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Supplementary algal feeding of a laboratory breeding stock of Ostrea edulis L. and its effect on the potential of larvae

by

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INTRODUCTION

A technique for conditioning Ostrea edulis to produce viable larvae in the laboratory has been described by Walne (1966). This procedure included supplementing the seawater supply to the breeding stock with cultured unicellular algae, but the effect of the extra food ration on the potential of the larvae subsequently released was not determined.

This paper reports some of the more important results obtained in two experiments which determined the value of providing oysters with an algal supplement during the conditioning process. Variations in the viability of the different broods of larvae produced have been investigated.

Since the value of natural sea water as a culture medium for oyster larvae varies considerably over short time periods at Conway (Helm 1971), both the larvae and the algal foods on which they were fed were cultured in Lyman and Fleming artificial sea water (from Sverdrup et al. 1949). This technique provided a standard environment for the culture of each brood of larvae, irrespective of the time of liberation from the breeding stock. Any variation in the vigour of the different broods could therefore be attributed either to genetic variability between broods or to the environment of the adults prior to the release of their larvae.

METHODS

Procedure for the conditioning of the breeding stock

Two experiments were made in the spring of 1970 and 1971, using 4- to 5-year-old small commercial grade oysters from the River Helford, Cornwall. In experiment 1, 50 oysters were held on a 1.25 cm polythene mesh frame, 10 cm off the bottom of each of a pair of 125 litre polythene tanks. Sea water was distributed to the tanks by a splash jet and run to waste through an overflow from the bottom of each tank. The stock-holding

tanks in the second experiment were of approximately the same proportions but of smaller capacity; 16 oysters were held in each tank so that the volume of water available to each individual was comparable to that of the first experiment.

The unfiltered sea water used in each experiment during the adult conditioning period was maintained at a temperature of 20 ± 1°C. The flow through each tank was adjusted to provide 20 ± 2 ml per oyster per minute. Stock given supplementary food received a flow enriched with 10 cells per microlitre of Tetraselmis suecica (Kylin) Butch., dosed continuously into the seawater distribution line by a peristaltic pump. The control population with no food supplement benefited only from the natural phytoplankton present in the unfiltered sea water. Samples of 50 and 100 oysters were conditioned under each regime in the first and second experiment respectively.

At the beginning of the experiments condition factor estimates were made on the individuals of a sample of 50 oysters. Approximately 50 individuals from each of the regimes were taken for analysis at the conclusion of the trials. The condition factor was determined as follows:

$$\text{Condition factor} = \frac{\text{dry meat weight (g)}}{\text{internal shell volume (ml)}} \times 1000,$$

where dry meat weight = weight of meat after oven-drying at 100°C for 24 hours, and

internal shell volume = total volume displaced by an individual minus volume of the shell.

Liberations of larvae were collected from the stock tanks with a polyvinyl chloride sieve with a nylon mesh base. The total number of larvae liberated by a female could not be accurately determined because some were lost through the tank overflow, and others were filtered from suspension by the adults or sank to the bottom of the tank and became entangled with accumulated faeces and pseudo-faeces. Those larvae retained on the sieve were sampled to enable an estimate to be made of the number, and a small sample was measured to determine the initial size.

Culture conditions of the larvae and spat

Samples of approximately 1500 larvae from each brood were set up in Lyman and Fleming artificial sea water in duplicate 2.5 litre hard glass beakers. The artificial medium was prepared by dissolving analytical grade reagents in deionized water and adjusting the salinity to 31‰. Each culture of larvae was treated with 50 i.u. of Penicillin G and 50 µg of

Streptomycin sulphate per millilitre, to control bacterial growth (Walne 1966), and fed with a mixture of 50 cells Isochrysis galbana Parke and 5 cells Tetraselmis per microlitre (Walne and Spencer 1968). Beakers were aerated by a single glass aerator tube at a constant rate of airflow and kept in a temperature-controlled water bath operating at 24 ± 0.5°C.

The algal foods were cultured in Roux flasks of 1 litre capacity in the artificial medium with the addition of nutrient salts, trace elements and vitamin supplements, as described in detail by Walne (1966). Inocula for the Roux flasks were maintained in Erdschreiber medium made up with the artificial sea water to acclimate the flagellates to the culture conditions. Cultures for feeding to the larvae were set up at regular intervals so that young, vigorous cultures were always available.

