustaceans. For the bicarbonate enclosures, the same size fractions were used to provide a separation of different sizes of autotrophs.

The enclosures were sampled approximately every second day. For size fractions <10 um, an integrated water sample was taken from the enclosures using 6 mm tygon tubing lowered to 1 metre above the bottom of the enclosures. The sample was placed in an acid-rinsed bottle and manipulation of the sample was commenced within two hours of collection in a lab located on the shore of the lake. Prior to fractionation, the samples were prescreened on 35 um screens to remove larger particles which can cause occasional sporadic counts on the smaller size fractions. Subsamples of the prescreened sample were filtered in parallel on the 0.2, 1.0, 3.0, and 10.0 um filters with a pressure not exceeding 12 cm of mercury. The following amounts of water were filtered: 1) 0.2 um, 5 ml; 2) 1.0 um, 5 ml; 3) 3.0 um, 10 ml; 4) 10.0 um, 50 ml. Filtering these volumes of water allowed me to detect low levels of radioactivity in some size fractions. This was especially critical for bicarbonate, where incorporation of label into particles was only a small percentage of the total added label. Each filter was placed in a glass scintillation vial and prepared for counting by adding 100 ul of distilled H_2O, 900 ul of NCS tissue solubilizer (Amersham corporation) and followed, 20 minutes later, by the addition of 0.34 ml of

glacial acetic acid and 12 ml of OCS fluor (Amersham corporation).

Dissolved carbon (DC), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), dissolved tritium compounds (DH), dissolved inorganic tritium (DIH), and dissolved organic tritium compounds (DOH) were measured from a 0.2 um filtrate. DC was determined by adding 60 ul of 1N NaOH to two ml of filtrate to retain DIC and followed by the addition of 12 ml of ACS II fluor (Amersham corporation). DH was simultaneously measured from this preparation. DOC was determined by adding 28 ul of 0.1 N HCl to 2 ml of 0.2 um filtrate, bubbled for 1 hour (Gachter et al. 1984) and followed by the addition of 12 ml of ACS II. DIC was determined as the difference between DC and DOC. DOH was measured by lyophilizing 2 ml of 0.2 um filtrate followed by the addition of 12 ml of ACS II. DIH was determined as the difference between DH and DOH.

Samples of water for the 35 and 120 um fractions were collected from the enclosures using an electric bilge pump (Jabsco Products, Costa Mesa, Cal.). Twenty-five liters were pumped from the enclosures, passed through a 35 um plankton net with the filtrate passing back into the enclosures. A 19 mm diameter tygon tubing was used on the pump with the tubing raised and lowered once through the water column while the 25 liters of sample was being collected. The concentrated fraction was removed from the plankton net and made up to 100

ml with unlabelled lake water filtered through a Whatman GF/C glass fiber filter. A subsample of the concentrate was filtered onto a 35 um screen. The volume filtered was varied to prevent clogging of the filter. The remainder of the 100 ml concentrate was filtered onto a 120 um screen. Nitex screens were prepared for scintillation counting as per the polycarbonate filters. Due to time limitations at the start of the experiment sampling of the >35 um fractions did not commence until the second day of the experiment. On day 1 radioactivity in the >35 um fraction was assumed to be zero.

The samples were counted using a dual label isotope program on a Packard Tri-Carb scintillation counter (model 2000ca). The external standards ratio method was used to correct for quench. Backgrounds were prepared by repeating the manipulations for the dissolved and particulate size fractions described above using lake water from outside the enclosures. Samples were counted for 20 minutes or until the standard deviation of the counts was $\pm$ 3%. For most analyses presented in this paper the amount of isotope per size fraction is expressed as a percentage of the total amount of isotope (ml^{-1}) added to the enclosures on the first day of the experiment.

As previously indicated, two experiments were performed, June and August, which differed slightly in several respects. Since <3% of the added [^{14}C] bicarbonate was incorporated in June the enclosures were enriched in August with nutrients to

increase uptake of label by algae. I could not perform experiments in a more eutrophic system, since Federal regulations permit in situ use of isotopes only on the property of Atomic Energy of Canada. Therefore, I chose to manipulate the enclosures to increase uptake rather than use a more eutrophic lake. The enclosures were enriched with 75 ug N l^{-1} as $NaNO_3$ and 7.5 ug P l^{-1} as K_2HPO_4 5 days prior to the beginning of the experiment. Subsequently, 10 ug N l^{-1} and 1 ug P l^{-1} were added to the enclosures approximately every 5 days. Additionally, to increase the amount of bicarbonate label available for uptake, enclosures were reduced in size to 4 m in August from 6 m used in June and the amount of [^{14}C] bicarbonate added per enclosure was increased from approximately 350 DPM ml^{-1} in June to 4800 DPM ml^{-1} in August. Sampling began 5 days after the initial addition of nutrients in August, while in June, sampling began 24 hours following deployment.

When sampling began, the [^{14}C] glucose activity was approximately 350 DPM ml^{-1} in June and 600 DPM ml^{-1} in August. [^{3}H] thymidine activity was approximately 650 DPM ml^{-1} in June and 1100 DPM ml^{-1} in August.

In June, the initial uptake of [^{14}C] glucose (0-7 hours) was measured in the lab on separate water samples inoculated with isotope. This was done as I felt that, due to the size of the enclosures, the label may not homogenously disperse over this time interval. Water samples were taken from the

enclosures just prior to inoculation with isotope and transported back to the lab. The [^{14}C] glucose was added to 200 ml samples at approximately the same specific activity as that of the enclosures. Subsamples were removed at 15 minutes, 1, 2, and 4 hours and filtered in parallel onto 0.2 um (2 ml), 1 um (2 ml), 3 um (4 ml), and 10 um (4 ml) polycarbonate filters. Additional 2 ml samples were taken to determine total activity. These samples were prepared for counting as above. In August I took samples directly from the enclosures over the first 8 hours. After counting, I felt that the similarities of the counts between enclosures after one hour was an indication of homogenous dispersal of the label. I therefore was able to determine initial uptake directly from the enclosures in this experiment.

Three of the nine enclosures were not inoculated with isotopes and were used to follow the dynamics of the plankton community. Integrated water samples were taken from the enclosures using 19 mm diameter tygon tubing for determination of chlorophyll a, and abundances of bacteria and heterotrophic flagellates. Samples for the determination of bacterial and flagellate abundances were preserved with glutaraldehyde (1% final concentration) and stored at 4°C. Crustacean abundances were determined from samples taken with the bilge pump. These samples were preserved with formalin (5% final concentration) and stored at 4°C.

Chlorophyll a was measured in June by filtering 300 ml

of water onto a GF/C Whatman glass fiber filter and extracting the chloropyll with ethanol following the procedure outlined in Ostrofsky and Rigler (1987). The filters were stored frozen at -10 to -15°C for two weeks prior to extraction of chl a. The formula for calculation of chl a in mg m^{-3} was from Bergmann and Peters (1980). In August, in addition to GF/C measurements of chl a taken over the course of the experiment, the chlorophyll a on each size fraction was determined at the time of deployment of the enclosures and on the day of inoculation. Water was pre-filtered (amounts filtered in parentheses) through either a 1.0 (800 ml), 3.0 (750 ml), 10.0 (600 ml), 35 (300 ml), or 120 (300 ml) um filter and the filtrate collected on a GF/F glass fiber filter with the chl a per size fraction determined by difference. As a GF/F filter has a 0.8 um effective pore size this may slightly underestimate the amount of chl a per size fraction but the error should be small. For determinations of algal biomass a C:chl a ratio of 30:1 (Strickland 1960) was assumed.

Bacteria were enumerated using DAPI fluorescence (Porter and Feig 1980). Final concentration of DAPI used for staining was 0.1 ug ml^{-1}. Five ml of sample were filtered onto 0.2 um Irgalan black stained Poretic filters and were counted at 1250x magnification using a Zeiss Ph3 100x neofluor lens and Zeiss filter set BP365/11, FT395, LP397. 800-1000 bacteria were counted on an average of 40 fields per

filter. Attached bacteria were not separately enumerated. Most slides were prepared within one month of collection and stored at -10 to -15°C for up to 3 months prior to counting. In some cases, due to failure of the fluorochrome to stain the bacteria, some slides were prepared 4 months following collection, however, this did not seem to affect the number of bacteria enumerated as counts were similar to those obtained from the first set of prepared slides. For analyses requiring bacterial biomass, a conversion factor of 9.2×10^{-15} g C per bacterial cell was assumed, obtained by taking the mean of the conversion factors used by Biddanda (1988), Coffin and Sharp (1987), Güde et al. (1985), Rublee et al. (1984), Williams (1984), Thompson et al. (1982), Fuhrman (1981), Watson et al. (1977), and Ferguson and Rublee (1976).

Heterotrophic flagellates were enumerated after primulin staining following Caron (1983), as modified by Bloem et al. (1986). Twenty-five ml of sample was filtered onto a 1.0 um Irgalan black stained Poretic filter. Slides were prepared within 2 weeks of collection and stored frozen at -10 to -15°C for up to 2 months. As above, some slides were not usable and were prepared again up to 3 months following collection. Although it has been reported that this may reduce the autofluorescence of autotrophic flagellates (Bloem et al. 1986) visible autofluorescence of cells was still evident and as above counts of flagellates were similar to

those determined from the first set of slides. Flagellates were counted at 1250x magnification using a Zeiss Ph3 100x neofluor lens and Zeiss filter set BP365/11, FT395, LP397 for visualization of primulin. Autotrophic flagellates were identified by red autofluorescence of cells using the Zeiss filter set BP546/12, FT580, LP590. Transects of the filter were counted until 60 to 100 animals had been enumerated.

Crustaceans were enumerated with a dissecting microscope. Cladocerans and copepods were counted separately. No distinction was made on the basis of size. As less than 2% of the enumerated crustaceans passed a 120 um screen I decided to confine my attention to the >120 um fraction in determining how much of the label was being transferred to large crustaceans. For purposes of presentation of the data, the small percentage of crustaceans in the 35-120 um fraction was added to the numbers in the >120 um fraction. The biomass of crustaceans at the commencement of both experiments was determined by filtering the >120 um fraction onto a GF/C filter and drying for 24 hours at 65°C. Carbon content was assumed to be 40% of dry weight (Riemann 1985).

In addition to the field experiments, several manipulations were carried out in microcosms in the lab. First I wished to investigate the behavior of [^{14}C] and [^{3}H] incorporated into bacteria in the absence of grazers in order to test the hypothesis that observed loss of label from

bacteria in the enclosures was due to grazing by bacteriovores. Bacteria were isolated from Maskinonge Lake by plating 100 ul of whole lake water on agar supplemented with nutrient broth. These isolates were resuspended in 250 ml of 0.2 um filtered and autoclaved Maskinonge water under sterile conditions. For the course of the experiment these cultures (3 replicates) were grown semi-continuously by replacing 10% of the culture daily with 0.2 um filtered and autoclaved Maskinonge water. Inoculation and sampling of the cultures began 5 days following inoculation of the bacteria. Cultures were incubated at room temperature. Approximately every two days subsamples were removed from the culture under sterile conditions and 8 ml filtered onto a 0.2 um filter. 2 ml samples of the filtrate were used for determination of DC, DH, DIC, DOC, DOH, and DIH and 2 ml samples taken directly from the culture for determination of TC and TH. These samples were prepared and counted as previously described. Activity of the isotopes per size fraction were expressed as a percentage of the total label added (ml^{-1}) at the commencement of the experiment.

Due to metabolism of [^{3}H]-thymidine during the course of the experiment, inorganic [^{3}H] compounds, presumably in the form of [^{3}H] H_2O, were released into the water. An experiment was conducted to determine if [^{3}H] H_2O is taken up by particles present in the water column. 200 ml water samples from Maskinonge Lake were inoculated with [^{3}H] H_2O to a

specific activity similar to that in the limnocorrals. Flasks were incubated at 20°C in an incubator using a 16l:8d light cycle. Subsamples were taken approximately every second day and 4 ml were filtered onto a 0.2 um Poretic filter. Two ml samples were also taken from the flask for determination of total activity and 2 ml of the 0.2 um filtrate were taken for dissolved activity. Samples were prepared and counted as previously described.

Several experiments were also conducted to detect possible sources of error in the experimental protocol. NaOH and HCl, used for the determination of DC and DOC respectively, were tested to determine if addition of these compounds to the water samples would affect the counting efficiency of [^{3}H] and [^{14}C]. An identical amount of [^{3}H] and [^{14}C] was added to each of nine scintillation vials containing 2 ml of distilled water. Three of these vials received 60 ul of 1 N NaOH, three received 29 ul of 0.1 N HCl and three acted as controls. 12 ml of ACS II fluor was added and vials were counted as described previously. ANOVA was used to test for differences among the mean radioactivity determined for each treatment and the control. Similarly, as nitex screen does not dissolve in the presence of NCS, an identical procedure was used to determine if the presence of these screens in the scintillation vial would alter counting efficiency. A t-test was used to identify any difference between the treatment and the control.

As [^{14}C] glucose or [^{3}H] thymidine could potentially bind to inert detrital particles present in the water column, which would appear as biotic uptake, an experiment was performed to determine if abiotic uptake of this kind would occur in Maskinonge Lake water. Prior to inoculation of the limnocorrals in both June and August 200 ml water samples were taken from the enclosures and formalin added to a final concentration of 5%. [^{14}C] glucose and [^{3}H] thymidine were added to these samples to give specific activities similar to that in the enclosures. One, two and four hours following addition of the isotope, 2 ml subsamples were filtered onto a 0.2 um filter to detect any binding of isotope to inert particles. 2 ml samples of whole water and 2 ml of 0.2 um filtrate were taken for determination of total and dissolved activity respectively. 60 ul of 1 N NaOH was added to retain any inorganic [^{14}C] and samples were subsequently prepared and counted as described above.

Data analysis was performed using SAS (SAS Institute 1982). Nutrient flux through the plankton was described with compartmental models, solved using the PAR program of BMDP (BMDP Statistical Software 1981, Ralston et al. 1988) which simultaneously solves a series of differential equations.

Results and Discussion

Control experiments

I found that the presence of NaOH or HCl in the scintillation vials prepared for counting had no effect on the counting efficiency of [^{14}C] ($p>.25$) or [^{3}H] ($p>.25$). Nitex screen also had no effect on [^{14}C] ($p>.25$) or [^{3}H] ($p>.25$). Additionally, I found no detectable abiotic uptake in Maskinonge water samples (table 1). Similarily, less than 0.3% of the total [^{3}H] H_2O label added was ever detected in particles >0.2 um over a period of 14 days (table 2) indicating that reuptake of respired [^{3}H] H_2O in the enclosures would not need to be taken into account in any data analyses.

Fig. 1 shows the tracer dynamics of label incorporated by bacteria in the absence of grazers. As much as 95% of the DIC was lost to the atmosphere by the end of the experiment, presumably due to the larger surface area to volume ratio in the flasks compared to the enclosures. Therefore in fig. 1, rather than present only the small amount of DIC measured in the water, DIC is shown as representing all carbon not in particles or in DOC. Bacterial respiratory losses are high during initial uptake while losses of both [^{14}C] and [^{3}H] are very small following depletion of organic substrate (ranging from 0.3 - 0.7% day^{-1} following initial uptake). Since loss

Table 1. Abiotic uptake in Maskinonge Lake samples over a 4 hour period following inoculation with isotopes. Total, dissolved and particulate (>0.2 µm) activity are shown (DPM ml^{-1}). These experiments were performed with samples fixed with 5% formaldehyde (final concentration). Samples were taken just prior to inoculation of the enclosures. Particulate activity was collected on a 0.2 µm filter. Prior to inoculation with isotopes, backgrounds were prepared with the same water samples. Standard errors are shown in parentheses (n=2).

Time (hours)	Total	Dissolved	Particulate
		[^{14}C] glucose	
		June	
0	203.41 (5.10)	192.57 (10.25)	0.18 (0.18)
1	195.19 (9.91)	187.68 (3.78)	0.22 (0.22)
2	194.78 (2.88)	200.79 (15.74)	0.0 (0.0)
4	199.54 (6.57)	-	0.0 (0.0)
		August	
0	635.85 (10.67)	625.95 (14.68)	0.0 (0.0)
1	648.13 (70.96)	635.89 (7.46)	0.0 (0.0)
2	-	-	-
4	613.48 (44.62)	671.81 (20.63)	0.01 (0.01)
		[^{3}H] thymidine	
		June	
0	614.19 (6.55)	612.10 (15.15)	0.87 (0.07)
1	639.39 (5.16)	608.54 (20.88)	0.77 (0.77)
2	617.89 (8.85)	622.75 (24.87)	0.0 (0.0)
4	647.65 (14.53)	-	0.0 (0.0)
		August	
0	5003.96 (31.05)	4835.50 (48.92)	0.0 (0.0)
1	4760.47 (70.52)	4967.87 (68.24)	0.0 (0.0)
2	-	-	-
4	5012.78 (32.62)	5114.30 (34.15)	0.01 (0.01)

Table 2. Uptake of [^{3}H] H_2O by particles in Maskinonge Lake samples. Shown are total, dissolved and particulate (>0.2 µm) activity (DPM ml^{-1}). Prior to inoculation of the samples backgrounds were prepared with the same water samples. Standard errors are shown in parentheses (n=3).

Time (Hours)	Total	Dissolved	Particulate
4	641.10 (10.07)	649.75 (31.25)	0.69 (0.69)
24	633.12 (7.55)	584.90 (11.37)	1.89 (1.89)
72	644.30 (7.55)	611.49 (23.19)	0.42 (0.42)
120	582.49 (45.58)	630.12 (10.96)	0.73 (0.73)
168	598.23 (8.38)	526.09 (9.61)	0.0 (0.0)
216	597.01 (12.48)	590.43 (13.43)	0.0 (0.0)
264	612.11 (7.43)	598.47 (1.75)	0.0 (0.0)
312	623.91 (5.58)	731.15 (20.38)	0.0 (0.0)

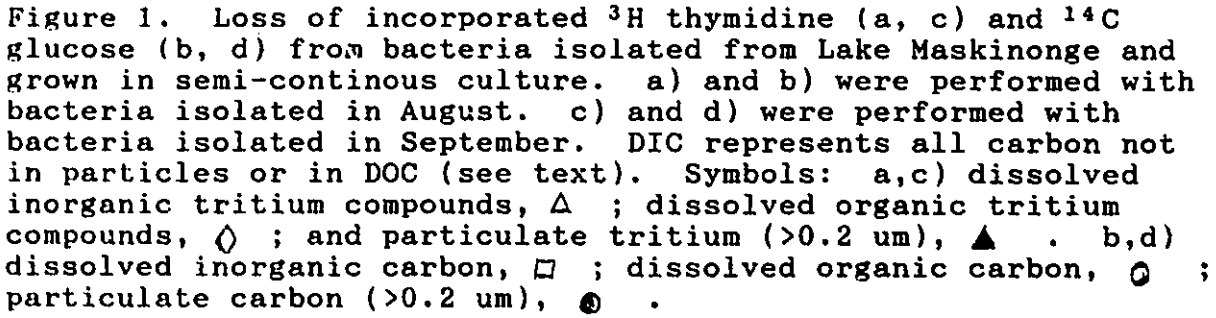


Figure 1. Loss of incorporated ^{3}H thymidine (a, c) and ^{14}C glucose (b, d) from bacteria isolated from Lake Maskinonge and grown in semi-continous culture. a) and b) were performed with bacteria isolated in August. c) and d) were performed with bacteria isolated in September. DIC represents all carbon not in particles or in DOC (see text). Symbols: a,c) dissolved inorganic tritium compounds, △ ; dissolved organic tritium compounds, ◇ ; and particulate tritium (>0.2 um), ▲ . b,d) dissolved inorganic carbon, □ ; dissolved organic carbon, ○ ; particulate carbon (>0.2 um), ● .

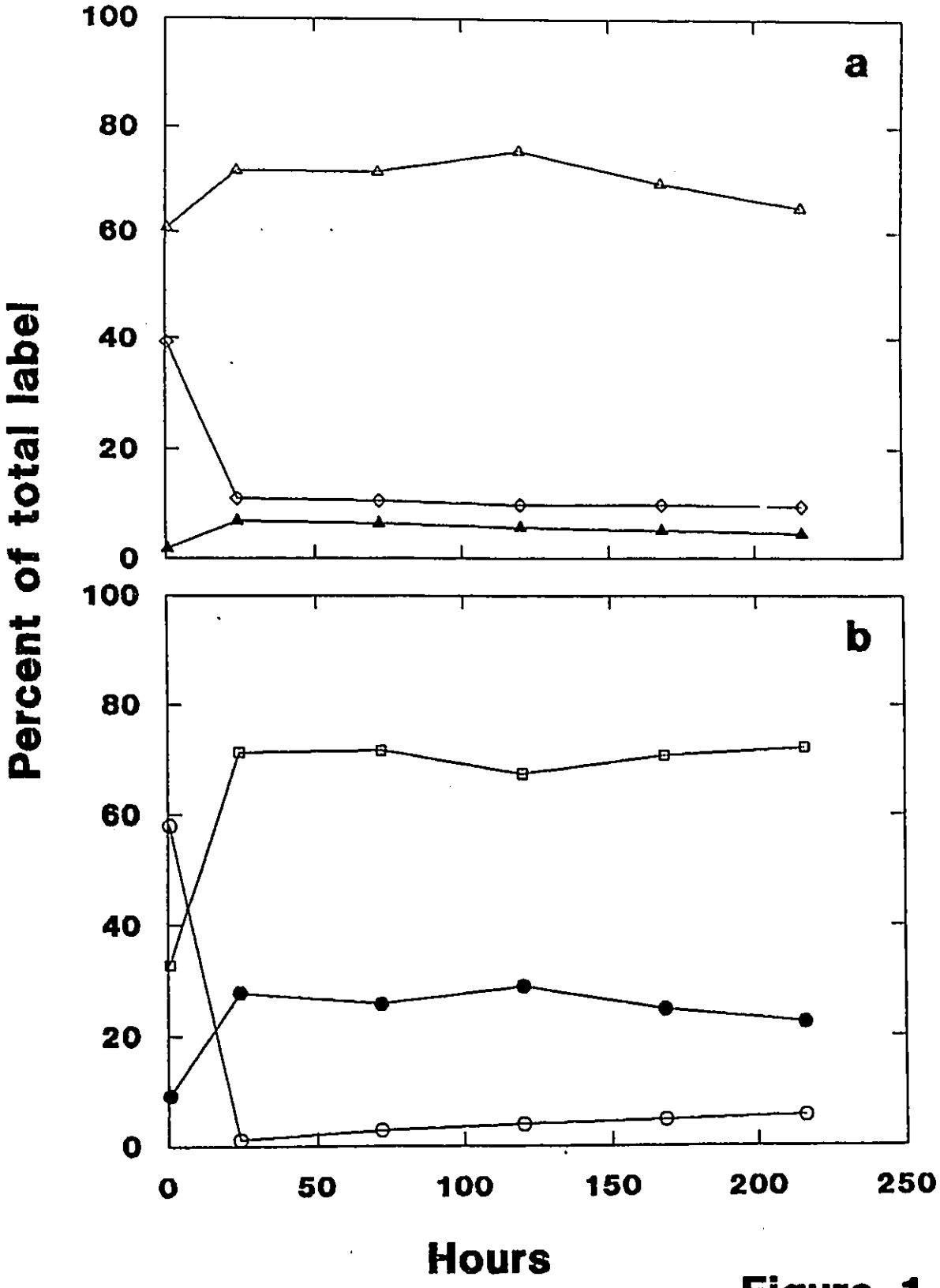

a
b
100
80
60
40
20
0
Percent of total label
0
50
100
150
200
250
Hours

Figure 1

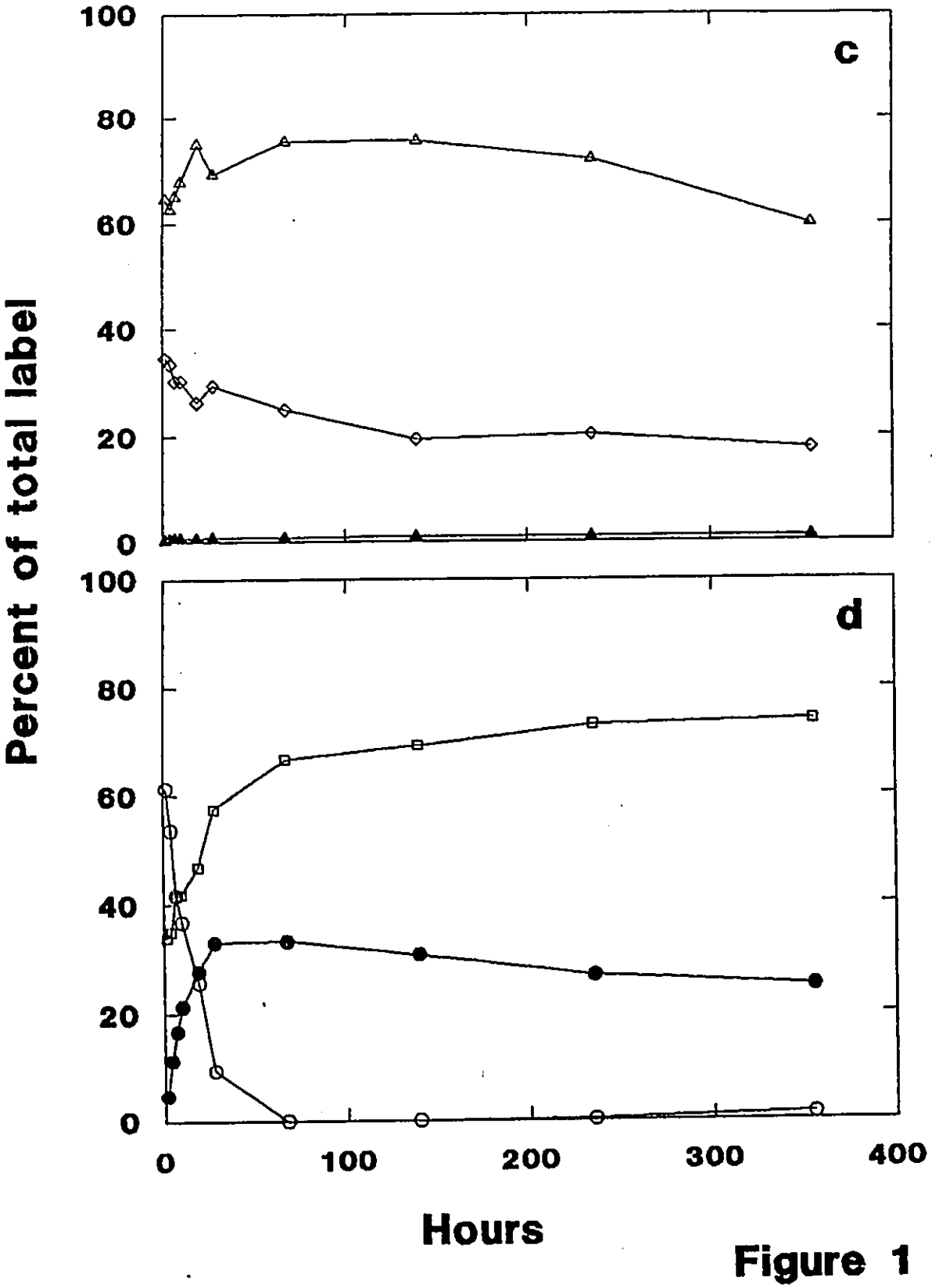

c
d
100
80
60
40
20
0
Percent of total label
0
100
200
300
400
Hours

Figure 1

of label from bacteria in the enclosures following initial uptake greatly exceeds this amount (fig. 2 and 3, discussed in the following section), these results suggest that grazing is the major loss process for bacterial carbon.

