BASTERIA

TIJDSCHRIFT VAN DE NEDERLANDSE
MALACOLOGISCHE VERENIGING

Vol. 34, No. 5–6, pp. 91–154 15–XII–1970

Radiotracer studies on the nutrition of the early larvae of Ostrea edulis

by

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INTRODUCTION

The catastrophic decline of oyster production in the United States and in some parts of Europe has focused attention to the need for artificial culturing of these organisms. Forty years of extensive attempts to rear bivalves from gametes to metamorphosis has only achieved significant progress, with repeatable results, within the past ten to fifteen years (Loosanoff & Davis, 1963). Accomplishing satisfactory mollusk larval growth is dependent upon rather complex techniques and good and sufficient food supplies. Of the latter, only a few planktonic organisms have been identified as being adequate sources of nutrition for several species of mollusks (Loosanoff & Davis, 1963). In these studies clams and oysters have received most of the attention due to their commercial value, and only scattered information is available on the ecology and physiology of the economically less important species. For a description of proven methods successful in conditioning and maintaining bivalves under

laboratory conditions, Loosanoff & Davis (1950, 1963) and Loosanoff (1954) should be referred to.

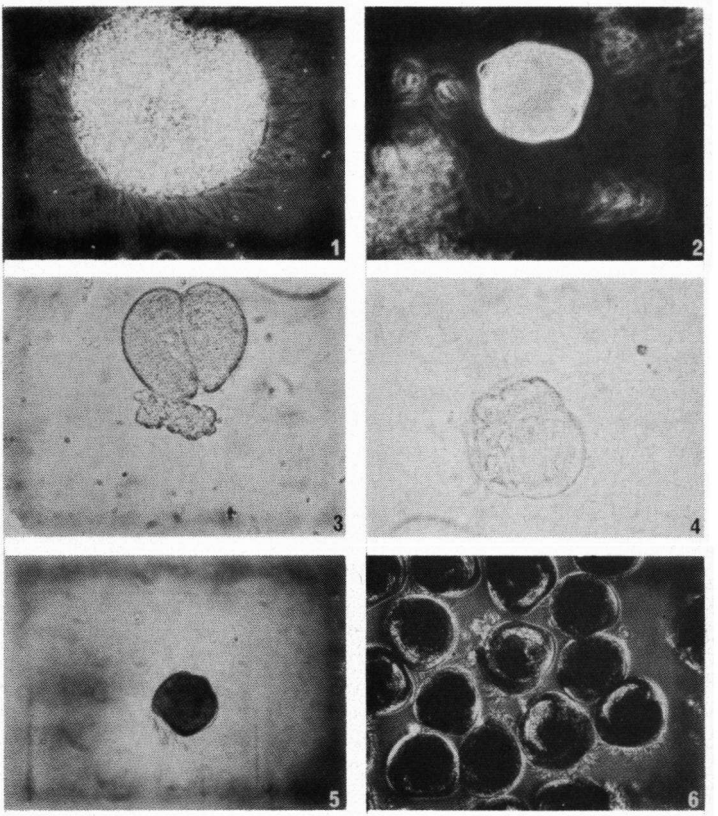
Earlier investigators studied the food preferences of selected bivalves (oysters and clams) by examining the contents of the stomach and feces. Savage (1925) carried out one of the most comprehensive studies of this kind comparing the seasonal variations in plankton composition with the stomach contents of oysters. The results of such a procedure are inconclusive since neither the relative nutritive value of the planktonic organisms to the oysters was determined, nor was it ascertained whether or not the organisms found were actually assimilated.

Recent investigations have followed a more precise path. Planktonic larvae have been raised on specific diets composed primarily of axenic cultures of algae. The value of the selected food organisms has been determined by counting the larvae reaching metamorphosis and measuring their growth rate (Walne, 1963).

The American oyster, *Crassostrea virginica* (Gmelin), and the hard-shell clam, *Mercenaria mercenaria* (L.), are the subjects of intense investigations (Galtsoff, 1964; Loosanoff & Davis, 1963) but the European oyster, *Ostrea edulis* (L.), though introduced into the United States (Galtsoff, 1964) has not been studied as extensively in this country. This species is more tolerant to lower temperatures than the American oyster, and success in propagating it in the cooler waters of Maine on the East Coast and Washington on the West Coast has been recorded (Loosanoff & Davis, 1963). In a series of papers, Walne (1956, 1958, 1959, and 1963) reported the value of different algal species as food to the planktonic larvae of *O. edulis*.

