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Some factors affecting the growth of the larvae of Ostrea edulis

by

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A technique for the large-scale culture of oyster larvae was described by Walne (1966). This was based on experiments made at Conway in 1963-64, and since then we have continued to examine factors which influence the growth of larvae under hatchery conditions. This paper describes two aspects which have received attention; they are respectively the shape and nature of the rearing vessel, and the food provided for the larvae.

Unless stated to the contrary, the larvae in these experiments have been treated by the standard procedures, including water treatment, aeration and culture of food, which have been described elsewhere (Walne 1966).

Shape and nature of vessel

Both the shape and fabric of the vessel can have a considerable influence on the growth of larvae. Two standard vessels have been used at Conway for a number of years: 1-litre glass beakers and 75-litre polythene bins. In a number of cases beakers have been set up with 200-400 larvae from a large-scale culture and these have been reared for four days with a change of water after the second day. These tests were comparable with the large cultures as regards larvae, water, food and antibiotics, but the density of the larvae was only about one-third of that in the bins. In the second type of experiment beakers of water were dipped out of the bins immediately after the water change and the larvae cultured for a further two days. In these, the larval density was identical in both vessels and was about 1000 per litre.

The following results show the average length increment which was obtained in the two types of comparison:

Density of larvae	Duration of experiment (days)	Food (cells per µl)	Average length increment (µ)		Number of experiments
			Bin	Beaker	
(a) high	2	100 Isochrysis	16.3	20.4	17
		10 Tetraselmis	16.8	20.0	14
(b) low	4	100 Isochrysis	31.6	48.4	12

In no case were the same larvae used in (a) and (b). In both the low-density and high-density beaker experiments the growth rate, at 12 μ and 10 μ per day respectively, was greater than the 8 μ per day found in the bin experiments. There are a number of reasons for the relatively poor result experienced in the bins, and two of these have been examined. Firstly, the shape is different and therefore the depth of the water column and the turbulence pattern caused by the aeration differed. Secondly, the materials of which the vessels were made differed.

To examine the depth of water column a number of rearing experiments have been made in hard-glass tubes. The influence of the length of the water column was investigated by bolting standard lengths of tubing together; the bottom was closed with a domed glass end and the tubes were held vertically. An aerator tube reached to the bottom. We used tubing of 10 cm bore, holding water depths of 80, 162 and 220 cm; these corresponded to volumes of 7, 14 and 21 litres respectively. Five series of experiments have been made in which the larvae were fed on a mixture of 50 cells of Isochrysis per μl and 5 cells of Tetraselmis per μl, and the results are analysed with respect to growth rate and development of the eyespot. The mean lengths of the larvae (in μ) during the ten-day growing period in the five experiments were as follows:

Days	Depth of water (cm)		
	80	162	220
2	204	206	205
4	223	229	229
6	237	246	249
8	253	259	264
10	263	269	274

The differences are small but they consistently show that the deeper the tube, the larger the average shell length of the larval population. This is confirmed by examining the mean shell length for the three tubes in each experiment on each of the even-numbered days (they were not measured on the odd-numbered days). From this the number of occasions on which the mean length of the larvae in tubes of different depth exceeded or was less than the average for the three replicates can be determined. The following table shows the results for 25 sets of measurements (five experiments on each of days 2, 4, 6, 8 and 10), given as the number of occasions on which the mean length of the larvae was below average, average, or above average:

Mean length of the larvae	Depth of water (cm)		
	80	162	220
Below average	19	9	3
Average	1	2	3
Above average	5	14	19

From this it can be concluded that the larvae in the longest water column were larger than average about 80 per cent of the time. A similar trend was found in the average proportion of the population which had developed eyespots (indicating that the larvae were mature):

Day	Depth of water (cm)		
	80	162	220
8	4.8%	7.2%	13.5%
10	17.4%	26.2%	31.0%

Comparisons have been made between the growth in tubes holding 12 litres of water (depth 140 cm) and bins and beakers. The results of five sets of comparisons between bins and tubes are shown in the following table, which sets out the situation on day 8:

	Bin		Tube	
	Mean length (μ)	% eyed	Mean length (μ)	% eyed
1	250	0	274	0
2	246	1	270	26
3	191	0	248	0
4	242	0	265	30
5	221	0	250	4
Average	230	<1	261	12

Six comparisons are also available between similar tubes holding 12 litres and 1-litre glass vessels. The comparison is not so strict as with the bins, because the larval densities were only 250-500 per litre in the beakers, while in the tubes they were at 800-1500 per μl. The mean growth increment in the four-day period was 40.0 μ in the tubes and 42.0 μ in the beakers. From all these tests it appears that tubes and beakers give similar results which are better than that from the 75-litre polythene bins. The next section is concerned with another source of variation - the material used.

Materials for culture vessels

We have suspected for some time that the nature of the material would be important, but this has been difficult to test because of the necessity of using vessels of similar shape and size, but made of different materials.