Overview of isotope dynamics

Figures 2 and 3 show the redistribution of label for the enclosures inoculated with [^{14}C] glucose for both field experiments. Initial uptake (approximately 0-8 hours for June and 0-24 hours for August) was dominated by the smallest size fractions (0.2-3 um). Subsequently, label was redistributed, mainly to dissolved inorganic or refractory organic compounds (considered refractory based on the assumption that since glucose is removed rapidly from solution any DOC measured in the water over the course of the experiment must be composed of complex molecules which are taken up slowly, if at all). Less than 6% of the label incorporated into particles following initial uptake is found in the >120 um fraction over the course of the experiment. The two major differences between the June and August experiments were the smaller percentage of label retained by particles following initial uptake in August and the difference in distribution of the label within particles.

Figures 4 and 5 show the redistribution of label for enclosures inoculated with [^{3}H] thymidine for these

Figure 2. Partitioning of labelled carbon originally inoculated as ^{14}C glucose for June. All values are expressed as a percentage of the total label added on day 1 (ml^{-1}). Average standard error for a given fraction shown in parentheses (n=3). Symbols: a) dissolved carbon (2.85%), ▲ ; dissolved inorganic carbon (1.03%), □ ; dissolved organic carbon (2.21%), O ; and particulate carbon (>0.2 um), (2.10%), ● . b) cumulative particulate size fractions; >0.2 um, (2.10%), ● ; >1.0 um (0.51%), △ ; >3.0 um (0.25%), ■ ; >10.0 um (0.20%), ◇ ; >35 um (0.11%), + ; and >120 um (0.21%), ★ .

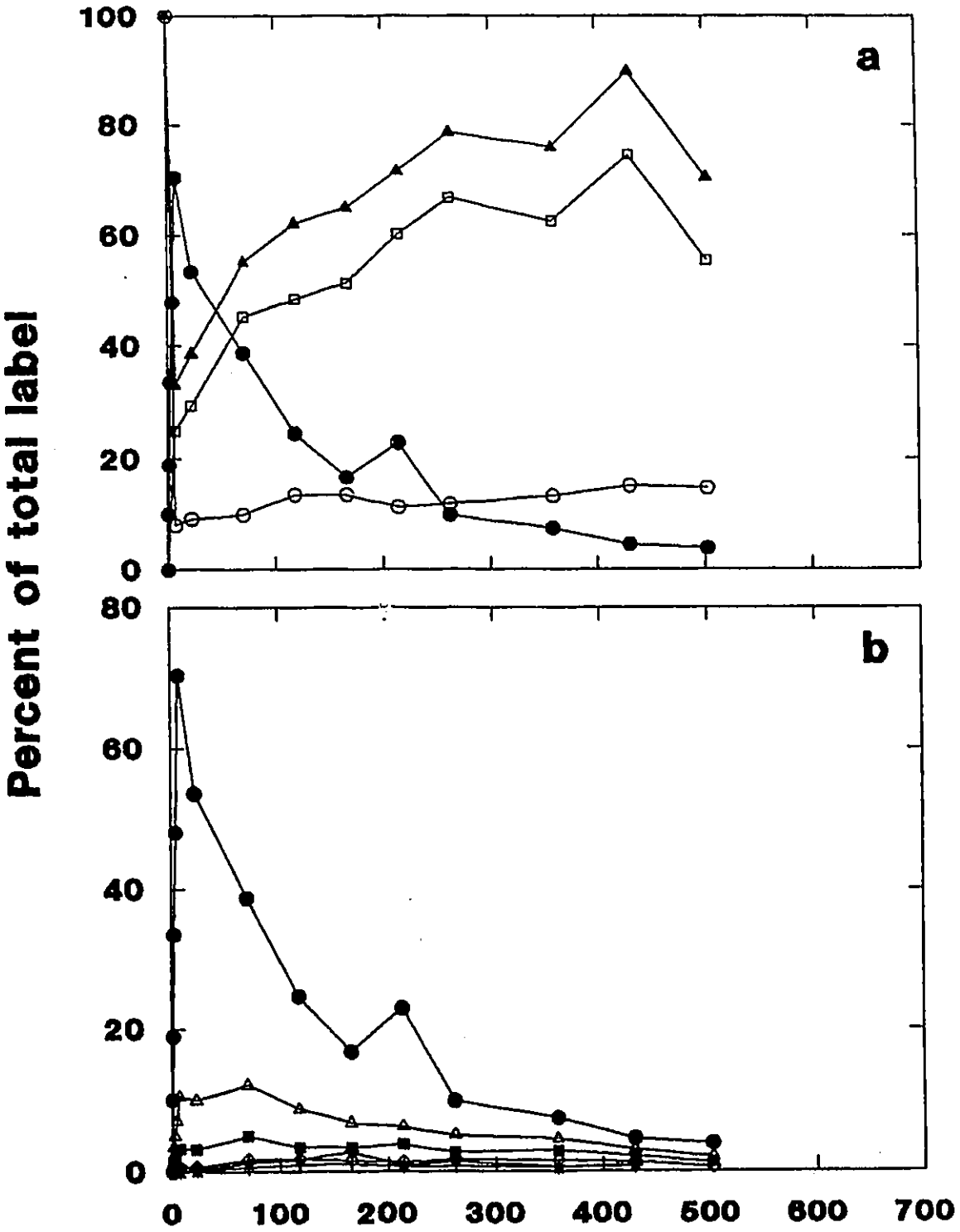

a
b
Percent of total label
Hours
0
20
40
60
80
100
0
100
200
300
400
500
600
700

Figure 2

Figure 3. Partitioning of labelled carbon originally inoculated as ^{14}C glucose for August. All values are expressed as a percentage of the total label added on day 1 (ml^{-1}). Several enclosures were lost during this experiment and, as a result points at 285 and 357 hours represent two enclosures and points at 429 and 525 hours represent one. Average standard error for a given fraction is shown in parentheses. Symbols: a) dissolved carbon (5.11%), ▲ ; dissolved inorganic carbon (4.08%), □ ; dissolved organic carbon (2.07%), ○ ; and particulate carbon (>0.2 um), (2.75%), ● . b) cumulative particulate size fractions; >0.2 um, (2.75%), ● ; >1.0 um (0.84%), △ ; >3.0 um (1.35%), ■ ; >10.0 um (0.64%), ◇ ; >35 um (0.15%), + ; and >120 um (0.12%), ✱ .

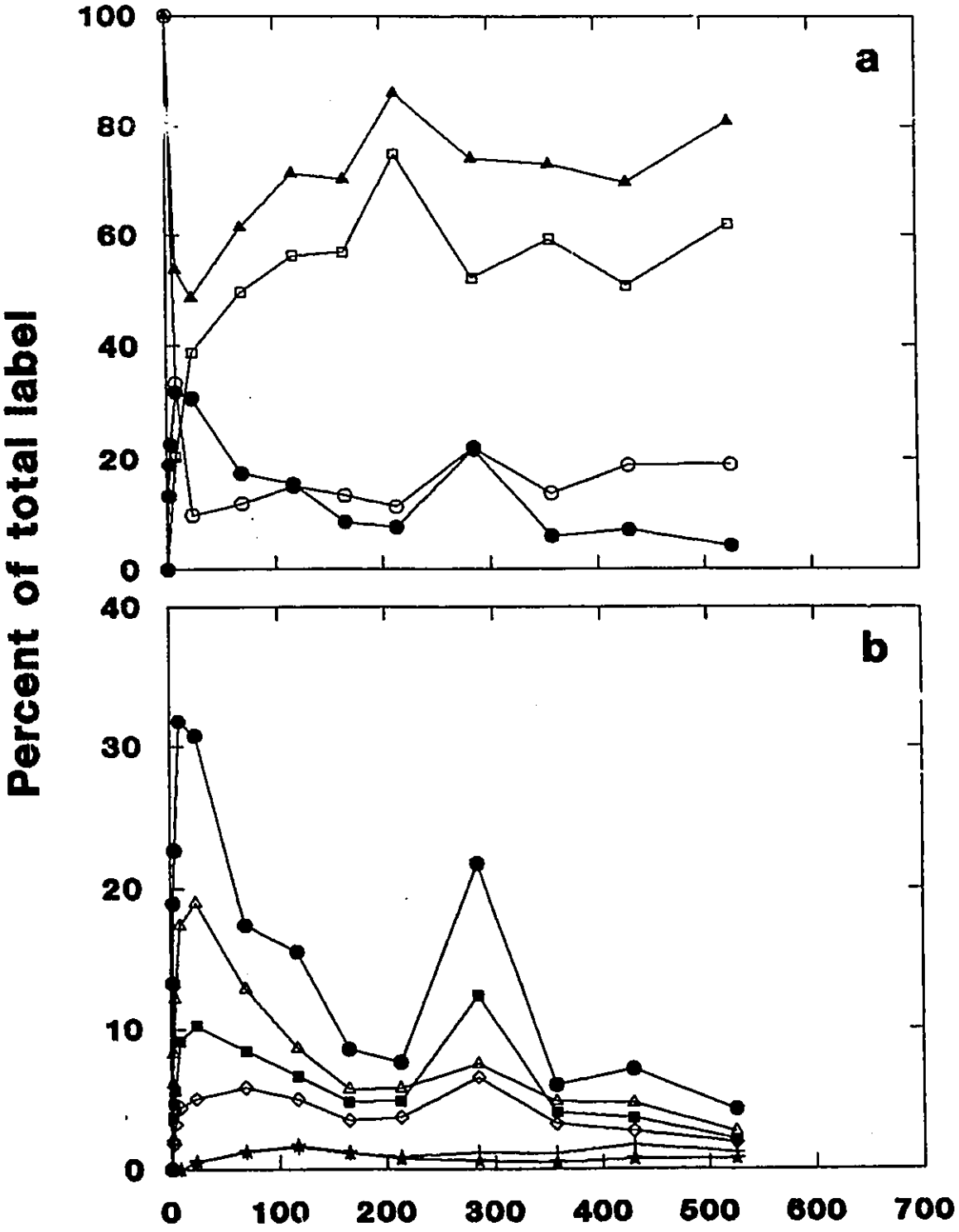

a
b
Percent of total label
Hours
100
80
60
40
20
0
40
30
20
10
0
0
100
200
300
400
500
600
700

Figure 3

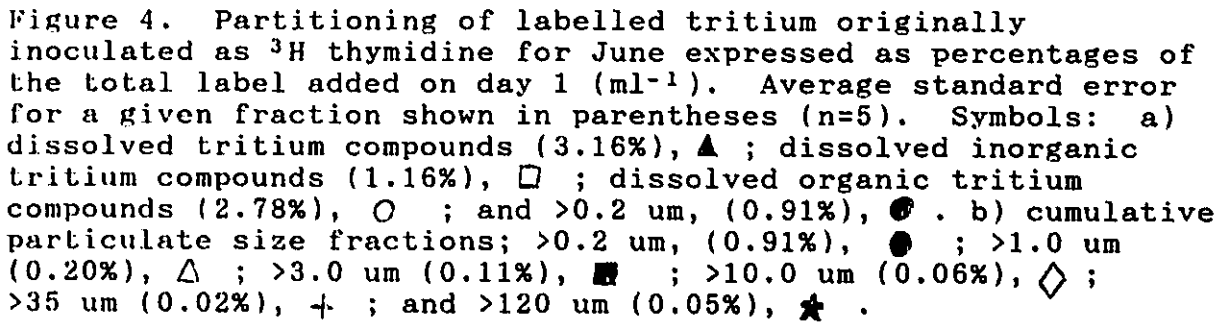


Figure 4. Partitioning of labelled tritium originally inoculated as ^{3}H thymidine for June expressed as percentages of the total label added on day 1 (ml^{-1}). Average standard error for a given fraction shown in parentheses (n=5). Symbols: a) dissolved tritium compounds (3.16%), ▲ ; dissolved inorganic tritium compounds (1.16%), □ ; dissolved organic tritium compounds (2.78%), ○ ; and >0.2 um, (0.91%), ● . b) cumulative particulate size fractions; >0.2 um, (0.91%), ● ; >1.0 um (0.20%), △ ; >3.0 um (0.11%), ■ ; >10.0 um (0.06%), ◇ ; >35 um (0.02%), + ; and >120 um (0.05%), ★ .

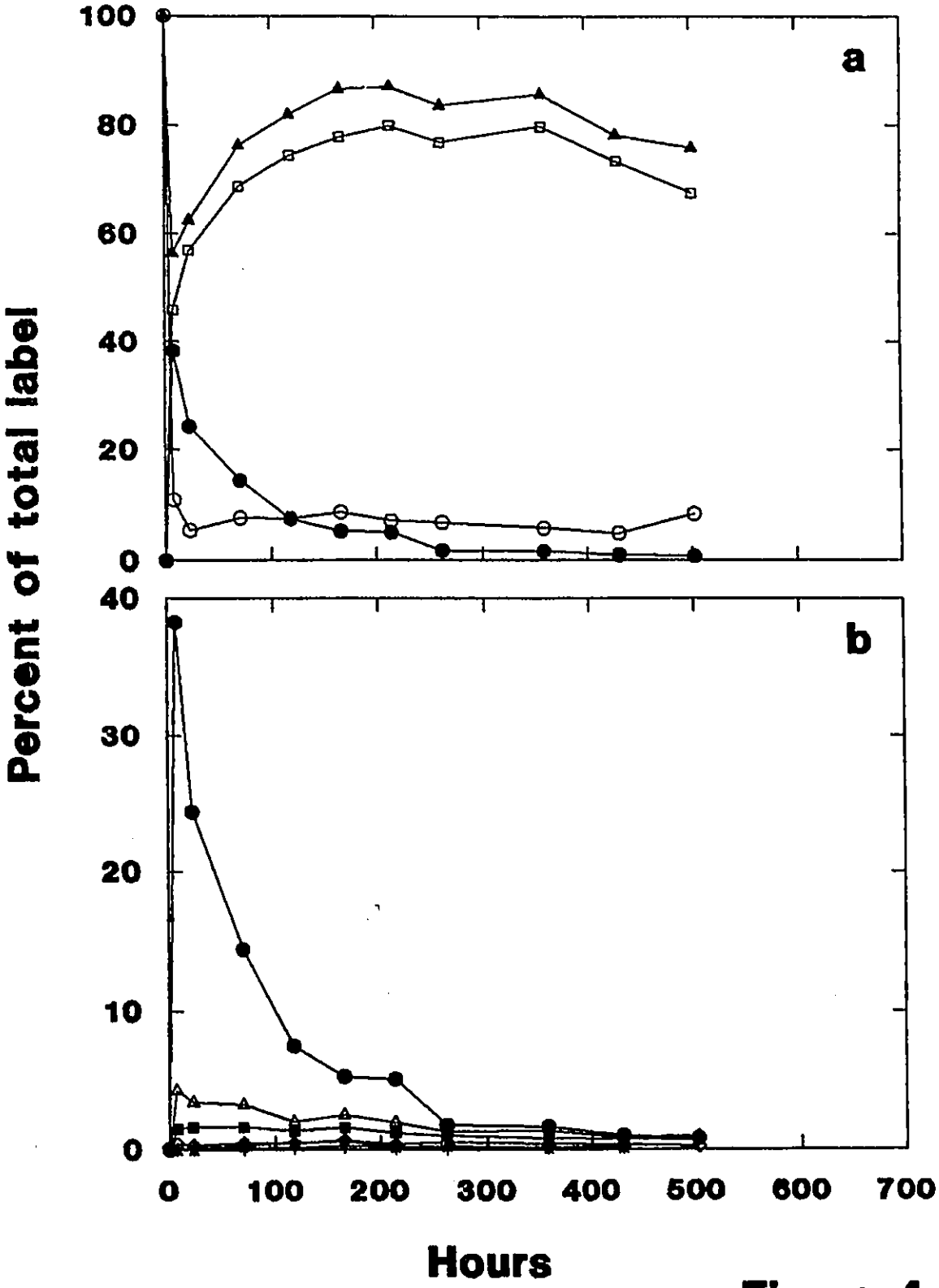

a
b
Percent of total label
Hours
100
80
60
40
20
0
40
30
20
10
0
0
100
200
300
400
500
600
700

Figure 4

Figure 5. Partitioning of labelled tritium originally inoculated as ^{3}H thymidine for August expressed as percentages of the total label added on day 1 (ml^{-1}). For August, 285 and 357 hours represent 5 enclosures while 429 and 525 hours represent 4 enclosures. Average standard error for a given size fraction are shown in parentheses. Symbols: a) dissolved tritium compounds (5.28), ▲ ; dissolved inorganic tritium compounds (6.63), □ ; dissolved organic tritium ccmpounds (4.98), ○ ; and >0.2 um (0.92%), ● . b) cumulative particulate size fractions; >0.2 um, (0.92%), ● ; >1.0 um (1.08%), △ ; >3.0 um (0.88%), ■ ; >10.0 um (0.67%), ◇ ; >35 um (0.16%), + ; and >120 um (0.15%), ★ .

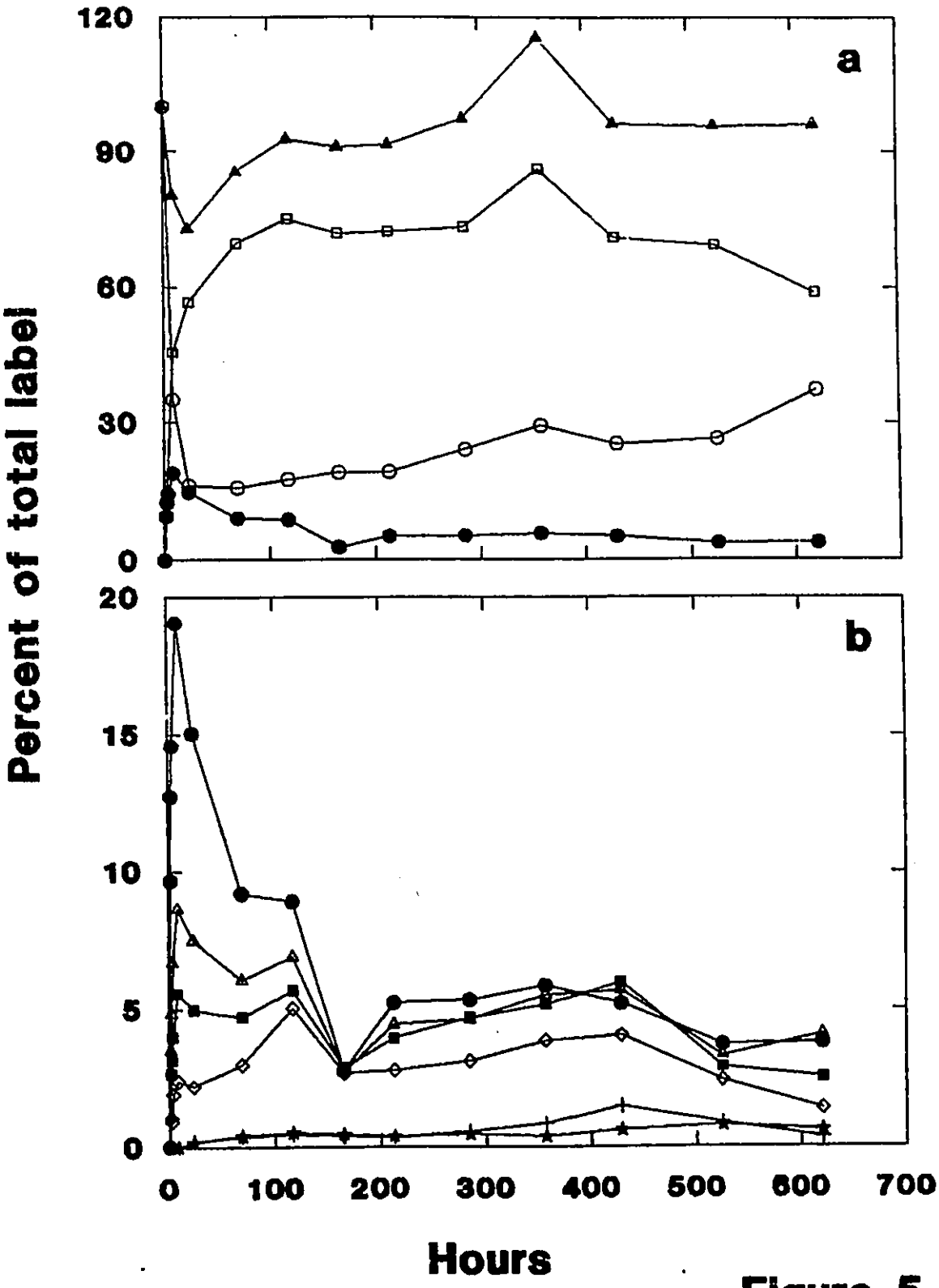

120
90
60
30
0
a
20
15
10
5
0
b
0
100
200
300
400
500
600
700
Percent of total label
Hours

Figure 5

experiments. These results differ from glucose in the rate of loss of [^{3}H] from bacteria in comparison to [^{14}C] (see section constraints on modelling) and in the lower percentage of label retained by particles at any given time. Otherwise tracer dynamics appear very similar and serve as a verification of the observed [^{14}C] glucose flux.

Figures 6 and 7 show the results from the bicarbonate labelled enclosures for the same experiments. In June, the majority of label taken up is by small autotrophic picoplankton (0.2-3 um), while in August it was taken up principally by organisms in the 10-35 um fraction. Over the course of the experiment less than 10% of the label incorporated into particles is found in the >120 um fraction.

In both June and August, only a small amount of the [^{14}C] glucose was transferred to the largest size fraction over the course of the experiment, similar to that observed by Ducklow et al. (1986). The present study was performed in freshwater as opposed to marine for that of Ducklow et al. and, additionally, took place at different times of the year with different water temperatures (15°-17° and 25°-27° compared to 8°-10° C in the case of Ducklow et al.) Given these differences, the similarity of the tracer dynamics suggest that these results are typical of aquatic environments. This supports the conclusion of Ducklow et al. that the components of the microbial loop appear to be a sink for carbon with most carbon being remineralized rather than

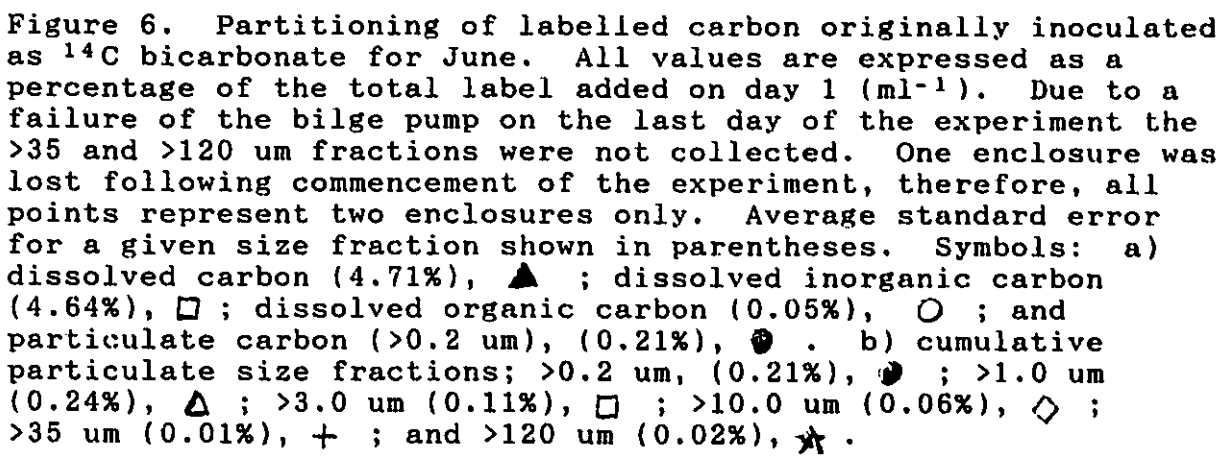


Figure 6. Partitioning of labelled carbon originally inoculated as ^{14}C bicarbonate for June. All values are expressed as a percentage of the total label added on day 1 (ml^{-1}). Due to a failure of the bilge pump on the last day of the experiment the >35 and >120 um fractions were not collected. One enclosure was lost following commencement of the experiment, therefore, all points represent two enclosures only. Average standard error for a given size fraction shown in parentheses. Symbols: a) dissolved carbon (4.71%), ▲ ; dissolved inorganic carbon (4.64%), □ ; dissolved organic carbon (0.05%), ○ ; and particulate carbon (>0.2 um), (0.21%), ● . b) cumulative particulate size fractions; >0.2 um, (0.21%), ● ; >1.0 um (0.24%), △ ; >3.0 um (0.11%), □ ; >10.0 um (0.06%), ◇ ; >35 um (0.01%), + ; and >120 um (0.02%), ✱ .

