THE TROPHIC RELATIONSHIPS BETWEEN SUSPENDED MARINE BACTERIA AND THE SUSPENSION-FEEDERS MYTILUS EDULIS AND ARTEMIA SALINA

by

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ABSTRACT

The trophic relationships, in terms of growth efficiencies, between suspended marine bacteria and the suspension feeders, the blue mussel, Mytilus edulis and the brine shrimp, Artemia salina, were established.

Mytilus edulis could not be supported on suspended bacteria. The growth efficiency of A.salina was dependent upon both the concentration of bacteria in the culture provided as a food source and on the size of the brine shrimp. At concentrations less than 1.5 X 10^6 cells/ml, young shrimp, less than 1 mm in length, died within a few days (zero growth efficiency). Correspondingly, low filtering rates (less than 1 ml/hour/organism) and low consumption rates (less than 0.1 μg/hour/organism) were observed for these organisms at such low concentrations. For brine shrimp greater in length than 1.0 mm, a bacterial concentration of 2.5 X 10^6 cells/ml was required before positive growth efficiencies were obtained. As the food concentration increased beyond this concentration, growth efficiencies steadily increased. An upper limit for the concentration of bacterial cells that could be converted into the biomass of A.salina was not detected; the growth efficiencies continued to increase to a maximum of 60% as the bacterial concentrations supplied increased to 10^7 cells/ml. The growth efficiencies were maximal when Artemia salina obtained a length of 2.5 mm, at which time the highest consumption rates and growth rates were also observed. Growth efficiencies for organisms larger or smaller than 2.5 mm were

lower.

Bacterial densities, expressed in terms of both cells per ml and the amount of ATP per ml, supported from various types of organic substrates, were determined under varying inorganic nutrient and oxygen regimes. The substrates studied included the seaweeds, Ulva lactuca and Fucus vesiculosus, and the vascular plants, Zostera marina and wood chips. Under nutrient-rich conditions (30 μM NO_3^-, 6.0 μM PO_4^{-3}), bacterial densities supported from 1 g (dry weight) of Ulva lactuca reached a maximum of 2×10^7 cells/ml or 12×10^{-3} μg ATP/ml. Based on the established trophic relationships, it was calculated that the amount of suspended bacteria per gram dry weight of substrate grown under these conditions can sustain a maximum weight of 46 μg of Artemia salina or adult brine shrimp.

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INTRODUCTION

Overview

The bacterial component of the detrital food chain is recognized as an important factor controlling the overall productivity of an estuarine ecosystem (Sibert and Naiman,1980). By assimilating organic matter and converting it into biomass, marine bacteria constitute an important route for the flow of matter and energy through marine food webs (Azam and Hodson,1977) and by grazing on this bacterial biomass, many marine organisms meet their nutritional requirements (Jorgensen,1966; Boucherand and Chamroux,1976).

Bacteria can exist as cells attached to particles, as aggregates, or as suspended cells (Jones and Jannasch,1956; Riley,1963). The importance of the attached bacteria as a food source for many grazers has been well established. By colonizing detrital particles, bacteria increase the nutritional value of the particles for the consumer (Newell,1965; Fenchel,1970; Odum et al.,1973). Detritus has minor food value, and the attached bacteria form the major source of high protein food (Frankenberg and Smith,1967; Fenchel,1972; Meyer-Reil and Faubel,1980; Findlay and Tenore,1982). This has been shown to be the case for many detritivores such as oligochaetes (Giere,1975; Coull,1973) and polychaetes (Tenore et al.,1979; Tenore and Hanson,1980). Bacterial aggregates have also been shown to be an important food source for many primary consumers

such as zooplankton (Provasoli et al.,1959; Seki,1966a; Seki and Kennedy,1969; Sorokin et al.,1970; Rieper,1978), sponges (Reiswig,1975), gastropods (Sorokin,1968), mussels (Schleyer,1981), and oysters (Sorokin,1968).

The importance of suspended bacteria as a food source for many organisms has not been investigated as thoroughly as that of attached and aggregated bacteria. In this study the term free-living bacteria refers to suspended bacteria and should not be confused with non-symbiotic bacteria. Researchers recognize that interactions between suspended bacteria and bacterial grazers exist, but emphasis is placed on successional changes in the number of bacterial cells and bacterial grazers over time, not on the specific trophic relationships (Hamilton,1973; Fenchel,1982a,1982b).

The Study Animals

Both Mytilus edulis(L) and Artemia salina(L) are suspension-feeders and obtain their food by filtering water and retaining the suspended particles (Jorgensen,1966). Artemia salina is classified as a non-filtering suspension-feeder since the production of water currents and food uptake are performed by the same organs. Mytilus edulis is a filtering suspension-feeder as these activities are performed by different appendages (Seki,1982).

A controversy exists in the literature regarding the ability of M.edulis to support itself with suspended marine bacteria as its only source of food. Hollibaugh et al.(1980)

showed that the blue mussel was capable of feeding on free-living bacteria as some bacterial retention was observed over time. However, Wright et al.(1982) demonstrated that this organism could not exist beyond a few days when supplied with free-living bacteria as its only food source.

Seki (1966b) developed a chemostat consisting of dissolved organic matter (DOM), bacteria and protozoa which were supplied to A.salina. In this particular system, the brine shrimp reached sexual maturity in 20 days. Seki (1966b) suggested that the brine shrimp were nourished chiefly by grazing the protozoa. However, the bacteria may have also sustained the growth of suspension-feeders as Linley and Newell (1981) suggested. Seki et al. (1968) did, however, demonstrate that suspended bacteria, grown on bactopeptone, enabled brine shrimp to grow, but the extent of the growth dependency was not established.

Since trophic relationships between free-living bacteria and the suspension-feeders, Artemia salina (L) and Mytilus edulis (L), have not been well established these organisms were chosen for this study.

Trophic Relationships

The term trophic relationship used in this study refers to the transfer of energy between organisms within successive trophic levels of the food web. A valuable tool in studies of trophic relationships is 'Growth Efficiency' (GE). GE measures the efficiency with which an organism converts the food available into its own body tissue (Reeve,1963a). The energy obtained

from the available food is assimilated into the organism's biomass (Parsons et al.,1977). The assimilated energy is directed into pathways for growth, metabolic requirements, and excretion. Growth is defined in this study as the increase in body weight over time and trophic relationships will be determined only in terms of gross GEs which only account for the energy used for growth.

It has been well established that GE depends on the concentration of the suspended particles supplied to the organism. The efficiency of the conversion of the food into the biomass of the suspension-feeder is a function of the food intake, since this determines the amount of food energy available for growth. Food intake will vary with the filtering rate of the suspension-feeder. Filtering rates measure the volume of medium that passes the food-catching apparatus within a certain period of time and, therefore, will affect the number of particles that potentially can be retained (Gauld,1951). A minimum number of particles may be required before filtering commences (Frost,1975) and the efficiency of particle retention at low food concentrations may be poor, as observed for Daphnia magna (Rigler,1961) and for Artemia salina (Reeve,1963b,c). Theoretically, as the food concentration increases, filtering rates decline. This was shown to be the case for copepods feeding on phytoplankton cells (Marshall and Orr,1955; Conover,1966; Mullin,1963; Frost,1972). As the filtering rate declines, the rate of food intake or consumption rate may either remain constant or decline (Parsons et al.,1977). However, as

Gaudy (1974) clearly shows, assimilation rate may increase with decreasing food intake, probably because at the low food concentrations, the suspended cells remain in the gut longer due to the lower flushing rate of the intestine (Reeve,1963d). The longer the food stays in the gut, the greater the digestive efficiency, and possibly growth assimilation. Therefore, at lower food concentrations peak GEs are observed. This relationship between food concentrations and GEs is found for both Artemia salina (Gilbor,1957; Reeve,1963a) and Mytilus edulis (Jorgensen,1952; Schuttle,1975) as well as other suspension-feeders, such as euphausiids (Lasker,1960) and copepods (Marshall and Orr,1955; Conover,1961) which were fed phytoplankton.

GEs also vary with the size of the organism, generally decreasing with age (Brody,1945; Richman,1958). The amount of energy used for growth in young and therefore smaller organisms is greater than the amount used in larger and older organisms (Parsons et al.,1977). A period of maximum GEs during the animal's life, may be observed (Makarova and Ye Zaika,1971). At this point a minimum amount of energy is required for metabolic functions and maximum growth rates can be obtained. As the organism approaches sexual maturity, the energy used for growth approaches zero, hence GE decreases and approaches zero. The period of increasing GE to a maximum may also be due to the morphological changes taking place in the organism over time. As the suspension-feeder increases in size, the filtering appendages become more complex and more efficient in retaining

particles (Gauld,1959). However, once the energy required for body maintenance of the organism exceeds that for growth, GEs will decrease despite the efficiency of the filtering apparatus.

Published GEs for brine shrimp and blue mussels have been primarily determined using a phytoplankton food source (Gilbor,1957; Reeve,1963a; Hollibaugh et al.,1980; Wright et al.,1982). Little emphasis has been placed on the importance of suspended bacteria in controlling growth.

Indicators of Bacterial Densities

Several methods are available to determine bacterial densities; however, the suspended bacterial densities in this study were determined from the amount of cellular adenosine triphosphate (ATP) and by direct counting under a microscope.

ATP Analysis

ATP has been used extensively as an indicator of microbial density since its development by Holm-Hansen and Booth in 1966. The analysis is based on a chemical reaction which involves the emission of light. In the presence of luciferin and the enzyme luciferase, ATP is hydrolyzed. For every molecule of ATP present, one photon of light is emitted. The reaction proceeds as indicated in the following equation:

$$\text{LUCIFERIN} + \text{ATP} + O_2 \xrightarrow{\text{luciferase}} \text{LUCIFERIN} + \text{AMP} + \text{P-P} + h\nu$$

(reduced) Mg^{2+} (oxidized)

When all reagents are in excess, the light intensity, hv, is directly proportional to the concentration of ATP. Magnesium is added to extend the time of peak light intensity which declines exponentially (McElroy and Strehler,1949). In order to obtain reproduceable results, the intensity of light produced from the samples should be measured after a constant time (Holm-Hansen,1973). The amount of ATP in the sample is determined by comparison with a set a standards. When using the crude enzyme extract of the firefly, the limit of detection is 10^{-4} μg ATP/ml (Holm-Hansen and Booth,1966).

Firefly luciferase was originally thought to be specific for ATP (McElroy and Strehler,1949) yet it is likely that other molecules containing high-energy phosphate bonds also cause some light emission. Adenosine diphosphate (ADP), guanosinetriphosphate (GTP), coenzyme A, plus a variety of other nucleoside triphosphates emit some light in the presence of the firefly extract (Holm-Hansen and Booth,1966); however, the authors conclude that these compounds are not of great importance when estimating bacterial densities by this technique since they are in low concentrations.

The reliability of the method is based on several assumptions.

1. ATP is a constituent of all living cells.

ATP is one of the most important low molecular weight compounds and is ubiquitous in all living organisms. The molecule undergoes dephosphorylation to release energy for such metabolic processes as glycolysis, fatty acid synthesis and oxidation, protein and nucleic acid synthesis (Bulleid,1977) and is also responsible for the energy-transfer process associated with light production in bioluminescent organisms.

2. ATP is not associated with dead cells.

Holm-Hansen (1973) found that when ATP was added to a culture of dead cells and the solution was filtered, ATP was not detected on the filter. He, therefore, concluded not only that ATP was not present in dead cells, but that it did not adsorb onto detrital particles.

3. The cellular concentration of ATP is constant during the analytical procedure.

Changes in the ATP pool of an organism can occur when cells are stressed. Hodson and Azam (1977) suggest that one form of stress could be filtration and that to minimize this, the filtration period should be quite short. This is achieved by filtering small volumes of medium (less than a litre) (Sutcliffe and Orr,1976). Lysing, another form of stress, is minimized by ensuring that the filtering vacuum pressure does not exceed 8 atm (Holm-Hansen,1973). The extraction procedure is designed to ensure that no changes in the levels of ATP occur. TRIS (tris hydroxymethyl-aminomethane) is the most effective

extracting reagent for seawater samples (Holm-Hansen and Booth,1966; Hamilton,1973; Sutcliffe and Orr,1976; Hodson et al.,1976). TRIS buffer effectively prevents the breakdown of ATP by denaturing any phosphorylating enzymes that are present (Hamilton and Holm-Hansen,1967). It is essential that the living cells are killed instantly. In order to do this the buffer must be close to 100°C in order to rupture the cell walls and release intracellular ATP (Bulleid,1977; Jones and Simon,1977). Holm-Hansen et al.(1968) suggest that the pH of the buffer must be mildly alkaline (pH 7.7) to prevent the precipitation of the TRIS buffer which lowers the luminescence (Bulleid,1978; Perry et al.,1979).

4. The carbon to ATP ratio is constant.

This is the most criticized assumption. The cellular weight ratio of carbon to ATP for bacteria has been shown to vary considerably between taxa, culture conditions and stages of growth. The ratio can vary as much as 50% between different species of bacteria (Karl,1980) but in marine bacteria the ratio is around 250±25 (Banse,1980). Nutrient-deficient cultures tend to have lower amounts of cellular ATP, hence the ratio is higher (Holm-Hansen,1969). ATP concentrations also vary depending on the stage of growth. Forrest (1965) found that the amount of ATP in Streptococcus faecalis reached a maximum during exponential growth and the carbon to ATP ratio per cell reached a minimum. However, when ATP concentration is expressed in terms of cell mass, no change in the ratio is observed

throughout the various phases of growth (Franzen and Binkley,1961; Atkinson and Walton,1967; Chapman and Atkinson,1977).

Epifluorescence Analysis

Direct counting is still believed by many researchers to be the most reliable method for estimating bacteria density. The best resolution is obtained when a fluorescent stain is used and the cells are viewed with epifluorescent illumination (Daley and Hobbie,1975). In 1979, Daley modified and refined the Acridine Orange Direct Counting (AODC) technique and verified that this method is the best available procedure for accurately counting native aquatic bacteria. To ensure reproduceable results the filter must remove all the cells (Bowden,1977). Hobbie et al. (1972) recommend using polycarbonate Nuclepore filters (0.2 μm) because they have a uniform pore size and a flat surface that retains all the bacteria on top of the filter. All the bacterial cells must be visible. Acridine Orange reacts with nucleic acids producing a red-orange glow when it binds with RNA, a compound more abundant in actively growing cells, and a green glow when it binds to DNA, found in inactive cells (Hobbie et al.,1972). The stained cells become clearly visible when placed against a dark background and for this reason Iragalan Black is used to stain the Nuclepore filters (Daley,1979). The cells must be non-aggregated prior to counting as accurate counts can only be obtained if the bacteria are evenly distributed on the filter. Bowden (1977) suggests that

aggregation can be reduced by placing a Millipore filter, type GS, under the Nuclepore filter.

Bacterial Densities

It has been well established that suspended bacteria are nutritionally dependent upon dissolved organic matter (DOM) (Khailov and Burlakova,1969; Schleyer,1981) and that the amount and type of DOM controls bacterial density (Jannasch,1967; Taga,1968; Sieburth,1971). By harvesting considerable quantities of DOM, bacteria may convert it into their own biomass (Paerl,1974,1978).

DOM consists of a pool of predominantly macromolecular and colloidal material which is very resistant to microbial degradation as well as a much smaller pool of lower molecular weight compounds which can be used directly by bacteria (Fenchel and Blackburn,1979). Thirty percent of the DOM present in an estuarine environment consists of carbohydrates, 70% of which is resistant to microbial degadation (Verlimrov et al.,1981). Greater than 50% of the DOM is represented by compounds such as amino acids and non-structural proteins which are readily utilized by bacteria (Rice and Tenore,1981).

The two major sources of DOM for bacterial uptake are particulate organic matter (POM) and zooplankton. The POM releases DOM primarily through leaching and bacterial decomposition and the zooplankton expel DOM by excretion.

Leaching is the major process for DOM release (Harrison and Mann,1975a,b) and takes place in the first few weeks after the

substrate is placed in seawater (Rice and Tenore,1981). Bacterial decomposition becomes the most important process for DOM release when leaching has subsided. The amount of DOM released by leaching depends on the type, age, state, and size of the POM.

Algal detritus is more susceptible to leaching than vascular plant detritus (Olha,1972; Tenore,1977b; Rice,1979; Tenore and Hanson,1980). Marine macroalgae lose about 60% of their gross production as DOM by leaching (Khailov and Burlakova,1969) compared to only 40% for vascular plants (Otsuki and Wetzel,1974) and 30% for deciduous leaf litter (Cummins,1974).

The age, state, and size of the detritus also determines substrate-susceptibility to leaching. Harrison and Mann (1975a) show that young Zostera marina loses more organic matter per day, expressed as a percentage of total organic matter (TOM) than old Z.marina and old eelgrass loses more than dead particles. Khailov and Burlakova (1979) report similar findings for macroalgae. Dried Zostera marina releases more DOM than fresh Zostera marina (Harrison and Mann,1975b) and the amount of DOM expelled from small particles is much greater than that released from larger particles (Gosselink and Kirby,1974; Harrison and Mann,1975b).

Once most of the leaching has ceased, algal detritus is decomposed more easily by microbes than is vascular detritus (Cummins et al.,1973; Gosselink and Kirby,1974; Tenore,1977a; Tenore and Hanson,1980). The red seaweed Gracilaria foliifera

is readily decomposed compared to the decay-resistant vascular plant, Spartina alterniflora. (Tenore,1977b). Rice and Tenore (1981) reported the total organic carbon (TOC) lost after 150 days when various types of substrates were placed in sterile seawater. Red algae lost 65%, the marshgrass, Spartina alterniflora, lost 20% and brown algae lost an intermediate amount of around 35%. These differences may be attributed to the amounts of lignin, total nitrogen, and phenolic residues (Newell and Lucus,1981).

The cell wall of the organic substrate is the major determinant of the resistance or susceptibility to microbial decomposition (Gunnison and Alexander,1975a). Resistance increases with increasing amounts of lignin present in the cell wall (Gunnison and Alexander,1975b).

Typically, vascular plants contain less total nitrogen than seaweeds (Tenore,1983). Substrates which are high in total nitrogen enable a greater initial rate of bacterial decomposition (Rice,1979; Tenore et al.,1979). The remineralization of organic nitrogen is considerably more rapid than that of organic phosphorus; both, however, are regenerated more rapidly than organic carbon (Seki et al.,1968). Therefore, substrates high in organic nitrogen enable the microbes to decompose the substrate at a greater initial rate than substrates low in organic nitrogen; hence total bacterial densities associated with nitrogen-rich substrates will be greater (Tenore,1983).

King and Heath (1967), Suberkropp et al.(1976) and Kaushik

and Hynes (1971) report that phenolic residues also affect microbial attack. Polyphenols are alkali- or alcohol-extractable polyhydroxybenzene derivatives formed by the oxidation of lignins (Hedges and Mann,1979). These compounds are extremely decay resistant (Fenchel and Blackburn,1979) and are present in large quantities in woody plant materials. Smaller quantities are found in non-woody vascular plants and the least amount is found among non-vascular substrates (Hedges and Mann,1979). Water-soluble extracts from detritus containing phenolic compounds have also been shown to inhibit the growth of many marine bacteria (Harrison,1982).

Small particles have a greater surface area which enhances microbial activity (Fenchel,1970; Hargrave,1972; Gosselink and Kirby,1974). Particle size thus plays an important role in determining the release of of DOM by microbial decomposition. As bacterial activity increases, more DOM is released (Odum and de la Cruz,1967).

Drying of the substrates causes structural and chemical changes which affect microbial attack. Harrison and Mann (1975b) find that the eelgrass, Zostera marina, decomposed less rapidly when dried but Zieman (1968) finds that drying of a similarly structured vascular plant, Thalassia testudinum, permits easier entrance by microbes, and hence, faster decomposition.

In systems which include primary consumers such as zooplankton, it has been shown that the associated bacterial population remains in an active metabolic state (Fenchel and

Harrison,1976; Fenchel,1977; Levington,1980). One reason this may result is that the grazers are excreting DOM which becomes available to the microbes (Zobel and Feltham,1938; Johannes,1968; Newell,1965; Hargrave,1970; Fenchel,1970,1975).

Heterotrophic bacteria require a ratio of carbon to nitrogen to phosphorus by weight of 200 to 10 to 1 for their conversion to microbial protoplasm (Thayer,1976). When the microorganisms are supported on nutrient-poor matter, their demands for nitrogen and phosphorus are met by assimilating dissolved inorganic nitrogen and phosphorus (Fenchel and Jorgensen,1977). Thus the concentration of dissolved inorganic nutrients can influence bacterial densities. The addition of nitrate and phosphate stimulates microbial growth thus increasing total biomass (Carlucci,1971; Fenchel and Harrison,1976; Fenchel,1977; Fenchel and Blackburn,1979). As the bacteria decompose and assimilate the structural compounds of the detritus, they utilize the dissolved inorganic nutrients present (Tenore,1977b). The amount of dissolved inorganic matter initially decreases as the microbial population grows, but once the substrate is attacked by the microbes, the concentration of the dissolved inorganic nutrients will increase as they are released through bacterial mineralization (Fenchel and Harrison,1976).

These factors, which affect bacterial densities, are those which operate in an aerobic system. In an anaerobic environment, the process of bacterial mineralization can be divided into a series of metabolic steps with each step

requiring a physiologically different type of organism (Fenchel and Jorgensen,1977). The initial process (hydrolysis of particulate matter into amino acids , disaccharides and long chain fatty acids, by extracellular enzymes) is similar to aerobic degradation. However, in the absence of oxygen, these molecules are then converted to other compounds by fermentation. These compounds, which include lactic, formic, acetic, propionic and butyric acids, then serve as substrates for further bacterial mineralization (Fenchel and Blackburn,1979). Although the mechanisms of anaerobic and aerobic degradation differ, the bacterial densities supported on the organic matter and the rate of decay are not significantly different (Seki and Yokohama,1978).

Purpose of Study

This study examines the the trophic relationships, in terms of GEs, between suspended marine bacteria and the suspension-feeders, the blue mussel, Mytilus edulis(L), and the brine shrimp, Artemia salina(L). The experimental conditions necessary to support bacterial densities which enable these suspension-feeders, of various sizes, to obtain the highest observed GEs will then be determined. The hypothesis to be tested is that suspended bacteria can be converted into the biomass of M.edulis and A.salina at some measureable, optimum growth efficiency.

METHODS AND MATERIALS

Bacterial Growth

Culture Techniques

Four types of organic substrate were collected on estuarine beaches around Vancouver, British Columbia. *Fucus vesiculosus* L. and wood chips were obtained from Point Grey and *Zostera marina* L. and *Ulva lactuca* L. were collected from the ferry jetty at Tsawwassen. For each experiment, the substrate was washed with fresh water to remove attached sediment particles and epiphytes and then was divided into two portions of equal wet weight. One portion was dried in an oven at 50°C for 12 h to determine its dry weight and the other underwent various preparations depending on the experimental design. The experimental sample either remained wet or was dried. It was then either left intact or ground into particles in a blender. Wet experimental substrates were autoclaved. The sizes of the substrate particles which were determined under a microscope are reported in Table 1. Table 2 shows the various treatments that were conducted.

A sample of each substrate was sent to 'The Canadian Microanalytical Service', in Vancouver, where the relative amounts of total carbon, hydrogen, and nitrogen were determined.

The prepared particulate matter was added to a 6 l flask containing 5 l of autoclaved synthetic seawater (50 g $MgSO_4$ $7H_2O$, 0.25 g $NaHCO_3$ H_2O and 155 g NaCl in 10 l of

distilled water). The media had a pH of approximately 7.5 (S.D.= 0.5) measured by a pH meter (model#320,Fisher Co.) and a salinity of 28 parts per thousand (ppt) measured by a YSI salinometer (model#33). Two hundred milliliters of a natural seawater sample, collected from the shoreline of Point Grey, was filtered through a 0.45 μm Millipore filter (type HA, diameter of 47 mm) and added to the flask.

Three levels of inorganic nutrient concentrations used in the experiments were created from the addition of nitrate-nitrogen and phosphate-phosphorous. Nutrient-rich media contained 6.0 μM phosphate and 30 μM nitrate, nutrient-poor media were at concentrations of 1.2 μM phosphate and 10 μM nitrate and nutrient-depleted media had no inorganic nutrients added.

The flasks were then placed in a cold room at 12°C and kept in darkness. Air was injected into the flasks for the aerobic experiments by inserting airstones which were connected to a laboratory air supply. For the anaerobic experiments, the flasks containing the autoclaved synthetic seawater were bubbled with nitrogen gas from a tank to remove all oxygen. The redox potential was monitored daily during this period. When the electrical potential (Eh) reached -200 mv (measured by a pH meter,model#150, Fisher Co.), which took approximately 7 days, the substrates were added to the flasks. The medium was purged with nitrogen gas for an additional two days. The redox potential was checked periodically throughout the experimental period to ensure that the media were still anaerobic (Eh was

less than -200 mv, Wetzel,1975).

