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GROWTH OF OYSTER LARVAE, *CRASSOSTREA VIRGINICA*, OF VARIOUS SIZES IN DIFFERENT CONCENTRATIONS OF THE CHRYSOPHYTE, *ISOCHRYSIS GALBANA*

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ABSTRACT

*Oyster larvae (*Crassostrea virginica*) of seven different size groups were fed different concentrations of* Isochrysis galbana. *The optimum concentration of* I. galbana *for each size group was determined by measuring the increase in mean length of larvae during the 48 hr test period. The optimum concentration increased with increasing larval size and ranged from 2.5 µl of packed cells per liter of larval culture for larvae 74 µ long to 32.5 µl of packed cells per liter of larval culture for larvae averaging 246 µ in length. It was found to be more efficient to increase the* Isochrysis *concentration as the larvae grew than to feed the larvae at constant rates.*

INTRODUCTION

There is much current interest in the culture of oyster larvae by large privately owned hatcheries and other organizations raising oyster larvae for their research programs. One of the most important factors in bringing large numbers of oyster larvae successfully to metamorphosis is the type and amount of food used during the rearing procedure.

Most studies of the food requirements of oyster larvae have been concerned primarily with the relative growth achieved with particular microorganisms. Cole (1937) was the first to demonstrate that pure cultures of naked flagellates could be used to produce significant growth of *Ostrea edulis* larvae under laboratory conditions. Bruce, Knight and Parke (1940) cultured six species of flagellated algae and found two, *Isochrysis galbana* and *Pyraminomonas grossi*, that were good foods for *O. edulis* larvae. Walne (1963) reported that *I. galbana*, among other algal species, was an acceptable food for *O. edulis* larvae. Davis (1950, 1953) tested a number of potential foods for *Crassostrea virginica* larvae and found that five flagellated species and *Chlorella* sp. were utilized. Later, 10 genera of microorganisms were tested by Davis and Guillard (1958) and they found that the chrysophytes, *I. galbana* and *Monochrysis lutheri*, were of approximately equal value and the best single foods for *C. virginica* larvae.

Some information is available on the quantitative aspects of feeding shellfish larvae. Loosanoff, Davis and Chanley (1953, 1955) studied the larvae of *Mercenaria mercenaria* and reported that heavy concentrations of *Chlorella* sp. killed larvae, that larval growth was abnormally slow when an insufficient amount of food was present, and that the optimum larval growth over a 12-day period occurred at concentrations of 50,000 large (8 µ) or 400,000 small (4 µ) *Chlorella* sp. cells/ml. Davis and Guillard (1958) found the optimum concentrations of *I. galbana* and *M. lutheri* for *M. mercenaria* larvae to be 200,000 and 250,000 cells/ml, respectively, with little difference in growth occurring over a wide range of concentrations.

Bayne (1965) reported that *Mytilus edulis* larvae exhibit a general increase in growth rate with increasing *I. galbana* concentrations up to 100,000 cells/ml, the highest cell concentration tested. Bayne's data also showed that the grazing rate and the number of cells caught per larva in 24 hr increased with an increase in larval size.

Walne (1956, 1963, 1965, 1966) investigated the quantitative aspects of feeding *O. edulis* larvae. Walne (1965) reported a rapid increase in assimilation of radioactively labeled *I. galbana* as food concentrations increased until at 50,000 cells/ml about 70% of the maximum assimilation is obtained. He further showed that at cell densities over 100,000/ml the increase in assimilation is slight for substantial increases in cell density. Walne also performed experiments which indicated that, as larval sizes increase from about 170 - 260 μ, the numbers of cells assimilated by a larva in 24 hr increase from 6,000 - 15,000. At larval densities of 1.0 - 1.5/ml Walne (1966) reported that it was necessary to add food to cultures more frequently than every 24 hr to maintain cell concentrations high enough for optimum growth of the grazing larvae.

