

AN ABSTRACT OF THE THESIS OF

Robert Edward Malouf for the Master of Science
(Name) (Degree)
in Fisheries and Wildlife presented on Aug 11, 1970
(Major) (Date)

Title: FOOD CONSUMPTION AND GROWTH OF THE LARVAE OF
THE PACIFIC OYSTER (CRASSOSTREA GIGAS)

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The food consumption and growth of Pacific oyster larvae were studied in three experiments making use of a constant flow apparatus. The apparatus maintained a continuous flow of various densities of algae through test chambers containing different numbers of larvae in a factorial design.

Three additional experiments were conducted in which a flow of algae was not used. These standing water experiments were conducted to study the effects of temperature, larval size, and algal density on the food consumption rate of oyster larvae.

Dichromate wet oxidations were conducted on samples of oyster larvae to establish the relationship between shell length and caloric content of the larvae. These data were used to estimate the total caloric content of test populations of larvae.

The constant flow experiments showed that larval growth rates increased as the density of algae flowing into the test chambers increased up to an optimum density. Subsequent increases in algal inflow density caused the larval growth rate to decline.

Larval food consumption in the three standing water experiments was measured as cells consumed per larva per hour and as an instantaneous coefficient of food consumption called grazing rate. Grazing rate is essentially a measure of the proportion of the algal population that is removed by the larvae.

The standing water experiments showed that larval food consumption increased rapidly with increases in temperature from 10°C to 24°C. Grazing rate more than doubled with each increase of 5°C. In other experiments, the grazing rate of Pacific oyster larvae was found to increase exponentially with increases in larval shell length and linearly with increases in the caloric content per larva. A third experiment showed that larval grazing rate was inversely related to algal density (i. e. , grazing rate declined with increased algal density). The number of algal cells consumed per larva per hour, on the other hand, was found to be directly related to the algal density.

The possible application of a constant flow feeding system to an oyster hatchery is discussed.

Food Consumption and Growth of the
Larvae of the Pacific Oyster (Crassostrea gigas)

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1971

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ACKNOWLEDGEMENTS

Many people in the Department of Fisheries deserve my thanks for their assistance and encouragement in the completion of this study. I owe thanks to Dr. Charles Warren and Dr. Robert Brocksen for their help in the early stages of the study, and to Mr. Jack McIntyre whose assistance in the preparation of the thesis was most helpful.

I owe special thanks to Mr. Thomas Thatcher who unselfishly gave his time in conducting biomass determinations of oyster larvae at the Oak Creek Fisheries Lab.

I am indebted to a number of people at the Marine Science Center for their cooperation and help, particularly to Professor Wilbur Breese for his assistance and for encouraging my interest in oyster culture. I would also like to thank Mr. Dennis Lund and Mr. Dean Satterlee for their help in designing the constant flow apparatus used in this study.

I wish to thank Dr. William McNeil whose guidance and enthusiasm were invaluable to me.

I owe special thanks to my wife, Judy, and to my son, Robby, for putting up with all this.

Financial support for this study came from the National Science Foundation through their Sea Grant Program.

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FOOD CONSUMPTION AND GROWTH OF THE LARVAE OF THE PACIFIC OYSTER (Crassostrea gigas)

INTRODUCTION

A dependable supply of high quality seed stock is essential to the development of any aquiculture system. The more advanced systems in aquiculture use hatcheries to produce seed stock whereas the less advanced systems obtain seed stock from natural waters. Oyster culture still depends largely upon the collection of juveniles from natural waters, but a transition to hatchery culture is beginning.

The principal commercial oyster grown in the Pacific Northwest, the Pacific Oyster (Crassostrea gigas), came originally from Japan. The industry remains dependent upon seed oysters imported from Japan, supplemented in most years with seed collected at a few locations in Washington and British Columbia. However, the price of imported seed is increasing rapidly, and there are periodic failures of natural spawning in Northwest waters. Lack of a dependable source of seed is one factor which has depressed the industry.

Studies are under way at the Oregon State University Marine Science Center, Newport, to determine the feasibility of commercial oyster hatcheries in the Pacific Northwest. This study of the food requirements of Pacific oyster larvae was intended to assist in gaining a better understanding of factors that will be important to a successful

hatchery operation. Financial support for the study came from the National Science Foundation's Sea Grant Program.

Efforts to develop techniques for the rearing of oyster larvae began more than 30 years ago. Early attempts to raise the larvae of the European oyster (Ostrea edulis) in large open tanks were only partially successful (Cole, 1936 and 1939). Greater success was obtained by introducing artificially cultured algae as a food source for European oyster larvae (Bruce et al., 1940).

Subsequently, efforts were directed toward isolating species of algae that could be easily cultured. Bruce et al. (1940) found great differences in food value among different species of algae for oyster and clam larvae. Walne (1956) reported excellent growth of European oyster larvae that were fed pure cultures of the naked flagellate Isochrysis galbana. Other workers later noted that I. galbana and Mono-chrysis lutheri gave particularly good results when fed to various species of oyster and clam larvae.

Other studies were conducted to determine the concentrations of algae that would permit adequate growth of larvae. Loosanoff et al. (1953 and 1955) experimented with various combinations of algal concentrations and larval density and concluded that, at a given algal concentration, the rate of growth of oyster larvae is inversely proportional to the number of larvae per unit volume. The authors also stated, on the basis of experiments with a fairly limited range of algal

concentrations, that as far as growth was concerned, an increase in larval density could not always be compensated for by an increase in algal concentration. The authors finally concluded that the optimum ration was dependent on the size and quality of the algae and on the density of the larvae.

Using I. galbana as a food organism for European oyster larvae, Walne (1956) studied the effect of increasing food density on larval growth. He found that growth rates increased to a maximum and then leveled off and even declined as food became extremely dense. Later, Walne (1963, 1965 and 1966) conducted detailed studies of the food consumption and growth rate of European oyster larvae using seven algal species including M. lutheri and I. galbana as food organisms.

After it had become apparent that I. galbana and M. lutheri were excellent food organisms for oyster and clam larvae, considerable attention was given to the mass culture of these two species. The contributions of Guillard (1957), Ukeles (1961), Davis and Ukeles (1961), and Matthiessen and Toner (1966) are especially noteworthy in this regard.

The value of approaching food consumption and growth problems by considering the basic relationships that exist between the density or total biomass of a predator and the biomass of its prey has been shown by Brocksen et al. (1968 and unpublished). Brocksen and

his colleagues based their conclusions on the results of work done with fish in laboratory streams. However, density dependent relationships may be valid for populations of planktonic communities as well. If so, they would provide a useful basis for studying the problem of food consumption and growth of oyster larvae.

We need to determine whether or not the growth rates of oyster larvae are simple functions of the density of their food organism, and conversely, that the density of the food organism is a function of the total biomass of the larvae. It is the purpose of this study, then, to define more precisely the relationships between the total biomass of the larval population, the average growth rate of the larvae, and the density of the algal food source. Larvae of the Pacific oyster were used in experiments to attempt to define some of the basic relationships that affect the food consumption and growth of oyster larvae. With previously perfected rearing techniques, I examined the food consumption and growth of individual larvae and of the total population under different combinations of larval density and algal concentration. In addition, I examined the effects that the population of larvae had on the density of their algal food source.

A better understanding of the relationships between oyster larvae and their algal food supply should assist the operation of an oyster hatchery. It may be possible, for example, to maximize the growth and survival of larvae through proper control of the feeding schedule.

The period of time during which the larvae are held in feeding tanks might be reduced and the number of juveniles collected for transplanting to growing grounds might be increased.

GENERAL METHODS

Algae were fed at known rates to known concentrations of larvae in two types of systems: flowing water and standing water.

Algae

M. lutheri was fed to oyster larvae in all experiments. This naked flagellate is easily cultured and can tolerate slightly higher temperatures than the other commonly used food organism, I. galbana. In addition, Monochrysis is reportedly less likely to become toxic at the culture temperatures employed (19-22°C.) than is Isochrysis (Ukeles, 1961). M. lutheri is approximately 6 microns in diameter (Droop, 1954) and is small enough for even the smallest oyster larvae to feed upon.

Algae was cultured in 5 gallon carboys under fluorescent lights. The carboys contained 16 liters of sea water that had previously been filtered, treated with ultraviolet light, chlorinated, and finally de-chlorinated by carbon filtration.

Nutrients and trace elements were added to the culture water from stock solutions prepared according to formulas slightly modified from Matthiessen and Toner (1966). Components of the four stock solutions are given in Table 1. One ml of each stock solution was added for each liter of water in the final culture medium with 2 liters

Table 1. Nutrient solutions used in the culture of Monochrysis
lutheri and Isochrysis galbana.

Nutrient	Formula	Stock Solution
1. sodium nitrate granular, refined	NaNO_3	150 gms/liter
2. sodium phosphate monobasic, certified	$\text{NaHO}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	10 gms/liter
3. trace metals:		
cupric sulfate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	1.96 gms/liter
zinc sulfate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	4.4 gms/liter
cobalt(ous) chloride	$\text{CoCl}_2 \cdot 5\text{H}_2\text{O}$	2.0 gms/liter
manganese(ous) chloride	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	36.0 gms/liter
sodium molybdate	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	1.26 gms/liter
4. vitamins:		
biotin-crystalline		1 mg/liter
vitamin B-12 crystalline		1 mg/liter
thiamine hydrochloride		200 mg/liter

of algae inoculum. They were not replenished.

The cultures generally reached a concentration of about two million cells per ml in about a week. Cultures of two million cells per ml or more were used for feeding experiments whenever possible to attain the desired test concentrations of algae with as little of the stock algae culture as possible. Cultures over five million cells per ml were not used since they contained a high proportion of senescent cells that tended to clump or settle out in the test containers.

Algae were counted in the cultures and in the experimental containers with a Coulter Counter model B, following procedures outlined by Sheldon and Parsons (1967). Several workers, including Mulligan and Kingsbury (1968), and Parsons et al. (1961), have demonstrated the applicability of the Coulter Counter to this type of work.

Larvae

The Pacific oyster larvae used in this study came from the pilot oyster hatchery at the Marine Science Center. The adults were spawned artificially (Loosanoff and Davis, 1963). Larvae used in any one experiment were drawn from a single hatchery tank after they had been thoroughly mixed. No attempt was made to control parentage.

Larvae were removed from the hatchery tanks by draining the water through a series of stainless steel screens stacked in order of

diminishing mesh size. This was done to separate clumped algae and other debris from the larvae and to produce samples of larvae of a more uniform size. Generally, larvae that had passed through the larger mesh screens but were retained on a 124 micron screen were used in the flowing water experiments.

It was found that reproducible counts could be obtained if 1 ml samples were drawn off with an automatic pipette while the water in the beaker was being gently agitated. Agitation was achieved by raising and lowering a perforated plexiglas disc in the beaker.

Preliminary 1 ml samples gave a rough approximation of the volume of water containing the desired number of larvae for each test chamber. Ten 1 ml subsamples were subsequently withdrawn from the aliquots assigned to each test chamber, and final adjustments were made in the number of larvae in each aliquot before the aliquot was added to the chamber.

Counts of larvae were also made while some of the experiments were in progress. The larvae were uniformly suspended with a plexiglas plunger before a piece of 11 mm diameter glass tubing was extended down into the chamber to collect larvae from the entire length of the water column. This procedure was repeated three times so that a total of 200 ml were withdrawn and placed in a small beaker. Ten subsamples of 1 or 5 ml each, (depending on the density of the larvae), were taken using the method described above. These larvae

were killed with AFA, counted and measured. The remainder of the 200 ml sample was returned to the test chamber.

The larvae were measured with an American Optical Company micrometer eyepiece calibrated in microns at 100X. All measurements were made across the greatest dimension of the shell parallel to the hinge or umbone (Figure 1).

Flowing Water Experiments

An apparatus was designed to maintain a constant inflow of algal cells at a known density to a test chamber containing a known number of larvae. By controlling the number of algal cells entering chambers containing known densities of larvae, different levels of food and consumer biomass were simulated. Only the algal densities and the number and size of the larvae varied. The volume of water passing through the test chambers was held constant.

The density of algae in the water leaving the test chambers was determined at least once every 24 hours. The difference in density of algae between the inflow and outflow of the test chambers provided measures of the feeding rate at different levels of food input. Measurements of the growth of the larvae in the test chambers provided information on the effect that various densities of algae had on larval growth.

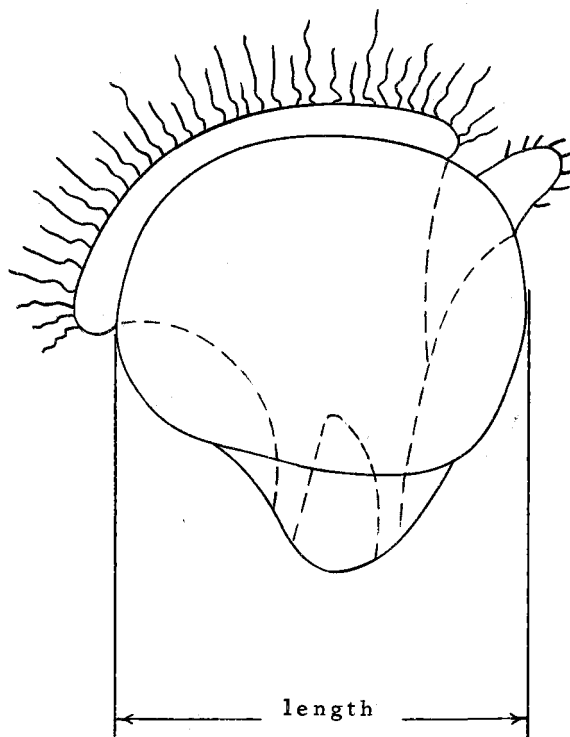


Figure 1. Simplified drawing of a large oyster larvae showing shell length.

The test chambers (Figure 2) were four-inch diameter polyvinyl chloride (PVC) pipe cut into 30-inch lengths. One end of the pipe was covered with a 116-micron monofilament nylon screen ("Nitex" - Tobler and Ernst Inc., Elmsford, New York). A coupling to which a piece of 1/4-inch PVC sheet fitted with a tubing adapter had been glued was placed over the screened end of the pipe. Tygon tubing was attached to the tubing adapter, extended back up parallel to the chamber, and attached with a rubber stopper to a glass elbow that served to maintain the water in the chambers at a constant level. The chambers were allowed to fill to a depth of 24 inches, a water depth equivalent to five liters.

