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Title: THE UTILIZATION OF NONLIVING ALGAL PRODUCTS BY LARVAL OYSTERS

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Harry K. Phinney

Although studies have shown that some marine invertebrates can obtain a portion of their nutrition from dissolved and nonliving particulate organic matter, the relative importance of such foods in their diet has not been established. The success of oyster hatcheries could be significantly improved if the diet of living algal cells can be replaced by one of soluble organics or nonliving particles.

The degree to which living algal cells, dissolved organic matter (DOM) and organic aggregates are taken up by young Crassostrea gigas larvae is compared in this study. The relative uptake of the three types of potential food is established by measuring the amount of ^{14}C-labeled food incorporated in 24 hours. DOM is prepared from the ^{14}C-labeled algal cells by cell disruption or enzymatic hydrolysis. Organic aggregates are produced from the DOM by bubbling or by precipitation.

Studies of larvae 80-90 μm in length demonstrate that at low food densities (0.5 mgC per liter), algal cells are incorporated at the highest rate. At concentrations of food above 1.8 mgC per liter, however, the rate of incorporation of DOM-fed larvae is greater than that of larvae fed on algal cells. Rates of incorporation by larvae fed organic aggregates may be higher or lower than rates of incorporation of algal cells depending on the biochemical composition, size and concentration of the aggregates.

By the time the oyster larvae attain a length of 120-140 μm their capacity to incorporate algal cells has increased greatly, suggesting a change in their feeding or digestive system has occurred. Although these larvae incorporate DOM and organic aggregates at high rates, the rate of incorporation of algal cells is even higher.

During the first 24 hours, up to 37% of the assimilated DOM and up to 35% of the organic aggregates are respired by the larvae. This implies that much of this food is entering the metabolic pathways. Other evidence suggests that some of the remaining assimilated DOM and organic aggregates is utilized for growth. Therefore, DOM and organic aggregates appear to be of nutritional importance to larval bivalves. Application of this work to oyster hatchery operations and future research is discussed.

The Utilization of Nonliving Algal Products by Larval Oysters

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INTRODUCTION

Crassostrea gigas Thunberg 1795, the oyster used in this study, has a larval period of from two to five weeks depending mainly upon temperature and availability of food. At the end of this period the free-swimming veliger larvae set or become attached to a substrate such as a rock or an old oyster shell. After metamorphosis they are called spat which first grow into juvenile and finally adult oysters. In many parts of the world natural oyster settings cannot meet the current demand for this economically important bivalve species. As a result, there is much interest in supplementing natural settings with oysters raised in commercial hatcheries. In addition, hatcheries offer the opportunity for genetic improvement of oyster production. Primarily through the efforts of Loosanoff and his co-workers (see Loosanoff and Davis 1963) at the National Marine Fisheries Service Biological Laboratory in Milford, Connecticut larval and juvenile bivalves can now be successfully cultured.

Despite extensive investigations over the past 75 years, it has not been established what constitutes the natural food of oysters. Ukeles (1971) reviewed the current status of research on the nutritional requirements of oysters. She discusses three categories of potential food for oysters: (1) living organisms (plants, animals and

bacteria), (2) dissolved organic matter, and (3) organic detritus.

For experimental purposes and in commercial hatcheries, living food (phytoplankton) is the main source of food used to rear oyster larvae. Progress in the use of various living organisms for oyster nutrition was reviewed by Ukeles (1971). Most studies of bivalve feeding have been to determine the algal species and algal densities that provide optimum growth of the larvae (Davis 1953, Guillard 1958, Davis and Guillard 1958, Walne 1963, 1965, 1966, 1970a, b, Malouf 1971). The best phytoplankton foods for rearing Crassostrea virginica and C. gigas consist of members of the Haptophyceae, Chrysophyceae, Bacillariophyceae, Prasinophyceae and Chlorophyceae which lack cell walls (or possess very thin ones), lack toxic metabolites and are less than 8 μm in diameter (Davis and Guillard 1958, Guillard 1958). Isochrysis galbana Parke and Monochrysis lutheri Droop are the two most commonly used algal flagellates for small larvae. Larger larvae (above 125 μm in length) are less restricted in the types of food they can utilize (Davis 1953). Temperature also plays an important part in determining the degree to which a certain food is utilized. Larvae grown at 20°C can ingest naked flagellates and Chlorella sp. but can only digest the naked flagellates. At 25°C and 30°C larvae are able to utilize Chlorella (Loosanoff and Davis 1963).

The optimum algal density for larval growth depends upon the algal cell volume. Walne (1970a) noted that these densities can be expressed in terms of the total volume of algae per unit volume of water. For juvenile bivalves, this optimum is approximately 5000 μm^3 of algal material per μl (for the median cell volume range of 10 μm^3 to 1000 μm^3 per cell). This is equivalent to about 80 Isochrysis galbana cells per μl or about 15 Tetraselmis seucica cells per μl (median cell volumes 60 μm^3 and 335 μm^3, respectively.

Even good food species such as Isochrysis galbana may not supply optimal levels of all nutrients. Some studies have demonstrated that mixtures of different algal species produce greater larval growth than any of the species fed individually (Davis and Guillard 1958, Walne 1970b). The reason why some algal food species produce better growth than others is not apparent. As was pointed out earlier, small larvae apparently cannot utilize algae possessing thick cell walls but many algae lacking thick cell walls are not utilized either. For example Dunaliella, which lacks a thick wall, is a comparatively poor larval food (Walne 1970b).

There have been several studies on the biochemical composition of phytoplankton. Parsons et al. (1961) found that marine phytoplankton, when cultured under similar chemical and physical conditions, have very similar organic composition regardless of the size of individuals or taxonomy of the organism. There is evidence, however,

that certain chemical and physical factors can alter the biochemical composition of phytoplankton (Taub and Dollar 1965, Easley 1969, Carell et al. 1970, Wallen and Geen 1971a and 1971b, Saddler and Taub 1972, and Morris et al. 1974). Differences in amino acid composition between phytoplankton foods have not correlated with differences in growth rates of clams (Walne 1970a) or zooplankton (Cowey and Corner 1966). Phytoplankton species belonging to the same genus frequently contain similar fatty acid compositions (Chuecas and Riley 1969). Fatty acid distributions differ significantly, however, in members of different Divisions or Classes. DeMort (1970) was unable to relate differences in amino acid or fatty acid composition with the nutritional value of phytoplankton for oyster larvae. It appears that other factors such as digestibility of macromolecules and the presence of biologically active substances such as vitamins may determine, to a large extent, the nutritive value of phytoplankton. There is some evidence that vitamins may play a role in the nutritional value of phytoplankton or may be a limiting factor in the culture media. Davis and Chanley (1956) found that vitamins significantly increased the growth rate of oyster larvae even when compared to controls receiving a mixed phytoplankton diet. Shiraishi and Provasoli (1959) demonstrated that a vitamin mixture could replace the requirement of using mixed algal species as food for Tigriopus japonicus. As a result, Tigriopus could be grown on either

Isochrysis or Chroomonas alone if given a vitamin supplement. D'Agostino and Provasoli (1970) provided evidence that vitamins increased the fertility of Daphnia magna and that this enhancement acted via the algal food. Kern (1973) observed that juvenile oysters fed on vitamin-enriched algae grew significantly faster than juveniles fed on algae receiving only the usual vitamins present in the culture media.

The role of bacteria as a food source for larval oysters is still a matter of conjecture. Some bacteria are pathogenic (Guillard 1959, Tubiash et al. 1965, Walne 1970b, Brown 1973) and thus any food value they may have is inconsequential. Other bacteria, while not pathogenic, retard or cause no increase in larval growth rate (Davis 1950, Davis 1953, Walne 1963). Hidu and Tubiash (1966) found that addition of the antibiotic mixture, Combistrep® actually caused an increase in bacterial numbers. The authors suggest that Combistrep®, while inhibiting pathogenic bacteria, causes an increase in certain other bacteria which appear to be of nutritional value to oyster larvae.

Dissolved organic matter (DOM) is generally considered to be that which passes through a 0.45 μm Millipore® filter or a Whatman® GF/C glass fiber filter. This is the definition of DOM which I will use in this paper. By this definition DOM includes colloidal (particles 1.0 μm to 1.0 nm in their smallest dimension) as well as truly dissolved material. Particulate organic matter is that retained by the

filter. Sharp (1973), however, has refined these definitions by recognizing four size classes of organic matter: Total, particulate and two colloidal classes. For oceanic water he found that about 1% was greater than 0.8 μm (particulate), 8% was in the 0.025 μm - 0.08 μm range (colloidal 1) and 16% was in the 0.003 μm - 0.8 μm range (colloidal 2). Total dissolved organic-carbon content of ocean waters generally is in the range of 0.3-3 mgC liter^{-1}, although values up to or exceeding 20 mgC liter^{-1} may be found in coastal waters as a result of phytoplankton activity and pollution from land. For reviews on the composition of dissolved organic compounds in seawater see Wagner (1969), Riley and Chester (1971) and Williams (1971).

Sources of DOM in coastal waters include extracellular products from algae, decay of dead organisms, addition from terrestrial runoff and excretions from animals. In the vicinity of oyster beds the greatest input of DOM is likely to be from the extracellular products of algae and the decay of algae and sea grasses. It has been firmly established that healthy phytoplankton release some of their photosynthates and macromolecules into the extracellular environment. Hellebust (1974) reviewed the nature of algal extracellular products and the processes by which they are released. Algal extracellular products include virtually all groups of biochemicals found in algal cells. The fraction released is quite variable and represents from 0 - 90% of the total primary production of the algae.

The idea that dissolved organic matter (DOM) might serve as a direct food source for aquatic organisms was advanced early in this century by Pütter who hypothesized that aquatic animals feed on the organic matter in solution in the surrounding water. He assumed that the DOM was largely derived from the phytoplankton which were believed to lose the greater part of their photosynthate through the cell surfaces into the surrounding water. However, as a result of a critical review by Krogh (1931) which indicated that the DOM is not present in great enough quantities to be a nutrient base for living organisms, Pütter's ideas were discredited. Recent evidence suggests that although Pütter's estimates were incorrect, his theory may be valid for some animals in coastal and estuarine waters.

Speculation over the significance of DOM as a food base continued for several decades. Stephens and Schinske (1961) provided the first clear evidence that dissolved materials can be taken up by a variety of invertebrates. They reported net movement of amino acids from solution in seawater into 35 genera of marine invertebrates representing 11 phyla. Subsequent research has demonstrated that marine invertebrates are able to take up basic nutrients such as amino acids and sugars from extremely dilute ambient solutions and concentrate them as much as several hundred fold (Stephens and Virkar 1966, Ferguson 1967a, b, Chapman and Taylor 1968, Stephens 1968, 1972, Bass et al. 1969, Reish and Stevens 1969, Southward and

Southward 1970, 1972a,b, Chien et al. 1972, Chia 1972, Péquignat 1972, 1973, Sorokin 1973, Sorokin and Wyshkwarzev 1973, Anderson and Bedford 1973, Shick 1973, Pearse and Pearse 1973).

Péquignat (1973) investigated the assimilation of amino acids and glucose by organs of Mytilis edulis by liquid scintillation and autoradiography. He noted that within one hour the gills and mantle can concentrate the dissolved compounds by a factor of several hundred. Additional DOM is trapped by mucus (produced by the gills and mantle) which subsequently can be utilized when it reaches the hepatic caeca. He concludes that DOM would provide a "significant portion of their normal diet" if the DOM was continually replenished. He also suggests that DOM may be an important nutrient source for veliger larvae and other ciliated plankton after their yolk supply is exhausted and while the gut is still rudimentary.

Investigating the nutritional importance of DOM to several marine animals, Sorokin and Wyshkwarzev (1973) compared uptake of DOM with uptake of living bacteria and algae. In animals having ciliary apparatus and extensive mucoid body surfaces assimilation of DOM (protein hydrolysate) was equal to or higher than that of bacteria or algae at equivalent concentrations. For example, Malleus sp. (a bivalve mollusc) fed on DOM assimilated about 1.8% per day of its body weight. Assimilation of bacteria and algae was 0.67% and 0.35% of the body weight per day, respectively. Assimilation of DOM by

pteropod larvae was about 300 times the assimilation of algae. The authors fail to give adequate descriptions of methods used to measure assimilation and details as to species and culture conditions of the bacteria and algae used in their experiments. Nevertheless, their report suggests that certain invertebrates can assimilate significant levels of DOM from dilute solutions.

The third possible source of nutrition to oysters, nonliving particulate matter, has received comparatively little attention. This fraction consists of detritus[1] and organic aggregates. The term organic aggregate was used by Riley (1963) to describe the more or less rounded amorphous masses of organic material with internal spaces that may harbor a flora of bacteria, fungi, and small algae. The aggregates comprise about 70% of the total particulate organic matter (Riley et al. 1964). Baylor et al. (1962) and Sutcliffe et al. (1963) discovered that organic particles (later called organic flakes) could be formed experimentally by bubbling air through seawater. The role of bubbles, inorganic particles and bacteria in the formation of organic flakes and aggregates was a matter of debate for several years (Riley 1970).

Khailov and Finenko (1970) describe a mechanism of aggregate formation that encompasses the other ideas. According to this scheme

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[1] Detritus as defined here is material of identifiable structure consisting of the remains of animal and plant bodies or their excreta.

organic aggregates form by the adsorption of surface-active macromolecules on solid-water or gas-water interfaces. Particles (bacteria, membranes, inorganic material, detritus, etc.) or bubbles may act as nuclei on which macromolecules adsorb. Bubbles may also facilitate aggregate formation by increasing bacterial numbers or by causing bacterial flocculation. Following aggregate formation, bacteria modify the adsorbed material through biochemical processes (Khailov and Finenko 1970), thereby hydrolyzing refractory materials and making them available to filter feeders. Thus it is not necessary for the animal itself to break down refractory materials, as was once thought, in order to obtain nutrition from detritus and organic aggregates. Figure 1 summarizes the mechanism by which various organic materials may be incorporated into food chains.

Baylor and Sutcliffe (1963), using organic aggregates produced from seawater by bubbling, found that the aggregates could support the growth of Artemia salina. The growth rates of the animals fed on the aggregates or on yeast were roughly the same for the first four days. After this time, growth rates of animals fed on aggregates was lower but the animals continued to increase in size. No measurement of the food levels was made, however, and the authors suggest the food level of the organic aggregates may have been lower than that of the yeast. Paffenhöfer and Strickland (1970) investigated the role of natural organic aggregates, artificial detritus (made from diatoms)

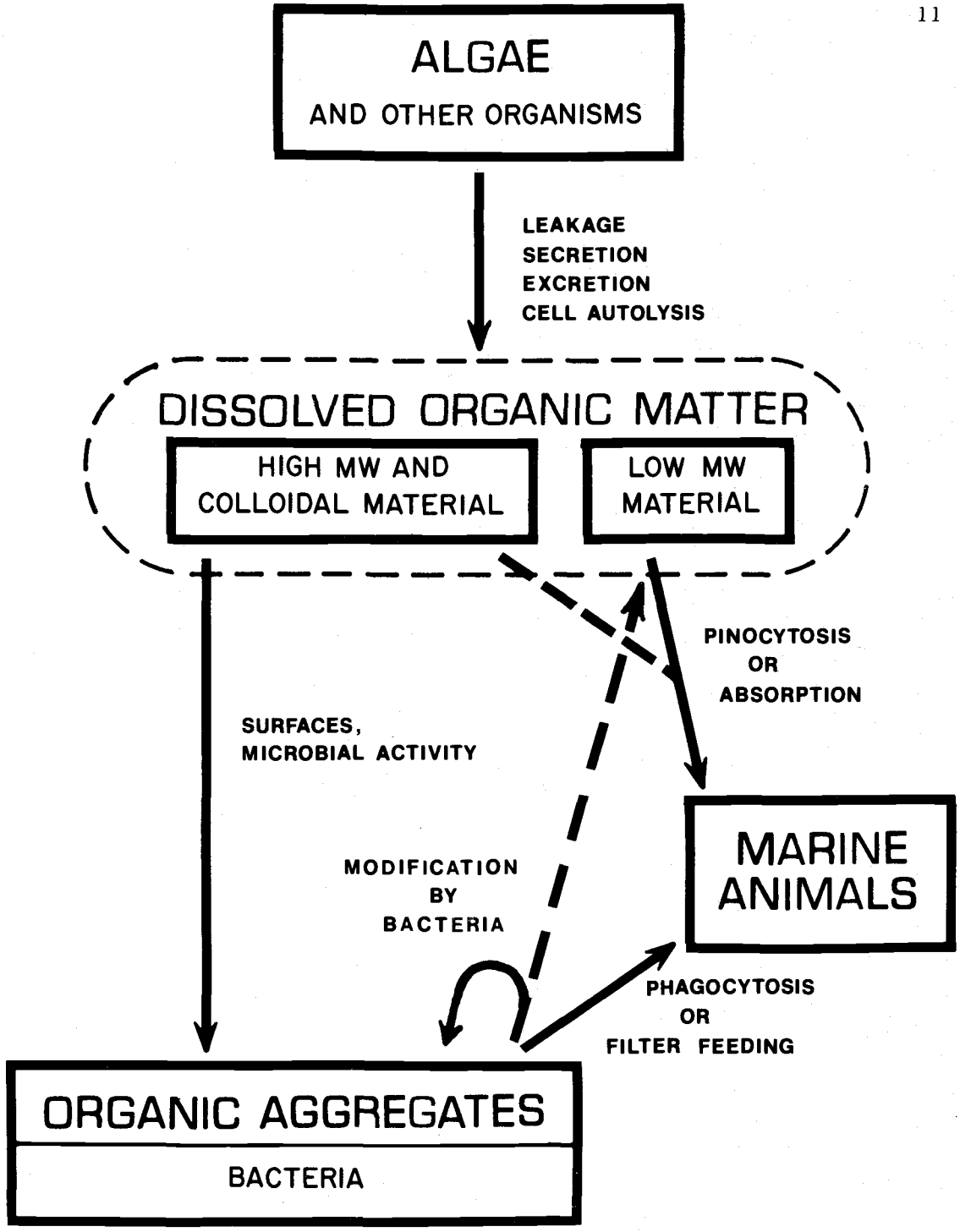

Figure 1. Scheme for the utilization of dissolved organic matter by marine animals.

and fecal material in the nutrition of Calanus helgolandicus. The dead diatoms and fecal material were really ingested but the organic aggregates were not consumed. Shrimp lived for months on dried pellets of natural detritus (Qasim and Sankaranarayanan 1972). Moulting occurred during this period indicating growth had taken place.

