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RELATIVE VALUE OF TEN GENERA OF MICRO-ORGANISMS AS FOODS FOR OYSTER AND CLAM LARVAE

By HARRY C. DAVIS AND ROBERT R. GUILLARD

(This report presents the findings of a cooperative project of the Fish and Wildlife Service, United States Department of the Interior, and the Oyster Institute of North America.)

FISHERY BULLETIN 136

From Fishery Bulletin of the Fish and Wildlife Service

VOLUME 58

UNITED STATES GOVERNMENT PRINTING OFFICE • WASHINGTON • 1958

For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C.
Price 15 cents

ABSTRACT

Twelve species of micro-organisms, representing ten different genera, were used in feeding experiments to determine their relative value as foods for larvae of oysters (*Crassostrea virginica*) and clams (*Venus mercenaria*). *Isochrysis galbana* and *Monochrysis lutheri* were approximately equal in value as foods for either species of larvae, and were the best single foods for oyster larvae. Together with *Chlorococcum* sp. they were also the best single foods for clam larvae. Somewhat more rapid growth of both oyster and clam larvae was obtained by feeding a mixture of *Isochrysis galbana*, *Monochrysis lutheri*, *Dunaliella euchlora*, and *Platymonas* sp. than was obtained by feeding equal quantities of any of these foods separately.

Growth of larvae of both oysters and clams was comparatively good in each of five concentrations of *Isochrysis* and *Monochrysis* tested. The rate of growth did not fall off rapidly nor was there any mortality at the higher concentrations of these foods, as there was when equally high concentrations of *Chlorella* sp. (Lewin's isolate) were used.

The presence, and perhaps thickness, of cell walls and the degree of toxicity of the metabolites are believed to be important factors in determining the usability of micro-organisms as foods for bivalve larvae.

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By HARRY C. DAVIS, *Fishery Research Biologist, Bureau of Commercial Fisheries*
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The increasing interest in pond and hatchery culture of lamellibranchs as a dependable source of seed stock has focused attention on the need for additional information on the effect of various micro-organisms on the survival and growth of larvae of these mollusks. We need to know not only which organisms the larvae can utilize as foods, but also which forms produce toxic metabolites that could interfere with growth or kill the larvae, should such micro-organisms become too numerous (Loosanoff et al., 1954). If we are to fertilize ponds to increase the number of food organisms, we need to know the optimum concentration of such organisms and the range of concentrations that the larvae can tolerate and still grow at an acceptable rate.

We are reporting the results of some feeding experiments designed to determine the relative food value to larvae of oysters (*Crassostrea virginica*) and clams (*Venus mercenaria*) of representatives from ten genera of micro-organisms, and of one experiment designed to test the effect on larval growth of five different concentrations of the two micro-organisms that proved to be of most value as foods.

We wish to express our gratitude to Dr. V. L. Loosanoff for assistance and constructive criticism throughout these experiments, to Mrs. Barbara Myers for the statistical treatment of our data, to C. A. Nomejko for preparing the figures, and to Miss Norma Pritchard for many of the larval measurements.

FOOD ORGANISMS TESTED

Four of the genera fed to oyster and clam larvae were representatives of the Chrysophyta. *Isochrysis galbana* Parke (1949), *Monochrysis lutheri* Droop (1953) and *Prymnesium parvum* Carter (1937) are motile unicells of the Order Chrysomonadales. For bacteria-free subcultures of these organisms we are indebted to Drs. L. Provasoli and J. McLaughlin of the Haskins Laboratories, New York. (Dr. Provasoli isolated bacteria-free cultures of *Isochrysis* from the original Parke cultures). The fourth organism, *Phaeodactylum tricornutum* Bohlin (1897), has been considered a diatom and has been widely used under the name of *Nitzschia closterium* (Ehrenberg) Wm. Smith forma *minutissima* Allen and Nelson (1910). Following Hendey (1954), we consider it a chrysophyte of undetermined systematic position.

The remaining six genera were representatives of the Class Chlorophyceae. *Dunaliella*, *Chlamydomonas*, and *Platymonas* are motile unicells of the Order Volvocales, while *Chlorococcum* and *Chlorella*, of the Order Chlorococcales, are nonmotile except that *Chlorococcum* has motile zoospores. *Stichococcus* is a nonmotile unicell of the Order Ulotrichales.

Isochrysis, *Monochrysis*, *Prymnesium*, and *Dunaliella* are naked and normally undergo cell division while motile. *Chlamydomonas* and *Platymonas* have cell walls and divide while nonmotile, producing 2, 4, and sometimes 8 small, thin-walled flagellated cells. *Chlorococcum*, although it has cell walls and is nonmotile, produces naked, motile zoospores. *Chlorella* produces autospores that are nonmotile and have cell walls from the time they are liberated from the parent cell. *Stichococcus*, like *Chlorella*, has neither a motile stage nor a naked stage. It belongs to an order that is essentially filamentous but the "filaments" of *Stichococcus* seldom consist of more than two cells. *Phaeodactylum* is nonmotile and has a cell wall that may be weakly siliceous (table 1).

NOTE—Approved for publication June 6, 1957. Fishery Bulletin 136.

TABLE 1.—*Micro-organisms used in feeding experiments with larval oysters and clams*

Organism	Source	Approximate size (microns)	Thousands of cells/ml. of larval culture	Cell walls
1. *Isochrysis galbana*	Parke (1949)	5.5×3×2.5	120	No
2. *Monochrysis lutheri*	Droop (1954)	7×2.5	110	No
3. *Prymnesium parvum*	Droop (1954)	9×4.5×3.5	30	No
4. *Phaeodactylum tricornutum*	[1]	To 3.5×27 [2]	55	[2] Yes
5. *Dunaliella euchlora*	[3]	11×6	40	No
6. *Dunaliella* sp.	[3]	11×6	50	No
7. *Chlamydomonas* sp. (Cambridge collection #11/35)	Lewin(?)	8.5×3.5	70	Yes
8. *Platymonas* sp. #1	Guillard at Woods Hole, Mass.	11×8.5×5	27	Yes
9. *Chlorococcum* sp.	Guillard at Milford, Conn.	6 to 14 (zoospores, 3–5)	20	[4] Yes
10. *Chlorella* sp. (Indiana U. collection #580)	Lewin at Milford, Conn.	To 13 (average 4–7)	110	Yes
11. *Chlorella* sp. "A"	Guillard at Milford, Conn.	To 8 (average 4.5)	100	Yes
12. *Stichococcus* sp.	Ryther (1954)	2×2 to 6	550	Yes

[1] Our subculture was obtained from Dr. John Ryther, Woods Hole Oceanographic Institution. It is believed to be derived from the Plymouth Laboratory culture described by Wilson (1946).
[2] Typical cultures had about 5 percent "oval" cells 7.5 microns in length, and less than ½ percent "triradiate" cells. Cell walls weakly siliceous.
[3] *Dunaliella euchlora* and *Dunaliella* sp. are very similar in appearance and in culture requirements. It is possible they are different clones of the same strain, the origin of which is not known. *D. euchlora* (Lerche 1937) was identified by Dr. R. A. Lewin, and is the organism used by Ryther (1956).
[4] Zoospores of *Chlorococcum* sp. are naked.