Bacterial growth over time was determined from a water sample that was taken either daily or every second day for a period of 18 days. The flasks under anaerobic conditions were tightly sealed with a rubber plug with three projecting tubes. One tube served as an entrance for the nitrogen gas. The second tube enabled excess nitrogen gas to escape (this tube was closed once nitrogen purging ceased). The third tube was designed to enable collection of water samples without allowing air to enter. This was achieved by having a continual vacuum in the tube. The sample was then extracted from the flask by opening a stop cock in the vacuum tubing and bleeding off the sample. Water samples from the oxygenated flasks were obtained by pouring the desired quantity of the medium into a beaker. One portion of this water sample was used for epifluorescence analysis and a second for ATP determination.

Epifluorescence Analysis

The epifluorescence or Acridine Orange Direct Counting (AODC) method was followed according to the procedure outlined by Hobbie et al. (1977). Five milliliters of the sample was preserved with 0.25 ml of formalin (37% formaldehyde). The sample was covered and placed in a refridgerator at 10°C until the analysis was to be conducted.

The analysis involved three steps: staining of the cells followed by their filtration and direct counting under a microscope. The fluorescent dye used was 1 mg acridine orange

(AO) (Fisher Scientific Company) per ml of distilled water. Once prepared, this solution was filtered through a 0.22 μm Millipore filter, type GS. A volume of 0.1 ml or 0.2 ml of this acridine orange solution was added to 1 ml or 2 ml, respectively, of the water sample. After two minutes, the sample was filtered under a vacuum pressure of approximately 0.8 atm. The filtration apparatus consisted of a 0.45 μm Millipore filter (type HA, 25 mm in diameter) which was placed underneath a Nuclepore filter (0.2 μm pore size, 25 mm in diameter). The Nuclepore filter was previously dyed in Iragalan Black (2 g Iragalan in 1 l of 2% acetic acid). When the filtration of the sample was complete, the Nuclepore filter was placed on a clean glass microscope slide. The blank had only the filtered AO stain. One drop of immersion oil was added and a cover slip was then placed on top. Slides were kept in the dark until ready for counting (always within 24 hours).

Bacteria within 10 fields of view were counted using a Zeiss microscope with epifluorescence attachments. The size of the field varied so that at least 20 cells per field were present. The filter combinations of this microscope were identical to those used by Hobbie et al. (1977).

The number of bacteria per ml was determined using the following formula:

cells/ml = (the mean bacterial count of 10 fields) (the area covered by filtered cells, 2.011 X 10^8 μm^2) (the area of the field, in $\mu m^2)^{-1}$ (the volume of sample filtered in ml)$^{-1}$.

ATP Analysis

The ATP content was determined in a manner similar to the method described by Holm-Hansen (1966). The technique involved two steps, the extraction of ATP and the quantitative analysis. Either daily or every second day, a sample for analysis was extracted from the bacterial cultures and frozen until analyzed.

Ten milliliters of autoclaved TRIS buffer (0.02 M tris hydroxymethyl aminomethane (THAM), adjusted to pH 7.7 with 1 N HCL) was heated to boiling (98°C) in a test tube by means of a block heater. A volume of the sample, which varied between 10 ml and 100 ml, depending on the density of the culture, was filtered onto a 0.22 μm Millipore filter (type GS, diameter of 47 mm). The filter was then rolled and placed in the boiling tris buffer for 7 minutes. After this period, the solvent was decanted into a clean test tube and cold tris buffer was added to bring the total volume to 10 ml. The test tube was then placed on ice until the solution reached room temperature (18°C). The samples were covered and frozen until the time of the analysis. Two replicates were processed from each extract. Blank or control samples consisted of the extract obtained from heating a Millipore filter in the TRIS buffer.

The percentage of ATP recovered from this extraction

procedure averaged between 85-95% for all the experiments. This percentage was determined by comparing the ATP content of a sample spiked with a known amount of ATP with that of a non-spiked sample using the following relationship:

$$\% \text{ Recovery} = 100\,[(\text{spike} + \text{sample}) - \text{sample}](\text{spike})^{-1}$$

(Geesey and Costerton,1979).

Quantitative analysis of ATP was determined using a Chem-Glow Photometer (model# J4-7441) and an Integrator Timer (model# J4-74622), both of which were purchased from the American Instrument Company. The firefly extract (Sigma Chemical Company) was reconstituted in 5 ml of distilled water to obtain a solution at pH 7.4 containing 0.05 M potassium arsenate and 0.02 M magnesium sulphate. This solution was then placed in the dark at 12°C for 2 to 3 hours to obtain stabilized activity. Following this period the firefly extract was diluted with 5 ml of 0.1 M sodium arsenate and 5 ml of 0.04 M magnesium chloride, both of which had a pH of 7.4. Immediately after the addition of these dilutants, this enzyme solution was mixed and placed on ice.

When the sample extracts thawed, 0.2 ml of this extract and 0.2 ml of the enzyme were injected into a cuvette, mixed and placed in the photometer cell holder. This procedure took place in exactly 15 sec. The number of light Intensity Units (IU) which accumulated in a period of 10 sec. was recorded.

In order to convert IU into μg of ATP, a set of ATP standards was prepared ranging in IU similar to those observed

from the extracts. The amount of ATP per extract was then converted to μg of ATP per ml of the experimental sample using conversions based on direct proportions.

Units for Bacterial Density

For most experiments bacterial density was determined in cells/ml and μg ATP/ml, and then expressed per gram dry weight of the substrate. These values were converted to μgC for only one experiment by assuming the mass of one bacterial cell was 2.2×10^{-7} μgC (Dale,1974). and that the carbon to ATP ratio was 250 (Holm-Hanson,1960).

Statistical Analysis

For each substrate under nutrient-poor conditions, bacterial densities were monitored in duplicate experimental flasks. A one-way analysis of variance was performed to determine the variance associated with experimental error. This was essential in order to determine significant differences at the 95% confidence level for the bacterial densities obtained under the different experimental conditions. This variance associated with experimental error was then assumed to be constant for all experiments undertaken in this study.

To determine significant differences between values of bacterial density, obtained from the various treatments, an F-test was performed using the variance calculated from the above experimental error. The null hypothesis stated that the

variance between experiments was equal to the variance associated with error or the variance within the experiments. If $F._{05}[x,y]$, defined as the mean square among experiments divided by the mean square within experiments (x and y refer to the degrees of freedom associated with the treatment and with the experimental error, respectively), was greater than or equal to the reported $F._{05}[x,y]$ then the null hypothesis was rejected (Sokal and Rohlf,1969). The conclusion in this case would then have been that the difference among experimental replicates was due to the fixed treatment effect.

Decomposition Rates

The percentage weight lost by the substrates during each experiment was used as an indication of decomposition rate. This loss was computed by dividing the initial dry weight of the substrate into the difference of the initial and final dry weights and multiplying by 100.

Growth and Feeding of Suspension-Feeders

Growth Experiments

(i) The Source of Food

The food supplied for the growth experiments on Artemia salina and Mytilus edulis consisted of the suspended bacterial cultures that were harvested from the bacterial growth experiments. These suspensions were filtered through a glass fibre filter to attempt to remove the bacteria attached to the substrate particles. The concentration of the bacterial cultures used for the growth experiments ranged from 10^5 cells/ml to 10^7 cells/ml.

Dunaliella tertiolecta Butcher was also provided as a food source for the growth experiments with Mytilus edulis. This culture was obtained from the Northeast Pacific Culture Collection (#1) at the University of British Columbia.

(ii) Mytilus edulis

The blue mussel, Mytilus edulis, was collected on rocks in the intertidal area on the north shore of Point Grey, British Columbia. A series of growth experiments were conducted in which three food sources were provided, bacteria supported from Ulva lactuca, Fucus vesiculosus and Dunaliella tertiolecta. Twenty-five mussels were placed in 1400 ml of the bacterial or phytoplankton cultures. The bacterial cultures were of two

different salinities, 14 ppt and 28 ppt. Continuous air was supplied by inserting airstones, which were connected to an air supply, and the growth experiments were conducted at 12°C. The mean length and width of these mussels were determined on the initial and final days of the experiments. Every third day, 100 ml of the food source was added to the flasks. The total amount of food added (the sum of all food provided throughout the experiment) was determined by epifluorescence for the bacterial cultures, and by a Coulter-Counter (model ZB1) for the phytoplankton cultures. The details of the experimental conditions are shown in Table 2. Mean growth rates of the mussels, in units of mm/day, were also determined.

(iii) Artemia salina

Dry eggs of A.salina(L), purchased from the Carolina Biological Supply Company, were added to an aquarium containing 2 to 3 l of synthetic seawater (28 ppt). This tank was well aerated and kept at room temperature (18°C). A desk lamp was placed at one end of the tank. The positive phototactic response of the hatched brine shrimp (Sogeloos,1973) allowed their easy removal with a pipette. Between 100 and 150 shrimp (all were in the first or second instar of growth) were added to a series of aerated flasks containing 50 ml of the bacterial cultures. Bacterial densities were monitored over time and ranged from 6.8×10^5 cells/ml (S.D.=1.5×10^5) to 9.33×10^6 cells/ml (S.D.=0.80×10^6).

Five brine shrimp were removed daily, as of day 0, from

each flask and the mean length and the 95% confidence limits were determined in mm under a dissecting microscope. The experiments lasted between 4 and 16 days.

Feeding Experiments

(i) Calculation of Filtering Rates

Filtering rates were calculated as follows:

$$ml/hr = 2.303V(\log C_0 - \log C_t)\ (t)^{-1},$$

where C_0 and C_t equal the number of cells/ml present before and after the specified time, t (in hours) and V (in ml) is equal to the volume of medium per organism (Hentig,1971).

(ii) Mytilus edulis

Filtering rates were determined only for the mussels feeding on phytoplankton. The reason for this will become evident in the discussion. One or two mussels, of a similar size, were placed in a series of beakers containing 100 ml of the culture of Dunaliella tertiolecta. The cultures were diluted with filtered synthetic seawater (28 ppt) to give final concentrations ranging from 30,000 to 40,000 cells/ml. The mussels used to determine filtering rates were those previously fed with Dunaliella tertiolecta in the growth experiments. Filtering rate experiments were conducted under the same conditions as the growth experiments. Control flasks, without

mussels, were monitored to determine natural increases in cell number during the experimental period. The number of cells, before and after the filtration period of 3 hours was determined using the Coulter-Counter.

(iii) Artemia salina

One to six brine shrimp varying in size from 0.5 mm to 3.0 mm were placed in a series of flasks containing 50 ml of a bacterial culture ranging in concentration from 10^5 cells/ml to 10^7 cells/ml. In all but one of the experiments, the cultures were inoculated with a bacterial-antibiotic 24 hours previous to the beginning of the experiment. The 2 ml inoculum used was taken from a solution containing 100 mg of penicillin and 50 mg of streptomycin dissolved in 10 ml of distilled water (Judy Acreman, personal communication). This inoculum was first filtered through a 0.22 μm Millipore filter before being added to the bacterial culture. The number of bacterial cells before and after the experimental time was determined using the Acridine Orange Direct Counting Technique. The filtration experiments varied between 3 and 4 hours. Changes in bacterial numbers within the control flasks, containing no organisms, were taken into account.

Growth Efficiencies

Growth efficiencies of *Artemia salina* when fed suspended bacteria were determined as follows:

> Growth Efficiency(%) = 100(the change in dry weight of *A.salina* per hour) (the dry weight of the bacterial cells consumed per hour)$^{-1}$
>
> Reeve, 1963a

The dry weight of *A.salina* was determined using the exponential relationship between brine shrimp length, in mm, and dry weight, in μg, reported by Reeve(1963a). The changes in dry weight of brine shrimp per hour at various bacterial concentrations were determined from the growth rates that were observed at those concentrations. Correspondingly, the cells consumed by organisms of this size were calculated from the filtering rate experiments. One bacterial cell was assumed to be 2.2×10^{-7} μgC (Dale, 1974) and the carbon to dry weight ratio was equal to 0.344 (Ferguson and Rublee,1975).

RESULTS

Bacterial Growth and Densities

The Growth Curve

After the bacterial inoculum was added to each flask the bacterial concentration on day 0 varied in cells/ml from 10^2 to 10^3, and in μg ATP/ml from 10^{-7} to 10^{-6}.

A pattern was generally observed in all experiments. The initial growth phase was represented by a rapid increase in bacterial density to a peak which was followed by a plateau in density which was either at the same level as the peak or lower. Figure 1 is an example of this general pattern.

When bacterial biomass was expressed in terms of μg of carbon per ml, the correlation between the epifluorescence and ATP data was poor (Figure 2). Bacterial densities were therefore determined in terms of 10^6 cells per ml and ng ATP per ml which were both expressed per gram dry weight of the substrate added to each experimental flask. The substrate amount had no effect on bacterial densities when expressed in these latter units (Figure 3).

The Nutrient Content of the Substrates

Table 4 indicates the relative percent of total carbon, hydrogen and nitrogen found within the various substrates. Fucus vesiculosus and Zostera marina had similar amounts of the

elements. Ulva lactuca possessed significantly more nitrogen and less carbon than the other three substrates and wood chips contained the least nitrogen but the most of carbon.

The Effect of Different Organic Substrates on Bacterial Densities

(i) Similar Size Particles, Same Subtrate Preparation

When autoclaved substrates were added to media under nutrient-rich conditions, bacterial densities were significantly higher during the growth period when the substrate was U.lactuca than when it was F.vesiculosus or Z.marina. All of these substrates supported greater bacterial densities than wood chips (Figure 4a). This was the general pattern observed under nutrient-poor conditions (Figure 4b). The ATP data supported this result (Figure 5). This same general trend was also found when the substrates were dried (Figure 6).

(ii) Similar Size Particles, Different Substrate Preparations

The epifluorescence data indicated that in flasks with either autoclaved or dried, large substrate particles under nutrient-poor conditions, bacterial numbers were not significantly different throughout most of the growth curve (Figure 7). However, for the same experiments, the amount of ATP was greater in flasks with the dried substrates (Figure 8).

When the substrate particles were small, significant

differences occurred at the peak phase of growth; autoclaved particles supported greater densities than dried particles (Figure 9).

(iii) Different Size Particles, Same Substrate Preparation

When the substrates were autoclaved, the size of the particles had no effect on bacterial densities nor the shape of the growth curve. Both the epifluorescence and ATP data supported this result (Figure 10).

When the substrates were dried, only bacterial densities associated with large particles of Ulva lactuca were greater than those associated with small particles during the peak phase of growth. No significant differences were observed, however, once the population reached the plateau stage of growth (Figure 11).

The Effect of Specific Experimental Conditions

Inorganic nutrients appeared to have a significant effect on the number of bacterial cells supported by Ulva lactuca and Fucus vesiculosus only (Figure 12)). The addition of nutrients also prolonged the period of maximal bacterial densities obtained with U.lactuca and F.vesiculosus.Media with varying concentrations of inorganic nutrients had no significant effect on the quantity of bacteria supported by either Z.marina or wood chips throughout the entire growth period (Figures 12).

Without the addition of inorganic nutrients, bacterial

populations supported by U.lactuca and F.vesiculosus steadily declined following the peak phase to the lowest values obtained in any experiment. Initially, more bacteria were supported by U.lactuca, but by the time the population levelled in density, there was no significant difference in the quantity of bacteria observed (Figure 13). Significant differences in densities were not observed for Z.marina and wood chips throughout the entire growth period.

During the final stage of growth, bacterial numbers supported from the various substrates under anaerobic conditions were not significantly different than those obtained from the aerobic experiments (Figure 14).

Decomposition Rates

Decomposition rates, expressed as percent of dry weight lost during the experiments, were a function of the substrate type and size, and the concentration of inorganic nutrients within the medium. Ulva lactuca decomposed faster than Fucus vesiculosus and Zostera marina, which in turn had a higher decomposition rate than wood chips (Table 5). The presence of inorganic nutrients enhanced decomposition. Small particles decomposed faster than large particles. These observations were found for all substrates except wood chips which demonstrated similar decomposition rates regardless of particle size or nutrient addition.

Growth of Bacterial Grazers

Mytilus edulis

Mytilus edulis was unable to survive when supplied with suspended bacteria as their source of food. Both small and large mussels provided with bacteria cultured with Fucus vesiculosus and Ulva lactuca died after 3 and 4 days, respectively, regardless of the salinity or bacterial concentration of the medium (Table 3). Mussels of similar sizes supplied with Dunaliella tertiolecta filtered the cells (Figure 15). Their growth rates and total number of cells consumed within the growth period are indicated in Table 3.

Artemia salina

The growth of Artemia salina was a function of the bacterial concentration supplied. A.salina grown in a medium containing higher concentrations of bacteria achieved a longer length during the experimental period than those grown in a medium containing lower bacterial concentrations. However significant differences in length occurred only after a week of growth (Figure 16). When the concentration of bacteria was less than 1.5×10^6 cells/ml, log 6.2, Artemia salina was not able to survive beyond a couple of days (Figure 17).

Growth rates were dependent upon the concentration of bacteria supplied; as the initial bacterial concentration increased, growth rates increased (Figure 18). Growth rates

also varied with the size of the shrimp; as the organism obtained a longer length, the growth rate increased to a maximum and then decreased (Figure 18). The type of organic substrate used to support the bacterial cultures had no effect on these patterns.

Artemia salina demonstrated filtering rates that were a function of the initial bacterial concentration as well as the age of the brine shrimp. Filtering rates increased to a maximum as the concentration of the bacterial cultures provided increased, but then steadily decreased as the bacterial concentration further increased. These filtering rates were greater with larger brine shrimp (Figure 19).

The growth efficiencies of Artemia salina, when supplied with suspended bacteria, increased to a maximum as the concentration of the bacteria increased beyond 2.5 X 10^6 cells/ml, log 6.4 (Figure 20). As the body length of A.salina increased, the shrimp was able to convert bacterial cells into its own biomass with a greater efficiency. This growth efficiency reached a peak at a length of 2.5 mm and after this, steadily declined (Figure 20). This peak correlated with the number of cells consumed. Brine shrimp of 2.5 mm consumed more cells than organisms either smaller or greater in size (Figure 21). When brine shrimp were supplied with bacteria at concentrations of less than 2.5 X 10^6 cells/ml, log 6.4, growth efficiencies dropped to zero (Figure 20).

The maximum size of brine shrimp that could be supported by the bacterial cultures increased in direct proportion to the

concentration of the bacteria (Figure 22) and the bacterial cell concentration was directly proportional to the total nitrogen content of the substrate (Figure 23). The weights of Artemia salina that could be obtained if supplied with the concentrations of bacteria supported from 1 g of substrate during the plateau phase of the bacterial growth experiments are reported in Figure 24.

DISCUSSION

Bacterial Growth and Densities

Epifluorescence versus ATP

The poor correlation between the epifluorescence and ATP data when converted to units of μg carbon was possibly due to the assumptions made in making these conversions. In order to convert cells/ml to μgC/ml, a constant weight for the bacterial cells was assumed. However, natural variation in both bacterial cell sizes and weights occur in time and space (Watson et al.,1977). Since the epifluorescence data were lower than the ATP data in terms of μgC/ml, one might speculate that the average weight of one bacterial cell was greater than 2.2×10^{-7} μgC, the weight assumed for this study, if the ATP data were correct.

For the conversion of μg ATP to μg C, it was assumed that the carbon to ATP ratio was constant. As mentioned previously, this is the most criticized assumption of the analysis (Karl,1980). In order for this ratio to be constant during growth, cellular ATP must increase proportionally with the increasing cellular carbon, and the production rate of ATP must equal the rate of utilization (Forrest,1965). Since the bacterial μg C calculated from ATP data exceeded the μgC calculated from epifluorescence data, one might speculate that the carbon to ATP ratio of 250 was too high or that the rate of production of ATP during the time of measurement exceeded the

rate of utilization, thus producing more ATP per cell. Caution should therefore be used when determining bacterial biomass in carbon units from ATP analysis. However, the use of ATP as a relative indication of bacteria growth is acceptable since this study demonstrated that the same bacterial growth trends were observed with both epifluorescence and ATP techniques.

The Growth Pattern

The standard pattern of bacterial growth over time obtained in this study was also found by Hargrave (1970), who used the oxygen consumption technique, by Fenchel (1970,1972,1977) and Seki and Yokohama (1978), who used the epifluorescence technique, and by Hamilton and Holm-Hansen (1967) who used the ATP technique. In all of these studies the bacterial population rose to a peak within a period of 1 or 2 days, and then either maintained high densities or decreased to a plateau by 6 to 8 days.

Bacterial densities may have increased to a maximum within a few days because the bacteria were utilizing the DOM that was initially leached from the substrate and assimilating these readily available compounds into their own biomass (Harrison and Mann,1975a). If the amount of these readily available nutrients was reduced, the population would maintain steady state. At this point, most of the DOM utilized was probably produced by bacterial decomposition of both dead bacterial cells and substrate (Robinson et al.,1982). This plateau or final phase was the stage at which the bacterial cultures were supplied to

the suspension feeders as the food source.

The Effect of Different Organic Substrates on Bacterial Growth and Densities

(i) Similar Size Particles, Same Substrate Preparation

The observed differences in bacterial densities supported from the various substrates could be attributed to differences in the quality and quantity of the DOM that was initially leached from the organic matter. Algal detritus has been shown to be more susceptable to leaching than vascular plant detritus (Tenore,1977a; Rice,1979; Tenore and Hanson,1980) and therefore releases more DOM for subsequent bacterial uptake (Tenore and Rice,1981). This would account for the greater densities with Ulva lactuca compared to those with Zostera marina and wood chips (Tenore and Rice,1981). Dissolved organic compounds may have been readily taken up by the bacteria enabling the population to reach greater densities when grown on U.lactuca. Phenolic residues may also have affected the bacteria numbers and ATP concentrations as these compounds are antibiotic (Conover and Sieburth,1963; Prakash et al.,1972). Both Zostera marina and wood chips release these compounds (Hedges and Mann,1979). The lower bacterial densities supported by these substrates could be a result of the presence of these compounds. One might suspect Fucus vesiculosus, also a seaweed, to have supported more bacteria than Zostera marina, but as this study demonstrated densities were not significantly different

throughout most of the growth period. A possible explanation for this is that F.vesiculosus contains and releases similar amounts of polyphenolic compounds as Z.marina (Hedges and Mann,1979).

The concentration of total nitrogen may also have dictated the amount of bacteria that could be supported by the detritus. Detritus high in total nitrogen may release more dissolved total nitrogen into the medium which would become available for microbial uptake and subsequent microbial growth (Tenore et al.,1979). Ulva lactuca not only was the most readily leached substrate, but also contained the most total nitrogen. Bacterial populations supported by U.lactuca therefore were capable of obtaining maximal levels. Fucus vesiculosus and Zostera marina had similar, but lower, amounts of total nitrogen and therefore supported lower bacterial densities. Wood chips contained the least amount of nitrogen and supported the lowest amount of bacteria.

The amount of organic nitrogen within the substrate appeared to be an important factor controlling the quantity of bacteria. The bacterial densities in flasks with substrates that were either autoclaved or dried were in direct proportion to the amount of substrate nitrogen. Ulva lactuca supported greater microbial densities than Fucus vesiculosus or Zostera marina, both of which supported greater densities than wood chips.

(ii) Different Size Particles, Same Substrate Preparation

Based on the literature, one would expect to find more bacteria per unit surface area associated with small particles than large particles. The smaller the particles, the larger the total surface area available for microbial activity and attachment, and hence greater potential DOM release (Odum and de la Cruz,1967; Fenchel,1970; Hargrave,1972). However, when the substrates were dried, the suspended bacterial densities were greater for a longer period of time at the peak growth phase in flasks containing large particles of U.lactuca. The reason for this could be that more bacterial cells were attached to the smaller particles than to the larger particles meaning that although total bacterial densities were probably greater in the flasks with the smaller particles, as the researchers suggest, the densities of free-living bacteria which were measured in this study were actually less. This idea is supported by the fact that during the initial and plateau phases of growth, the amount of bacteria supported from large and small particles was not significantly different. Initially, the amount of DOM released from large and small particles was the same, thus giving rise to a similar bacterial densities. However, as time progressed, more cells colonized the smaller particles and the number of suspended cells declined to a concentration less than that associated with the larger particles. Once the small particles were covered by the attached bacteria, more DOM was released, which enabled the number of free-living bacteria to increase to values that were no longer significantly different

than those supported from the larger particles.

The size of particles of Ulva lactuca had no effect on the bacterial numbers when autoclaved. It would appear that autoclaving enabled more DOM to be released from the small particles maintaining the free-living bacterial population at high concentrations regardless of microbial particle attachment.

No differences in bacterial densities were observed for Fucus vesiculosus, wood chips, and Zostera marina of different particle sizes. This was probably because bacteria tend not to adhere to vascular plants or woody tissues as readily as to algal detritus (Tenore and Hanson,1980) and the possibilty that these substrates release phenolic compounds regardless of their state.