Twelve-gallon carboys fitted with a siphon were used to maintain a constant flow of algae into the test chambers. The carboys were covered with translucent plastic to reduce light and to prevent the algae from reproducing in the carboys while an experiment was in progress. The density of algae in the carboys was determined each time that the algae were counted in the outflow from the test chambers. The algal density in the carboys was found to remain fairly constant, although there was a slight decrease in density due to settling as the water level in the carboys became low.

Preliminary tests showed that strong aeration would maintain a uniform dispersion of algae in the test chambers. Hence, the density of algae in the outflow was an accurate measure of the algal

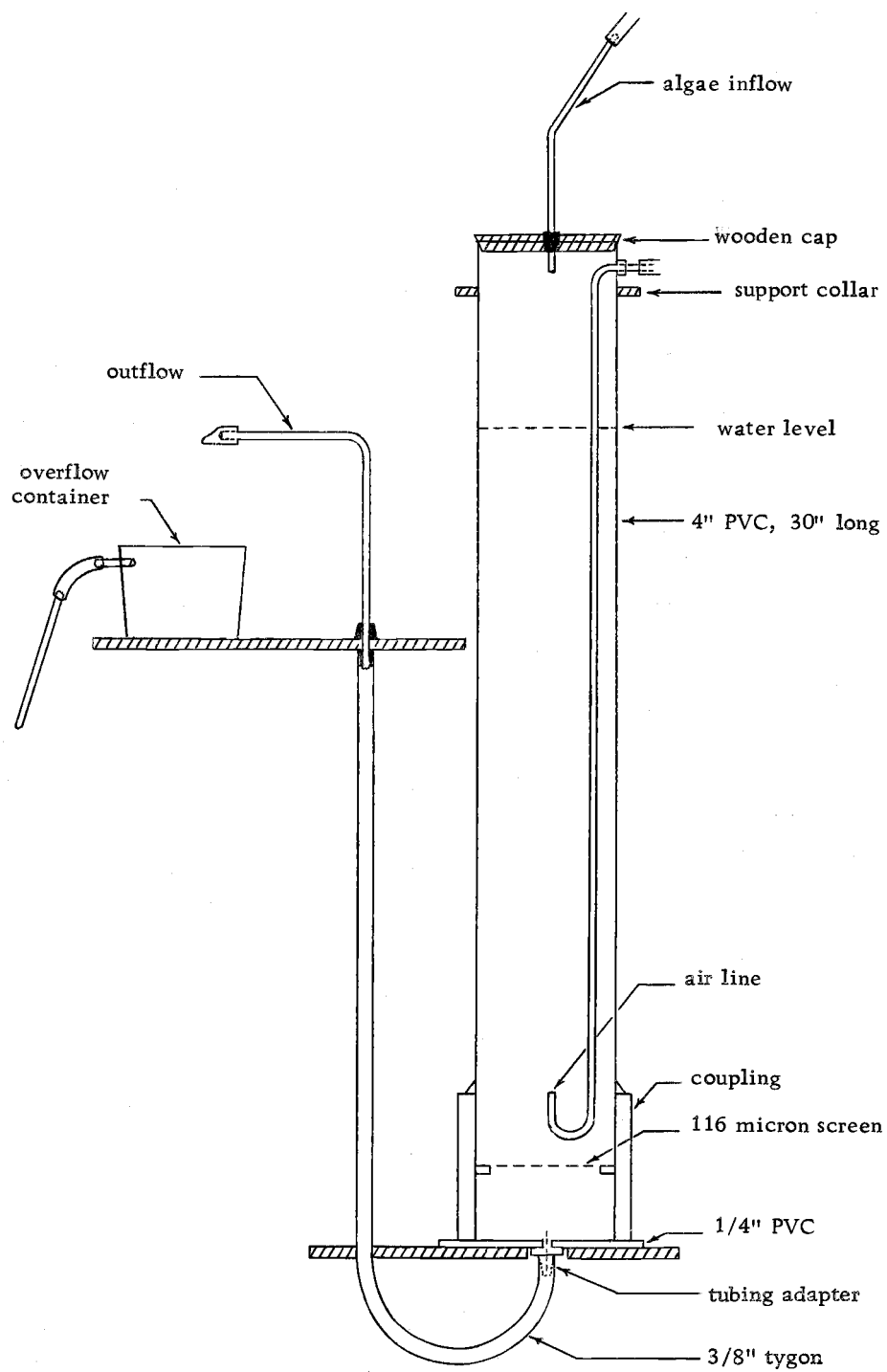


Figure 2. Apparatus used for constant flow experiments.

density within the test chamber. Preliminary tests also showed that the algal density in the outflow was essentially the same as the inflow if no larvae were present in the system.

In other preliminary tests, the Coulter Counter model J plotter was used in conjunction with the counter to make size frequency determinations of cells from stock algal cultures and from test chambers containing larvae. No differences in particle sizes were evident between the stock cultures and algal populations that had been grazed upon by oyster larvae for several hours in a flowing water system. The similarity of the histograms indicated that the counts made on samples taken from chambers in which grazing was being measured did not contain a significant amount of cell fragments and detritus that were within the size range of the Coulter Counter.

Standing Water Experiments

There were a number of specific questions on the effects of temperature, larval size, and food availability on the feeding rate of oyster larvae which were best studied in static, rather than running, water systems. Standing water experiments were conducted in one gallon containers which provided an extremely simple and stable system in which conditions could be easily controlled for short term experiments.

Standing water experiments were 24 hours or less in duration and were concerned only with the rate of food consumption of the larvae. Growth was found to be insignificant over these restricted time periods.

BIOMASS DETERMINATION

The growth of larvae was most easily observed from increases in average shell length. Such measurements were easily obtained since larvae were sampled and sacrificed periodically to determine their density in flowing water chambers and standing water containers.

Shell length is a measure of surface only and does not adequately reflect an animal's mass. It is difficult to compare the relative biomasses of populations consisting of larvae of different sizes where shell length is the only criterion. In an attempt to overcome this problem, I determined the relationship between shell length and biomass in terms of the organic content of oyster larvae.

The method used is a modification of the dichromate chemical oxygen demand determination described in the 10th edition of Standard Methods for the Examination of Water, Sewage, and Industrial Wastes (American Public Health Association, 1955). A known excess of oxidant (potassium dichromate) was added to a sample of a known number of larvae. After oxidation was completed, the amount of oxidant remaining was determined. The amount of reacting oxygen was calculated from the difference between the initial and final quantity of oxidant. Caloric values are then calculated from the amount of oxygen consumed during oxidation of the larvae.

Larvae used in the biomass determinations were taken from the hatchery, washed, and carefully counted. A sub-sample of 50 was measured to give an estimate of shell length for the sample. The remaining larvae were placed in 2 liters of sea water without food and held overnight.

Three groups of between 4,000 and 16,000 larvae each were removed by screening, washed with distilled water, and placed in 30 ml. of double distilled water in reaction flasks to which 7.5 g. of mercuric sulfate and five silicone carbide boiling chips had previously been added. Fifteen ml. of 0.25N potassium dichromate and 45 ml. of concentrated sulfuric acid containing silver sulfate were added to each flask. The mixture was refluxed for three hours, cooled, and diluted to 210 ml. with distilled water. Ferroin indicator was added, and the mixture was titrated with ferrous ammonium sulfate of known normality.

In addition to the three flasks containing larvae, three flasks containing only distilled water were treated in a like manner. The three "blank" flasks gave a measure of the amount of dichromate reduced by impurities other than the organic matter of the oyster larvae.

From the results of the titrations, the amount of oxygen consumed by the oxidation of the larvae was calculated from the following equation:

$$\text{mg. /L. COD} = \frac{(a-b)c \times 8000}{d}$$

where a is the mean amount of ferrous ammonium sulfate used in titrating the blank flasks in milliliters; b is the amount of ferrous ammonium sulfate used in titrating the sample flask in ml ; c is the normality of the ferrous ammonium sulfate; and d is the milliliters of sample that was oxidized (30 ml. in this case).

Titration of the samples with ferrous ammonium sulfate after dichromate oxidation gave a measure of the amount of oxygen consumed in mg. O₂ per liter of sample. These values were then multiplied by an oxycalorific coefficient of 3.42 calories per mg. O₂ to obtain an estimate of the caloric content of the samples. The oxycalorific coefficient used here is similar to the value of 3.4 suggested by Maciolek (1962) as representative coefficient for carbohydrates, proteins, and lipids, and is also within the range of values 3.33-3.49 found by Ivlev (cited by Maciolek, 1962) for 16 invertebrates. Conversion of oxygen consumed values to calories involves fewer assumptions than conversions to weight or organic carbon values (Maciolek, 1962).

Determinations of the caloric content of seven batches of Pacific oyster larvae are given in Table 2 and are shown graphically in Figure 3.

Table 2. The results of dichromate oxidations showing the relationship between larval shell length and the caloric content per larva of Pacific Oyster larvae.

Length of larvae in microns ^{1/}	96 ₊₂	100 ₊₂	133 ₊₅	189 ₊₄	249 ₊₇	274 ₊₅	306 ₊₅
Number of larvae used per sample	7,400	5,360	8,325	16,550	10,280	9,080	4,200
Calories per larva (sample No. 1)	.00033	.00039	.00075	.0019	.00372	.00581	.00655
Calories per larva (sample No. 2)	.00038	.00039	.00077	.0019	.00369	.00578	.00666
Mean caloric content per larva	.00036	.00039	.00076	.0019	.00371	.00579	.00661

^{1/}Means with 95 percent confidence interval estimate.

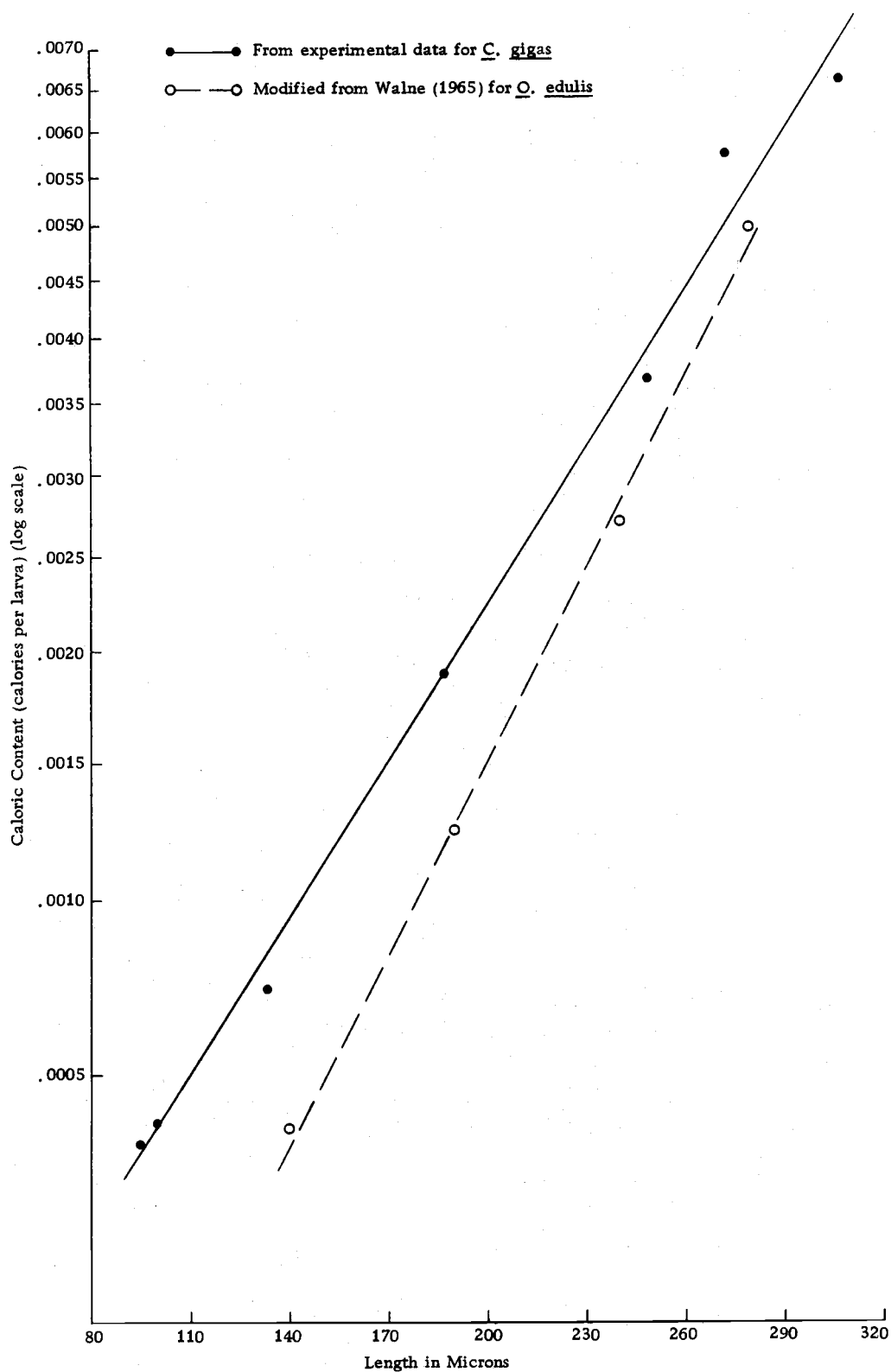


Figure 3. The relationship between shell length and caloric content of Pacific and European oyster larvae.

These data are the means of values obtained from two samples of 4,000 to 16,000 larvae each. The measurements of shell length are the mean values of 50 larvae.

Larval length in microns when plotted against the caloric content per larvae on a semi log scale (Figure 3) yields a straight line. This shows that there is an exponential increase in caloric content of Pacific oyster larvae with increases in shell length. Walne (1965) found a similar relationship between the shell length of European oysters and their total dry weight. He also found that the shell of oyster larvae includes a constant 75 percent of the total dry weight. Millar and Scott (1967) confirmed that the shell of European oyster larvae makes up about 75 percent of the total dry weight. However, they pointed out that the percentage will vary somewhat depending on the condition of the larvae.

Walne's (1965) data for the total dry weight of European oyster larvae can be multiplied by .25 to obtain an approximation of the dry weight of organic matter per larva and then by 5,000 calories per gram to estimate the caloric content of the animal tissue (Davis and Warren, 1968; and Maciolek, 1962). These values can then be compared with data obtained in this study for the Pacific oyster larvae (Figure 3).

Considering the differences in the life cycles of the European oyster larvae used by Walne and the larvae of the Pacific oyster used

in this study, differences in the caloric content of the larvae, particularly at the 145-175 micron size range, are not surprising. The European oyster, which broods its young, releases larvae that are perhaps ten days old and are 140-170 microns long. These larvae seem to be quite restricted in their feeding prior to release (Walne, 1965). The eggs of the Pacific oyster, on the other hand, are fertilized externally. The larvae feed actively for several days before they reach 140 microns in length. One might expect the larvae of the Pacific oyster to have a greater biomass compared to shell length than European oyster larvae less than 175 microns long. Later, as the larvae of both species approach setting size, it appears that their biomasses become more nearly the same.