METHODS

Food Production

The algal cultures were grown under fluorescent white and cool-white lamps providing a light intensity of roughly 500 F. C. Culture temperatures were maintained between 19.0° and 23.0° C. Bacteria-free, 75-ml. liquid stock cultures were maintained in 250-ml. Ehrlenmeyer flasks, while cultures used for feeding were grown in volumes of 500 ml. to 1 liter either in 2-liter Ehrlenmeyer or in 2,800-ml. Fernbach flasks. The latter cultures attained suitable densities in 5 to 15 days, so that the volume required for feeding was only 5 ml. to 33 ml. per liter of sea water in the containers in which the larvae were grown.

All algae were grown in Long Island Sound sea water (salinity 22.0–27.0 p. p. t.) enriched as follows:

MAJOR ELEMENTS

NaH_2PO_4	20	mg./l.
Ferric sequestrine (NaFeEDTA)—13% Fe)	10	mg./l.
and either		
$NaNO_3$	150	mg./l.
or		
$NaNO_3$	150	mg./l.
plus		
NH_4Cl	50	mg./l.

TRACE ELEMENTS

Cu	0.005	mg./l.
Zn	0.01	mg./l.
Co	0.005	mg./l.
Mn	0.1	mg./l.
Mo	0.01	mg./l.

VITAMINS

Thiamine HCl	0.2	mg./l.
Biotin	1	μg./l.
B_{12}	1	μg./l.
Folic acid	2	μg./l.
Paba	10	μg./l.
Nicotinic acid	0.1	mg./l.
Inisotol	1.0	mg./l.
Calcium pantothenate	0.2	mg./l.
Pyradoxine HCl	0.1	mg./l.

Although the requirements of the different micro-organisms for the various ions and vitamins have not been established, the medium either with or without NH_4^+ was suitable for all, and it was desired to standardize the medium as much as possible. Only *Monochrysis* and *Chlamydomonas* were supplied NH_4^+, although a few others may utilize it more readily than NO_3^- as a source of nitrogen. The medium both with and without ammonium has been found nontoxic to clam and oyster larvae in the concentrations used in feeding.

Larval Culture

The technique for obtaining fertilized eggs, permitting them to develop to straight-hinge larvae, and of handling the larvae were the same as previously described by the senior author (Davis 1953), except that 3-liter polyethylene containers were used for the experimental larval cultures rather than the cumbersome 20-liter earthenware jars used previously. We attempted to set up the cultures with 30,000 to 45,000 straight-hinge larvae per culture or 10 to 15 larvae per ml. Actually the average numbers of larvae per ml. were as follows:

	Oyster larvae	*Clam larvae*
Experiment 1	10	13
Experiment 2	16	14
Experiment 3	9	10

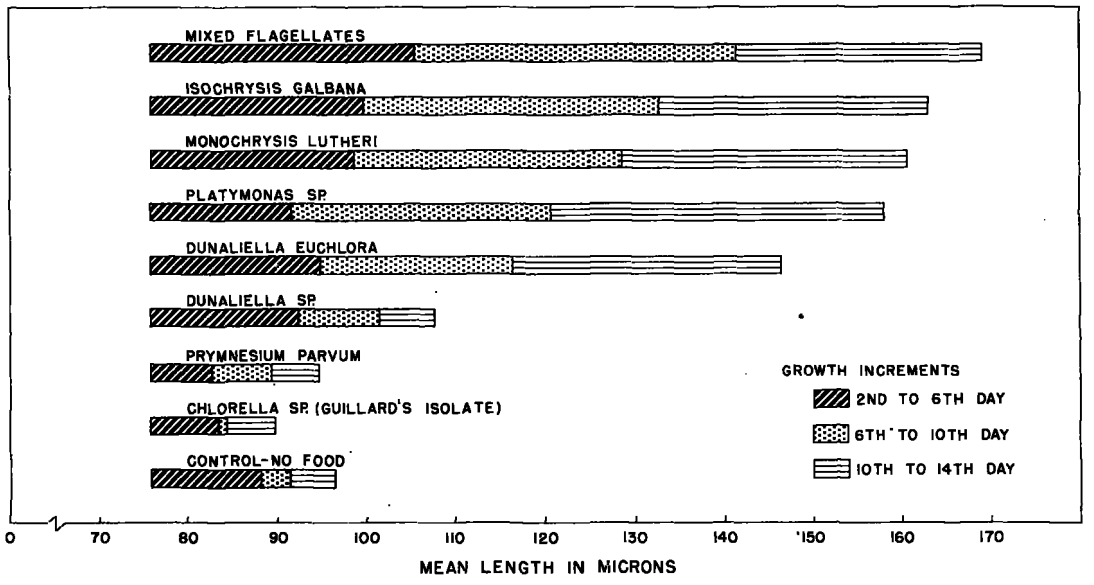

FIGURE 1.—Growth of larvae of oysters (*Crassostrea virginica*) in cultures receiving different micro-organisms at the rate of 10×10^{-3}mm.^{3} of packed cells per milliliter per day (experiment 1). Concentration of oyster larvae averaged 10 per ml. Plots were based on mean length of 100 larvae from each of the duplicate cultures. Mixed flagellates consisted of equal quantities (by packed cell volume) of *Isochrysis*, *Monochrysis*, *Dunaliella euchlora*, and *Platymonas*.

The foods were tested on oyster and clam larvae simultaneously so that the condition of the foods and of the sea water was the same for larvae of both species. Two cultures of oyster larvae and two cultures of clam larvae were used to test each food. To find whether a mixture of food organisms might give better growth of larvae than any species separately, one pair of cultures of each species of larvae in the first and second experiments received a mixture consisting of equal quantities of *Isochrysis galbana*, *Monochrysis lutheri*, *Dunaliella euchlora*, and *Platymonas* sp. In addition, in each experiment, one pair of cultures of each species of larvae served as controls and received no supplemental food.

To equate the quantities of food given, since the average size of individual cells varies with the species of food organisms (table 1), we fed at a rate of 10×10^{-3}mm.^{3} of packed cells per milliliter of larval culture per day. The wet-packed cell volume of a 10-ml. sample of each food culture was measured using Hopkins tubes and a relative centrifugal force of about 1,000 for 25 minutes. The volume of each food culture required to give 10×10^{-3}mm.^{3} of packed cells per ml. of larval culture was then calculated, and this amount was fed daily.