(iii) Similar Size Particles, Different Substrate Preparation

For small particles greater bacterial densities were obtained when Fucus vesiculosus and Zostera marina were autoclaved as opposed to dried. This supports the previously stated theory that autoclaving releases more DOM into the medium enabling the population to peak at greater concentrations. However, autoclaving may not have been an effective device to release DOM from the large particles as no significant differences were observed in bacterial densities associated with large particles of autoclaved and dried F.vesiculosus and Z.marina.

The Effect of Specific Experimental Conditions

(i) Inorganic Nutrients

The change of nutrient conditions from poor to rich enabled bacterial populations, supported from the seaweeds F.vesiculosus and U.lactuca, to increase in concentration by one order of magnitude. This result suggests that suspended bacteria are capable of rapid uptake of inorganic nutrients, as Fenchel (1977) found, as well as efficient conversion of inorganic nutrients into their own biomass. These differences were still observed at the final growth phase even though the nutrients were probably depleted by this time, as shown by Fenchel (1977) and Fenchel and Blackburn (1979). This implies that the bacterial populations sustained their differences in densities by utilizing the DOM released from bacterial decomposition.

Under nutrient-depleted conditions, the populations supported on Ulva lactuca and Fucus vesiculosus declined to the lowest values observed in all the experiments. This demonstrates the importance of dissolved inorganic nutrients in controlling bacterial densities since the DOM component alone, under nutrient-depleted conditions, was not capable of maintaining concentrations greater than 10^6 cells/ml.

The concentration of inorganic nutrients had no effect on bacterial densities obtained from Zostera marina and wood chips. Generally, inorganic nutrients in the medium are important to bacteria supported on nutrient-poor detritus (Fenchel and Jorgensen,1977). Bacteria then require an exogenous supply of

nutrients. Since both Zostera marina and wood chips contained low amounts of total nitrogen, it may be suggested that these substrates limited the number of bacteria from the low amount of DOM they released. The concentration of inorganic nutrients added may not have been enough to obtain detectable differences.

(ii) Oxygen

Dissolved organic compounds released under aerobic conditions may be different from those released under anaerobic conditions. This is because algal components mineralize completely in an aerobic environment and not in an anaerobic environment and lignins, waxes, phenols, and aromatic compounds demand the presence of oxygen for biologically catalized cleavage (Fenchel and Blackburn,1979). Despite these differences in the type of DOM released from the substrate, it appeared that aerobic and anaerobic conditions had no effect on the quantity of bacteria supported. The results of Seki and Yokohama (1968) support these data. Therefore, one might expect that DOM was equally available to the bacteria in the oxygenated and deoxygenated flasks (as Knauer and Ayers,1977) suggest) and therefore similar concentrations of suspended bacteria may be supported.

Decomposition Rates

Decomposition rates varied with substrate type possibly due to the differences in the amount of organic nitrogen (Seki et al.,1968), lignin (Gunnison and Alexander,1975a) and polyphenols (King and Heath,1967) contained within them. Ulva lactuca was more readily decomposed compared to Zostera marina since microbes process algal detritus more easily than vascular detritus (Tenore,1975a; Rice,1979; Tenore and Hanson,1980). Fucus vesiculosus and Zostera marina decomposed at similar rates possibly because of the similar amounts of organic nitrogen and polyphenolic compounds that were released from these substrates; both may have been decomposed more slowly than Ulva lactuca because of the greater amount of organic nitrogen and no phenolic residues released from this substrate.

Similar decomposition rates were observed for wood chips for all treatments. This was probably due to the high amount of lignin that was present in the cell walls (Gunnison and Alexander,1975a) which prevented microbial attack.

The decomposition rates were a relative indication of the amount of suspended bacteria that was supported by the various substrates under different experimental conditions. Exceptions to this observation were small particles of Ulva lactuca which had a 2-fold increase in decomposition rate over large particles, yet the suspended bacterial densities supported by the large and small particles were similar. Higher decomposition rates associated with small particles have also observed by Gosselink and Kirby (1974), Hargrave (1972) and

Harrison and Mann (1975a). Fenchel (1977) attributes the enhanced decomposition of small particles to greater microbial attachment. As suggested previously, the total microbial biomass (attached and suspended bacteria) associated with the small particles may have been greater than that with large particles, and therefore could have given rise to this greater decomposition rate.

The Growth of Suspension-Feeders

Mytilus edulis

Toxicity, laboratory conditions, or starvation are possible reasons for the observed death of Mytilus edulis when supplied with the suspended bacterial cultures as their only source of food. Polyphenols are toxic metabolites released by Fucus vesiculosus (Tenore and Rice,1980). The presence of these compounds in the bacterial culture medium supported by F.vesiculosus could have caused the mussels to die. However, it was unlikely that a toxic response was the only cause of death since M.edulis also died when supplied with a culture of suspended bacteria grown from Ulva lactuca, a substrate which does not produce these toxic metabolites. The laboratory conditions to which the mussels were adapted were not related to their death. Similarly conditioned mussels were capable of growing and feeding on the phytoplankton culture, Dunaliella tertiolecta. Wright et al. (1982) describe the blue mussel, Mytilus edulis, as having a "course filtering apparatus" as this

organism has greater spaces between the cirri along its gill filaments than other molluscs. Geukensia demissa and Mytilis californianus are both capable of filtering free-living bacteria (Vahl,1972; Jorgensen,1975). Because of this morphological feature, M.edulis may not have been able to remove bacteria from the suspension with any measureable efficiency. Their death was probably a result of starvation. It cannot be implied that no bacterial cells were filtered, since some bacterial retention may have taken place as found by Hollibaugh et al.(1980). However, even if there was some retention, the energy assimilated from this source of food was sufficient to support the mussels for only a few days. The concentration of free-living bacterial cells supplied as a source of food was not a contributing factor in the mussels' death as media of similar bacterial concentrations have been shown to support Mytilis californianus for several months (Zobel and Feltham,1938).

Artemia salina

The GEs of Artemia salina were a function of the concentration of suspended bacteria supplied as food. A minimum concentration of 2.5×10^6 cells/ml (log 6.4) was required to obtain a detectable GE. It could be that concentrations less than this value did not enable the parapodia of the brine shrimp to efficiently filter the medium clear of cells, as Provasoli and D'Agostino (1969) suggested, since low filtering rates were observed. The number of particles collected and then consumed was not great enough to enable the organism to grow, and hence

GEs were zero.

Once bacterial concentrations exceeded 2.5 X 10^6 cells/ml, the shrimp efficiently filtered the media, retained and digested the suspended bacteria and were then able to convert the food into their own biomass. Observed GEs then increased above zero because of the enhanced consumption rates. As the food concentration increased, filtering rates decreased, which enabled efficient retention of bacteria. Since growth rates increased it seems reasonable to assume that most of the ingested food was assimilated for growth.

Belkemishev (1954) and Raymont and Gross(1954) observed an upper limit to the number of particles that could be converted into the biomass of an organism. At high food concentrations, the digestive efficiency of brine shrimp has been observed to decrease (Reeve,1963b) due to the greater pressure on the particles as they pass through the gut. Cells are therefore expelled before they could be digested (Reeve,1963d). This upper limit was not observed during the study and may therefore occur at cell concentrations above 10^7 cells/ml, the highest concentration used in the growth experiments. Despite the fact that this upper limit for GEs occurred at cell concentrations greater than 10^7 cells/ml, maximum consumption occurred at concentrations less than this value. Other researchers have also found a maximum threshold for prey consumption (Parsons and LeBrasseur,1970; Frost,1975). In addition, however, this study found that this peak in consumption was followed by a decline as prey concentration was further increased; a trend also observed

by Mullin (1963) and Nassogne (1970). This decline may be explained by examining the feeding processes of brine shrimp that were observed by Reeve (1963b). As brine shrimp filter the medium, a clump of food particles accumulate behind the labrum before being passed to the maxillae. At high food concentrations, the ball of cells increases in size too quickly to be brought to the mouth for consumption and it is disposed of by the first thoracic limbs. This process of discarding the excess collected cells may account for the lower consumption rates observed at high bacterial concentrations. However, cells that were consumed must have been efficiently digested as the shrimp continued to grow. This gave rise to the increased GEs observed at high food concentrations.

GEs also increased as the size of Artemia salina increased. However, significant differences occurred only after the organism obtained a length of 1 mm. Once the brine shrimp were larger than 1 mm, higher filtering rates were observed. This probably occurred because their filtering appendages were more complex and therefore more efficient in retaining bacteria cells. Gauld (1959) noted that the number of thoracic limbs of Artemia salina increased and the setae became more finely developed in the 4th and 6th instars, the growth stages which correspond to brine shrimp of sizes 1 mm and 1.5 mm, respectively. The marked increase in GEs continued as the organism increased to a length of 2.5 mm but then decreased. This could be due to the organism's energy requirements for growth and body maintenance. Young A.salina have a lower energy

cost for body maintenance and most of the ingested food is converted directly into biomass. As they continue to grow, the feeding appendages become more complex and therefore more efficient in collecting food particles, thus increasing GEs. However, once the organism reaches 2.5 mm, a balance could be reached between the energy requirements for growth, metabolism, and physical activities. Therefore, less energy would be converted into their own biomass, and GEs would decrease. The lower GEs for larger shrimp may also have been a result of the lower consumption rates observed. Corresponding filtering rates, however, were higher, meaning that the volume of medium passed by the filaments was greater but cell retention was less. The decreased ration might be a result of the free-living bacterial cells being too small for the large brine shrimp to retain. The distance between the filtering setules of adult brine shrimp are 5.7 μm (Ussing,1938), and the bacterial cells, were 2-4 μm in diameter.

The GEs calculated for Artemia salina supported from suspended bacteria are comparable to those reported by Gilbor (1957) and Reeve (1963a) for A.salina fed with phytoplankton cultures. A maximum growth efficiency of 65% was found by Reeve (1963a) at a concentration of $3X10^4$ cells/ml. Such an efficiency was obtained in this study at a concentration of 10^7 bacterial cells/ml. Free-living bacteria and phytoplankton cells are therefore comparable food sources in terms of potential GEs for A.salina.

Summary

GEs for the blue mussel, Mytilus edulis were not measureable; however, GEs were detected for Artemia salina supported on this food source and definite relationships existed between growth and ration. The length of A.salina that was supported or maintained on suspended bacterial cultures was linearly proportional to bacterial concentration as shown in Figure 22. Since experimental conditions as well as substrate type dictated bacterial densities obtained per gram (dry weight) of the substrate provided, the weight, in μg, of one brine shrimp that can be supported from these bacterial cultures was determined. The results of this calculation are reported in Figure 24. Bacteria supported by 1 g (dry weight) Ulva lactuca under nutrient-rich conditions can support one adult brine shrimp which weighs 46 μg. Free-living bacterial cultures supported by wood chips can only maintain A.salina that are in the first instar growth stage or weigh 0.5 μg. Similar, yet intermediate, sizes of brine shrimp could be sustained from bacterial cultures grown with Fucus vesiculosus and Zostera marina. Under nutrient-rich conditions, bacteria are at sufficient concentrations to support brine shrimp greater in weight than 2 μg or 2.0 mm in length. Bacterial cultures grown under nutrient-poor conditions will only sustain A.salina that are less than 2μg.

CONCLUSIONS

Based on the findings of this study, the following conclusions can be made.

1. Epifluorescence and ATP techniques are comparable indicators of bacterial growth.

2. The growth of bacteria when supplied with a detrital substance follows a distinct pattern. It is suggested that this growth pattern is a result of the amount of DOM released from the detritus by leaching and bacterial decomposition.

3. The type of the organic substrate supplied to seawater is the most important factor controlling suspended bacterial densities that can be supported; Ulva lactuca supports more than Fucus vesiculosus and Zostera marina which support more than wood chips. The amounts of organic nitrogen, polyphenolic compounds, and lignin, are probably responsible for these observed differences.

4. Decomposition rates of organic substrates generally reflect the suspended bacterial biomass that is associated with the substrate.

5. The addition of inorganic nutrients to media with the seaweeds Ulva lactuca and Fucus vesiculosus enables greater quantities of suspended bacteria to be obtained. However,

inorganic nutrients have no effect on bacterial biomass supported from Zostera marina and wood chips.

6. Aerobic and anerobic conditions yield bacterial cultures that are not significantly different in densities and therefore are not considered important in determining suspended bacterial densities.

7. Bacterial densities supported by Ulva lactuca under nutrient-rich aerobic conditions enable A.salina to obtain an optimal GE of 60%. Under these conditions, 1 g (dry weight) of Ulva lactuca can support 10^7 cells/ml, which in turn can sustain the growth of adult brine shrimp which are 3 mm in length. Bacteria grown on 1 g of large or small particles of Fucus vesiculosus or Zostera marina, either autoclaved or dried, are only capable of supporting brine shrimp less than 2 mm in length. Bacterial densities obtained from bacterial cultures grown with wood chips can only support brine shrimp which are less than 0.5 mm in length.

8. Mytilus edulis cannot convert suspended bacteria into their own biomass with any measureable efficiency. Furthermore, they are not capable of existing with suspended bacteria as their only source of food. It is suggested that this is a result of their inability to filter the small suspended bacterial cells with their relatively course filtering apparatus.

9. Artemia salina are capable of converting suspended bacteria into their own biomass. However, a minimum number of available cells is required before measureable growth efficiencies are observed. Beyond this concentration growth efficiencies of A.salina depend on the concentration of available cells; GEs increase as the food available increases. The upper limit of the bacterial concentration that can be converted into A.salina biomass exceeds 10^7 cells per ml. GEs also vary with the size of Artemia salina. Maximum GEs are observed for brine shrimp that are 2.5 mm in length. Brine shrimp of this length demonstrate higher growth rates and consumption rates than smaller or larger organisms. It is suggested that this results because the filtering apparatus is completely developed during this stage of growth. The shrimp are thus capable of filtering and retaining bacterial cells most efficiently and therefore greater rations are obtained. In addition, the energy obtained from the cells consumed may be primarily assimilated for growth; hence greater growth rates result.

10. This study shows that some marine suspension-feeders are capable of obtaining measureable GEs when supplied with suspended bacteria. Unless this is taken into account, the importance of suspended bacteria as a source of food for suspension feeders may be underestimated when the transfer of energy within the detrital food chain is studied.

REFERENCES

Atkinson, O.E. and G.M. Walton. 1967. Adenosine triphosphate conservation in metabolic regulation. J. Biol. Chem. 242: 3239-3241.

Azam, F. and R.E. Hodson. 1977. Size disribution and activity of marine microheterotrophs. Limnol. Oceanogr. 22:492-501.

Banse, K. 1980. Microzooplankton interference with ATP estimates of plankton biomass. J. Plankton Res. 2:235-238.

Beklemishev, C.W. 1954. The feeding of some common plankton copepods in Far-Eastern Seas. J. Zool. 33:1210-1229.

Boucher, G. and S. Chamroux. 1976. Bacteria and meiofauna in an experimental sand ecosystem. Material and preliminary results. J. Environ. Mar. Biol. Ecol. 24:237-249.

Bowden, W.B. 1977. Comparison of two direct counting tecniques for the enumeration of aquatic bacteria. Appl. Environ. Microbiol. 33:1229-1232.

Brody, S. 1945. Bioenergetics and growth. Reihold Publishing Co., New York. pp. 104-117.

Bulleid, N.C. 1977. Adenosine triphosphate analysis in marine ecology. A review and manual. Division of Fisheries and Oceanography, Report No. 75. 14pp.

Bulleid, N.C. 1978. An improved method for the extraction of adenosine triphosphate from marine sediment and seawater. Limnol. Oceanogr. 23:174-178.

Carlucci, A.F. 1971. Nutrients and microbial response to nutrients in seawater. In: Marine Microbiology. C.D. Litchfield (ed.), Dowden, Hutchinson and Ross Inc., Stroudsburg, Penn. pp. 245-247.

Chapman, A.G. and D.E. Atkinson. 1977. Adenine nucleotide concentrations and turnover rates. Their correlation with biological activity in bacteria and yeast. Adv. Microbiol. Physiol. 15:254-300.

Conover, R.J. 1961. Metabolism and growth in Calanus hyperboreus in relation to its life cycle. Zooplankton Production Symposium, Cons. Int. Mer., unpublished paper no. 11.

Conover, R.J. 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11:338-354.

Conover, R.J. and J.M. Sieburth. 1963. Effect of Sargassum distribution on its epibiota and antibacterial activity. Bot. Mar. 6:147-157.

Coull, B.C. 1973. Estuarine meiofauna: A Review: Trophic relationships and microbial interactions. In: Estuarine Microbial Ecology. L.H. Stevenson and R.R. Coull (eds.), Univerisity of Carolina Press, Columbia. pp. 499-512.

Cummins, K.W. 1974. Structure and function of stream ecosystems. Bio. Sc. 24:631-641.

Cummins, K.W., R.C. Peterson, F.D Howard, J.C. Wuycheck and V.I. Holt. 1973. The utilization of leaf litter by stream detritivores. Ecology 54:336-345.

Dale, N.G. 1974. Bacteria in sediments: Factors related to their distribution. Limnol. Oceanogr. 19:519-524.

Daley, R.J. 1979. Direct epifluorescence enumeration of native aquatic bacteria: uses, limitations and comparative accuracy. In: Native Aquatic Bacteria: Enumeration, Activity and Ecology. J.W. Costerton and R.R. Colwell (eds.), American Society for Testing Materials. pp. 29-45.

Daley, R.J. and J.E. Hobbie. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr. 20:875-882.

Fenchel, T. 1970. Studies on the decomposition of organic detritus derived from the turtle grass Thalassia testudinum. Limnol. Oceanogr. 15:14-20.

Fenchel, T. 1972. Aspects of decomposer food chains in marine benthos. Verh. Deutsch Zool. Ges. 65, Fahresuersamml 14: 14-22.

Fenchel, T. 1975. The quantitative importance of the benthic microfauna of an arctic tundra pond. Hydrobiologia 46:445-464.

Fenchel, T. 1977. The significance of bactivorous protozoa in microbial community of detrital particles. In: Aquatic Microbiological Communities. J. Cairns (ed.), Garland Publishing Inc., London. pp. 529-543.

Fenchel, T. 1982a. Ecology of heterotrophic microflagellates. III. Adapatations to heterogenous environments. Mar. Ecol. Prog. Ser. 9:25-33.

Fenchel, T. 1982b. Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9:35-42.

Fenchel, T. and T.H. Blackburn. 1979. Bacteria in detritus food chains. In: Bacteria and Mineral Cycling. Institue of Ecology and Genetics, Academic Press, London. pp. 52-78.

Fenchel, T. and P. Harrison. 1976. The significance of bacterial grazing and mineral cycling for the decomposition of particulate detritus. In: Role of Terrestrial and Aquatic Organisms in Decompositional Processes. J.M. Anderson and A. Macfudyen (eds.), Blackwell Scrent Publ., Oxford. pp. 285-299.

Fenchel, T. and B.B. Jorgensen. 1977. Detritus food chains of aquatic ecosystems: The role of bacteria. In: Advances in Microbial Ecology. M. Alexander (ed.), Plenum Press, New York. pp. 1- 57.

Ferguson, R.L. and P. Rublee. 1975. Contribution of bacteria to standing crop of coastal plankton. Limnol. Oceanogr. 21: 141-144. .

Finday, S. and K.R. Tenore. 1982. Nitrogen source for a detritivore: Detritus substrate versus associated microbes. Science 218:371-373. .

Forrest, W.W. 1965. Adenosine triphosphate pool during the growth cycle in Streptococcus faecalis. J. Bacteriol. 90: 1013-1018.

Frankenberg, D. and K.L. Smith. 1967. Coprophagy in marine animals. Limnol. Oceanogr. 12:443-450. .

Franzen, J.S. and S.B. Binkley. 1961. Comparison of the acid-soluble nucleotides in E. coli at different growth rates. J. Biochem. 236:515-519.

Frost, B.W. 1972. Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod Calanus pacificus. Limnol. Oceanogr. 17:805-815.

Frost, B.W. 1975. A threshold feeding behaviour in Calanus pacificus. Limnol. Oceanogr. 20:263-266.

Gaudy, R. 1974. Feeding four species of pelagic copepods under experimental conditions. Mar. Biol. 25:125-141.

Gauld, D.T. 1951. The grazing rate of planktonic copepods. J. Mar. Biol. Assn. UK. 29:695-706.

Gauld, D.T. 1959. Swimming and feeding in crustaceans larvae: The nauplius larva. Proc. Zool. Soc. Lond. 132:31-50.

Geesey, G.G. and J.W. Costerton. 1979. Bacterial biomass determinations in a silt-laden river: Comparison of direct epifluorescence microscopy and extractable ATP techniques. In: Native Aquatic Bacteria: Ennumeration, Activity and Ecology. American Society for Testing and Material. pp.117-127.

Giere, O. 1975. Population structure, food relations and ecological role of marine oligochaetes, with special reference to meiobenthic species. Mar. Biol. 31:139-156.

Gilbor, A. 1957. Conversion of phytoplanton to zooplankton. Nature 179:1304.

Gosselink, J.G. and C.J. Kirby. 1974. Decomposition of salt marsh grass, Spartina alterniflora. Limnol. Oceanogr. 19: 825-832.

Gunnison, D. and M. Alexander. 1975a. Basis for the resistance of several algae to microbial decompostion. Appl. Microbiol. 29:729-738.

Gunnison, D. and M. Alexander. 1975b. Basis for the susceptability of several algae to microbial decomposition. Can. J. Microbiol. 21:619-628.

Hamilton, R.D. 1973. Interrelationships between bacteria and protozoa. In: Estuarine Microbial Ecology. L.H. Stevenson and R.R. Coull (eds.), University of South Carolina Press, Columbia. pp. 491-497.

Hamilton, R.D. and O. Holm-Hansen. 1967. Adenosine triphosphate content of marine bacteria. Limnol. Oceanogr. 12:319-324.

Hargrave, B.T. 1970. The effect of a deposit feeding amphipod on the metabolism of benthic microflora. Limnol. Oceanogr. 15:21-30.

Hargrave, B.T. 1972. Aerobic decomposition of sediment and detritus as a fuction of particle surface area and organic content. Limnol. Oceanogr. 17:583-596.

Harrison, P.G. 1982. Control of microbial growth and of amphipod grazing by water soluble compounds from leaves of Zostera marina. Mar. Biol. 67:225-230.

Harrison, P.G. and K.H. Mann. 1975a. Chemical changes during the seasonal cycle of growth and decay in eelgrass (Zostera marina) on the Atlantic coast of Canada. J. Fish. Res. Bd. Can. 32:615-621.

Harrison, P.G. and K.H. Mann. 1975b. Detritus formation from eelgrass (Zostera marina L.): The relative effects of fragmentation, leaching, and decay. Limnol. Oceanogr. 20: 924-934.

Hedges, J.I. and D.C. Mann. 1979. The decomposition of plant tissues by their lignin oxidation products. Geochemica et Cosmochimica Acta 43:1803-1807.

Hentig, R.Von. 1971. Influence of salinity and temperature on the development, growth, reproduction, and energy budget of Artemia salina. Mar. Biol. 9:145-182.

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225-1228.

Hobbie, J.E., O. Holm-Hansen, T.T. Packard, L.R. Pomeroy, R.W. Sheldon, J.P. Thomas and W.J. Wiebe. 1972. A study of the distribution and activity of microorganisms in ocean water. Limnol. Oceanogr. 17:544-555.

Hodson, R.E. and F. Azam. 1977. Determination and biological significance of dissolved ATP in seawater. In: Second Bi-Annual ATP Methodology Symposium. G.A. Bourn (ed.), SAI Technology Co., San Diego. pp. 127-137.

Hodson, R.E., O. Holm-Hansen and F. Azam. 1976. Improved methodology for ATP determination in marine environments. Mar. Biol. 34:143-149.

Hollibaugh, J.T., J.A. Fuhrman and F. Azam. 1980. Radioactive labeling of natural assemblages of bacterioplankton for use in trophic studies. Limnol. Oceanogr. 25:172-181.

Holm-Hansen, O. 1969. Determination of microbial biomass in ocean profiles. Limnol. Oceanogr. 14:740-747.

Holm-Hansen, O. 1973. Determination of total microbial biomass by measurement of adenosine triphosphate. In: Estuarine Microbial Ecology. L.H. Stevenson and R.R. Coull (eds.), University of South Carolina Press, Columbia. pp. 73-89.

Holm-Hansen, O. and G.R. Booth. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11:510-519.

Holm-Hansen, O., M.B. Shattuck and D.M. Karl. 1977. Effects of luciferin concentration on light emmision from luciferase-ATP preparations. In: Second Bi-Annual ATP Methodology Symposium. G.A. Bourn (ed.), SAI Technology Co., San Diego. pp. 81-99.

Holm-Hansen, O., O.W. Sutcliffe and J. Sharp. 1968. Measurement of deoxyribonucleic acid in the ocean and its ecological significance. Limnol. Oceanogr. 13:507-514.

Jannasch, H.W. 1967. Growth of marine bacteria at limiting concentrations of organic carbon in seawater. Limnol. Oceanogr. 12:264-271.

Jannasch, H.W. and G.E. Jones. 1959. Bacterial populations in seawater as determined by different methods of ennumeration. Limnol. Oceanogr. 4:128-139.

