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Further experiments on the growth of the spat of *Ostrea edulis* L. in a closed water system

by

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ABSTRACT

A comparison was made in four experiments of the growth of oyster spat at various levels of feeding, number of spat per litre of water, number of spat per tray and flow through the tray. A factorial design was adopted so as to test all levels of each factor against the other factors.

Food level had the greatest influence on growth; the density per litre was important but only because of its effect on food supply; the number per tray had some effect, while the effect of changes in flow rate were very small.

INTRODUCTION

This paper presents the results of further experiments on the techniques of handling very small oyster spat. The spat are removed from the collector within 24 hours of metamorphosis. This alleviates the problem of overcrowding and eliminates the difficulties in handling large areas of collectors until the spat reach 10 mm in size.

Previous experience has shown that holding the spat in trays fitted with a mesh base and allowing the water to flow into the tray from an overhead spray is effective for clams (Walne and Dean 1967) and oysters (Spencer 1970). The four experiments described below outline the influence of flow-rate of the water, number of spat per tray, number of spat per litre and food level on the growth of spat. As there was no detectable mortality in any of the experimental treatments, this is not discussed any further.

METHOD

The method used was based on a re-circulation system, since we find this the most useful way of handling these small spat for the first three

weeks after metamorphosis. A series of 50 litre plastic tanks was adopted, six in the first two experiments and eight in the remainder. Four polystyrene trays with a base size of 32 x 16.5 cm (525 cm^2) hung in each tank; the base material was a nylon net, with a mesh size of 440 µm. A small plastic centrifugal pump sucked water from the base of the tank at 4 litres per minute and delivered it through a perforated PVC distribution pipe (1.43 cm hose), suspended 8 cm above the water surface. ~~Four~~ Sixteen 2.4 mm holes were drilled in the pipe so that four sprays of water entered the top of each tray, to give a flow-rate of 1 litre per minute per tray.

The tanks stood together in a controlled temperature room which was either in darkness or at a low level of artificial illumination. Each tank was filled with sea water which had been coarsely screened through a 61 µm nylon mesh. The particulate content of the sea water used at each water change was estimated both by drying a sample retained after filtration through a glass fibre paper and by counting the abundance of suspended particles 2.5-5.0 µm in diameter with a Coulter counter. The mean results of these and other indications of water condition are given in Table 1.

Table 1 Average condition of sea water, algal food and spat in the four experiments

	Experiment			
	4	7	8	10
Mean number of 2.5-5.0 µm particles per ml in sea water	110 x 10^3	66 x 10^3	39 x 10^3	47 x 10^3
Dry matter in sea water (mg/l)	4.81	3.97	2.33	1.28
Mean salinity (‰)	31.2	33.1	33.7	33.6
Mean dry weight of 10^6 Tetraselmis cells (mg)	0.219	0.224	0.213	0.245
Mean temperature of experiment (°C)	21.8	22.8	24.2	22.7
Initial weight of spat (mg)	0.22	0.16	0.12	0.43

Cultures of the flagellate Tetraselmis suecica (Kylin) Butch. obtained from the laboratory's large-scale culture system (Walne 1966) were used as food. The average weight of the cells is given for each experiment in Table 1. The water in the experimental tanks was changed twice a week and the tanks were thoroughly cleansed. The concentration of food was checked daily and sufficient culture added to restore the food to the required level.

Each of the experiments was started with spat 5-6 days old. Each lasted for 21 days and every 7 days an assessment was made of the number and size of the spat. To do this the spat were washed into a tared 5 cm PVC sieve which served as a weighing dish. Surplus water was removed through the mesh base of the sieve by standing it on a dry cloth. After weighing, a sub-sample was removed, weighed, and the number of spat counted. Throughout this paper the live weight is taken as a measure of the size of the spat.

A factorial experimental design was adopted so that in any one trial all levels of each factor under examination were tested against each other. The various levels of spat per litre were obtained by varying the number of trays in a tank containing the selected levels of spat per tray. In all cases four trays were used in each tank, but on occasion the experimental design necessitated one or more of the trays being empty.

RESULTS

Table 2 shows for the four experiments the mean size of spat at the various levels of each factor. An analysis of variance has been carried out and the significance of the differences in response to the main effects is indicated, as well as those first-order interactions which were also significant at the 5 per cent level. To reduce the complexity of the data we have only considered the mean weight after 21 days in this paper, and we indicate in the text where the mean weights showed important differences in the course of the experiment. Some levels of the various factors tested were repeated in more than one experiment and these results can be abstracted and treated as replications. The similarity of growth by these four batches of spat makes possible a preliminary assessment of the growth response over a wider range of levels than would have been the case if each experiment had been considered in isolation.