EFFECTS OF MICRO-ORGANISMS ON OYSTER LARVAE

The relative value of the different micro-organisms as foods for oyster larvae was studied in three experiments. Seven species individually and a mixture of four of them were tested in the first experiment (fig. 1). The second experiment was a replica of the first except that three species not previously tested were also included (fig. 2, upper part of graph). In a third experiment, two additional species not available for use in the first and second experiments were tested and *Monochrysis* was repeated for comparison (fig. 2, lower part of graph).

The ranking of the various foods remained remarkably constant throughout, although the growth of oyster larvae in all cultures of the second experiment was somewhat slower than in either of the other experiments. A statistical comparison of the ranking of the eight foods common to the first and second experiment shows a correlation in their order of rank of 0.87, or less than 1 chance in 100 that such good agreement between their order of rank in the two experiments could occur by chance.

In the two experiments in which the mixture of *Isochrysis*, *Monochrysis*, *D. euchlora*, and *Platy-*

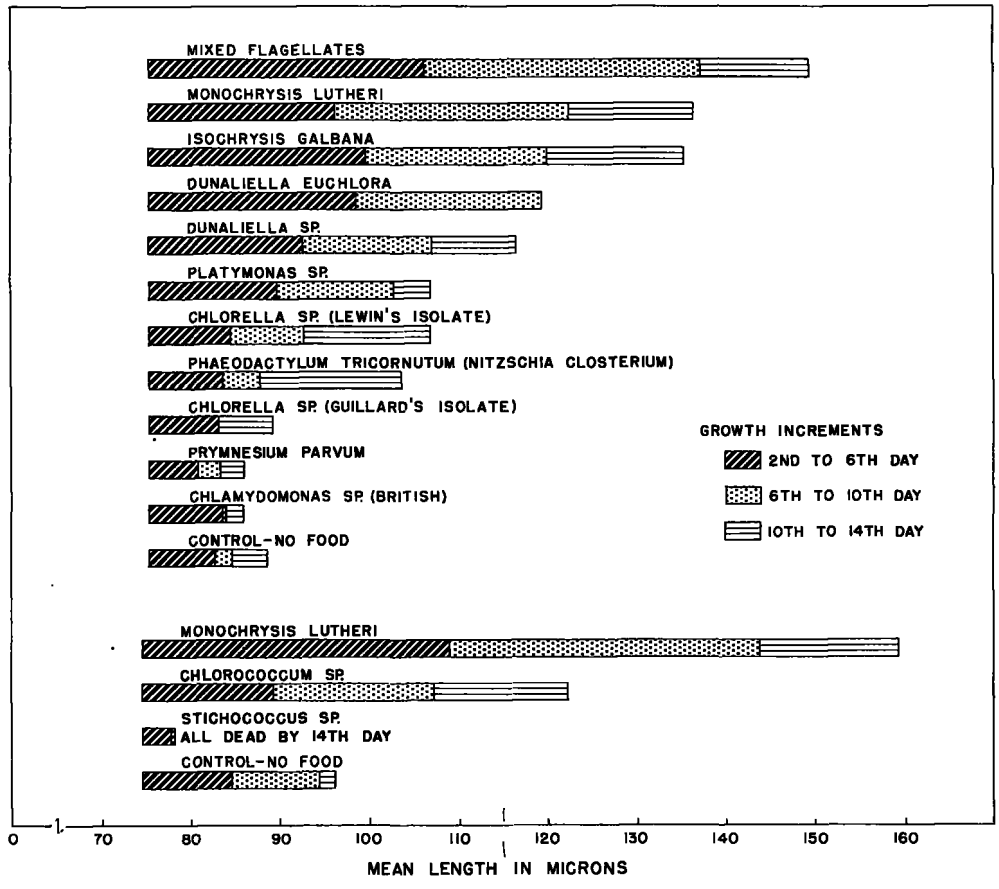

FIGURE 2.—Growth of larvae of oysters (*Crassostrea virginica*) in cultures receiving different micro-organisms at the rate of $10x10^{-3}$mm.^{3} packed cells per milliliter per day (experiment 2, upper part of graph; experiment 3, lower part of graph). Concentration of oyster larvae averaged 16 per ml. in second experiment and 9 per ml. in third experiment. Plots were based on mean length of 100 larvae from each of the duplicate cultures. Mixed flagellates consisted of equal quantities (by packed cell volume) of *Isochrysis*, *Monochrysis*, *Dunaliella euchlora*, and *Platymonas*.

monas was tested, it proved to be the best food (figs. 1 and 2). At each measuring period, oyster larvae receiving this food were significantly larger in both experiments than larvae receiving any of the individual foods separately.

Isochrysis and *Monochrysis* proved to be the best of the individual foods for oyster larvae, and were of almost equal value. Thus, while *Isochrysis* gave slightly better growth of larvae than did *Monochrysis* in the first experiment (fig. 1), *Monochrysis* proved to be slightly the better food in the second experiment (fig. 2, upper part of graph). Larvae receiving either of these two foods were significantly larger at 14 days, in both experiments, than larvae receiving any of the other single foods, except that larvae receiving *Platymonas* were almost as large in the first experiment.

Both species of *Dunaliella* appear identical microscopically, but oyster larvae receiving *D. euchlora* were significantly larger than larvae receiving *Dunaliella* sp. at 6 and 10 days in both experiments, and in the first experiment, were much larger than those receiving *Dunaliella* sp. at 14 days. In the second experiment, although oyster larvae receiving *D. euchlora* grew almost as rapidly as larvae receiving *Isochrysis* for the first 10 days, they did not grow at all between the 10th and 14th days. Such a radical change in the food value of *D. euchlora* appears strange, but we believe we may have been feeding very young cultures of *D. euchlora* for the first 10 days and much older cultures, in which toxic metabolites may have become highly concentrated, during the last 4 days of the experiment.

Oyster larvae receiving *Platymonas* were consistently even smaller at 6 days than larvae receiving *Dunaliella* sp., and they continued throughout the second experiment to grow less rapidly than

larvae receiving the latter food (fig. 2). In the first experiment, however, by the 10th day larvae receiving *Platymonas* were larger than larvae receiving either species of *Dunaliella*, and by the 14th day were almost as large as larvae receiving *Monochrysis* (fig. 1). It was also noted that the digestive gland of larvae receiving *Platymonas* was dark olive green in the first experiment, whereas in the second experiment the digestive gland of larvae receiving this food was more nearly reddish brown. We believe, therefore, that the difference in food value of *Platymonas* in the two experiments reflected a difference in the physiological state of the *Platymonas* cultures that probably included not only a difference in thickness of cell walls but also a difference in chemical composition.