We have been able to obtain 1-litre beakers made from four materials. Borosilicate glass, polythene and polypropylene were commercially available, and we had a set moulded from the same grade of fibreglass as has been used for the larval culture bins at the White Fish Authority hatchery, Conway. Five trials were made, each material being tested in triplicate. All four materials were not always tested together, but the glass beakers were used throughout as a reference. In each test about 250 larvae were grown for four days (the water was changed on the second day) on a mixture of Isochrysis and Tetraselmis as food. The results were as follows:

Initial length (μ)	Length increment in glass vessels (μ)	Increment in other vessels as a % of that in glass vessels		
		Polythene	Polypropylene	Fibreglass
182	38	84	58	-
195	60	97	53	-
191	45	104	78	93
194	42	114	48	100
186	33	94	61	91
203	38	-	-	100
183	36	-	-	97
Average		99	60	96

These clearly demonstrate that poor growth was obtained with polypropylene vessels, but there was little to choose between those made of the other materials. It must be stressed that these results do not mean that polythene is always superior; they apply only to these batches of material.

It has been possible to obtain polyethylene bins, all of similar shape and each holding 75 litres, from four manufacturers. They had the following characteristics:

A Black, rigid polythene; our standard vessel.

B Grey, rigid polythene.

C Black, rigid, with a 'bloom' on the surface.

D White, virgin polythene.

Five experiments were made in which the growth of larvae in the standard bin 'A' was compared with growth in bins 'B', 'C' and 'D'. The mean size and the percentage eyed after 12 days were as follows:

Experiment	Manufacturer	Size at day 12 (μ)	% eyed, day 11-12
1	A	267	11
	B	281	34
2	A	257	8
	B	272	42
3	A	256	28
	C	260	22
4	A	246	0
	D	272	17
5	A	278	32
	D	280	23

Although the number of trials described above is small, they do indicate that variations in the suitability of vessels made of the same material, but by different manufacturers, do exist. In addition, the quality of a vessel from a given manufacturer is likely to vary from time to time, according to the proportion and nature of scrap polyethylene, the nature of the antioxidant added (this may be up to 2 per cent of polythene vessels), and the mould release agent which may be difficult to remove by cleaning.

Tetraselmis as food for oyster larvae

In a study on the food value of a variety of algal species to oyster spat it was found that exceptionally good growth was obtained with Tetraselmis suecica. As this species is easy to grow in large-scale culture, these results stimulated examination of its food value to larvae. The initial trials showed that it was approximately equal to Isochrysis and therefore more detailed tests of its value, both on its own and in combination with Isochrysis, were made.

In four series of experiments larvae were grown in 1-litre beakers for four days in a range of concentrations; all the series were duplicated, but each concentration was not tested every time. The following table shows the mean length increment obtained:

Cells of Tetraselmis per μl	Average length increment (μ)	Number of tests made
2.5	30.2	2
5	36.1	4
7.5	45.0	2
10	41.9	3
20	37.0	4
30	46.4	2
40	37.7	1

These results show that Tetraselmis supported growth in excess of 10 μ per day; this is comparable to that obtained with Isochrysis.

Further trials which compared directly the growth of larvae fed on Isochrysis and Tetraselmis in 1-litre beakers during 48 hours showed the foods to be equally matched. The larvae fed with 100 cells per μl of Isochrysis grew, on average, 19.6 μ, while those fed on 10 cells per μl of Tetraselmis grew 18.8 μ. In nineteen comparative trials better growth was obtained with Isochrysis than with Tetraselmis in ten, and in nine the reverse was the case. From these results we can conclude that the two species are very similar in food value in short-period experiments. This is extended in the data presented below, which show that Tetraselmis will support a satisfactory level of growth throughout the larval life.

A mixture of Tetraselmis and Isochrysis as food for oyster larvae

Preliminary tests suggested that a mixture of the two species was a better food than either species on its own, and this has been examined in detail in small- and large-scale experiments.

Six combinations of food were tested in five series of experiments. Each series was in duplicate 1-litre beakers and the larvae were grown for four days. The following table shows the food combinations tested and the average growth increment obtained in the five series of experiments:

Cells per μl		Average growth increment (μ)
Isochrysis	Tetraselmis	
100	-	9.8
80	2	10.9
60	4	13.5
40	6	10.8
20	8	10.7
-	10	8.9

From these results a combination of 50 cells of Isochrysis and 5 cells of Tetraselmis per μl was selected for study in large-scale cultures where the larvae were reared until mature. The growth of larvae fed on this mixture when cultured in the glass tubes holding 12 litres, which were described previously, was compared with duplicate cultures in which they were fed only on Isochrysis (100 cells per μl) or Tetraselmis (10 cells per μl).

In six experiments which lasted for 9 to 16 days the growth of larvae fed with the mixture was compared with those fed on Isochrysis. Little difference in the average size was found until they were approaching maturity; when the mean size of those fed on Isochrysis was in the range

271-290 μ, those fed on the mixture were, on average, 9.5 μ larger. The most marked difference occurred in the proportion of the population which had eyespots; eyed larvae were generally twice as numerous amongst those fed on mixed food, compared with those fed only on Isochrysis. Experiments in tubes were not usually continued until spatfall, but presumably those with the more substantial number of eyed larvae would yield an earlier and more prolific spatfall.

In three similar experiments the value of Tetraselmis has been compared with the mixture. Little difference was found in the growth rate, but again the proportion of eyed larvae was higher amongst those fed on the mixed foods.

Day	Percentage eyed	
	Tetraselmis	Mixed foods
7	6	10
9	35	46
11	52	76

From these results it seems reasonable to suggest that since mixed foods enhance the development of mature larvae, they may well increase the vigour with which metamorphosis is carried through and the subsequent viability of the spat.

Reference

Walne, P. R., 1966. Experiments in the large-scale culture of the larvae of Ostrea edulis L. Fishery Invest., Lond., Ser. 2, 25(4).