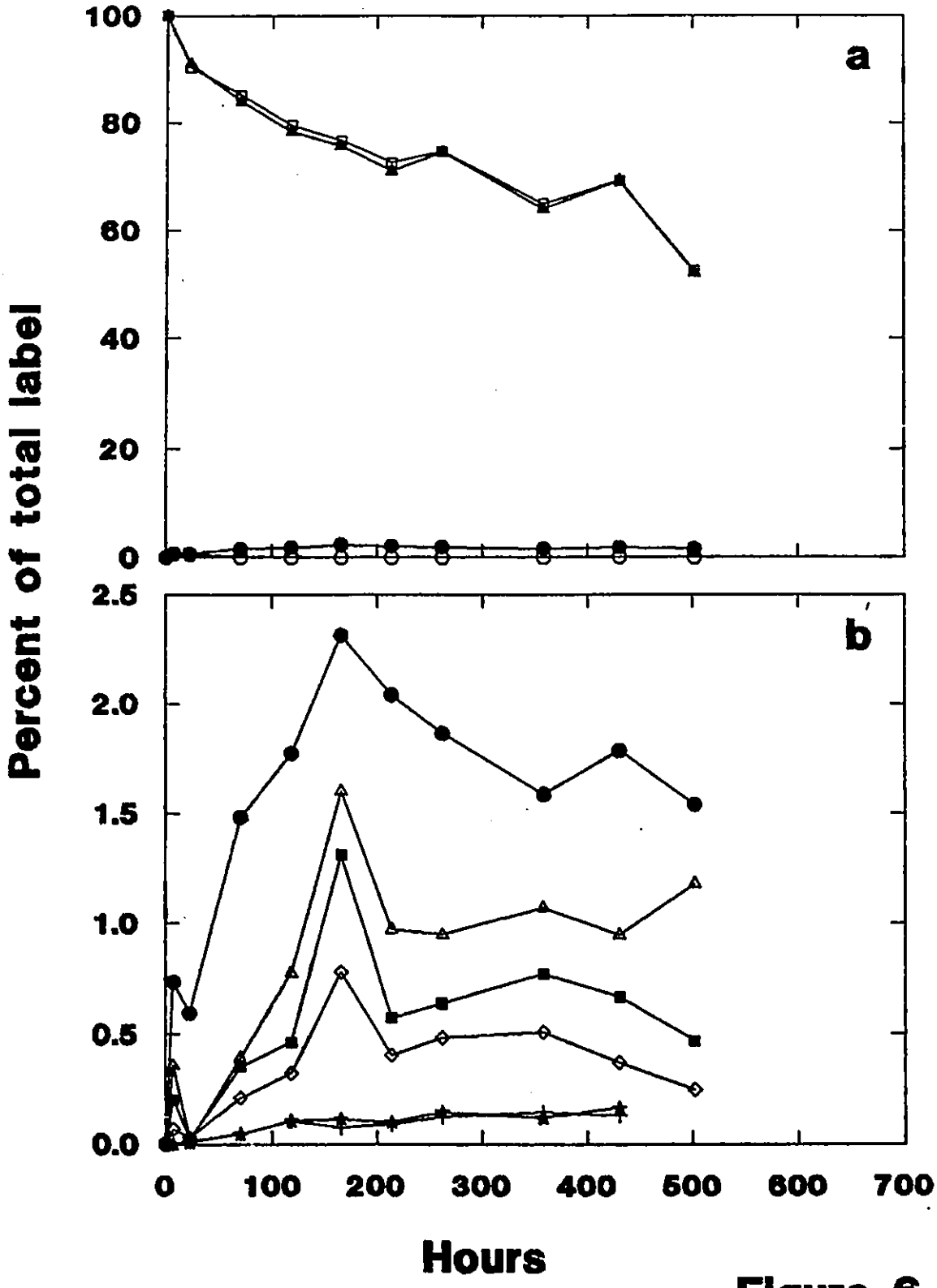

a
b
100
80
60
40
20
0
2.5
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
700
Percent of total label
Hours

Figure 6

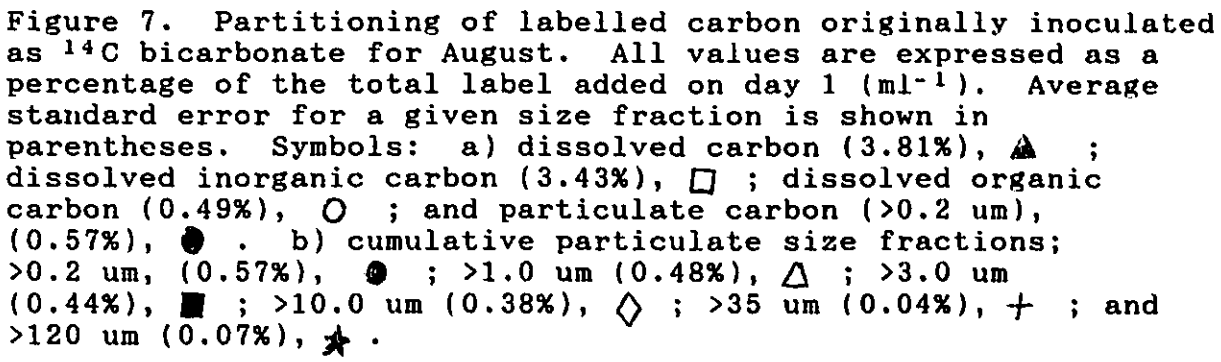


Figure 7. Partitioning of labelled carbon originally inoculated as ^{14}C bicarbonate for August. All values are expressed as a percentage of the total label added on day 1 (ml^{-1}). Average standard error for a given size fraction is shown in parentheses. Symbols: a) dissolved carbon (3.81%), ▲ ; dissolved inorganic carbon (3.43%), □ ; dissolved organic carbon (0.49%), ○ ; and particulate carbon (>0.2 um), (0.57%), ● . b) cumulative particulate size fractions; >0.2 um, (0.57%), ● ; >1.0 um (0.48%), △ ; >3.0 um (0.44%), ■ ; >10.0 um (0.38%), ◇ ; >35 um (0.04%), + ; and >120 um (0.07%), ★ .

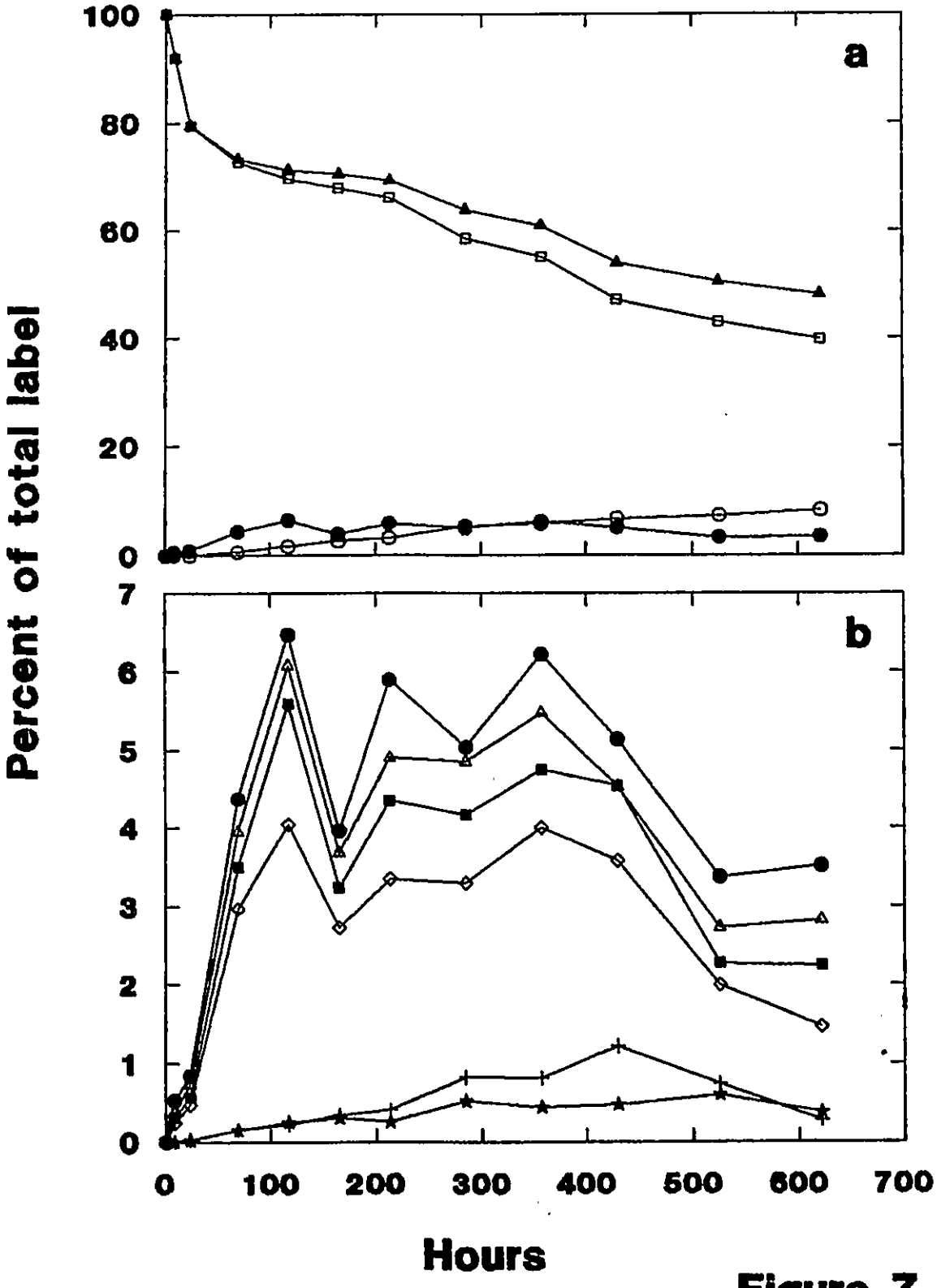

Percent of total label
100
80
60
40
20
0
a
7
6
5
4
3
2
1
0
b
0
100
200
300
400
500
600
700
Hours

Figure 7

passed to larger crustaceans.

Sherr et al. (1987b) felt that singling out bacteria as a sink may be misleading, based on the results of Davies (1984) in which only 1.2% of a bicarbonate label was recovered in crustaceans. In the present study, in both June and August, only a small fraction of the bicarbonate label was transferred to the largest crustacean fraction. This suggests that although bacteria appear to be a sink, Sherr et al. (1987b) were correct in stating that, in comparison, autotrophs can also be considered a sink. Grazing of autotrophs by crustaceans appears to be inefficient with most nutrients presumably lost either to respiration or sedimentation of autotrophs, or grazing by smaller heterotrophs such as protozoans. These results are similar to those of Forsberg (1985) who showed that in freshwater lakes (temperate and tropical) grazing generally accounts for less than 20% of total algal carbon losses. To my knowledge, the present study is the first of its kind to verify this observation by comparing carbon transfer to higher trophic levels from both heterotrophic bacteria and autotrophs in the same system.

Given that carbon flow from both heterotrophic and autotrophic sources appears to be inefficient, do heterotrophic bacteria actually contribute significant amounts of the total carbon flow to crustaceans? Simple comparisons of the percentages of label incorporated by the >120 um

fraction from bacteria and algae, such as described above, are not sufficient for obtaining comparative quantitative estimates of the flux of carbon to crustaceans from both bacterial and algal sources for two reasons. First, if bacterial and algal standing stocks differ, then an identical percentage of tracer incorporated by crustaceans could still represent different amounts of total carbon due to the differing specific activities. Second, most of the added [^{14}C] bicarbonate remains in this form over the course of the experiment (figs. 6 and 7) and therefore is always available for algal uptake, unlike the [^{14}C] glucose which is removed by the bacterioplankton within the first few hours of the experiment (figs. 2 and 3). All else being equal, the crustaceans could receive a greater influx of label from the autotrophs due to the constant uptake of additional label.

To determine the significance of heterotrophic bacteria as a carbon source, quantitative comparisons of the flux of carbon to crustaceans from bacterial and algal sources must be obtained. To accomplish this I have used compartmental modelling (Riggs, 1963). This analysis will be addressed in the following sections.

Compartmental modelling

This section will deal with a general overview of compartmental modelling, centering on the methodology and

assumptions of this approach to tracer dynamics. Fig. 8 shows an example of a compartmental model. Compartments of the model are represented as squares lettered A-C. Arrows represent the transfer of a substance into or out of a compartment. The rate of transfer of a substance out of a compartment is assumed to be proportional to the amount of that substance present in the compartment at any given time. The proportion of material lost per unit time (h^{-1} in this paper) is described by a rate constant (k_1-k_3 in fig. 8). The change in the amount of a substance can therefore be described in terms of differential equations which, for the model of fig. 8, are

$$d[A]/dt = -(k_1 + k_2)\,[A]$$
$$d[B]/dt = k_2\,[A]$$
$$d[C]/dt = k_1\,[A] - k_3\,[C]$$

where A and C on the right hand side of the equations represents the amount of a substance present in compartments A and C at a given time and k_1-k_3 represent the rate constants.

In the case of the present study, the compartments represent planktonic size fractions based on the assumption that aquatic food webs can be described in terms of distinct pools of carbon (i.e. different sizes of heterotrophs and autotrophs and dissolved pools). Rate constants will describe the rate of transfer of carbon within the plankton.

Figure 8. Example of compartmental model. k_1-k_3 represent rate constants describing the rate of transfer of a substance between compartments. Squares lettered A-C represent the compartments of the model.

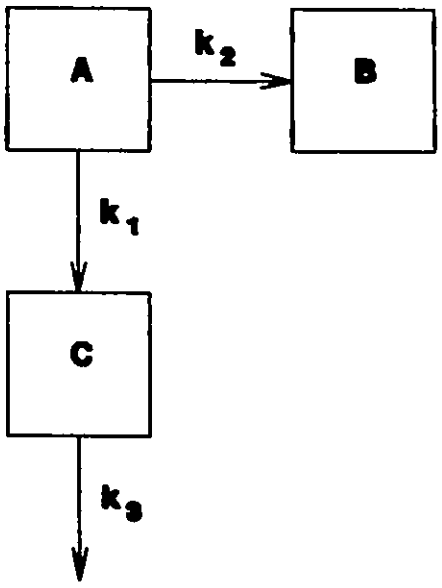


Figure 8

The procedure followed was to first solve the differential equations for a given model to obtain the rate constants describing the flow of carbon between compartments. The data used for solving these equations are the observed changes in radioactivity in a compartment over time, i.e. figs. 2, 3, 6, and 7. Models which minimize bias and residual sums of squares between predicted [^{14}C] content and observed [^{14}C] content of a compartment were taken to most closely approximate the flow of carbon through the system. This is most easily visualized by graphing predicted and observed values, therefore graphs of this type will be shown for each model. Residual sums of squares will also be given for each model as a relative means of comparing the fit of the models. In some cases, comparisons of the generated rate constants to corresponding estimates of grazing, respiration, etc., obtained from the literature, will serve as additional means of assessing the models.

Having generated rate constants describing [^{14}C] flux, total carbon flow to crustaceans was estimated by substituting these rate constants back into the differential equations of the model. The standing stock biomass of either bacteria or algae, depending on the model, was inserted into the corresponding compartment of the equations, replacing the [^{14}C] data originally used to calculate the rate constants. The differential equations were iterated, (from 0 to 24 hours was used in this paper), which produced estimates of the mass

of carbon that would be transferred to the various compartments of the model over this period of time. This process was carried out for both bicarbonate and glucose data, and a comparison of the results indicates the relative contribution of bacterial and algal carbon to the total carbon input to crustaceans.

Compartmental modelling is based on several simplifying assumptions which must be verified if this approach is to be applied to this study. First, compartments are assumed to be in steady state. Dynamics of the plankton in the enclosures were followed over the course of the experiment to identify departures from this assumption (figs. 9 and 10). Bacteria in both experiments and flagellates in June show no systematic departure from steady state. Crustaceans appear to decrease over the course of both experiments, however, this may not be critical as grazing on both heterotrophs and autotrophs would be reduced simultaneously. In August, flagellates show an increase and decrease in numbers which does not seem to have any large effect on bacterial carbon flow since it is not reflected in a change in bacterial numbers. There is a rapid decrease in chl a in June and a less pronounced decrease in August which may have some bearing on the results. These latter two departures from steady state will be considered following calculation of carbon flow in June and August.

Second, [^{14}C] is assumed to be homogenously dispersed

Figure 9. Crustacean, heterotrophic flagellate, bacteria, and phytoplankton (chl a) dynamics for June. Some crustacean samples were lost due to spillage of samples or failure of the pump.

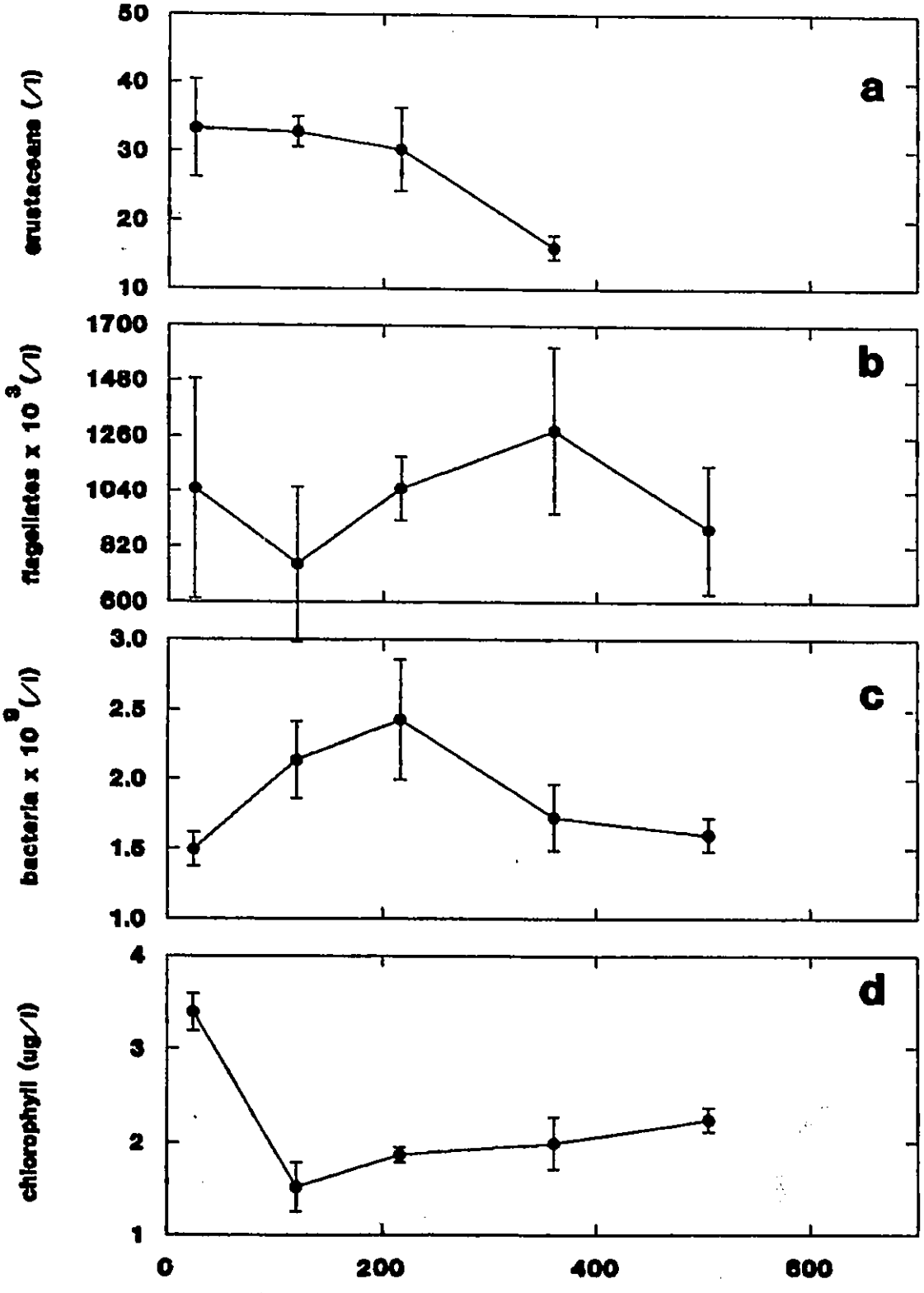

crustaceans (/l)
50
40
30
20
10
a
flagellates x 10 3 (/l)
1700
1480
1260
1040
820
600
b
bacteria x 10 9 (/l)
3.0
2.5
2.0
1.5
1.0
c
chlorophyll (ug/l)
4
3
2
1
d
0
200
400
600
Hours

Figure 9

Figure 10. Crustacean, heterotrophic flagellate, bacteria, and phytoplankton (chl a) dynamics for August. Time of inoculation was taken as 0 hours. Enclosures were enriched 120 hours prior to this time.

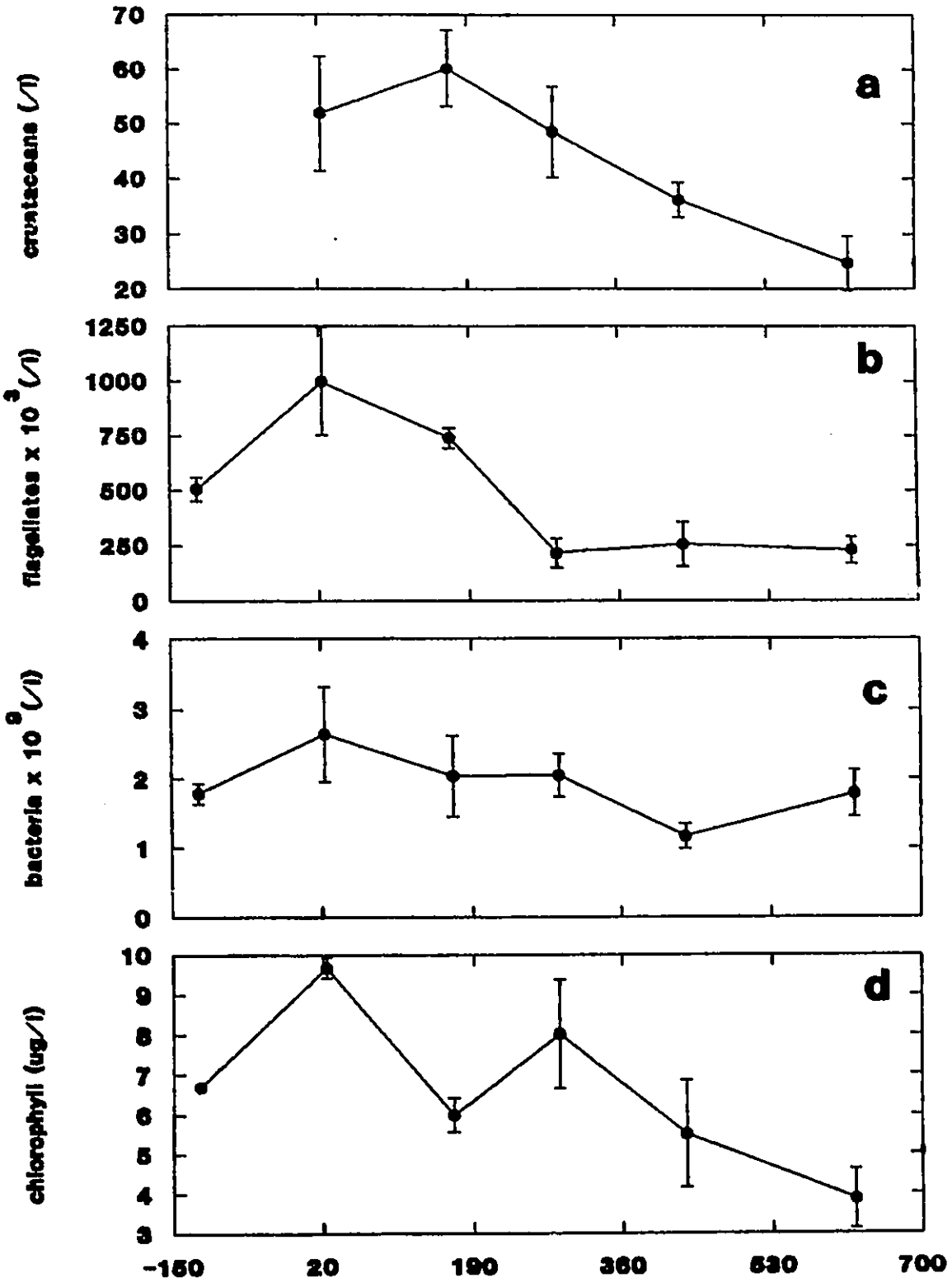

crustaceans (/l)
70
60
50
40
30
20
a
flagellates x 10^3 (/l)
1250
1000
750
500
250
0
b
bacteria x 10^9 (/l)
4
3
2
1
0
c
chlorophyll (ug/l)
10
9
8
7
6
5
4
3
d
-150
20
190
360
530
700
Hours

Figure 10

within a given compartment. On a short time scale, i.e. minutes, this assumption may be violated, but the length of time between samplings in this study should allow for even labelling of the plankton.

Additionally, modelling the results of enclosure studies, such as this, assume that label remains in the pelagic system for a similar length of time as would be found for the open lake. If label is removed faster than would normally occur, label transferred in the enclosures may underestimate the carbon which is actually being transferred by grazing in the open lake. Vézina (1986), working in the same lake with ^{32}P labelled enclosures, found sedimentation values similar to those of open lake environments and, additionally, found little loss of label to periphyton growth on the walls. During the course of my experiments recovery of isotope varied from 70% to over 100% of the added glucose and thymidine labels (figs. 11 and 12) which, coupled with Vézina's results, suggest that most label remains available to crustaceans in the pelagic system and does not quickly sediment from the water column.