GROWTH

Three experiments with the constant flow apparatus provided information on relationships between larval growth and algal density. The experiments will be described individually after some general comments on the calculation of growth rates.

Growth rates were determined from periodic measurements of shell length for a sample of larvae. The mean length was converted to caloric content by use of the upper curve in Figure 3.

Instantaneous growth coefficients, k , were calculated for each test group from the formula:

$$k = \frac{\log_e C_2 - \log_e C_1}{t}$$

where: C_1 = the initial caloric content per larva

C_2 = the final caloric content per larva

and t = the time in days.

This same equation was applied to mussel larvae by Bayne (1965) and to oyster larvae by Walne (1963). However, both authors used shell length rather than caloric content as a criterion for growth.

Mortality in some test chambers appeared to be selective for smaller larvae. Such selective mortality tended to shift the average size of the larvae upward, thus exaggerating the estimated growth of the population. This effect was compounded by the increased food

supply to the surviving larvae. Therefore, I will omit test chambers having more than 50 percent mortality from growth analyses.

Because of inherent differences among different broods of larvae and procedural differences among the three constant flow experiments, growth data from the three experiments were analyzed separately.

My experiments were intended to describe general relationships between larval density and algal inflow density as reflected by the growth of larvae. My procedures did not involve replication of individual experiments, and a valid error term for testing differences in responses was not available; nor could such a term be obtained from curve fitting since the curves resulting from different combinations of larvae and algae varied greatly in their general shapes. My conclusions were reinforced, however, by the smoothness of the resulting curves (to be described) since a small error term would normally be associated with any appropriately fitted smooth curve.

Experiment I

This experiment was planned as a 4 x 4 factorial design with the following algal inflow concentrations and larval densities:

<u>Larvae per ml</u>	<u>Algal cells per ml (inflow)</u>			
	<u>5,000</u>	<u>10,000</u>	<u>20,000</u>	<u>40,000</u>
1	(1)	(5)	(9)	(13)
2	(2)	(6)	(10)	(14)
4	(3)	(7)	(11)	(15)
8	(4)	(8)	(12)	(16)

Numbers in parentheses identify individual test chambers.

The volume of water used in each chamber was 5,000 ml. The total number of larvae used were 5,000, 10,000, 20,000, and 40,000. Their initial length was 189 microns. This experiment lasted 11 days.

Mortality exceeded 50 percent in five test chambers (Table 3). Fungus or bacterial infections are possible causes of mortality (see Loosanoff and Davis, 1954), but no pathogens were observed in this experiment.

K-values ($K=1000 \times k$) calculated for each group of larvae in Experiment I are given in Table 3. The table also gives the mean density of the algae that flowed into and out of each chamber during the experiment. These data show that there is a definite relationship between the density of algae flowing into each chamber and the growth rate of the larvae (Figure 4). Growth rate increased with increasing algal density up to 20,000 cells per ml. Growth rates declined at an algal inflow density of 40,000 cells per ml. Maximum growth in Experiment I was achieved at a mean algal outflow density of 11,000 to 14,000 cells per ml. This algal outflow density was the result of an inflow density of about 20,000 cells per ml through a test chamber initially stocked with one and two larvae per ml (chambers 9 and 10). The largest K value (118.9 in chamber 9) is 36 times the smallest (3.3 in chamber 4). Hence, a 36-fold difference in the growth rate of larvae was observed in this experiment.

Because of unpredicted high mortality in four test chambers, an analysis of variance test was not used. Instead, I calculated 95 percent confidence interval estimates of the mean sizes of the larvae at the end of the experiment (Table 4). From Table 4 it is possible to make general comparisons among algal inflow density, initial larval density, and growth of larvae.

At an algal inflow density of 5,000 cells per ml (chamber 2) two-fold increases in the initial larval density from two larvae per ml

Table 3. Stocking rate, mortality, final mean length, and instantaneous growth coefficient of Pacific oyster larvae fed at four different algal densities (Experiment I).

Chamber number	Initial no. of larvae/ml.	Percent mortality	Length of larvae (microns)		Mean algal inflow density	Mean algal outflow density	Instantaneous growth rate (K)
			Final mean value	Standard error			
1	1	43	268	5.18	6,474	4,350	82.1
2	2	21	263	3.79	6,783	2,751	75.0
3	4	16	222	3.90	6,845	2,192	37.6
4	8	18	195	4.45	6,714	1,641	3.3
5	1	97	---	---	11,762	10,738	---
6	2	53	237	5.77	11,812	9,760	50.5
7	4	94	---	---	11,704	10,302	---
8	8	30	208	3.50	11,747	2,026	22.2
9	1	49	309	4.33	21,318	13,869	118.9
10	2	23	303	6.51	22,077	11,359	114.9
11	4	100	---	---	22,079	17,782	---
12	8	39	240	5.09	21,834	3,338	76.6
13	1	38	254	5.45	40,482	33,097	26.8
14	2	18	283	6.03	41,388	25,868	36.4
15	4	95	---	---	41,630	26,098	---
16	8	40	276	4.66	42,286	11,834	34.4

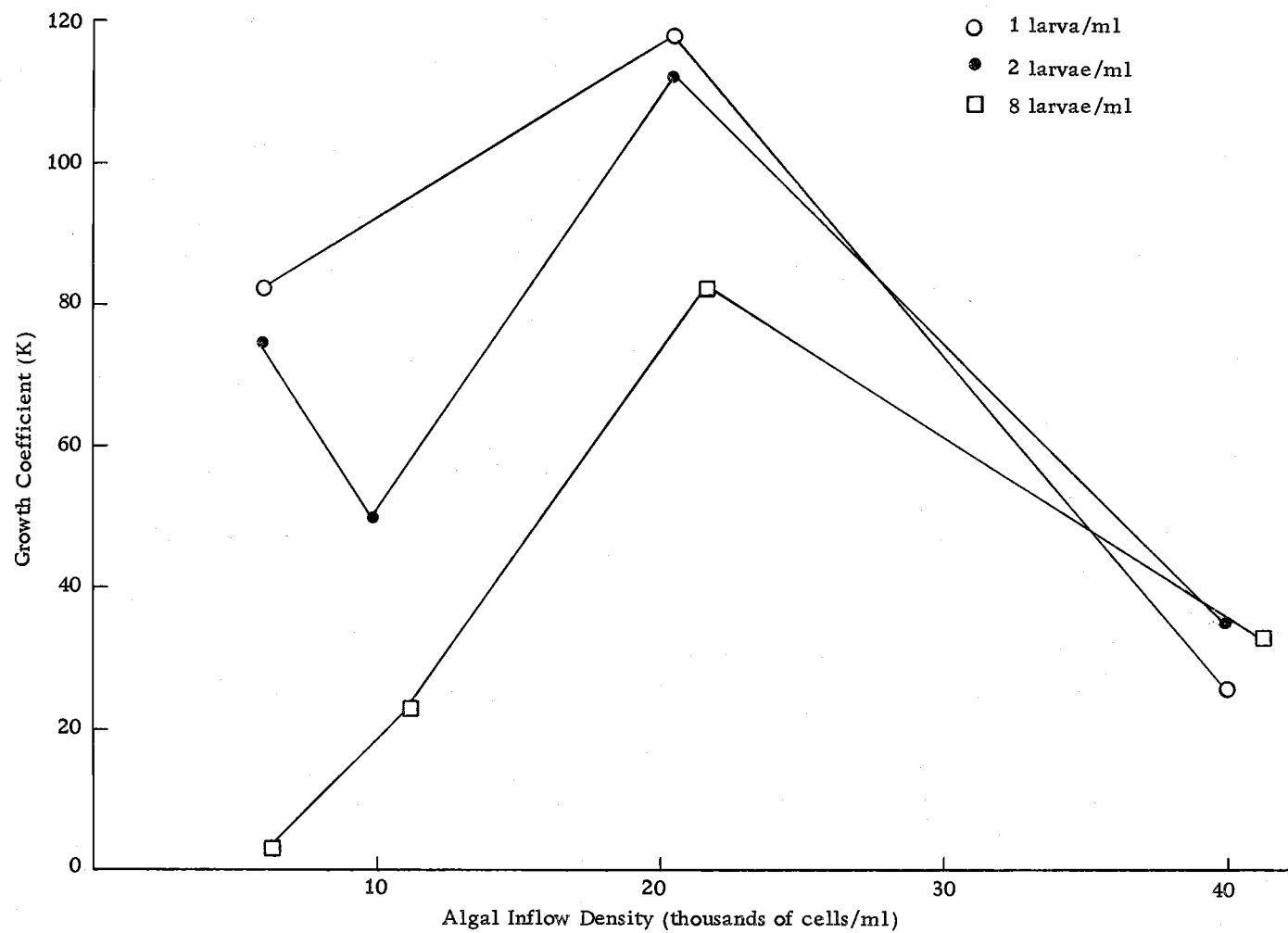


Figure 4. General relationship between algal inflow density and larval growth rate at four larval densities (Experiment I).

Table 4. The final mean length in microns of samples of 20 larvae each and the associated 95 percent confidence interval estimates of the population mean length (Experiment I).

		Algal inflow density (cells/ml)			
		5, 000	10, 000	20, 000	40, 000
Initial larval density (larvae per ml)	1	268 $\pm$ 10	*	309 $\pm$ 9	254 $\pm$ 11
	2	263 $\pm$ 8	237 $\pm$ 11	303 $\pm$ 13	283 $\pm$ 12
	4	222 $\pm$ 8	*	*	*
	8	196 $\pm$ 9	208 $\pm$ 7	* (240 $\pm$ 13)**	277 $\pm$ 9

*These test cells were omitted from the experiment because of high larval mortality.

**Larvae were measured three days earlier in this test cell than in the other test cells.

(chamber 2) to four per ml (chamber 3) and to eight per ml (chamber 4) was associated with reduced larval growth. At an algal inflow density of 40,000 cells per ml, differences in initial larval density (chambers 14 and 16) did not appear to affect larval growth.

Experiment II

This experiment was planned as a 2 x 4 factorial design with the following algal concentrations and larval densities:

<u>Larvae per ml</u>	<u>Algae per ml (inflow water)</u>			
	<u>10,000</u>	<u>20,000</u>	<u>40,000</u>	<u>80,000</u>
2	(1)	(3)	(5)	(7)
16	(2)	(4)	(6)	(8)

Numbers in parentheses identify individual test chambers.

The larvae used in Experiment II had an initial length of 193 microns.

Since only eight carboys were used in Experiment II, the eight remaining carboys were filled with sterile sea water to allow the water to come to room temperature before a new batch of algae was introduced. In Experiment I there was a period of four hours while the carboys were washed during which no algae entered the chambers. This was then followed by a period of about eight hours during which the inflow water was entering at a temperature less than 20° C.

Two of the eight chambers used in Experiment II showed high mortality early in the experiment (Table 5). Chambers 5 and 6, both at a feeding rate of 40,000 cells per ml, experienced about 50 percent mortality in two days and nearly 100 percent mortality by the end of the experiment. A flow of algae was maintained through chambers 5 and 6 for the duration of the experiment in spite of the heavy mortality. This was done to measure the effects of a large population of an unidentified protozoan that developed in the two chambers as the larval population declined. It was found that the density of the algae in the outflow did not differ significantly from the inflow density in spite of the presence of many thousands of protozoa. As in Experiment I, no cause for the extreme mortality in the two chambers was determined.

It is apparent that the growth rate of the larvae is directly related to the density of algae in both the inflow and the outflow in Experiment II (Table 5). In all cases higher algal inflow densities resulted in higher growth rates for a given larval density in this experiment (Figure 5). Maximum growth was achieved at an inflow density of 80,000 cells per ml through a test chamber containing two larvae per ml (chamber 7).

Table 5. Stocking rate, mortality, final mean length and instantaneous growth coefficient of Pacific oyster larvae fed at two different algal inflow densities (Experiment II).

Chamber number	Initial number of larvae/ml.	Percent mortality	Length of larvae (microns)		Mean algal inflow density	Mean algal outflow density	Growth rate (K)
			Final mean value	Standard error			
1	2	0	242	3.04	10,927	3,389	83.7
2	16	9	199	2.23	10,943	1,237	9.4
3	2	0	272	3.15	20,923	6,397	131.7
4	16	12	202	2.03	20,770	2,143	18.2
5	2	100	---	---	---	---	---
6	16	100	---	---	---	---	---
7	2	6	296	2.34	79,477	49,588	153.4
8	16	17	247	2.72	77,997	3,500	88.2

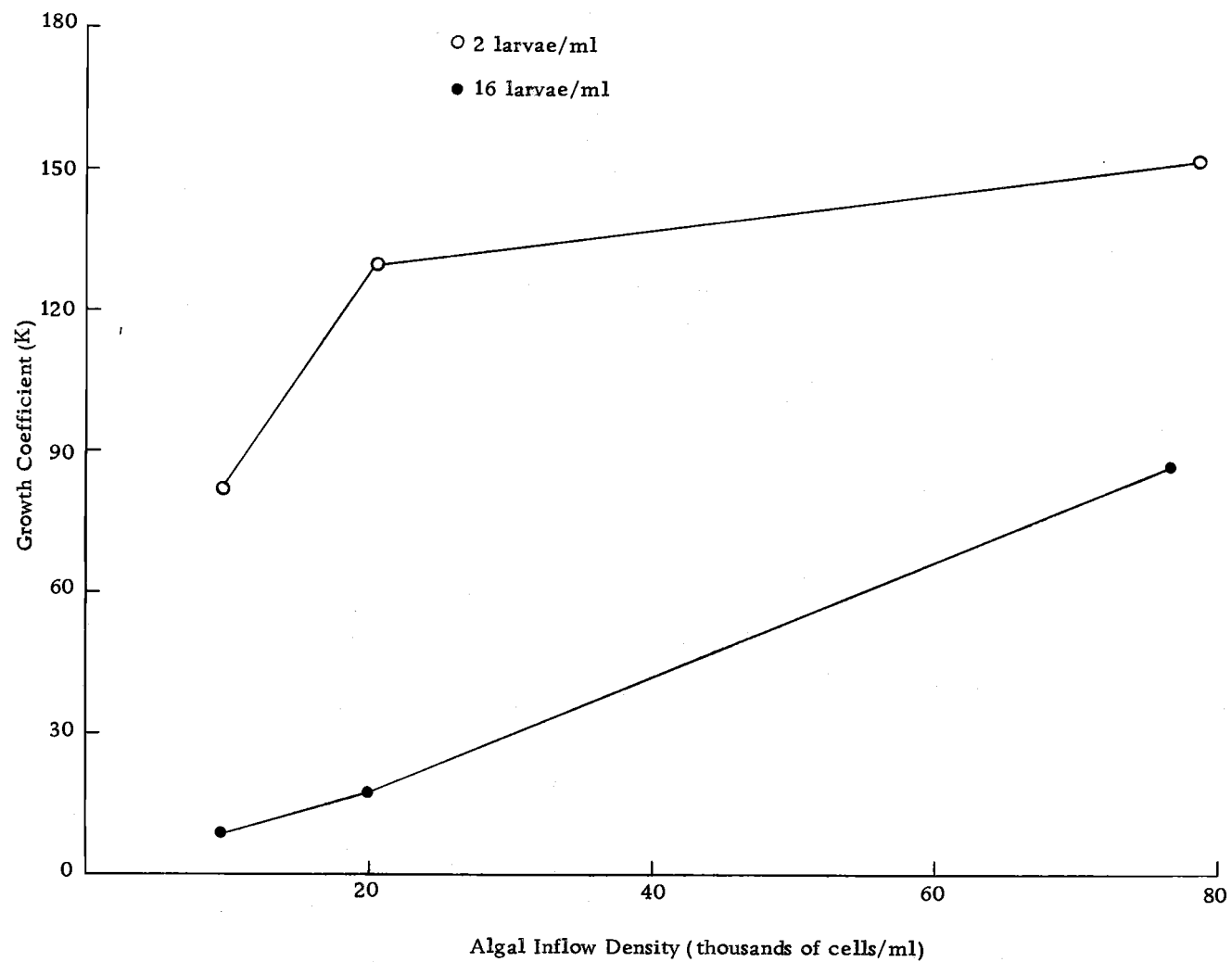


Figure 5. Relationship between algal inflow density and larval growth rate for two larval densities. (Experiment II).