An inspection of Table 2 shows that growth was strongly influenced by food level and the number of spat per litre, while the effect of varying the number of spat per tray and the flow-rate per tray generally had a weaker effect.

Table 2 The mean weight in mg of spat grown for 21 days in various conditions. In Experiment 4 all trays received 1 litre/minute. In Experiment 7 all tanks held 50 spat per litre

Experiment 4	
Spat/litre	
80	2.9
164	1.7**
Spat/tray	
1 462	2.6
2 604	2.0**
Food/µl	
2.5	1.1
5.0	2.3
10.0	3.5†

Experiment 7	
A Spat/tray	
1 266	5.6
873	6.0
358	6.9†
B l/min	
0.5	6.6
1.0	5.8†
C Food/µl	
5	4.4
10	7.9†
B.C**	

Experiment 8	
A Spat/tray	
1 480	8.3
877	9.5
418	11.0**
B Spat/l	
84	6.9
56	9.8
28	12.4†
C Food/µl	
10	8.7
20	10.5**
B.C†	

Experiment 10	
Food/µl	
10	11.3
20	14.3**
Spat/tray	
799	12.8
408	12.8
Spat/l	
49	10.1
24	15.5†
l/min	
0.5	12.5
1.0	13.1

** = significant (P < 5% > 1%)

† = highly significant (P < 1%)

Number of spat per litre and food level

In the first experiment we restored the cell density of Tetraselmis to the required level each day and therefore the amount of food added to the tanks varied according to the grazing pressure. In the remaining experiments this procedure was adopted only during the first week. After this period we found that the density of Tetraselmis was so low as to be negligible after 24 hours' grazing by the spat, and we therefore daily added the full food ration to each tank. In Experiments 7, 8 and 10 one of the treatments was a second ration of Tetraselmis added at the end of the afternoon for the last two weeks of the experiment. The amount was 5 cells per µl during the second week and 10 cells per µl during the third week. For comparative purposes it is most useful to consider the total quantity of Tetraselmis, expressed as cells per µl, added per tank over the three-week period. This can be converted to dry weight (Table 1) or to cells per spat (Table 2).

Tetraselmis has several major advantages for hatchery use, but it has the unfortunate characteristic that some of the cells settle to the bottom of the tank when the algal culture is first diluted. The extent to which this happens and the length of time before the cells become motile again is variable. This characteristic reduces the accuracy of the estimated food consumption of the spat.

In the first two experiments the level of the ration fed daily was varied. By the end of the first week significant differences were found in the growth rate. After three weeks the differences were highly significant ($P < 0.01$, Table 2). These results showed that a daily ration of 10 cells per µl was better than smaller quantities, even at spat concentrations as low as 50 per litre. In the next two trials two feeding levels were compared: 10 cells/µl daily and 10 cells/µl plus an additional ration in the last two weeks (described as 20 cells/µl in Table 2), and in both cases the higher level of food produced significantly larger spat.

The food available to a spat depended on the amount added and the volume of water available to each spat. An analysis of the data from the last three experiments shows how growth was closely related to food supply. In Figure 1 the total number of cells of Tetraselmis available to a spat over the 21-day period is related to the size of the spat at the end of the period. Only those experiments in which there were about 800 spat per box and each box had a flow of 1 litre per minute were selected for this analysis; six examples came from Experiment 7 and eight from each of Experiments 8 and 10. Clearly, total food had a

dominating influence. The spat per litre varied between 24 and 84, but Figure 1 demonstrates that the significant differences found at different levels of spat concentration (Table 2) could be completely explained on the basis of food available, and it is not necessary, under the conditions of these experiments, to suggest that the accumulation of excretory products had an important influence.

The weight increment obtained for a unit amount of food added declined steadily as the food level increased (Fig. 2). This decrease in assimilation efficiency with more abundant food is to be expected on general biological grounds.

Experiments 7, 8 and 10 provide a triplicated comparison of two levels of feeding, at two levels of tray density, at a constant flow-rate of 1 litre/tray/minute and 49-56 spat per litre. The results (Table 3) indicated a reasonable consistency between experiments set up at different times with different batches of spat, and show how much more important is food level compared with density in the trays.