Johannes, R.E. 1968. Nutrient regeneration in lakes and oceans. Adv. Microbiol. 1:203-213.

Jones, G.E. and H. W. Jannasch. 1956. Aggregates of bacteria in seawater as determined by treatment with surface active agents. Limnol. Oceanogr. 4:269-276.

Jones, J.G. and B.M. Simon. 1977. Increased sensitivity in the measurement of ATP in freshwater samples with a comment on the adverse effect of membrane filtration. Freshwater Biol. 7:253-260.

Jorgensen, C.B. 1952. Efficiency of growth in Mytilus edulis and two gastropods. Nature 170:714.

Jorgensen, C.B. 1966. Biology of suspension feeders. Pergamon Press Ltd. Great Britain. pp. 212-220.

Jorgensen, C.B. 1975. Comparative physiology of suspension feeding. Ann. Rev Physiol. 37:57-79.

Karl, D.M. 1980. Cellular nucleotide measurements and applications in microbial ecology. Microbiol. Rev. 44:740-785.

Kaushik, N.K. and H.B.N. Hynes. 1971. The fate of dead leaves that fall into streams. Arch. Hydrobiol. 68:465-515.

Khailov, K.M. and Z.P. Burlakova. 1969. Release of dissolved organic matter and distribution of their total organic production to inshore communities. Limnol. Oceanogr. 14: 521-527.

King, H.G.C. and G.W. Heath. 1967. The chemical analysis of small samples of leaf material and the relationship between the disappearance and composition of leaves. Pedobiologia 7:192-197.

Knauer, G.A. and A.V. Ayers. 1977. Changes in carbon, nitrogen, adenosine triphosphate, and chlorophyll a in decomposing Thalassia testudinum leaves. Limnol. Oceanogr. 22:408-414.

Lasker, R. 1960. Utilization of organic carbonby a marine crustacean: Analysis with carbon-14. Science 131:1098.

Levington, J.S. 1980. Particle feeding by deposit feeders: models, data, and prospectives. In: Marine Benthic Dynamics. K.R. Tenore and B.C. Coull (eds.), University of South Carolina Press, Columbia. pp. 423-439.

Linley, E.A.S. and R.C. Newell. 1981. Microheterotrophic communities associated with the degradation of kelp debris. Kieler Meeresforsch., Sonderth 5:345-355.

Makarova, N.P. and V. Ye Zaika. 1971. Relationship between animal growth and quantity of assimilated food. Hydrobiol. 7:1-8.

Marshall, S.M. and A.P. Orr. 1955. On the biology of Calanus finmarchicus. VIII. Food uptake, assimilation, and excretion in adult and stage V Calanus. J. Mar. Biol. Assn. 34:495-529.

McElroy, W.D. and B.L. Strehler. 1949. Factors influencing the response of the bioluminescent reaction to adenosine triphosphate. Arch. Biochem. 22:420-433.

Meyer-Reil, L.A. and A Faubel. 1980. Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. Mar. Ecol. 3:251-256.

Mullin, M. 1963. Some factors affecting the feeding of marine copepods of the genus Calanus. Limnol. Oceanogr. 8:239-250.

Nassogne, A. 1970. Influence of food organisms on the development and culture of pelagic copepods. Helogolander wiss. Meeresunters. 20:333-345.

Newell, R.C. 1965. The role of detritus in the nutrition of two marine deposit feeders, the prosobranch, Hydrobia ulvae and the bivalve, Macoma baltica. Proc. Zool. Soc. Lond. 144:25-45.

Newell, R.C. and M.I. Lucas. 1981. The quantitative significance of dissolved and particulate organic matter released during fragmentation of kelp in coastal waters. Kieler Meeresforsch., Sonderth 5:356-369.

Odum, E.P. and A.A. de la Cruz. 1967. Particluate organic detritus in a Georgia salt marsh estuarine ecosystem. In: Estuaries. G.H. Lauff (ed.), Publ. Amer. Assn. Adv. Sci. pp. 383-388.

Odum, W.E., J.C. Zieman and E.J. Heuld. 1973. The importance of vascular plant detritus to estuaries. In: Proceedings of the Coastal Marsh and Estuary Management Symposium. R.H. Chabreck (ed.), L.S.U. Div. of Continuing Education, Baton Rouge. pp. 91-135.

Olah, J. 1972. Leaching, colonization and stabilization during detritus formation. Mem. Ital. Idrobiol. 29:105-127.

Otsuki, A. and R.G. Wetzel. 1974. Release of organic matter by autolysis of a submersed macrophyte Scirpus subterminalis. Limnol. Oceanogr. 19:842-845.

Paerl, H. 1974. Bacterial uptake of dissolved organic matter in relation to detrital aggregation in marine and freshwater systems. Limnol. Oceanogr. 19:966-972.

Paerl, H. 1978. Microbial organic carbon recovery in aquatic ecosystems. Limnol. Oceanogr. 23:927-935.

Parsons, T.R. and R.J. LeBrasseur. 1970. The availability of food to different trophic levels on the marine food chain. In: Marine Food Chains. J.H. Steele (ed.), Oliver and Boyd, Edinburough. pp. 325-343.

Parsons, T.R., M. Takahashi and B. Hargrave. 1977. Biological oceanographic processes. (Second Edition) Pergamon Press, Oxford. pp. 124-138.

Perry, W.B., J.T. Boswell and J.A. Stanford. 1979. Critical problems with extraction of ATP for bioluminescence assay of plankton biomass. Hydrobiol. 65:155-163.

Prakash, A., A. Jenen and M.A. Rashid. 1972. Humic substances and aquatic productivity. In: Humic Substances. D. Provoledo and H.L. Golterman (eds.), Center for Agricultural Publishing and Documentation, Wageningen, Netherlands. pp. 259-268.

Provasoli, L. and A. D'Agostino. 1969. Development of artificial media for Artemia salina. Biol. Bull. 136:434-453.

Provasoli, L., K. Shiraishi and J.R. Lance. 1959. Nutritional idiosyncrasies of Artemia and Tigriopus in monoxenic culture. Ann. N.Y. Acad. Sci. 77:260-261.

Raymont, J.E.G. and F. Gross. 1954. On the feeding and breeding of Calanus finmarchicus under laboratory conditions. Proc. Roy. Soc. Edin. 61:267-287.

Reeve, M.R. 1963a. Growth efficiency in Artemia under laboratory conditions. Biol Bull. 125:1330145.

Reeve, M.R. 1963b. The filter feeding of Artemia. I. In pure cultures of plant cells. J. Exp. Biol. 40:195-205.

Reeve, M.R. 1963c. The filter feeding of Artemia II. In suspensions of various particles. J. Exp. Biol. 40:207-214.

Reeve, M.R. 1963d. The filter feeding of Artemia. III. Faecal pellets and their associated membranes. J. Exp. Biol. 40: 215-221.

Reiswig, H.M. 1975. Bacteria as food for temperate water marine sponges. Can. J. Zool. 53:582-589.

Rice, D.L. 1979. Trace element chemistry of aging marine detritus derived from coastal macrophytes. Ph.D. Thesis, Georgia Institute of Technology, Atlanta, Georgia.

Rice, D.L. and K.R. Tenore. 1981. Dynamics of carbon and nitrogen during the decomposition of detritus derived from estuarine macrophytes. Estuarine, Coastal and Shelf Sci. 13:681-690.

Rieper, M. 1978. Bacteria as food for marine harpacticoid copepods. Mar. Biol. 45:337-345.

Richman, S. 1958. The transformation of energy by Daphnia pulex. Ecol. Monogr. 28:273-291.

Rigler, F.H. The relation between concentration of food and feeding rate of Daphnia magna. Can. J. Zool. 39:857-868.

Riley, G.E. 1963. Organic aggregates in seawater and the dynamics of their formation and utilization. Limnol. Oceanogr. 8:372-381.

Robinson, J.D., K.H. Mann and J.A. Novitisk. 1982. Conversion of the particulate fraction of seaweed detritus to bacterial biomass. Limnol. Oceanogr. 27:1072-1079.

Schleyer, M.H. 1981. Microorganisms and detritus in the water column of a subtidal reef of Natal. Mar. Ecol. 4:307-320.

Schuttle, E.G. 1975. Influence of algal concentration and temperature on the filtration rate of Mytilus edulis. Mar. Biol. 30:331-341.

Seki, H. 1966a. Role of bacteria as food for plankton. Information Bull. on Planktology in Japan 13:54-62.

Seki, H. 1966b. Studies on microbial participation to food cycle in the sea. III. Trial cultivation of brine shrimp to adult in a chemostat. J. Oceanogr. Soc. Japan 22:105-110.

Seki, H. 1982. Assimilation and decomposition of organic solutes by microorganisms. In: Organic Materials in Aquatic Ecosystems. C.R.C. Press Inc., Boca Raton, Florida. pp. 95-114.

Seki, H., and O.D. Kennedy. 1969. Marine bacteria and other heterotrophs as food for zooplankton in the Strait of Georgia during the winter. J. Fish. Res Bd. Can. 26:3165-3173.

Seki, H., J. Skelding and T.R. Parsons. 1968. Observations on the decomposition of a marine sediment. Limnol. Oceanogr. 13:440-447.

Seki, H. and Y. Yokohama. 1978. Experimental decay of eelgrass (Zostera marina) into detrital particles. Arch. Hydrobiol. 84:109-119.

Sibert, J.R. and R.J. Naiman. 1980. The role of detritus and the nature of esturarine ecosystems. In: Marine Benthic Dynamics. K.R. Tenore and B.C. Coull (eds.), University of South Carolina Press, Columbia. pp. 311-323.

Siebuth, J. 1971. Distribution and activity of oceanic bacteria. Deep Sea Res. Oceanogr. Abst. 18:1111-1121.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co., San Francisco. pp. 192-201.

Sorgeloos, P. 1973. First report on the triggering effect of light on the hatching mechanism of Artemia salina dry cysts. Mar. Biol. 22:75-76.

Sorokin, J.I. 1968. The use of ^{14}C in the study of nutrition of aquatic animals. Mitt. Int. Verin. Theor. Angew. Limnol. 16:1-41.

Sorokin, J.I., T.S. Petipa and Ye V. Pavlova. 1970. Quantitative estimate of marine bacterioplankton as a source of food. Okeanologiya 10:253-260.

Suberkropp, K., G.L. Godshalk and M.J. Kug. 1976. Changes in the chemical composition of leaves during processing in a woodland stream. Ecology 57:720-727.

Sutcliffe, W.H. and E.A. Orr. 1976. Difficulties with ATP measurements in inshore waters. Limnol. Oceanogr. 21:145-149.

Taga, N. 1968. Some ecological aspects of marine bacteria in the Kuroshio current. Bull. Misaki Mar. Biol. Instit. Kyoto Univ. 12:65-76.

Tenore, K.R. 1977a. Utilization of aged detritus derived from different sources by the polychaete Captiella capitata. Mar. Biol. 44:51-55.

Tenore, K.R. 1977b. Growth of the polychaete Capitella capitata cultured under different levels of detritus derived from various sources. Limnol. Oceanogr. 22:936-941.

Tenore, K.R. 1983. What controls the availability to animals of detritus derived vascular plants: organic nitrogen enrichment of caloric availability? Mar. Ecol. Prog. Ser. 10:307-309.

Tenore, K.R. and R.B. Hanson. 1980. Availability of detritus of different types and ages to a polychaete consumer, Capitella capitata. Limnol. Oceanogr. 25:553-558.

Tenore, K.R. and D.L.Rice. 1980. A review of the trophic factors affecting secondary production of deposit feeders. In: Marine Benthic Dynamics. K.R. Tenore and B.C. Coull (eds.), University of South Carolina Press, Columbia. pp. 325-340.

Tenore, K.R., R.B. Hanson, B.E. Dornseif and C.N. Wiederhod. 1979. The effect of organic nitrogen supplement on the utilization of different sources of detritus. Limnol. Oceanogr. 24:350-355.

Thayer, G.W. 1976. Identity and regulation of nutrients limiting phytoplankton production in the shallow estuaries near Beaufort, N.C. Oecologia 14:75-92.

Ussing, H.H. 1938. The biology of some important plankton animals in fjords of East Greenland. Medd. Gronland 100:1-108.

Vahl, O. 1972. Efficiency of particle retention in Mytilus edulis. Ophelia 10:17-25.

Verlimrov, B., J.A. Ott and R. Novak. 1981. Microorganisms on macrophyte debris: Biodegradation and its implication in the food web. Kieler Meeresforsch., Sonderth. 5:333-344.

Wetzel, R.G. 1975. Limnology. W.B. Saunders Co., Philadelphia. pp. 248-256.

Wright, R.T., R.B. Coffin, C.P. Ersing and D. Pearson. 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27:99-111.

Zieman, J.C. 1968. A study of the growth and decompostion of the sea grass Thalassia testudinum. M.S. Thesis, Univ. Miami. 50p.

Zobel, C.E. and C.B. Feltham. 1938. Bacteria as food for certain marine invertebrates. J. Mar. Res. 1:312-327.

TABLES

Table 1. The surface area of the substrate particles. These data show the surface area, in cm^2, of the various substrates used in the bacterial growth experiments.

		Surface Area (cm^2)			
Substrate		Ulva sp.	Fucus sp.	Eelgrass	Wood chips
Whole plant (wet or dry)		25-100	30-150	15-50	0.5-1.0
Blended Plant	wet	0.25-0.5	0.25-1.0	0.25-1.0	-
	dry	0.02-0.08 $X10^{-4}$	0.24- 1.12 $X10^{-4}$	0.24-0.72 $X10^{-4}$	0.48-1.20 $X10^{-4}$

Table 2. A summary of the conditions of the bacterial growth experiments with different sizes and states of substrates under different nutrient and oxygen regimes.

Substrates (a)	Inorganic Nutrients (b)	Detritus State (c)	Detritus Size (d)	Aerated
U,F,E,WC	N-	wet	large	yes
U,F,E,WC	Nt	wet	large	yes
U,F,E,WC	Ntt	wet	large	yes
U,F,E	Nt	wet	small	yes
U,F,E,WC	Nt	dry	small	yes
U,F,E,WC	Nt	dry	large	yes
U,F,E,WC	Nt	wet	large	no

(a) U=Ulva sp.
F=Fucus sp.
E=Eelgrass
WC=Wood chips

(b) N-=no inorganic nutrients added
Nt=nutrient-poor
Ntt=nutrient-rich

(c) wet=autoclaved
dried=oven-dried

(d) see Table 1 for sizes

Table 3. The experimental conditions used to determine the growth rates of Mytilus edulis when supplied with suspended bacterial cultures grown with Fucus vesiculosus and Ulva lactuca, and the phytoplankton, Dunaliella tertiolecta.

Food Source	Fucus sp.		Ulva sp.		Dunaliella sp.	
Salinity (ppt)	28	14	28	14	28	28
Temperature (^{0}C)	12	12	12	12	12	12
Length of Experiment (days)	3*	3*	4*	4*	16	16
initial width (mm)	4.7	11.6	4.4	11.0	17.0	7.7
S.D.	0.4	0.8	0.5	0.9	1.2	0.6
initial length (mm)	1.7	6.6	1.6	6.6	10.1	4.5
S.D.	0.3	0.5	0.2	0.6	0.8	0.4
Cells supplied (10^{6}cells/ml)	5.28	4.98	6.13	5.56	0.06	0.07
Growth Rate (mm/day) (length/ width)	0/0	0/0	0/0	0/0	.01/ .03	.05/ .003
S.D.	-	-	-	-	.008/ .01	.009/ .001

*length of experiment indicates the day the mussels die
S.D. represents Standard Deviations

Table 4. The relative percents of total carbon, hydrogen and nitrogen within the substrates used in this study.

Substrate	Carbon (%)	Hydrogen (%)	Nitrogen (%)
Ulva sp.	29.96	5.38	3.98
Fucus sp.	36.20	5.28	1.62
Eelgrass	36.60	5.86	1.82
Wood Chips	46.62	6.29	0.20

Table 5. Decomposition rates in terms of percent dry weight lost of large and small substrate particles which are autoclaved or dried under different nutrient and oxygen regimes (Standard Deviations: wood chips=0.3, Eelgrass=1.1, Fucus sp.=0.8 and Ulva sp.=2.1).

Substrate	Inorganic Nutrients (a)	Detritus State (b)	Detritus Size (c)	Aerated	% Weight Lost
Wood Chips	N-	wet	large	yes	1.4
	N+	wet	large	yes	1.2
	N++	wet	large	yes	1.7
Eelgrass	N-	wet	large	yes	20.0
	N+	wet	large	yes	23.3
	N++	wet	large	yes	37.8
Fucus sp.	N-	wet	large	yes	10.6
	N+	wet	large	yes	24.3
	N++	wet	large	yes	36.9
Ulva sp.	N-	wet	large	yes	20.0
	N+	wet	large	yes	25.5
	N++	wet	large	yes	40.0
Wood chips	N++	wet	large	yes	1.7
Eelgrass	N++	wet	large	yes	37.8
Fucus sp.	N++	wet	large	yes	36.9
Ulva sp.	N++	wet	large	yes	40.0
Wood chips	N+	dried	large	yes	1.1
	N+	dried	small	yes	1.2
Eelgrass	N+	dried	large	yes	35.1
	N+	dried	small	yes	33.7
Fucus sp.	N+	dried	large	yes	38.3
	N+	dried	small	yes	40.0
Ulva sp.	N+	dried	large	yes	29.9
	N+	dried	small	yes	66.4

Wood chips	N+	wet	large	no	1.1
Eelgrass	N+	wet	large	no	22.2
Fucus sp.	N+	wet	large	no	28.3
Ulva sp.	N+	wet	large	no	36.9

(a) N-=nutrient-depleted
N+=nutrient-poor
N++=nutrient-rich

(b) wet=autoclaved
dried=oven-dried

(c) see Table 1 for sizes

FIGURES

Figure 1. A typical bacterial growth curve . These data show bacterial densities over time when supplied with autoclaved Zostera marina under nutrient-poor conditions.

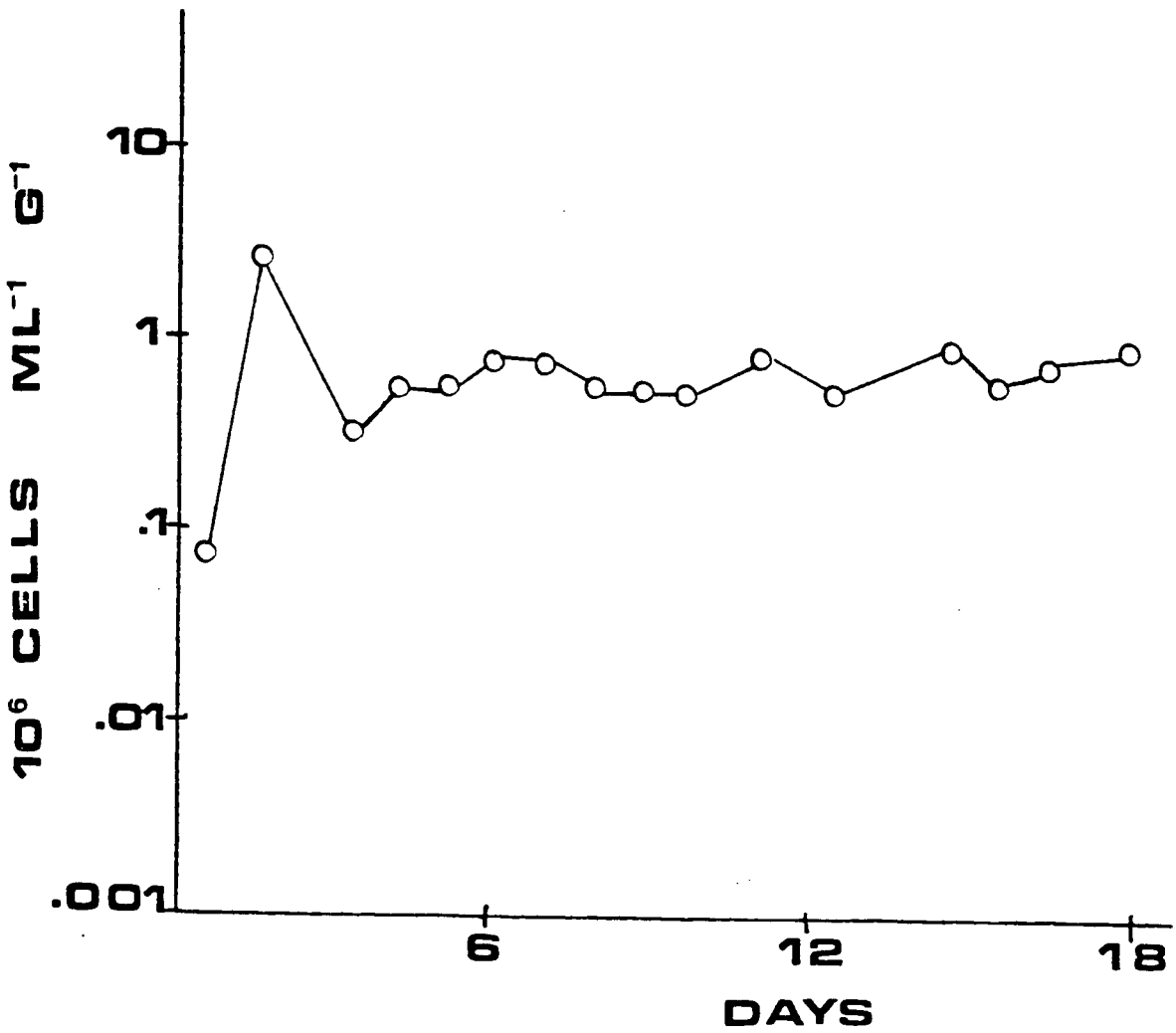
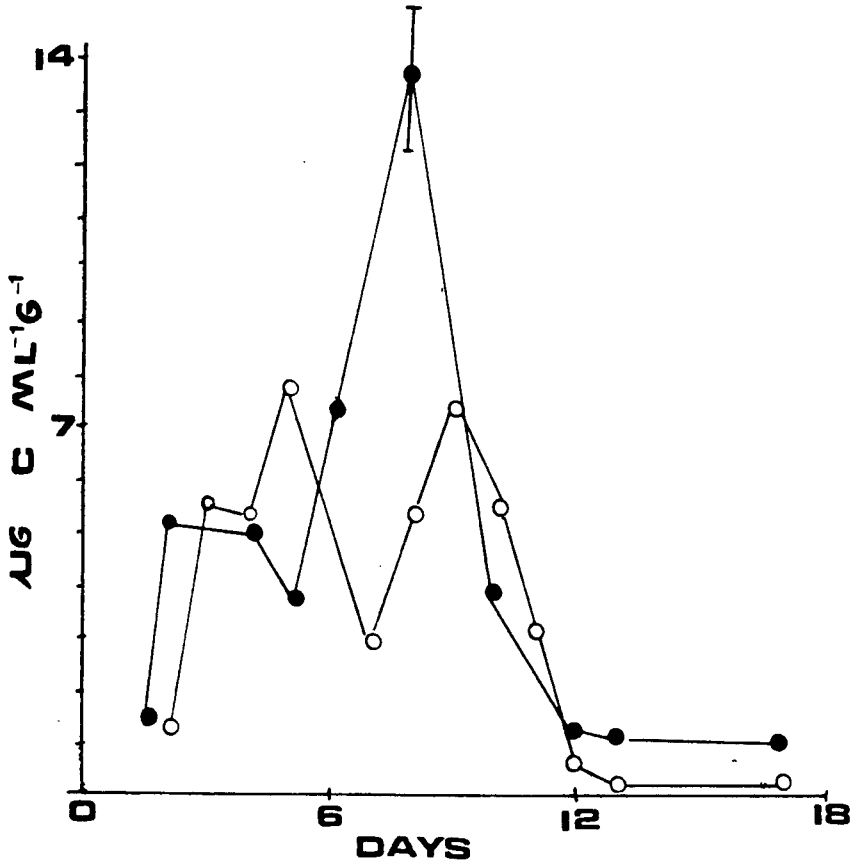
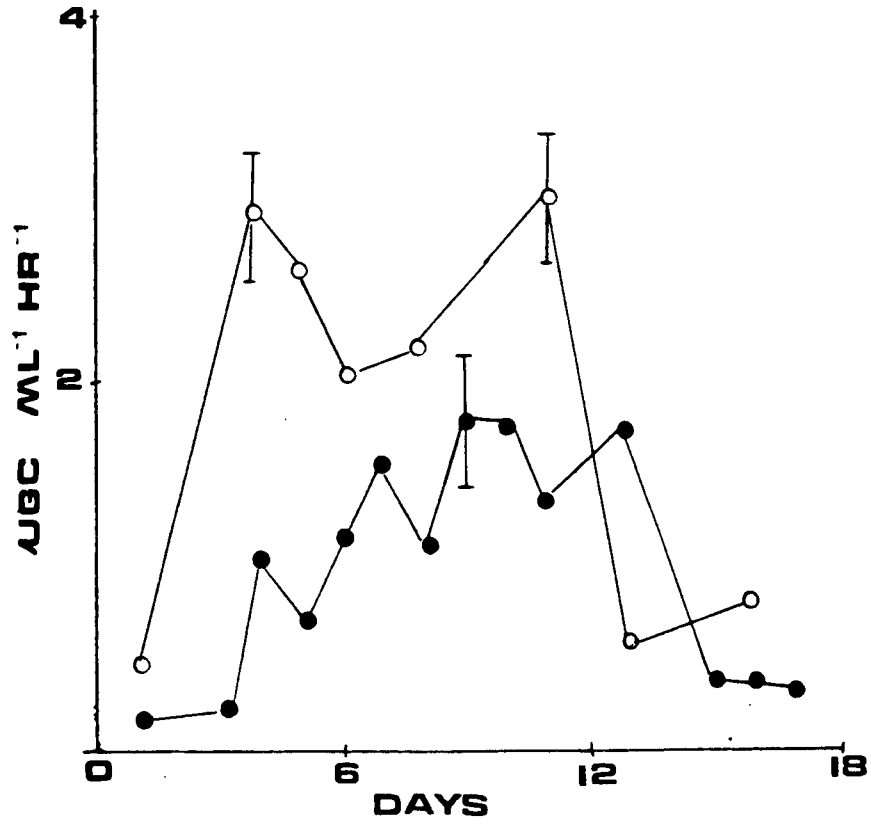

10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS

Figure 2. The suspended bacterial biomass in μgC over time that is supported from
(a) Ulva lactuca
(b) Fucus vesiculosus
(ATP data○; Epifluorescence data●).