Davis and Guillard (1958) reported some information on the relatonship between algal concentration and the growth of *C. virginica* larvae. These workers fed five different concentrations of *I. galbana* and *M. lutheri* to oyster larvae. They found that a concentration of 250,000 *M. lutheri* cells/ml was optimum at each sampling in a 14-day experiment. With *I. galbana* young larvae grew best at 100,000 cells/ml, whereas older larvae grew fastest at 400,000 cells/ml. The data of Davis and Guillard, however, do not reveal the quantity of *I. galbana* to feed to larvae of specific sizes to obtain maximum growth.

Ukeles and Sweeney (1969) also reported some food concentration data for *C. virginica* larvae. They fed ^{14}C-labeled *M. lutheri* to straight-hinge *C. virginica* larvae and found that retention is most efficient at a food concentration of about 200,000 cells/ml or 13,000 cells/larva. At these food concentrations approximately 150 - 250 *M. lutheri* cells were taken up and retained per larva in 24 hr. No data are reported for older larvae.

In the present work the concentrations of *I. galbana* necessary to effect maximum growth of *C. virginica* larvae of various sizes are reported, and some comparisons are made between feeding at constant rates and feeding on a graduated schedule according to larval size.

METHODS

Algal Culture

I. galbana was chosen for this study because it has been found to be one of the best foods for *C. virginica* larvae (Davis and Guillard, 1958) and because similar studies have been performed using this species with *O. edulis* larvae (Walne, 1956, 1963, 1965, 1966). The *Isochrysis* used in these experiments was grown in semicontinuous unialgal cultures (not bacteria free) in a heat-sterilized, enriched seawater medium following the methods described by Ukeles (1971). The *Isochrysis* required was harvested daily and the density of the culture determined by centrifuging a 10-ml sample in a Hopkins tube for 15 min at 1,000 g. The resulting packed cell volumes were used to determine the appropriate quantities of algal suspension to feed to the larval cultures. The food concentrations reported, therefore, are expressed as microliters of packed cells per liter of larval culture.

Feeding Concentration Experiments

All of the feeding concentration experiments were short-term, acutely measured tests, molded after the methods of Walne (1965). For each series of experiments a stock population of oyster larvae, consisting of the pooled progeny from a number of Long Island Sound parents, was reared according to the methods of Loosanoff and Davis (1963). The stock populations were reared at 28°C in 15-liter polyethylene containers containing filtered seawater to which 100 ppm sodium sulfamethazine (Sulmet, American Cyanamid Co.)[1] had been added, and were fed exclusively on a diet of *Isochrysis*. To obtain larvae of a uniform size for an individual experiment and to make the results more applicable to commercial hatcheries where larvae are separated and grown by size, the entire stock population was screened through a series of nylon mesh screens and the desired size group selected. The nylon screens used had square openings of 54, 75, 100, 135, 151, 180 and 216 μ. Mesh size refers to the screen in this series which retained larvae after a 3 min seawater rinse. Straight-hinge larvae were not screened for size, but were rinsed on a 36 μ nylon screen before use in the tests.

In the first series of experiments eight groups of *C. virginica* larvae in four basic size categories were tested in duplicate 1-liter cultures to which *Isochrysis* concentrations of 0, 2.5, 5.0, 10.0, 20.0 and 40.0 μl/l were added daily. The cultures of about 15,000 larvae each were maintained in Pyrex glass beakers at 28°C in filtered and ultraviolet-treated seawater to which 100 ppm of

[1] Trade names mentioned in this paper do not imply endorsement by the National Marine Fisheries Service.

TABLE 1. *Average optimum* Isochrysis *concentrations (μl/l) for maximum growth of oyster larvae of various sizes in 48 hr.*

Average Initial Larval Size (μ)	Initial Larval Mesh Size	Average Optimum Feeding Concentration
74.4	S-H	2.5
80.1	54	5.6
107.0	75	13.8
139.8	100	17.5
170.0	135	22.5
200.4	151	32.5
246.4	180	32.5

sodium sulfamethazine had been added. The experiments were sampled and terminated at 48 hr. Growth data consisted of 100 larval measurements for each sample. Larval lengths were measured to the nearest 5 μ with an ocular micrometer.