Unlike the American oyster, *O. edulis* is a protandric mollusk being either functionally male or female. Yonge (1966) has estimated the egg size to be approximately 150μ in diameter. Spawning occurs when the eggs pass from the genital opening through the gill filaments into the inhalent chamber of the mother where they are fertilized by indrawn spermatozoa after which early larval development takes place. Swarming occurs after six to ten days. The diameter of normal swarming larvae is from 199μ to 212μ (Loosanoff & Davis, 1963) (cf. figs. 1-10). On the other hand the American oyster, *C. virginica*, expels ripe eggs and spermatozoa into the surrounding water where fertilization occurs, and the smallest straight-hinged larvae measure approximately 68μ x 55μ (Loosanoff & Davis, 1963).

The present investigation deals with the feeding of isotopically labeled algal food to the larvae of *O. edulis* during their incubation



Figs. 1-6. Micro-photographs taken during the identification of gravid oysters using a Nikon microscope with a Polaroid attachment. 1. Spermatozoa united into a sperm ball (phase contrast). 2. Live fertilized egg (phase contrast). 3. Early cleavage. 4. Do. 5. Umbo larva showing ciliary circles (phase contrast). 6. Early straight hinge larvae. Figs. 1-4 130 x, figs. 5-6 85 x.

period. Two possibilities exist for the larvae to obtain food: 1) they may directly utilize food entering the parent's shell through siphoning action, or 2) the parent digests the available food entering the shell and secretes nutrients for larval consumption. The necessity to differentiate which of the two possibilities is indeed the case is obvious since the maximum size nannoplankton that the incubated larvae could utilize is limited to only a few microns.

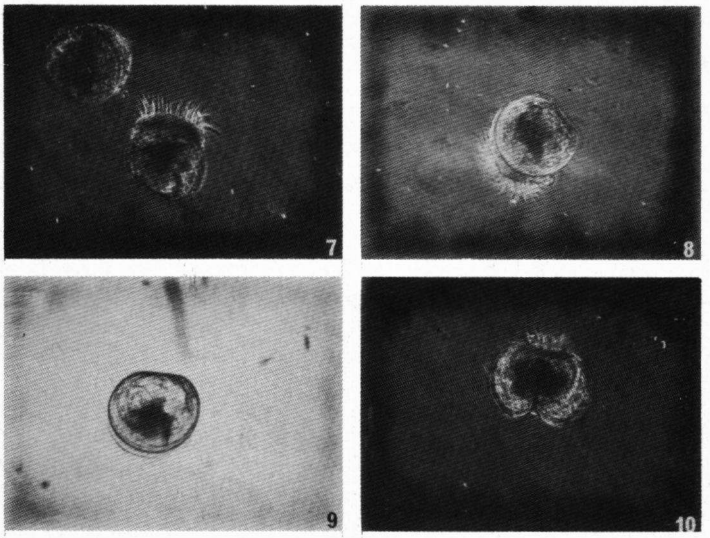
MATERIALS AND METHODS

The European oyster, under natural conditions, begins spawning during the summer months when temperatures rise above 15°C (Yonge, 1966); therefore, prior to the start of the experiments, 24 European oysters of approximately equal dimensions were thermally conditioned by raising the temperature of their holding trays from 10°C to 18°C. During this procedure, running sea water passed through the trays at 2-3 gallons per hour. Twenty-three days later it was noted that spawning was taking place, and the appearance of gravid females occurred on the twenty-fifth day.

Gravid females were selected for the experiment by randomly taking several oysters and placing them in trays of filter sterilized sea water. After a period of approximately 20 minutes, if left undisturbed, the oysters resumed siphoning. They were then quickly removed from the trays and part of their internal contents spilled into petri dishes. These samples were examined under the medium power of a microscope for the presence of ripe eggs, spermatozoa, or larvae. Since both fertilization and incubation occur in the inhalent chamber of the animals, and since some of the contents of their chambers may be easily removed, gravid females can be identified without difficulty. Two animals of approximately equal weight, 42.00 g, and 43.02 g, and equal sizes 7.3 x 6.4 cm and 7.6 x 6.9 cm respectively, were placed in separate pyrex trays with dimensions of 30 x 22 x 5 cm.