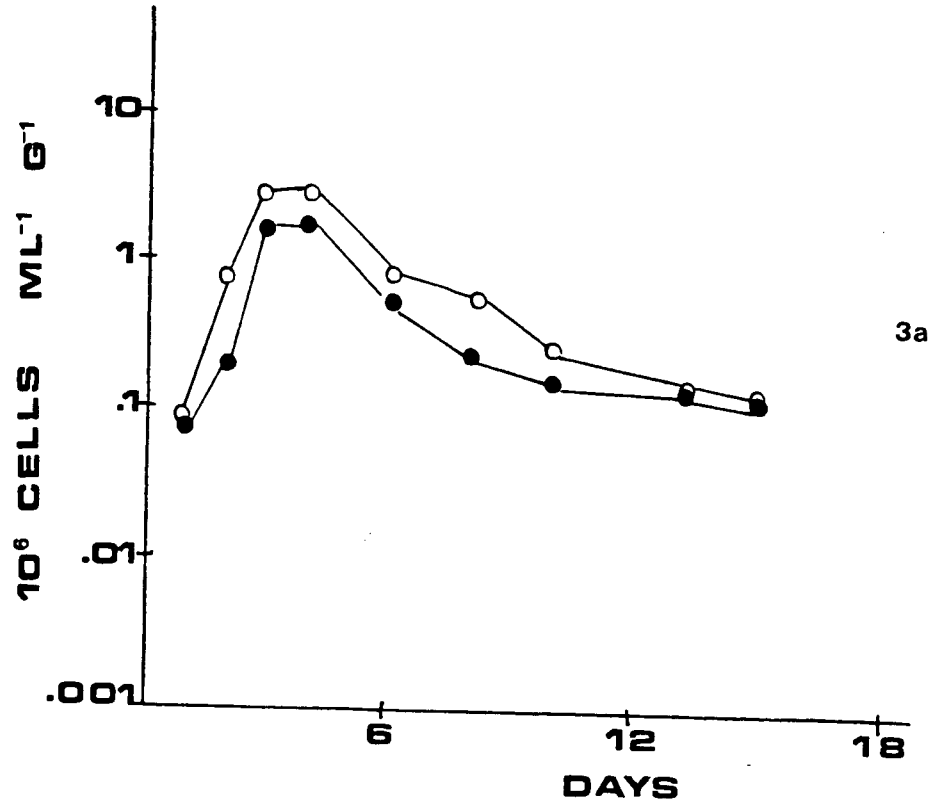


2a

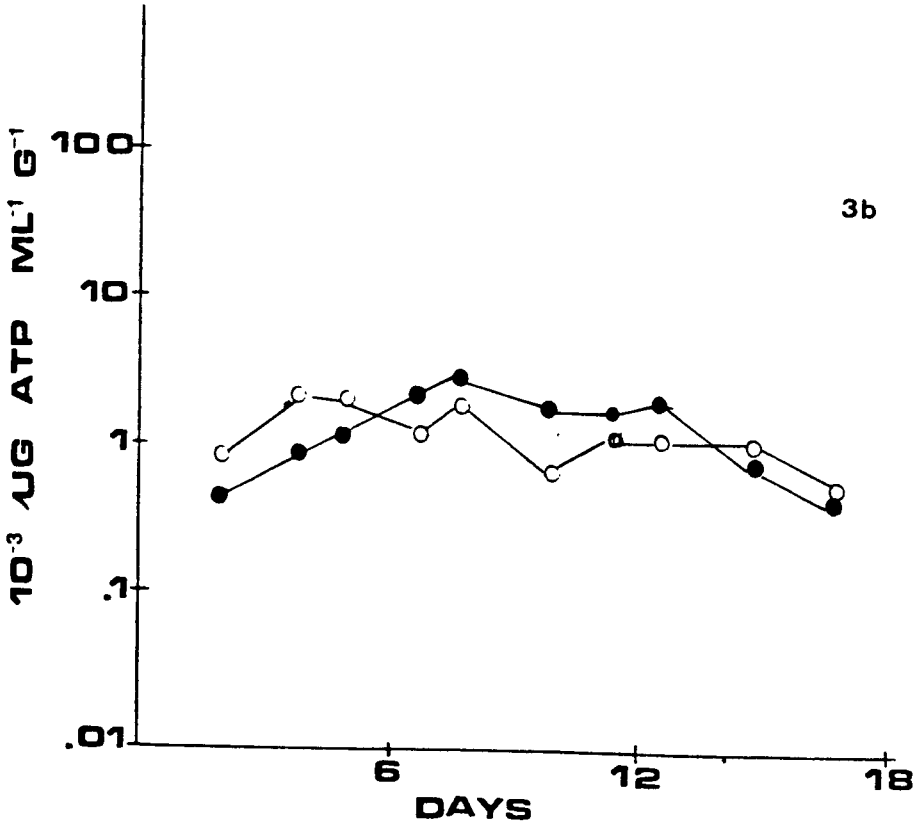


2b

Figure 3. Bacterial densities per gram of substrate that can be supported with different amounts of Zostera marina
(a) Epifluorescence data
(b) ATP data
(20g○; 60g●).

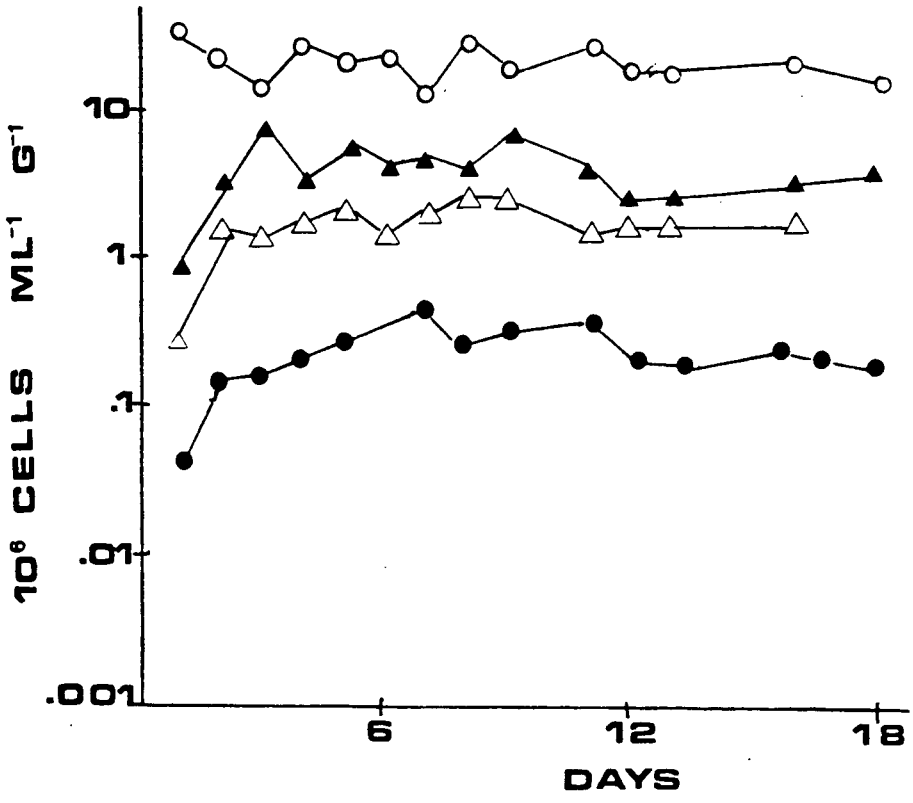

10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS

3a

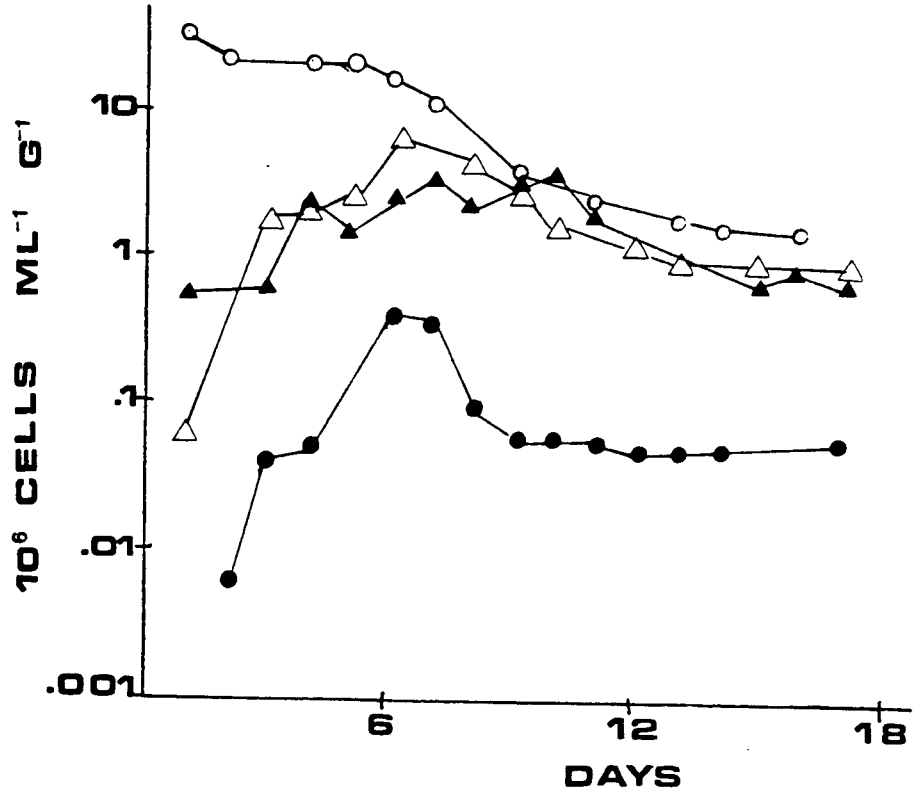

100
10
1
.1
.01
10^{-3} UG ATP ML^{-1} G^{-1}
6
12
18
DAYS

3b

Figure 4. The effect of autoclaved substrates on the number of bacteria
(a) nutrient-rich conditions
(b) nutrient-poor conditions
(*Ulva lactuca* O; *Fucus vesiculosus* ▲; *Zostera marina* ▲; wood chips ●).


10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS

4a


10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS

4b

Figure 5. The effect of autoclaved substrates on bacterial ATP under nutrient-poor conditions (*Ulva lactuca* o; *Fucus vesiculosus* ▲; *Zostera marina* ▲; wood chips ●).

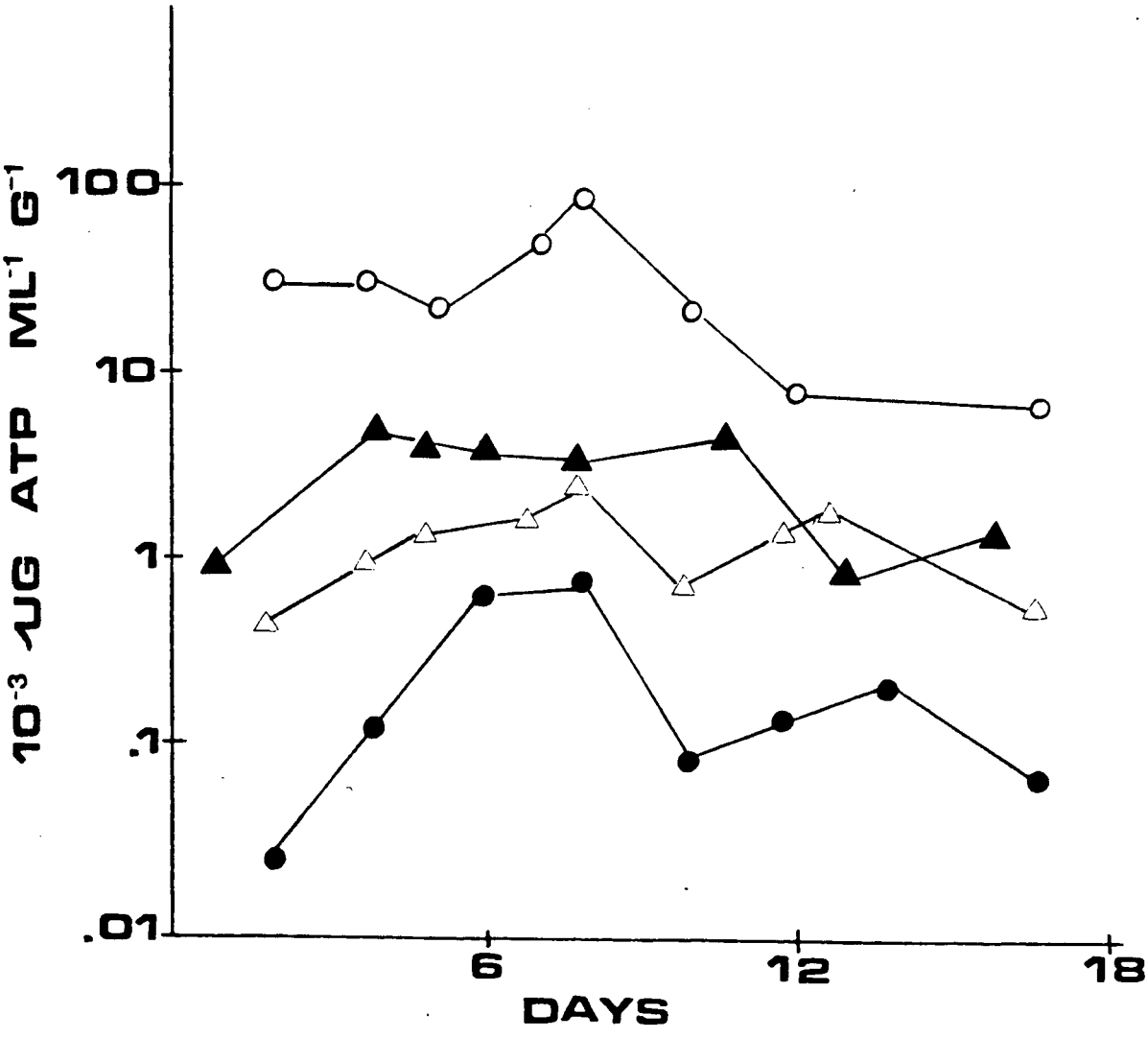
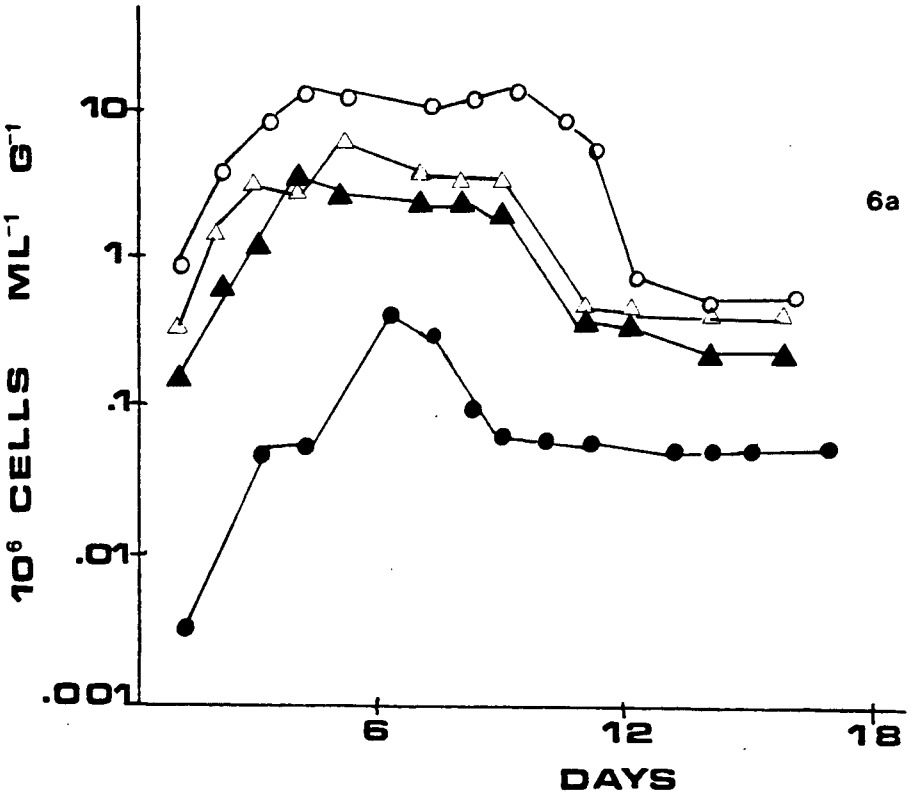
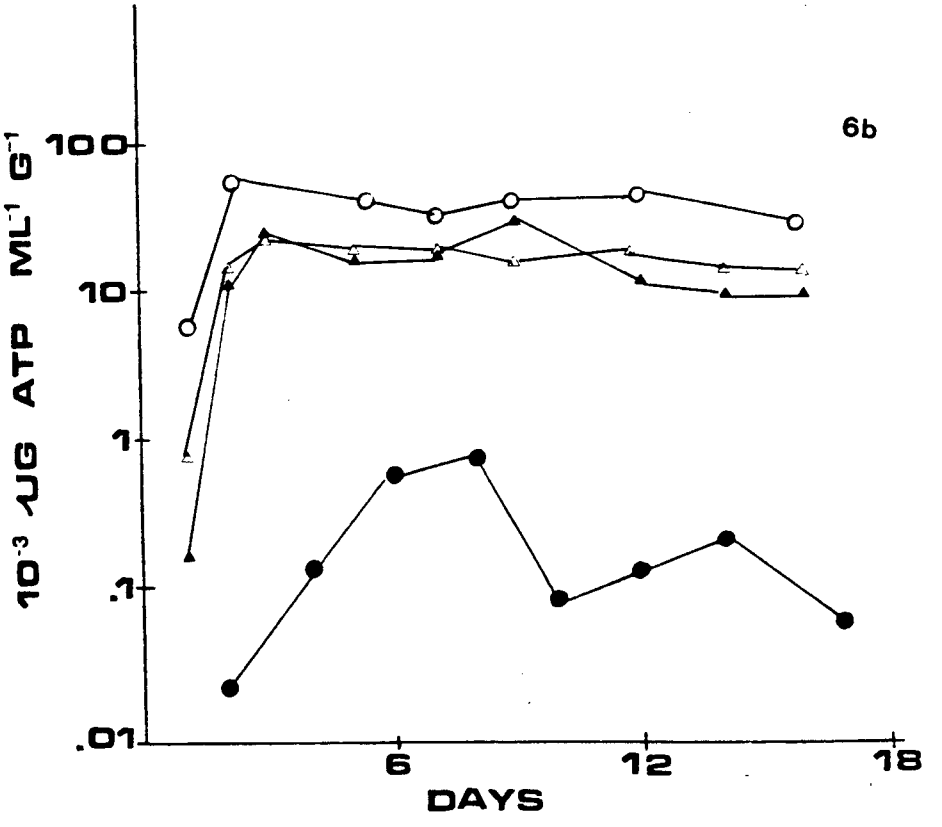

10^{-3} UG ATP ML^{-1} G^{-1}
100
10
1
.1
.01
6
12
18
DAYS

Figure 6. The effect of dried substrates on bacterial densities under nutrient-poor conditions
(a) Epifluorescence data
(b) ATP data
(*Ulva lactuca* o; *Fucus vesiculosus* ▲; *Zostera marina* ▲; wood chips ●).

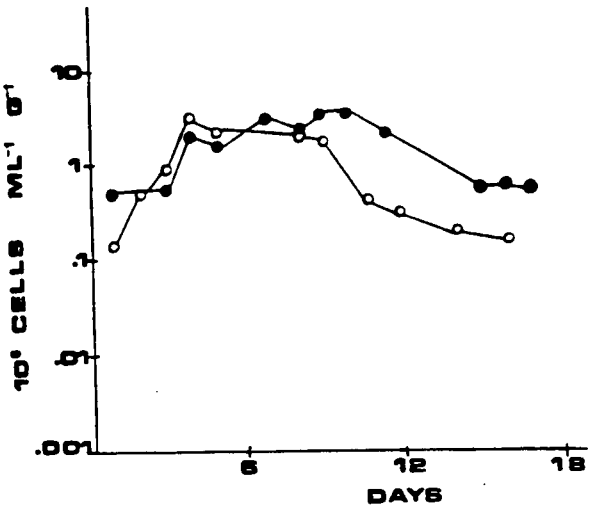

10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS

6a

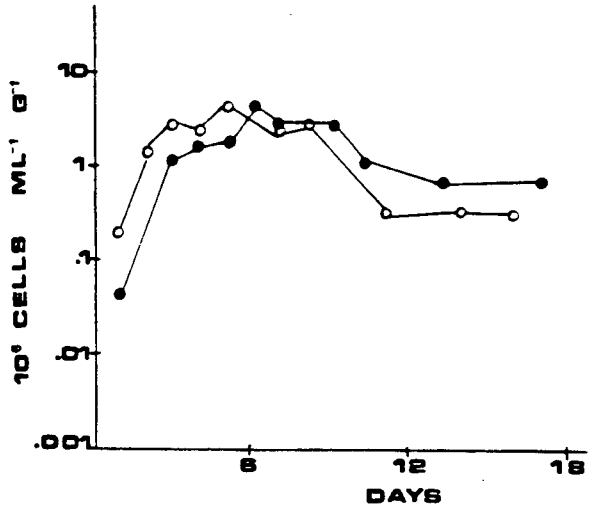

100
10
1
.1
.01
10^{-3} UG ATP ML^{-1} G^{-1}
6
12
18
DAYS

6b

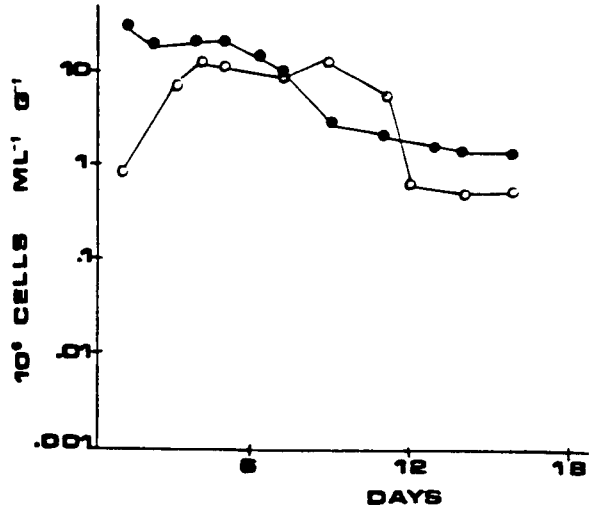
Figure 7. The effect of autoclaved and dried, large substrate particles on the number of bacteria under nutrient-poor conditions
(a) Fucus vesiculosus
(b) Zostera marina
(c) Ulva lactuca
(autoclaved●; dried○).