Using the results from the first series of experiments, series of seven *Isochrysis* concentrations were selected for testing each of seven larval size groups. Each size group was tested in two 48 hr experiments, and duplicate 1-liter cultures were used at each of the seven concentrations of food tested in each experiment. Experimental methods were identical to those above. In the two experiments involving larvae larger than 237 μ in initial length a clean oyster shell was added to each beaker to provide a suitable substrate for larvae that might attain a size sufficient for metamorphosis.

Feeding Schedule Experiments

After the feeding concentration experiments had revealed the optimum feeding rate for the seven sizes of larvae, two experiments were performed in which the growth of oyster larvae using a graduated feeding schedule and several constant feeding rates were compared. The average optimum food concentrations, which comprised the graduated feeding schedule, are indicated in Table 1. The constant feeding rates were 0, 2.5, 5.0, 10.0, 20.0 and 40.0 μl/l. The tests were set up with straight-hinge larvae and were terminated when eyed larvae were observed. Larvae in all treatments were screened from the cultures and resuspended in clean seawater every two days. Larvae in one set of cultures in the first feeding schedule experiment were separated by size every two days, set up in beakers according to mesh size at a density of 15 larvae/ml, and the beakers fed at the concentrations of *Isochrysis* indicated in Table 1. All other methods and materials were identical to those above.

RESULTS AND DISCUSSION

Food Concentrations and Larval Growth

The results of the first series of experiments are presented in Table 2. These data show generally the concentrations of *Isochrysis* necessary for good growth of oyster larvae of various sizes. However, in the first and seventh experiments reported in Table 2 the maximum growth was achieved in the lowest and highest food concentrations tested, respectively, making it necessary to expand the ranges of concentrations used in later tests. Because our initial food concentrations were widely spaced, we also wanted to test some intermediate concentrations. Therefore, for the remainder of the

TABLE 2. *The average growth increments (μ) of oyster larvae of various sizes after being fed different concentrations of* Isochrysis *in Experimental Series 1.*

Food Concentration (μl/l)	Initial Larval Length (μ)							
	77.1	77.7	104.1	104.2	139.4	145.8	200.6	204.2
40.0	3.3	10.1	17.1	22.0	5.9	31.9	30.9	8.4
20.0	4.1	13.1	19.2	33.4	3.4	41.0	19.9	27.0
10.0	7.9	14.9	19.5	38.4	11.8	20.9	8.0	14.6
5.0	11.7	18.8	15.1	28.8	9.0	7.1	3.8	4.9
2.5	13.8	19.3	9.1	16.1	4.7	1.4	0.0	4.2
Unfed	3.9	4.6	2.7	5.5	0.0	0.0	0.0	1.2

TABLE 3. *The average growth increments (μ) of oyster larvae of various sizes after being fed different concentrations of* Isochrysis *in Experimental Series 2.*

Food Concentration (μl/l)	Initial Larval Length (μ)						
	74.2	80.1	104.6	137.1	168.1	200.4	255.0
60.0							9.7
50.0							17.1
45.0						38.1	16.8
40.0						38.1	11.9
35.0					20.5	35.6	16.3
30.0					27.6	38.1	17.6
25.0				43.1	30.6	37.7	11.1
20.0			41.9	42.9	34.2	26.7	
17.5			43.8	41.9	33.6	28.0	
15.0		17.0	45.1	43.6	33.2		
12.5		13.2	41.7	39.3	27.5		
10.0	11.7	14.7	39.0	25.8			
7.5	12.3	17.7	34.7	15.5			
5.0	15.0	14.2	18.6				
3.8	15.6	12.3					
2.5	14.6	12.7					
1.2	7.5						
0.6	5.6						
Unfed	2.2	0.0	0.0	0.0	2.3	3.4	0.0

food concentration experiments reported here we used the data in Table 2 to select a series of food concentrations to be tested against larvae of specific sizes.