Two species of algae were used, the silicious, centric diatom *Cyclotella nana* Hustedt (Hustedt, 1957) and the naked chrysophyte *Monochrysis lutheri* Droop (cf. Droop, 1955). They were obtained as axenic cultures from Milford Laboratories and Woods Hole Oceanographic Institute, respectively. In all procedures to bring the algal cultures to the desired cell concentration, sterile conditions were maintained. The algae were grown in a culture medium described by Loosanoff & Davis (1963), composed of two stock solutions of which 10 ml aliquots were added to 1 liter of sea water respectively. The culture medium was then autoclaved for 15 minutes at 15 pounds pressure. *C. nana* and *M. lutheri* were added to the medium, installed in 2 liter Erlenmeyer flasks, and stoppered with sterile cotton plugs. Both cultures were continuously aerated and illuminated under 80 watt fluorescent light, maintained at 22°C, and their concentrations brought to the following: *Monochrysis* 5×10^6 cells/ml, and *Cyclotella* 7.5×10^5 cells/ml.

Carbon-14 labeled sodium carbonate, sp. act. 48.3mC/mM, was



Figs. 7-10. Micro-photographs taken during the identification of gravid oysters using a Nikon microscope with a Polaroid attachment. 7. Early straight hinge larvae with velum protruding (phase contrast). 8. Early straight hinge larva with velum protruding (phase contrast). 9. Early straight hinge larva withdrawing its velum. 10. Early straight hinge larva with valves opened. All figures 85 x.

introduced into the cultures at 0.5mC/1. During labeling the algae were continuously illuminated, aerated, and maintained at 22°C ± 1° for 48 hours in the radioactive medium. Cell counts were made every 12 hours employing a Sedgwick-Rafter cell. After reaching the desired cell concentrations, the algae were centrifuged for three minutes in a Sharples Super Centrifuge at 1.3×10^4 g's and washed three times with sterile sea water. The algae were then transferred into filter sterilized sea water and placed into 2 liters Erlenmeyer flasks. Cell counts were made and the *Monochrysis* culture was adjusted to the cell concentration of *Cyclotella*. The final counts of both cultures were approximately 1.25×10^6 cells/ml. The flasks were aerated, and the agitation supplied by the air bubbles maintained a homogeneous suspension of the cells also causing the algae to drip steadily (40 ml/hr) into the pyrex trays holding the oysters.

The introduction of the algal cells was maintained for 52 hours. Assuming the approximate larval count, made at the termination of feeding, to be 1 million per oyster, slightly more than 1200 algal

cells were available to each larva per day. This number of *Monochrysis* cells is below that found by Walne (1959, 1963) to provide optimum growth for the larvae of *O. edulis*; however, since food consumption will be determined by the amount of radioactive uptake by the oysters and not by measuring growth induced by algal ingestion, this number seems to be sufficient for both cultures.

One ml of the cultures was drawn off at the start of the experiment at 0 hours, 24 hours, and at 52 hours (the time of completion of the experiment). The samples were fixed with 75% formaldehyde and were set aside for radioactive determinations. At the termination of feeding, the oysters were removed from the pyrex trays, opened, and the larvae washed into sterile sea water. Both the parent oysters and the larvae were washed three times in sterile sea water and fixed in a 7% formaldehyde solution.

For estimating the number of larvae from each oyster, the method of Walne (1964) was followed with minor modification. The fixed larvae were washed into a 1 liter graduated cylinder and agitated to provide even distribution. At the same time, 0.5 ml samples were drawn off and counted on squared slides under a microscope. Five such counts were made with 340-650 larvae per sample. The mean of the five counts was 512 and 467 for the larvae fed *Cyclotella* and *Monochrysis*, respectively.

The oysters, larvae, and algal samples were thoroughly dried under an infrared lamp for 72 hours. After drying, the parent oysters were ground into homogeneous powders, and three 10 mg samples of each oyster were weighed out. Similar 10 mg samples of the larvae and 1 ml of the algal suspensions were also weighed, and all of these samples were prepared for combustion in a Schöniger combustion flask.

After combustion, the flasks were placed in a bath of dry ice and acetone. A toluene-naphthalene scintillation liquid (Wang & Willis, 1965 : 167) was prepared, and 12 ml was introduced into the flasks in order to entrap any available Carbon-14 bound in carbon dioxide. Ten ml samples of the mixture were pipetted from the flask into counting vials designed for liquid scintillation.

Combustion in all but two cases went to completion and these samples were eliminated from the series. Counting was performed in a Nuclear-Chicago Mark I External Standard Liquid Scintillator employing channels ratio technique. The scintillator was programmed to count each vial for 20 minutes and to determine the efficiency of each count. Background was automatically subtracted.