Attempts to rear oyster larvae (Davis 1950) and clam larvae (Loosanoff et al. 1951) on detritus were unsuccessful. Clam larvae grew well on lyophilized Isochrysis galbana (Hidu and Ukeles 1962) and on frozen I. galbana (Chanley and Normandin 1967) but oyster larvae displayed poor growth. Clam larvae were also able to utilize pulverized Ulva lactuca (Loosanoff and Davis 1963, Chanley and Normandin 1967).

The experimental approach used in the study of the nutrition of Tigriopus and Artemia (Shiraishi and Provasoli 1959, Provasoli and Shiraishi 1959, Provasoli and D'Agostino 1969, D'Agostino and Provasoli 1970) has not been used for oysters. Instead, a wide variety of organic substances not available to bivalves in their natural environment has been fed to oysters on a trial and error approach. These artificial foods include corn meal, rice hominy, yeast, corn starch (Dunathan et al. 1969), milk, bouillon, pablum, various plant and animal extracts (Chanley and Normandin 1967) and human erythrocytes (Claus and Adler 1970). None of these artificial foods has consistently supported the growth of young oyster larvae.

Radiotracers have been an extremely useful tool in the study of feeding rates and food utilization in many filter feeding organisms. Carbon-14 has proven especially useful in this regard. Many of these methods are described in detail by Sorokin (1968). Additional methods and problems with interpretation of results obtained from ^{14}C incorporation studies are described by Johannes and Satomi (1967), Schindler (1968), Bell and Ward (1970) and Conover and Francis (1973).

Walne (1965) studied the nutrition of Ostrea edulis L. by using ^{32}P-labeled algal foods. He investigated the effects of temperature and algal density on the assimilation of several species of phytoplankton by different sizes of larvae. He demonstrated that assimilation by oyster larvae is proportional to cell density up to about 25 (Isochrysis galbana) cells μl^{-1}. Above 50 cells μl^{-1} substantial increases in cell density cause only small gains in assimilation. Feeding and assimilation are increased by temperature increases in the range of $14^{o}C$ - $30^{o}C$. Most important, he showed a high positive correlation between ^{32}P incorporation and larval growth.

Ukeles and Sweeney (1969) investigated the uptake of ^{14}C-labeled Monochrysis lutheri by 48 hour Crassostrea virginica larvae. This is the first, and only study, so far as I am aware, of the nutrition of very young oyster larvae. Incorporation increased rapidly during the first few hours of incubation and then leveled off. The authors suggested this to be a result of continuous feeding with an equilibrium established

between food entering and leaving the gut. Although ingestion increased with an increase in food density, utilization did not show a similar increase. They also demonstrated that large numbers of small particles such as trichocysts, non-nutritive algae and bacteria may interfere with normal feeding and digestion.

With the exception of the radiotracer experiments, the feeding experiments previously described measured growth chronically, that is, for a relatively long period of time. In such studies survival, shell growth or percent of the larvae setting was used as a measure of utilization of the food. Unfortunately the foods tested were generally used as a sole source of nutrition. Therefore, a minor dietary deficiency would result in poor growth and the food would be interpreted as having little value to the animal. A better approach is to supplement a known good food in several proportions with the food being tested. Considerable amount of time and effort is required to perform such experiments and it is difficult to compare several types of food.

In acutely measured feeding experiments, larvae are fed for up to 48 hours. Assimilation is frequently measured using radiotracers. This type of experimentation could overestimate food value by failing to detect toxic effects or by failing to note a nutritional deficiency which may not retard assimilation but may retard growth. Such experiments may underestimate food value by failing to account for rapid metabolism of assimilated foods or by not allowing sufficient time for

the larvae to adapt to the food or culture conditions. This method does, however, allow a number of factors to be tested within a relatively short time.

My investigation is designed to acutely measure the relative degree to which living algal cells, DOM, and organic aggregates are taken up by the larval oysters for growth and respiration. It is not an energy budget study. Absolute values for assimilation and production, therefore, are not determined in this study. Johannes and Satomi (1967) have discussed methods and problems of measuring assimilation or retention of organic matter in aquatic invertebrates. The term incorporation is used in place of production in this study. I define incorporation as that portion of the consumed food that is retained by the organism at the end of the time period of the experiment. I define respiration as that portion of the consumed food that is respired (as measured by $^{14}CO_2$ evolution) during the experiment. Specifically, I have examined the nutrition of young oyster larvae soon after their development from trochophores to veligers. At this stage the larvae apparently have poorly developed feeding and digestive systems and are therefore very restricted in the types of food they can utilize. The relative importance of the three types of food is established using a radiotracer (^{14}C) to label the food.

METHODS

Seawater

The seawater used in all experiments was obtained from the seawater supply at the Oregon State University Marine Science Center, Newport, Oregon. Water was taken after it passed through a large settling basin, sand filters and an ultraviolet light water sterilization unit. For Experiments 1-6, seawater of salinity 33.5°/oo was obtained on November 7, 1973. For Experiments 7-13, seawater of salinity 33.0°/oo was obtained on April 10, 1974. Water was stored at 15°C in the dark in either sterile borosilicate or polypropylene carboys, or disposable polyethylene containers. Approximately one week before an experiment the salinity of the seawater was adjusted to 28.0-28.5°/oo with glass distilled water. It was then pressure-filtered through a glass fiber prefilter and a 0.45 μm Millipore® membrane filter and finally through a sterile 0.22 μm Millipore® or a 0.20 μm Nucleopore® membrane filter into autoclaved flasks or borosilicate carboys. This seawater, used for culturing the algae and for rearing the oyster larvae, will be referred to as membrane filtered seawater.

Algae

Isochrysis galbana Parke 1949 was obtained from the Indiana Culture Collection (LB 987). The Pseudoisochrysis paradoxa (VA 12), which was obtained (via the Oregon State University Marine Science Center) from Dr. John Dupuy of the Virginia Institute of Marine Science, is an alga isolated and provisionally named by Dupuy. It is currently being used by the Oregon State University pilot oyster hatchery as well as many of the West Coast commercial oyster hatcheries as a substitute for Isochrysis or Monochrysis for raising Crassostrea gigas. The two cultures were freed of bacteria by the use of antibiotic solutions containing penicillin G, streptomycin sulfate and chloramphenicol using the methods of Guillard (1973). Sterility was checked periodically using either 0.1% (w/v) of bacteriological peptone or STP medium (Shiraishi and Provasoli 1959). Both of these media were made up with enriched seawater containing 1% agar.

All algae were cultured in an enriched seawater medium, a modification of Provasoli's ES (Provasoli 1968). The composition of this enrichment is shown in Table 1. Stock cultures were maintained in cotton stoppered 125 ml flasks at 15°C. "Cool White" fluorescent tubes on a 16 hour light (8 hour dark) cycle provided an incident light intensity of 1,500 lux. These cultures were swirled twice per day and transferred every 5 days. Unlabeled algal cultures used for larval

Table 1. Composition of the algal culture medium.

Major Elements	
$NaNO_3$	70 mg
Na_2 · glycerophosphate	10 mg
Fe EDDHA[a]	7.5 mg (450 μg Fe)
Trace Elements	
H_3BO_3	5.70 mg
$FeCl_3 \cdot 6H_2O$	0.245 mg
$MnSO_4 \cdot 4H_2O$	0.820 mg
$ZnSO_4 \cdot 7H_2O$	0.110 mg
$CoSO_4 \cdot 7H_2O$	0.024 mg
Na_2EDTA	5.00 mg
$Na_2MoO_4 \cdot 2H_2O$	0.013 mg
Vitamins	
B_{12}	167 μg
Thiamine·HCl	167 μg
Biotin	167 μg

This enrichment is made up as a concentrated stock solution, filter sterilized and added to 1 liter of membrane filtered seawater.

[a]CIBA GEIGY Corp., Ardsley, NY

food were grown at 19°C in 2 liter flasks containing 1 liter of culture medium. Continuous illumination from "Cool White" fluorescent tubes provided an incident light intensity of 6000 lux. Filter sterilized sodium bicarbonate (200 mg liter^{-1}) was added aseptically to this media to provide additional carbon. These cultures were fed to oyster larvae only when in the exponential phase of growth.

Algal cell numbers and median cell volumes were measured using an Electrozone/Celloscope® Model 112Tu particle counter (Particle Data, Inc.) following the procedures established by Sheldon and Parsons (1967). All counts were made below the 5% coincidence level. Median cell volume was measured as an estimate of mean cell volume. Since both of the species of algae used in this report have a cell size distribution which is nearly normal, the median is a good approximation of the mean. Paper mulberry pollen and polystyrene spheres (Particle Information Service) of various diameters (3.5 μm, 5.2 μm, 8.0 μm, 10.3 μm, and 15.0 μm) were used as calibration standards. Cell numbers and median cell volumes were checked periodically with haemocytometer counts and with a Coulter Counter® Model ZBI equipped with a 64 channel distribution analyzer.

Preparation of Labeled Foods

Labeled algal foods were prepared by adding 80 μCi of $NaH^{14}CO_3$ (Amersham Searle) to each 500 ml of recently innoculated algal culture.

For Experiments 4 and 5, 250 μCi of $NaH^{14}CO_3$ were added to each 500 ml of algal culture. Generally, a culture was started on day 0 with 3×10^5 cells per ml and used on day 3 during log growth phase, when its density was about 2.5×10^6 cells per ml. For most experiments 1 liter of algal culture was grown in a 2 liter erlenmeyer flask under continuous light (6000 lux) at 19°C.

All of the ^{14}C-labeled foods were prepared from the ^{14}C-labeled algae. The first step was to centrifuge the labeled algae using a Foerst continuous flow centrifuge. The algal cells were then resuspended in membrane filtered seawater and centrifuged again at 755 x g for about 10 minutes. The algal pellet was resuspended in membrane filtered seawater and samples taken to determine median cell volume, cell density, carbon and nitrogen content and radioactivity. Preliminary experiments demonstrated that Isochrysis galbana and Pseudoisochrysis paradoxa cells treated in this manner were not damaged appreciably. Growth rate and photosynthetic capability decreased by less than 5% as compared to control cultures not centrifuged. Isochrysis galbana cells did, however, tend to clump after centrifugation. Pseudoisochrysis paradoxa cells did not clump after centrifugation. It was for this reason that the switch from Isochrysis to Pseudoisochrysis was made part way through the study.

DOM was prepared from the algal cells by suspending the algal pellet in 20 ml of a 33 mM phosphate buffer containing 1 mM Na_2EDTA

made up in double distilled water. This suspension was then placed in a rosette cell and sonicated 4 minutes at 5°C. After centrifugation for 20 minutes at 4°C and 20,000 x g, the centrifugate was filtered through a 0.22 μm Millipore® filter. At this point, samples were taken for carbon and nitrogen analysis and for radioactivity measurement. The DOM was diluted to 100 ml with membrane filtered seawater and then fed to the larvae. The DOM used in Experiments 12 and 13 was prepared by enzymatic hydrolysis rather than sonication. An 8 liter culture of Pseudoisochrysis paradoxa was innoculated with 500 μCi of $NaH^{14}CO_3$ and grown for 3 days in an 18 liter borosilicate carboy at 20°C and 6000 lux of continuous illumination. This culture was centrifuged down as before but diluted with 100 ml of 33 mM phosphate buffer containing:

10 mM Na_2EDTA	10 ml
Streptomycin SO_4	25 mg
Penicillin G	100 mg
Pronase®	5 mg
Cellulinase®	5 mg

This mixture was incubated at 37°C on a shaker. After 24 hours, an additional 5 mg each of Pronase® and Cellulinase® (Calbiochem) were added to the mixture. At the end of 40 hours the mixture was centrifuged 20 minutes at 20,200 x g at 4°C. A portion of the centrifugate was filtered through a 0.45 μm membrane filter and designated

DOM (0.45 μm). Another portion of the centrifugate was filtered through a 0.05 μm membrane filter and was designated DOM (0.05 μm).

Larvae

Adult oysters (Crassostrea gigas) were obtained from the pilot oyster hatchery at the Marine Science Center or were purchased from the Oregon Oyster Company, Newport, Oregon. Larvae used in all experiments (except Experiments 8-11)[2] were provided by the pilot oyster hatchery. Adults were spawned artifically according to the methods of Loosanoff and Davis (1963). No attempt was made to control parentage.

When the larvae were approximately 24 hours old they were sampled to determine the percentage which had developed normally, i.e., developed normal "D" shaped shells. If over 75% of the larvae developed normally they were transported to Corvallis in carboys. There, the larvae were separated from most of the bacteria and detritus by screening them on to a 44 μm mesh Nitrex® screen and washing with membrane filtered seawater. The larvae were then placed into 8 liter polyethylene tanks or borosilicate carboys containing membrane filtered seawater. The larval density was adjusted to about 15 larvae per ml. Approximately 50 algal cells per μl were

[2] I spawned the gametes for these larvae in Corvallis using the same techniques as used at the pilot oyster hatchery.

added to the tanks using the species of algae which would later be used in the experiment. Sufficient algae were added daily to return the density to 50 cells per µl. Larvae were grown in the dark at 24-26°C until the day of the experiment.

With the exception of Experiments 12 and 13, larvae were screened onto 60 µm mesh Nitex® screens at the end of 3-5 days and washed with membrane filtered seawater. Subsequently, they were placed into filtered seawater and starved for about 4 hours at 24-26°C. At the end of this period they were again screened, washed, and placed into a 1000 ml beaker at a density of about 200 larvae per ml. While gently agitating the larvae in the beaker with an acrylic plunger, the larvae were distributed into the experimental containers using a 5 ml Oxford Macroset® pipette. After each container had received an aliquot of larvae the procedure was repeated at least twice more to assure that each beaker received equal numbers of larvae. The containers (400 ml polypropylene beakers) were then filled to about 250 ml with membrane filtered seawater. Appropriate amounts of ^{14}C-labeled food were then added and the volume brought to 320 ml with membrane filtered seawater. The beakers were covered loosely with polypropylene lined covers and incubated in the dark for the duration of the experiment. For experiments lasting 24 hours, larvae were agitated at least twice during the experiment with an acrylic plunger.

At the end of the experimental period larvae were screened onto a 53 μm Nitex® screen. (The interval between feeding and screening was carefully timed so that all beakers were incubated for the same period of time.) In Experiments 1-5, the larvae were then placed back into the beakers, seawater and unlabeled food added, and allowed to clear their digestive systems for intervals ranging from 0-24 hours. At the end of this period the larvae were again screened onto 53 μm mesh Nitex® screen and then washed into concentration tubes. A concentration tube consists of a 5 ml disposable Oxford pipette tip with a 1 cm length of polyethylene tubing slipped over the tip. The other end of the tubing is plugged. After the larvae were washed into the concentration tube, a drop of $HgCl_2$ (40 mg ml^{-1}) was added. After the larvae had settled, the liquid was carefully withdrawn with a syringe. The polyethylene tubing, containing the larvae, was removed and placed into a scintillation vial containing 10 ml of Aquasol®.