Tables 3 and 4 present the results of the second and third series of experiments. In Figure 1 the *Isochrysis* concentrations which produced the most rapid growth of larvae of various sizes are plotted against initial larval length. Duncan's multiple range tests (Steel and Torrie, 1960) were performed to determine in each experiment which growth increments were not significantly different from the maximum increment obtained (95% confidence level), and these are indicated as vertical lines in Figure 1. The average optimum *Isochrysis* concentrations for larvae in the seven size groups tested are presented in Table 1.

These feeding experiments indicate that, as oyster larvae grow, their food requirements increase substantially. A 13-fold increase in *Isochrysis* concentrations was found necessary to support maximum growth of the larvae over the range of sizes tested. Straight-hinge larvae 74 μ in length grew

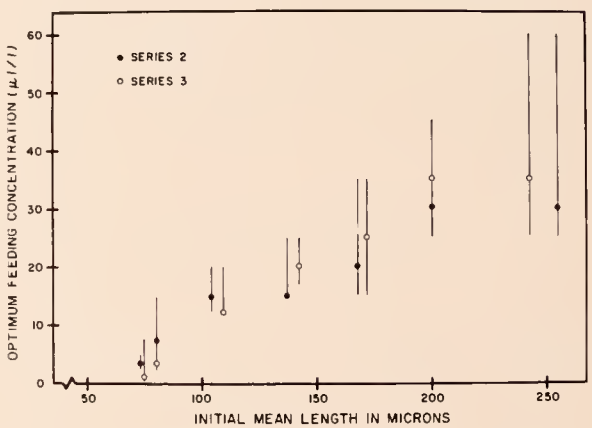

FIG. 1. *The optimum* Isochrysis *concentrations for* C. virginica *larvae of various initial mean lengths. Points indicate concentrations in which the greatest growth increment was obtained in the raw data. Vertical lines indicate concentrations in which growth increments were not statistically different from those obtained in the concentration producing the greatest growth increment.*

TABLE 4. *The average growth increments (μ) of oyster larvae of various sizes after being fed different concentrations of* Isochrysis *in Experimental Series 3.*

Food Concentration (μl/l)	Initial Larval Length (μ)						
	74.7	80.1	109.4	142.4	172.0	200.4	237.7
60.0							0.0
50.0							0.0
45.0						20.7	0.5
40.0						22.9	0.0
35.0					20.4	25.3	4.5
30.0					20.4	22.4	0.0
25.0				36.8	23.3	21.6	1.3
20.0			22.6	38.1	22.6	15.1	
17.5			24.7	37.7	20.2	11.2	
15.0		17.3	26.2	28.1	18.8		
12.5		18.8	27.4	23.6	14.1		
10.0	6.7	20.6	20.5	19.1			
7.5	7.9	19.8	18.6	11.0			
5.0	9.4	14.0	14.8				
3.8	9.5	21.6					
2.5	8.5	17.7					
1.2	10.3						
0.6	8.5						
Unfed	3.7	4.7	1.7	0.0	0.0	0.0	0.0

fastest at an average concentration of 2.5 μl/l, while larvae 200 μ in length required an average *Isochrysis* concentration of 32.5 μl/l for maximum growth.

Larvae longer than 237 μ grew slower than the other groups tested (Tables 3 and 4). There are no data in the literature to suggest that oyster larvae grow more slowly as they approach metamorphosis in the presence of a substrate suitable for setting. We suspect that this slow growth was due to a toxic substance associated with the *Isochrysis* cultures used for these tests since the larvae in the fed beakers failed to swim actively, while the larvae in the unfed beakers did swim actively.