RESULTS

The obtained radioactivity determinations, both on the mother and on the larvae which utilized the two different algae, are compiled in Table I. This table also shows the radioactive counts for the algae at zero hours and at fifty-two hours of the feeding experiment. By knowing the initial activity, the labeling efficiency of the cultures was calculated to be 1.3 percent for *Monochrysis* and 0.12 percent for *Cyclotella*. All values have been corrected for background.

Table I
A comparison of the radioactivity of both parent and oyster larvae

Experimental organisms	cpm	% Efficiency	dpm	
Parent oyster fed *M. lutheri*	285.3	79.0	361.2	10 mg Samples
Parent oyster fed *C. nana*	9.14	79.5	11.50	
Larvae fed *M. lutheri*	92.77	79.0	117.43	
Larvae fed *C. nana*	10.26	78.3	13.11	
M. lutheri	1,138.85 (0 hr)	79.0	1,441.58	1 ml Samples
	2,449.95 (52 hr)	79.0	3,101.20	
C. nana	105.59 (0 hr)	79.0	133.66	
	45.38 (52 hr)	79.4	57.15	

Since the counts shown in Table I refer to the dry weights of the different starting materials, the following reasoning has been employed in order to be able to more meaningfully compare them with each other. The counts obtained for the algae at 52 hours represent 30 mg of dry weight. As a generalization, one can state that the algal cells contain approximately 80 percent water, thus the wet weight of *Cyclotella* and *Monochrysis* would be 150 mg each. For the sake of simplicity, the weights were transformed into kilos and the corresponding dpm calculated. Thus 1 kg wet weight of *Cyclotella* at fifty-two hours corresponds to 3.8×10^5 dpm/kg and the same wet weight of *Monochrysis* equals 2.1×10^7 dpm/kg.

The *Cyclotella* fed larvae, when treated in the same manner, would yield a count of 2.62×10^5 dpm/kg wet weight; whereas, the *Monochrysis* fed larvae would yield 2.34×10^6 dpm/kg wet weight.

The parent oyster fed *Cyclotella* shows an activity of 2.3×10^5 dpm/kg wet weight and the one fed on *Monochrysis*, 7.2×10^6 dpm/kg wet weight. These numbers are compiled in Table II.

Table II
The corresponding dpm for the wet weights of the algae, larvae and parent oysters

Parent fed *M. lutheri*	7.2×10^6 dpm/kg	
Parent fed *C. nana*	3.3×10^5 dpm/kg	
Larvae fed *M. lutheri*	2.34×10^6 dpm/kg	
Larcae fed *C. nana*	2.62×10^5 dpm/kg	Larvae
M. lutheri at 52 hours	2.1×10^7 dpm/kg	
C. nana at 52 gours	3.8×10^5 dpm/kg	52 hours

In order to ascertain the uptake ratios, the radioactivity counts of the larvae and parent oysters were divided by the radioactivity shown for the algae at fifty-two hours. Ratios were also calculated for comparing the relative uptake of the two algal species by the larvae and parents. These calculations are given in Table III.

Table III
Uptake ratios

$$\frac{\textit{C. nana} \text{ fed larvae}}{\textit{C. nana}} = \frac{2.62 \times 10^5 \text{ dpm/kg}}{2.8 \times 10^5 \text{ dpm/kg}} = 0.69$$

$$\frac{\textit{M. lutheri} \text{ fed larvae}}{\textit{M. lutheri}} = \frac{2.34 \times 10^6 \text{ dpm/kg}}{2.1 \times 10^7 \text{ dpm/kg}} = 0.11$$

$$0.69 \div 0.11 = 6.26$$

$$\frac{\textit{C. nana} \text{ fed parent}}{\textit{C. nana}} = \frac{2.3 \times 10^5 \text{ dpm/kg}}{3.8 \times 10^5 \text{ dpm/kg}} = 0.61$$

$$\frac{\textit{M. lutheri} \text{ fed parent}}{\textit{M. lutheri}} = \frac{7.2 \times 10^6 \text{ dpm/kg}}{2.1 \times 10^7 \text{ dpm/kg}} = 0.34$$

$$0.61 \div 0.34 = 1.79$$

Finally, in order to find out whether or not an adequate number of algal cells were available to the larvae, the following calculations were performed. The wet weight of a single larva was estimated, from its measurements, to be 3.56×10^{-4} mg presuming a density of 1.02. In the same manner, a single cell of *Cyclotella* was determined to weigh 2.4×10^{-5} mg. A comparison of these weights shows that approximately 15 cells of *Cyclotella* equal the weight of a single larva. Since the amount of algal cells introduced into the culture trays is known to be 5×10^{7} cells/hour and each tray contained approximately 1×10^{6} larvae, each larva had fifty algal cells/hour available for consumption. This number of cells approximates three times the weight of an individual larva.