Table 3 The weight in mg of spat after 21 days grown under similar conditions in Experiments 7, 8 and 10. The means of the triplicated results are shown underlined

	Food level (cells/µl/day)	
	10	20
800 spat/tray	7.0, 8.6, 8.5 = <u>8.0</u>	10.8, 10.9, 12.6 = <u>11.4</u>
400 spat/tray	7.9, 9.2, 8.9 = <u>8.7</u>	11.0, 13.1, 13.0 = <u>12.4</u>

Number of spat per tray

Significant differences in growth were observed in those experiments where at least one level of this factor exceeded 1 000/tray (equivalent to 190 spat per 100 cm^2). The individual results from Experiments 7, 8 and 10, where the numbers per tray were altered but the flow was 1 litre/minute, the food 10 cells/µl daily, and spat in the range 49-56/litre, are shown in Figure 3. Table 3 also indicates the small effect obtained by changing the number per tray from 400 to 800, compared with the effect of changing the food level.

Flow rate per tray

Flows of 0.5 and 1.0 litre/minute/tray were tested in Experiments 7 and 10. The results are confusing, because a significant difference was obtained in Experiment 7 but not in 10. A portion of the two experiments

can be combined (Table 4) and the means of the duplicated results are consistently in favour of the lower level of flow-rate, although the differences are very small. The present information suggests that the effect of the two levels of this factor is either none or very small.

Table 4 The weight in mg of spat after 21 days grown under similar conditions in Experiments 7 and 10. The means of the duplicated results are shown underlined

	Flow-rate per tray (litre/min)	
	1.0	0.5
800 spat/tray	7.0, 8.5 = <u>7.8</u>	8.5, 8.3 = <u>8.4</u>
400 spat/tray	7.9, 8.9 = <u>8.4</u>	9.2, 8.5 = <u>8.8</u>

CONCLUSION

The experiments reported in this paper point to the overwhelming importance of food supply, a lesser influence of density in the tray and a negligible influence from flow-rate on the growth rate of oyster spat, and at the levels of each of the factors tested. A very good growth rate, leading to a size of 10-15 mg (3.9-4.6 mm) after 21 days, can be obtained under the following conditions:

<u>Food</u> 10 cells per µl of <u>Tetraselmis</u> added daily for the first seven days;
15 cells per µl added daily for the second seven days and 20 cells per µl for the last seven days.

<u>Spat concentration</u> Up to 50 per litre. Higher concentrations will probably require continuous feeding instead of two rations per day.

<u>Spat per tray</u> The effect of different densities suggests that the effect is small up to 1 000 per tray (190 per 100 cm^2).

<u>Flow-rate</u> The difference between 0.5 and 1.0 litre per minute per tray is small. The lower flow-rate of 0.5 litre is equivalent to 95 ml per 100 cm^2 of tray/minute.

In round figures a suggested stocking density is 200 spat per 100 cm^2 of tray in 4 litres of water which is changed twice a week, and circulated at a rate of 100 ml per minute.

A check on the growth rates obtained in 1971 can be obtained by comparison with those given by Spencer (1970) on the basis of the food ration given to each spat, since some of the other conditions overlapped.

The lowest tray density tested was 440 spat/100 cm^2 and the number per litre was 73 and 133. The calculated food ration per spat in the first 21 days was 2.8 and 1.4 x 10^6 cells respectively. The weight (mg) at the end of 21 days was:

	Spencer 1970	This paper
2.8 x 10^6 cells	5.0	6.9
1.4 x 10^6 cells	4.8	3.7

The slightly reduced growth in the 1970 experiment is explained by the higher number of spat per unit area of tray (see Fig. 3). These comparative results confirm that the technique can give very similar growth rates with different batches of spat.