The experiments of Davis and Guillard (1958), although not specifically designed to reveal optimum feeding concentrations for larvae of various sizes, did indicate a general increase in food requirements as oyster larvae grow. The data of these workers showed the optimum *Isochrysis* concentration for 75 μ larvae to be 10μl/l, whereas 140 μ larvae grew best at 40 μl/l. These concentrations are somewhat higher than those found to be optimum in the present study. For the most part, larvae in the present study grew faster and at lower concentrations of food than did those of Davis and Guillard. The lower temperatures (21 - 23°C) used by Davis and Guillard and differences in the quality of the *Isochrysis* cultures used may account for this discrepancy.

Guillard (1958) considered the data of Davis and Guillard (1958) and the levels of food organisms encountered by shellfish larvae in natural situations and suggested that an algal concentration of 10 μl/l be used as a guide in feeding oyster larvae at densities of 3 - 15/ml. The data from the present study show that this concentration of cells is less than optimum for larvae over 100 μ long.

The studies of Ukeles and Sweeney (1969) showed that about 150 - 250 *M. lutheri* cells/larva were taken up and retained by straight-hinge larvae in 24 hr at a concentration of 13,000 cells/larva, the most efficient feeding concentration. Assuming that 100,000 *Isochrysis* cells/ml is equal to 10 μl of packed cells/liter (Davis and Guillard, 1958), in our study only about 1,600 *Isochrysis* cells were available to each straight-hinge larva in a 24-hr period at the concentrations that produced the most rapid growth. Although we made no final algal counts, significant clearing of the cultures was observed and most of the those cells available

were probably utilized. The slower growth of straight-hinge larvae which we obtained at higher food concentrations (Table 2) shows that under the conditions in the present experiments the lower feeding rate is superior.

Walne (1956, 1963, 1965, 1966) provided much information on the feeding behavior of *O. edulis* larvae. Because this species is larviparous and releases larvae averaging 170 μ in length, no comparisons of food requirements are possible for small larvae, but some can be made for larger ones. Walne (1965) reported that *O. edulis* larvae averaging 219 μ in length catch an average of 24,000 *Isochrysis* cells in 24 hr. The growth data in the present study indicate maximum growth of similar size *C. virginica* larvae at a concentration of about 20,000 cells/larva in 24 hr. Walne (1965) also reported that, as *O. edulis* larvae grow from about 170 - 260 μ in the planktonic phase, the assimilation of *Isochrysis* cells increases 2.5 fold. The data presented here indicate an approximate doubling of optimum cell concentrations for *C. virginica* larvae of similar sizes.

One of the prime considerations in evaluating a feeding schedule is the number of larvae that can be reared per unit volume. The present experiments indicate that acceptable growth can be achieved with proper feeding concentrations over a wide range of larval sizes at a density of 15 larvae/ml. Walne (1965) obtained rapid growth at a density of 140 *O. edulis* larvae/l and a cell concentration of 123,000/ml; but to get similar growth at a larval concentration of 5,000/l the *Isochrysis* concentration had to be tripled. In the first case the small number of larvae grazing did not significantly reduce the *Isochrysis* concentration, while at the higher larval density food became a limiting factor. Davis (1953) observed an inverse relationship between larval density and growth at various *Chlorella* sp. concentrations for *C. virginica* larvae. Loosanoff *et al.* (1955) who fed various amounts of *Chlorella* sp. to clam larvae, *M. mercenaria*, concluded that an increase in larval densities beyond a certain limit cannot be compensated for by a proportionate increase in the quantity of food. The limits in this situation appear to result from the mechanical interference with feeding at high algal concentrations (Loosanoff *et al.*, 1955), the possible occurrence of toxins produced by the algal cells or present in the algal suspension from some other source, and the accumulation of inhibiting quantities of metabolic wastes from the larvae at high densities.