DISCUSSION

The experiments have shown that both the parent oyster and larvae do take up the tested algae. There are, however, remarkable differences among the six variables that cannot be ascribed to experimental error. In spite of the fact that the labeling efficiency of the *Cyclotella* cultures was only one-tenth of that shown by *Monochrysis*, considerably larger uptake of *Cyclotella* occurred both in the parent oyster (1.79 times) and the larvae (6.26 times) fed with this organism. Why this difference in labeling efficiency occurred between the two algal species is not known. It is plausible to suppose that *Cyclotella* had a faster metabilism than *Monochrysis* and by the end of the forty-eight hours in the radioactive medium this alga had already given off considerably quantities of the activity. It is more probable, on the other hand, that since *Cyclotella* exhibited more sluggish growth than *Monochrysis* in our experiment (it was difficult to reach the desired cell concentration) owing to their slower metabolism, they did not take up the radioactivity.

The calculated uptake ratios show that the largest amounts of algae were utilized by the larvae fed on *Cyclotella*. During the fifty-two hour period these larvae consumed approximately 70 percent of their own body weight. The *Monochrysis* fed larvae, on the other hand, ate approximately 11 percent of their weight in the same period, i.e., the utilization of *Cyclotella* was about six times more than that of *Monochrysis*. The diatom fed parent took up approximately six-tenths of its body weight during the experiment and the *Monochrysis* fed parent approximately 0.34 of its own

weight. The utilization of *Cyclotella* was 1.8 times better than that of *Monochrysis* in the adults.

If one compares the metabolism of the parent with their larvae (both fed the same food organism), it becomes evident that the larvae ate about the same amounts as their parent on the *Cyclotella* diet and about three times less on the *Monochrysis* diet. Finally, since the difference in the ratios of the parent oysters consuming the two algal species and those of the larvae is very small, 1.79 versus 6.26 (equals 3.45) and 0.32 versus 1.1 (equals 3.49) apparently there is no contrast in selectivity of the food uptake by the parents and larvae.

Because the total number of larvae ($\sim 10^6$) within the parent ingests approximately the same weight of *Cyclotella* and one-third the weight of *Monochrysis* than the parent itself ingests, the radiotracer experiments have decidedly shown that the larvae are able to efficiently utilize both kinds of algae tested and that their food supply does not originate through a secretory mechanism of the parents. In biological transformation of materials, usually a 10 percent efficiency is found; thus, if the larvae would have been fed by the parent, a ratio of at least 1 : 10 should have occurred.

In earlier studies, Loosanoff & Davis (1963) and Walne (1963), have demonstrated that *Monochrysis* is a good food organism for the planktonic larvae of both the American and European oysters. Thus, the relatively poor uptake of this organism, both by the parent and its larvae in the present experiment could possibly be explained by the fact that the algae might have clumped, resulting in too large particle sizes for ingestion. It was noted that during the feeding period a significant amount of pseudofeces was produced by the animal kept on *Monochrysis*, and this phenomenon may explain why this otherwise excellent food was not sufficiently utilized.

The results obtained on the larvae feeding on *Cyclotella* is worthy of mention. The possibility that diatoms with silicified walls serve as excellent food for oyster larvae has been reported (Matthiessen & Toner, 1966; Nelson, 1921). This may be explained by the structure of the diatom. The shells of silicious diatoms are composed of two unfused valves, and the destruction of their protoplasmic contents may occur, following their being taken up by the amoebocytes, through the intracellular enzymes entering the pores of the valves or by the walls being pulled apart, exposing the cytoplasm to digestion.

SUMMARY

C-14 labeled algae (*Cyclotella nana* and *Monochrysis lutheri*) were fed to the parents and incubant larvae of *O. edulis*. Through liquid scintillation counting, it was established that both the parent and the larvae take up particulate food during incubancy, thus the parent does not seem to supply the food requirements of the larvae. Food utilization in the larvae was shown to be approximately the same in *Cyclotella* and one-third in *Monochrysis* as in the parents. Substantial C-14 uptake was found in larvae on *C. nana* proving its ingestion by the animals while *M. lutheri* seemed to be of lesser value to the incubatory larvae of *O. edulis* in this experiment.

ACKNOWLEDGEMENT

We would like to express our appreciation to Mr. George Vanderborgh of Vanderborgh-Redel Oyster Laboratories, Oyster Bay, New York, for his kind permission to use his facilities during the feeding experiments.

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