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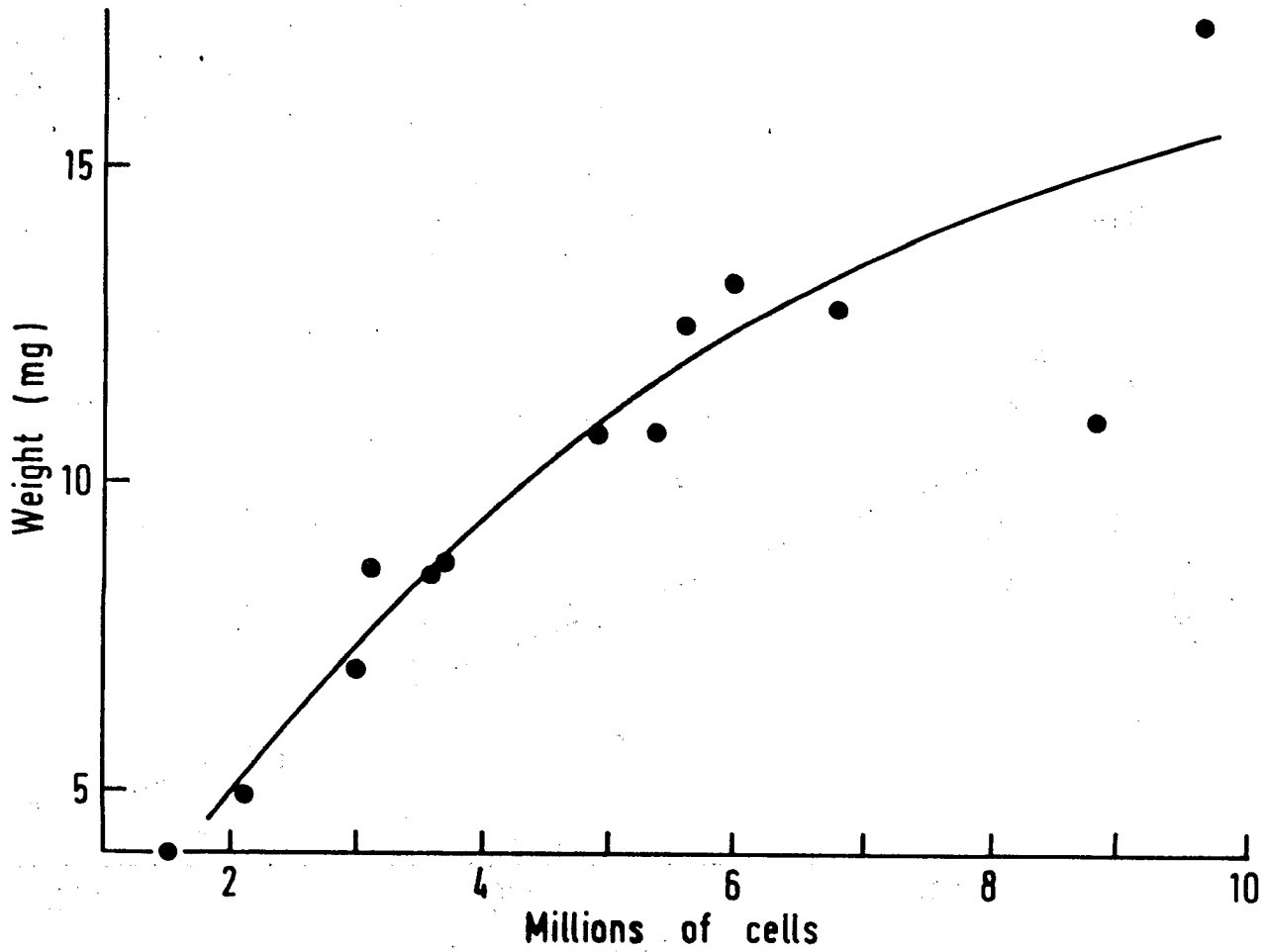

Figure 1 The weight of spat after 21 days related to the number of Tetraselmis cells/spat fed during that period. The curve is calculated from the straight line in Figure 2.