For purposes of modelling, the activity of the individual fractions in this study were grouped into 0.2-3, 3-120 and >120 um. In this way, models were kept as simple as possible, yet still allowed a distinction to be made between food items which would be directly accessible to crustaceans, and those which would generally be accessible only by passing

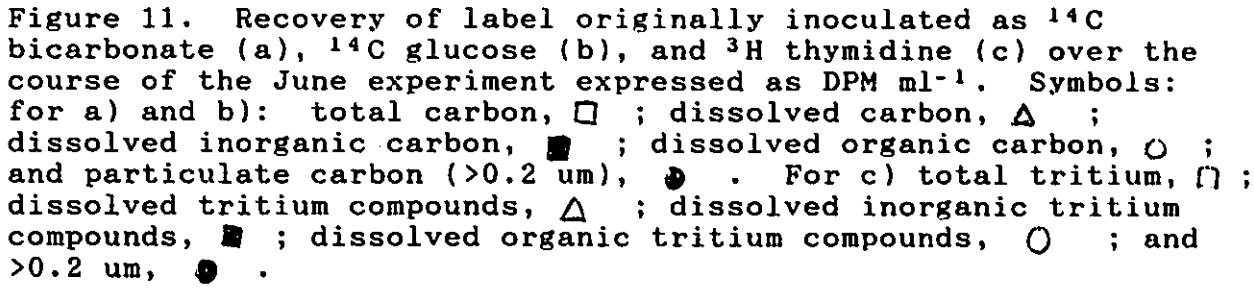


Figure 11. Recovery of label originally inoculated as ^{14}C bicarbonate (a), ^{14}C glucose (b), and ^{3}H thymidine (c) over the course of the June experiment expressed as DPM ml^{-1}. Symbols: for a) and b): total carbon, □ ; dissolved carbon, △ ; dissolved inorganic carbon, ■ ; dissolved organic carbon, ○ ; and particulate carbon (>0.2 um), ● . For c) total tritium, ⊓ ; dissolved tritium compounds, △ ; dissolved inorganic tritium compounds, ■ ; dissolved organic tritium compounds, ○ ; and >0.2 um, ● .

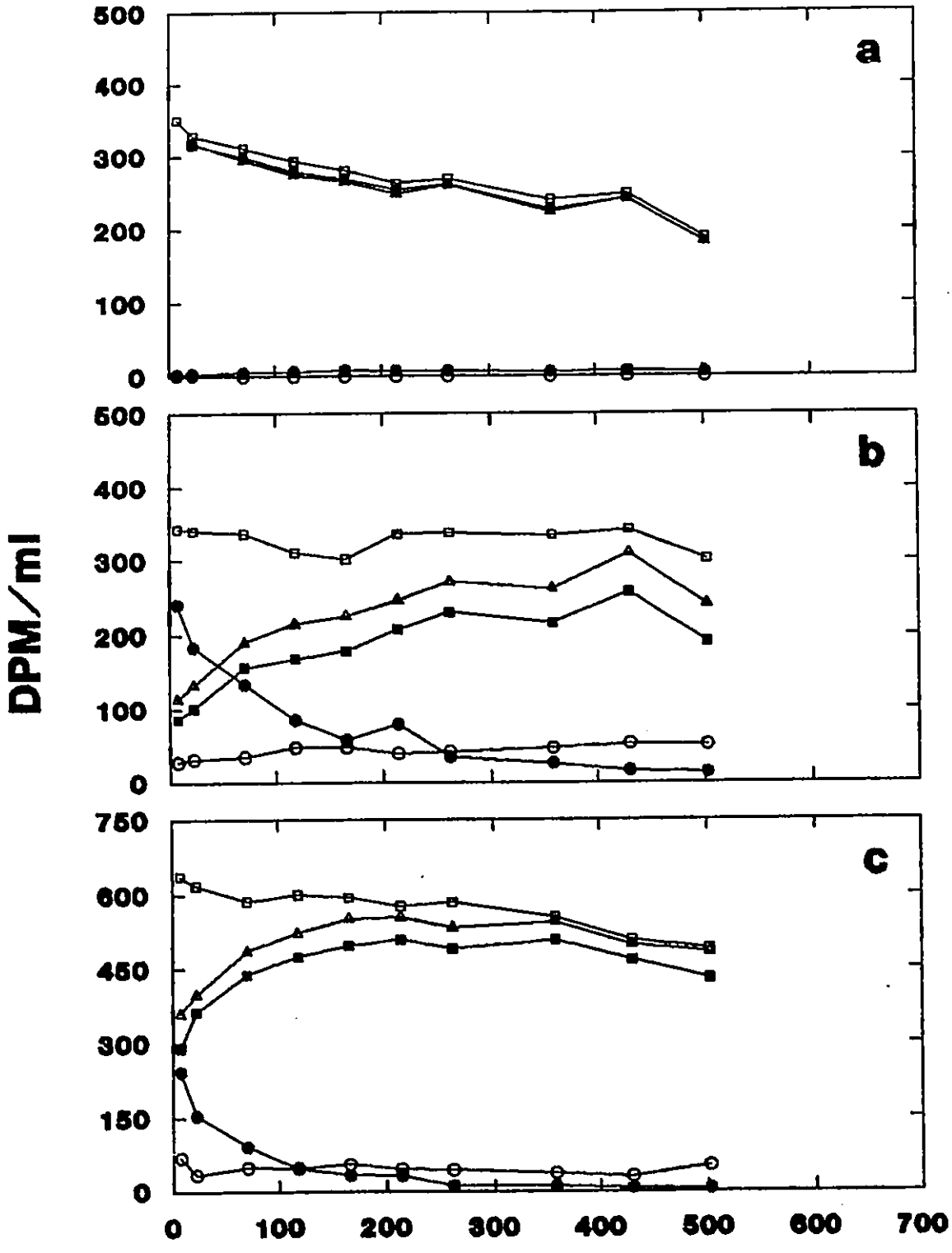

a
b
c
500
400
300
200
100
0
500
400
300
200
100
0
750
600
450
300
150
0
0
100
200
300
400
500
600
700
DPM/ml
Hours

Figure 11

Figure 12. Recovery of label originally inoculated as ^{14}C bicarbonate (a), ^{14}C glucose (b), and ^{3}H thymidine (c) over the course of the August experiment expressed as DPM ml^{-1}. Symbols: for a) and b): total carbon, □ ; dissolved carbon, △ ; dissolved inorganic carbon, ■ ; dissolved organic carbon, ○ ; and particulate carbon (>0.2 um), ● . For c) total tritium, □ ; dissolved tritium compounds, △ ; dissolved inorganic tritium compounds, ■ ; dissolved organic tritium compounds, ○ ; and >0.2 um, ● .

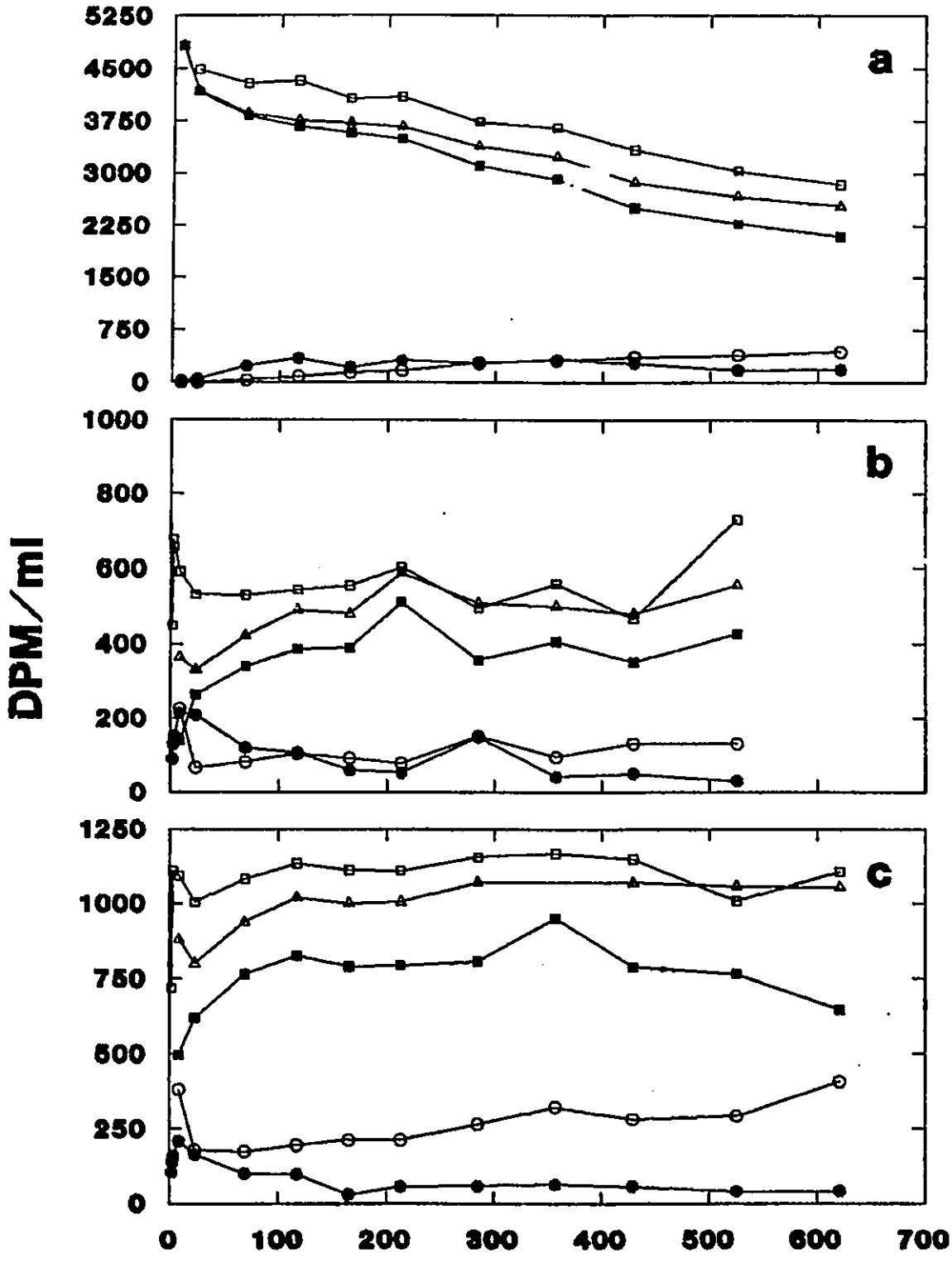

a
b
c
DPM/ml
Hours
5250
4500
3750
3000
2250
1500
750
0
1000
800
600
400
200
0
1250
1000
750
500
250
0
0
100
200
300
400
500
600
700

Figure 12

through the microbial loop involving at least one intermediate trophic level. The 0.2-3 um fraction (heterotrophic bacteria and autotrophic picoplankton) represents the latter food items, while the 3-120 um fraction (larger phytoplankton or heterotrophs such as protozoans and rotifers) represents the former. The means of the [^{14}C] data from the replicate enclosures were used in modelling based on the observation that the isotope dynamics for each enclosure were similar (figs. 13-16).

When analyzing flux of label originally inoculated as [^{14}C] glucose, only events following initial uptake are modelled. Since most DOC measured over the course of the experiment is refractory, this compartment differs prior to and following incorporation of glucose. This results in some changes in carbon dynamics which is most clearly shown by the rate of production of DIC by plankton which decreases by an order of magnitude following uptake of glucose by plankton (fig. 17). Therefore all models for describing [^{14}C] flux through heterotrophs are set with the initial values for the compartments equal to the values when all the original [^{14}C] glucose has been removed from the system. This is taken to be 7 hours for June, and 23 hours for August based on the observation that at these points the initial decrease in DOC ceases and is replaced by a slow increase in DOC over the course of the experiment (figs. 2 and 3). This should correspond approximately to the time when the original glucose

Figure 13. Partitioning of labelled carbon originally inoculated as ^{14}C glucose for June for each individual replicate. The size fractions shown here were used for compartmental modelling. All values are expressed as a percentage of the amount of isotope added to the enclosures on the first day of the experiment. Symbols in a, b, c, and d each correspond to the same enclosure. a) dissolved inorganic carbon, upper curves; dissolved organic carbon, lower curves. b) >120 um fraction. c) pooled 3-120 um fraction (obtained by summing the activities in the 3-10, 10-35, and 35-120 um fractions). d) pooled 0.2-3 um fraction (obtained by summing the activities in 0.2-1 and 1-3 um fractions).

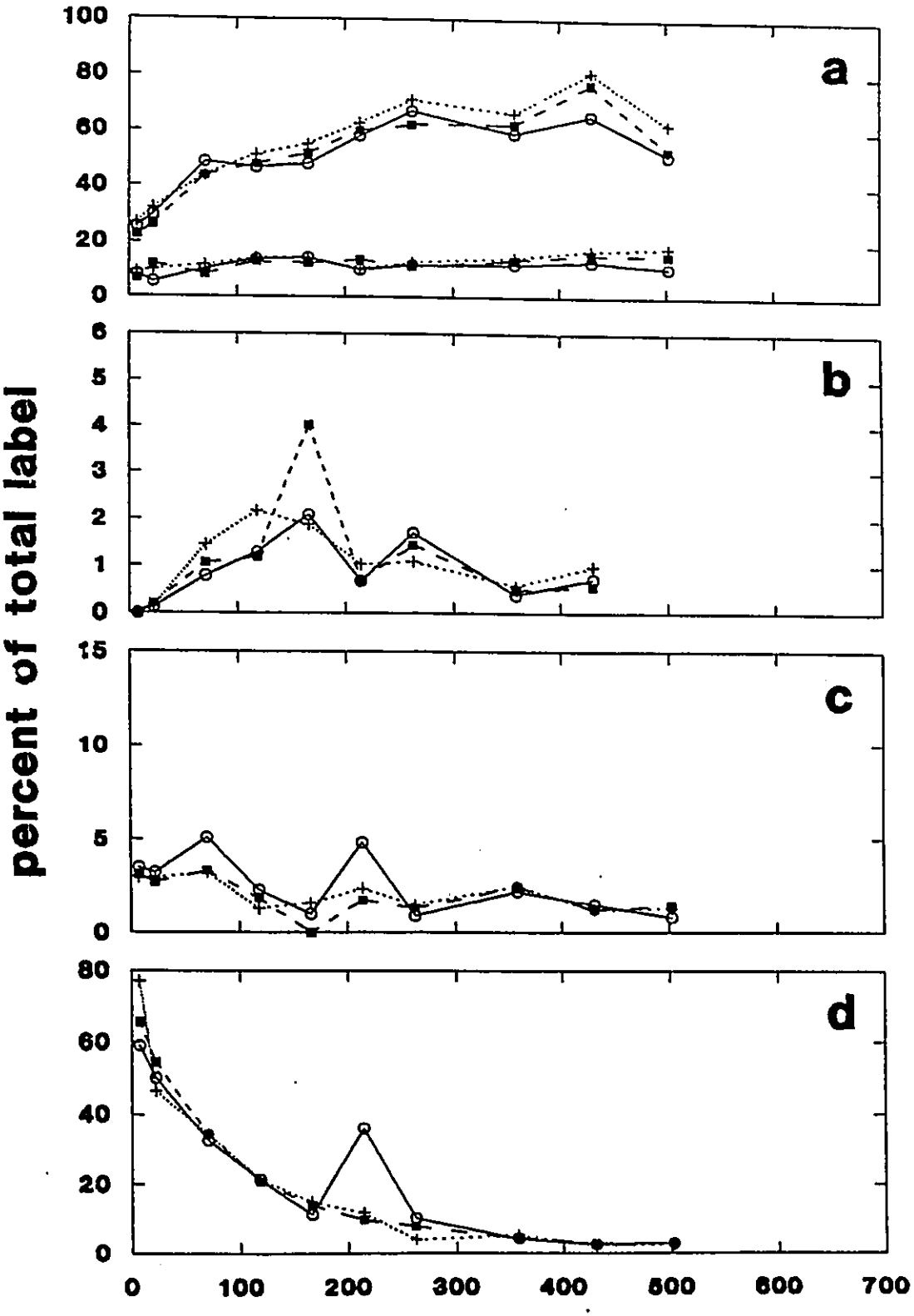

a
b
c
d
percent of total label
Hours
0
100
200
300
400
500
600
700

Figure 13

Figure 14. Partitioning of labelled carbon originally inoculated as ^{14}C glucose for August for each individual replicate. The size fractions shown here were used for compartmental modelling. All values are expressed as a percentage of the amount of isotope added on the first day of the experiment. Symbols in a, b, c, and d each correspond to the same enclosure. a) dissolved inorganic carbon, upper curves; dissolved organic carbon, lower curves. b) >120 um fraction. c) pooled 3-120 um fraction. d) pooled 0.2-3 um fraction.

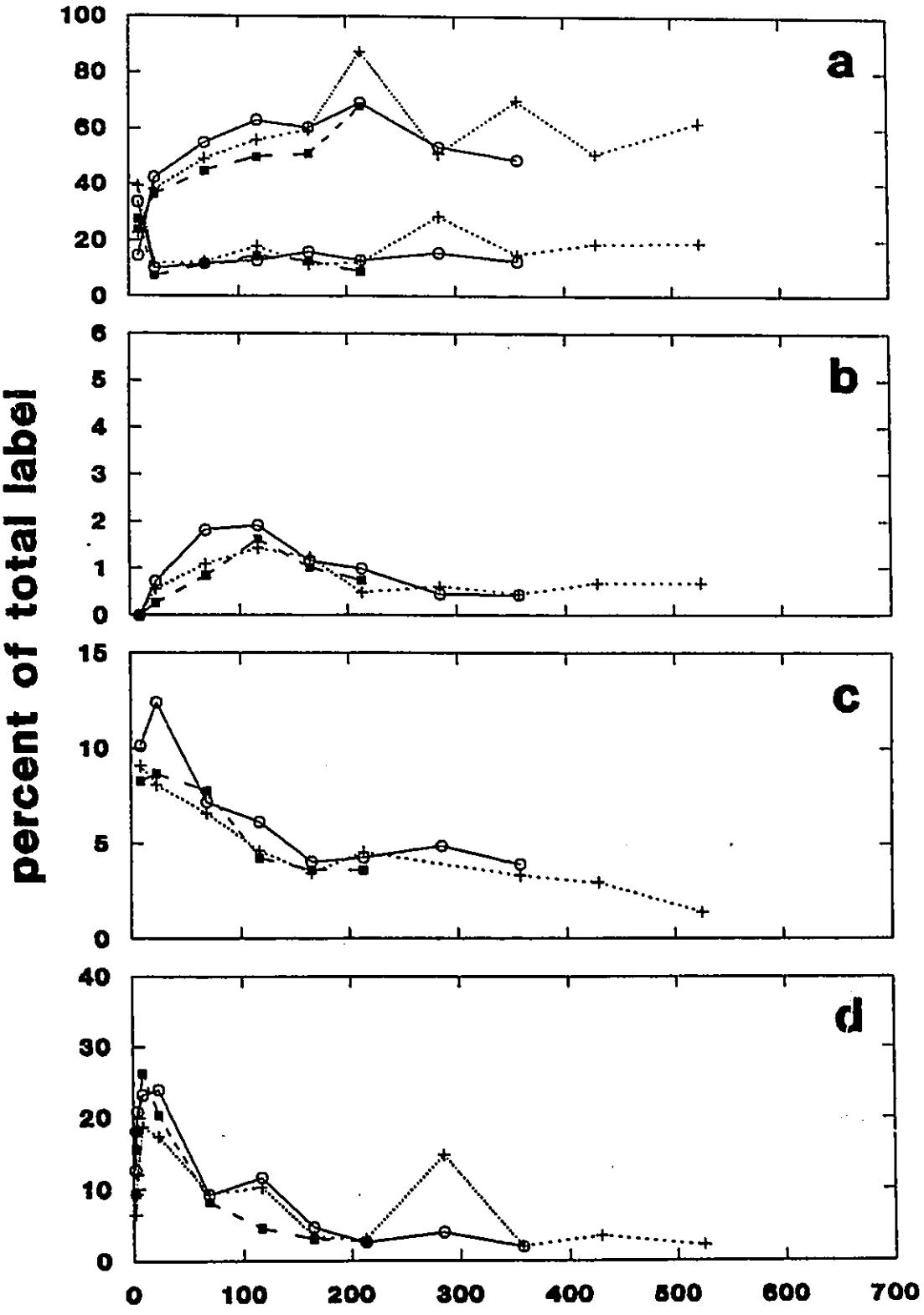

a
b
c
d
percent of total label
Hours
0
100
200
300
400
500
600
700

figure 14

Figure 15. Partitioning of labelled carbon originally inoculated as ^{14}C bicarbonate for June for each individual replicate. The size fractions shown here were used for compartmental modelling. All values are expressed as a percentage of the amount of isotope added on the first day of the experiment. Symbols in a, b, and c each correspond to the same enclosure. One enclosure was lost following the commencement of the experiment. a) >120 um fraction. b) pooled 3-120 um fraction. c) pooled 0.2-3 um fraction.

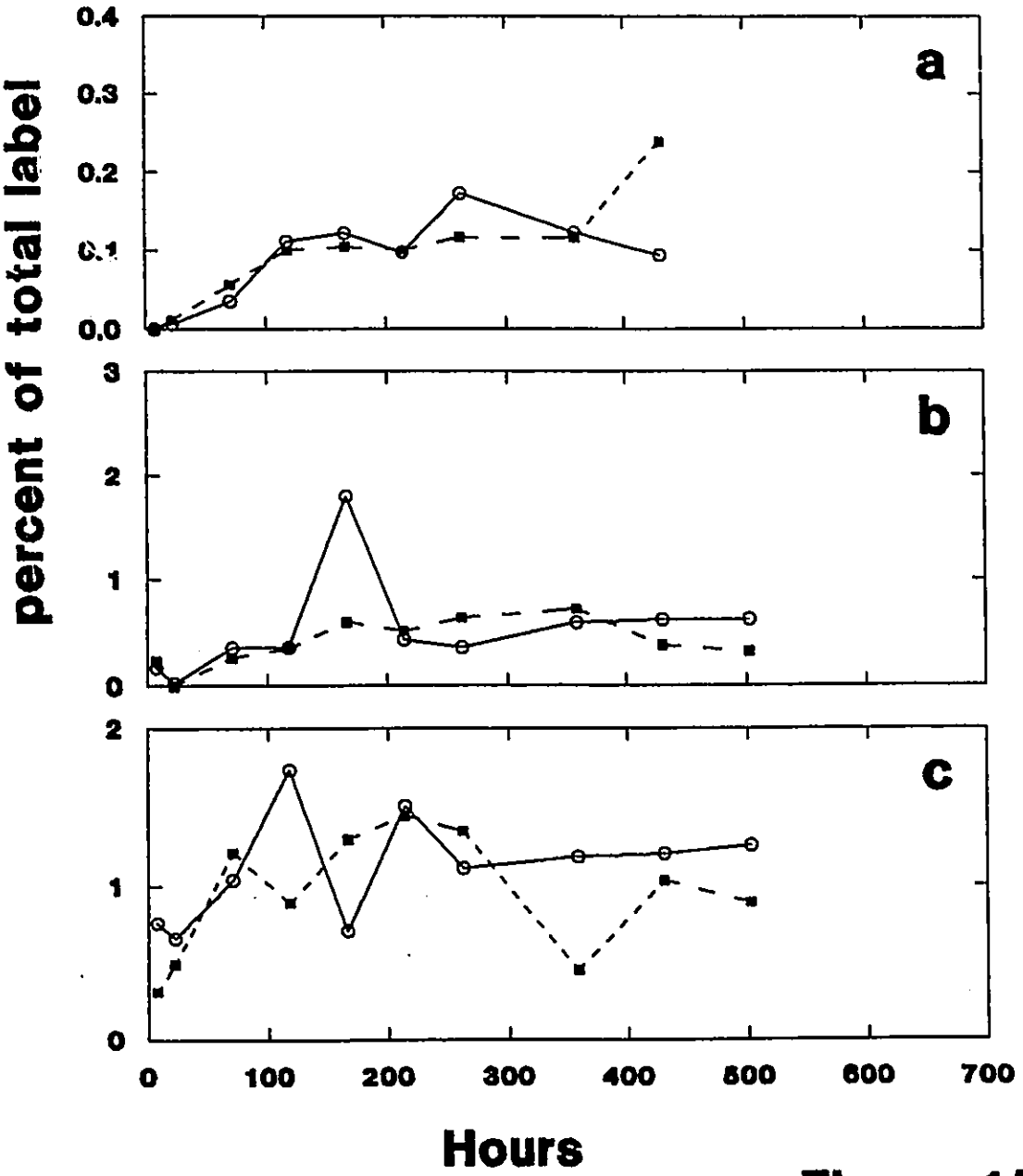

percent of total label
0.4
0.3
0.2
0.1
0.0
a
3
2
1
0
b
2
1
0
c
0
100
200
300
400
500
600
700
Hours

Figure 15

Figure 16. Partitioning of labelled carbon originally inoculated as ^{14}C bicarbonate for August for each individual replicate. The size fractions shown here were used for compartmental modelling. All values are expressed as a percentage of the amount of isotope added on the first day of the experiment. Symbols in a, b, c, and d each correspond to the same enclosure. a) dissolved organic carbon. b) >120 um fraction. c) pooled 3-120 um fraction. d) pooled 0.2-3 um fraction.