10³ CELLS ML⁻¹ G⁻¹
10
1
.1
.01
.001
6
12
18
DAYS

7a

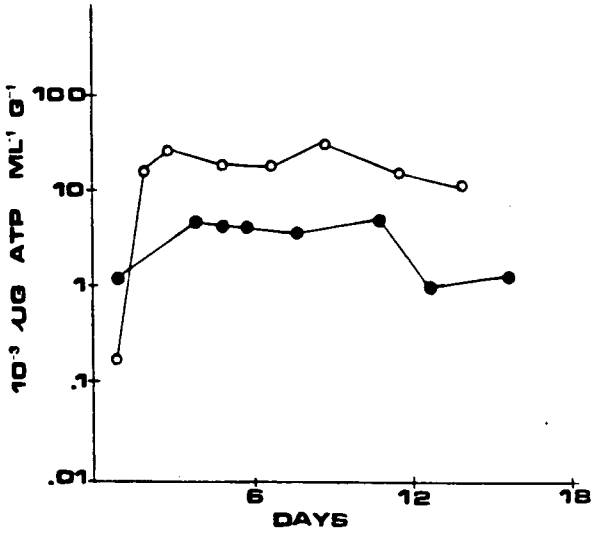

10³ CELLS ML⁻¹ G⁻¹
10
1
.1
.01
.001
6
12
18
DAYS

7b

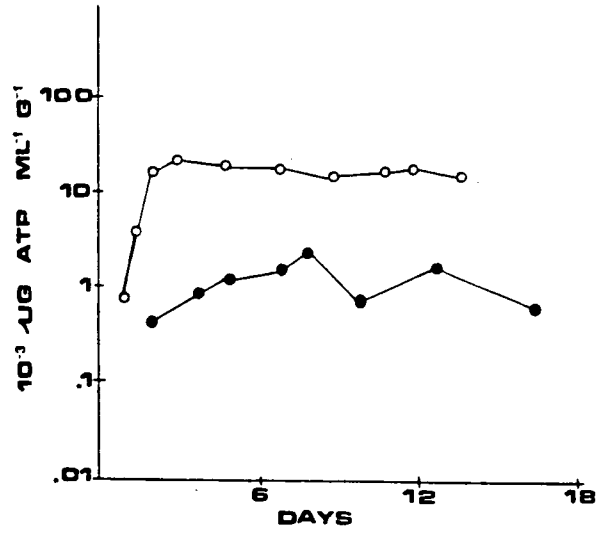

10³ CELLS ML⁻¹ G⁻¹
10
1
.1
.01
.001
6
12
18
DAYS

7c

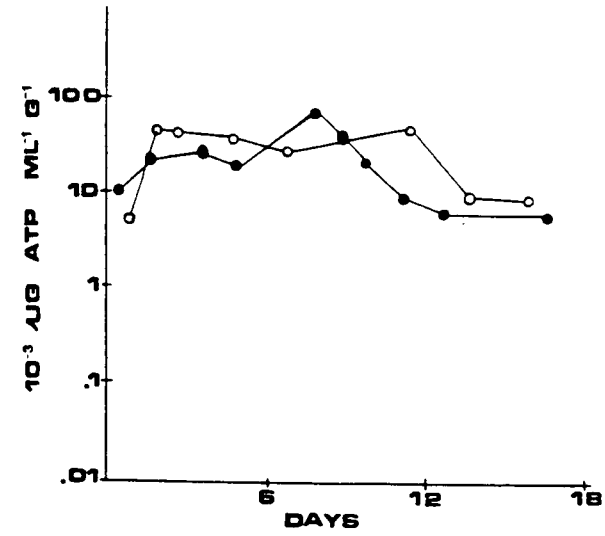
Figure 8. The effect of autoclaved and dried, large substrate particles on bacterial ATP under nutrient-poor conditions
(a) Fucus vesiculosus
(b) Zostera marina
(c) Ulva lactuca
(autoclaved●; driedO).


100
10
1
.1
.01
6
12
18
DAYS
10^{-3} UG ATP ML^{-1} G^{-1}

8a

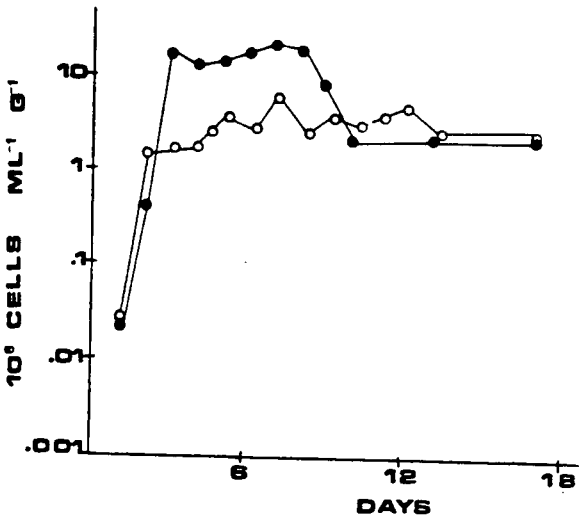

100
10
1
.1
.01
6
12
18
DAYS
10^{-3} UG ATP ML^{-1} G^{-1}

8b

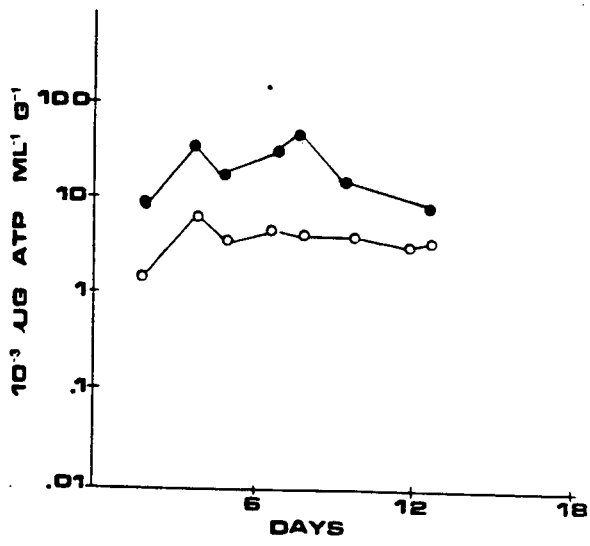

100
10
1
.1
.01
6
12
18
DAYS
10^{-3} UG ATP ML^{-1} G^{-1}

8c

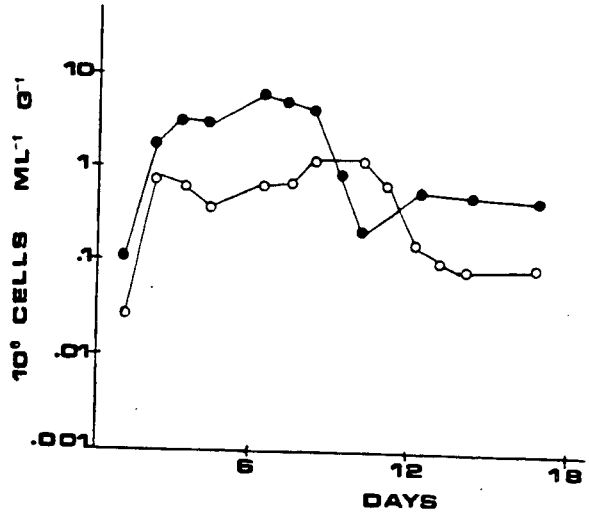
Figure 9. The effect of autoclaved and dried, small substrate particles on bacterial densities supported under nutrient-poor conditions
(a) Epifluorescence data for Fucus vesiculosus
(b) ATP data for Fucus vesiculosus
(c) Epifluorescence data for Zostera marina
(d) ATP data for Zostera marina
(auotoclved●; dried○).


10^6 CELLS ML^{-1} G^{-1}
10
1
.1
.01
.001
6
12
18
DAYS

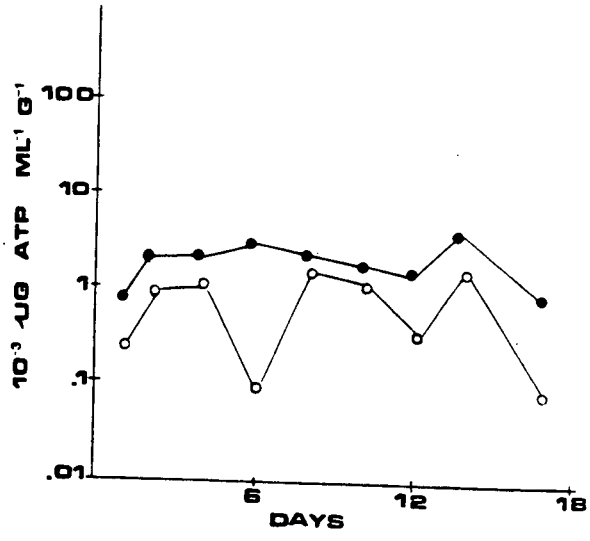
9a


10^{-3} UG ATP ML^{-1} G^{-1}
100
10
1
.1
.01
6
12
18
DAYS

9b

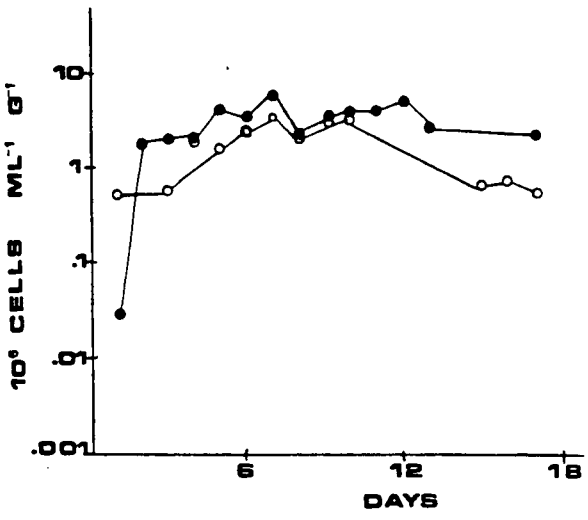

10^6 CELLS ML^{-1} G^{-1}
10
1
.1
.01
.001
6
12
18
DAYS

9c

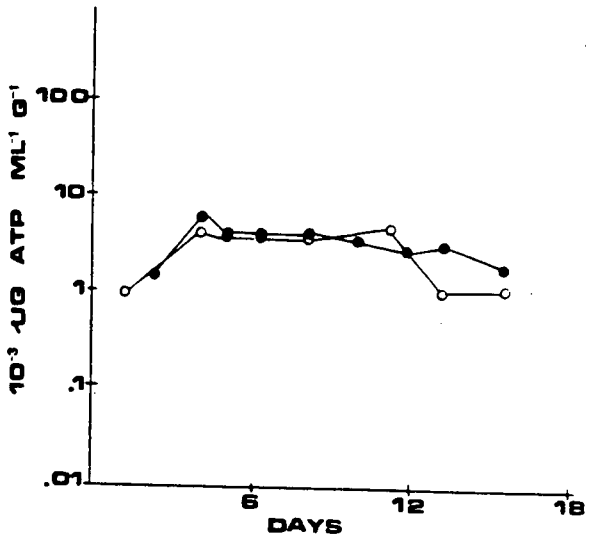

10^{-3} UG ATP ML^{-1} G^{-1}
100
10
1
.1
.01
6
12
18
DAYS

9d

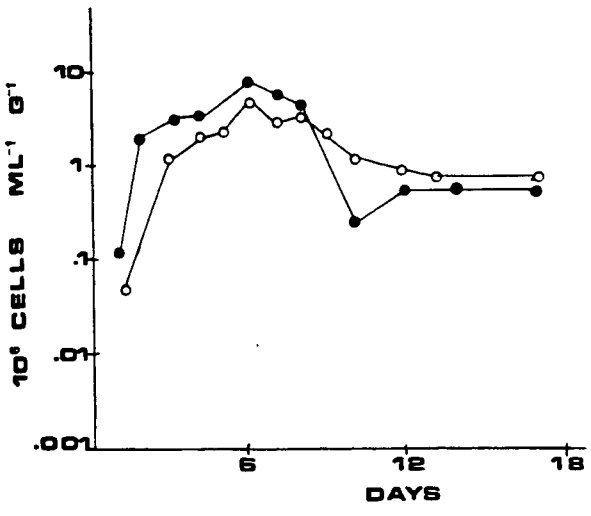
Figure 10. The effect of small and large, autoclaved substrate particles on bacterial densities under nutrient-poor conditions
(a) Epifluorescence data for Fucus vesiculosus
(b) ATP data for Fucus vesiculosus
(c) Epifluorescence data for Zostera marina
(d) ATP data for Zostera marina
(small particles○; large particles●).



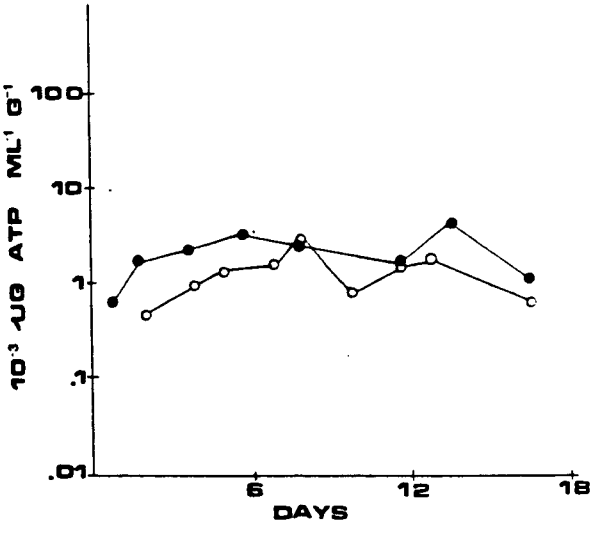
10a



10b

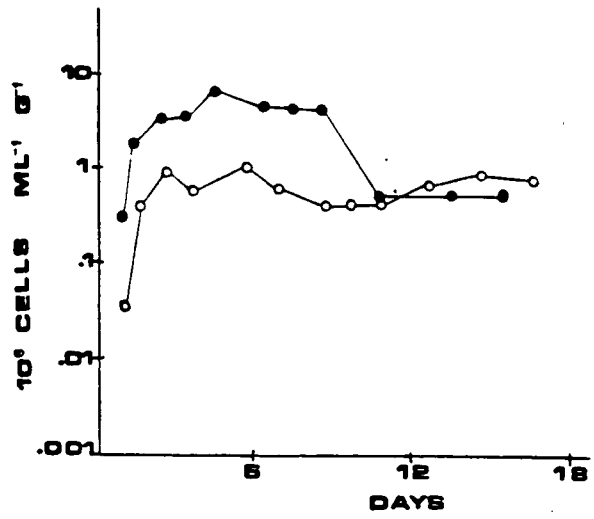


10c

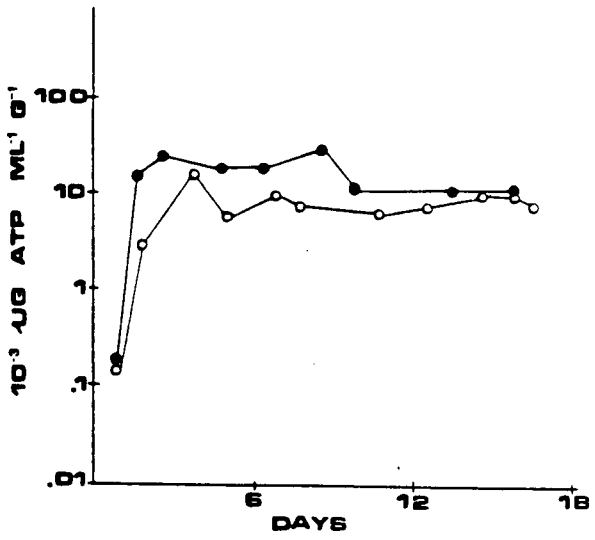


10 d

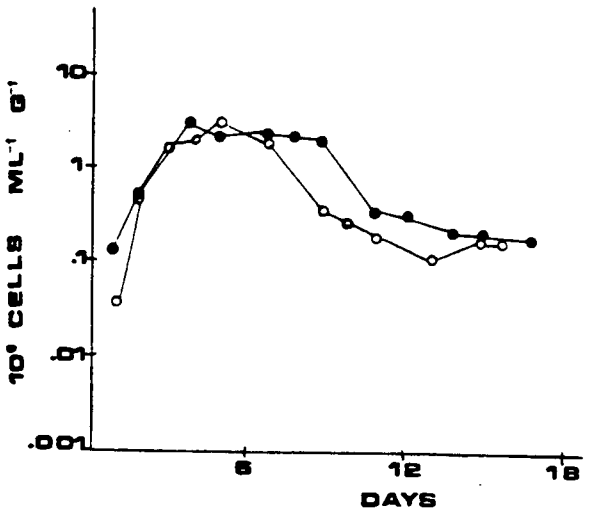
Figure 11. The effect of small and large, dried substrate particles on bacterial densities under nutrient-poor conditions
(a) Epifluorescence data for Ulva lactuca
(b) ATP data for Ulva lactuca
(c) Epifluorescence data for Fucus vesiculosus
(d) ATP data for Fucus vesiculosus
(e) Epifluorescence data for Zostera marina
(f) ATP data for Zostera marina
(g) Epifluorescence data for wood chips
(h) ATP data for wood chips
(small particles○; large particles●).



11a



11b



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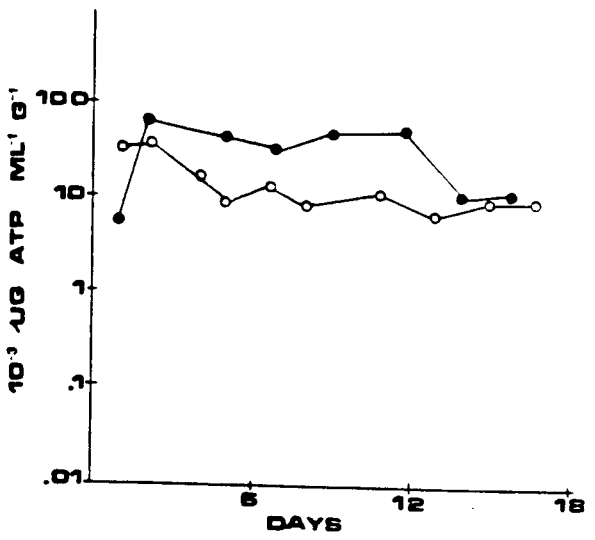

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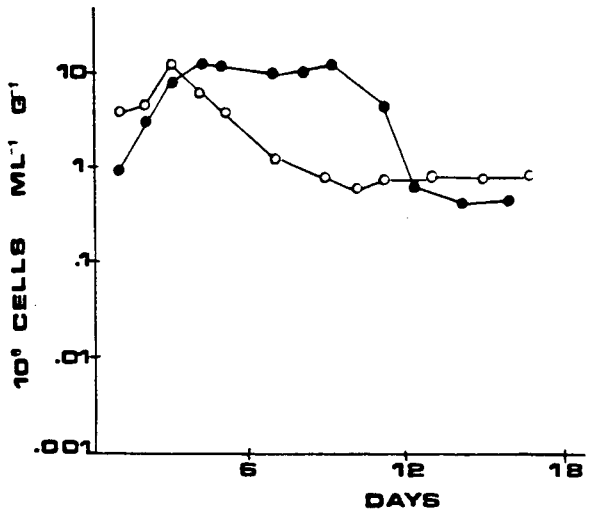

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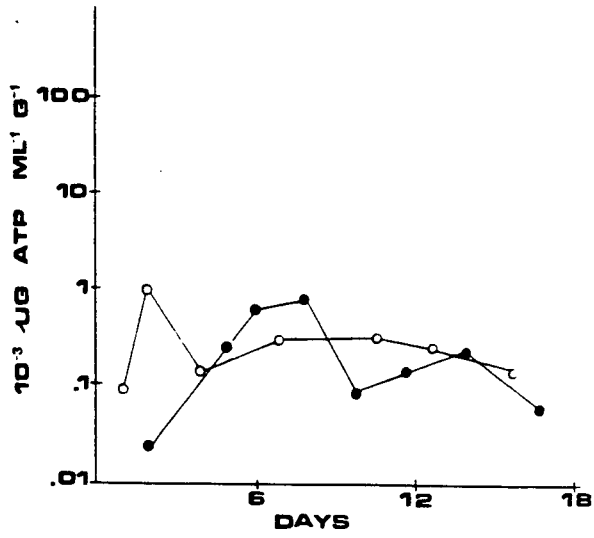

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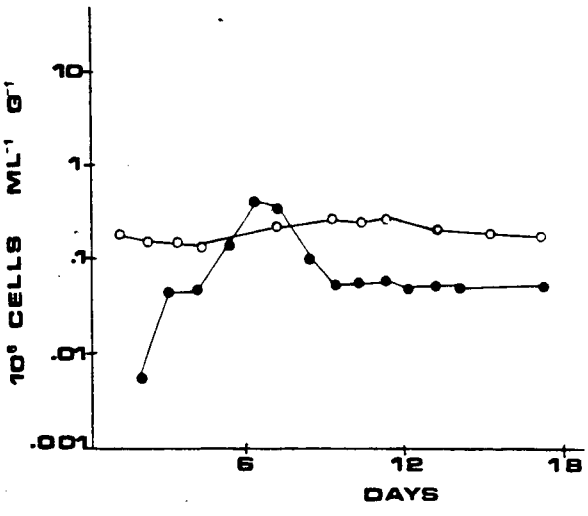
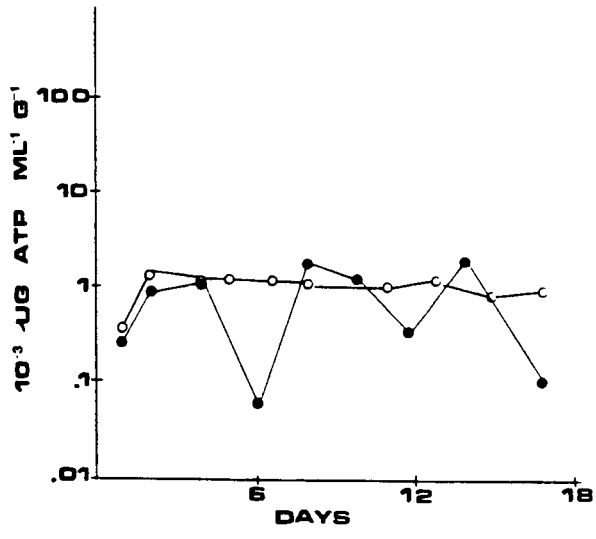
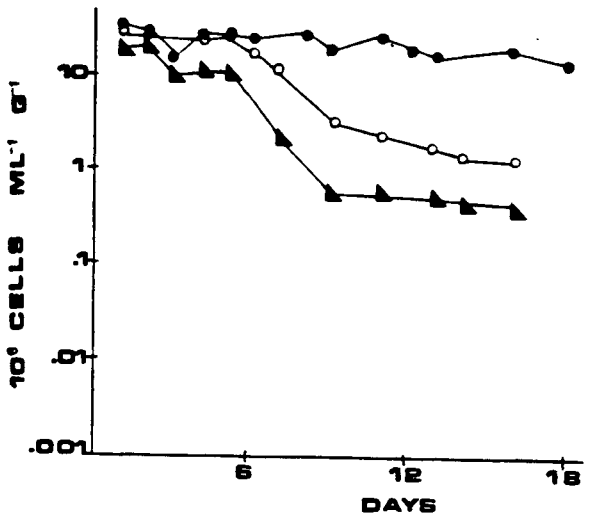


11g

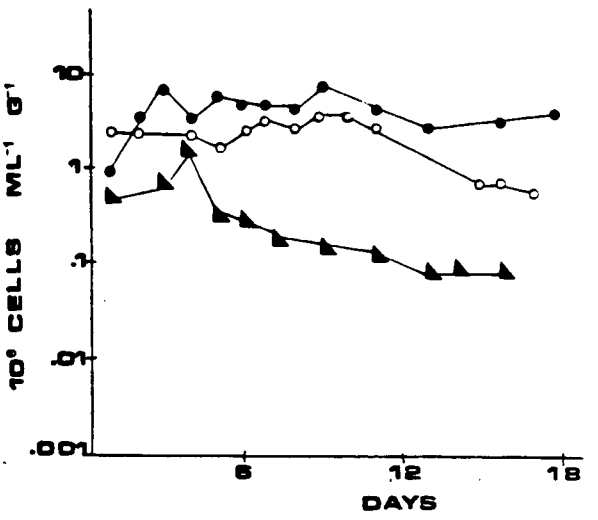


11h

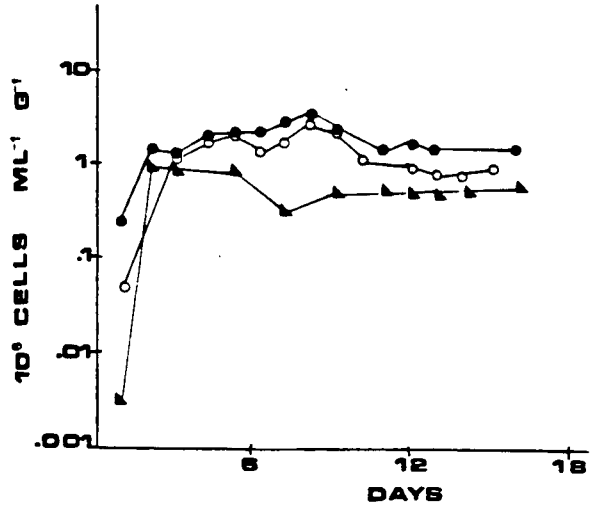
Figure 12. The effect of nutrient conditions with autoclaved sustrates on bacterial densities
(a) Epifluorescence data for Ulva lactuca
(b) Epifluorescence data for Fucus vesiculosus
(c) Epifluorescence data for Zostera marina
(d) Epifluorescence data for wood chips
(e) ATP data for wood chips
(nutrient-rich●; nutrient-poor○;
nutrient-depleted▶).