The results from Experiment II differed in several respects from those of Experiment I. There was no indication of reduced larval growth at algal densities above 20,000 cells per ml (Table 6). Where larvae were at a density of 2 per ml, their growth rate appeared to level off above an algal inflow density of 20,000 cells per ml (Figure 5). However, where the larvae were at a density of 16 per ml, the growth rate continued to climb at algal densities beyond 20,000 cells per ml.

In Experiment I, I used a maximum algal inflow of 40,000 cells per ml and a maximum larval density of 8 per ml. The results of Experiment II, with its higher densities of algae (up to 80,000 cells per ml) and larvae (up to 16 larvae per ml), suggest that the relationship between the density of larvae and their food supply is very complex. The results further suggest that the quantitative relationships between the growth of the larvae and the density of their food supply vary substantially with the density of the larvae. I therefore planned a third constant flow experiment to examine the effects of higher algal inflow densities on the growth of larvae. Table 6 shows that the growth of larvae in these two chambers did not differ significantly. Chambers 2 and 4 were fed at a low level and growth in both chambers was minimal.

Table 6. The final mean length in microns of samples of 75 larvae each and the associated 95 percent confidence interval estimates of the population mean length (Experiment II).

		Algal inflow density (cells per ml)			
		10,000	20,000	40,000	80,000
Initial larval density (larvae/ml)	2	242 $\pm$ 6	272 $\pm$ 6	*	296 $\pm$ 4
	16	199 $\pm$ 4	202 $\pm$ 4	*	247 $\pm$ 5

*These test cells were omitted from the experiment because of high mortality of larval oysters.

Experiment III

Experiment III was designed as a 2 x 4 factorial similar to

Experiment II:

Larvae per ml	Algae per ml (inflow water)			
	20,000	40,000	80,000	160,000
2	(1)	(3)	(5)	(7)
16	(2)	(4)	(6)	(8)

Numbers in parentheses identify individual test chamber.

The initial size of larvae used in the experiment was 199 microns. Procedures followed were the same as Experiment II.

Survival was high in all chambers except chamber 6 (Table 7).

More than 50 percent mortality occurred in chamber 6 in which most

Table 7. Stocking rate, mortality, final mean length, and instantaneous growth coefficient of Pacific oyster larvae fed at four different algal inflow densities (Experiment III).

Chamber number	Initial number of larvae/ml.	Percent mortality	Length of larvae (microns)		Mean algal inflow density (cells/ml)	Mean algal outflow density (cells/ml.)	Growth rate (K)
			Final mean value	Standard error of the mean			
1	2	29	267	4.99	20,749	8,193	107.5
2	16	11	215	2.85	21,008	1,514	25.8
3	2	25	285	3.63	40,916	21,453	113.4
4	16	8	223	3.82	41,472	2,458	39.2
5	2	24	265	4.05	80,402	58,906	104.4
6	16	58	233	5.01	80,716	14,951	53.6
7	2	29	233	4.39	164,353	138,897	56.6
8	16	14	249	4.60	166,582	28,051	82.3

of the larvae died during the last two days of the experiment. Careful examination of dead and dying larvae from chamber 6 revealed what appeared to be a fungus infection. Stained with neutral red, the fungus was very similar to one described and tentatively identified as Sirolpidium sp. by Davis and Loosanoff (1954) and Vishniac (1955). They reported that the fungus caused heavy mortality in some of their cultures but that rapid decay and consumption of larval tissues by protozoa made it easy to miss the fungus. No zoospores were seen in the present instance. The most prominent structure of the fungus was what Davis and Loosanoff described as tubes leading from internal sporangia. These tubes extended as much as 200 microns beyond the shell of the infected larva and in some cases appeared to be composed of very small segments. Sporangia tubes were seen extending out of both dead and dying larvae. There also appeared to be a fungus growth on clumps of pseudofeces and other detritus. From these observations, I cannot be certain if the observed fungus was the direct cause of mortality or if it produced toxic metabolites.

The calculated instantaneous growth coefficients, K , for Experiment III, are shown in Table 7. The data show that larval growth rate increased with increases in algal inflow density for those chambers

having a larval density of 16 per ml (Figure 6). Increases in algal inflow density did not result in higher larval growth rates in those test chambers that had an initial larval density of two per ml. In fact, as Figure 6 shows, larval growth rate declined as algal inflow density exceeded 40,000 cells per ml in chambers containing two larvae per ml.

Chambers 1 and 8 are of particular interest in this experiment. Chamber 1 contained 10,000 larvae that were fed an algal inflow density of 20,000 cells per ml, while chamber 8 contained 80,000 larvae that were fed at approximately 160,000 cells per ml. That is, chambers 1 and 8 were fed the same amount of algae per larva. Yet larvae in chamber 1 showed significantly better growth than those in chamber 8.

The results from Experiment III were similar to those from Experiment II at similar algal inflow densities. However, it appears that the maximum larval growth rate at a density of 16 larvae per ml may occur at an algal density of 160,000 cells per ml or higher. The 95 percent confidence interval estimates of the mean length of larvae in the test chambers are presented in Table 8.

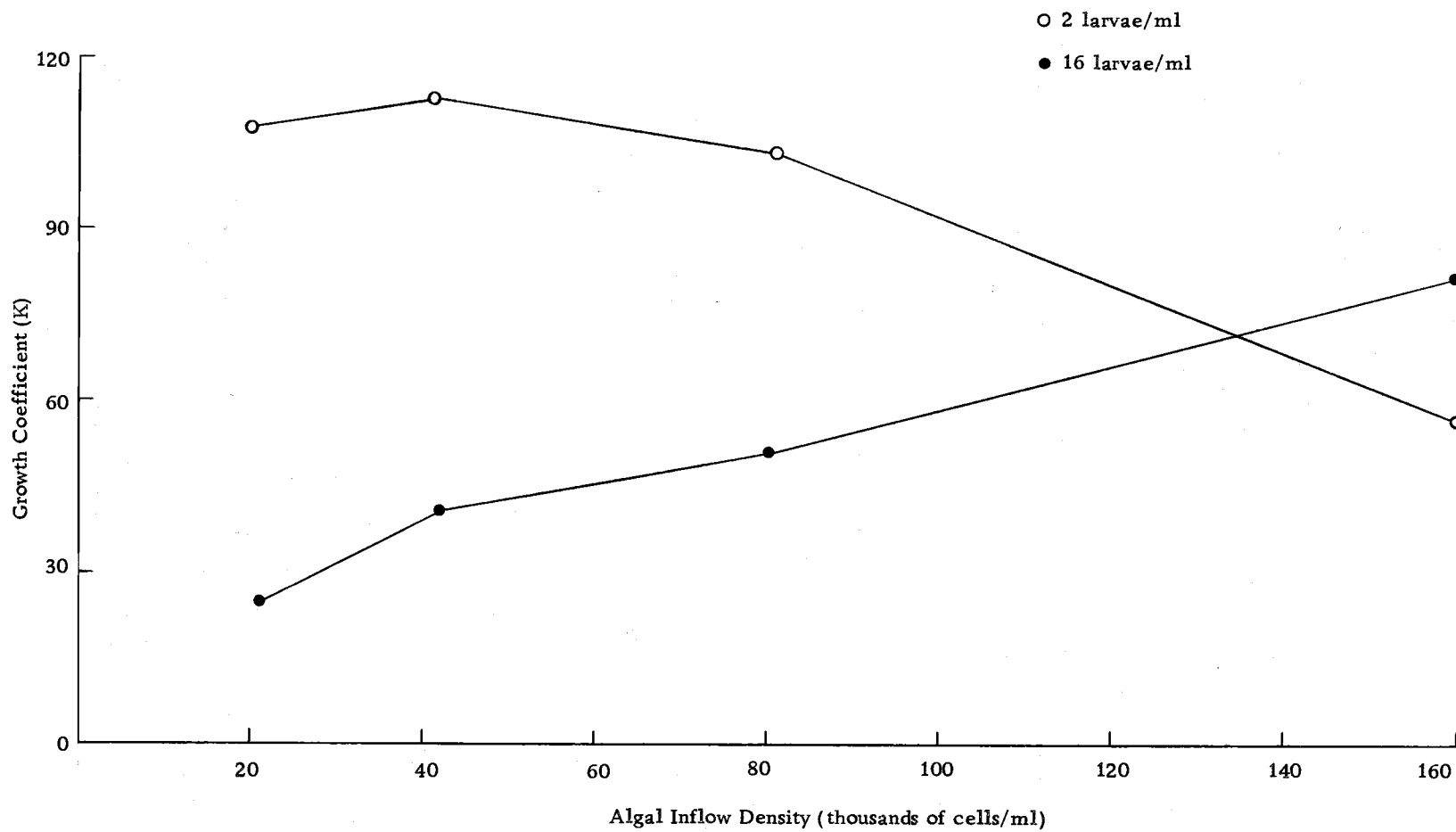


Figure 6. General relationship between algal inflow density and larval growth rate for two larval densities (Experiment III).

Table 8. The final mean length in microns of samples of 75 larvae each and the associated 95 percent confidence interval estimates of the population mean length (Experiment III).

		Algal inflow density (cells/ml)			
		20,000	40,000	80,000	160,000
Initial larval density (larvae/ml)	2	267 $\pm$ 10	285 $\pm$ 7	265 $\pm$ 8	234 $\pm$ 9
	16	214 $\pm$ 6	223 $\pm$ 8	233 $\pm$ 10	249 $\pm$ 9

Interpretation of Growth Results

There are certain similarities in the general shapes of the curves relating larval growth and algal inflow densities. It would appear from the information at hand that the curves relating larval growth rate to algal density are dome shaped.

The results of Experiment I showed a maximum larval growth rate at an algal inflow density of 20,000 cells per ml at larval densities up to eight per ml. Further increases in algal density brought about a marked decline in larval growth for all larval densities tested.

In Experiment II the curves relating larval growth rate to algal inflow density continued to ascend at 80,000 cells per ml (16 larvae/ml) or flatten out (2 larvae/ml). In Experiment III, where algal inflow

densities were increased to 160,000 cells per ml, there was a marked decline in larval growth rate at a larval density of two per ml, but growth of larvae at a density of 16 per ml continued to increase.

There are a number of possible explanations for the growth differences among the three experiments. The relatively low maximum growth rate and rapid decline in growth rate at higher algal densities in Experiment I may have been due in part to the procedures employed in that experiment. The larvae in Experiment I were subjected to slightly colder water than was used in Experiments II and III. The profound effect of temperature on larval growth has been shown by a number of workers including Bayne (1965), Davis and Calabrese (1964 and 1969), Loosanoff and Davis (1963), and Walne (1956 and 1965). All of these workers found that larval growth rates increased rapidly with increases in temperature.

In part, the difference in growth rates among the three experiments may have been due to differences in some growth-determining characteristic of the larvae used in these experiments. Chanley (1954), on the basis of a series of experiments conducted with clam larvae, concluded that there is a wide range of inherited differences even among sibling larvae that affect their growth rates. Personal observations of larval growth in hatchery tanks suggest that progeny from different parental crosses produce widely different growth rates even though they are treated identically.

Since the experiments were not conducted concurrently, differences in the quality of the algal cultures among the experiments were unavoidable and may have affected growth rates of the larvae. Taub and Dollar (1965) reported that the chemical composition of the alga Chlorella varied significantly as light intensity and the composition of the culture medium were varied experimentally. It is possible that the composition and, therefore, the food value of the M. lutheri varied in the present experiments.

Despite the existence of variation in larval growth among the three experiments, there are certain features that are common to all three of the growth curves. Growth rates of the larvae increased as the mean algal inflow density was increased to 20,000 cells per ml. in all three experiments. Subsequent increases in algal density yielded less significant increases in larval growth rates. These results indicate that at larval densities of eight per ml. or less there was little or no advantage to an algal inflow density of more than 20,000 cells per ml.

The reduction of larval growth rates at high algal densities that is indicated by the curves in Figures 4 and 6 is similar to results reported by other workers. Loosanoff et al. (1954) reported that the growth of clam larvae was retarded by overfeeding. The larvae of the mussel, Mytilus edulis, grow best at an intermediate algal density, and any increase beyond the optimum density will

reduce their growth rate (Bayne, 1965). Davis and Guillard (1958) found reduced growth rates for larvae of the Eastern oyster, Crassostrea virginica at high concentrations of M. lutheri. They concluded that at high algal concentrations the larvae were adversely affected by metabolic products of the algae. Walne (1966) reported similar results in his experiments with batch-feeding of I. galbana to the European oyster. He concluded that there was no advantage to batch-feeding at a concentration of 120,000 cells per ml. compared to 30,000 per ml. under the conditions of his experiments.