Respiration of the ^{14}C incorporated was measured, in Experiments 1, 5, 6, and 8-10, in specially designed beakers. Polypropylene beakers (800 ml) were fitted with a small propropylene funnel inserted in the side of the beaker near the bottom. The funnel opening (on the inside of the beaker) was covered with 53 μm mesh Nitex® screen. The beaker was covered with a polypropylene lined cover. A latex tube attached to the stem of the funnel could be closed with a pinch clamp. This apparatus permitted water samples to be taken at

any time without much agitation of the water or contact with air. (Preliminary experiments, in which known amounts of $NaH^{14}CO_3$ was added to seawater in these beakers, established that the loss of $^{14}CO_2$ to the atmosphere was less than 3% in 24 hours.) These beakers were treated the same as other experimental beakers except that the contents were not agitated after the experiment had commenced. At the end of the experiment a 50 ml aliquot was carefully drained into a 250 ml serum bottle. The bottle was immediately sealed with a serum stopper that had a plastic cup (K 882320 Kontes Glass Co., Vineland, N. J.) suspended from it, containing a 25 x 60 mm piece of accordian-folded chromatographic paper (Whatman ® No. 1). Then, 2 ml of 2 N H_2SO_4 was injected through the septum. Next, 0.35 ml of phenethylamine was slowly added to the filter paper and the bottle placed on a shaker at room temperature. At the end of two hours, when essentially all of the CO_2 evolved by acidification had been trapped in the phenethylamine, the paper was removed and placed in a scintillation vial as discussed in the section on radiotracers. This procedure was modified from that of Hobbie and Crawford (1969). Respiration blanks consisting of beakers containing food but lacking larvae were used to correct for inorganic ^{14}C in the food and for algal and bacterial respiration.

Larval density was determined by withdrawing aliquots from the beakers during agitation and collecting the larvae on a 25 mm

Millipore® EAWG filter with grid. Approximately 3 ml of saturated $CaCl_2$ was added to the filter, rendering it almost transparent (Millipore 1973). Larvae on the filter were then counted under a dissecting microscope. A total of about 1000 larvae were counted. Larval length was measured on the same filters using a compound microscope equipped with an ocular micrometer. Larval length is defined as the greatest dimension of the shell parallel to the straight hinge.

Radiotracers

The radioactivity of algal cells and organic aggregates was determined by filtration onto 25 mm Millipore® membrane filters (1.2 μm pore size) or Whatman® GF/C glass fiber filters. These filters were placed directly into scintillation vials containing 10 ml Aquasol®. Radioactivity of the DOM was measured by pipetting 50 μl or 100 μl directly into scintillation vials containing 10 ml Aquasol®. Filter papers from respiration experiments were placed directly into scintillation vials containing 15 ml toluene/Triton X-100® (3:1 v/v) with 0.4% (w/v) of P.P.O. and 0.01% (w/v) of P.O.P.O.P.

Samples were counted 2-10 days later on a Packard Tri-Carb® Model 2405 liquid scintillation spectrometer. The samples were counted to a preset level of 100,000 registered counts. All samples

were corrected to 100% counting efficiency using channels ratio or external standard quench correction.

Carbon-Nitrogen Analysis

Organic carbon and nitrogen content of the algal cells and organic aggregates was determined by filtering the particles onto 13 mm Whatman ® GF/C glass fiber filters. Samples of the DOM were taken by spotting a total of 100 µl of the DOM on a 13 mm Whatman ® GF/C glass fiber filter. All filters were dried at 60 °C for 24 hours and stored in a dessicator at -10 °C until analyzed.

Samples from preliminary experiments and from Experiment 6 were analyzed on a Carlo Erba Model 1100 CHNO analyzer. The remaining samples were analyzed on a Hewlett Packard Model 185B CHN analyzer. Acetanilide was used as a standard for carbon and nitrogen. Appropriate blanks were also analyzed and the readings subtracted from those of the samples.

The concentration of the natural DOM used in Experiments 12 and 13 was too low to be measured using the above technique. Organic carbon from these samples was measured on an OIC carbon analyzer.

The food concentrations used in the experiments are expressed as mgC per liter. This refers to the organic carbon present in the added food only and does not include organic carbon present in the membrane filtered seawater.

Data Analysis

The inverse specific carbon radioactivity (C_r) can be calculated from the radioactivity and carbon content of a food. C_r is the amount of organic carbon in the food which corresponds to 1 cpm of radioactivity. Similarly, the inverse specific algal radioactivity (A_r) is the number of algal cells corresponding to 1 cpm of radioactivity.

The raw data for ^{14}C incorporation are expressed in cpm per beaker. These ^{14}C incorporation values are divided by the number of larvae per beaker and by the time interval in days to give the rate of incorporation, D, in cpm larva^{-1} day^{-1}. The rate of incorporation can also be expressed in terms of organic carbon and the number or volume of algal cells incorporated:

$I_c = D \cdot C_r$ = Rate of incorporation in ngC larva^{-1} day^{-1}

$I_a = D \cdot A_r$ = Rate of incorporation in cells larva^{-1} day^{-1}

$I_v = I_a \cdot V$ = Rate of incorporation in volume (of algal cells) larva^{-1} day^{-1} (V = median algal cell volume)

The raw data for ^{14}C respiration are expressed in cpm per bottle. These ^{14}C respiration values are divided by number of larvae per bottle and by the time interval in days to give the rate of respiration in cpm larva^{-1} day^{-1}. The rate of respiration can also be expressed in terms of organic carbon respired:

$R_c = R \cdot C_r$ = Rate of respiration in ngC larva^{-1} day^{-1}

In some experiments the percent of the assimilated food that is respired during the experiment ($\%R_c$) is calculated:

$$\%R_c = \frac{R_c}{I_c + R_c} \times 100$$

In Experiments 12 and 13 the Daily Index of Incorporation ($I_c/W \times 100$) is calculated. W is the organic carbon content (see Experimental Procedures section) of an oyster larva. The Daily Index of Incorporation is the percent of its body weight that the larva incorporates each day.

Experimental Procedures

Twenty-two separate experiments were performed in this study. Some preliminary experiments were omitted from this report because they were incomplete. Other experiments were omitted due to larval mortality in control cultures during or soon after an experiment. Results of 13 experiments are reported here. Table 2 shows dates of the experiments larval culture conditions, parameters of the algal foods, and characteristics of the DOM and organic aggregates used in the experiments.

Experiments 1-3 were designed to determine the optimum algal food concentration for young oyster larvae (80-90 μm in length). In all three experiments the larvae were fed labeled Isochrysis galbana for 4 hours, screened, and placed back in membrane filtered seawater

Table 2. Culture conditions and characteristics of the foods used for larvae in Experiments 1-13. Isochrysis galbana was fed in Experiments 1-6, and Pseudoisochrysis paradoxa was fed in Experiments 7-13. Symbols are explained in the Methods section.

		Larval Culture Conditions				Algal Foods					DOM		Aggregates	
Exp.	Date	Age	Length	Density	Temp	Cell Volume	A_r	C_r	Carbon Content	C/N[a]	C_r	C/N	C_r	C/N
		days	μm	ml^{-1}	°C	μm^3	Cells cpm^{-1}	pgC cpm^{-1}	pgC $cell^{-1}$		pgC cpm^{-1}		pgC cpm^{-1}	
1	11/23/73	3	83.9	26.0	26.0	58	6.3							
2	12/1/73	4	87.8	22.7	26.0	67	3.3							
3	1/19/74	4	91.6	19.2	26.0	70	14.8							
4	11/10/73	3	85.9	14.9	26.0	58	1.6							
5	2/11/74	3	83.2	8.0	26.0	58					11.2			
6	2/12/74	4	88.3	7.2	26.0	59	4.4	65.2	14.8	6.1	162.6			
7	6/22/74	4	83.2	6.3	14/24	45	14.4	192.8	13.4	8.2	165.9	6.0	165.9	
8	7/5/74	3	83.0	8.2	26.0	39	10.2	110.0	10.8	7.5	102.1	4.5	102.1	
9	7/15/74	4	85.1	11.8	26.0	37	7.4	91.1	12.3	8.6	105.0	5.4		
10	7/26/74	3	80.8	11.1	26.0	32	14.5	117.4	8.1	7.6	122.5	5.2		
11	10/2/74	10	119.1	2.2	24.0	32	10.0	80.1	8.0	8.4	77.7	5.5	102.4	6.0
12	10/27/74	12	139.2	2.2	24.0	33	12.9	104.1	8.7	8.3	[b]	3.5	222.6	26.9
13	10/27/74	4	85.3	10.5										

[a] Carbon:Nitrogen ratio by weight

[b] C_r = 581.9 pgC cpm^{-1} for the 0.05μm filtered DOM, 446.8 pgC cpm^{-1} for the 0.45μm filtered DOM, and 87.7 pgC cpm^{-1} for the natural algal extracellular products.

containing unlabeled Isochrysis galbana cells for an additional 4 hours to clear their digestive systems of labeled but unused algal material.

The clearing times used in the preceding experiments were based on values for Daphnia (Schindler 1968, Bell and Ward 1970). Experiments 4 and 5 were designed to determine the length of time necessary for oyster larvae to clear their digestive system of unused algal cells and DOM, respectively. In Experiment 4, larvae were fed labeled algal cells (50 cells μl^{-1}) for a period of one hour. Larvae were screened quickly and placed into beakers containing the same concentration of unlabeled algae and allowed to void unused materials for periods of from 0-24 hours. At the end of this period, larvae were screened and counted for radioactivity remaining. Respiration was measured for the 24 hour clearing period only. In Experiment 5 larvae were fed labeled DOM (1.0 mgC $liter^{-1}$) for one hour and allowed to clear for periods up to 24 hours in water containing the same concentration of unlabeled DOM.

Larvae were fed either algal cells or DOM in Experiment 6. The amount of the assimilated food which was incorporated and that respired was measured. The DOM in this experiment was produced by sonicating the algal cells in seawater rather than in the phosphate buffer containing EDTA as described in the section on Preparation of Labeled Foods.

Experiment 7 compared incorporation of algal cells (Pseudo-isochrysis paradoxa), DOM and organic aggregates at 14°C and at 24°C. Organic aggregates were prepared by adding 15 ml of the DOM to 50 ml seawater (pH raised to 10 with NaOH) and homogenizing with a Tissumizer® Model SDT-182N (Tekmar Company, Cincinnati, Ohio). The production of bubbles by the Tissumizer® together with precipitation of organic matter by the high pH caused the formation of organic aggregates. The aggregates were not separated from the DOM by centrifugation prior to feeding.

In Experiment 8 larvae were fed either algal cells, DOM or organic aggregates. Incorporation and respiration of the assimilated food were compared. Organic aggregates were prepared by adding DOM to 100 ml seawater containing 50 mg calcium carbonate and mixing with the Tissumizer®. The aggregates were separated from the unprecipitated organic matter by centrifugation and were resuspended in membrane filtered seawater prior to feeding.

Incorporation and respiration of the assimilated food (algal cells or DOM) were examined in Experiment 9. The algal cells and DOM were prepared as described in the section on the Preparation of Labeled Foods.

Antibiotics were used in Experiment 10 to determine whether bacterial activity influences measurements of respiration and incorporation. Beakers designated "A" contained chloramphenicol (33 mg

liter^{-1}), streptomycin (50 mg liter^{-1}) and penicillin G (50,000 IU liter^{-1}). Beakers designated "AA" contained twice the concentration of antibiotics present in "A." The DOM was disrupted in the phosphate buffer except that it was made up with half strength seawater (14% salinity).

The incorporation of algal cells, DOM and organic aggregates by larger larvae (119 μm in length) was measured in Experiment 11. Aggregates were formed by adding 130 ml DOM to 670 ml seawater and bubbling in a bubble tower (Wallace and Wilson 1969). The aggregates were then centrifuged and the pellet resuspended in membrane filtered seawater. The suspension was homogenized with the Tissumizer® to reduce particle size before feeding (Figure 2).

Experiments 12 and 13 were performed together in an attempt to compare incorporation by small (85.3 μm) and large (139.2 μm) larvae. The foods included algal cells, organic aggregates, "natural" DOM and DOM filtered through two different pore diameter filters. The natural DOM was prepared from the natural extracellular products of Pseudoisochrysis paradoxa, which were separated from the algal cells by centrifugation and filtration. Inorganic ^{14}C was removed from the filtrate by acidifying to pH 3 and stirring for 5 hours. The pH was adjusted to 7.8 prior to feeding. No dilution of this food was made prior to feeding. The enzymatic preparation of the DOM has been described in the section on the Preparation of Labeled Foods.

The soluble organic matter produced by the enzymatic hydrolysis was separated from the particulate matter by centrifugation. The centrifugate was the fraction used as DOM. The pellet was homogenized with the Tissumizer® to form the artificial detritus. The particle size distribution of the detirtus is shown in Figure 2. Streptomycin and penicillin G were added to yield 50 mg liter^{-1} of each in all larval cultures. The degree to which particles form from DOM either through bacterial action or spontaneously was examined in Experiment 12. DOM was added to beakers containing membrane filtered seawater and antibiotics and incubated at 24°C. At the end of 24 hours the contents were filtered onto a 0.45 μm membrane filter and counted for ^{14}C activity.

The carbon and nitrogen content of the larvae[3] used in Experiments 12 and 13 was determined by filtering larvae onto Whatman® GF/C glass fiber filters. For organic carbon values, larvae were acidified (2 ml of 6 N HCl in 25 ml seawater) for 2 hours to remove inorganic carbon. These larvae were then filtered onto Whatman® GF/C glass fiber filters as described before. Microscopic examination revealed the presence of an organic skin surrounding the larvae but the carbonaceous shell had dissolved following the acidification step.

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[3] Larvae were starved 5 hours prior to analysis.

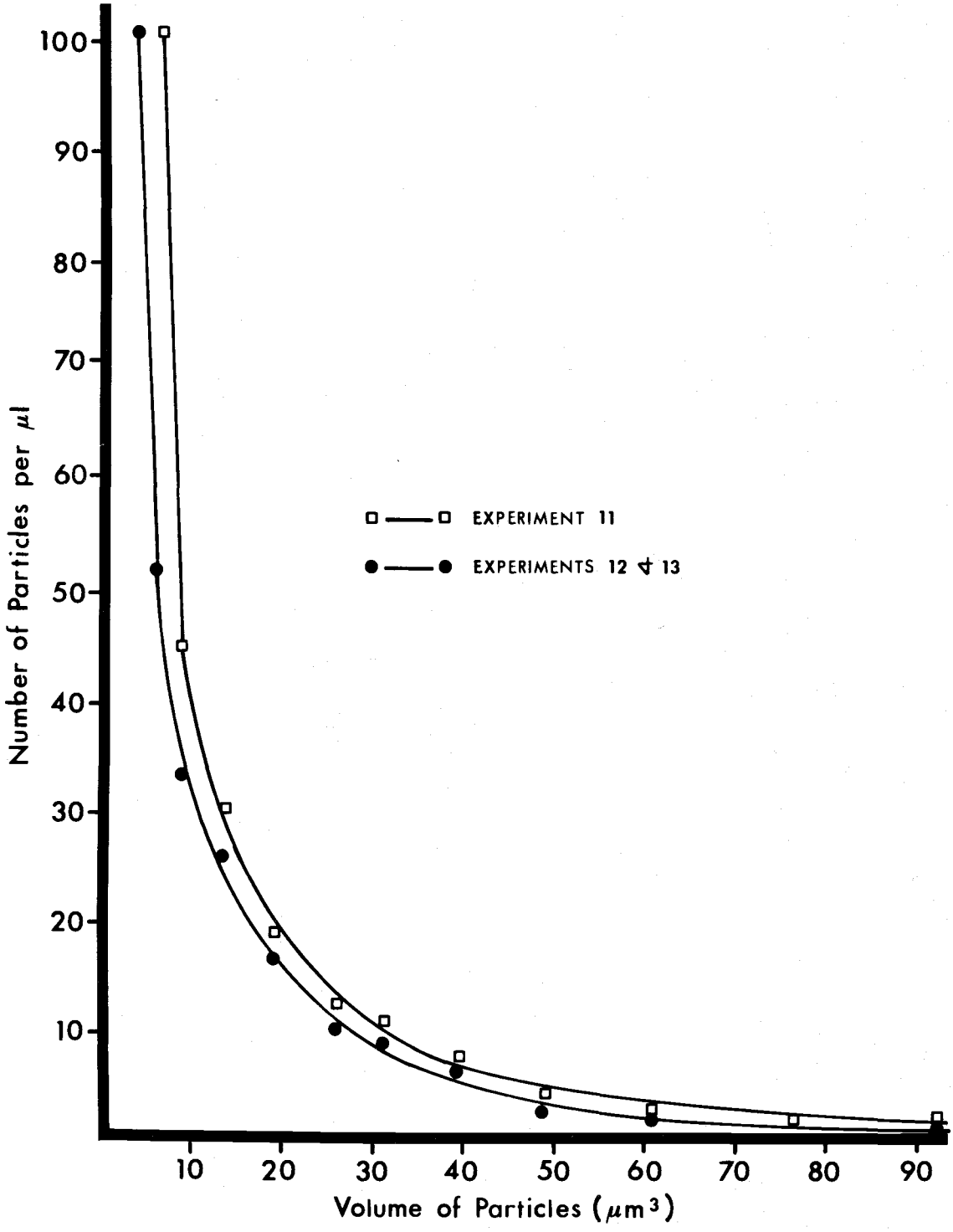

Figure 2. Size distribution of the organic aggregates used in Experiments 11, 12 and 13.