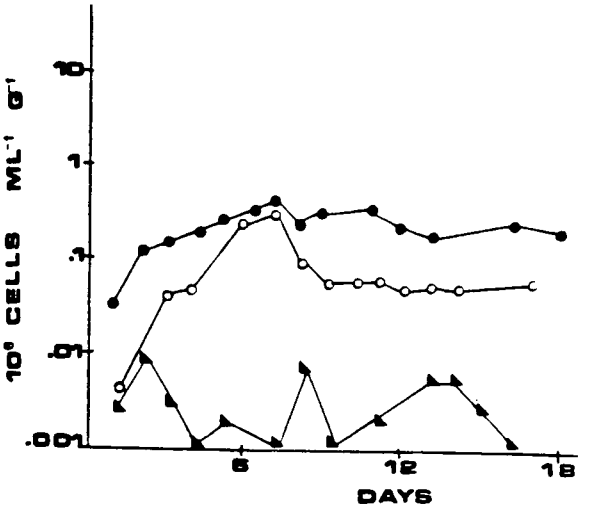
12a



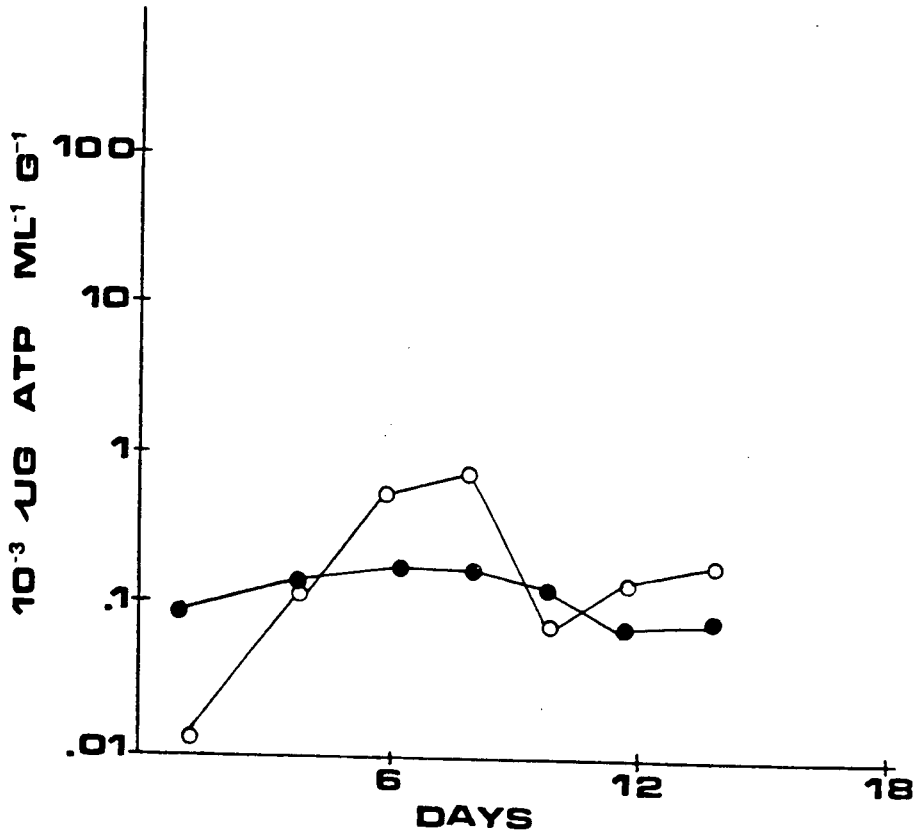
12b



12c



12d

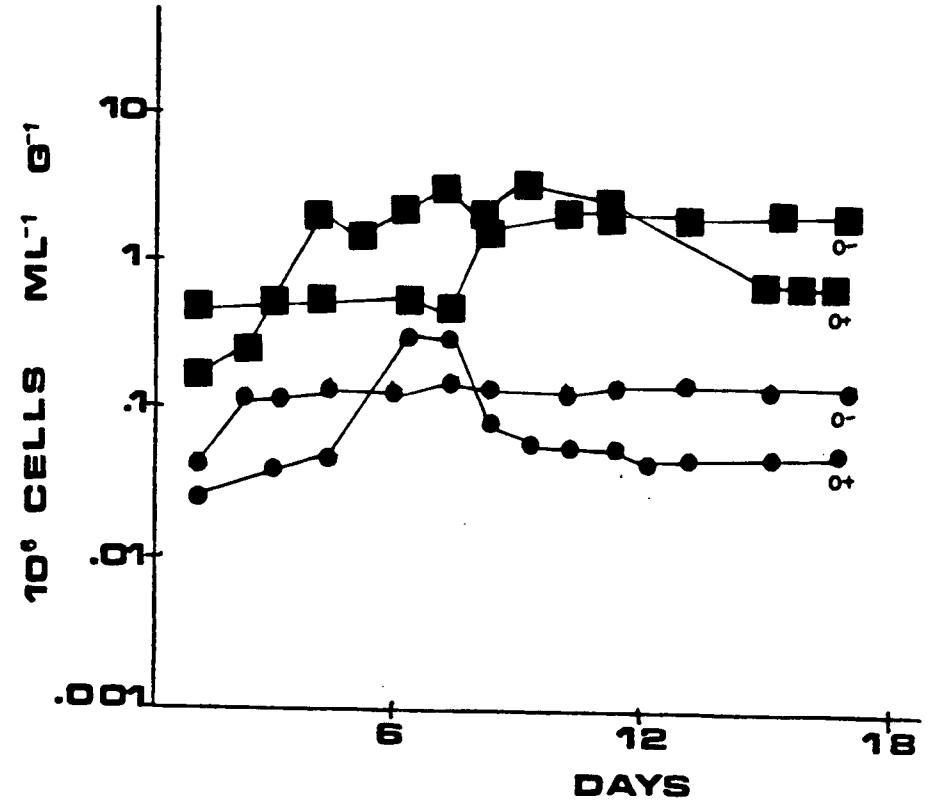


12e

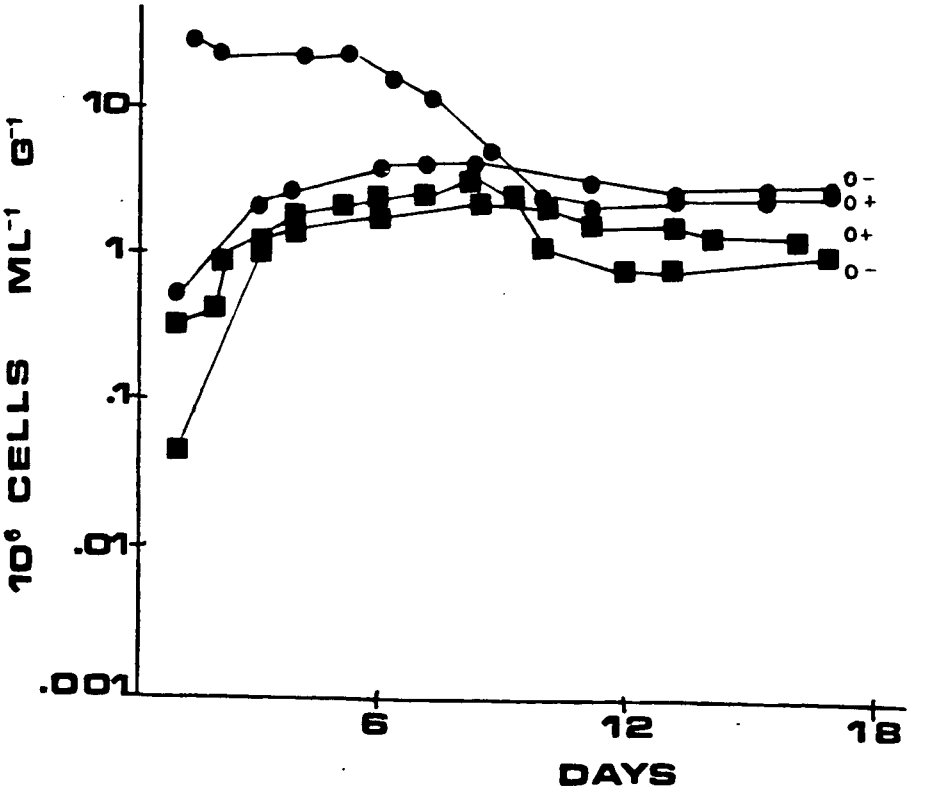
Figure 13. The effect of nutrient-depleted conditions on the number of bacteria supported from various substrates (*Ulva lactuca* o; *Fucus vesiculosus* ▵; *Zostera marina* ▲; wood chips ●).

10^6 CELLS ML^{-1} G^{-1}
100
10
1
0.1
0.01
DAYS
6
12
18

Figure 14. The effect of aerobic and anaerobic conditions on the number of bacteria supported from various substrates (a) Fucus vesiculosus■;wood chips● (b) Ulva lactuca●;Zostera marina■ (aerobic O+; anaerobic O-).


10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS
0-
0+
0-
0+

14a


10
1
.1
.01
.001
10^6 CELLS ML^{-1} G^{-1}
6
12
18
DAYS
0-
0+
0+
0-

14 b

Figure 15. Filtering Rates of Mytilus edulis as a function of the concentration of the phytoplankton culture supplied (large mussels■; small mussels◤).

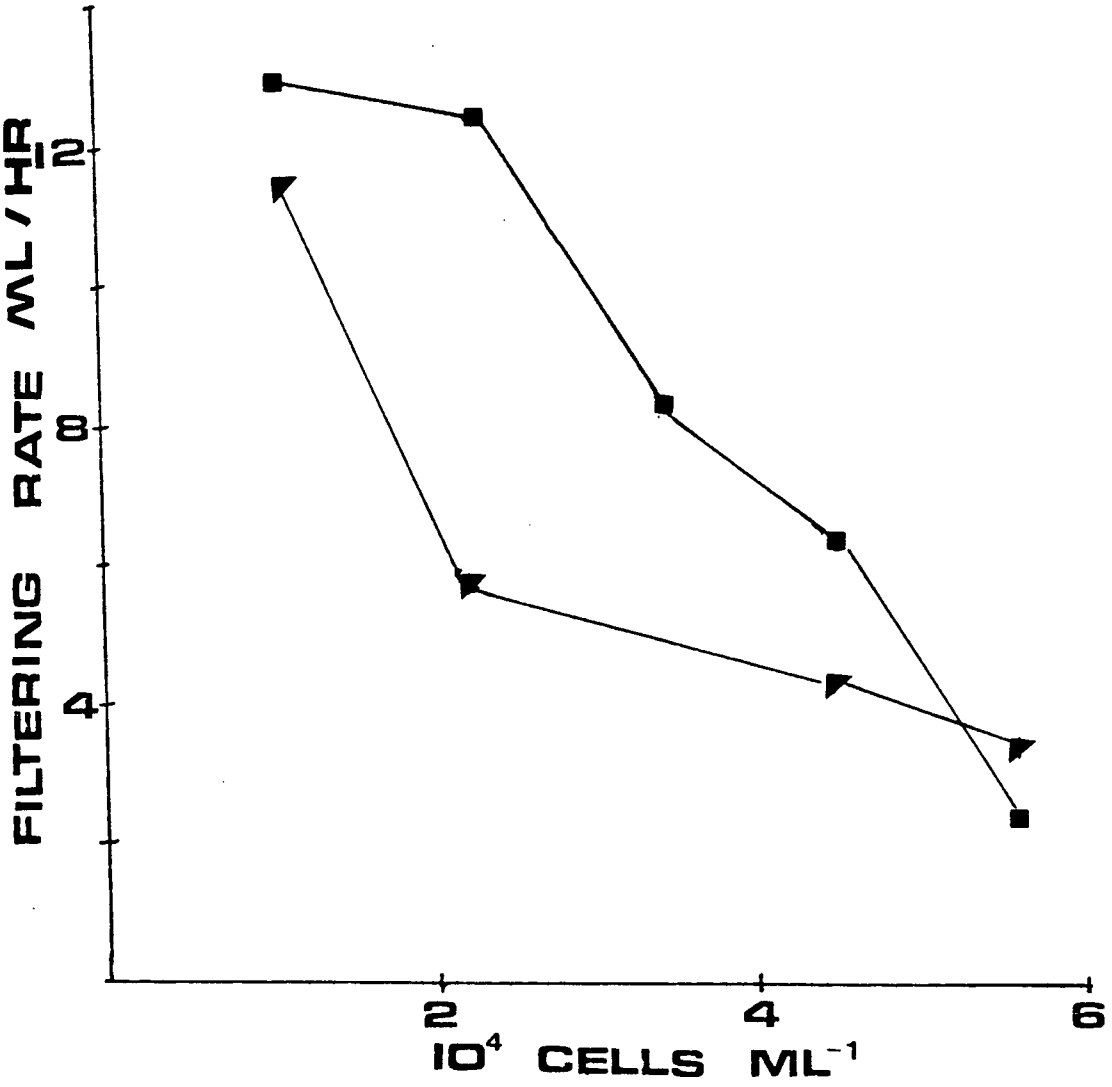

FILTERING RATE ML / HR
12
8
4
2
4
6
10⁴ CELLS ML⁻¹

Figure 16. Growth of Artemia salina when supplied with bacterial cultures of cell concentrations greater than log 6.2 (the log concentration of the bacteria is indicated beside the curves; 95% Confidence Intervals).

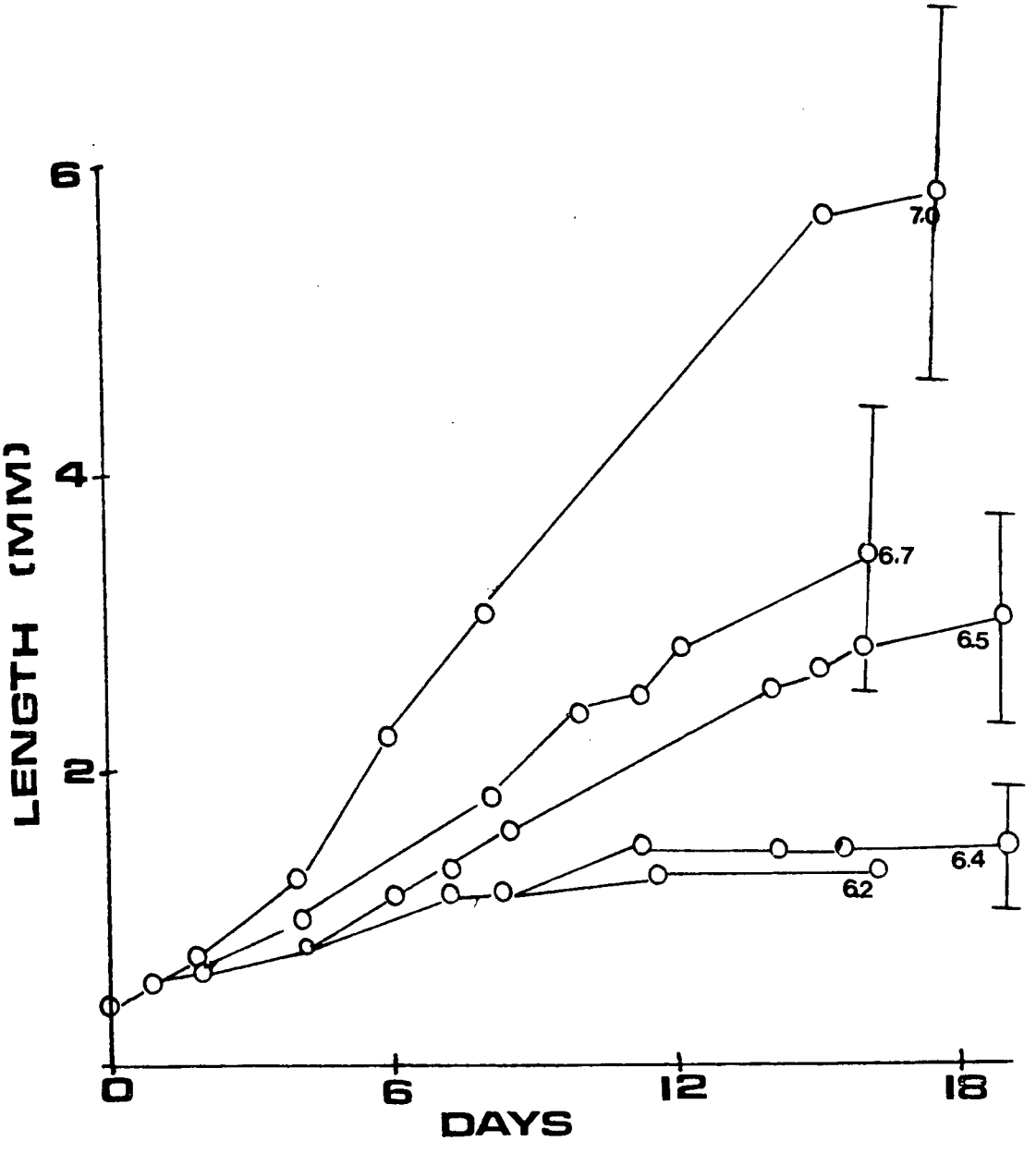

6
4
2
LENGTH (MM)
7.0
6.7
6.5
6.4
6.2
0
6
12
18
DAYS

Figure 17. Growth of Artemia salina when supplied with bacterial cultures of cell concentrations less than log 6.2 (the log concentration of the bacteria is indicated beside the curves; 95% Confidence Intervals).

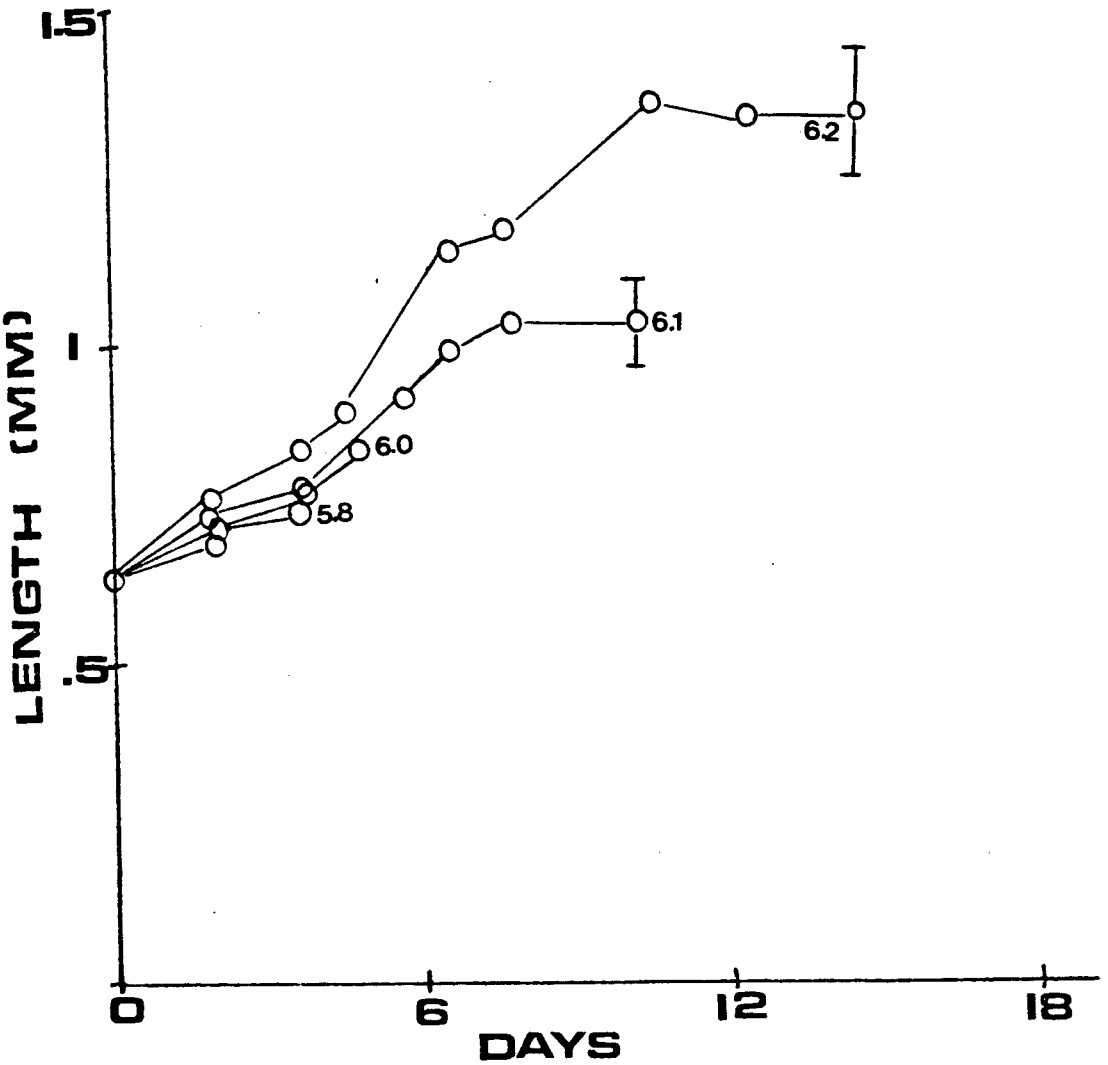

1.5
1
.5
LENGTH (MM)
6.2
6.1
6.0
5.8
0
6
12
18
DAYS

Figure 18. Growth Rates of Artemia salina (the log concentration of the bacteria supplied and the substrate source is indicated in the brackets beside the curves; (F) Fucus vesiculosus;(U) Ulva lactuca;(E) Eelgrass.