Clam larvae in heavier than optimum concentrations of algal cells have fewer algal cells in their stomachs than larvae in lighter concentrations of algae (Loosanoff et al., 1953). These authors also reported that larvae left in dense algal concentrations for long periods of time lost the ability to regulate the intake of food and became choked with algae. I also observed this reaction in oyster larvae kept in extremely heavy concentrations of algae. The larvae seemed to become clogged with algae and eventually pulled in their velum and stopped feeding.

My own observations and data indicate that another important cause of reduced growth at high algal densities is the excessive formation of pseudofeces. Yonge (1926) described the pseudofeces of oyster larvae as long strings of mucus with algal cells embedded singly and in clumps along the length of the mucus string. These

strings trail behind and often entangle and trap a swimming larvae.

A normally feeding larva produces a continuous flow of mucus in which food particles are trapped and are carried to the mouth to be ingested (Yonge, 1926). Normally, the bulk of the mucus that is produced is reingested. A larva that is producing excessive pseudofeces is not only removing its food supply from suspension and making it unavailable for consumption, but is producing and losing large amounts of mucus.

The consequences of this type of superfluous feeding is apparent in the growth data from Experiment III. Chamber 8 had an inflow density of 160,000 cells per ml. and an initial larval density of 16 per ml. The larvae reduced the algal concentration to 28,000 cells per ml. Despite a food consumption rate of 1,389 cells per larva per hour, growth in chamber 8 was only fair ($K = 82$). Chamber 1 had an algal inflow density of 20,000 cells per ml. and an initial larval density of two larvae per ml. These larvae removed approximately 989 cells per larva per hour in reducing the algal density to 8,193 cells per ml., but they grew faster ($K = 108$) than the larvae in chamber 8.

The removal of a relatively large number of algal cells from suspension by the larvae in chamber 8 did not result in a correspondingly high growth rate. These results indicate that many of the cells that were removed from suspension by the larvae in chamber 8 were not ingested and assimilated, but were either ingested and not

assimilated or were rejected as pseudofeces.

Millar (1955) described the mechanisms of food movement in the gut of the larvae of the European oyster. He described a muscular pulsation of the digestive diverticula, the site of all absorption, that withdrew partially digested food materials from the stomach. He concluded that it was entirely a matter of chance whether food particles drawn off into the midgut and to the rectum had been in the stomach and digestive diverticula for a short or long time, and therefore, to what extent they had been digested and assimilated. It appears that a larva that was exposed to a very high concentration of algae could ingest algal cells and pass them through its system without gaining any nutritional benefit from them.

FOOD CONSUMPTION

An understanding of the factors that influence food utilization is essential to a complete understanding of the growth of oyster larvae. The food consumption of oyster larvae was studied in two types of experiments. The flowing water experiments have already been described in some detail. Standing water experiments offered a more precise measure of food consumption over short periods of time than the flowing water experiments. These experiments consisted of determining the density of algae in a small container with a known number of larvae at the beginning and the end of a short period of time. The difference between the two counts provided an estimate of the food consumed and was the basis for calculating an instantaneous rate of grazing (to be described later).

It should be pointed out that I determined the number of cells destroyed or removed by the larvae and not necessarily the number of cells that they actually consumed. It became apparent from some of the experiments that there is a significant difference between the amount of algae that the larvae destroyed and the amount that they actually consumed under some conditions.

Larval Food Consumption in a Flowing System

The three constant flow experiments discussed previously permitted a study of the relationship between algal density and larval growth, and, at the same time, provided an opportunity to determine the effect that various larval biomasses had on the algal density in the system.

Samples of larvae were counted and measured every other day during each flowing water experiment. These data were then converted to total larval biomass using the results of previous larval biomass determinations (Table 2, page 19). I then determined the density of algae in the outflow from each test chamber and related that outflow density to the algal inflow density and to the total larval inflow density and to the total larval biomass in each chamber.

The density of algae in the outflow was fairly constant among the three experiments for a given larval biomass. The outflow densities were not determined until at least 24 hours after a water change in Experiment I. Since the carboys containing algae were at room temperature by that time, the procedural differences between Experiment I and Experiments II and III apparently did not affect the food consumption data for Experiment I.

Experiment I

The results of Experiment I show that larval biomass had a very definite effect on algal density. Fluctuations in larval biomass due to the effects of mortality and growth are reflected in changes in the algal density existing in the test chambers (Appendix 1). Chambers 9 and 10, for example, both of which nearly doubled in larval biomass, during the course of the experiment, showed a marked decline in algal density. Because high mortality reduced the larval biomass, chambers 7 and 12 showed an increase in algal density.

Experiment II

Larval biomass again had a profound effect on algal density in Experiment II. Outflow densities in chambers 3 and 7, for example, decreased considerably as the total larval biomass increased during the experiment (Appendix 2). In cases where the algal density was low at the beginning of the experiment such as in chambers 1, 2, and 8, increases in larval biomass, which were considerable in chambers 1 and 8, had little effect on the algal density. In general, when algal density was reduced to 5,000 cells per ml. or less, very large increases in larval biomass were required to further reduce the algal density.

Experiment III

Results of this experiment, as in Experiments I and II, show that the various larval biomasses had a pronounced effect on the algal outflow density. Increases in larval biomass occasioned by the growth of the larvae were reflected by a reduction in the outflow density (Appendix 3). Chambers 4 and 8, for example, showed a marked decline in algal density as the mean size and, thus, the total biomass of the larvae increased. Chamber 6 showed an increase in algal outflow density as larval growth was more than compensated for by a high mortality rate causing a decline in the total larval biomass.

In general, the algal outflow densities obtained in Experiment III were very much the same as those obtained for Experiments I and II for similar larval biomasses and algal inflow rates.

Effect of Larval Biomass on Algal Density

Increases in larval biomass, due either to a high initial larval density or to the growth of larvae, resulted in a decrease in the density of algae in the outflow. Figure 7 shows the effects of different larval biomasses on the density of algae in the test chambers for all three constant flow experiments. Each point represents the measured algal density from a chamber having a known algal inflow density and larval biomass. Because the three experiments included different

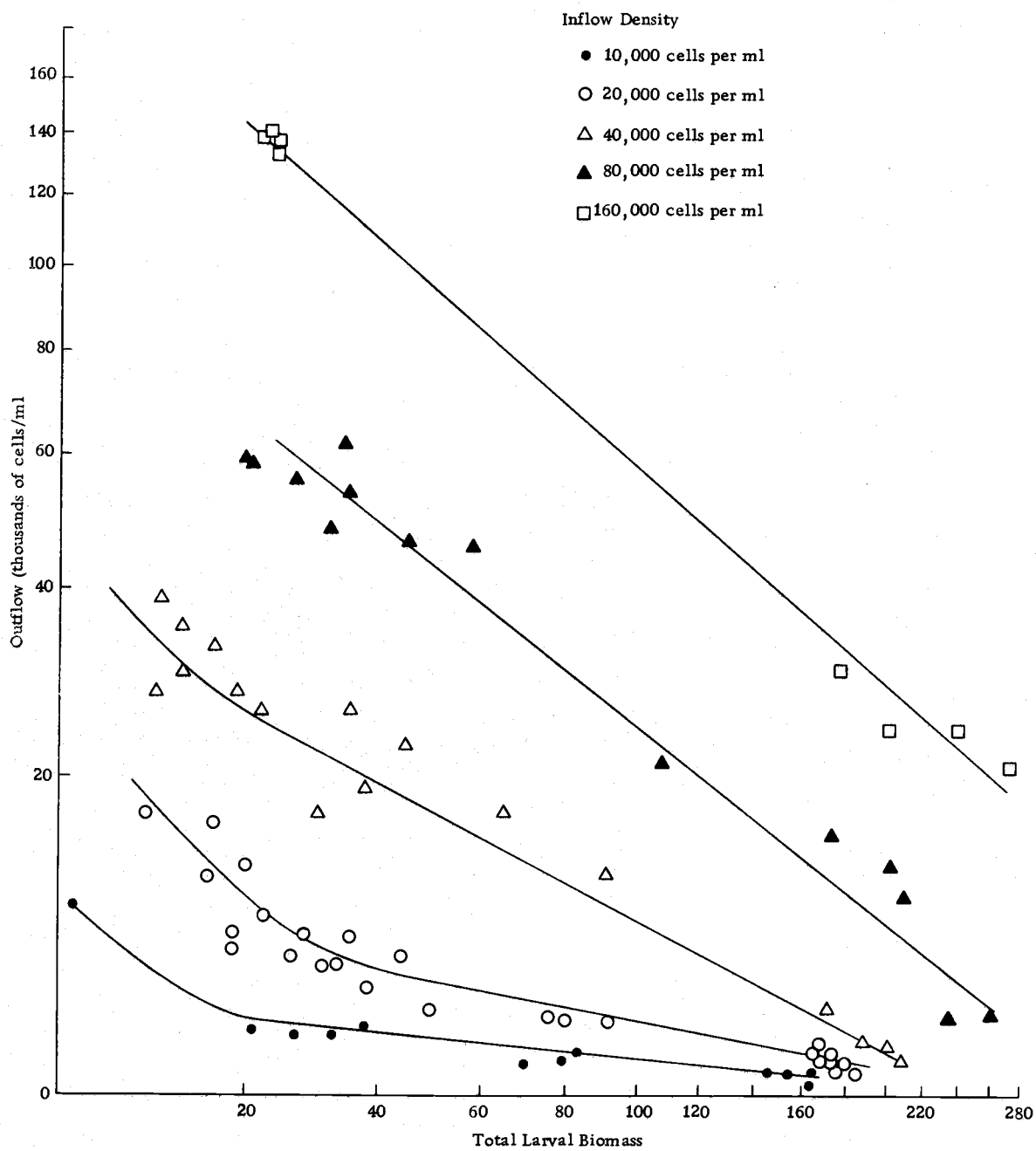


Figure 7. The relationship between total larval biomass in calories and the density of algae in the outflow from constant flow systems (data from Experiments I, II, and III).

ranges of algal inflow densities, four of the five curves include data from two or more experiments. Only the 160,000 cells per ml. inflow curve is from a single experiment, Experiment III.

Below outflow densities of 5,000 cells per ml., large increases in larval biomass were necessary to reduce the algal density by a measurable amount (Figure 7). Counts made on the filtered sea water used in these experiments showed a background of 800 to 1,500 counts per ml. As the algal densities became low, this background count became a more significant percentage of the count and prevented experimental food densities below 1,500 cells per ml. in most cases. Moreover, as the algal density becomes very low, the number of algal cells consumed per larva per unit time approaches zero (Walne, 1965). It becomes more difficult for larvae to trap and consume algal cells when the algal density is reduced to a low level. Therefore, only relatively great increases in larval biomass may be reflected in changes in algal density when the algal density is already low.

Measurement of Food Consumption in Standing Water

Standing water experiments overcame the difficulties involved in varying the temperatures used in the constant flow experiments and the questionable precision of measuring food consumption rates in the complex constant flow apparatus. In these experiments, the density of algae in one-gallon plastic containers was initially determined.

After some known period of time, usually 18 or 24 hours, the algal density was determined again. These data were then used to calculate an instantaneous coefficient of food removal called the grazing rate.

Calculation of Grazing Rate

Grazing rate (G) is defined by Cushing (1968) and has been applied to studies of bivalve larvae by Bayne (1965) and by Walne (1965), among others. G is an instantaneous coefficient intended to describe the change in the number of algal cells due to the simultaneous effects of algal reproduction and the destruction of algal cells due primarily to the presence of some grazing animal. The rate of change in the number of algal cells in a closed container is equal to the rate of reproduction of the algae, R, minus the rate of destruction of algal cells, G, times the number of algal cells present at time t, N_t .

That is:

$$\frac{dN}{dt} = (R - G) N_t$$

$$\text{or} \quad \frac{dN}{N_t} = (R - G)dt$$

$$\text{integrating} \quad \log_e N_t = (R - G)t + c$$

$$\text{at } t=0, N=N_0$$

$$\text{so} \quad \log_e N_0 = (R - G)0 + c$$

$$\text{and} \quad \log_e N_0 = c$$

$$\begin{array}{ll}
\text{substituting} & \log_e N_t = (R-G)t + \log_e N_o \\
\text{or} & \log_e N_t - \log_e N_o = (R-G)t \\
\text{and} & \frac{1}{t} \log_e (N_t/N_o) = R-G \\
\text{subtracting} & \frac{1}{t} \log_e (N_t/N_o) - R = -G \\
\text{so} & G = R - \frac{1}{t} \log_e (N_t/N_o)
\end{array}$$

where N_o is the number of algal cells present per ml. at $t=0$, i. e., the initial algal density; N_t is the algal density per ml. after time t ; t is the time in days; and R is the reproductive rate of the algae under the conditions of the experiment in the absence of larvae. R was measured and found to be negligible for each standing water experiment.

$$R = \frac{1}{t} \log_e (N_t/N_o)$$

Note that in calculating grazing rate in this way no consideration is made for the number of larvae present in the container. In order to make the grazing rate values that I obtained from any one experiment comparable, I was careful to insure that each test container within an experiment received the same number of larvae.

Effects of Temperature on Grazing Rate

Water baths of 10, 16, 19, and 24°C. were employed to create temperature differences. Three translucent plastic containers were used at each temperature. Three liters of sterile sea water adjusted to 25 ppt. salinity were added to each container. Three-thousand

six-hundred (3,600) larvae that averaged 229 microns in length were added to two containers and the third, with no larvae, was used to measure the reproductive rate of the algae at each temperature.

Enough I. galbana from a pure culture was added to each container to make an initial concentration of about 40,000 cells per ml. Counts were made for each container immediately after the algae were added. After 18 hours, the algal densities were again determined. These data were then used to calculate the grazing rate for the larvae under each of the conditions described (Table 9 and Figure 8).

In this experiment, grazing rate more than doubled with every increase of five degrees centigrade between 10° and 25° C. Other workers, including Walne (1965) and Ukeles and Sweeny (1969) found similar relationships between food consumption and temperature for other species of oysters. No attempt was made in the present study to relate the increases in food consumption occasioned by high temperatures with increases in growth rate. It has been shown by others that larval growth does increase rapidly with increases in temperature (Bayne, 1965, and Davis and Calabrese, 1964).