RESULTS

Experiments 1-3 (Figure 3) demonstrate the relationships between the rate of incorporation of algal cells by small oyster larvae and the concentration of the algal food. The rate of incorporation is proportional to concentration up to about 1500 μm^3 of algal cells μl^{-1}. The maximum rate of incorporation is attained at about 3000 μm^3 of algal cells μl^{-1}. The rate of incorporation does not increase with further increases in algal concentration.

The uniformity of results of these three experiments indicates the measurements of incorporation are reproducible. Malouf (1971) found that the lowest algal density at which maximum larval growth occurred was about 20 Monochrysis cells μl^{-1}. Walne (1970) found this value to be about 50 Isochrysis cells μl^{-1}. Ukeles and Sweeney (1969) found that the lowest density at which maximum ^{14}C incorporation occurred was about 50 Monochrysis cells μl^{-1}. When differences in the cell volumes of these algae are taken into consideration, the optimum algal densities reported agree closely with mine.

The clearing rates of small larvae were determined in Experiments 4 and 5. The oyster larvae require about 2-3 hours to excrete or defecate unused labeled algal materials (Figure 4). Further decreases in activity during the clearing procedure can be explained by respiratory losses. Oyster larvae also require about 2 hours to

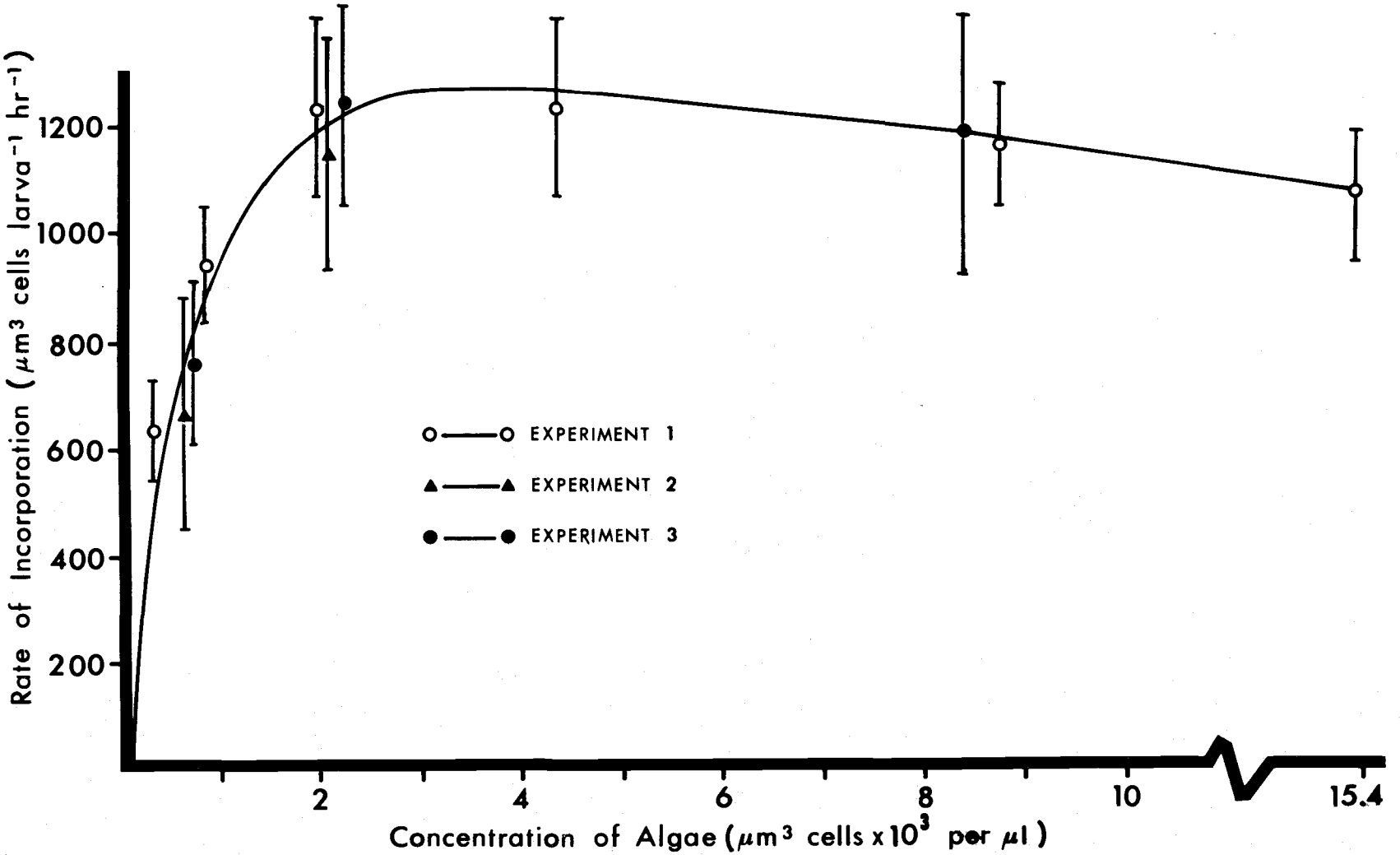

Figure 3. Relationship between the concentration of algal cells and the rate of incorporation by small oyster larvae (Experiments 1-3). The vertical bars represent the 90% confidence intervals.

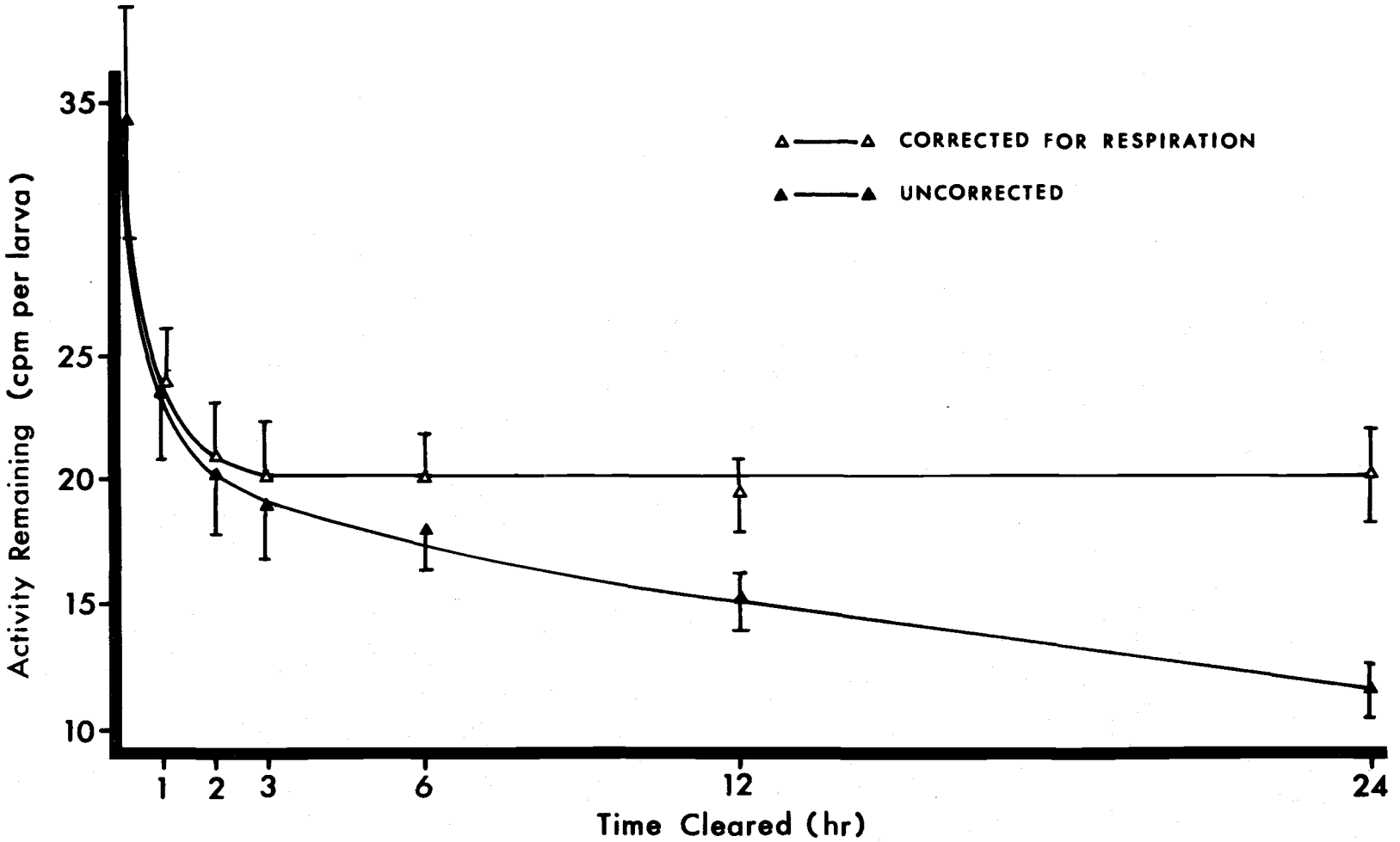

Figure 4. Clearing rates of small oyster larvae fed algal cells (Experiment 4). Rates of respiration equal to 8.79 cpm larva^{-1} day^{-1} (measured for the 24 hour clearing period only) were used to correct for that part of the decrease in activity attributable to respiration. Vertical bars represent the 90% confidence intervals.

"fix" the absorbed DOM (Figure 5). Further decreases in activity can again be explained by respiratory losses.

At this point, I switched from the 4 hour incorporation period (Experiments 1-3) to a 24 hour incorporation period and omitted the clearing step. The overestimation of incorporation resulting from omitting a clearing step in a 24 hour experiment is less than 5%. I base this estimate on a consumption rate of 480 cells larva^{-1} day^{-1} with 22 cells eliminated per larva during the clearing period.

In addition to eliminating the necessity for clearing the larvae, the 24 hour experiments with a longer incorporation time permit larvae to become acclimated to the culture conditions. If the food is toxic, the effects will become evident during the longer period. Biochemical and physiological systems have more time to adapt to the physical and chemical nature of the food. A 24 hour experiment is less apt to overestimate consumption or incorporation due to a high initial food uptake. For all of these reasons, the remainder of the experiments were 24 hours in duration with no clearing period.

The relationships between concentration of food (algal cells and DOM) and the rate of incorporation and the rate of respiration by small larvae were determined in Experiment 6 (Figure 6 and Appendix Table I). The rate of incorporation of algal cells is proportional to algal concentration below approximately 0.2 mgC per liter. Above 0.5 mgC per liter a large increase in the concentration of algal cells

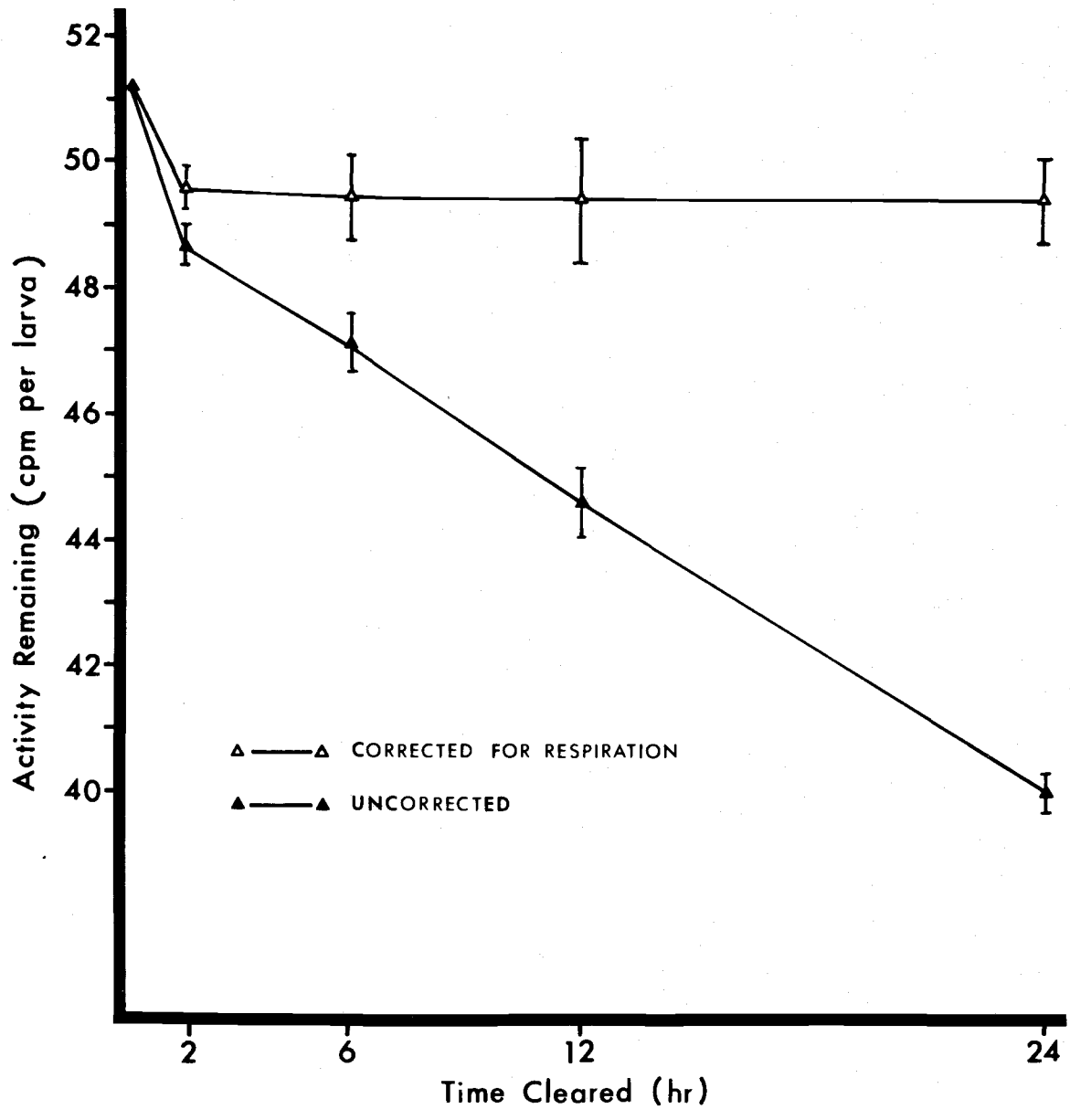

Figure 5. Clearing rates of small oyster larvae fed DOM (Experiment 5). Rates of respiration equal to 9.3 cpm larva^{-1} day^{-1} (measured for the 24 hour clearing period only) were used to correct for that part of the decrease in activity attributable to respiration. Vertical bars represent the 90% confidence intervals.