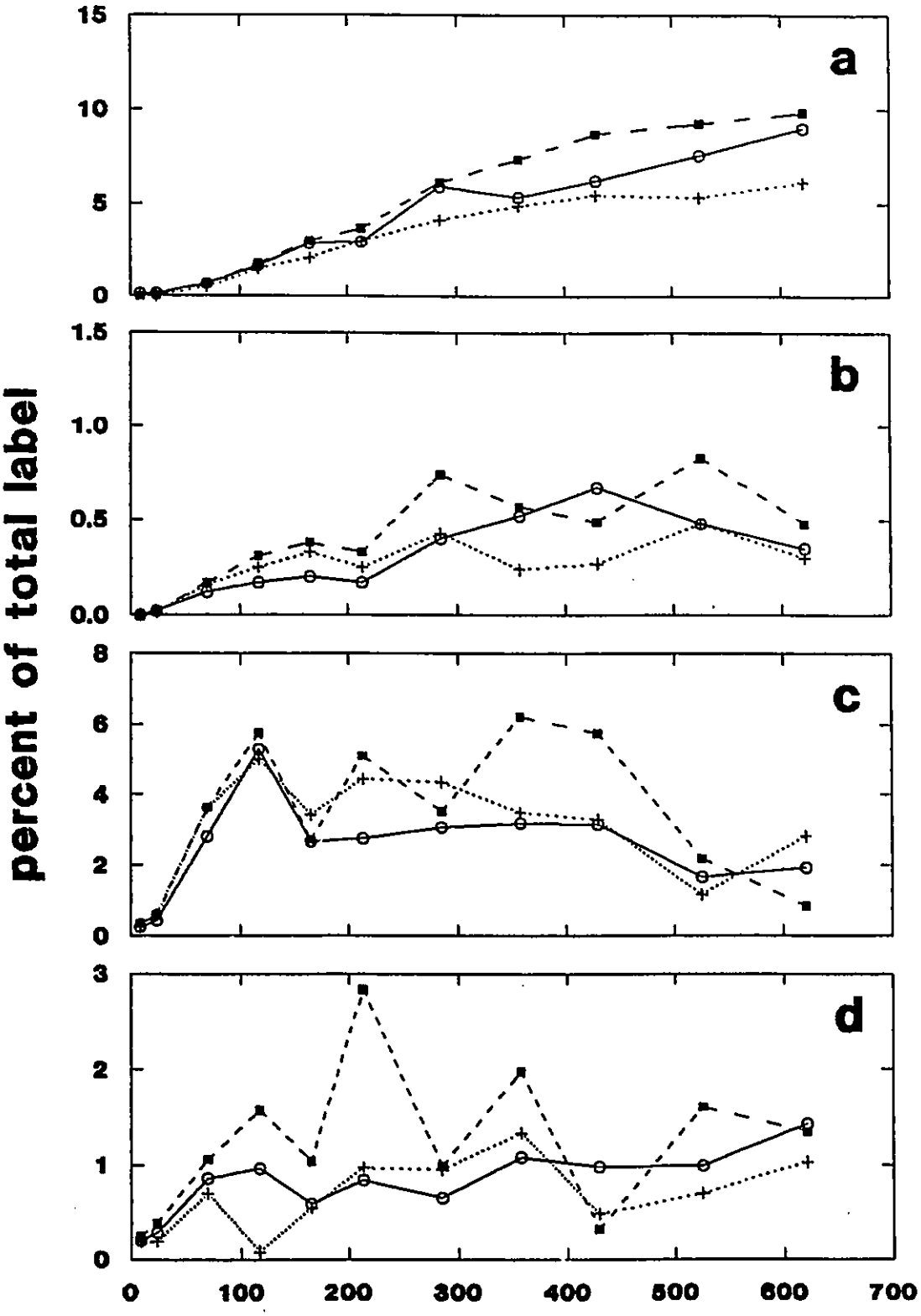

a
b
c
d
15
10
5
0
1.5
1.0
0.5
0.0
8
6
4
2
0
3
2
1
0
0
100
200
300
400
500
600
700
percent of total label
Hours

Figure 16

Figure 17. Compartmental models showing rate of production (proportion h^{-1}) of dissolved inorganic carbon (DIC) from particles (>0.2 um) during initial uptake (a, August; c, June) and following depletion (b, August; d, June) of ^{14}C glucose (DOC). DOC was depleted at approximately 7 hours for June and 23 hours for August. Numbers are the calculated rate constants showing the drop in DIC production following initial uptake.

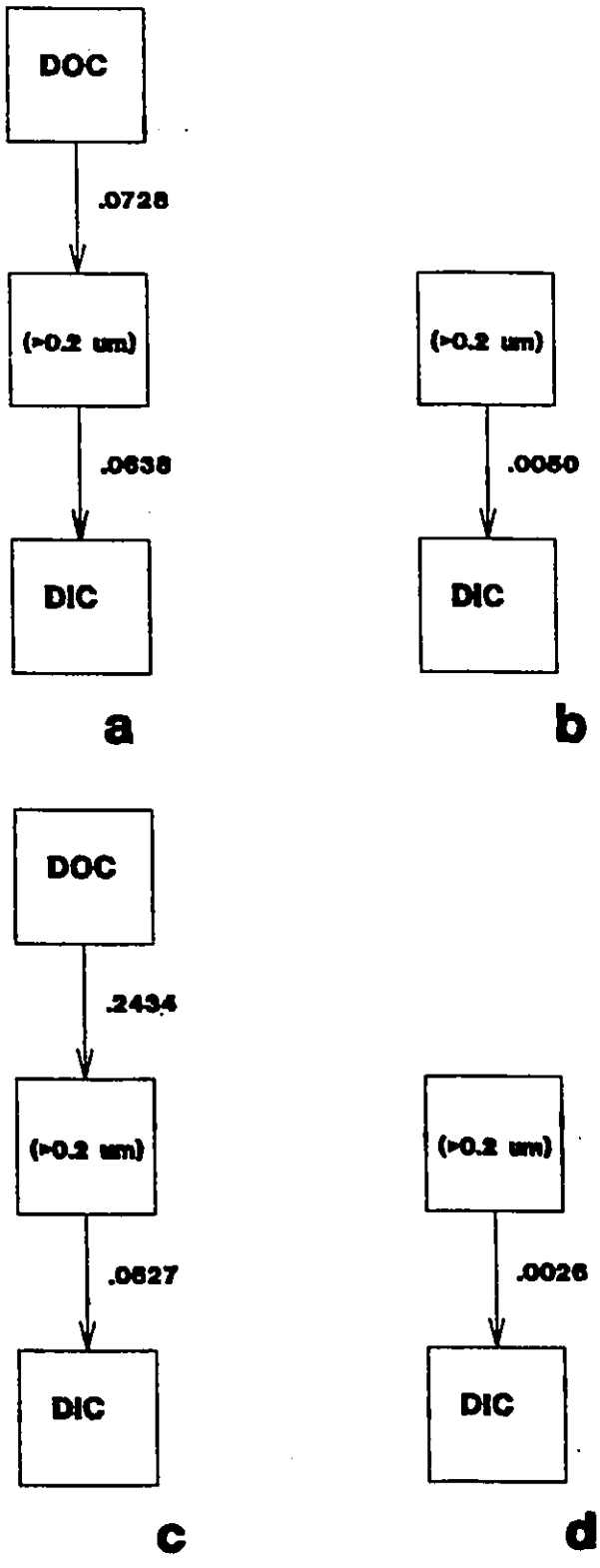

DOC
.0728
(>0.2 um)
.0638
DIC
a
(>0.2 um)
.0050
DIC
b
DOC
.2434
(>0.2 um)
.0627
DIC
c
(>0.2 um)
.0026
DIC
d

Figure 17

label has been removed and refractory DOC compounds are beginning to be released.

Models developed for the glucose enclosures are considered to represent heterotrophic dynamics (i.e. heterotrophic bacteria, bacteriovores and crustaceans) while the bicarbonate enclosures are taken to represent autotrophic dynamics (various sizes of autotrophs grazed by crustaceans). The terms heterotrophic and autotrophic models will be equivalent to glucose and bicarbonate models, respectively.

Constraints on modelling

In formulating and testing models to describe carbon flux, I found that two major constraints were placed on model structure. Given that these constraints restrict the possible structure of most of the models, they will be discussed separately in this section prior to presenting any of the individual models used to describe carbon flux. They can be summarized as: 1) a given compartment generally could not be depicted as simultaneously receiving carbon from more than one compartment. For example, DIC could not be modelled as arising from all three particulate fractions simultaneously and crustaceans could not be modelled as simultaneously grazing on both bacteria and bacteriovores. 2) recycling of DOC could not be incorporated into the models.

The first constraint results from having no information

within the data set itself on the relative contribution of carbon to a given compartment from individual particulate size fractions. If one postulates such a model, the simplest statistical solution of the differential equations predicts that all transfer of carbon to a compartment arises from one compartment rather than two or three.

A potential way of circumventing this problem would be to independently determine some of the rate constants in question and fix them in the model. In this way a specific amount of label would be transferred through these routes and excess label would be forced through alternate routes. Values from the literature could have been used for this, however, I chose not to do this to avoid possible sources of error resulting from using rates not specifically derived from this study site.

The solution generally used was to structure two models to depict two extremes of a given carbon flux. For example, for the August glucose dynamics, two models were formulated, the first depicted crustaceans as grazing exclusively on bacteriovores (3-120 um) while the second incorporated direct grazing of bacteria (0.2-3 um) by crustaceans. In this way a range of values was produced with the actual value expected to fall somewhere between these extremes. Prior to proceeding with this approach I verified that intermediate models which depicted carbon flow to a compartment arising from several compartments simultaneously did not change the overall results

to any extent. I arbitrarily fixed rates in several models such that carbon flow to a given compartment was forced through several routes simultaneously. As expected, carbon flow to crustaceans did fall within the calculated range of values. Therefore, having determined this, intermediate models were not considered further.

For the specific case of DIC production the results from the [^{3}H] thymidine enclosures were intended to provide information on respiration as a means to avoid this modelling constraint. This was based on the assumption that the [^{3}H] label would be incorporated into bacterial DNA and would be released only through some form of cell death, presumably grazing. I had assumed the [^{14}C] label would be lost at a faster rate than [^{3}H] and the difference between the two rates would be an indication of the rate of bacterial respiration. I could have then fixed this rate in the model prior to solving for the remaining rate constants. The opposite results were found, however, in that [^{3}H] was lost from the bacteria faster than [^{14}C] (table 3 and also seen by contrasting loss of [^{3}H] from particles in fig. 4 and 5 with loss of [^{14}C] in fig. 2 and 3, respectively). Consequently, these results did not provide me with information on bacterial respiration.

Two explanations are conceivable for the difference in loss of [^{14}C] and [^{3}H]. Recycling of [^{14}C] to the bacteria may have occurred through reuptake of labelled organic

Table 3: Comparison of rates of loss of [^{3}H] and [^{14}C] from 0.2-3 um fraction for June and August (proportion h^{-1}). These rates are calculated from simple models involving a single compartment (0.2-3 um) and a one way loss from this compartment. Initial values for the models was taken as 7 hours for June and 23 hours for August representing the points when the original glucose and thymidine have been depleted.

	June	August
Rate of loss of [^{3}H]	.0178	.0152
Rate of loss of [^{14}C]	.0095	.0107

molecules excreted by grazers. This could have resulted in a slower rate of loss of [^{14}C] from particles. Although this prevented estimation of bacterial respiration, as just outlined, it could potentially have provided the means to estimate the amount of recycling occurring in the plankton. However, an alternative explanation for the observed [^{14}C] and [^{3}H] results is related to increasing evidence that RNA and protein, in addition to DNA, may be labelled during uptake of [^{3}H] thymidine (Carman et al. 1988, Hollibaugh 1988, Servais et al. 1987, Wicks and Robarts 1987). If labelled RNA and protein is broken down faster than [^{14}C] labelled molecules, or more simply if the [^{14}C] is conserved and recycled to a greater extent as Carman et al. (1988) suggests, then faster loss of [^{3}H] from bacteria could have resulted. Without specific information on the metabolic activity of bacteria at this study site I cannot determine whether recycling of carbon, respiration of protein and RNA or both is occurring. Therefore, this led to the second constraint that recycling of organic nutrients could not be incorporated into the models. Instead the models were depicted as simply the net loss and accumulation of label over time. Given these constraints the models are presented in the following sections.

June models

This section is divided into two main parts. First,

models for quantifying carbon flux are presented for both the June glucose and bicarbonate data, centering on the reasons for choosing a particular structure for a given model. The second part deals with calculating carbon flow to crustaceans using the formulated models. This same format will be used for presentation of the August data.

Glucose - Figures 18 (model 1) and 19 (model 2) show the models chosen to quantify carbon flux to crustaceans from bacteria (residual sums of squares for these and all other models considered in the following sections are given in table 4).

Neither model 1 nor 2 incorporates direct grazing of crustaceans on bacteria. Copepods comprised 72% of the >120 um fraction and, since they are not considered to effectively graze bacteria (Nival and Nival 1976, Kibby 1971), would generally acquire bacterial carbon only through indirect grazing on bacteriovores. Although the cladocerans present may have directly ingested some bacteria (Pace et al. 1983, Porter et al. 1983, Peterson et al. 1978) this should be insignificant owing to the small numbers of cladocerans present at this time.

Due to the constraints outlined previously, models 1 and 2 depict respiration and excretion of DIC and DOC from either bacteria (model 1) or bacteriovores (model 2). I considered the >120 um fraction contribution to DIC and DOC insignificant

Figure 18. Model 1 used to describe June heterotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). B'vore refers not only to bacteriovores but also larger ciliates or rotifers which may ingest these bacteriovores prior to being consumed by crustaceans. DIC is dissolved inorganic carbon and DOC is dissolved organic carbon. b) and c) shows the fit of the regression lines to the experimental data. For clarity the separate planktonic size fractions, 0.2-3, 3-120 and >120, have been expressed as cumulative particulate size fractions >0.2, >3, and >120 um. This allows points for several size fractions to be plotted on the same graph without any points overlapping. Symbols: b) cumulative particulate size fractions for >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; dissolved organic carbon, □ ; >0.2 um, + .

model 1

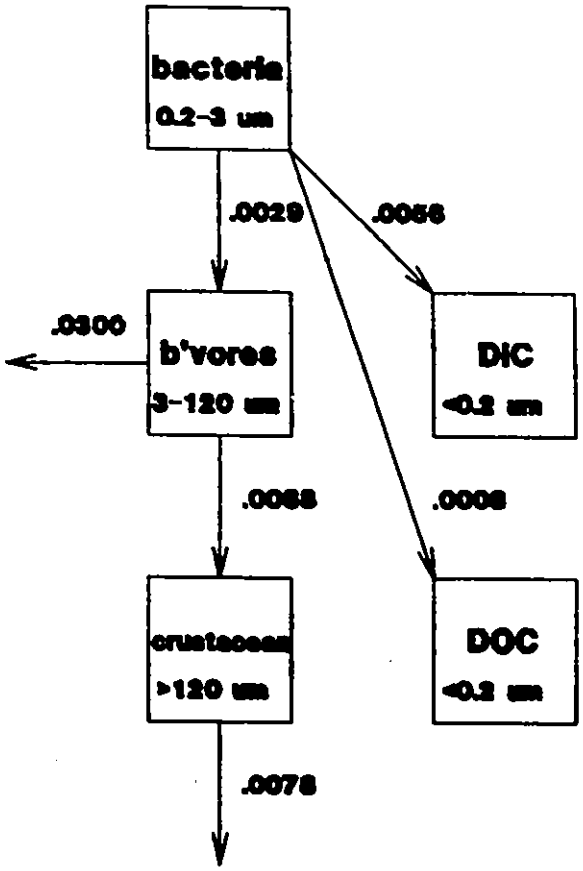


Figure 18a

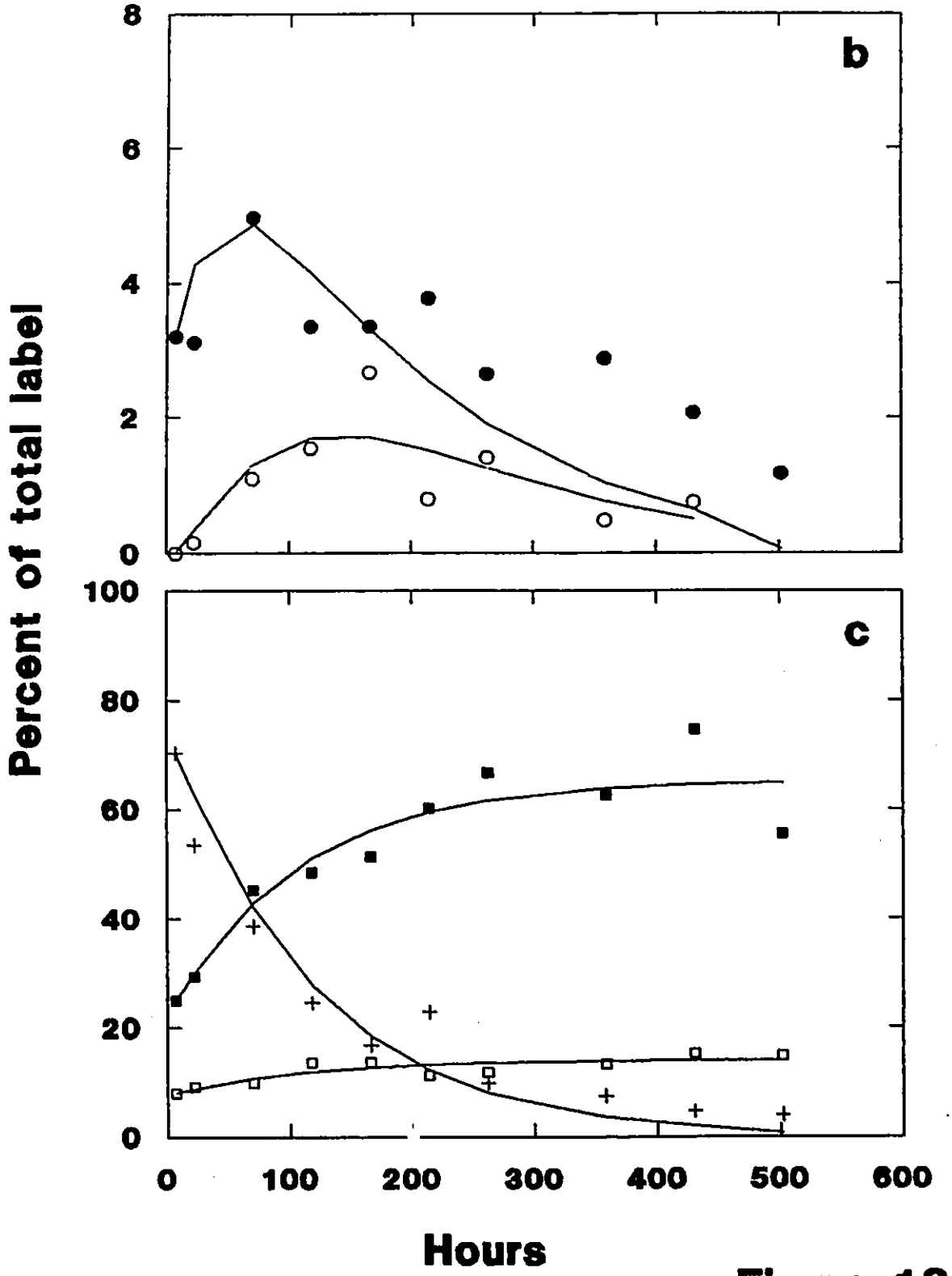

b
c
Percent of total label
Hours
0
2
4
6
8
0
20
40
60
80
100
0
100
200
300
400
500
600

Figure 18

Figure 19. Model 2 used to describe June heterotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; dissolved organic carbon, □ ; >0.2 um, + .

model 2

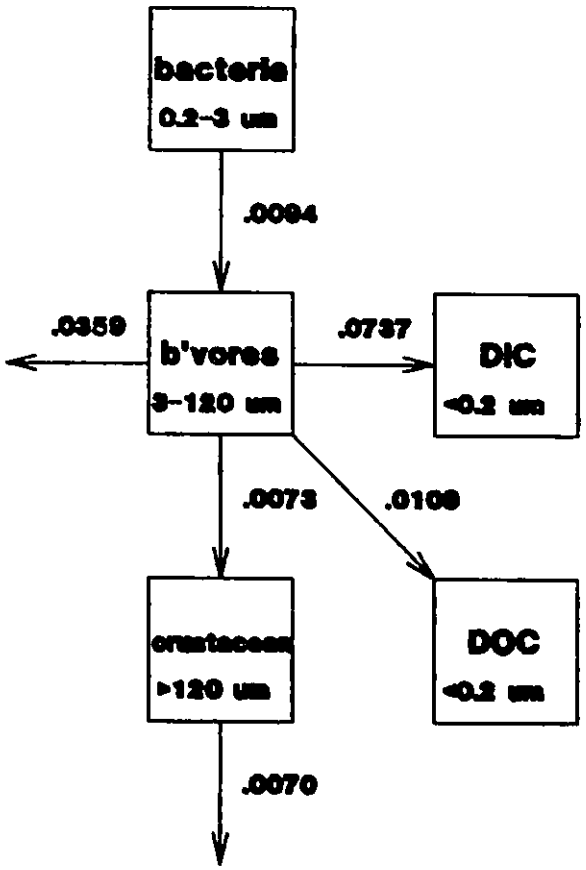


Figure 19a

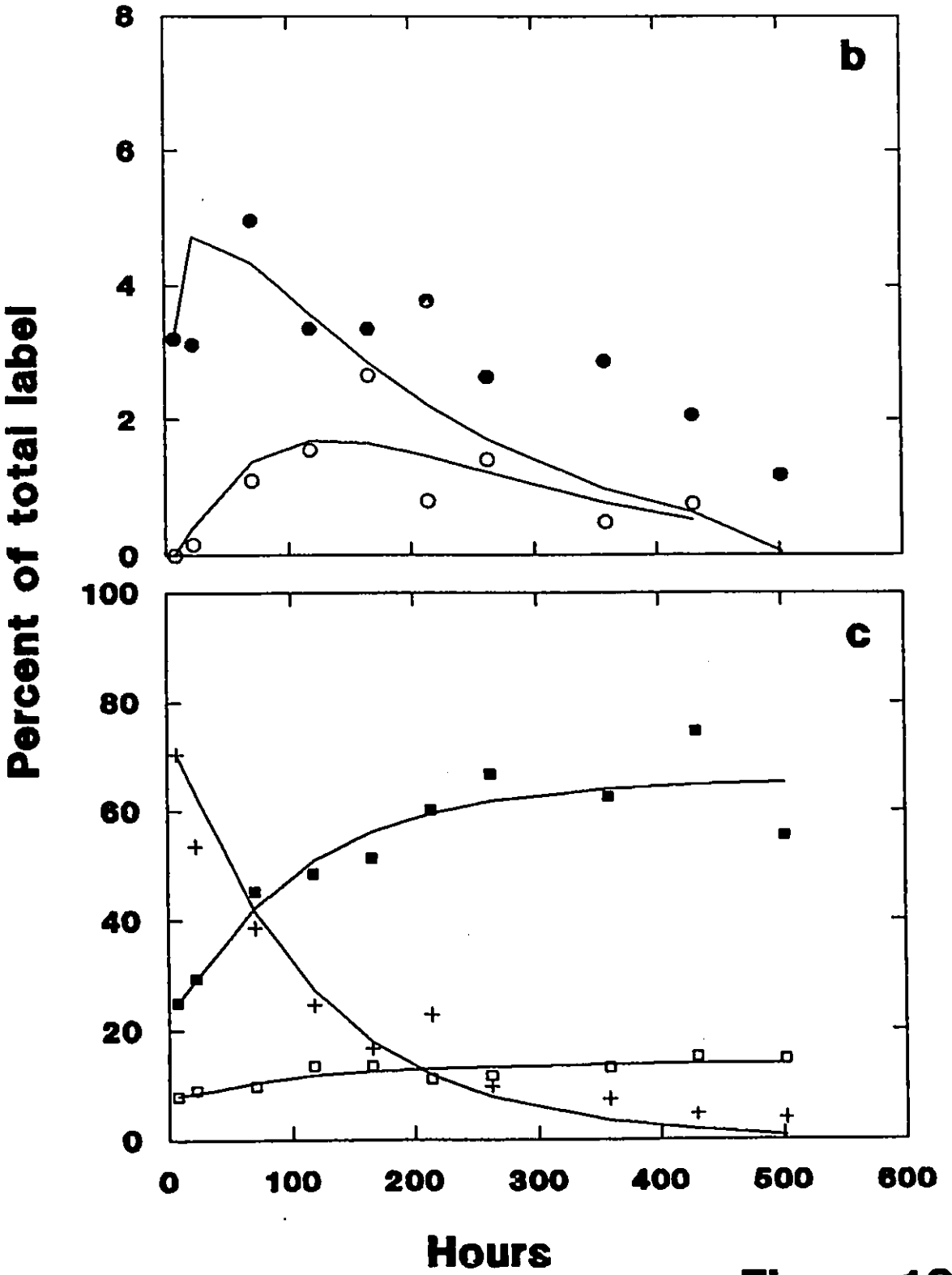

b
c
Percent of total label
Hours
0
2
4
6
8
20
40
60
80
100
0
100
200
300
400
500
600

Figure 19

Table 4. Residual sums of squares for the models used to calculate heterotrophic and autotrophic carbon flow.

Model 1	0.0466
Model 2	0.0468
Model 3	0.0475
Model 4	0.0258
Model 5	0.0258
Model 6	0.0554
Model 7	0.0554
Model 8	0.0734

compared to microbial processes and losses from this compartment are depicted simply as a one way loss representing sedimentation of faecal pellets, mortality etc.