At the larval density of 15/ml used in the present study the *Isochrysis* concentrations in the larval cultures were substantially reduced by grazing in the 24 hr between feedings. The ideal feeding situation should probably include provisions for continuous feeding so that an optimum concentration of cells would be present in the culture vessel at all times.

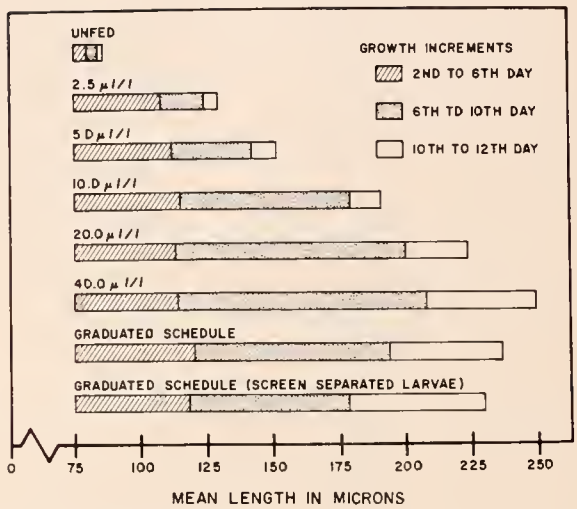

FIG. 2. *The growth of* C. virginica *larvae on different feeding schedules. Experiment 1.*

Feeding Schedules and Larval Growth

The results of the first feeding schedule experiment are presented in Figure 2. From 65 - 90% of the original larval population were alive in the different treatments on the twelfth day. A Duncan's multiple range test of the data from the final

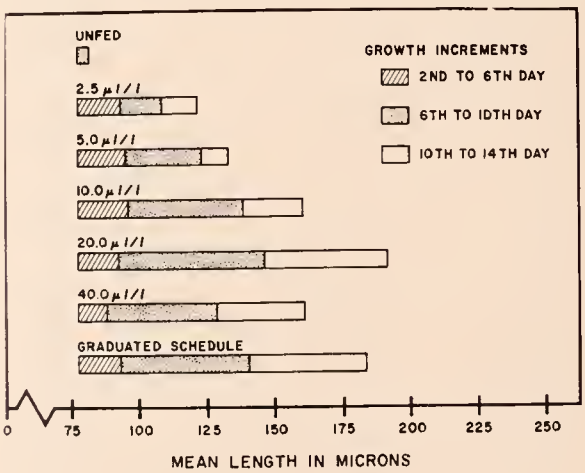

FIG. 3. *The growth of* C. virginica *larvae on different feeding schedules. Experiment 2.*

sampling date showed all treatment means to be significantly different from each other (95% confidence level), except between the means representing 20 µl/l and the graduated feeding schedule for screened larvae. The best growth occurred at 40 µl/l, but the cultures fed according to the graduated feeding schedule produced larvae only 5% smaller and required only 46% of the total *Isochrysis* used to feed the 40 µl/l cultures. The larvae that were screen-separated into size groups, adjusted to 15/ml, and fed according to size, were fed 56% of the food required to maintain the larvae in the fastest growing treatment.

The growth data from the second feeding schedule experiment are presented in Figure 3. All treatment means for the final sample are statistically different from each other (Duncan's multiple range test, 95% confidence level), except those representing 10 µl/l and 40 µl/l. On the 14 day of the experiment 70% of the original larval population were alive in the best two treatments. The larvae at 20 µl/l were 4% larger than those fed according to the graduated feeding schedule, but to effect this increase 63% more *Isochrysis* was required. These results show that using constant feeding rates *Isochrysis* concentrations of 20 to 40 µl/l are required to effect maximum growth rates of *C. virginica* larvae at densities of 10 - 15/ml. Similar high rates of growth can be achieved by starting at much lower feeding rates and then increasing the *Isochrysis* concentration as the larvae grow. This latter method requires a smaller volume of algae than the constant concentration method and could yield significant savings to organizations rearing substantial numbers of oyster larvae.

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