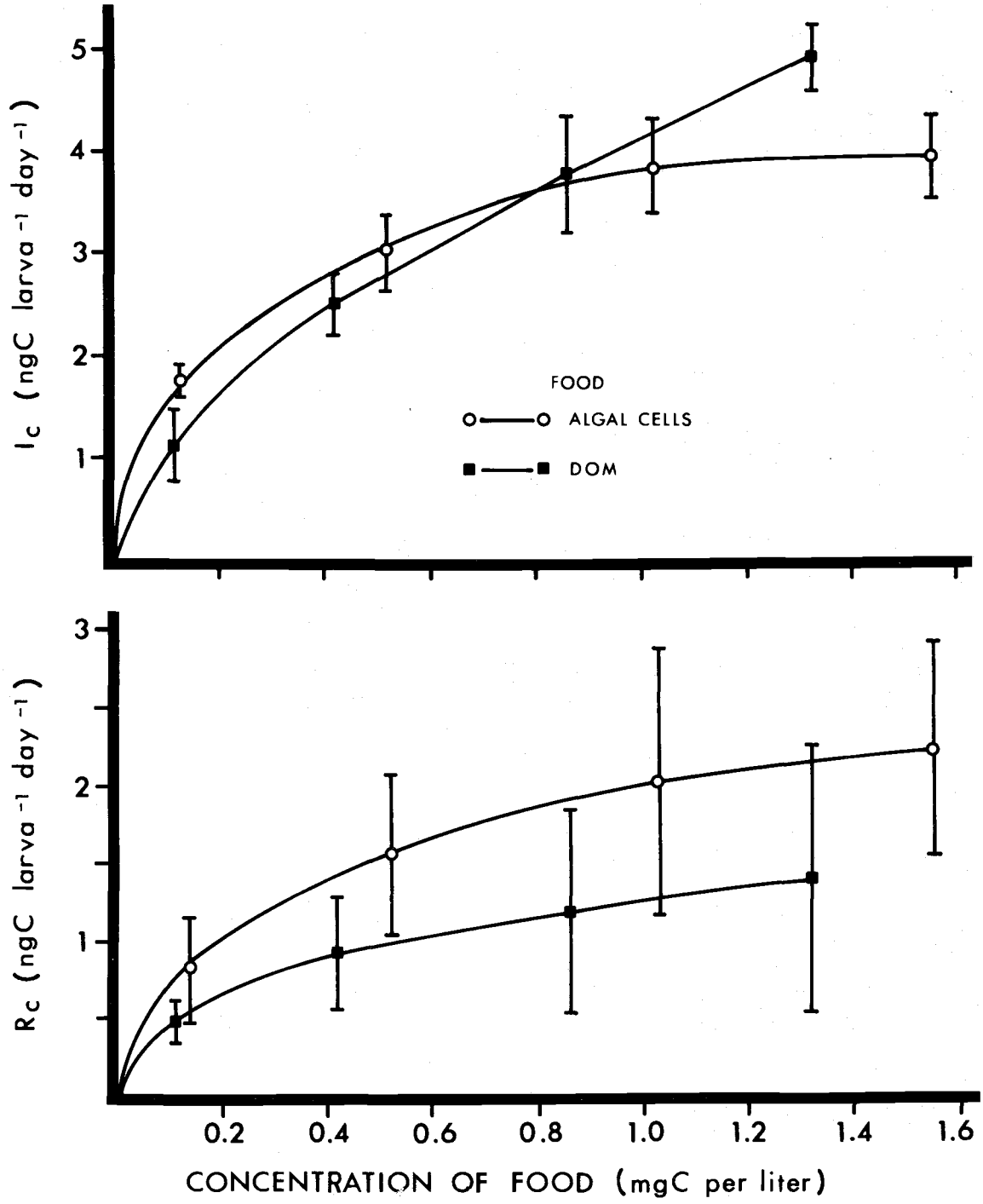

Figure 6. Relationship between concentration of food (algal cells and DOM), the rate of incorporation (I_c) and the rate of respiration (R_c) by small oyster larvae (Experiment 6). Vertical bars represent 90% confidence intervals.

results in only a slight increase in the rate of incorporation of the algal cells. The rate of incorporation of DOM is proportional to DOM concentration below 1.3 mgC per liter (the highest concentration of DOM fed to the larvae). The rate of respiration of the larvae fed both types of food increases with increasing food concentration, although the increase in the rate of respiration is small at food concentrations above 1.0 mgC per liter.

The relationships between food concentration (algal cells, DOM and organic aggregates) and the rate of incorporation of the foods by small oyster larvae at 24°C and at 14°C (Experiment 7) are shown in Figure 7 and Appendix Table II. At 24°C the rates of incorporation by larvae fed algal cells is similar to that observed in Experiment 6 except that the maximum rate of incorporation in Experiment 7 is attained at a lower algal concentration (about 0.7 mgC per liter). At low concentrations of DOM the rate of incorporation increases in proportion to food concentration but at a rate considerably lower than for larvae fed algal cells. At concentrations of DOM above 1.5 mgC per liter the rate of incorporation exceeds that of larvae fed algal cells. At low food concentrations rate of incorporation by larvae fed organic aggregates is intermediate between the rate of incorporation of algal cells and DOM. Above 0.8 mgC per liter, however, larvae fed organic aggregates have the highest rates of incorporation. The incorporation rates at 14°C follow a pattern similar to that at 24°C

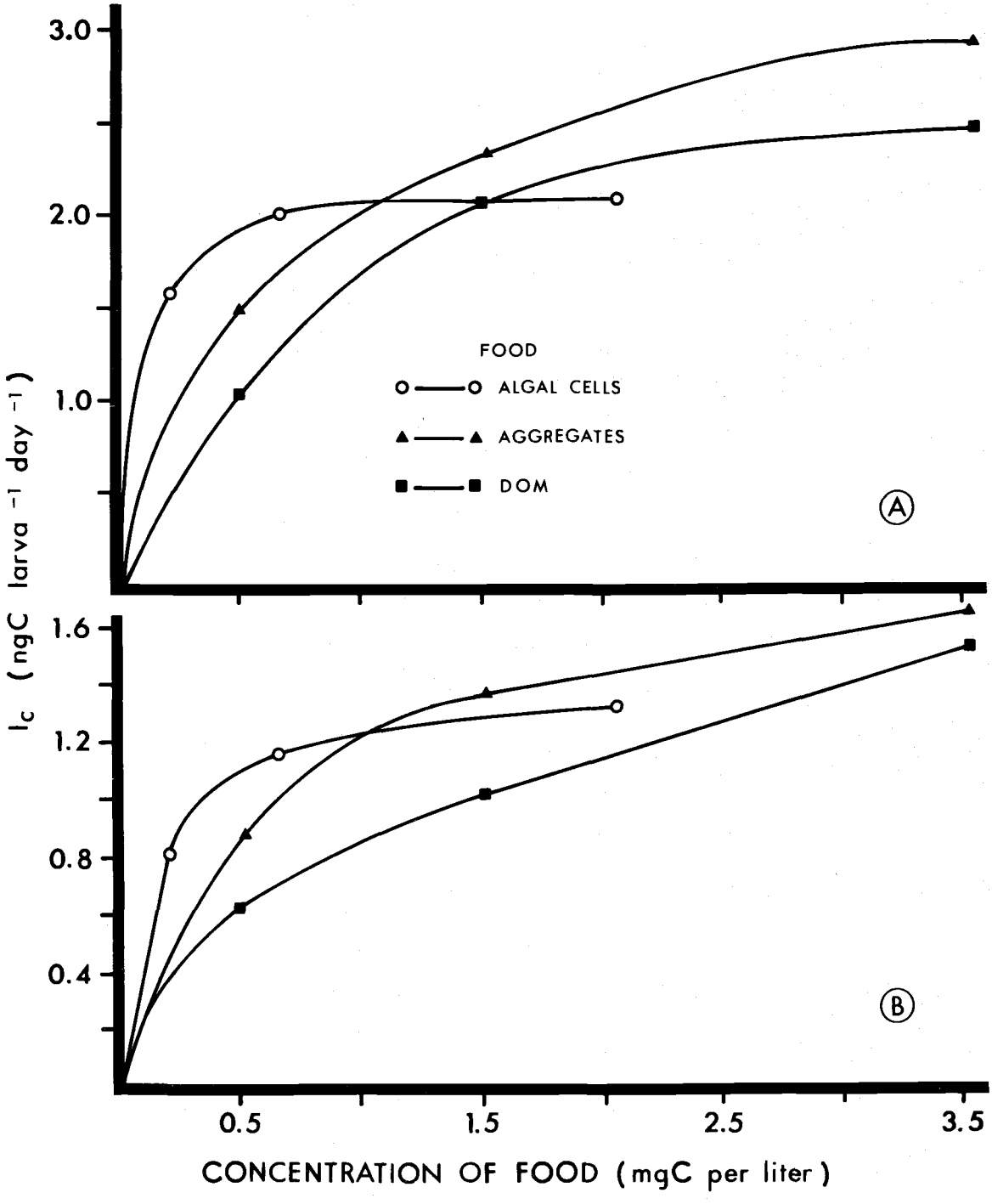

Figure 7. Relationships between concentration of food (algal cells, DOM and organic aggregates) and the rate of incorporation (I_c) by small oyster larvae (Experiment 7) at 24°C (A) and at 14°C (B).

except that the rates are reduced by about 58%

The relationships between the concentration of food (algal cells, DOM and organic aggregates) and the rates of incorporation and respiration by small oyster larvae (Experiment 8) are shown in Figure 8 and Appendix Table III. The rate of incorporation exhibited by larvae fed algal cells or DOM follows a pattern similar to that in previous experiments. At low concentrations of carbon, algal cells are incorporated at the higher rate but at concentrations above 0.6 mgC per liter DOM is incorporated at the higher rate. DOM, at a concentration of 2.0 mgC per liter, is incorporated at almost twice the rate at which algal cells are incorporated. Larvae incorporated organic aggregates at rates higher than they incorporated algal cells at all concentrations of carbon tested. At concentrations above 0.75 mgC per liter little change in incorporation rate occurs with increases in the concentration of the aggregates. The rates of respiration are about the same for the three food types and represent a substantial portion of the assimilation rate.

The rates of incorporation and respiration observed in Experiment 9 (Figure 9 and Appendix Table IV) were similar to those observed in Experiments 6-8. The rate of respiration of the larvae fed DOM was considerably lower than that of larvae fed DOM in the previous experiments.

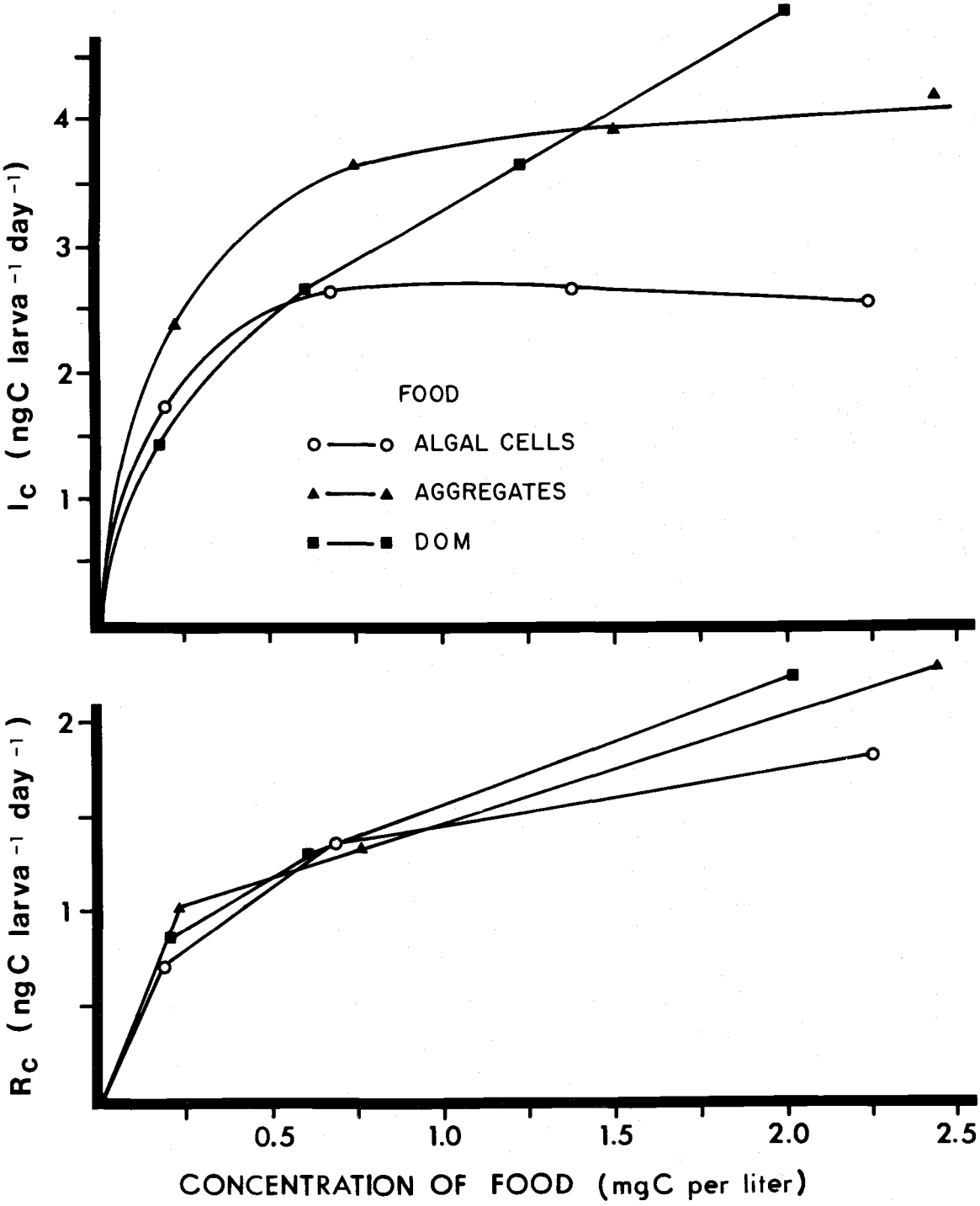

Figure 8. Relationships between concentration of food (algal cells, DOM, and organic aggregates), the rate of incorporation (I_C) and the rate of respiration (R_C) by small oyster larvae (Experiment 8).

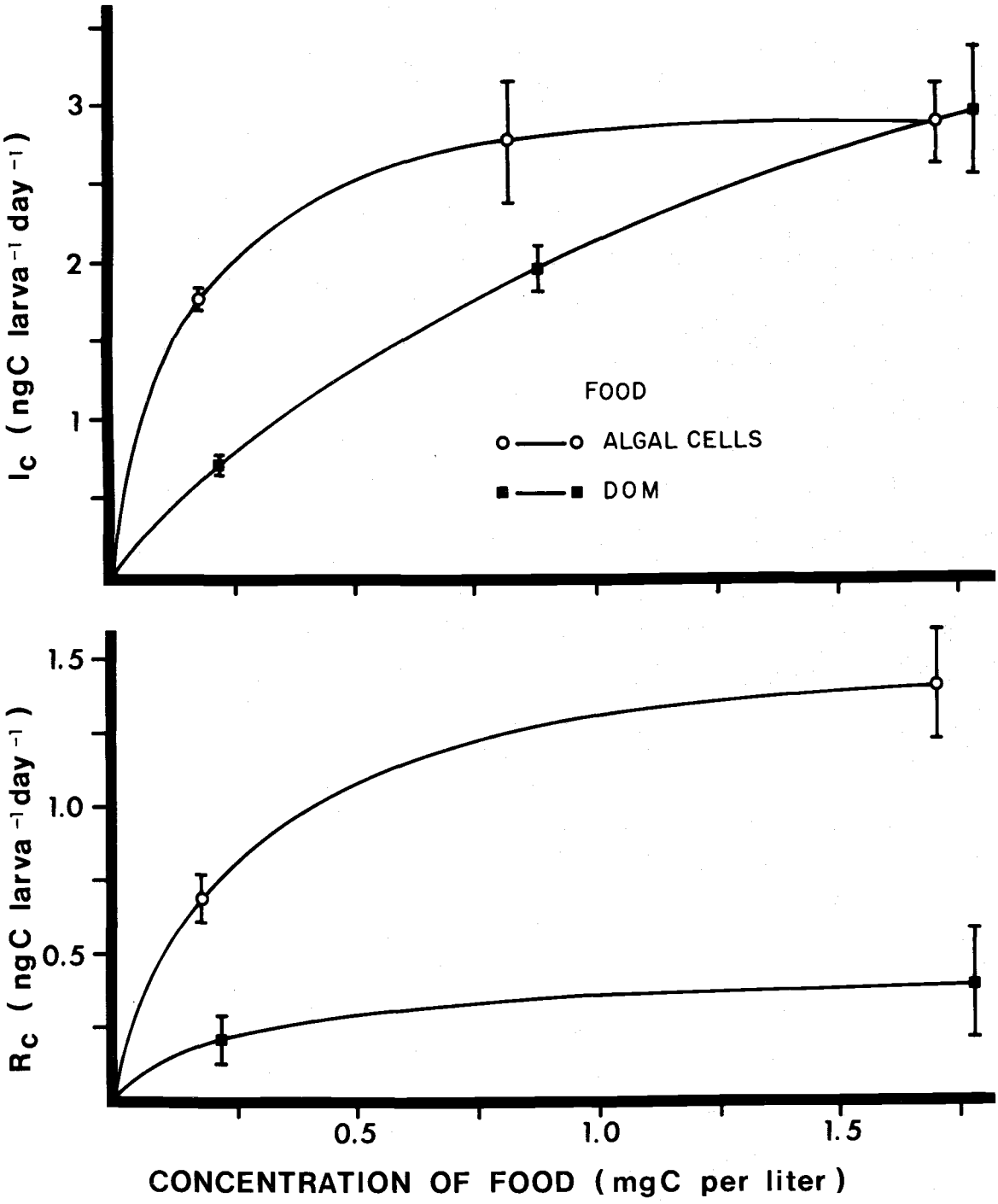

Figure 9. Relationships between concentration of food (algal cells and DOM), the rate of incorporation (I_c) and the rate of respiration (R_c) by small oyster larvae (Experiment 9). Vertical bars represent 90% confidence intervals.