The open-ended pathway emanating from the bacteriovores in models 1 and 2 (figs. 18 and 19) represents unspecified losses (e.g. sinking). Without this pathway (e.g. model 3, fig. 20) all label not transferred to the closed DIC or DOC compartments was constrained to pass through the only open ended compartment, the crustaceans. This resulted in a poor fit of predicted to observed values for both the 3-120 um and >120 um fractions (fig 20b). Additionally, it resulted in poor agreement between comparisons of grazing rates and loss rates of ingested carbon for the crustacean compartment in model 3, to values obtained from the literature. Specifically, the rate constant from crustaceans, representing mortality, egestion, etc., is equivalent to crustaceans losing ingested carbon at a rate of 170% d^{-1} (.0726 h^{-1} x 24 hours x 100, from fig. 20) compared to losses estimated by Pace et al. (1984) of 37% d^{-1} (these authors gathered information on rate constants for marine systems from the literature but as a first approximation should give some indication of expected rates for freshwater systems). Additionally, the rate constant from bacteriovores to crustaceans (.0424 from fig. 20) is equivalent to crustaceans grazing over 100 % of the bacteriovore biomass per day (.0424 x 24 x 100). Using estimated clearance rates for Diaptomus gracilis and Daphnia

Figure 20. Model 3 showing the effects of a closed bacteriovore compartment in a June heterotrophic model. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; dissolved organic carbon, □ ; >0.2 um, + .

model 3

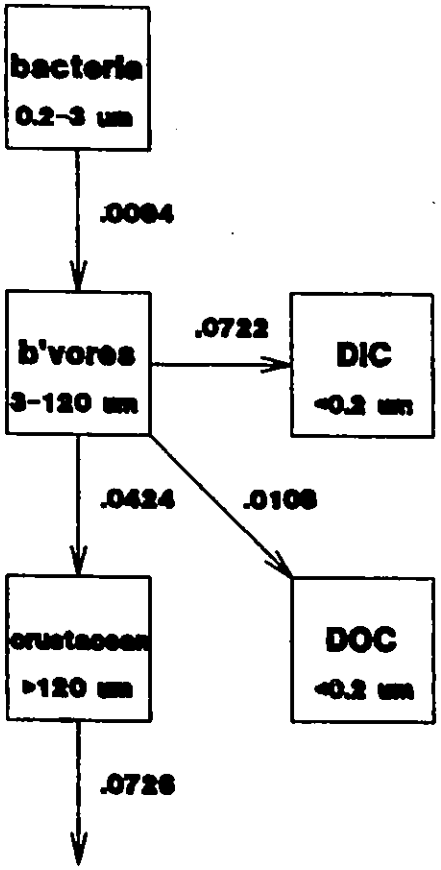


Figure 20a

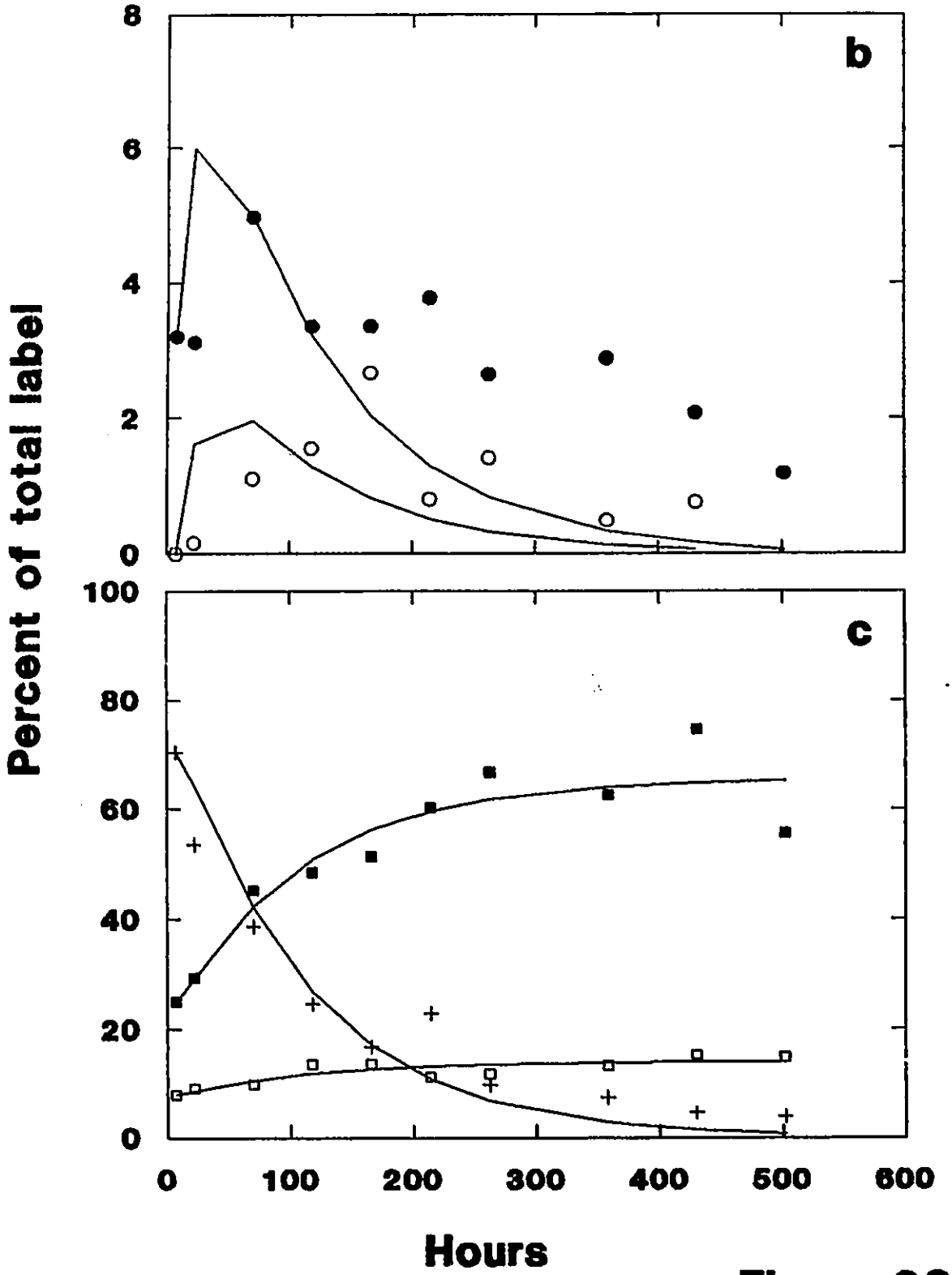

b
c
Percent of total label
Hours
0
2
4
6
8
0
20
40
60
80
100
0
100
200
300
400
500
600

Figure 20

hyalina from Thompson et al. (1982) the crustaceans in the enclosures could clear only approximately 370 mls l^{-1} d^{-1}, equivalent to 37% of the water column, compared to the 100% predicted by the model. Therefore, based on these comparisons, an open ended bacteriovore compartment was incorporated into the models.

Structuring the bacteriovore compartment in this way improves the fit to the >120 um data (fig 18b and 19b) and provides more realistic rates of grazing and losses of carbon for the crustacean fraction. Grazing is reduced from 100% to between 16% (.0068 x 24 x 100 from fig. 18) and 18% (.0073 x 24 x 100 from fig. 19) and losses of ingested carbon to between 17% (.0070 x 24 x 100 from fig. 19) and 19% (.0078 x 24 x 100 from fig. 18) from 170%, both of which agree reasonably well with the literature values.

The rate constants of models 1 and 2 which result from the assumption that respiration is either entirely bacterial or entirely protozoan suggest that model 1 is closer to representing actual carbon dynamics. From Pace et al. (1984) planktonic protozoans are estimated to lose 40% d^{-1} of ingested carbon while model 2 estimates over 300% d^{-1} ((.0737+.0109 +.0073+.0359) x 24 x 100 from fig. 19). In model 1, where most bacterial carbon is lost directly from this size fraction, bacteriovore losses are reduced to 88% from 300%, within the same order of magnitude as values from Pace et al. (1984) yet still provides a good fit for the

>120 um data.

Although model 2 may therefore not accurately represent carbon dynamics in June both models 1 and 2 were used to quantify carbon flow as model 2 provided an upper estimate for the amount of bacterial carbon transferred to crustaceans. This is due to the carbon content of the crustacean compartment being determined by the amount of carbon transferred to the bacteriovores. This is maximized in model 2 since all bacterial carbon is forced into this compartment.

If these comparisons are accurate and model 1 is more representative of carbon flow than 2, it suggests that the results of the control experiment designed to determine if incorporated label is lost from bacteria in the absence of grazers (fig. 1), may not be valid for these enclosures. This experiment had indicated that, in the absence of grazers, label retained following uptake was not lost to any great extent. This suggests that respiration by bacteria is negligible with grazing as the major loss process for bacterial carbon. However, model 1 indicates that bacteria may directly respire much of their incorporated carbon. This difference may reflect altered bacterial metabolism due to the culture conditions. Clearly the slower uptake of label and the lower percentage of label retained following uptake by cultured bacteria differs from that seen for bacteria in the enclosures (contrast uptake and percentage retained in figs. 1 and 2). As an initial conclusion this suggests that bacteria

will contribute only small amounts of carbon to higher trophic levels since most carbon appears to be lost directly as DIC and therefore would never become available to crustaceans.

Bicarbonate - Two models, based on different assumptions, are consistent with the June bicarbonate data. Model 4 (fig. 21) is based on the assumption that crustaceans acquire algal carbon exclusively through direct grazing on the 3-120 um fraction. This assumption is based on the dominance of copepods in the enclosures which would not effectively graze autotrophic picoplankton. For purposes of this model, carbon fixed in the 0.2-3 um fraction is assumed to sediment as particles or be respired with no losses occurring due to grazing.

In contrast, model 5 (fig. 22) is based on the assumption that label could be acquired in the >120 um fraction through an additional process. As above, crustaceans could graze directly on 3-120 um sized algae. Additionally, bacteriovores in the 3-120 um fraction could graze on autotrophic picoplankton and in turn be grazed by crustaceans. The tracer in the 3-120 um fraction would, in this case, represent both autotrophs and heterotrophs as depicted in fig 22. In this way model 5 attempts to incorporate carbon from autotrophic picoplankton passing through the microbial loop. Since the picoplankton dominate primary production in June (fig. 6b) significant amounts of carbon could pass through

Figure 21. Model 4 used to describe June autotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >0.2 um, + ; >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; >0.2 um, + .

model 4

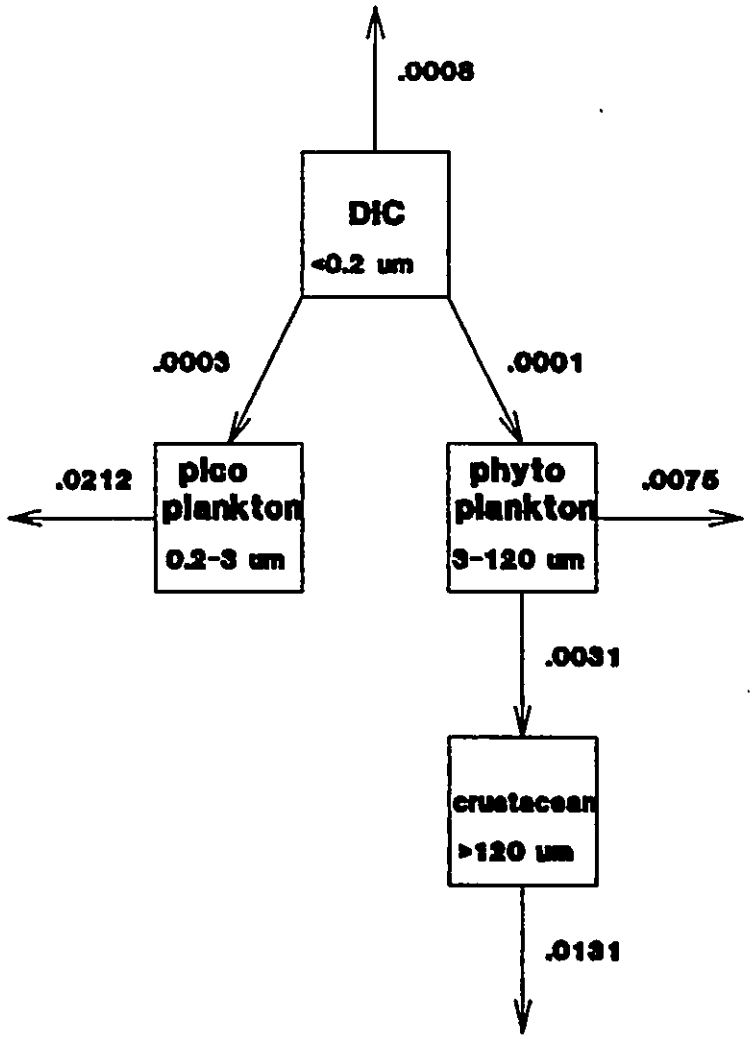


Figure 21a

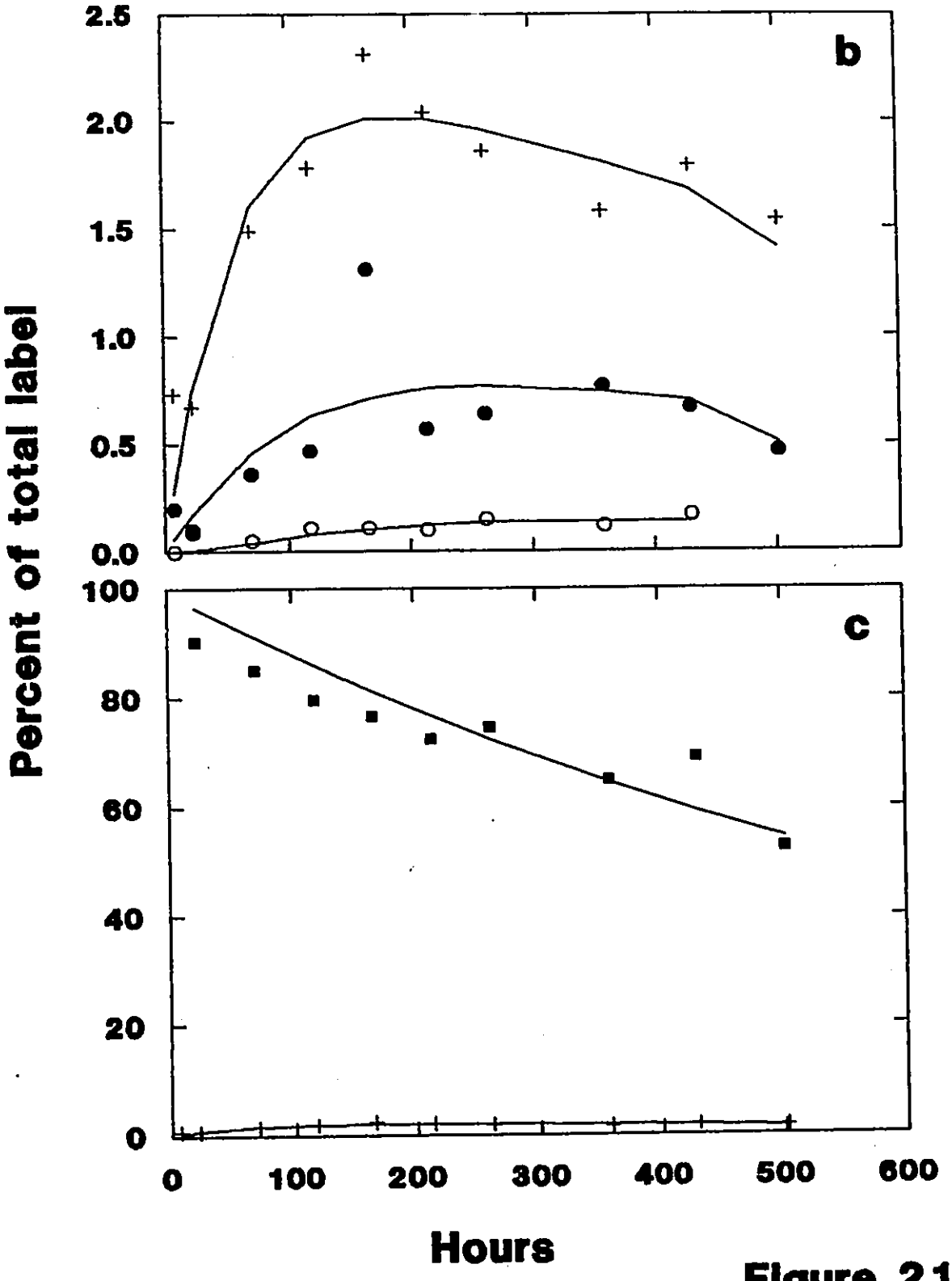

b
c
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
0
100
200
300
400
500
600
Percent of total label
Hours

Figure 21

Figure 22. Model 5 used to describe June autotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >0.2 um, + ; >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; >0.2 um, + .

model 5

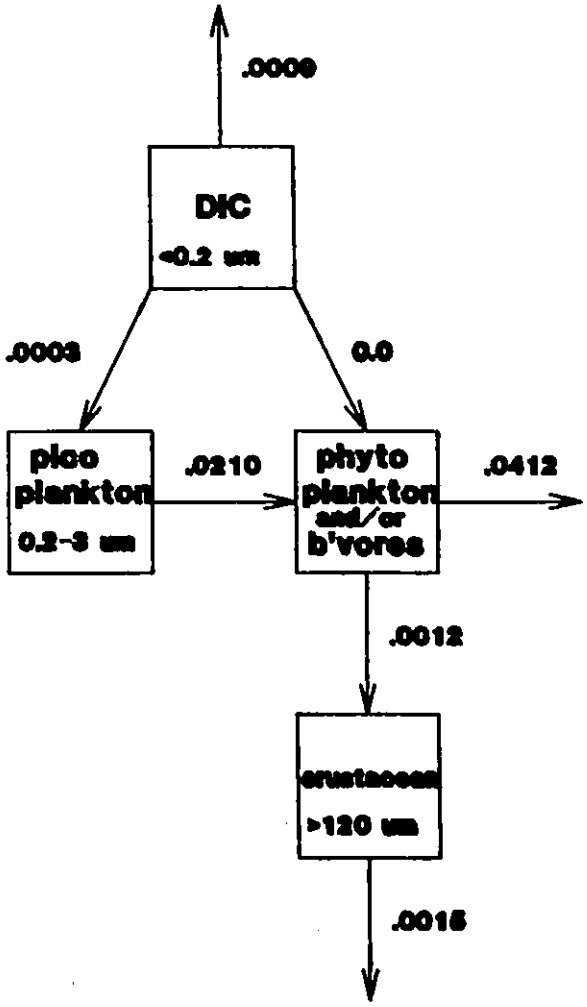


Figure 22a

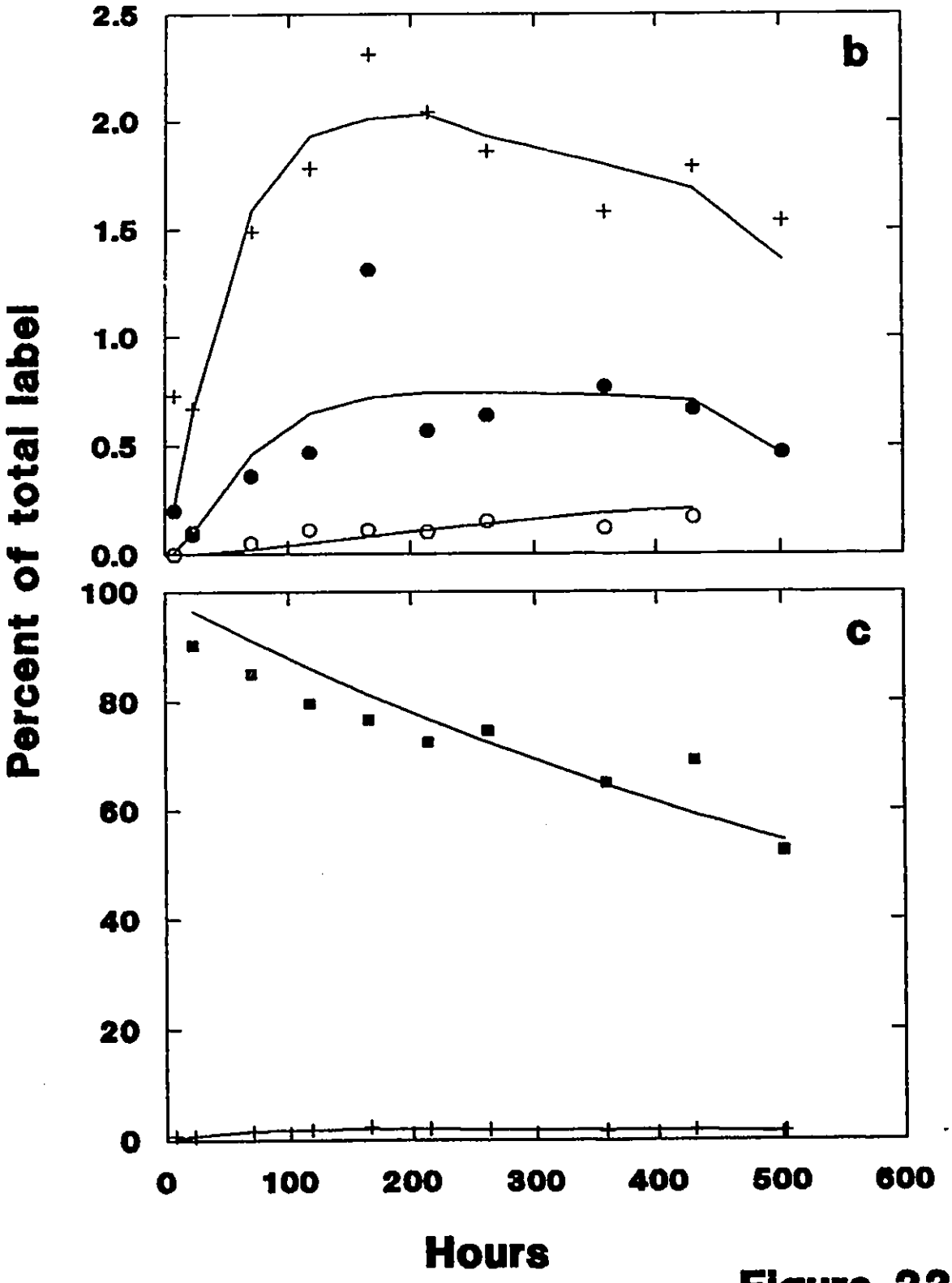

b
c
Percent of total label
Hours
2.5
2.0
1.5
1.0
0.5
0.0
100
80
60
40
20
0
0
100
200
300
400
500
600

Figure 22

this latter route.

Due to the difficulties of modelling the transfer of carbon to a given compartment from more than one compartment simultaneously, solving for the rate constants for model 5 results in uptake of DIC by the 3-120 um fraction being computed as zero. This indicates that, within the constraints of the model, grazing on autotrophic picoplankton by bacteriovores is sufficient to provide all of the [^{14}C] label measured in the 3-120 um fraction. Although this modelling constraint probably overemphasizes carbon flow from autotrophic picoplankton, it does suggest that, in June, much of the carbon acquired by crustaceans could potentially have arisen from these autotrophs by passing through non-crustacean grazers. Since both models provide good fits to the data (figs. 21b,c and 22b,c), reasonable estimates of losses of carbon from crustaceans, and, in particular, because of the potential implications for the microbial loop, both models will be considered when analyzing total carbon flow to crustaceans.

June carbon flow - As indicated (see section **compartmental modelling**), using the rate constants generated from the models described above, total carbon flow to crustaceans from both algal and bacterial sources can be calculated. For example, for model 2 (fig. 19) the equation describing the change in the carbon content of the 3-120 um fraction is

$$[3\text{-}120]_{t+1} = k_1 * [0.2\text{-}3]_t - (k_2 + k_3 + k_4 + k_5) * [3\text{-}120]_t * t$$

where $[3\text{-}120]_{t+1}$ equals the carbon content of the 3-120 um fraction at t+1 hours. $[0.2\text{-}3]_t$ and $[3\text{-}120]_t$ is the carbon content of these fractions at time t. k_1 to k_5 are the rate constants computed from the [^{14}C] data in h^{-1} and would be equal to .0094, .0737, .0109, .0073 and .0359 respectively in this case. A t value of 1 hour was used such that the time scale would be similar to that used to calculate the rate constants. Similarly, the other compartments of the model can be represented in the same way. For the starting values the standing stock biomass of algae or bacteria is inserted in the appropriate compartment with the remaining compartments set at zero. The calculation is then iterated from 0h to 24h.

When determining carbon flow to crustaceans in this manner the rate constant describing loss of carbon from crustaceans was set to zero, rather than using the computed value. This gross accumulation gives a more accurate depiction of total carbon input since differences in the rate of loss of carbon from crustaceans varies slightly from model to model and may give misleading results.

In June, algal biomasses of the different size fractions had not been measured directly by size fractionation of water samples. Therefore, percentages of the total algal biomass in each size fraction, prior to enrichment, for the August

experiment were used for the June data. This is based on the assumption that the relative contribution to total biomass of each size fraction did not change appreciably over this time period.

Table 5 shows the amount of carbon transferred from bacteria and algae to crustaceans for the June models. These results indicate that the significance of heterotrophic bacteria as a food source depends on which autotrophic model most closely approximates the actual carbon dynamics of the enclosures. If model 5, incorporating the transfer of carbon from autotrophic picoplankton through the microbial loop, is correct then 45-65% of the carbon consumed by crustaceans originates from heterotrophic bacteria with the remaining 35-55% from autotrophic picoplankton. Alternatively, if model 4, involving only direct grazing of 3-120 um algae by crustaceans, is accurate, then 1-2% of the total carbon flow to crustaceans originated from heterotrophic bacteria with the remaining from 3-120 um algae. In this case, due to the design of this model, all carbon from autotrophic picoplankton is lost through sedimentation or respiration.

These different answers are due to the 6 fold difference in biomass of the two autotrophic fractions (0.2-3 um, 13.7 mg C m^{-3}; 3-120 um, 87.9 mg C m^{-3}) and the necessity for carbon from autotophic picoplankton to pass through an intermediate trophic level before becoming accessible to crustaceans. Consequently, this reduces autotrophic carbon flow d^{-1} through

Table 5. Summary of the bacterial contribution to the total carbon input to crustaceans based on the June carbon dynamics. Samples for determining the bacterial and algal biomasses were taken 24 hours following inoculation of the enclosures.

1. bacterial biomass	13.8 mg C m^{-3}
algal biomass (3-120 µm)	87.9 mg C m^{-3}
(0.2-3 µm)	13.7 mg C m^{-3}
2. a) carbon input to crustaceans from bacteria over 24 hour period:	
model 1	0.05 mg C m^{-3}
model 2	0.11 mg C m^{-3}
b) carbon input to crustaceans from algae over 24 hour period:	
model 4	5.80 mg C m^{-3}
model 5	0.06 mg C m^{-3}
3. percentage of total carbon input to crustaceans from bacteria:	
a) assuming models 1 and 4 describes carbon dynamics	1%
b) assuming models 2 and 4 describes carbon dynamics	2%
c) assuming models 1 and 5 describes carbon dynamics	45%
b) assuming models 2 and 5 describes carbon dynamics	65%

model 5 in comparison to model 4 which, as a result, increases accordingly the percentage of the total carbon originating from heterotrophic bacteria.