This experiment shows the relationship between food consumption and temperature for a single batch of larvae of only one size and at one initial algal density. For that reason, the conclusions that may be drawn from the results are necessarily limited. However, there

Table 9. Effect of temperature on the rate of food consumption of Pacific oyster larvae¹

Temperature (C)	24	24	19	19	16	16	10	10
Initial algal concentration per ml	42,110	43,232	42,890	43,332	43,714	43,626	44,210	42,938
Final algal concentration per ml	11,304	11,104	25,930	31,186	37,342	36,500	42,378	40,538
Cells caught per larva per hour	1,069	1,116	589	422	220	247	64	83
Grazing rate	1.240	1.286	.659	.487	.215	.236	.034	.049
Mean grazing rate	1.26		.573		.225		.041	

¹ Mean length, 229 microns; 3,600 larvae in 3 L. of water.

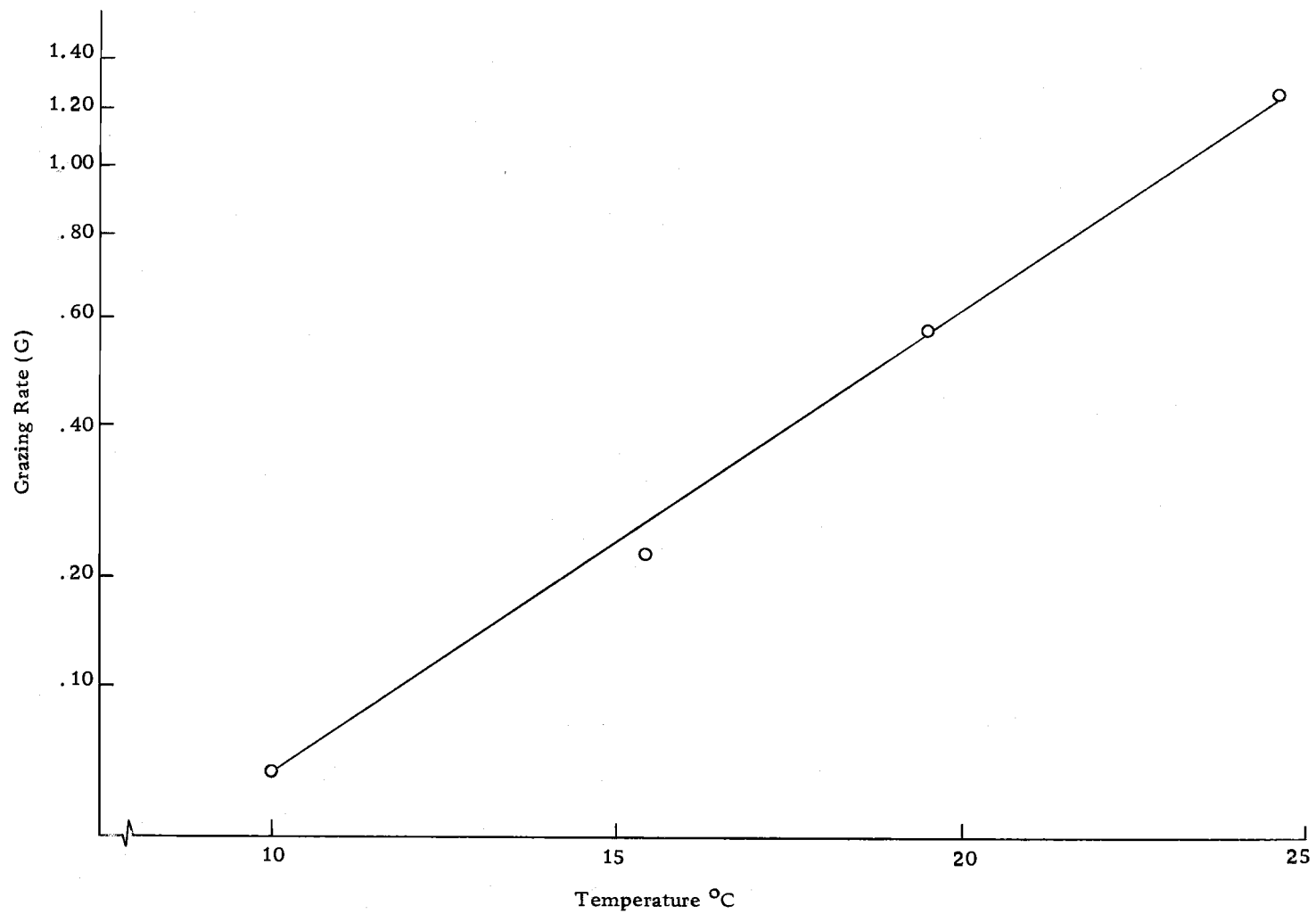


Figure 8. Effect of temperature on the grazing rate of Pacific oyster larvae.

can be little doubt that larval grazing rate increases rapidly with increases in temperature.

Effect of Larval Size on Grazing Rate

This experiment was conducted over a period of several months using larvae from a number of different parental crosses. Each test was conducted using translucent plastic jars of one gallon capacity. A constant temperature of 20°C. and an initial concentration of 40,000 cells of M. galbana per ml. was used in all cases. Three thousand larvae were added to three liters of sterile sea water at 25 ppt. salinity. An additional container with no larvae was used in each test to measure the reproductive rate of the algae.

The grazing rate of oyster larvae was found to increase exponentially with shell length (Figure 9) and linearly with caloric content per larva (Figure 10 and Appendix 4). These data support the contention that caloric content offers a more realistic measure of the size and, therefore, of the food requirements of oyster larvae than shell length. For example, from Figure 10, doubling the caloric content per larva from .002 to .004 calories per larva resulted in a doubling of the grazing rate of from .51 to 1.06.

Walne (1956) reported that large larvae of the European oyster did not seem to remove any more algae than small ones. He later reported (Walne, 1966) that 180 micron larvae consumed about 20,000

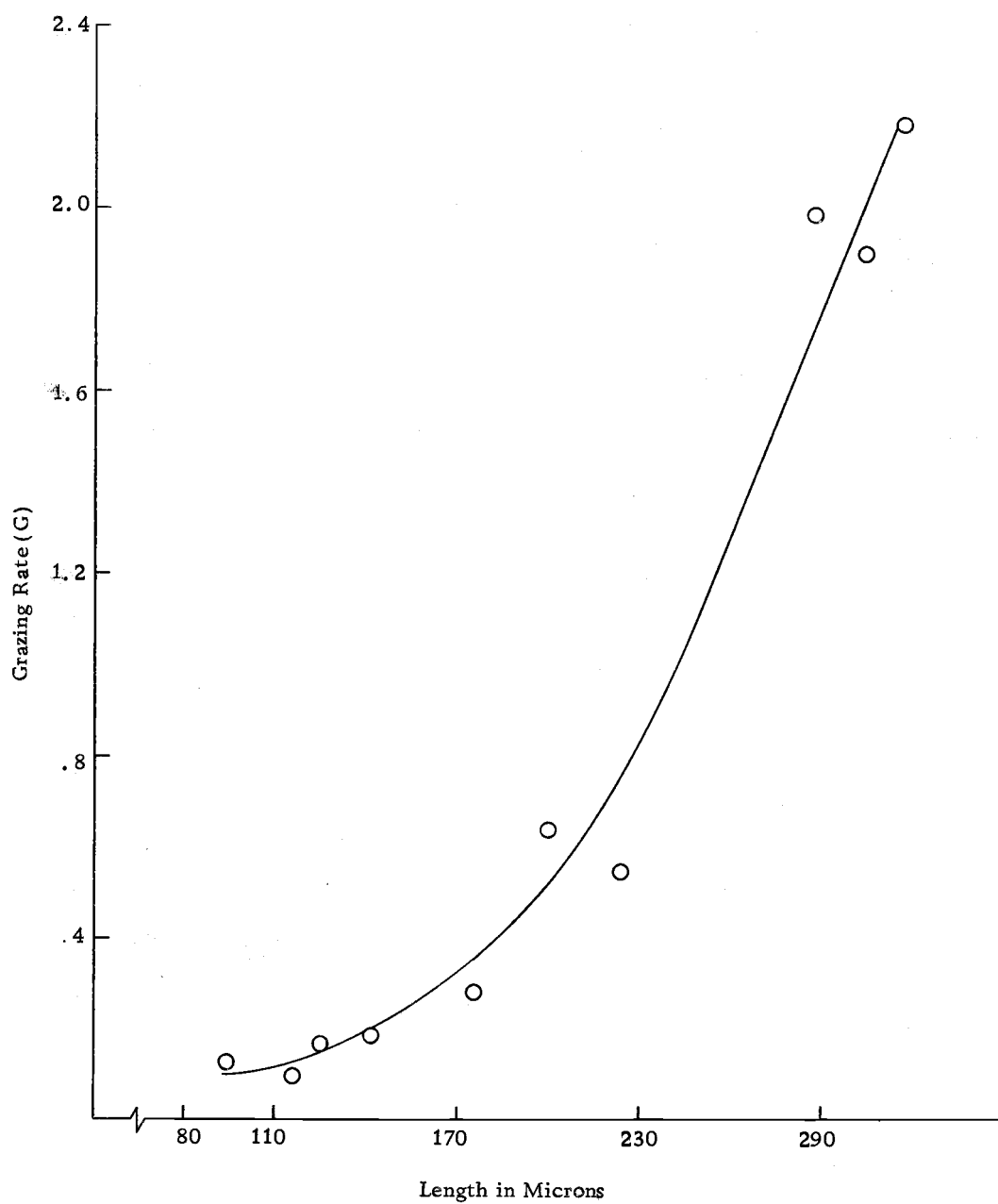


Figure 9. The relationship between larval shell length and grazing rate.

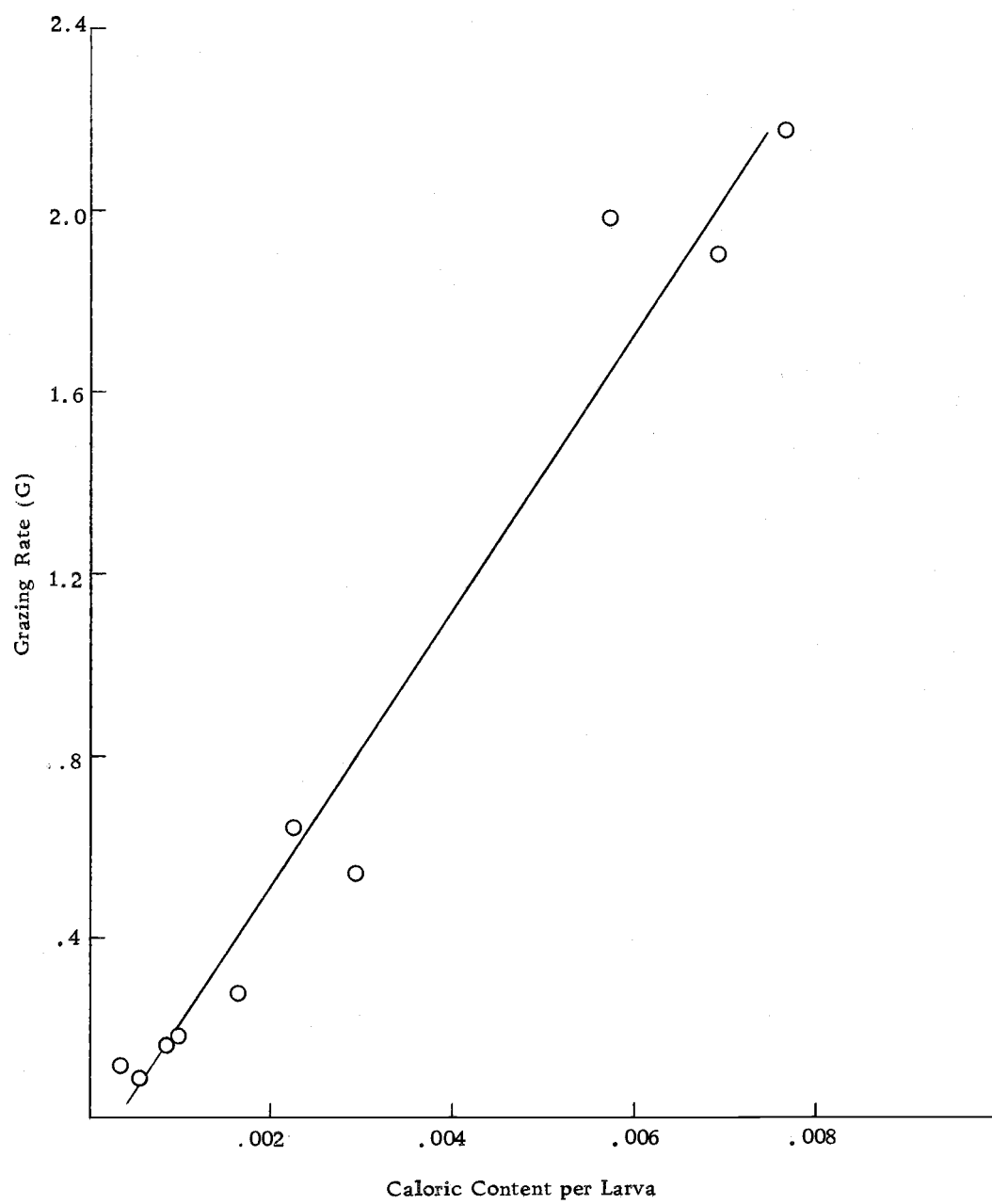


Figure 10. The relationship between caloric content per larva and grazing rate.

cells per larva per day, while 280 micron larvae consumed 60,000 cells per larva per day. Converting to approximate caloric content per larva, this means that he found a three-fold increase in food consumption as the caloric content per larva increased from .0017 to .0054, also a three-fold increase.

These experiments as well as other less rigorous observations indicate that the food consumption rate of very small larvae is extremely low and is, in fact, not measurable with the Coulter Counter until the larvae are about 90 microns in length. Ukeles and Sweeny (1969), using ^{14}C labeled algae, reported that 75 micron larvae of the Eastern oyster, C. virginica, consumed roughly 190 cells per larva per day. Even in high larval densities a food consumption rate of that magnitude would not be measurable using the techniques of the present study.

Effect of Algal Concentration on Grazing Rate

Three thousand 229 micron larvae were added to eight containers that contained 1,500 ml of sterile sea water adjusted to 25 ppt. salinity at a constant temperature of 20°C . Two containers were adjusted to each of four concentrations of M. galbana, 7,000, 25,000, 50,000, and 100,000 cells per ml. A ninth container at 25,000 cells per ml without larvae was used to measure the reproductive rate of the algae under the conditions of the experiment. Counts were made for each

container immediately after the algae were added. The algal concentration was again determined after 18 hours. From these data, grazing rates were calculated for each of the four initial algal concentrations. The results are given in Table 10 and Figures 11 and 12.