In Experiment 10, in the beakers lacking antibiotics, the rates of incorporation of the larvae fed algal cells and the larvae fed DOM were similar to those observed in previous experiments (Table 3). In beakers containing antibiotics the rates of incorporation increased in each case, but doubling the concentration of antibiotic decreased the rate of incorporation. This suggests that the larvae were taking up the labeled DOM directly rather than taking up bacteria which had incorporated the label. If they had been utilizing bacteria the addition of antibiotics should have lowered the rate of incorporation of label by the larvae.

The incorporation of labeled inorganic carbon by the larvae was also measured in Experiment 10. This incorporation was insignificant.

In Experiment 11 oyster larvae 119 μm in length were used to determine the relationships between concentrations of food (algal cells, DOM and organic aggregates) and the rate of incorporation (Figure 10 and Appendix Table V). The concentration of algae remaining in the beakers at the termination of the experiment indicated that the larvae had removed 75% of the cells. In previous experiments the larvae had removed less than 8% of the algal cells. To correct for this change in the concentration of algal cells during the experiment the exponential mean concentration is used in Figure 10 and Appendix Table V.

Table 3. Results of Experiment 10. The rates of incorporation and respiration are reported as the mean ± 1 standard deviation (4 replicates). Beakers designated "A" contain 33 mg liter^{-1} chloramphenicol, 50 mg liter^{-1} streptomycin and 50,000 IU liter^{-1} penicillin G. Beakers designated "AA" contain twice the concentration of antibiotics as "A".

Food Type	Food Density mgC liter^{-1}	Rate of Incorporation (D) cpm larva^{-1} day^{-1}	Rate of Incorporation (I_c) ngC larva^{-1} day^{-1}	Rate of Incorporation (I_a) cells larva^{-1} day^{-1}	Rate of Respiration (R) cpm larva^{-1} day^{-1}	Rate of Respiration (R_c) ngC larva^{-1} day^{-1}	Rate of Respiration (R_a) cells larva^{-1} day^{-1}
Cells	1.42[a]	21.3 ± 0.5	2.50 ± 0.06	308 ± 7	13.3 ± 2.3	1.56 ± 0.41	192.8 ± 33.4
Cells A	1.42	30.4 ± 1.4	3.57 ± 0.16	440 ± 20	8.6 ± 2.0	1.01 ± 0.35	124.7 ± 29.0
Cells AA	1.42	25.5 ± 2.6	2.99 ± 0.31	369 ± 38			
DOM	0.98	15.0 ± 0.5	1.84 ± 0.06		9.4 ± 2.1	1.15 ± 0.26	
DOM A	0.98	16.8 ± 0.4	2.06 ± 0.05		3.0 ± 1.8	0.37 ± 0.22	
DOM AA	0.98	13.5 ± 0.7	1.65 ± 0.09				
$NaH^{14}CO_3$	(10^5 cpm)	0.07 ± 0.05					
$NaH^{14}CO_3$	(10^7 cpm)	0.04 ± 0.03					

[a] Corresponds to 175 algal cells per μl.

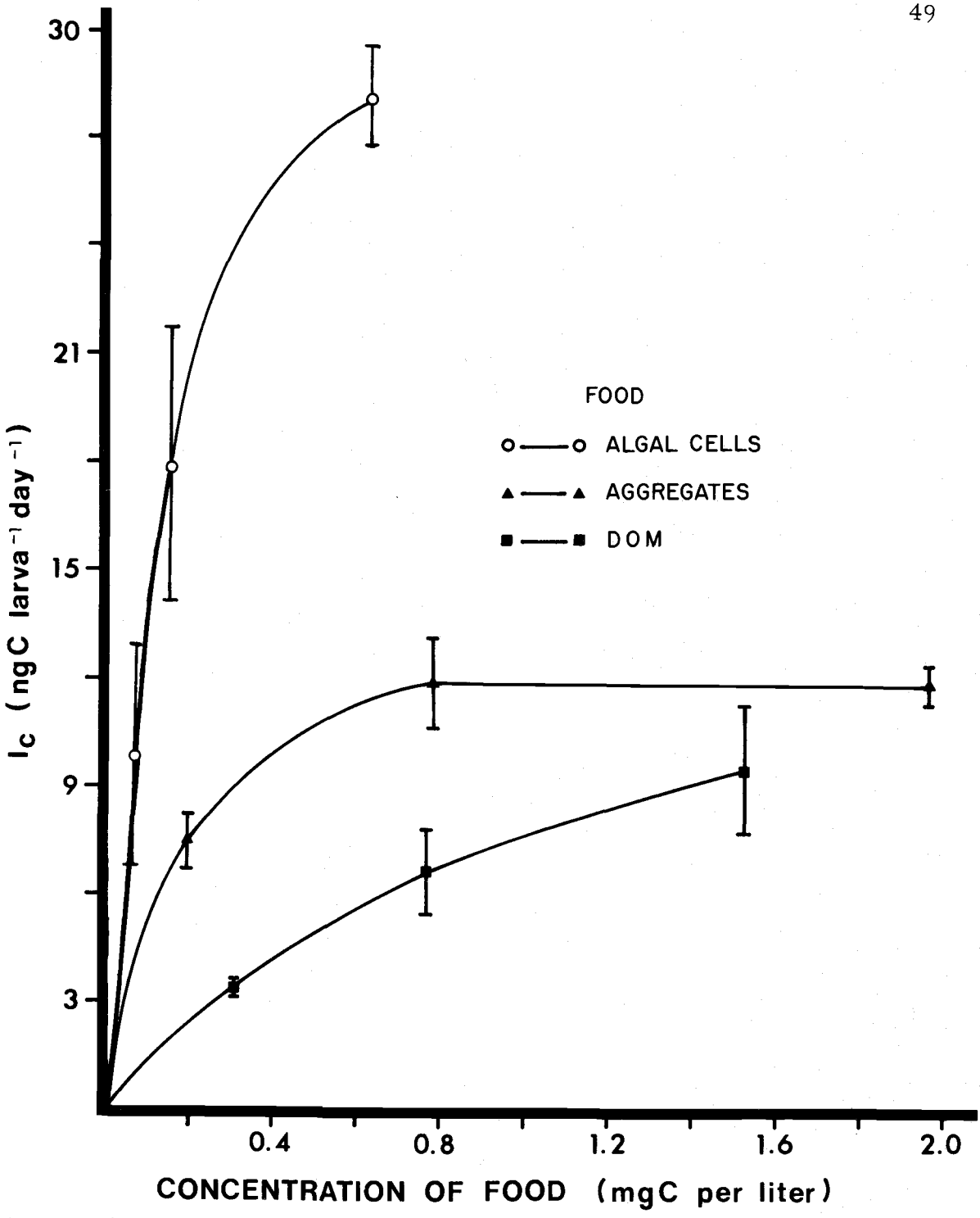

Figure 10. Relationships between concentration of food (algal cells, DOM and organic aggregates) and the rate of incorporation (I_c) by oyster larvae 119 μm in length (Experiment 11). Vertical bars represent the 90% confidence intervals.

The rate of incorporation of algal cells by these larger larvae is at least ten times the rate by small oyster larvae. The maximum rate of incorporation of algal cells by the large larvae was not attained at the highest algal density tested. The rate of incorporation of DOM is proportional to its concentration up to 1.5 mgC per liter. The rate of incorporation of organic aggregates, at low concentrations, approximates the rate of incorporation of algal cells; however, above 0.8 mgC per liter the rate does not increase with increases in the concentration of food.

Oyster larvae 139 μm in length were used in Experiment 12. Figure 11 (and Appendix Table VI) shows the rates of incorporation of algal cells, DOM (0.45 μm and 0.05 μm filtered) and organic aggregates by these larvae.

The rate of incorporation of the algal cells follows a pattern similar to that described under Experiment 11. The rate for the organic aggregates is comparatively high at concentrations of carbon below 1 mgC per liter but increases only slightly at higher concentrations of carbon. Compared to the rate of incorporation of algal cells, the rate of incorporation of DOM is very low at concentrations of carbon below 1 mgC per liter. The rate of incorporation of the DOM continues to increase with increases in the concentration of carbon. At DOM concentrations of 20 mgC per liter rates of incorporation equals the maximum rates of incorporation of algal cells.

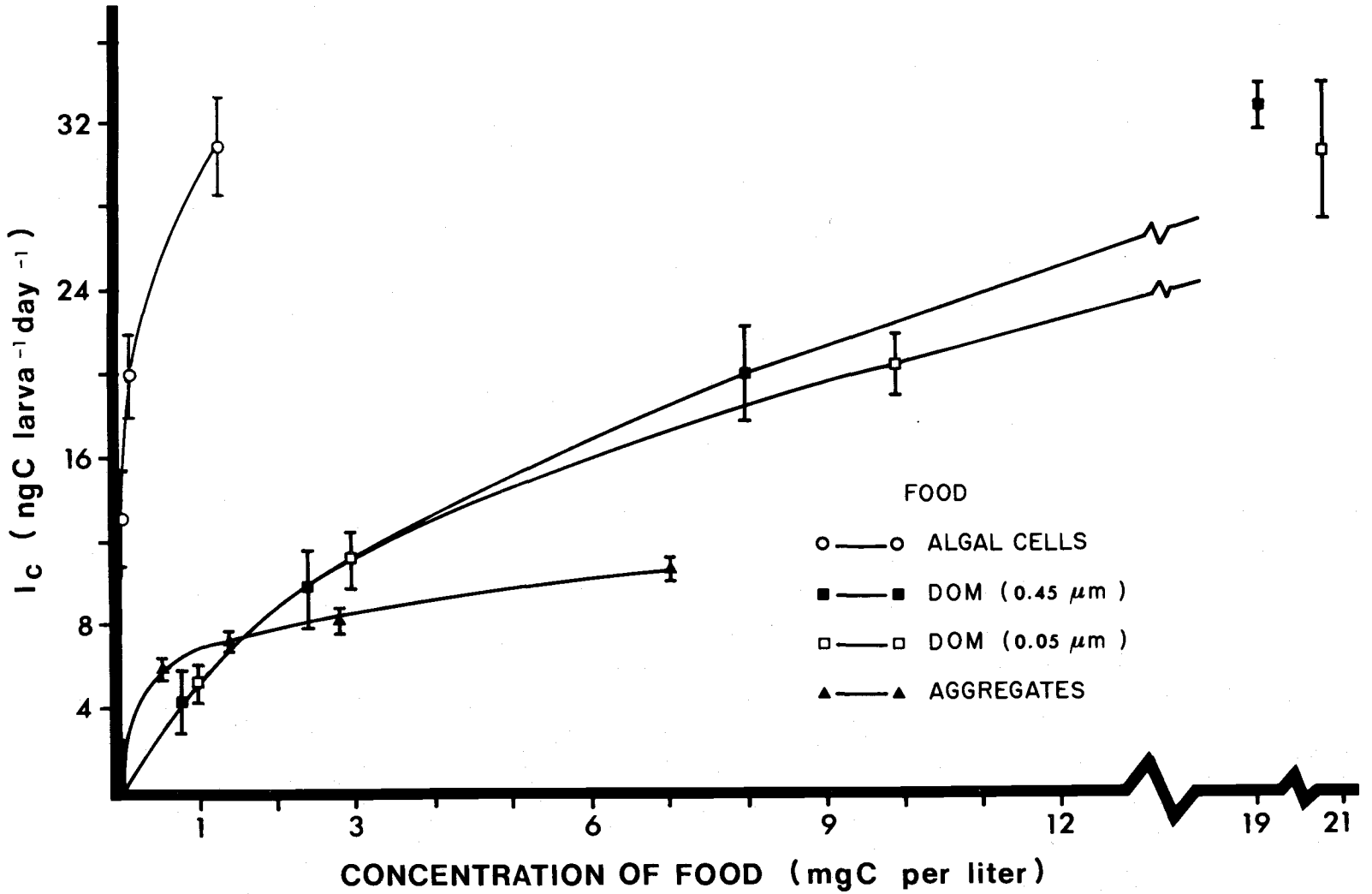

Figure 11. Relationships between concentration of food (algal cells, 0.45 μm filtered DOM, 0.05 μm filtered DOM, and organic aggregates) and the rate of incorporation (I_c) by oyster larvae 139 μm in length (Experiment 12). Vertical bars represent 90% confidence intervals.

Oyster larvae 85 μm in length were used in Experiment 13 (Figure 12, Appendix Table VII) to measure rates of incorporation of algal cells, DOM (0.45 μm and 0.05 μm filtered) and organic aggregates by these small oyster larvae. The rate of incorporation of the algal cells is similar to the rates measured in Experiments 6-10. The rate of incorporation of the aggregates increases with increases in concentration up to a concentration of carbon of about 3 mgC per liter. The rate of incorporation of the DOM continues to increase with increases in concentration. At a concentration of about 20 mgC per liter the rate of incorporation of DOM is about five times the maximum rate at which the small larvae incorporate algal cells.

Less than 0.2% of the DOM used in Experiments 12 and 13 formed particles during the experiment (either by bacterial action or spontaneously) large enough to be filtered out on a 0.45 μm membrane filter (Table 4).

The carbon and nitrogen content of the larvae used in Experiments 12 and 13 was determined and values (Table 5) were used in computing the index of incorporation in Appendix Tables VI and VII.

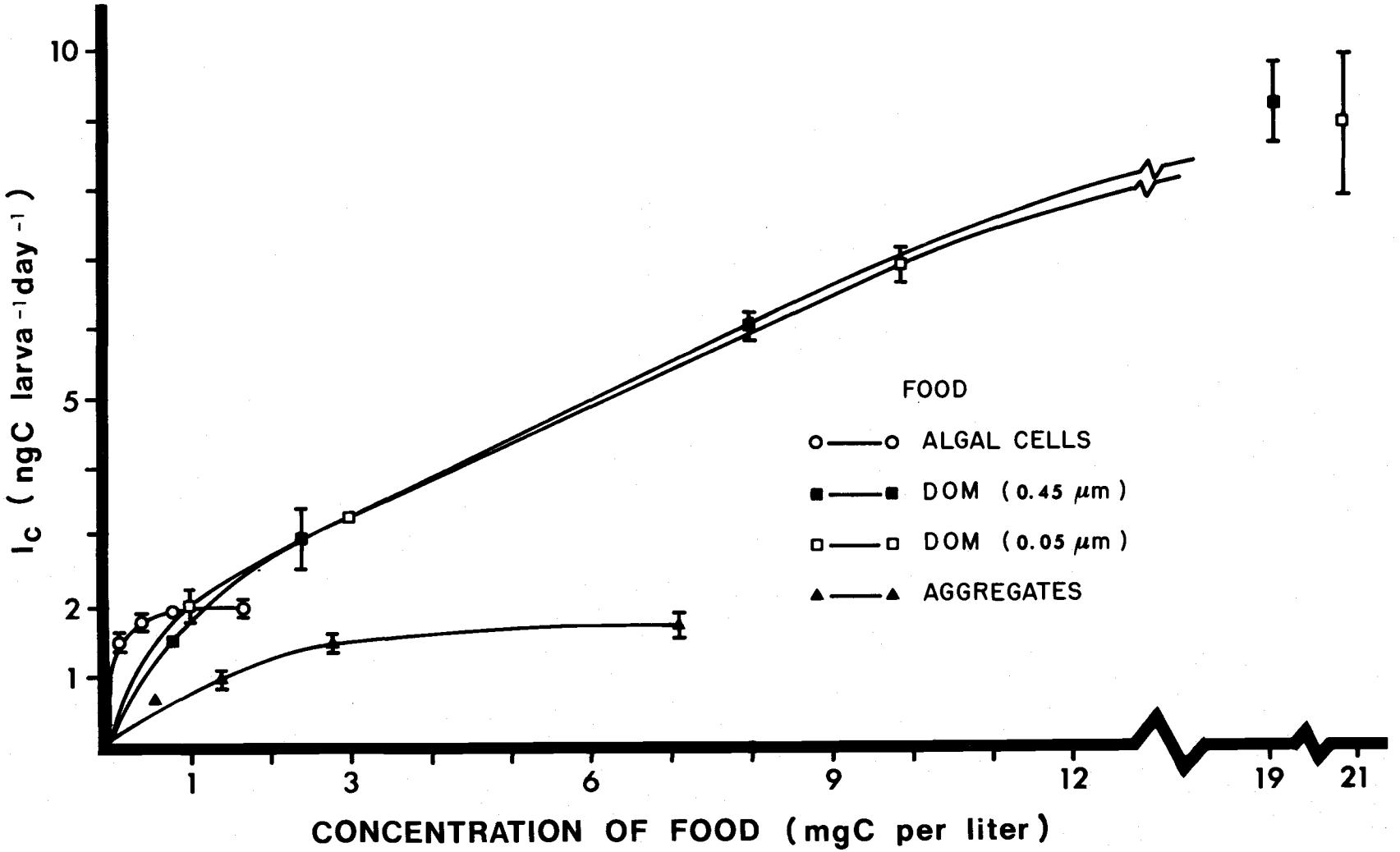

Figure 12. Relationships between concentration of food (algal cells, 0.45 μm filtered DOM, 0.05 μm filtered DOM, and organic aggregates) and the rate of incorporation (I_C) by small oyster larvae (Experiment 13). Vertical bars represent the 90% confidence intervals.