At 48-hour intervals the water, food and antibiotics of each beaker were changed and the larvae retained on a nylon mesh-based sieve on which they were thoroughly rinsed with fresh, filtered, ultra-violet-sterilized sea water. At the 96th hour of culture a sample of larvae from each beaker was retained for the measurement of 100 individuals. The total number of larvae removed from each population for measurement was recorded. By day 8 a proportion of the larvae in each culture was approaching maturity, indicated by the development of a pair of darkly pigmented eye-spots in the mantle. Commencing on the tenth day, beakers were illuminated at an intensity of approximately 1000 lux by a fluorescent lamp, and a single 9 cm square, frosted-glass spat collector coated with a clarified extract of adult oyster flesh was placed on the base of each vessel (Bayne 1969). The spat collector and the water, food and antibiotics of each beaker were changed at 24-hour intervals for a period of 6 days, and the number of spat settling on each day was recorded.

Spat which settled in the first 24-hour period were used in growth experiments. The pair of spat plates ~~in a pair~~ from the duplicate beakers set up with each brood of larvae were fixed back to back and suspended in aerated 2.5 litre beakers of artificial sea water. The spat were fed with the same food mixture as the larvae. No antibiotics were used during the two-week period of spat culture and the water and food were changed three times each week. After 24 hours the length of the prodissoconch (larval shell) of a sample of 50 spat from each brood was measured to determine the size of larvae at settlement. The spat on each collector were thinned randomly to a maximum of 30 and the growth of individuals followed by measuring the initial spat size and the size on days 7 and 14.

In the first experiment the majority of larval broods were obtained by artificially removing them from the mantle cavities of the brooding females at the conclusion of the experiment. As the period of time during which these broods were obtained was very short, it was not considered necessary to culture them under the artificial conditions described above. In these cases the larvae were grown in natural sea water and fed with food cultured in this medium.

RESULTS

Condition factor changes in the breeding stock

Mortalities of adults during the conditioning period did not exceed 5 per cent in either experiment.

Table 1 shows the results of the condition factor estimates in the two experiments. On both occasions there was a significant loss of condition during the experimental period by the food-supplemented and unsupplemented stock ($P = <0.05$). In each experiment the condition factor of the supplemented population was significantly higher than that of the control at the end of the conditioning period (Exp. 1, $P = <0.1$; Exp. 2, $P = <0.05$).

Table 1 Changes in condition factor in the two experiments (the 95 per cent confidence limits are shown in parentheses)

Experiment number	Condition factor		
	Initial	Final, supplemented	Final, control
1	88.2 (± 7.0)	73.4 (± 6.8)	61.2 (± 7.1)
2	112.3 (± 7.9)	74.8 (± 6.2)	56.9 (± 5.6)

Production and viability of larvae and spat

The control population in the first preliminary experiment produced fewer broods of larvae than the supplemented stock (Table 2).

Table 2 The number of broods of larvae obtained in the two experiments

Experiment number	Number of broods					
	Naturally liberated		Artificially stripped		Total	
	Supplemented	Control	Supplemented	Control	Supplemented	Control
1	3	2	8	4	11	6
2	13	8	5	5	18	13
Total					29	19

More far-reaching was the indication that the supplementary feeding of the stock influenced the potential of the larvae. The mean yield of spat (number of spat settled expressed as a percentage of the initial number of larvae) of the broods produced by stock provided with the extra food ration was significantly greater than the control ($P = <0.01$, Table 3).

Table 3 The mean yield of spat, as percentage of the initial number of larvae, in the first experiment (+ indicates that cultures collapsed prior to settlement. These broods contained many abnormal larvae)

Brood number	Supplemented	Control
1	+	11.2
2	32.5	10.7
3	51.7	2.8
4	43.4	+
5	44.1	+
6	50.6	+
7	57.9	
8	17.2	
9	29.9	
10	43.6	
11	16.2	
Mean	38.7	8.2

Significance tested by the Student's t-test, $P = < 0.01$.

Figure 1 shows the cumulative number of broods produced in the second experiment related to the number of days of stock conditioning. In experiments 1 and 2 the first brood of larvae was liberated by the supplemented stock after 33 and 39 days respectively. The first broods obtained from the control population were liberated after 46 days in the first experiment and 54 days in 1971. The subsequent pattern of liberations in the second experiment from both of the experimental populations was similar. Between days 58 and 76 only one liberation occurred. It is suggested that during this pause of 18 days those individuals which spawned as males in the early part of the experiment were completing their development as females, and that it was these individuals which produced subsequent female spawnings. Similarly those animals which functioned as females up to day 58 spawned as males later in the experiment. The occurrence of two peaks of female spawning has been recorded in field populations by Cole (1942) and Millar (1963).

In each experiment the total number of broods of larvae produced by the supplemented stock exceeded that of the control, although the differences were not significant. Considering both experiments together the control populations produced 31 per cent fewer broods than the supplemented adults.