Grazing rate, which is actually a measure of the effect of the larval population on the algal population, was found to be high at low algal densities and to decrease with increases in initial algal concentration (Table 10 and Figure 11); at low algal densities the larvae destroy a larger proportion of the algal population than they do at high algal densities. However, the number of algal cells destroyed per larva per hour increases with increased algal density (Table 10 and Figure 12). There is no apparent inhibition of the food trapping ability of the larvae at the algal densities tested. I did not distinguish between those algal cells that were ingested and wholly or partially assimilated and those that were rejected as pseudofeces.

There are indications that grazing rate reaches a minimum at high algal densities and then begins to level off. Bayne (1965) reported that he found no change in the grazing rate of mussel larvae with changes in the density of I. galbana. However, he was working with algal densities for the most part that exceeded 40,000 cells per ml. I found that above 40,000 cells per ml, the relationship is indeed much less apparent (Figure 11). Walne (1965) found that the grazing rate of European oyster larvae declined with increases in algal density up to

Table 10. The effect of initial algal density on the food consumption rate of Pacific oyster larvae¹

Initial algal density (cells/ml.)	Final algal density (cells/ml.)	Grazing rate (G)	Mean grazing rate	Number of cells consumed per larva per hour	Mean number of cells consumed per larva per hour
7,788	2,790	1.322	1.364	139	142
7,916	2,656	1.406		146	
26,290	8,554	1.451	1.18	492	455
29,428	14,384	0.908		417	
51,486	31,568	0.607	0.68	553	594
50,816	27,904	0.753		636	
102,368	68,466	0.491	0.47	942	921
104,108	71,698	0.451		900	

¹ Larvae were 229 microns long; 3,000 larvae were placed in 1,500 ml. of seawater in each case, temp. = 20°C.

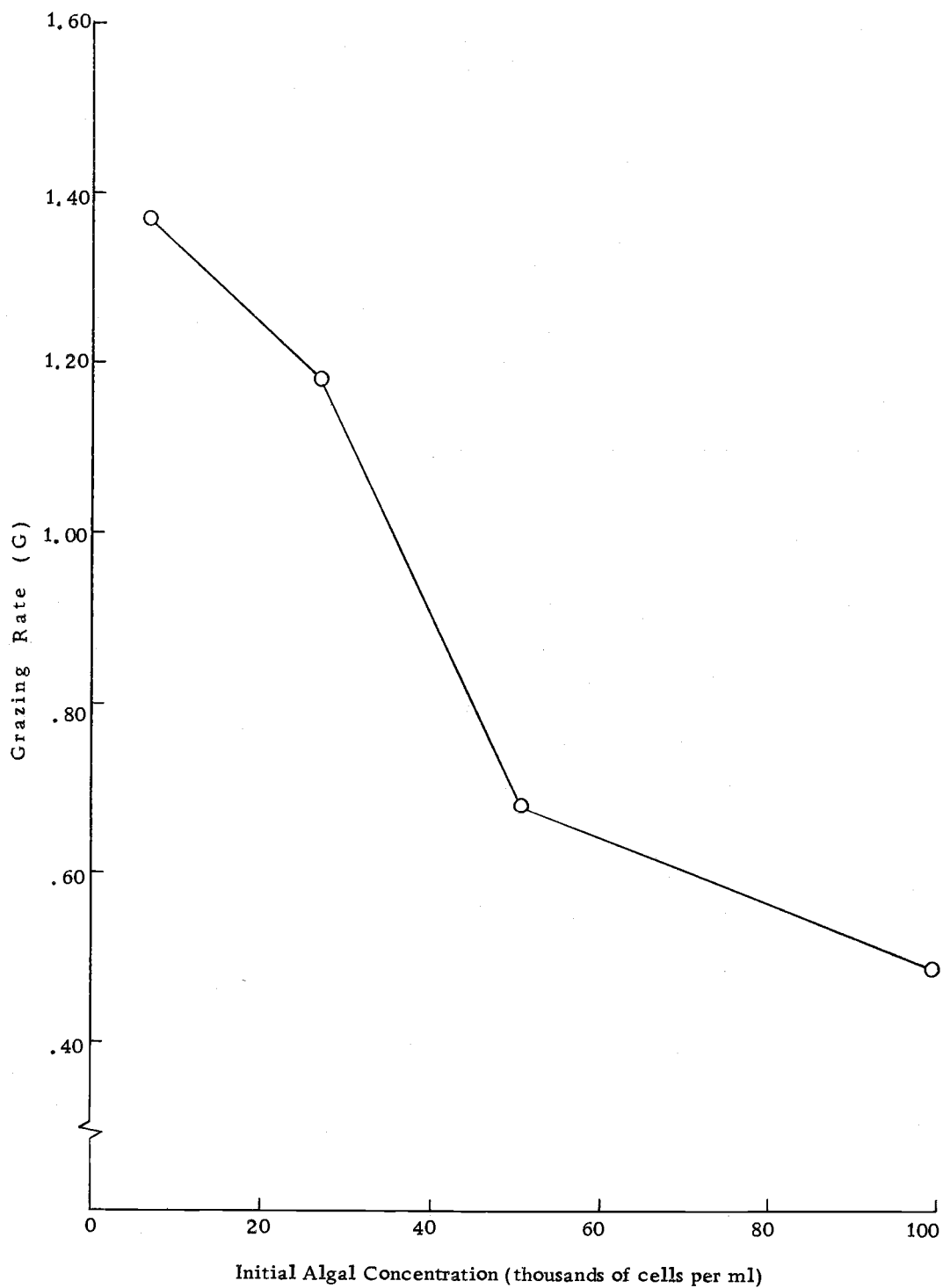


Figure 11. The relationship between initial algal concentration and grazing rate of Pacific oyster larvae.

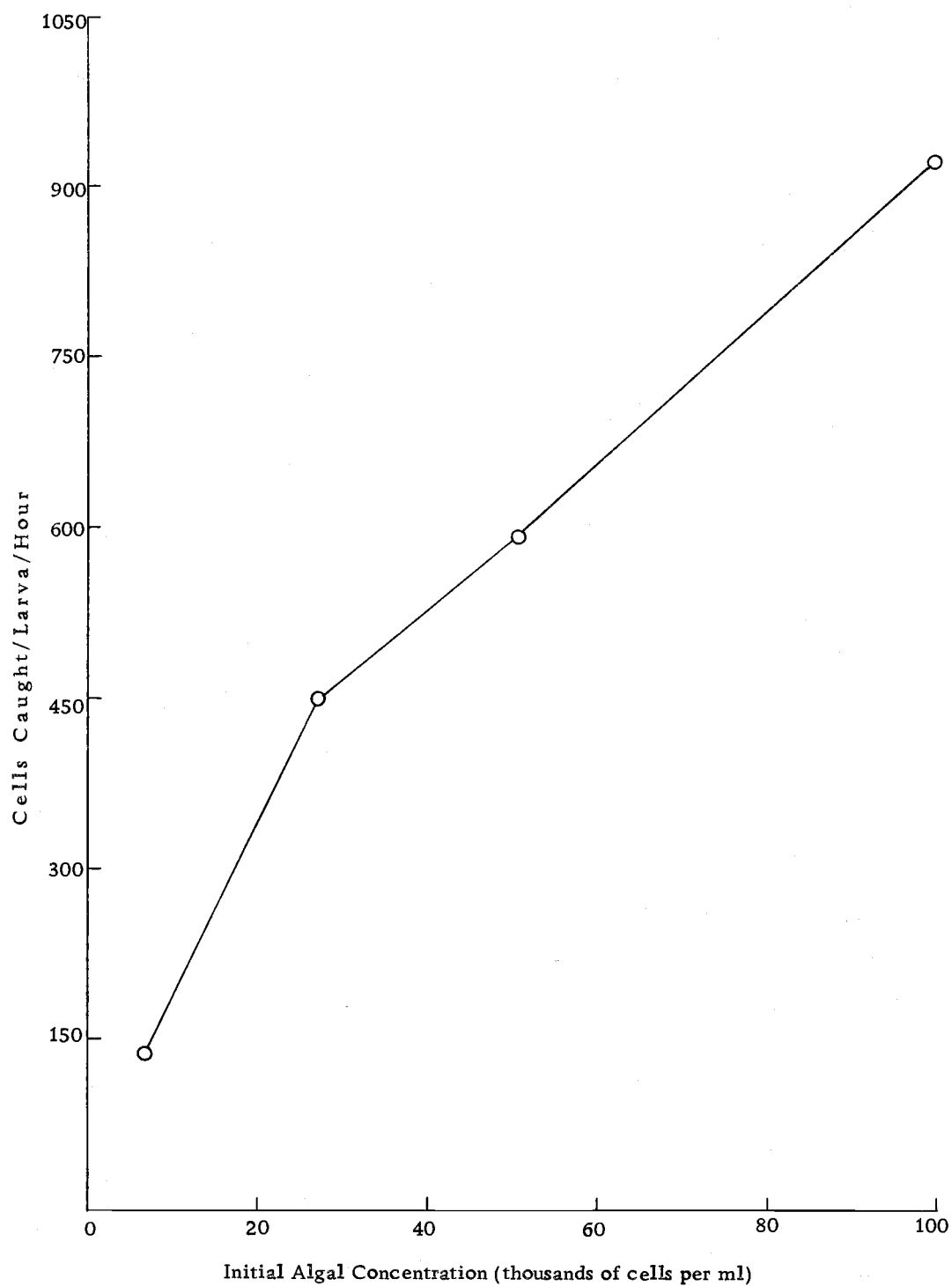


Figure 12. The relationship between initial algal concentration and the number of cells caught per larva per hour.

about 50,000 cells per ml. Above 50,000 cells per ml., he found very little difference in grazing rates as algal density increased.

In order for the grazing rate to level off as algal density increases, the number of algal cells destroyed per larva per unit time must continue to increase so that the fraction of the algal population that the larvae destroy becomes a constant. I found that the number of algal cells destroyed per larva per hour increased steadily with increasing algal density. Similar results have been reported for European oyster larvae (Walne, 1965).

It is difficult to compare the absolute values obtained for the grazing rate of larvae by other workers because the conditions and the duration of the experiments strongly influence the results. As the larvae consume algal cells, the algal density declines, so it becomes increasingly difficult for the larvae to obtain food. The number of cells consumed per larva per hour declines as the experiment progresses. If the experiment is terminated in 18 hours, a higher hourly consumption rate will be obtained than if the experiment were allowed to run for 24 hours. Walne's 1965 experiments lasted from 20 to 27 hours. Bayne conducted his experiments for 24 hours with small volumes of water and only one larva per container. My tests were conducted for a period of 18 hours using relatively large containers and high larval densities.

APPLICATION TO HATCHERY OPERATIONS

My studies have shown that a constant flow feeding system can be regulated to maintain an algal density that is most favorable for larval growth. At larval densities up to eight per ml, larval growth rate increases to a maximum with increases in algal density, but growth declines as algal density exceeds the optimum level. I illustrate this relationship in Figure 13 with data from Experiment I. Under the conditions of this experiment, the maximum growth rate of larvae was achieved at about 20,000 algal cells per ml in the inflowing water where the larval density ranged from 0.5 to about 6.0 larvae per ml.

The technique of batch-feeding oyster larvae which is commonly used is subject to a number of disadvantages. In order to supply larvae with enough food to last them 24 or 48 hours in one feeding, it is necessary to raise the algal concentration to a high level. At the initially high algal density, the larvae feed actively but an undetermined amount of their food supply is tied up as pseudofeces. Then, as the algal density declines with time, the larvae become less able to obtain food. In other words, it is virtually impossible not to overfeed the larvae part of the time and to starve them part of the time with batch-feeding.

Since the conditions of the constant flow system tested in this study differed in so many ways from the batch-fed hatchery tanks, it is difficult to compare the growth rates obtained with those commonly

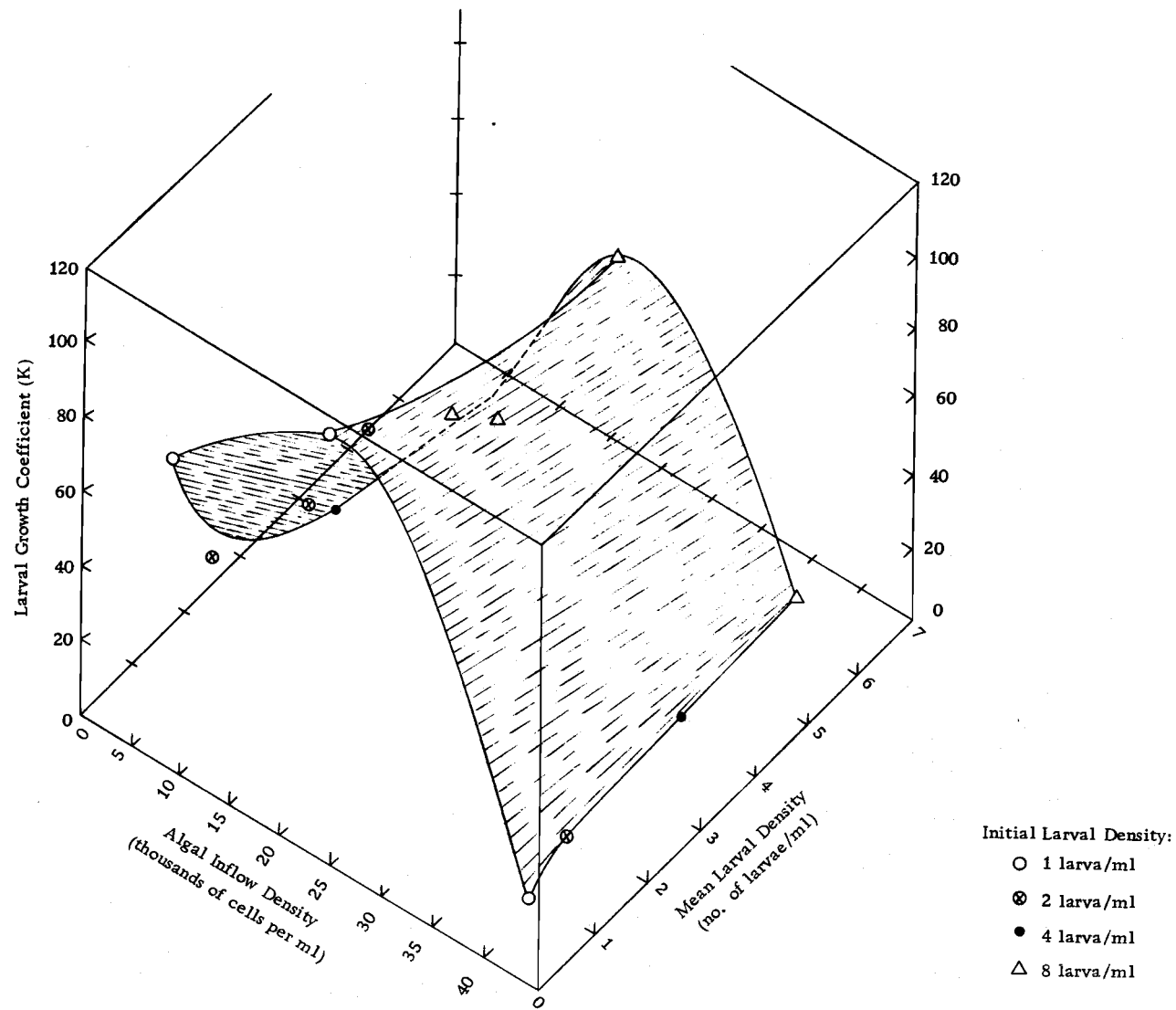


Figure 13. The relationship between algal inflow density, mean larval density and larval growth rate (data from Experiment I).

experienced in the hatchery. In addition to the higher temperature (these experiments were conducted at 20°C., hatchery tanks are maintained at 24°C.), the hatchery-reared larvae had the advantage of being fed both Monochrysis and Isochrysis. For reasons discussed previously, I used only Monochrysis in the constant flow systems. It has been shown that better growth can be obtained with a mixture of these two species than with either one of them alone (Davis and Guillard, 1958; and Bayne, 1965).