Table 4. Formation of particles from DOM. Retention of ^{14}C on a 0.45 μm membrane filter is used as an indicator of the spontaneous formation of particles from DOM. The % retention is the percentage of the DOM that forms aggregates large enough to be trapped on the filter.

Food Type	Food Density		Retention	% Retention
	mgC liter^{-1}	cpm x 10^{6} beaker^{-1}	cpm x 10^{3} beaker^{-1}	
DOM (0.05 μm)	9.92	5.45	4.95 ± 0.89[a]	0.09
DOM (0.05 μm)	23.79	13.08	17.00 ± 2.38	0.13
DOM (0.45 μm)	7.78	5.72	6.29 ± 0.99	0.11
DOM (0.45 μm)	19.15	13.72	20.58 ± 4.34	0.15

[a]Mean ± 1 standard deviation (4 replicates)

Table 5. Carbon and nitrogen content of oyster larvae. Values are reported as the mean ± 1 standard deviation (4 replicates).

Larval Length	Total Carbon	Organic Carbon	Nitrogen (before acidification)	Nitrogen (after acidification)
μm	ngC larva^{-1}	ngC larva^{-1}	ngN larva^{-1}	ngN larva^{-1}
85.3	37.8 ± 0.9	21.5 ± 0.8	3.6 ± 0.0	3.6 ± 0.1
139.2	181.7 ± 8.4	98.5 ± 2.7	17.0 ± 0.4	17.4 ± 0.6

DISCUSSION

Incorporation by Small Larvae

Figure 13 is a composite of the rates of incorporation of algal cells by small larvae in several of the 24 hour experiments. There is a direct relationship between the concentration of algal food and rates of incorporation for larvae of approximately the same size and age. The rate of incorporation is proportional to food density up to approximately 0.15 mg algal carbon per liter (about 14 Pseudo-isochrysis cells per µl), while densities greater than 0.6 mg algal carbon per liter (about 56 Pseudoisochrysis cells per µl) cause only a slight increase in the rate of incorporation. At a concentration of approximately 0.23 mg algal carbon per liter (about 22 Pseudo-isochrysis cells per µl), 80% of the maximum rate of incorporation is obtained. A slight decrease in the rate of incorporation is sometimes observed at high algal densities (Experiments 1 and 8). This inhibition may be the result of increased metabolic losses at high food densities or the result of toxicity to the larvae caused directly or indirectly by the high algal density.

At concentrations of food below 0.7 mgC liter^{-1}, small larvae incorporate DOM at a much lower rate than they incorporate algal cells. When the food concentration is raised to 0.8-1.8 mgC liter^{-1}, however, small larvae incorporate DOM at about the same rate as

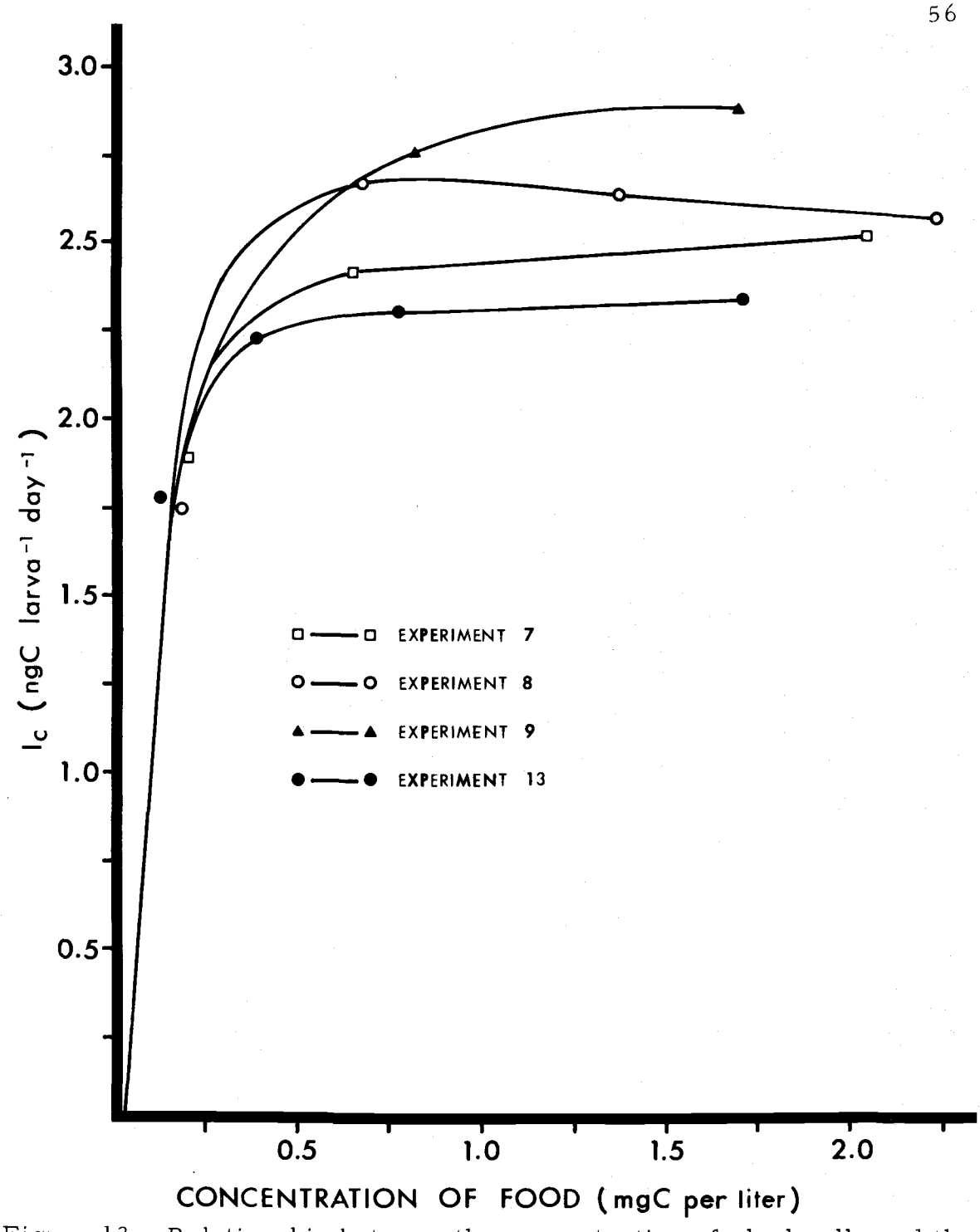

Figure 13. Relationship between the concentration of algal cells and the rate of incorporation by small oyster larvae (Experiments 7, 8, 9, and 13). Experiments 7 and 13 were performed at 24°C while Experiments 8 and 9 were performed at 26°C. To correct for the temperature difference, the rates of incorporation in Experiments 7 and 13 were multiplied by 1.2.

they incorporate algal cells. At food concentrations above 1.8 mgC liter^{-1}, small larvae incorporate DOM at rates considerably greater than they incorporate algal cells. For example, in Experiment 13 (Figure 12), at a DOM concentration of 19 mgC per liter, larvae incorporated DOM at a rate 4.7 times the maximum rate at which they incorporated algal cells. The 0.45 μm filtered and the 0.05 μm filtered DOM were incorporated at approximately the same rates. The organic carbon content of the natural extracellular products used in this experiment was so low that it was not possible to compare its rate of incorporation with the other foods.

Compared to rates of incorporation of algal cells and DOM, the organic aggregates showed the least consistent rates of incorporation. In Experiment 7 (Figure 7), the rate of incorporation by small larvae fed on the aggregates followed a pattern similar to that of larvae fed on DOM although the rates of incorporation were somewhat higher for the aggregates. Since these aggregates were not separated from the soluble organic matter the larvae were able to incorporate carbon from both dissolved and particulate organic matter. The rate of incorporation by larvae fed on the aggregates in Experiment 8 (Figure 8) is much greater than that of larvae fed on algal cells. These aggregates were formed by precipitation of the DOM with $CaCO_3$ resulting in particles 1-8 μm in diameter. The increase in rate of incorporation of aggregates over that of algal cells is puzzling.

Perhaps only certain compounds, such as proteins, were precipitated by the $CaCO_3$ yielding a particle which can be assimilated with greater efficiency than algal cells. In Experiment 11, in which the C:N ratio of organic aggregates (formed from DOM) was determined, the ratio was much lower than the C:N ratio of the algal cells.

The nonliving particulate food fed in Experiment 13 was not produced from DOM as in previous experiments. Instead it consisted of algal cell fragments from which the protein had been previously hydrolyzed by enzymatic action. The C:N ratio of these particles (Table 2) indicates that they contained a low percentage of protein. Only at food densities of 7 mgC per liter were these particles incorporated by small larvae at a rate comparable to that of algal cells (Figure 13).

Generally at concentrations of food below 1 mgC per liter small larvae incorporate algal cells at higher rates than they incorporate DOM or aggregates. At higher concentrations of food (above 2 mgC per liter) small larvae incorporate DOM at higher rates than they incorporate algal cells. Small larvae incorporate a maximum of 9.1% of their body weight each day when fed algal cells but incorporate up to 42.6% of their body weight each day when fed DOM (Appendix Table VII.

Incorporation by Larger Larvae

The larger larvae removed more cells from suspension than I had anticipated. As a result all of the algal densities I used were below the optimum for maximum incorporation. Nevertheless, it has been determined that the rate at which oyster larvae 119-139 μm in length incorporate algal cells is substantially greater than that by 80-90 μm larvae. For example, in Experiments 12 and 13, 85.3 μm and 139 μm larvae were fed the same densities of algal food. The maximum rate of incorporation by the 85.3 μm larvae was 1.95 ngC larva^{-1} day^{-1}, while the maximum rate of incorporation by the larger (139 μm) larvae was at least 30.99 ngC larva^{-1} day^{-1}.

Although the 139 μm larvae were less than 5 times larger (in terms of organic carbon content) than the small larvae they incorporated at least 15 times as many algal cells per day. The 139 μm larvae incorporated over 31% of their body weight each day. Walne (1965) calculated that Ostrea edulis assimilates from 13% (260 μm larvae) to 25% (170 μm larvae) of its body weight each day (based on carbon assimilation).

The rate of incorporation of DOM by the 139 μm larvae is approximately 3.5 times greater than that of the small larvae although the large larvae are 5 times larger (in terms of organic carbon).

Respiration of Assimilated Food

From the rates of respiration measured in Experiments 6, 8 and 9, it is estimated that between 28% and 41% of the algal cells consumed in 24 hours are respired during that period (Appendix Tables I, III and IV). In the same experiments it is estimated that between 11% and 37% of the DOM absorbed in 24 hours and between 27% and 35% of the aggregates incorporated in 24 hours is respired during that period. Respiration of labeled food begins 16-24 hours after initial consumption in Daphnia (Schindler 1968). My data indicate that respiration begins within 4 hours after consumption in oyster larvae.

A greater proportion of the assimilated food is respired at high concentrations of particles (algal or aggregate) than at low concentrations. The inverse relationship is seen for DOM. A smaller proportion of the absorbed food is respired at high DOM concentrations than at low concentrations. Perhaps the larvae are forced to expend a large amount of energy to process high densities of algae while the energy they expend during the assimilation of DOM does not increase proportionately at high DOM concentrations.

The differences in the mechanisms for uptake of particulate and soluble materials by the larvae support this explanation. Veliger larvae feed on small suspended particles that cilia of the velum collect

and direct to ciliary tracts leading to the mouth. The energy expended in collecting and digesting the particles increases with the number of particles processed. At high particle concentrations the larvae may (1) stop feeding, (2) reject excess particles as pseudofaeces and/or (3) pass much of the material through the gut without digesting it. Whichever occurs, the larvae waste some energy in handling high concentrations of particles. Bamford and Gingles (1974) suggest that glucose and galactose are taken up by active absorption at low substrate concentrations and by passive absorption at high concentrations. Amino acids may also be taken up at low substrate concentrations by active absorption involving several carriers. Stevens (1968) has calculated that the energy requirements for such uptake would be only a small percentage of that made available to the animal by this process. Based on these mechanisms for uptake it would be expected that a greater amount of energy is required to take up particulate food than soluble food at least at high substrate concentrations.

Incorporation: Meaning and Significance

The study, reported herein, was the second phase of a continuing investigation under the direction of Dr. H. K. Phinney to define the nutritional requirements of larval and adult bivalves. Specifically I have investigated the physical nature of the food required by larval oysters. I have demonstrated that oyster larvae can take up, or consume, and incorporate dissolved organic matter and nonliving

particulate organic matter. These foods are incorporated at a rate equal to and sometimes surpassing that of living algal food. Up to 37% of the assimilated DOM and up to 35% of the organic aggregates are respired during the first 24 hours. This implies that much of this food is entering the metabolic pathways.

To relate the significance of these findings to the nutrition of larval oysters it is necessary to define some bioenergetic terms. These terms, slightly modified from those used by IBP (Crisp 1971) are defined as follows:

Consumption - Total intake of food or energy.

Rejecta - That portion of the consumption that is voided as faeces, excretion, or leakage.

Assimilation - That part of the consumption that is utilized for physiological purposes, namely for production and respiration, but excluding rejecta.

Respiration - That part of the assimilated energy that is converted into heat either directly or through mechanical work performed by the organism.

Production (Growth) - That part of the assimilated food or energy that is retained in the biomass of the organism.

Respiration, as defined above, is essentially equivalent to respiration as I measured it. My incorporation values (minus about 5% for the inclusion of some rejecta in my experiments) are

essentially equivalent to production. Assimilation is then equal to incorporation + respiration. The problem with these bioenergetic definitions is that they pertain only to the consumed food. Respiration, leakage, and excretion of previously assimilated (unlabeled) organic matter are not included in these definitions. As a result, it is difficult to determine from my data the net production or growth which occurred during my experiments.

In order to relate incorporation and growth it is necessary to make the assumption that the amount of unlabeled material lost via excretion, leakage, and respiration is less than the amount of material incorporated by the larvae during the experiment. To determine this, I included in my experiments some beakers of larvae which were fed unlabeled Isochrysis or Pseudoisochrysis. The concentration of algal cells was maintained at about 0.7 mgC per liter. In each experiment the larvae had grown 18-32 μm in length by the end of five days. The fact that the larvae grew on the algal cells demonstrates that the amount of food assimilated exceeded losses due to excretion, respiration and leakage. Therefore, at least some of the algal food incorporated in the radiotracer experiments is, in all probability, utilized for growth.

There is little reason to suggest that losses due to excretion, leakage or respiration of previously assimilated foods would necessarily be greater for larvae fed DOM or aggregates than for larvae fed

algal cells. Stephens (1972) has shown that the rates of leakage and/or excretion increase at high DOM concentrations but only at concentrations at least 50 times greater than those used in my experiments. If growth occurs in larvae fed on a diet of algal cells, then it seems to me that larvae fed on DOM or organic aggregates, which I have shown are incorporated at rates equal to or exceeding the incorporation rate of algal cells, would also grow. In order for this growth to be sustained on a particular diet, however, it is essential that: (1) all essential nutrients be present in that diet and (2) that the larvae are capable of growth, i.e., they are healthy.

Practical Applications

In the next phase in this investigation I will compare the growth of oyster larvae fed algal cells, DOM or nonliving particulate matter (and combinations thereof) using long term feeding studies. Under the conditions of the present study, the highest rates of incorporation by young larvae were obtained using DOM. As a result I suggest that DOM might improve the growth rate of young bivalve larvae.