For these two autotrophic models neither the fit of the regression lines to the experimental data nor a comparison of the rate constants could be used to determine whether model 4 or 5 more closely approximates the actual carbon dynamics. Since both models, in combination with the heterotrophic models, yield estimates of daily carbon flow to crustaceans, an alternative approach for determining the accuracy of the models is to compare these estimates with the expected carbon requirments of the crustacean community present in the enclosures. Using estimates from Pace et al. (1984) and data from Boak and Goulder (1983) daily carbon requirements of crustaceans range from 10%-100% of their standing stock biomass, equivalent to 4.96 - 49.6 mg C m^{-3} for the present investigation. Carbon flow determined using model 4 is within the same order of magnitude as the lower estimate while model 5 underestimates carbon requirements by one to two orders of magnitude. This suggests that model 4, which does not incorporate the microbial loop, is the more accurate of the two models.

The observation that estimated carbon flow calculated from model 4 is at the lower range of estimated carbon requirments may be related to the drop in crustacean numbers in the enclosures over the course of the experiment (fig. 9).

By closing the bags to the sediments I have prevented any vertical migration of the crustaceans to the sediments (1-2 metres below the enclosures in this area). If part of the food requirments of crustaceans in this part of the lake was derived from benthic food webs this would no longer be available and hence the pelagic phytoplankton present may not have been sufficient to meet crustacean carbon requirements. Therefore carbon flow to crustaceans may have been reduced and numbers may have dropped as a result.

Model 4 is also superior to model 5 on other grounds. Calculating rate constants for model 5 had resulted in uptake of DIC by the 3-120 um fraction being computed as zero (fig. 22). However, if short term uptake is calculated for the algal fractions, the rate for the 3-120 um fraction is approximately 40% of that of the 0.2-3 um fraction (.0003 h^{-1} compared to .0008 h^{-1}, calculated as the slope of the initial uptake for bicarbonate data for 0-7 hours). Although some of this carbon flux may be the result of protozoans grazing on autotrophic picoplankton, much of this observed uptake would presumably be by 3-120 um size algae. This evidence suggests that the computed uptake rate of zero for the 3-120 um algae in model 5 is unrealistic, further justifying the conclusion that, of the two, model 4 most accurately depicts carbon flow. Based on these observations in support of model 4, I feel that the June data does not provide any strong support for the hypothesis that significant carbon flow is transferred through

the microbial loop to the crustaceans.

One error which may have some bearing on the conclusions reached here is the apparent departure from steady state shown by algae over the first few hours of the June experiment following deployment (shown by drop in chl a, fig. 9). This could be due to increased grazing pressure following confinement (de Lafontaine and Leggett 1987, Peterson et al. 1978) or increased sedimentation of algae due to a decrease in vertical mixing in the enclosures. This drop in chl a suggests that, rather than using a chl a value of 3.39 to calculate algal biomass, a value of 1.52 (fig. 9) may be more appropriate. If the calculation of carbon flow is repeated using an algal biomass calculated from this chl a value, the results suggest that bacteria still contribute only a small percentage of total carbon flow (2%-4%). Although the potential significance of bacteria is increased the overall conclusion that algae are the dominant food source of crustaceans is not changed.

August models

Glucose - For these data, I formulated two models in an attempt to incorporate carbon flow to crustaceans arising from direct grazing on bacteria as well as indirect flow by grazing on bacteriovores (model 6, fig. 23 and model 7, fig. 24, respectively). Unlike the June data, cladocerans comprised 71% of the total crustacean numbers in August and may have

Figure 23. Model 6 used to describe August heterotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >0.2 um, + ; >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; dissolved organic carbon, □ ; >0.2 um, + .

model 6

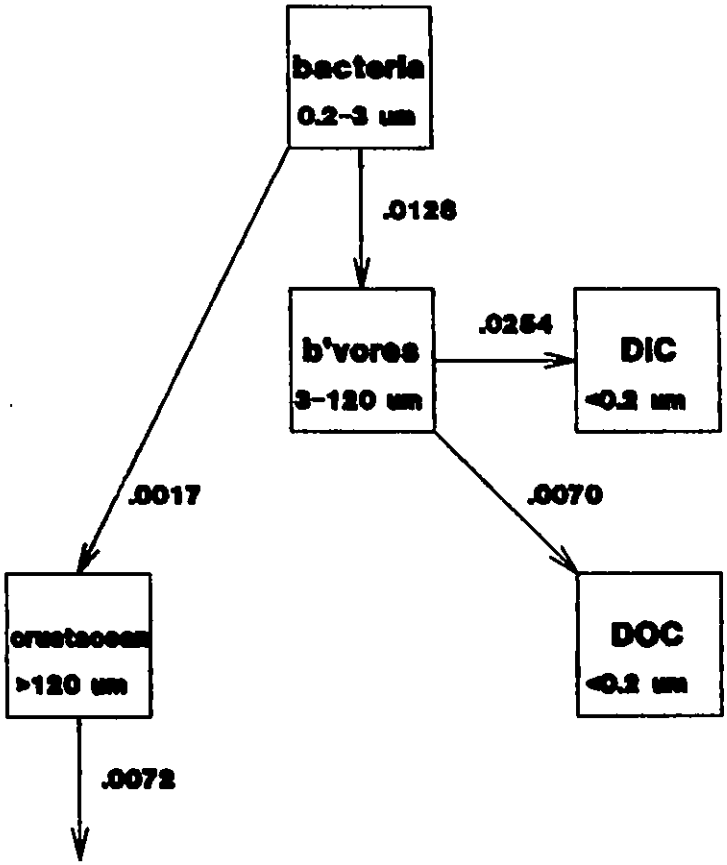
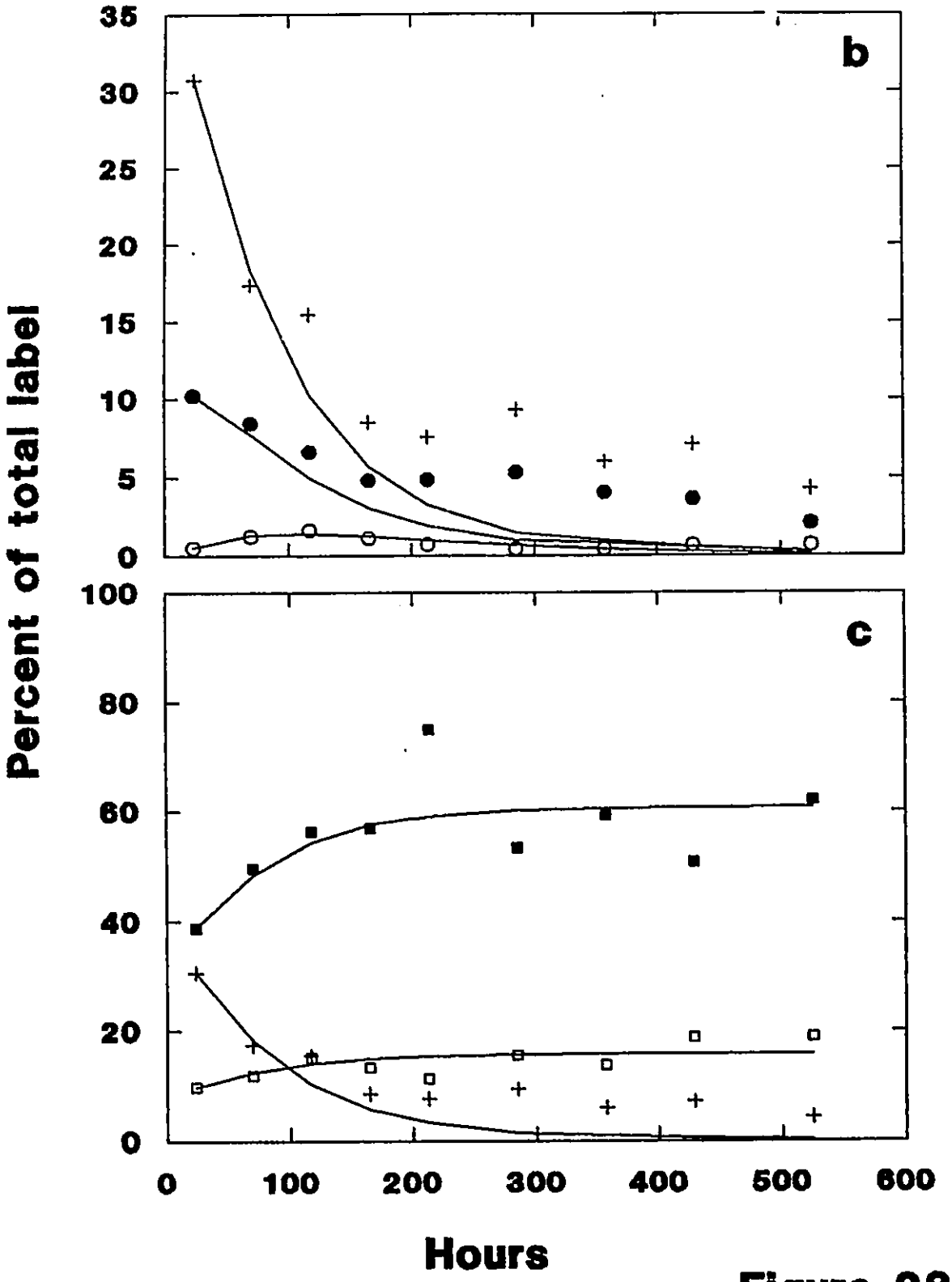


Figure 23a


b
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Percent of total label
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Figure 23

Figure 24. Model 7 used to describe August heterotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >0.2 um, + ; >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; dissolved organic carbon, □ ; >0.2 um, + .

model 7

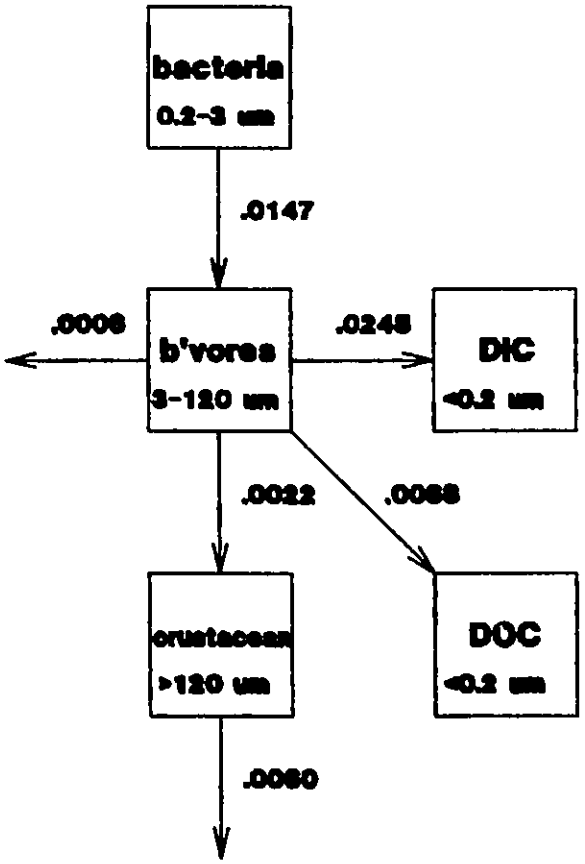
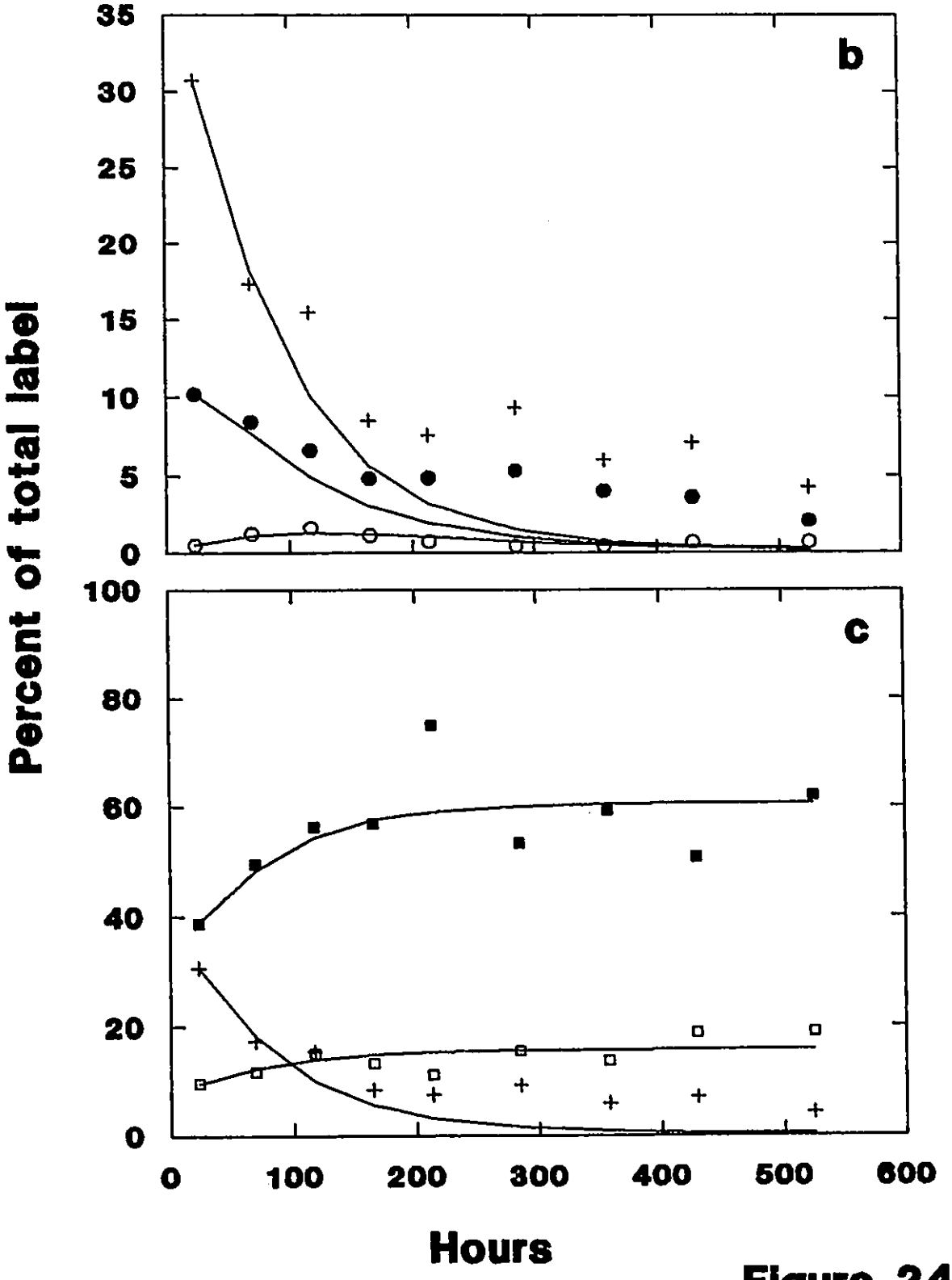


Figure 24a


b
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500
600
Percent of total label
Hours

Figure 24

obtained a significant portion of label through direct grazing on bacteria. This is based on evidence suggesting that cladocerans can effectively ingest bacterial sized particles (Pace et al. 1983, Porter et al. 1983, Peterson et al. 1978).

I formulated two separate models, since one model, simultaneously incorporating grazing on the 0.2-3 and 3-120 um fractions, could not be computed as one grazing rate was always calculated as zero. Therefore models 6 and 7 were formulated which, within the constraints of the models, will provide maximum and minimum estimates of carbon flow to crustaceans.

For the August glucose data, I have shown the label in the DIC and DOC pools as arising from only the bacteriovore fraction. In June, carbon flow estimates produced by model 1 and 2, in which DIC and DOC arose from either bacteria or bacteriovores separately, varied from .05-.11 mg C m^{-3} (table 5). Since these estimates vary only slightly, August models are shown only with bacteriovore respiration and excretion. Within the constraints of model 7, bacteriovore respiration will maximize crustacean carbon flow, since all bacterial carbon is forced through the bacteriovore compartment and, hence, can potentially pass to the crustacean compartment. The origin of label transferred to the dissolved compartments has no effect on carbon flow to crustaceans in model 6, since in this model all bacterial carbon at any given time will be available to the crustacean compartment, regardless of which

compartment produces DIC and DOC.

Bicarbonate - Model 8 (fig. 25) is based on the assumption that the label acquired by the crustaceans in these enclosures would originate largely from the 3-120 um fraction since most label taken up by phytoplankton in August is by this fraction (fig. 7b). DOC is assumed to originate by autotrophic excretion. Although some may arise from crustacean excretion or feeding, if this is portrayed in the model the crustacean rate constant for excretion is computed as zero with all label arising from autotrophs. Again, this is due to the limited information within the data set.

August carbon flow - Total carbon flow, using these models, is presented in table 6. Heterotrophic bacteria contributed from 5-23% of total carbon flow to crustaceans. The actual value should fall within this range since the lower figure underestimates carbon flow as it results from a model which forces all bacterial carbon through an intermediate trophic level prior to consumption by crustaceans. This would not accurately represent the fate of bacterial carbon at this time as the dominance of cladocerans over copepods would result in much direct grazing of bacteria by the >120 um fraction. Additionally, chl a drops over the course of the experiment which suggests that a lower algal biomass may be appropriate for estimating carbon flow. As in June, this could increase

Figure 25. Model 8 used to describe August autotrophic dynamics. Numbers are the calculated rate constants (proportion h^{-1}). b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >0.2 um, + ; >3 um, ● ; and >120 um, ○ . c) dissolved inorganic carbon, ■ ; dissolved organic carbon, □ ; >0.2 um, + .

model 8

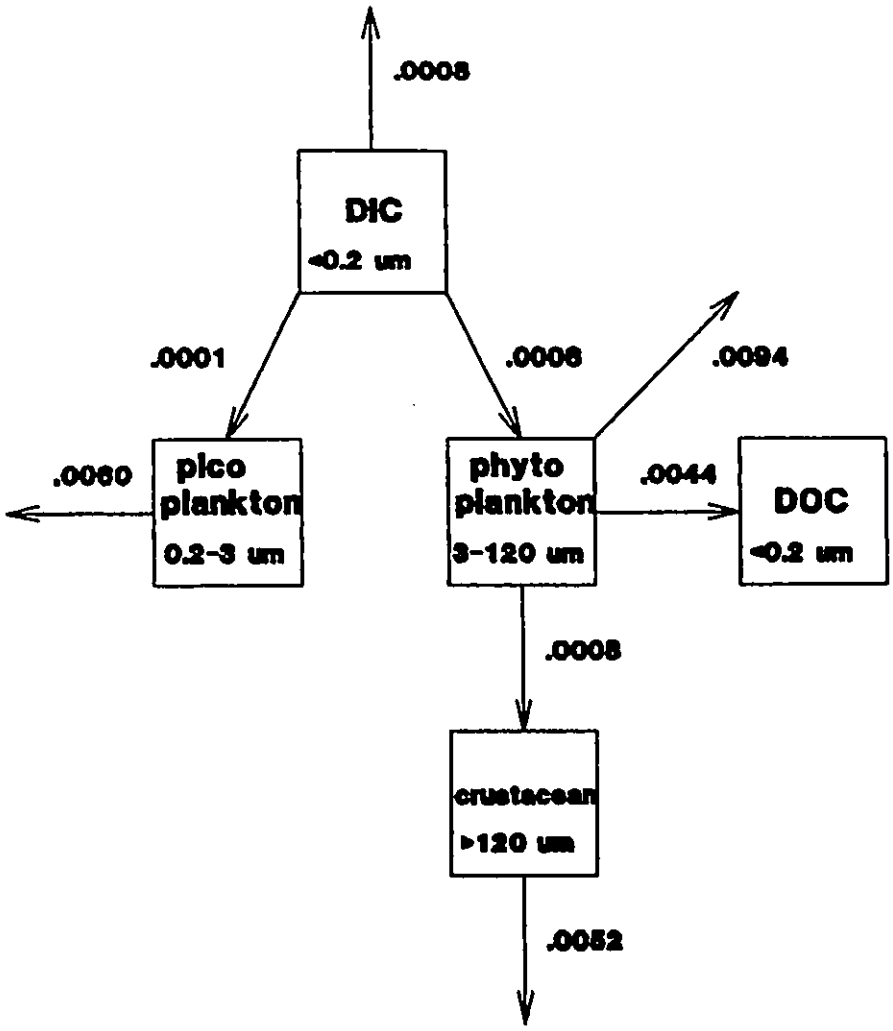
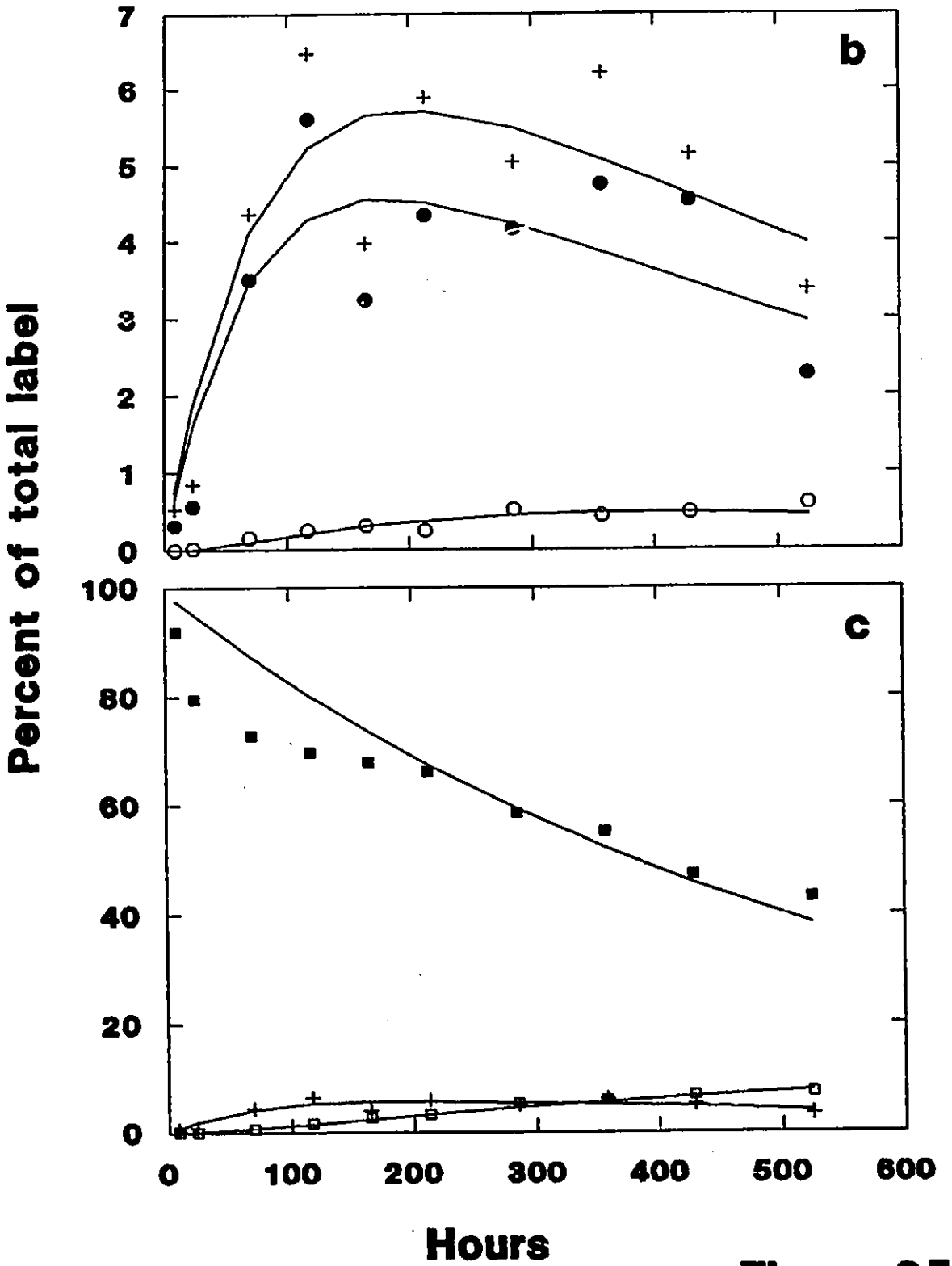


Figure 25a


b
c
Percent of total label
Hours
0
1
2
3
4
5
6
7
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40
60
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300
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500
600

Figure 25

Table 6. Summary of the bacterial contribution to the total carbon input to crustaceans based on the August carbon dynamics. Samples for bacterial and algal biomasses were taken 24 hours following inoculation of the enclosures, 148 hours following enrichment.

1. bacterial biomass	24.3 mg C m^{-3}
algal biomass (3-120 µm)	170.1 mg C m^{-3}
2. a) carbon input to crustaceans from bacteria over 24 hour period:	
model 6	0.84 mg C m^{-3}
model 7	0.15 mg C m^{-3}
b) carbon input to crustaceans from algae over 24 hour period:	
model 8	2.77 mg C m^{-3}
3. percentage of carbon input to crustaceans originating from bacteria:	
a) assuming models 6 and 8 describes carbon dynamics	23%
b) assuming models 7 and 8 describes carbon dynamics	5%

slightly the bacterial contribution to crustacean nutrition.

The upper estimate of 23%, from model 6 designed to incorporate the potential significance of direct grazing by cladocerans, is an overestimate based on the following observations: 1) since cladocerans filter larger particles more efficiently than smaller particles (Güde 1988, Pace 1988, Porter et al. 1983, Peterson et al. 1978) it is unrealistic that they would feed directly on bacteria to the exclusion of all larger particles as depicted in the model. 2) At this time some copepods were present which would not directly ingest bacteria to any great extent, so some of the bacterial carbon flow to crustaceans would pass indirectly through bacteriovores. Although 23% may be an overestimate, these results suggest that when cladocerans, rather than copepods, dominate zooplankton communities, the significance of bacteria as a carbon source will be increased. In June, when copepods dominated, only 1-2% of carbon flow originated from bacteria, in contrast to the 5-23% calculated for August.