In spite of these differences, growth rates obtained from the experimental systems were found to be only slightly lower than those commonly experienced in the hatchery. These results indicate that the use of a constant flow feeding system utilizing higher temperatures and a mixture of algal species in the hatchery could result in improved growth rates.

SUMMARY

1. This study of the food consumption and growth of Pacific Oyster larvae was conducted in conjunction with the development of a pilot oyster hatchery at Oregon State University's Marine Science Center. It is the broad aim of this work to determine the feasibility of commercial oyster hatcheries in the Pacific Northwest.
2. Dichromate wet oxidations were performed on samples of Pacific Oyster larvae of various sizes. From these data, the caloric content per larva was shown to increase exponentially with increases in shell length.
3. A constant flow apparatus was used in three experiments in which algal inflow concentrations and larval density were varied in a factorial design. By determining the density of algae flowing into and out of each test chamber and by monitoring the growth and survival of the larvae in the chambers, I studied the effects of the various algal concentrations and larval densities on the food consumption and growth of the larvae.
4. In the constant flow experiments larval growth rates apparently increased with increases in algal inflow density up to about 20,000 cells per ml. Larval growth rates increased less significantly with further increases in algal density. In most cases, larval growth declined at algal inflow densities over 40,000 cells per ml. Only fair growth of larvae in dense cultures (16 larvae per ml) was

attained despite heavy feeding (up to 160,000 cells per ml inflow).

5. The food consumption of oyster larvae was studied in the constant flow experiments and in three standing water experiments.

6. Increases in total larval biomass in the constant flow test chambers, due either to a high initial larval density or to the growth of the larvae, resulted in a decrease in the density of algae flowing out of the chambers. Below algal outflow densities of 5,000 cells per ml, large increases in larval biomass were necessary to reduce the algal density by a measurable amount.

7. Larval food consumption in the standing water experiments was measured both as cells consumed per larva per hour and as grazing rate, G .

$$G = R - (1/t) \log_e (N_t / N_o)$$

where N_o is the algal density per ml at the beginning of the experiment, N_t is the algal density at the end of the experiment, t is the time in days, and R is the reproductive rate of the algae.

8. Larval grazing rate was found to increase exponentially with increases in temperature from 10°C. to 24°C. Grazing rate more than doubled with each increase of 5°C.

9. The grazing rate of Pacific Oyster larvae was found to increase exponentially with increases in shell length and linearly with caloric content per larva.

10. Larval grazing rate was found to be high at low algal densities and to decrease with increases in algal density. However, the number of algal cells destroyed per larva per hour increased with increases in algal density.

11. A constant flow feeding system has the advantage of being able to maintain food density at an optimum level and thus to maximize larval growth using a minimum of algae. It is impossible to control food density over long periods of time with batch-feeding. It may be that a modified constant flow feeding system could be adapted for use in the hatchery to improve larval growth.

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APPENDICES

Appendix 1. Algal inflow density, larval size in microns and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment I).

Chamber number	Date	Algal inflow density (cells/ml)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larvae	Total caloric content of larval population	Algal outflow density (cells/ml)
1	11/3	5,724	203	4,080	.0023	7.1	4,488
1	11/7	6,162	231	2,650	.0033	8.6	4,080
1	11/10	5,484	268	2,850	.0048	13.7	3,022
2	11/3	5,700	227	7,900	.0031	24.5	2,520
2	11/7	5,182	248	7,050	.0039	27.6	2,892
2	11/10	5,346	263	7,550	.0045	33.6	2,222
3	11/3	5,394	193	15,140	.0021	31.8	1,850
3	11/7	5,310	213	14,950	.0026	39.0	1,912
3	11/10	4,990	222	16,400	.0030	48.4	1,470
4	11/3	5,166	192	38,800	.0021	81.1	1,192
4	11/7	5,322	196	33,750	.0021	71.9	1,183
4	11/10	5,136	196	33,000	.0021	70.3	1,458
5	11/3	11,838	199	900	.0022	1.9	11,234
6	11/3	11,114	192	7,640	.0021	15.9	9,776
6	11/7	10,012	212	6,100	.0026	15.6	9,662
6	11/10	10,630	237	4,315	.0034	14.7	6,796

Appendix 1. Algal inflow density, larval size in microns and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment 1). (Cont.)

Chamber number	Date	Algal inflow density (cells/ml.)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larvae	Total caloric content of larval population	Algal outflow density (cells/ml.)
7	11/3	11,144	195	14,140	.0021	29.7	7,914
7	11/7	11,718	---	---	---	---	11,032
7	11/10	11,854	208	1,145	.0025	2.9	11,448
8	11/3	11,884	202	37,165	.0023	84.7	1,910
8	11/7	11,260	205	33,450	.0024	78.9	1,778
8	11/10	11,784	208	28,500	.0025	71.0	1,414
9	11/3	22,104	239	4,700	.0035	16.6	16,722
9	11/7	21,032	287	2,700	.0058	15.7	12,810
9	11/10	22,216	309	2,565	.0072	18.5	8,412
10	11/3	22,726	207	8,165	.0025	20.2	13,012
10	11/7	21,192	252	6,850	.0041	27.7	8,684
10	11/10	22,392	303	6,800	.0068	46.2	7,460
11	11/3	23,166	204	---	---	--	21,430
11	11/7	21,770	---	0	---	0.0	18,630
12	11/3	22,350	216	34,500	.0027	93.5	3,430
12	11/7	21,668	239	23,000	.0035	80.7	3,142
12	11/10	22,268	269	6,050	.0048	29.1	14,448

Appendix 1. Algal inflow density, larval size in microns and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment I). (Cont.)

Chamber number	Date	Algal inflow density (cells/ml.)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larvae	Total caloric content of larval population	Algal outflow density (cells/ml.)
13	11/3	41,522	207	5,000	.0025	12.4	35,944
13	11/7	40,652	212	3,830	.0026	10.0	28,160
13	11/10	42,206	254	3,100	.0041	12.8	30,536
14	11/3	42,944	216	7,250	.0027	19.7	28,028
14	11/7	42,184	239	7,350	.0035	25.9	17,696
14	11/10	42,307	282	7,450	.0055	41.1	---
15	11/3	42,798	200	15,665	.0022	34.9	29,546
15	11/7	41,256	223	11,550	.0030	34.3	26,998
15	11/10	43,308	282	11,950	.0055	65.9	17,406
16	11/3	43,052	207	36,580	.0025	90.4	12,635
16	11/7	41,552	244	30,350	.0037	110.8	5,792
16	11/10	43,638	277	24,650	.0053	129.4	6,016

Appendix 2. Algal inflow density, larval size in microns and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment II).

Chamber number	Date	Algal inflow density (cells/ml.)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larvae	Total caloric content of larval population	Algal outflow density (cells/ml)
1	1/11	10,348	193	10,063	.0021	20.7	3,278
1	1/13	11,352	218	9,800	.0028	27.1	3,122
1	1/15	10,820	235	10,000	.0034	33.5	3,690
1	1/17	12,200	243	10,300	.0037	38.1	3,798
2	1/11	11,844	193	79,516	.0021	163.8	866
2	1/13	10,988	198	75,000	.0022	164.3	1,386
2	1/15	10,644	200	65,500	.0023	146.7	1,186
2	1/17	11,670	199	70,500	.0022	155.1	1,128
3	1/11	19,058	193	9,599	.0021	19.8	7,758
3	1/13	20,752	226	10,300	.0030	31.0	8,594
3	1/15	21,672	253	9,300	.0041	38.1	5,470
3	1/17	22,466	276	9,600	.0052	49.7	4,032
4	1/11	19,558	193	81,950	.0021	168.8	1,596
4	1/13	20,074	203	74,000	.0023	173.2	2,078
4	1/15	21,084	201	76,000	.0022	171.8	2,746
4	1/17	23,674	203	72,000	.0023	168.5	2,256

Appendix 2. Algal inflow density, larval size in microns and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment II). (Cont.)

Chamber number	Date	Algal inflow density (cells/ml.)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larvae	Total caloric content of larval population	Algal outflow density (cells/ml.)
5	1/11	39,206	193	9,744	.0021	20.5	36,068
5	1/13	44,614	---	2,200	---	---	43,668
5	1/15	39,342	---	0	---	---	39,366
5	1/17	35,366	---	0	---	---	33,460
6	1/11	39,162	193	81,366	.0021	170.9	26,106
6	1/13	40,658	209	38,500	.0025	96.3	32,522
6	1/15	41,148	219	18,000	.0028	50.4	38,008
6	1/17	---	236	8,500	.0034	28.9	---
7	1/11	77,340	193	9,947	.0021	20.5	59,366
7	1/13	82,832	231	10,100	.0032	32.7	48,500
7	1/15	78,666	270	9,200	.0049	45.1	46,728
7	1/17	81,206	296	9,300	.0063	58.6	46,424
8	1/11	78,924	193	81,950	.0021	168.2	3,342
8	1/13	81,620	219	77,500	.0028	217.0	3,040
8	1/15	74,572	232	72,000	.0033	234.0	3,858
8	1/17	71,284	247	68,500	.0038	261.7	3,864

Appendix 3. Algal inflow density, larval size in microns, and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment III).

Chamber number	Date	Algal inflow density (cells/ml.)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larvae	Total caloric content of larval population	Algal outflow density (cells/ml.)
1	2/24	22,088	199	10,349	.0022	22.9	9,898
1	2/28	21,040	219	9,400	.0028	26.7	7,130
1	3/2	20,814	243	9,300	.0037	33.9	8,714
1	3/4	20,376	267	7,500	.0047	35.3	9,476
2	2/24	21,498	199	78,523	.0022	174.3	1,472
2	2/28	21,852	206	74,500	.0024	175.8	1,442
2	3/2	21,504	210	70,000	.0025	175.0	1,206
2	3/4	20,472	215	70,000	.0027	186.2	1,946
3	2/24	41,608	199	9,954	.0022	22.1	26,742
3	2/28	43,052	235	9,200	.0034	30.8	17,022
3	3/2	41,022	250	9,500	.0039	37.5	19,954
3	3/4	40,936	285	7,700	.0057	43.9	23,346
4	2/24	43,242	199	77,480	.0022	172.0	4,374
4	2/28	40,238	211	75,000	.0025	188.3	2,584
4	3/2	42,538	218	72,500	.0028	200.1	1,788
4	3/4	40,972	223	71,000	.0029	207.3	1,686

Appendix 3. Algal inflow density, larval size in microns and calories, number of larvae present, total caloric content of the larval population, and the density of algae in the outflow for each test chamber (Experiment III). (Cont.)

Chamber number	Date	Algal inflow density (cells/ml)	Mean length of larvae (microns)	Number of larvae present	Caloric content per larva	Total caloric content of larval population	Algal outflow density (cells/ml)
5	2/24	79,450	199	9,875	.0022	21.9	58,582
5	2/28	80,862	229	8,800	.0032	27.8	56,510
5	3/2	83,274	249	8,900	.0039	34.8	62,130
5	3/4	77,422	265	7,700	.0046	35.5	54,482
6	2/24	81,344	199	79,050	.0022	175.5	15,324
6	2/28	80,858	216	75,000	.0027	203.3	13,124
6	3/2	81,112	226	68,500	.0030	207.6	14,366
6	3/4	77,384	233	33,500	.0032	108.2	21,016
7	2/24	165,064	199	9,875	.0022	21.9	138,056
7	2/28	163,248	219	8,600	.0028	24.1	132,752
7	3/2	162,640	220	8,400	.0028	23.8	139,480
7	3/4	162,064	234	7,000	.0033	23.1	140,176
8	2/24	164,512	199	80,850	.0022	179.5	30,850
8	2/28	170,648	220	73,500	.0028	206.5	24,958
8	3/2	165,488	231	74,500	.0032	240.6	38,750
8	3/4	162,816	249	69,500	.0039	274.5	20,942

Appendix 4. The relationship between larval size expressed as mean length and as the estimated caloric content per larva and the rate of larval food consumption.¹

Size of larvae		Initial algal density (cells/ml.)	Final algal density (cells/ml.)	Grazing rate (G)	Mean grazing rate	Number of cells consumed per larva per hour	Mean number of cells consumed per larva per hour
Mean length (microns)	Caloric content per larva						
94	.00035	42,912	39,122	.089	.122	79	107
94	.00035	44,166	37,458	.156		134	
116	.00056	43,932	39,500	.088	.097	92	101
116	.00056	44,802	39,458	.108		111	
127	.00087	42,898	36,070	.154	.162	142	149
127	.00087	43,534	36,022	.171		157	
142	.00097	43,618	35,484	.202	.197	169	163
142	.00097	42,600	35,002	.193		158	
176	.00165	40,882	28,768	.329	.327	252	253
176	.00165	41,354	29,198	.325		253	
200	.00225	43,062	23,720	.577	.648	403	437
200	.00225	43,340	20,712	.719		471	
224	.00295	44,658	25,538	.555	.553	398	388
224	.00295	42,484	24,406	.552			
288	.00572	41,472	4,638	2.166	1.98	767	744
288	.00572	41,286	6,618	1.809			
306	.00691	41,466	5,984	1.914	---	735	---
315	.00765	44,604	3,162	2.628	2.18	863	809
315	.00765	43,962	7,694	1.724		756	

¹ 6,000 larvae in 3L. of water were used in all cases.