Chlorella sp. (Lewin's isolate) was not tested in the first experiment. In the second experiment it proved to be a very much better food than *Chlorella* sp. (Guillard's isolate) and almost equal in value to *Platymonas*. It should be noted, however, that at 6 days neither *Chlorella*-fed culture was appreciably better than the unfed control culture, while larvae receiving *Platymonas* were significantly larger than those in the control culture (fig. 2). Larvae receiving *Chlorella* sp. (Guillard's isolate) grew less rapidly than larvae in the control culture in the first experiment (fig. 1), but did not significantly differ in size from larvae in the controls in the second experiment. However, larvae receiving this food did not grow at all between the 6th and 10th day in the second experiment (fig. 2), and suffered a significantly higher mortality than the control in both experiments.

Chlorococcum sp. was tested in the third experiment (fig. 2, lower part of graph). Since the unfed control and the *Monochrysis*-fed control cultures both grew faster than similarly treated cultures in the second experiment, direct comparison with foods used in the previous experiment is impossible. *Chlorococcum* would appear to have about the same food value for oyster larvae as Lewin's *Chlorella*, although the fairly good growth of larvae receiving *Chlorococcum* from the 2d to 6th day may indicate that the value of this food is more similar to that of *Platymonas*.

Oyster larvae receiving *Phaeodactylum* had about the same mean length as those receiving Lewin's *Chlorella* (fig. 2), but mean length is somewhat misleading in this instance. The majority of the oyster larvae in cultures receiving *Phaeodactylum* were apparently unable to utilize this food and did not grow at all. The comparatively few larvae that could utilize it did grow quite well, however, and by the 14th day several had reached 200 microns in length or were almost as large as the largest larvae in cultures receiving our best foods. Moreover, survival of larvae receiving this food was excellent and there was no indication that *Phaeodactylum* produced toxic metabolites.

Three organisms, in addition to Guillard's *Chlorella*, proved useless or toxic to oyster larvae. *Chlamydomonas* sp. (British isolate) proved to be of even less food value to oyster larvae than Guillard's *Chlorella* (fig. 2) and, as indicated by larval mortality, was almost as toxic. Larvae in cultures receiving *Prymnesium parvum* did not grow as rapidly as larvae in the unfed controls in either experiment 1 or 2 (figs. 1 and 2). Moreover, mortality of larvae in cultures receiving this "food" was significantly higher than in the control cultures in both experiments. *Stichococcus* sp. was the poorest of any of the foods tested (fig. 2), and was also the most toxic.

Experiments With Older Larvae

In two short experiments we compared the rate of growth of older oyster larvae when fed *Chlorella* with their rate of growth when fed mixed flagellates. In experiment A, in which the initial mean length of the larvae was 165.41 μ, measurements after 4 days of feeding indicated that the mean increase in length of the larvae fed *Chlorella* was less than one-half that of larvae fed mixed flagellates (table 2).

Both cultures were continued until setting, and the spat was collected. We counted only the spat that occurred on the white inner face of the test shells. Although each culture had an equal number of larvae at the beginning of the experiment when the larvae were 14 days old, we collected 2,180 spat (18th to 27th day) from the culture fed mixed flagellates, and only 680 (18th to 35th day) from the culture fed *Chlorella* sp.

In experiment B, in which the initial mean length was 149.75 μ (table 2), the differences in the rate of growth of older oyster larvae fed cultures of *Chlorella* and mixed flagellates were even more striking. However, these cultures were infested with rotifers from the start, and probably the data should only be considered as in general agreement with the first experiment.

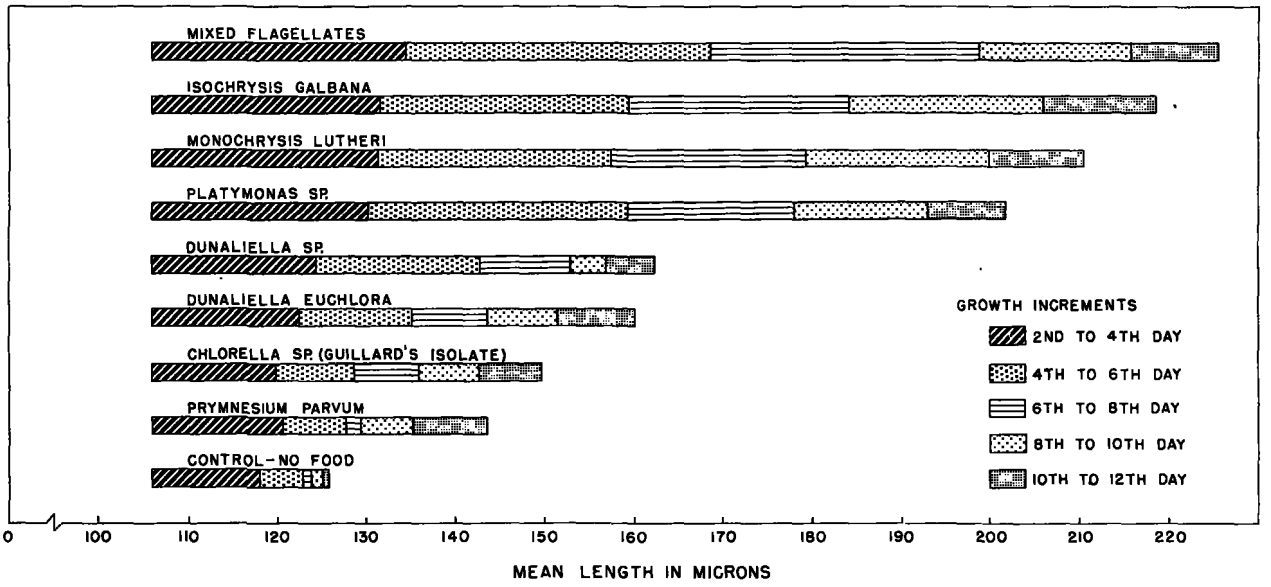

FIGURE 3.—Growth of clam larvae (*Venus mercenaria*) in cultures receiving different micro-organisms at the rate of 10×10^{-3} mm.^{3} of packed cells per milliliter per day (experiment 1). Concentration of clam larvae averaged 13 larvae per ml. Plots were based on the mean length of 100 larvae from each of duplicate cultures. Mixed flagellates consisted of equal quantities (by packed cell volume) of *Isochrysis*, *Monochrysis*, *Dunaliella euchlora*, and *Platymonas*.

TABLE 2.—*Growth of older oyster larvae fed cultures of* Chlorella *and of mixed flagellates*

Culture	Experiment A Mean length of larvae (microns)			Experiment B [1] Mean length of larvae (microns)		
	Initial	After 4 days	Increase	Initial	After 6 days	Increase
Chlorella sp.	165.41	188.65	23.24	149.75	152.90	3.15
Chlorella (Guillard's)				149.75	157.30	7.55
Mixed flagellates	165.41	216.35	50.94	149.75	182.50	32.75

[1] Initial larval culture heavily contaminated with rotifers which reduced rate of growth of all cultures in this experiment.