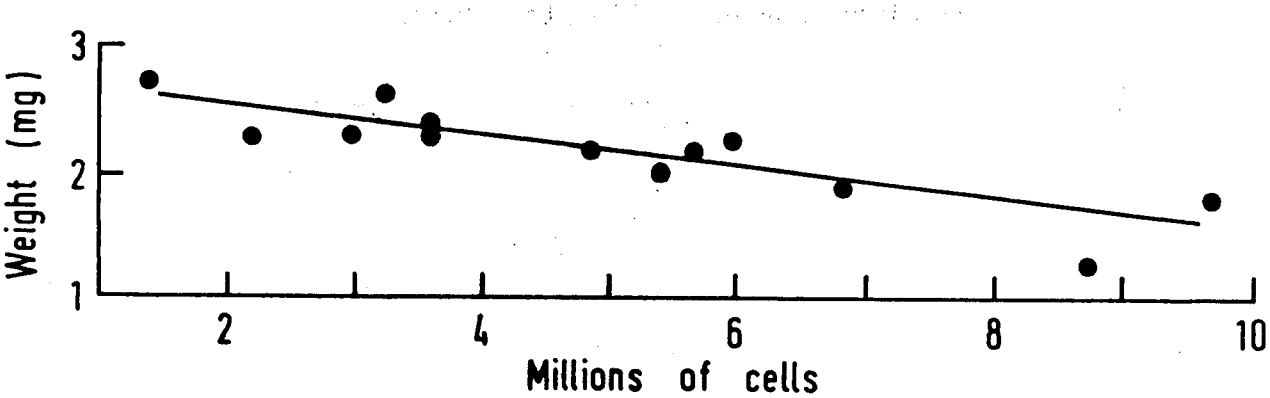

Figure 2 The weight of spat obtained in 21 days for each million cells fed related to the total number of cells fed in that period (800 spat/tray; flow 1 litre/minute).

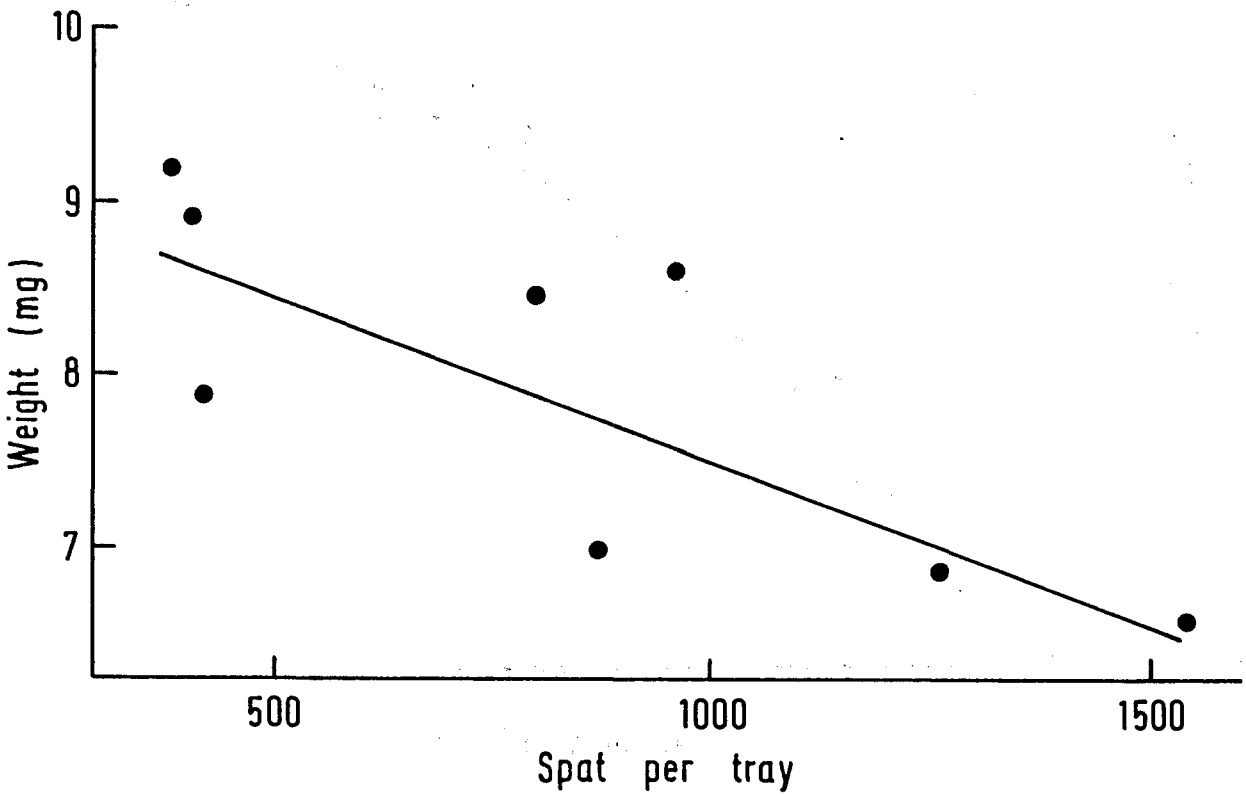

Figure 3 The weight of spat after 21 days related to the number of spat per tray. Combined results from Experiments 7, 8 and 10. All fed 10 cells/μl; flow 1 litre/minute; 49-56 spat per litre.