Overall, in both June and August, these models provide good fits to the data, with certain exceptions. In particular the 3-120 um fraction for June models (fig. 18b and 19b) and both the 3-120 and 0.2-3 um for August (fig. 23b and 24b) are systematically underestimated during the second half of the experiment. This suggests that some aspects of carbon flux are not accurately portrayed by the models. Two explanations are likely for this. First, some error may be statistical in

nature. For most of the experiment, the particulate fractions have only a small amount of activity in comparison to the DIC pool. The largest compartment (in terms of [^{14}C] activity) has proportionately the most influence on the fit of the models, such that poor fit of other compartments may have little effect on the residual sums of squares. Removing DIC from the June data and recalculating these heterotrophic models does result in improvement of fit without greatly altering the rate of DIC production (fig. 26 shows the fit of model 1 recalculated without DIC data). In this case, the observed lack of fit appears to have little influence on the conclusions reached from the models. The rate constant from bacteria to bacteriovores is reduced in the recalculated model, hence, carbon flow from bacteria to crustaceans is lower. This indicates that the conclusion that bacteria supply only small amounts of carbon to crustaceans still holds. For August data, this approach also reduces lack of fit somewhat (fig. 27 shows model 7 recalculated without DIC). For model 7 bacterial carbon flow to crustaceans remains similar (increasing to 6% from 5%). This approach does not work for model 6 as the rate of DIC production drops to near zero (.0002 from .0254), indicating that in this latter case inclusion of DIC is necessary to accurately model [^{14}C] dynamics. Overall, these results suggest that in June lack of fit may be largely statistical in nature and has little effect on the results. In August, since lack of fit was not wholly

Figure 26. Recalculation of model 1 with DIC data removed (indicated by the unlabelled compartment). Numbers are the recalculated rate constants. b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >3 um, ● ; and >120 um, ○ . c) dissolved organic carbon, □ ; >0.2 um, + .

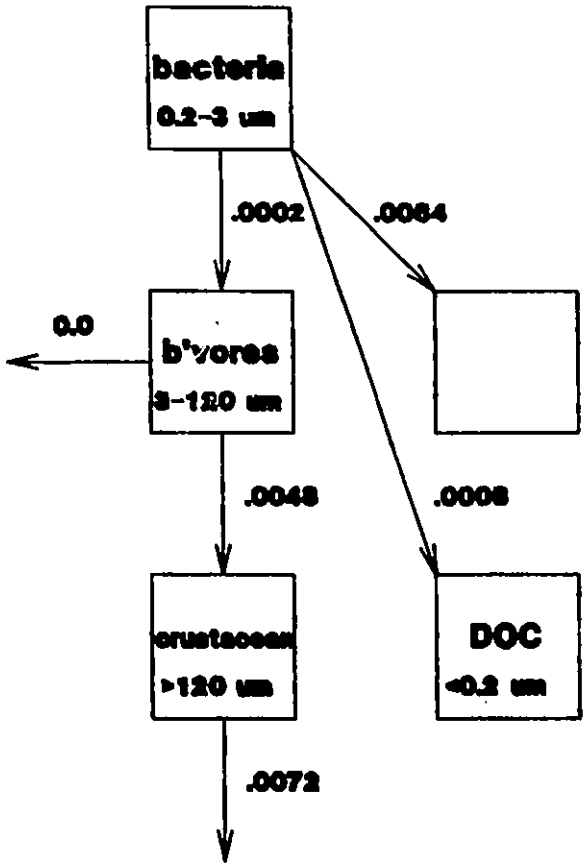
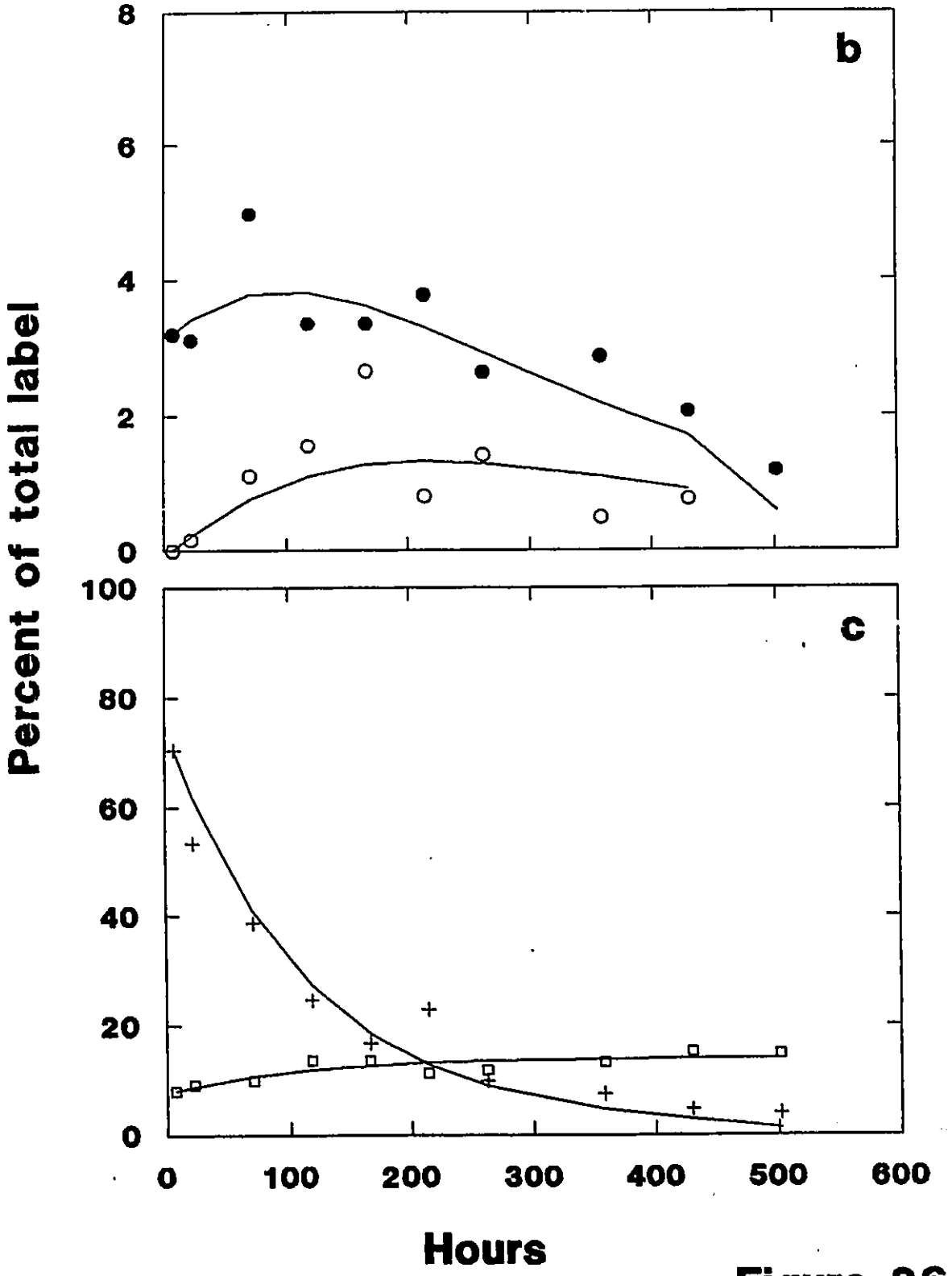

bacteria
0.2-3 um
.0002
.0064
0.0
b'vores
3-120 um
.0048
.0008
crustacean
>120 um
DOC
<0.2 um
.0072

Figure 26a


b
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Percent of total label
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Figure 26

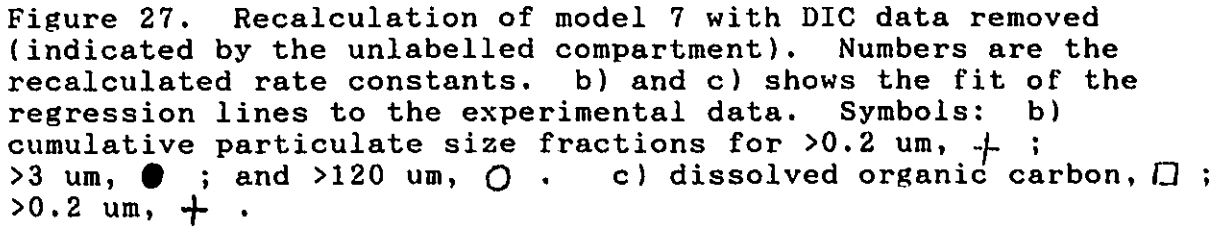


Figure 27. Recalculation of model 7 with DIC data removed (indicated by the unlabelled compartment). Numbers are the recalculated rate constants. b) and c) shows the fit of the regression lines to the experimental data. Symbols: b) cumulative particulate size fractions for >0.2 um, + ; >3 um, ● ; and >120 um, ○ . c) dissolved organic carbon, □ ; >0.2 um, + .

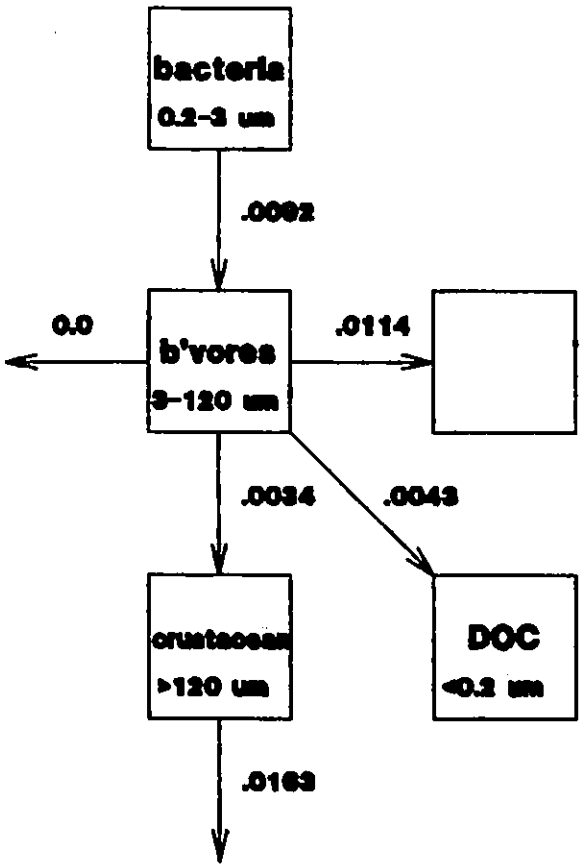
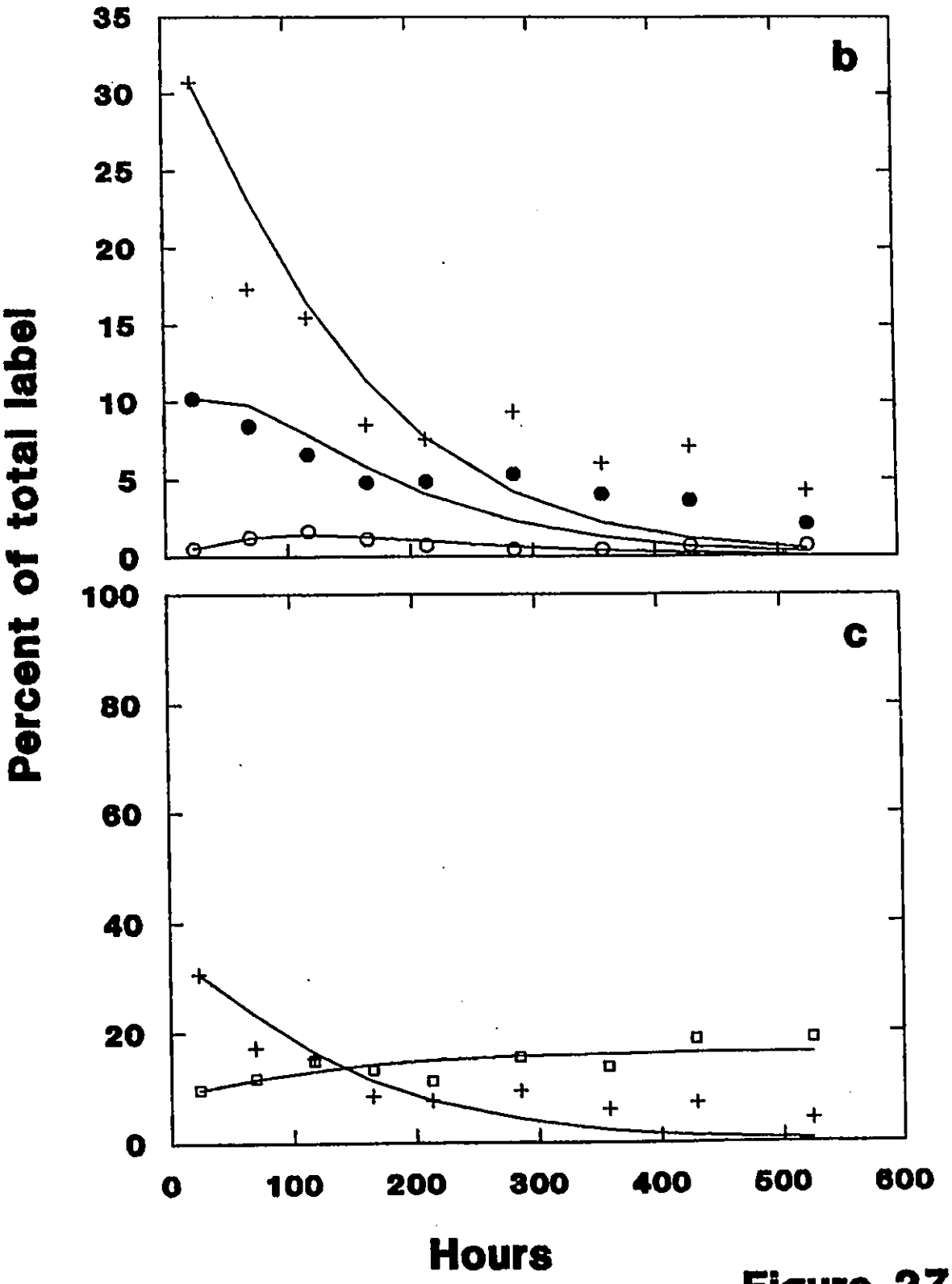

bacteria
0.2-3 um
.0092
0.0
b'vores
3-120 um
.0114
.0034
.0043
crustacean
>120 um
DOC
<0.2 um
.0163

Figure 27a


b
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Percent of total label
Hours
35
30
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15
10
5
0
100
80
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100
200
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Figure 27

corrected, additional factors may be involved.

These additional factors may be biological processes not accurately portrayed in the models. Autotrophic processes could occur in the glucose enclosures as a result of remineralization of glucose to DIC. Some label may also become associated with detrital particles and not be further utilized to any great extent. Additionally, recycling of carbon or selective grazing could also influence carbon dynamics. I assessed the possibility of autotrophic uptake by modelling [^{3}H] dynamics in which autotrophic uptake of [^{3}H] H_2O would not occur. The same lack of fit was observed suggesting that, although some uptake of DIC could occur, it probably is not responsible for the difficulties encountered during modelling. In the process of testing and selecting models, I formulated several models incorporating detrital compartments (e.g. fig. 28) or recycling (by fixing some rates such that carbon was returned to bacteria or bacteriovores). I found that the fit of the model was either not improved or, if improved, reduced crustacean carbon flow to near zero. Although this could potentially indicate that these processes were not occurring to any great extent, it is more likely to be related to difficulties associated with modelling processes for which I have no specific information.

Although lack of fit for August data could not be fully resolved using any of the above approaches, this may not affect estimates of crustacean carbon flow, since carbon flow

Figure 28. Example of a heterotrophic model incorporating a compartment representing detrital particles 3-120 um in size.

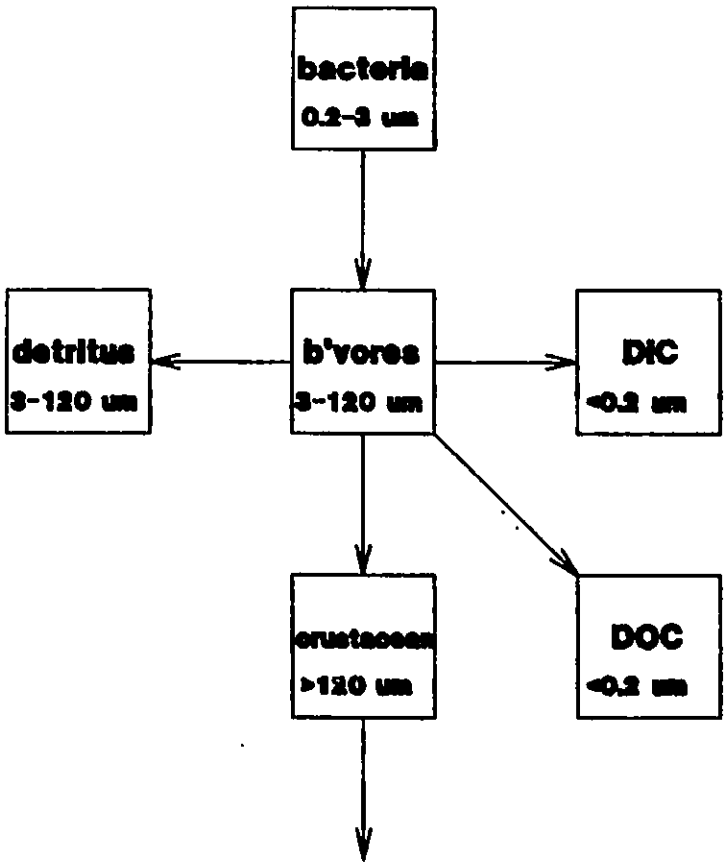
bacteria
0.2-3 um
detritus
3-120 um
b'vores
3-120 um
DIC
<0.2 um
crustacean
>120 um
DOC
<0.2 um

Figure 28

to this trophic level remained similar under a wide variety of model designs.

General discussion

Ducklow et al. (1986) concluded that bacteria were a sink with insignificant amounts of carbon being transferred to crustaceans. The results of the present investigation, with certain reservations, are consistent with this conclusion. However, the results presented here also suggest that in this same sense, autotrophs can also be considered a sink. I have verified this by comparing simultaneously, in the same system, the transfer of carbon flow from both autotrophs and heterotrophs to crustaceans. In both cases over a similar time span only a small percentage of label was transferred to the crustacean trophic level. In the case of heterotrophic bacteria most carbon is remineralized, presumably through respiration by bacteria or protozoan bacteriovores with little carbon reaching crustaceans. For autotrophs both sedimentation and respiration have been identified as major loss processes (Forsberg 1985) such that little carbon may be consumed by herbivores. Taken from this perspective, since both heterotrophs and autotrophs can be considered a sink, a comparison of carbon flow from both sources was necessary to verify the significance of bacteria as a carbon source for crustaceans.

The results of June and August can be used to predict when and where the microbial loop may be important as a potential carbon source for higher trophic levels. In June, the plankton is characterized by a dominance of copepods over cladocerans, most primary production by autotrophic picoplankton and overall, very low levels of primary production. This is characteristic of many freshwater and marine oligotrophic environments and, based on measured carbon flow in June, suggests that in these areas the microbial loop would not be a major carbon source for higher trophic levels.

In August, cladocerans predominate, and primary production is greater and takes place mainly in algae >3 um in size. Under these circumstances the significance of bacteria as a carbon source was increased. Carbon originating from bacteria may have accounted for as much as 23% of total carbon flow to crustaceans, depending on the extent of direct grazing of bacteria by cladocerans. Although, further experimental work is necessary to determine the extent to which this may be an overestimate, this does suggest that at times carbon flow from bacteria may be greater than the results of Ducklow et al. imply. Although direct grazing of bacteria by cladocerans can increase the efficiency of the microbial loop, the greater biomass of algae in comparison to bacteria still resulted in most crustacean carbon originating from algae. A greater algal biomass is typical of both freshwater and marine environments (based on bacterial biomass, chl a regression

from Bird and Kalff (1984)) suggesting that similar results would be found in other areas where cladocerans are present in large numbers.

These results suggest that the potential importance of bacteria as a carbon source may be maximized at the end of an algal bloom when bacterial biomass can equal algal biomass or, alternatively, when allochthonous inputs of carbon to a lake are high which could increase bacterial biomass. The estimated specific rate of carbon flow from bacteria to bacteriovores is approximately equal to that from algae to herbivores (.0029, .0017, models 1 and 6 compared to .0031, .0008, models 4 and 8). This indicates that if bacterial biomass were similar to algal biomass, carbon flow from bacteria would be of a similar magnitude. Further studies of the kind presented here would be required at different times of the year to verify these possibilities.

The size of the particle is probably the critical factor when considering the production of a higher trophic level. Under most circumstances, copepods, by effectively grazing only on larger particles would presumably be unable to acquire significant amounts of carbon from either bacteria or autotrophic picoplankton since the carbon must pass through an intermediate trophic level. In areas dominated by cladocerans which can directly acquire carbon from these small particles, the results from August suggest that, although the significance of bacteria and picoplankton increases, most

crustacean carbon originates from larger phytoplankton. This is due to phytoplankton biomass exceeding the biomass of bacterial sized particles.

In general, recent investigations into the microbial loop have yielded similar results to those of the present study. Parsons et al. (1981), by adding 1-5 mg glucose l^{-1} to large marine enclosures, was able to demonstrate a 3-4 fold increase in copepod numbers which suggests a transfer of bacterial carbon to copepods. As Ducklow et al. (1987) correctly point out, this increase in copepod biomass in comparison to the amount of glucose added is actually equivalent to an inefficient transfer of carbon of a similar magnitude as that found in the present study for June when copepods dominated the crustacean community.

Turner et al. (1988) postulated that the dominance of the marine cladoceran Penilia avirostris in some areas may be related to an ability to utilize bacterioplankton as a food source at times of low phytoplankton biomass. If true this would potentially indicate the importance of bacteria as a carbon source for this cladoceran. However, although it had previously been reported that this cladoceran could graze on cultured bacteria (references in Turner et al. 1988), these authors were unable to demonstrate feeding on natural bacterioplankton populations. They were able to demonstrate selective feeding on certain algal species which suggests that cladoceran/copepod shifts may be related to presence/absence

of favoured algal food species rather than differential abilities to graze bacteria. Bacteria may yet prove important to some marine cladocerans if other species are verified as being able to feed directly on bacteria.

Zooplankton abundance probably depends strongly upon their food supply (Sommer et al. 1986, Briand and McCauley 1978). This suggests that changes in zooplankton dynamics would correlate with changes in the dynamics of particles which are providing most of the carbon requirements of zooplankton. McCauley and Kalff (1981) have shown that crustacean biomass is better related to nannoplankton (particles 10-50 um in size) than to total phytoplankton biomass, indicating that these particles have the strongest influence on zooplankton dynamics. Although suggesting that these particles may be the major food source of zooplankton, this study did not include bacterial dynamics, so it cannot be determined from this data if a correlation between bacteria and zooplankton would also occur.

Güde (1988), measuring both chl a and bacterial abundance, has shown that chl a concentration decreases by almost two orders of magnitude in response to cladoceran grazing compared to a 2-3 fold decrease in bacteria. This suggests that most of the grazing impact, and by implication the major source of carbon, occurred on algae rather than bacteria.

Recently there have been several investigations

suggesting that cladocerans may consume significant amounts of bacterial secondary production (Güde 1988, Geertz-Hansen et al. 1987, Riemann 1985, Riemann and Bosselman 1984). For these studies, as much as 84% of the bacterial secondary production was consumed by cladocerans (Güde 1988), which could potentially amount to significant flows of carbon from this source. Of these investigations, Geertz-Hansen et al. and Riemann and Bosselman made some calculations designed to determine the potential significance of bacteria as a carbon source for crustaceans. Although cladocerans consumed from 23-66% and 27% of the bacterial production in their respective studies, these authors concluded that this amounted to an insignificant amount of the total carbon ingested by Daphnia, Thus, although cladocerans may influence bacterial dynamics, the influence of bacteria on crustaceans is probably overshadowed by the effect of algal food sources.

Pomeroy and Wiebe (1988) and Coffin and Sharp (1987), by considering the number of trophic transfers and assimilation efficiency per transfer, have also concluded that any intermediate trophic levels between crustaceans and bacteria would result in only a small percentage of the bacterial carbon reaching crustaceans.

The major difference between the present investigation and the cited studies is that none of these studies have directly measured carbon flow simultaneously from both bacterial and algal sources. McCauley and Kalff (1981)

considered only correlations with phytoplankton. Güde (1988), although measuring both chl a and bacteria, concentrated on the effects of crustaceans on bacteria. Geertz-Hansen et al. (1987) and Riemann and Bosselman (1984) based their work only on crustacean and bacterial dynamics, while Pomeroy and Wiebe (1988) and Coffin and Sharp (1987) considered trophic efficiencies within the microbial loop itself. All are valid approaches with the results suggesting either the significance of algae (McCauley and Kalff 1981) or the insignificance of bacteria (Pomeroy and Wiebe 1988, Coffin and Sharp 1987, Geertz-Hansen et al. 1987, Riemann and Bosselman 1984) as a food source for crustaceans. However, there always remains the question of whether errors inherent in a given methodology, coupled with no information on alternate food sources could result in misinterpretation of results.

The present investigation, by considering carbon flow to crustaceans from bacteria and algae simultaneously, has verified that greater algal biomass generally overshadows the amount of carbon originating from bacteria. However, the results of this study have also indicated that at times bacterial carbon flow may be increased, particularly when cladocerans are present. These results also suggest when and where the potential importance of bacteria would be maximized which could be addressed through similar experimental approaches to that used here.

Conclusions

1. Transfer of carbon from bacteria to crustaceans is highly inefficient (fig. 2 and 3), especially under conditions characteristic of oligotrophic environments. This suggests that the major role of the microbial loop appears to be remineralization of organic carbon.

2. Transfer of label from algae to crustaceans is also very inefficient (fig. 6 and 7), suggesting that it is misleading to single out bacteria as the only major sink for carbon in aquatic food webs.

3. When copepods dominate zooplankton communities, they acquire insignificant amounts of carbon from bacteria, presumably due to the necessity for bacterial carbon to pass through at least one intermediate trophic level.

4. The prescence of cladocerans, able to directly graze bacteria, increases the flow of carbon from bacterial sources. Greater inputs of carbon still originate from algal sources due largely to the greater algal biomass in comparison to bacterial biomass in most areas.

5. Conclusion 4 (above), implies that bacteria could supply large amounts of carbon when bacterial biomass equals algal biomass, but presumably only when cladocerans are present to

directly graze bacteria, bypassing all the intermediate trophic levels of the microbial loop. This situation may occur following the decline of an algal bloom or when allochthonous inputs of DOC to lakes are high which, as a result, would increase the substrate levels supporting bacterial growth.

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