EFFECTS OF MICRO-ORGANISMS ON CLAM LARVAE

The relative value of the different micro-organisms as foods for clam larvae was tested in experiment 1 (fig. 3), experiment 2 (fig. 4, upper part of graph), and experiment 3 (fig. 4, lower part of graph) simultaneously with the tests on oyster larvae.

Growth of clam larvae, like that of oyster larvae, was considerably slower in the second experiment than it was in the first. As with oyster larvae, however, the ranking as foods for clam larvae, of the eight micro-organisms tested in both experiments, was almost precisely the same. The correlation in the order of ranking of the foods in these two experiments was 0.98, the only difference being that *Monochrysis* and *Isochrysis* had exchanged places.

With clam larvae, as with oyster larvae, the mixture of *Isochrysis*, *Monochrysis*, *Dunaliella euchlora*, and *Platymonas* was the best food. In the first experiment clam larvae receiving this mixture were significantly larger, at each measuring period, than larvae receiving any of the food organisms separately (fig. 3). In the second experiment the mixture ranked below *Monochrysis* on the 6th and 8th days; but was in first place again by the 12th day (fig. 4).

Isochrysis and *Monochrysis* were of approximately equal value to clam larvae, and were the best two of the single foods tested in both the first and second experiments (figs. 3 and 4). However, *Chlorococcum*, by comparison with *Monochrysis* in the third experiment, appears to be at least as good for clam larvae as *Monochrysis* and *Isochrysis* and, perhaps, as good as the mixed flagellates (fig. 4). *Platymonas* also, for the first 8 days of the first experiment, was of approximately the same value for clam larvae as was *Monochrysis*. By the 12th day, even in the first experiment, larvae receiving *Platymonas* were significantly smaller than those receiving *Monochrysis* and throughout the second experiment *Platymonas* was a significantly poorer food than either *Monochrysis* or *Isochrysis*. As was noted in oyster larvae, the coloring of the digestive gland of the clam larvae receiving *Platymonas* was dark olive

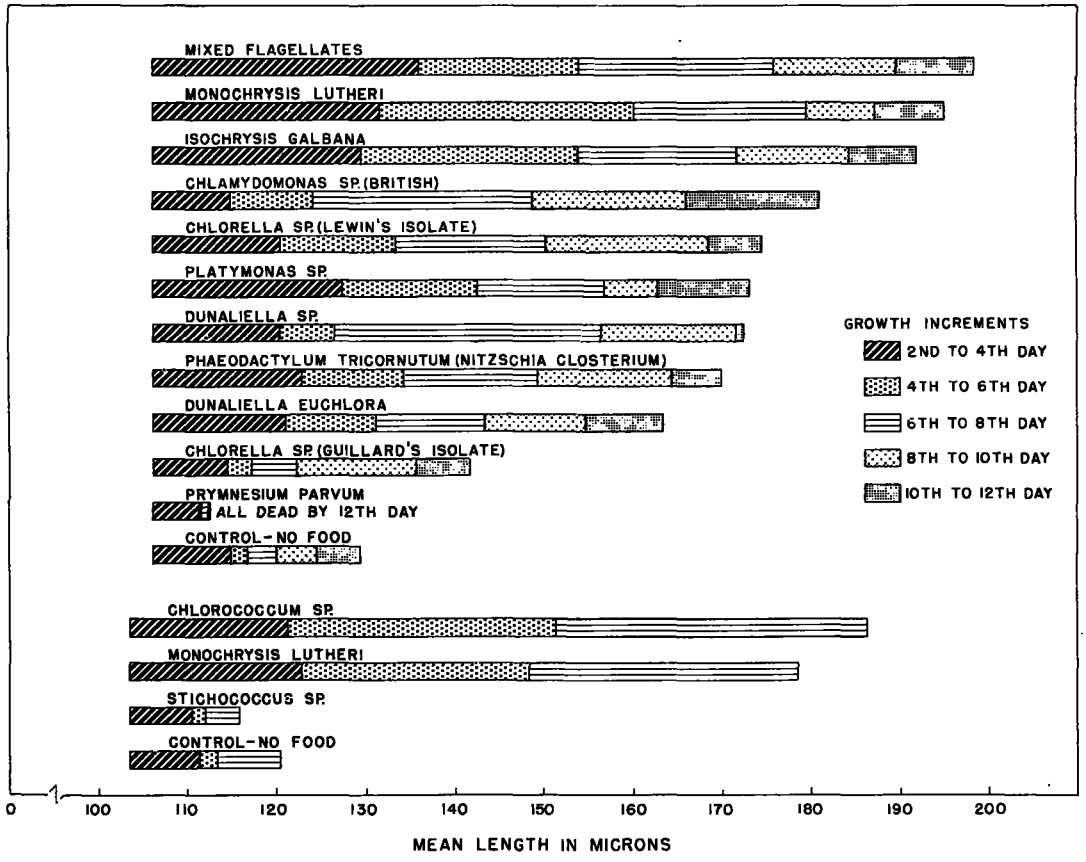

FIGURE 4.—Growth of clam larvae (*Venus mercenaria*) in cultures receiving different micro-organisms at the rate of 10×10^{-3} mm.^{3} of packed cells per milliliter per day (experiment 2, upper part of graph; experiment 3, lower part of graph). Concentration of clam larvae averaged 14 per ml. in the second experiment and approximately 10 larvae per ml. in the third experiment. Plots were based on mean length of 100 larvae from each of duplicate cultures. Mixed flagellates consisted of equal quantities (by packed cell volume) of *Isochrysis*, *Monochrysis*, *Dunaliella euchlora*, and *Platymonas*.

green in the first experiment and more nearly reddish brown in the second.

Two food organisms not tested in the first experiment, *Chlamydomonas* and Lewin's *Chlorella*, ranked above *Platymonas* as foods for clam larvae by the end of the second experiment (fig. 4). After only 2 days of feeding, it was observed that in each of the cultures receiving *Chlamydomonas*, almost precisely 50 percent of the clam larvae had dark green digestive glands and were growing. The remaining 50 percent of the larvae were almost completely colorless, had not grown, and showed no evidence of utilizing *Chlamydomonas*. This colorless group of larvae apparently never did utilize this food and eventually almost all of them died. Those larvae that did utilize *Chlamydomonas* showed a good rate of growth and by the 12th day larvae receiving this food ranked fourth in mean length but, due to the mortality among those larvae that never did utilize it, these cultures now contained only about one-half as many larvae as were present in cultures receiving other foods. Clam larvae receiving Lewin's *Chlorella*, as in previously reported experiments, grew at a fairly uniform rate but, as shown by this experiment, there are several better foods, even for clam larvae.