The growth rate of hatchery reared Crassostrea gigas larvae is often very slow during the first 6 days. By feeding the larvae DOM either along or in combination with particulate food the growth rate may be improved. I suggest that the DOM initially be prepared from several species of algae known to produce good larval growth. DOM

can be prepared quite easily either by cell disruption or by enzymatic hydrolysis. Although it is difficult to tell from only one experiment, it appears that DOM produced by enzymatic hydrolysis is incorporated at a higher rate than DOM produced by cell disruption. It would probably be necessary to change water every other day or to use antibiotics to control the growth of detrimental microorganisms in larval cultures fed on DOM. My results indicate that the larvae might be able to utilize algal cells efficiently when they reach a length of approximately 120 μm. At this time they could be reared on algal cells alone or in combination with DOM or particulate organic matter.

Organic aggregates offer another potential food for oyster larvae. My results suggest the particles incorporated at the highest rate are those having a low C:N ratio and a diameter of about 2-8 μm. The results of Experiments 11-13 suggest that larvae can utilize particulate matter less than 2μm in diameter, or even colloidal particles. In other words they may be able to utilize all sizes of organic matter from a diameter of about 8 μm down to and including dissolved organic matter.

The use of organic aggregates could present problems in the hatchery, however. The surface active DOM in natural seawater, which forms organic aggregates, has also been shown to concentrate heavy metals (Duce et al. 1972). This problem could probably be avoided in the hatchery by using aggregates produced from algal

products or other sources of organic matter. Another problem with the use of nonliving particulate matter is microbial contamination occurring when unused food settles out. Small particles (less than about 4 μm in diameter) can be kept in suspension quite easily. Specially designed tanks may be necessary in order to rear larvae on nonliving particulate matter greater than 4μm in diameter.

The fact that living phytoplankton have been shown to be a good diet for larval oysters in oyster hatcheries does not necessarily mean that the larvae are obtaining all of their nutrition from the algal cells. The larvae could be obtaining nutritive substances from the algal extracellular products. Davis and Calabrese (1964) found that larvae had a much higher growth rate at a temperature (30°C) at which cells of Isochrysis galbana (and probably other algal species) lyse, releasing their contents into the seawater. The resultant dissolved, colloidal and particulate matter could possibly be utilized at a higher rate than living algal cells.

Walne (1965) pointed out that densities of phytoplankton sufficient to account for the high growth rates of larvae in nature have not been observed in the field. Larvae frequently grow faster in the field than they do under "optimum" laboratory culture conditions. For example, Crassostrea gigas larvae grow to setting size in as little as 10 days in the field. In the laboratory, larvae occasionally start setting in about 12 days but 20-25 days is more typical. The results of my

investigation suggest that DOM and nonliving particulate matter are a source of nutrition for oyster larvae and may be an important supplement to the nutrition obtained from living algal cells.

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APPENDIX

Appendix Table I. Results of Experiment 6. The rates of incorporation and respiration at 26°C by larvae 88 μm in length are reported as the mean ± 1 standard deviation. There are 4 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density mgC liter^{-1}	Rate of Incorporation (D) cpm larva^{-1} day^{-1}	Rate of Incorporation (I_c) ngC larva^{-1} day^{-1}	Rate of Incorporation (I_a) cells larva^{-1} day^{-1}	Rate of Respiration (R) cpm larva^{-1} day^{-1}	Rate of Respiration (R_c) ngC larva^{-1} day^{-1}	Rate of Respiration (R_a) cells larva^{-1} day^{-1}	$\%R_c$ %
Cells	0.13[a]	26.94 ± 2.51	1.76 ± 0.16	118.5 ± 11.0	12.42 ± 3.42	0.81 ± 0.21	54.6 ± 14.3	31.5
Cells	0.52[a]	46.08 ± 6.04	3.00 ± 0.39	202.8 ± 26.6	23.76 ± 5.28	1.55 ± 0.34	104.5 ± 23.2	34.1
Cells	1.03[a]	58.86 ± 7.52	3.84 ± 0.49	259.0 ± 33.1	30.96 ± 8.10	2.02 ± 0.53	136.2 ± 35.6	34.5
Cells	1.55[a]	60.30 ± 6.80	3.93 ± 0.44	265.3 ± 29.9	33.84 ± 6.41	2.21 ± 0.42	148.9 ± 28.2	36.0
DOM	0.12	6.96 ± 2.15	1.13 ± 0.35		2.94 ± 0.54	0.48 ± 0.09		29.8
DOM	0.42	15.36 ± 1.88	2.50 ± 0.31		5.58 ± 1.38	0.91 ± 0.22		26.7
DOM	0.86	23.16 ± 3.57	3.77 ± 0.58		7.23 ± 2.46	1.18 ± 0.40		23.8
DOM	1.32	30.12 ± 5.43	4.90 ± 0.88		8.46 ± 3.24	1.38 ± 0.53		22.0

[a]These densities correspond to 8.9, 35.1, 69.6 and 104.8 algal cells per μl, respectively.

Appendix Table II. Results of Experiment 7. The rates of incorporation at 14°C and 26°C by larvae 83 μm in length are reported as the mean ± 1 standard deviation. There are 3 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density	Rate of Incorporation 14°C			24°C		
		(D)	(I_c)	(I_a)	(D)	(I_c)	(I_a)
	mgC liter^{-1}	cpm larva^{-1} day^{-1}	ngC larva^{-1} day^{-1}	cells larva^{-1} day^{-1}	cpm larva^{-1} day^{-1}	ngC larva^{-1} day^{-1}	cells larva^{-1} day^{-1}
Cells	0.20[a]	4.16 ± 0.11	0.81 ± 0.02	49.0 ± 1.2	8.19 ± 0.51	1.58 ± 0.10	117.9 ± 7.3
Cells	0.65[a]	6.00 ± 0.41	1.16 ± 0.08	70.0 ± 4.7	10.36 ± 0.34	2.01 ± 0.06	149.2 ± 4.9
Cells	2.04[a]	6.94 ± 0.43	1.33 ± 0.08	80.5 ± 4.7	10.48 ± 0.71	2.09 ± 0.14	156.1 ± 10.2
DOM	0.50	3.68 ± 0.66	0.61 ± 0.12		6.21 ± 0.40	1.03 ± 0.07	
DOM	1.51	6.13 ± 0.74	1.01 ± 0.12		12.47 ± 0.51	2.07 ± 0.08	
DOM	3.53	9.17 ± 0.55	1.53 ± 0.10		14.93 ± 1.08	2.47 ± 0.18	
AGG	0.50	5.34 ± 0.44	0.88 ± 0.07		9.03 ± 0.61	1.49 ± 0.10	
AGG	1.51	8.25 ± 1.21	1.37 ± 0.19		14.02 ± 0.94	2.33 ± 0.16	
AGG	3.53	9.91 ± 0.82	1.64 ± 0.13		17.76 ± 1.47	2.95 ± 0.25	

[a]These densities correspond to 14.9, 48.5, and 152.2 algal cells per μl, respectively.

Appendix Table III. Results of Experiment 8. The rates of incorporation and respiration at 26°C by larvae 83 μm in length are reported as the mean ± 1 standard deviation. There are 3 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density mgC liter^{-1}	Rate of Incorporation (D) cpm larva^{-1} day^{-1}	Rate of Incorporation (I_c) ngC larva^{-1} day^{-1}	Rate of Incorporation (I_a) cells larva^{-1} day^{-1}	Rate of Respiration (R) cpm larva^{-1} day^{-1}	Rate of Respiration (R_c) ngC larva^{-1} day^{-1}	Rate of Respiration (R_a) cells larva^{-1} day^{-1}	%R_c %
Cells	0.18[a]	15.90 ± 2.02	1.75 ± 0.22	162.2 ± 20.6	6.41 ± 0.41	0.70 ± 0.05	65.3 ± 4.2	28.6
Cells	0.68[a]	24.30 ± 1.93	2.67 ± 0.21	247.9 ± 19.7	12.27 ± 0.82	1.35 ± 0.09	125.2 ± 8.4	33.6
Cells	1.37[a]	24.03 ± 0.90	2.64 ± 0.10	244.8 ± 9.2				
Cells	2.24[a]	23.30 ± 2.09	2.56 ± 0.23	237.7 ± 21.3	16.46 ± 1.41	1.81 ± 0.16	167.9 ± 14.4	41.4
DOM	0.19	13.67 ± 1.77	1.40 ± 0.18		8.02 ± 0.63	0.82 ± 0.06		36.9
DOM	0.60	26.10 ± 3.05	2.66 ± 0.31		12.53 ± 0.95	1.28 ± 0.10		32.5
DOM	1.22	36.50 ± 2.46	3.72 ± 0.25					
DOM	2.00	46.23 ± 1.12	4.72 ± 0.11		21.94 ± 1.82	2.24 ± 0.19		32.2
AGG	0.23	23.17 ± 1.51	2.37 ± 0.15		9.80 ± 0.83	1.00 ± 0.08		29.7
AGG	0.74	35.87 ± 3.15	3.66 ± 0.32		13.06 ± 1.33	1.33 ± 0.14		26.6
AGG	1.49	38.27 ± 2.47	3.91 ± 0.25					
AGG	2.44	41.37 ± 1.57	4.22 ± 0.16		22.63 ± 1.56	2.29 ± 0.16		35.2

[a]These densities correspond to 16.3, 62.8, 126.7 and 207.9 algal cells per μl, respectively.

Appendix Table IV. Results of Experiment 9. The rates of incorporation and respiration at 26°C by larvae 85 μm in length are reported as the mean ± 1 standard deviation. There are 4 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density mgC liter^{-1}	Rate of Incorporation (D) cpm larva^{-1} day^{-1}	Rate of Incorporation (I_c) ngC larva^{-1} day^{-1}	Rate of Incorporation (I_a) cells larva^{-1} day^{-1}	Rate of Respiration (R) cpm larva^{-1} day^{-1}	Rate of Respiration (R_c) ngC larva^{-1} day^{-1}	Rate of Respiration (R_a) cells larva^{-1} day^{-1}	$\%R_c$ %
Cells	0.18[a]	19.20 ± 0.49	1.75 ± 0.04	142.1 ± 3.6	7.42 ± 0.55	0.68 ± 0.05	47.2 ± 3.5	28.0
Cells	0.81[a]	30.18 ± 2.64	2.75 ± 0.24	223.3 ± 19.5				
Cells	1.70[a]	31.49 ± 1.74	2.87 ± 0.16	233.0 ± 12.9	15.31 ± 1.20	1.40 ± 0.11	97.4 ± 7.6	32.8
DOM	0.22	6.58 ± 0.40	0.69 ± 0.04		1.91 ± 0.51	0.20 ± 0.05		22.5
DOM	0.88	18.58 ± 0.87	1.95 ± 0.09					
DOM	1.78	28.18 ± 2.34	2.96 ± 0.25		3.62 ± 1.04	0.38 ± 0.11		11.4

[a]These densities correspond to 14.6, 65.8, and 138.2 algal cells per μl, respectively.

Appendix Table V. Results of Experiment 11. Rates of incorporation at 24°C by larvae 119 μm in length are reported as the mean ± 1 standard deviation. There are 4 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density		Rate of Incorporation		
	cells μl^{-1}	mgC $liter^{-1}$	(D) cpm $larva^{-1}$ day^{-1}	(I_c) ngC $larva^{-1}$ day^{-1}	(I_a) $cells_{-1}$ $larva^{-1}$ day^{-1}
Cells	8.72	0.07	124.7 ± 23.8	9.99 ± 1.91	1247 ± 238
Cells	19.93	0.16	222.0 ± 29.5	17.96 ± 2.36	2220 ± 295
Cells	79.70	0.64	312.0 ± 11.0	28.11 ± 0.88	3120 ± 111
DOM		0.31	42.8 ± 1.7	3.32 ± 0.13	
DOM		0.77	84.1 ± 9.6	6.53 ± 0.75	
DOM		1.53	121.2 ± 14.0	9.41 ± 1.09	
AGG		0.20	72.6 ± 4.5	7.43 ± 0.46	
AGG		0.79	116.0 ± 7.5	11.87 ± 0.77	
AGG		1.97	115.0 ± 3.0	11.77 ± 0.31	

Appendix Table VI. Results of Experiment 12. Rates of incorporation at 24°C by larvae 139 μm in length are reported as the mean ± standard deviation. There are 3 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density	Rate of Incorporation			Index of Incorporation
		(D)	(I_c)	(I_a)	
	mgC liter^{-1}	cpm larva^{-1} day^{-1}	ngC larva^{-1} day^{-1}	cells larva^{-1} day^{-1}	%
Cells	0.09[a]	73.00 ± 5.79	7.60 ± 0.60	942 ± 75	7.7
Cells	0.10[a]	126.50 ± 13.44	13.18 ± 1.40	1633 ± 173	13.4
Cells	0.15[a]	191.06 ± 12.21	19.88 ± 1.27	2464 ± 157	20.2
Cells	1.30[a]	297.69 ± 14.24	30.99 ± 1.48	3840 ± 182	31.5
AGG	0.55	25.80 ± 1.27	5.74 ± 0.30		5.8
AGG	1.39	31.41 ± 1.27	6.99 ± 0.30		7.1
AGG	2.80	36.32 ± 1.63	8.08 ± 0.36		8.2
AGG	7.04	47.13 ± 1.41	10.49 ± 0.31		10.6
DOM (0.05 μm)	0.99	8.66 ± 1.04	5.04 ± 0.61		5.1
DOM (0.05 μm)	2.98	18.78 ± 2.64	10.93 ± 0.95		11.1
DOM (0.05 μm)	9.92	34.58 ± 1.63	20.12 ± 0.94		20.4
DOM (0.05 μm)	20.79	52.19 ± 3.53	30.37 ± 2.04		30.8
DOM (0.45 μm)	0.79	9.49 ± 2.14	4.24 ± 0.96		4.3
DOM (0.45 μm)	2.39	20.95 ± 2.63	9.61 ± 1.18		9.8
DOM (0.45 μm)	7.98	44.51 ± 3.32	19.88 ± 1.48		20.2
DOM (0.45 μm)	19.15	73.01 ± 1.41	32.61 ± 0.63		33.1
DOM (Nat)[b]	0.50	21.7 ± 1.2	1.9 ± 0.1		0.2

[a]These densities correspond to 10.3, 11.5, 17.2 149.4 algal cells per μl, respectively.

[b]Five replicates.

Appendix Table VII. Results of Experiment 13. Rates of incorporation at 24°C by larvae 85 μm in length are reported as the mean ± 1 standard deviation. There are 3 replicates of each treatment. Symbols are explained in the Methods section.

Food Type	Food Density	Rate of Incorporation			Index of Incorporation
		(D)	(I_c)	(I_a)	
	mgC larva^{-1}	cpm larva^{-1} day^{-1}	ngC larva^{-1} day^{-1}	cells larva^{-1} day^{-1}	%
Cells	0.13[a]	14.24 ± 0.86	1.48 ± 0.09	184 ± 12	6.9
Cells	0.39[a]	17.85 ± 0.54	1.86 ± 0.06	230 ± 6	8.7
Cells	0.78[a]	18.33 ± 0.25	1.91 ± 0.03	236 ± 3	8.9
Cells	1.71[a]	18.72 ± 0.41	1.95 ± 0.04	241 ± 5	9.1
AGG	0.56	2.80 ± 0.09	0.64 ± 0.02		3.0
AGG	1.41	3.91 ± 0.40	0.92 ± 0.09		4.3
AGG	2.82	6.24 ± 0.26	1.45 ± 0.06		6.7
AGG	7.04	7.39 ± 0.51	1.73 ± 0.11		8.0
DOM (0.05 μm)	0.99	3.42 ± 0.27	1.99 ± 0.16		9.3
DOM (0.05 μm)	2.98	5.60 ± 0.01	3.26 ± 0.01		15.2
DOM (0.05 μm)	9.92	11.78 ± 0.26	6.86 ± 0.15		31.9
DOM (0.05 μm)	20.79	15.29 ± 1.13	8.90 ± 0.65		41.4
DOM (0.45 μm)	0.79	3.20 ± 0.01	1.45 ± 0.00		6.7
DOM (0.45 μm)	2.40	6.65 ± 0.64	2.95 ± 0.29		13.7
DOM (0.45 μm)	7.98	13.47 ± 0.26	6.01 ± 0.12		28.0
DOM (0.45 μm)	19.15	20.52 ± 0.84	9.16 ± 0.38		42.6
DOM (Nat)[b]	0.50	5.5 ± 0.31	0.48 ± 0.03		2.2

[a]These densities correspond to 15.0, 45.0, 90.1, and 197.5 algal cells per μl, respectively.

[b]Five replicates.