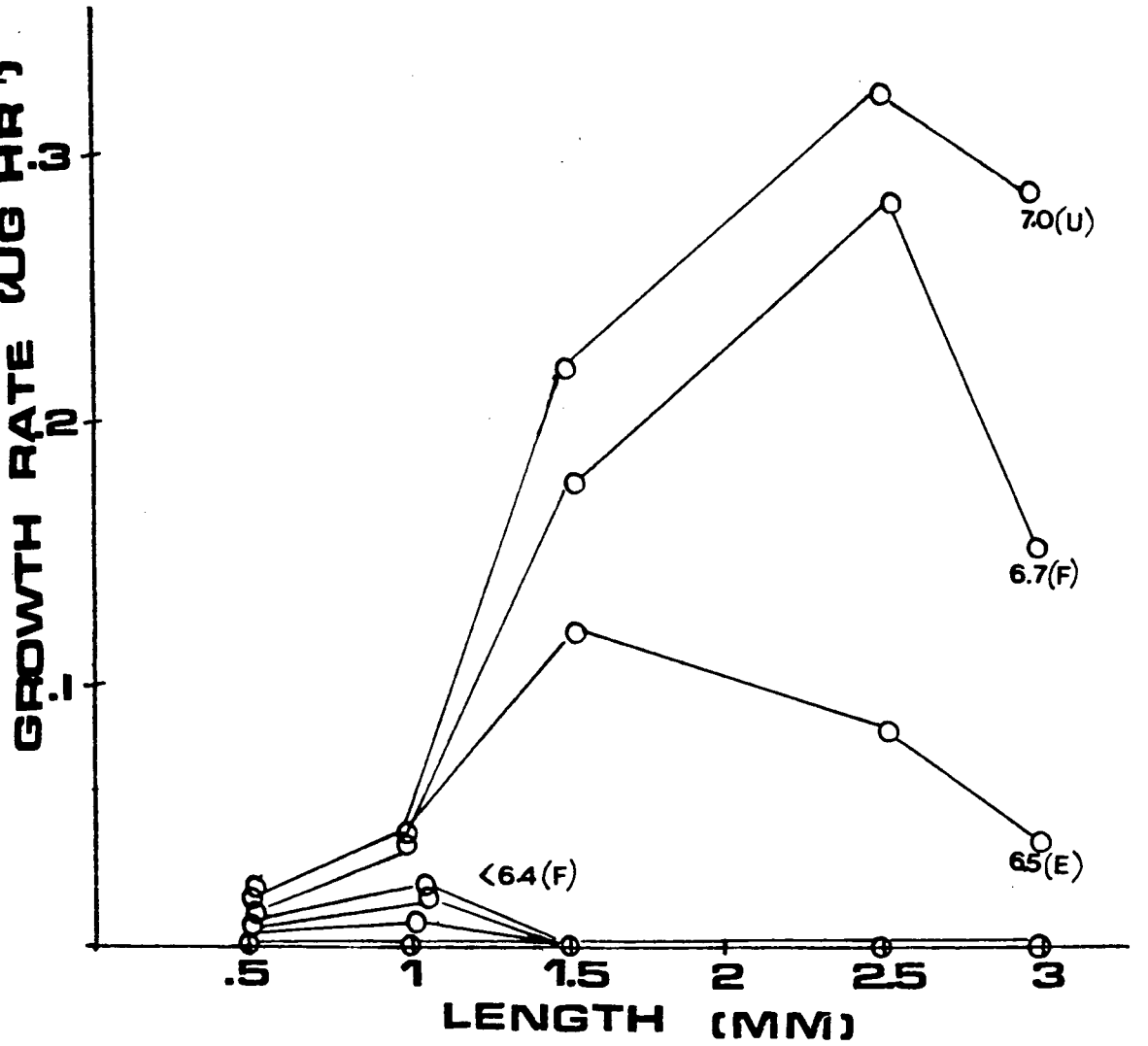

GROWTH RATE (UG HR⁻¹)
.3
.2
.1
7.0(U)
6.7(F)
6.5(E)
<6.4(F)
.5
1
1.5
2
2.5
3
LENGTH (MM)

Figure 19. Filtering Rates (FR) of Artemia salina as a function of the log concentration of the bacterial culture supplied. (the length, in mm, is indicated beside the curves)

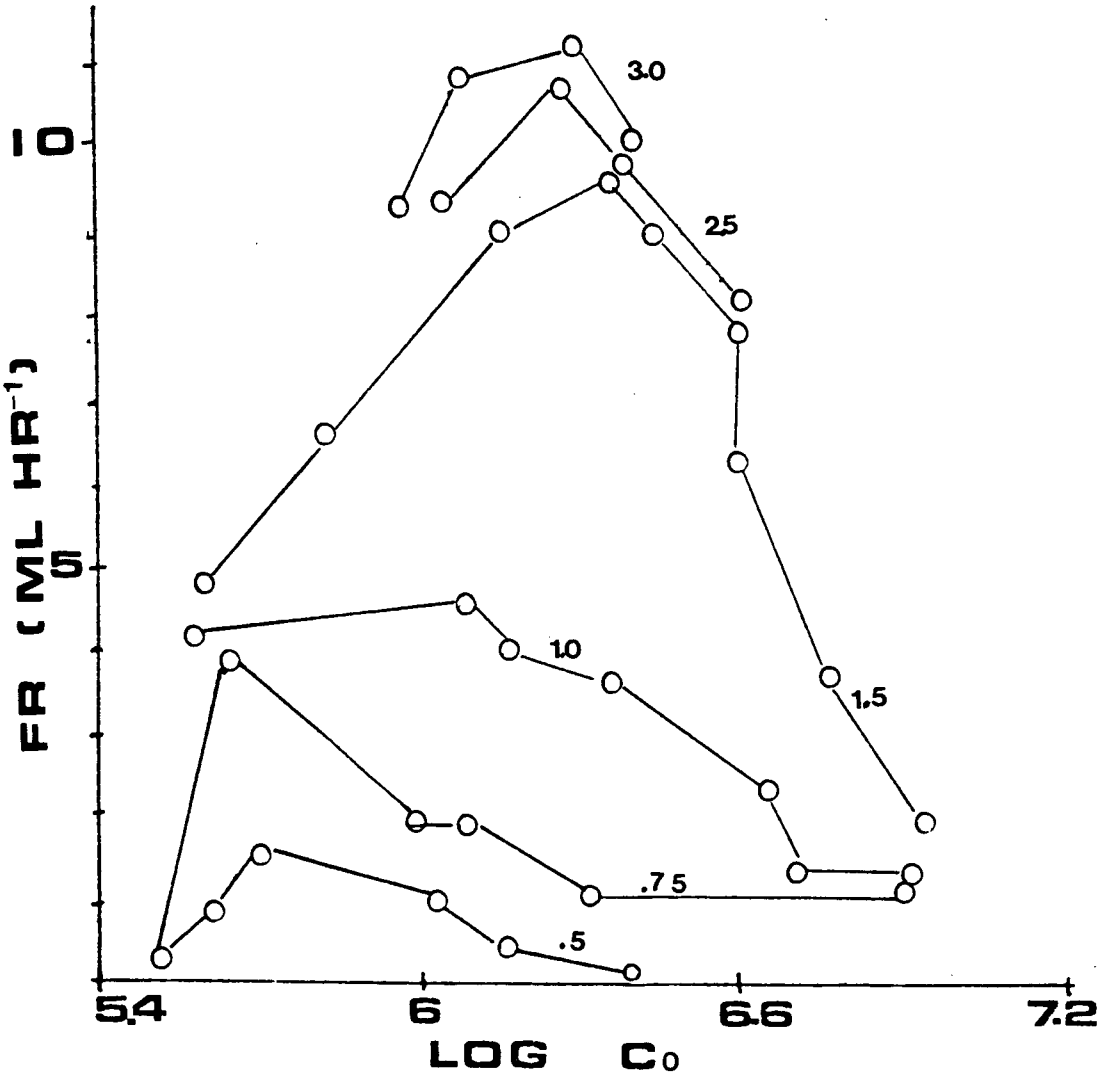

10
5
FR (ML HR⁻¹)
3.0
2.5
1.0
1.5
.75
.5
5.4
6
6.6
7.2
LOG C0

Figure 20. Growth Efficiencies of Artemia salina as a function of the size of the organism (the log concentration of the bacterial culture provided is indicated beside the curves).

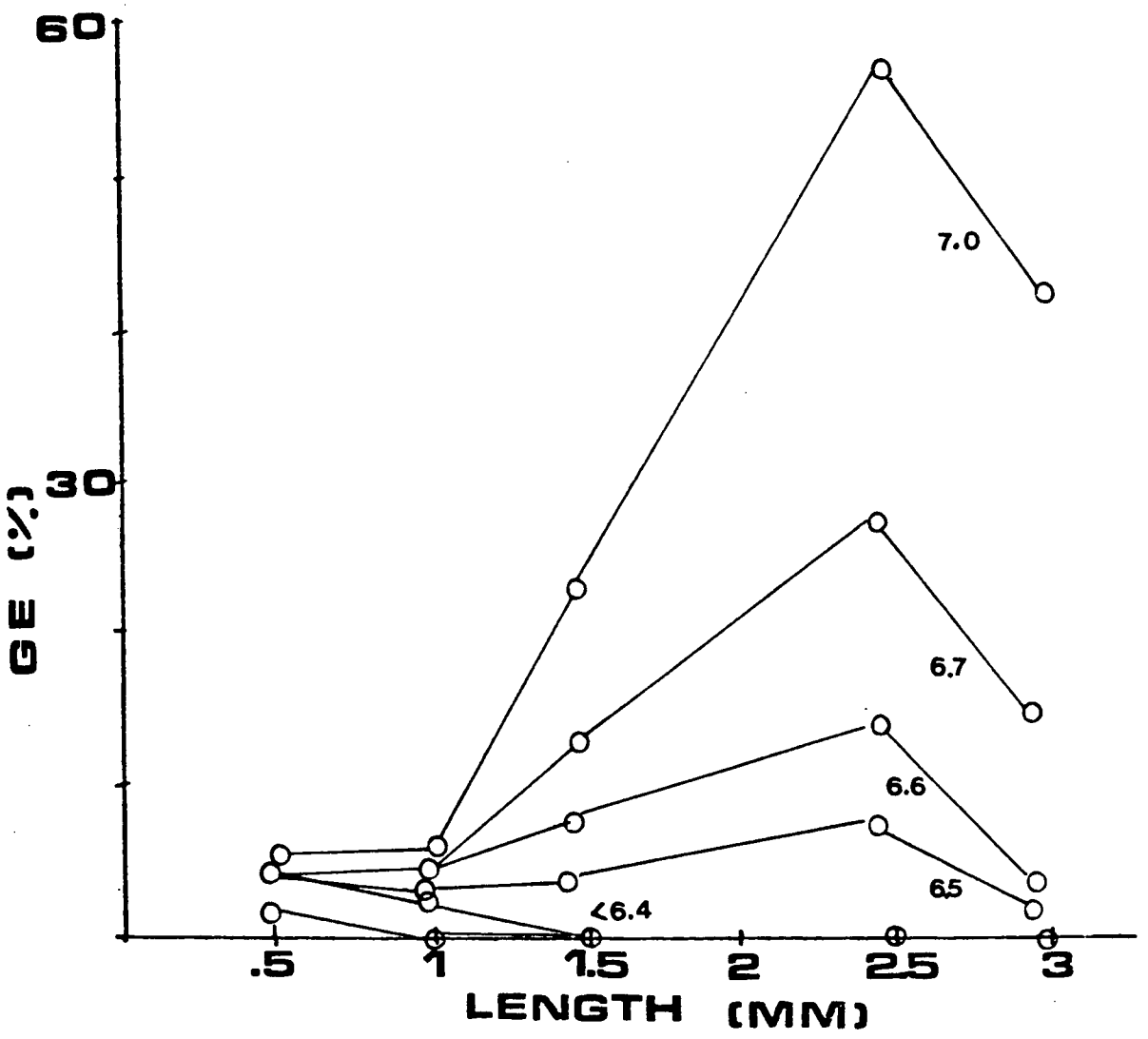

60
30
GE (%)
7.0
6.7
6.6
6.5
<6.4
.5
1
1.5
2
2.5
3
LENGTH (MM)

Figure 21. Consumption Rates of Artemia salina as a function of the size of the organism and the concentration of bacteria supplied (the length of Artemia salina, in mm, is indicated beside the curves).

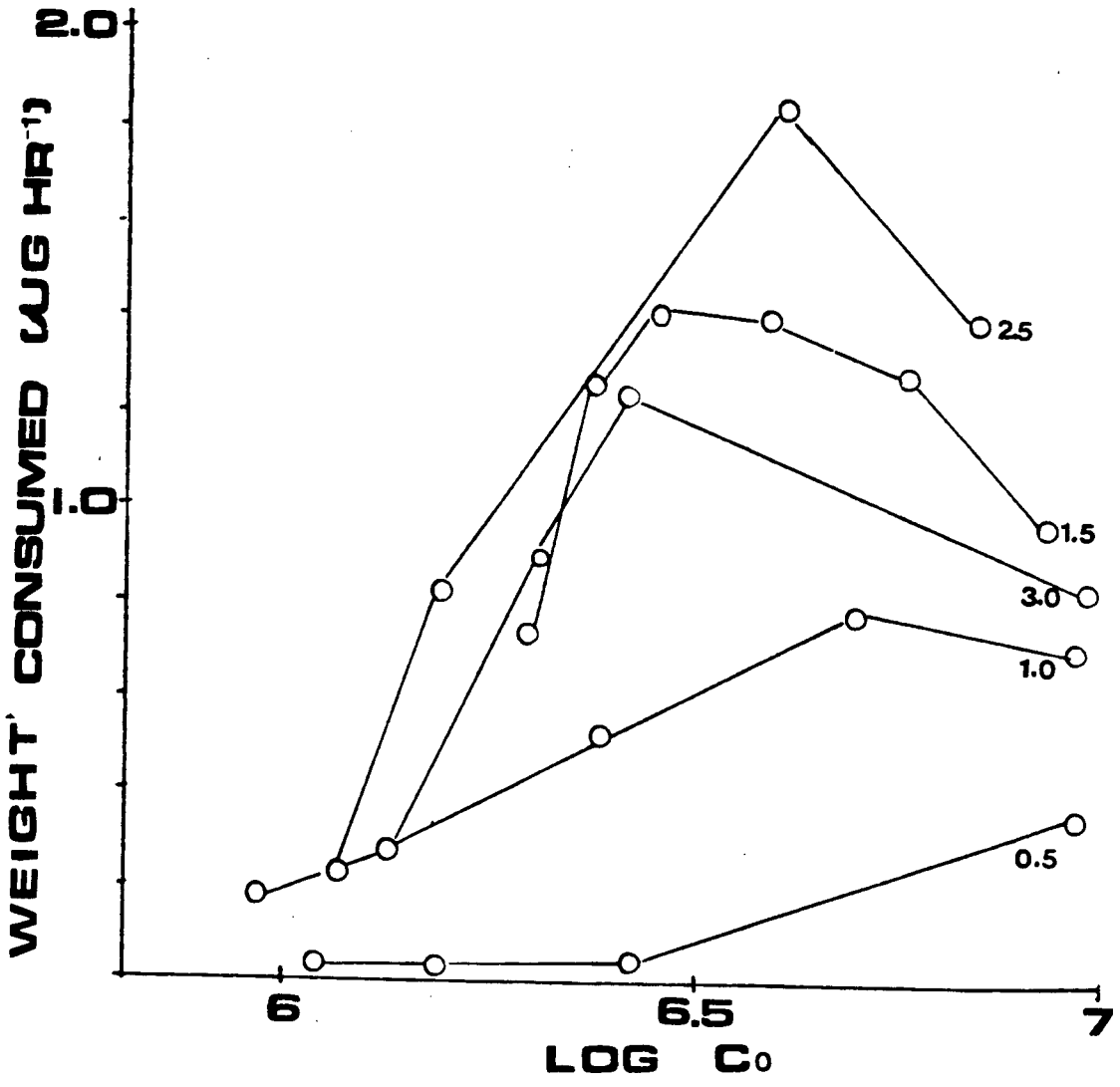

2.0
1.0
WEIGHT' CONSUMED (UG HR⁻¹)
2.5
1.5
3.0
1.0
0.5
6
6.5
7
LOG C0

Figure 22. The length of Artemia salina that can be obtained when supplied with different concentrations of suspended bacteria (95% Confidence Limits).

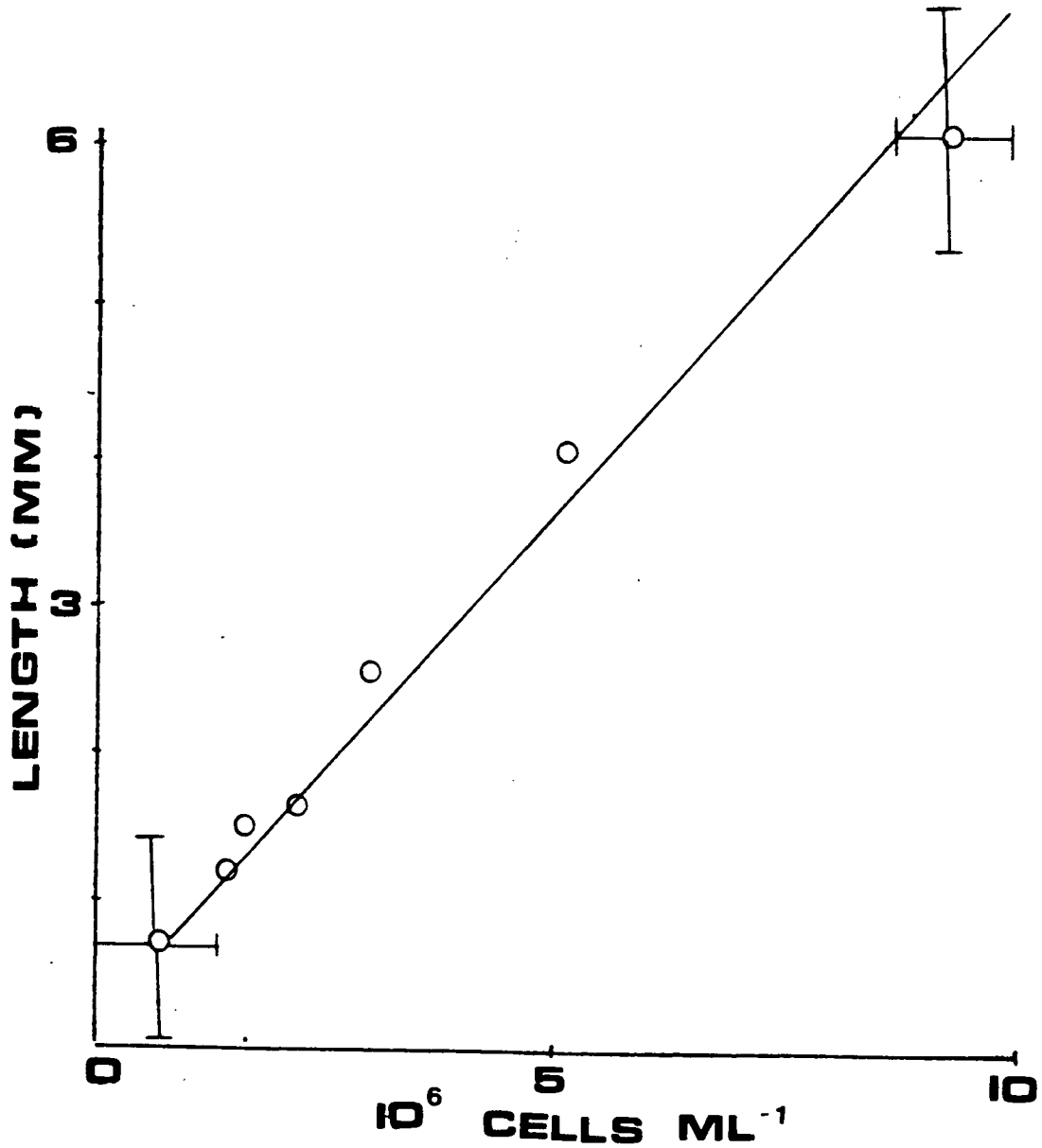

6
3
0
5
10
LENGTH (MM)
10^6 CELLS ML^{-1}

Figure 23. The relationship between total nitrogen content of the substrate and the number of bacteria supported when sustrates are autoclaved(▲) and dried(Δ) ; (U) Ulva lactuca,(F) Fucus vesiculosus,(E) Eelgrass,(WC) Wood Chips

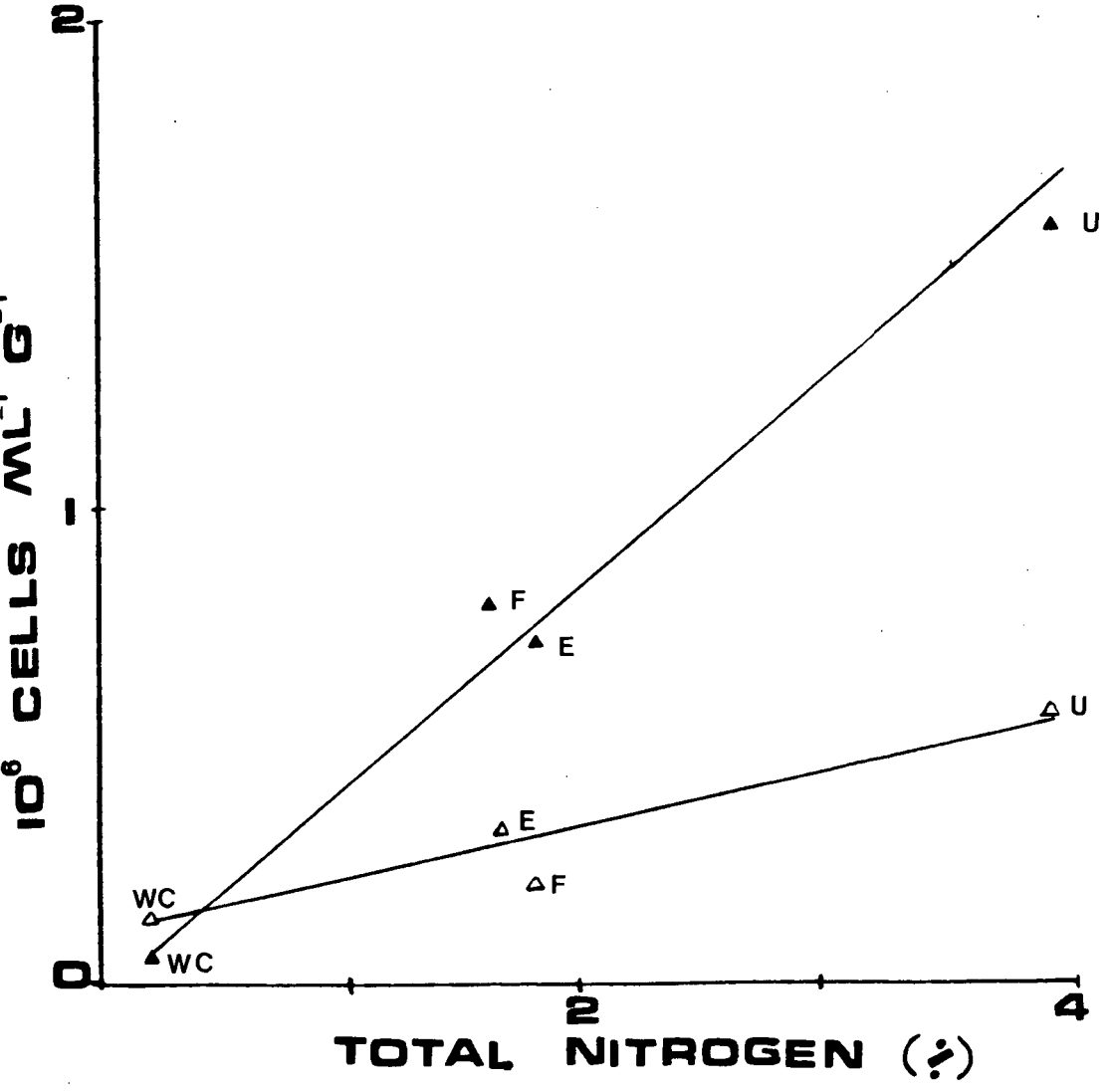
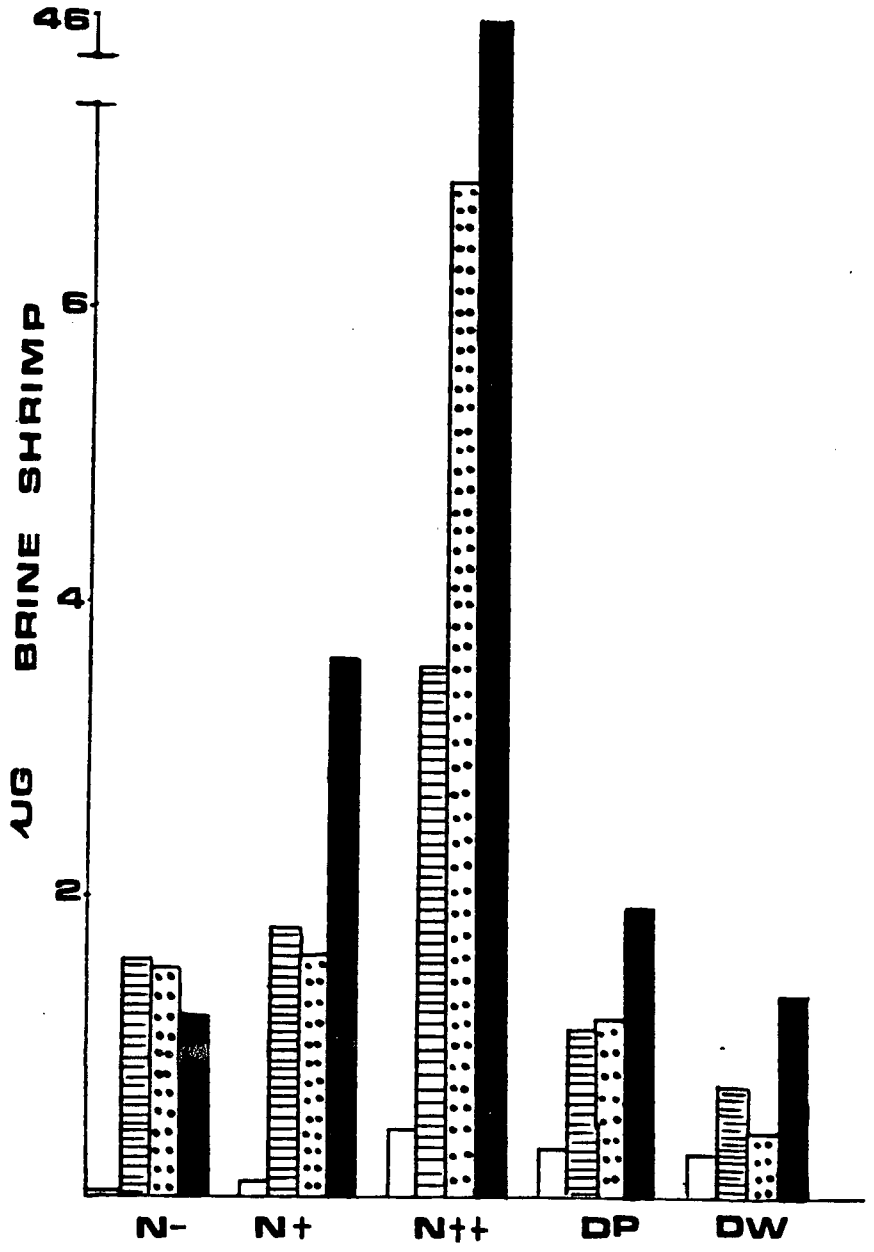

2
1
0
10^6 CELLS ML^{-1} G^{-1}
U
F
E
U
E
F
WC
WC
2
4
TOTAL NITROGEN (%)

Figure 24. The weight of Artemia salina that can be obtained from bacterial cultures grown under various experimental conditions .

N- = nutrient-depleted
N+ = nutrient-poor
N++ = nutrient-rich

Wood Chips = □
Eelgrass = ▤
Fucus sp. = ▦
Ulva sp. = ■

DW = dried substrates left intact
DP = dried substrates of small particles sizes


46
6
4
2
UG BRINE SHRIMP
N-
N+
N++
DP
DW