Phaeodactylum (*Nitzschia closterium* var. *minutissima*) has been used extensively as a food for clam larvae by Turner (personal communication). As shown by our second experiment, this food, like Lewin's *Chlorella*, gave a reasonably good rate of growth of clam larvae and the rate of growth was fairly uniform. On the basis of equal packed-cell volumes, however, as a food for clam larvae *Phaeodactylum* ranked eighth in the group of food organisms tested in these experiments (fig. 4).

Both species of *Dunaliella* were comparatively poor foods for clam larvae in the first experiment (fig. 3). In both experiments (1 and 2), growth

of larvae receiving *Dunaliella* sp. varied considerably from one measuring period to the next, while larvae receiving *Dunaliella euchlora* grew at a fairly uniform rate. *Dunaliella* sp., however, was the better of the two as a food for clam larvae in both experiments, and was almost as good as *Platymonas* in the second. *Dunaliella euchlora*, on the other hand, was the poorest of the really usable foods for clam larvae thus far tested (fig. 4).

Chlorella sp. (Guillard's isolate) was utilized by clam larvae in both experiments and did not appear toxic in the concentration used (table 1). Nevertheless, the rate of growth of clam larvae receiving this food was so slow that it is not considered a practical food to use.

The other two organisms tested, *Prymnesium parvum* and *Stichococcus* sp., both probably produce metabolites that are quite toxic to bivalve larvae. In the first experiment, all the clam larvae in one culture receiving *Prymnesium* were dead by the 12th day and in the second experiment all larvae in both cultures receiving this food were dead by the 12th day. *Prymnesium*, however, can be ingested and digested by clam larvae. This is indicated by the coloration of the digestive glands of some larvae, and by the survival of clam larvae in one culture of the first experiment and their significantly larger size compared with the controls at 12 days. *Stichococcus* did not cause excessive mortality of clam larvae, but larvae receiving this food grew even more slowly than those in the unfed control cultures. Gibor (1956) found that neither the larvae of *Artemia salina* nor those of *Tigriopus* grew at a normal rate when fed his *Stichococcus* sp. Walne (1956) working with larvae of *Ostrea edulis* also found *Prymnesium parvum* to be toxic although, in general, organisms of the Class Chrysophyceae, such as *Isochrysis galbana* and *Chromulina pleiades*, were more readily utilized than representatives of the other classes that he had tested. He found *Chlorella stigmatophora* gave poor growth of larvae and concludes that "those members of the Chlorococcales which have a thick cell wall are poor food for oyster larvae."

EFFECTS OF DIFFERENT CONCENTRATIONS OF *ISOCHRYSIS* AND *MONOCHRYSIS*

Since *Isochrysis* and *Monochrysis* were the two best foods for both oyster and clam larvae, an experiment was designed to determine the optimum concentration of these foods for most rapid growth of these larvae. Concentrations of 2.5×10^{-3} mm.^{3}, 5×10^{-3} mm.^{3}, 10×10^{-3} mm.^{3}, 20×10^{-3} mm.^{3}, and 40×10^{-3} mm.^{3} of packed wet cells per milliliter of larval culture were chosen to make the data as comparable as possible to the data on clam larvae reported by Loosanoff, Davis, and Chanley (1953) using *Chlorella* sp. (Lewin's isolate) as the food.

On Oyster Larvae

As shown by comparison with the controls, growth of oyster larvae was quite good at all the concentrations of *Isochrysis* and *Monochrysis* tested (fig. 5). Although on the 6th day, a concentration of 100,000 cells per ml. of *Isochrysis* was optimum by a slight margin, by the 10th day this concentration was slightly poorer than either of the two higher concentrations tested. At 14 days, the oyster larvae receiving 400,000 cells of *Isochrysis* per ml. had the greatest mean length. At this time, the mean lengths of larvae were in the same order as the concentrations of food, and the differences in mean length of larvae between successive concentrations were highly significant.

Oyster larvae that received 250,000 cells of *Monochrysis* per ml. were significantly larger, at each measuring period, than larvae that received the next higher or the next lower concentration of this food. Oyster larvae grew quite well, however, over a relatively wide range of concentrations of either *Monochrysis* or *Isochrysis*.

The slightly poorer growth of oyster larvae at the two highest concentrations of *Isochrysis*, during the first 6 days, was probably the result of mechanical interference with feeding; there is no indication of toxic metabolites with this food. The slightly slower growth of oyster larvae at the highest concentration of *Monochrysis* may indicate that *Monochrysis* produces slightly toxic external metabolites, since this concentration seems to retard growth somewhat, even during the later larval stages.

On Clam Larvae

With clam larvae we found that, within the range tested, there was no rapid falling off of the rate of growth after passing the optimum concentration of either *Monochrysis* or *Isochrysis*, as there was with *Chlorella* (fig. 6). Although 200,000 cells per milliliter of *Isochrysis* appears to be the optimum concentration, there was little difference in the rate of growth of clam larvae over the

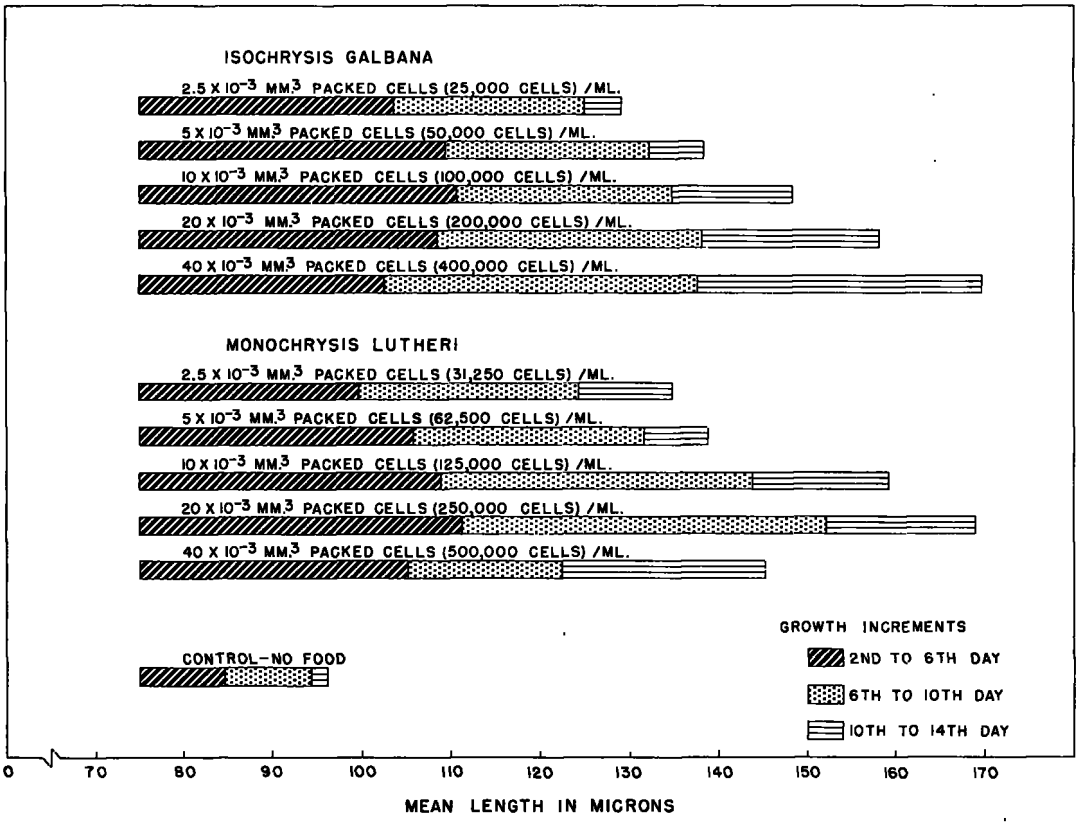

FIGURE 5.—Growth of oyster larvae (*Crassostrea virginica*) in cultures receiving five different concentrations of *Isochrysis* and of *Monochrysis*. The concentration of oyster larvae averaged 9 per ml. Plots were based on the mean length of 100 larvae from each of duplicate cultures.

range of 50,000 to 400,000 cells per ml. of this food. Likewise, although 250,000 cells per ml. appears to be the optimum concentration of *Monochrysis*, there was little difference in the rate of growth of clam larvae in concentrations of this food ranging from 125,000 to 500,000 cells per ml., at least through the 8th day. Clam larvae grew normally in concentrations of either *Isochrysis* or *Monochrysis* equal to the concentration at which Lewin's *Chlorella* was lethal. We believe this may indicate that the external metabolites of *Monochrysis* or *Isochrysis* are much less toxic to clam larvae than are those of *Chlorella*.

DISCUSSION

It is probably significant that in these experiments the four best foods for oyster larvae—*Isochrysis*, *Monochrysis*, and the two species of *Dunaliella*—were all naked flagellates. If we exclude *Prymnesium*, a naked flagellate that was toxic, even the poorest of the naked flagellates, *Dunaliella* sp., was consistently a better food, during the first 6 days, than any of the organisms that had cell walls. Moreover, *Platymonas* and *Chlorococcum* were the only two organisms tested having cell walls that gave appreciably better growth of oyster larvae than no food during the first 6 days. Apparently, oyster larvae cannot readily utilize organisms having cell walls, particularly during the early larval stages. That older oyster larvae can utilize forms having cell walls was demonstrated in the first experiment by the rapid growth after the 6th day of larvae receiving *Platymonas*, and by the reasonably good growth of older oyster larvae receiving *Chlorococcum*, Lewin's *Chlorella*, or *Phaeodactylum*.

Clam larvae, unlike oyster larvae, were able to utilize several forms having cell walls from the earliest larval stages. Thus, even at 4 days clam larvae receiving *Platymonas*, *Phaeodactylum*, *Chlorococcum*, or Lewin's *Chlorella* were significantly larger than the unfed controls, and as large or larger than larvae receiving the two species of *Dunaliella*, which are naked flagellates. Moreover, *Chlorococcum*, a form having cell walls, was possibly the best food yet tested for clam larvae.

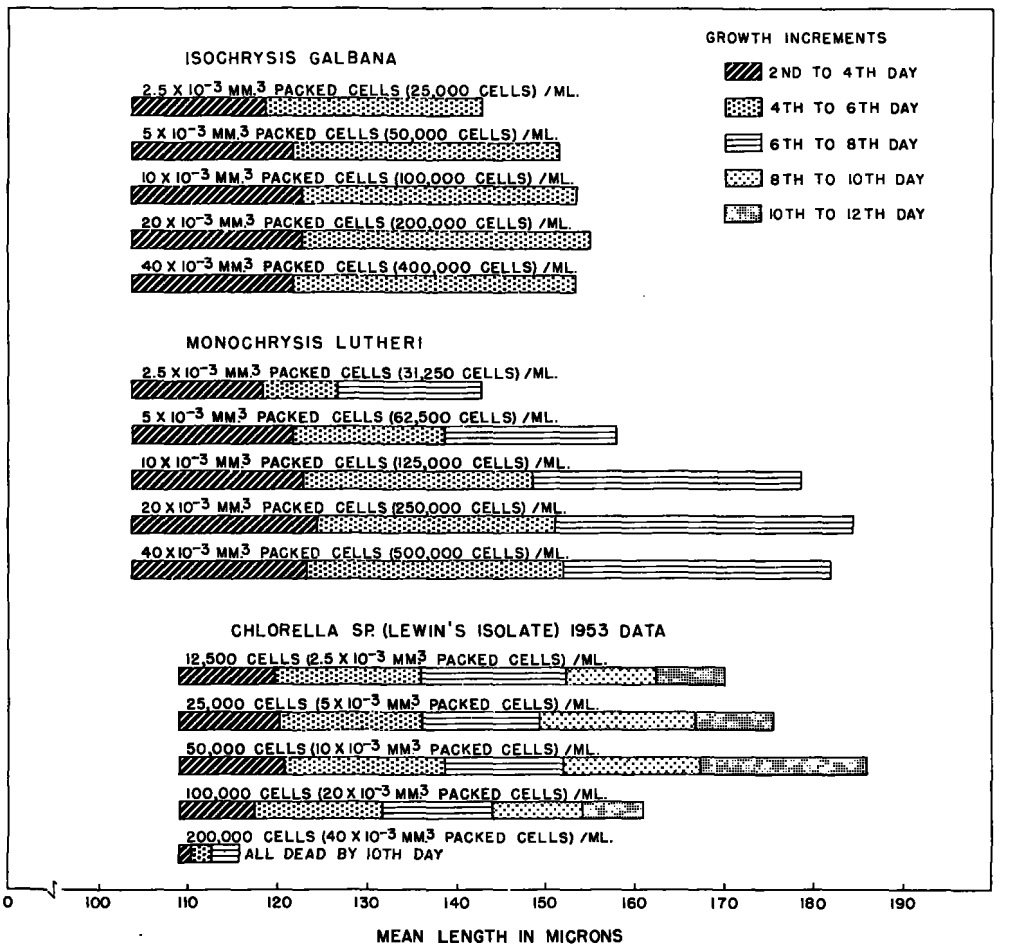

FIGURE 6.—Growth of clam larvae (*Venus mercenaria*) in cultures receiving five different concentrations of *Isochrysis*, of *Monochrysis*, and of *Chlorella* sp. (Lewin's isolate). The concentration of clam larvae averaged 10 per ml. Plots were based on the mean length of 100 larvae from each of duplicate cultures.

Nevertheless, at 4 days larvae receiving *Monochrysis* and *Isochrysis*, the two best naked flagellates, were significantly larger than larvae receiving any of the organisms with cell walls, except *Platymonas*. This may indicate that the presence, or perhaps the thickness of the cell wall, affects the utilization of a food even by clam larvae during the very early larval stages.

Gibor (1956), similarly, found that growth of larvae of *Artemia salina* was faster when fed any one of three Polyblepharidaceae (*Stephanophora gracilis*, *Dunaliella viridis*, and *Dunaliella salina*) than when these larvae were fed either his *Platymonas* sp. or his *Stichococcus* sp. He also postulates that this may have been due to the presence of a rigid cellulose cell wall in the latter two species.

Another factor that appears to be important in determining the value of micro-organisms as foods for bivalve larvae is the toxicity of their metabolites and the quantity of such metabolites they produce. As was shown by Loosanoff, Davis, and Chanley (1954), *Chlorella* sp. (Lewin's isolate) produced toxic metabolites that killed clam larvae when a sufficient volume of the filtrate from a *Chlorella* culture was added to the larval culture. Moreover, Davis and Chanley (1955) showed that the first evidence of the toxic effect of several substances on clam larvae was a reduction in the rate of growth of these larvae. Lucas (1955) has pointed out that metabolites of a given micro-organism may affect other aquatic organisms in quite different ways, promoting growth of some while hindering growth of others.

Gibor (1956) considered the possibility that his *Stichococcus* sp. was producing an "inhibitor to the growth" of *Artemia* larvae, but in the experiment in which he attempted to test this hypothesis he

apparently had no control for comparison and, perhaps erroneously, concluded that "Good development of the larvae showed that no inhibitor was produced."

Obviously, to be of food value to bivalve larvae, a micro-organism must be small enough, or break up into fragments small enough, to be ingested by the larvae, and must contain the necessary food elements in its body or in its storage products. Nevertheless, we believe that much of the difference in food value of closely related micro-organisms is directly attributable to differences in the quantity or toxicity of their metabolites. Thus, *Prymnesium*, which is fairly closely related to *Monochrysis* and *Isochrysis*, is small enough to be ingested and is, apparently, readily digested, but it is of little or no value as a food because of its toxicity. We have some evidence, moreover, that the toxic metabolites of *Prymnesium* are not primarily external, but are retained in the organism itself. It would seem highly probable also, from observations on the rate of growth of the larvae and on their mortality, that one of the primary differences between *Chlorella* sp. (Lewin's isolate) and *Chlorella* sp. (Guillard's isolate) as a larval food is the greater toxicity of the metabolites of the latter.

The differences between the rates of growth of clam and oyster larvae receiving the two species of *Dunaliella* were also possibly only reflections of differences in the toxicity of the metabolites of these two foods. In this case, however, it would be necessary to assume that the metabolites of *Dunaliella* sp. were more toxic to oyster larvae than were those of *D. euchlora*, and that the reverse was true for clam larvae. *D. euchlora* also reversed the usual trend in that it was the only food thus far tested that appeared to be a comparatively good food for oyster larvae but a comparatively poor one for clam larvae. Gibor (1956) also found that the food value of *Dunaliella viridis* differed from that of *D. salina* and that the food value of *D. salina* varied from one experiment to another.

Chlamydomonas, likewise, appeared to produce metabolites toxic to oyster larvae, but the pattern of growth shown by clam larvae receiving this food suggests that both the toxicity of metabolites and an inability of many of the clam larvae to digest the cell walls of *Chlamydomonas* were involved. *Phaeodactylum*, unlike *Chlamydomonas*, did not appear to produce toxic metabolites and the failure of the majority of oyster larvae to utilize these organisms probably reflects an inability of the majority of the larvae to digest the cell walls of *Phaeodactylum*.

In pond or hatchery practice, it is the numbers of such organisms as *Monochrysis* and *Isochrysis* that we would want to increase by fertilization. Since both clam and oyster larvae grow quite well throughout a wide range of concentrations of these micro-organisms, and since the metabolites of *Monochrysis* and *Isochrysis* appear to be very low in toxicity, it would not be necessary to control their numbers as rigidly as would be necessary with organisms such as *Chlorella*.

SUMMARY

1. Representatives of 10 genera of micro-organisms were tested in feeding experiments to determine their relative value as foods for larvae of oysters and clams.

2. *Isochrysis galbana* and *Monochrysis lutheri* were of approximately equal value and were the best single foods for oyster larvae.

3. A mixture of *Isochrysis* and *Monochrysis* of the Class Chrysophyceae, with *Platymonas* sp. and *Dunaliella euchlora* of the Class Chlorophyceae provided better growth of oyster larvae than did any of these foods singly.

4. With the exception of *Prymnesium parvum*, which is toxic, even the least valuable of the naked flagellates was a better food for young oyster larvae than were any of the micro-organisms with cell walls, but older oyster larvae can utilize some forms having cell walls.

5. *Stichococcus* sp., *Prymnesium parvum*, and *Chlamydomonas* sp. (British isolate) reduced the rate of growth of oyster larvae below that of the unfed controls, and *Chlorella* sp. (Guillard's isolate) was slightly poorer than no food in one experiment and slightly better in the other.

6. The mixture of flagellates provided better growth of clam larvae than did equal quantities of any of the single foods tested.

7. *Chlorococcum*, *Isochrysis*, and *Monochrysis* were the three best single foods for clam larvae.

8. Clam larvae can utilize several forms having cell walls even from the earliest larval stages.

9. *Stichococcus* sp. and *Prymnesium parvum*, both highly toxic, were the only two micro-organisms tested that were of no food value to clam larvae.

10. *Isochrysis* gave no evidence of toxicity even

in the highest concentration tested and the optimum concentration of *Isochrysis* for either oyster or clam larvae was at least double the optimum concentration of *Chlorella* sp. (Lewin's isolate).

11. Both oyster and clam larvae grew normally even in the highest concentration of *Monochrysis* tested and the optimum concentration of this food for either species of larvae was also at least double that for *Chlorella* sp. (Lewin's isolate).

12. We believe from these observations that *Isochrysis* and *Monochrysis* produce very little, if any, toxic metabolites.

13. The presence, or perhaps thickness, of cell walls and the degree of toxicity of the external metabolites are probably important factors in determining the usability of micro-organisms as foods by bivalve larvae.

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U. S. GOVERNMENT PRINTING OFFICE: 1958 O—458617