Table 4 shows a summary of the data collected for each brood of larvae successfully cultured in the second experiment. (An unexplained decrease in the food value of the algae for larval feeding prevented results of comparative value from being collected late in the experiment.) The mean 96 hour growth increment and the percentage spat yield of larvae from the food-supplemented stock were significantly greater than those of the control population at the 5 and 10 per cent levels respectively. No significant differences were obtained by comparing the mean prodissoconch size or the mean spat growth of the experimental populations. The mortality of spat was negligible in all cases.

In the hatchery situation interest lies primarily in producing a high yield of potentially vigorous spat, and it is of interest to consider the relationships of these characters to other parameters which may be predictive of them.

The size of larvae at liberation was positively correlated with their growth during the first 96 hours of culture ($P=<0.05$), as was the 96 hour growth increment with percentage spat yield ($P = <0.01$), and prodissoconch size at settlement with the growth of spat ($P = <0.05$) (see Figure 2, A, B and C).

Further inter-relationships of significance, correlating the number of days of adult conditioning to various parameters of larval viability and potential, together with levels of significance and calculated regression lines, are shown in Figure 3, A, B, C and D. Progressively lower spat yields and reduced rates of spat growth can be expected as the number of days of conditioning that a female requires prior to spawning increases. The levels of significance of the various inter-relationships shown in the figures are summarized in Table 5.

Table 4 96 hour larval growth, % yield of spat, prodissoconch size, and spat growth for broods of larvae reared in Experiment 2 (NS = not significant)

Number of days of conditioning	96 hour larval growth (μm)	% yield of spat	Prodissoconch size (μm)	Spat growth (mm)	
39	49.8	62.3	329.6	3.46	)
46	60.7	100.2	324.3	3.09	)
48	54.7	33.2	315.7	2.88	)
53	45.8	28.8	310.7	3.11	) From food-supplemented adults
54	45.9	38.0	303.8	2.39	)
58	44.4	44.2	292.9	2.97	)
58	50.8	41.4	300.3	2.36	)
54	35.8	26.0	312.0	2.83	)
54	38.2	25.7	302.0	2.75	) From non-supplemented
58	43.0	24.8	294.5	2.66	) adults
76	33.1	8.9	-	-	)
Mean value for food-supplemented population	50.3	49.7	311.0	2.75	
	P = < 0.05	P = < 0.1	NS	NS	Level of significance of difference between food-supplemented and non-supplemented adults
Mean value for non-supplemented population	37.5	21.3	302.8	2.75	

Table 5 Summary of the levels of significance of the inter-relationships of the various parameters of larval and spat viability as measured in Experiment 2 (NS = not significant)

Number of days of conditioning	96 hour larval growth (μm)	% yield of spat	Prodissoconch size (μm)	Spat growth (mm)
Number of days of conditioning	5%	5%	0.1%	5%
	96 hour larval growth (μm)	1%	NS	NS
		% yield of spat	10%	NS
			Prodissoconch size (μm)	5%
				Spat growth (mm)

DISCUSSION

The results obtained demonstrate the benefit of enriching the sea-water supply to oyster breeding stocks with laboratory-cultured Tetraselmis. More broods can be expected by following this procedure, and the results obtained suggest that the larvae produced will grow more rapidly and provide greater yields of spat than the larvae from stock not receiving a food supplement.

Various parameters of larval and spat viability correlate significantly. The results indicate that the growth of larvae in the first 96 hours after release is predictive of spat yield, and the size of larvae at the time of settlement is a guide to their growth potential as spat.

It is to be expected that adults will lose condition under laboratory conditioning if the environment provided is one of high ambient temperatures and low food supply (Bayne and Thompson 1970). The high ambient temperature is a pre-requisite of rapid gonad development and so is essential to the conditioning environment. Data provided would seem to indicate the need for adequate food supplies to the adults during the period of conditioning. From the commercial point of view the question raised is: should the food required be supplied by algal supplements to

relatively small volumes of heated sea water, or by providing a greater flow of unsupplemented sea water through the stock tanks.

The decline in larval vigour observed as the period of conditioning increased may be associated with the production of eggs of reduced quality, or a decline in the conditioning environment as the conditioning period progressed. Biochemical analyses of newly-liberated larvae which are to be made (by Dr D. Holland, NERC, Marine Invertebrate Biology Unit, Menai Bridge) may indicate which of these hypotheses is correct.

Further work is required to determine the optimum level of supplementary feeding for oyster breeding stocks.

SUMMARY

1. A technique is described for culturing oyster larvae in an artificial environment.
2. Preliminary experiments on the effects of supplementary algal feeding of adult Ostrea edulis on larval viability and potential are reported.
3. Larval viability and potential were found to be improved by the supplementary feeding of adult oysters during conditioning.
4. Progressively lower spat yields and reduced rates of spat growth were observed as the number of days that a female required for conditioning prior to spawning increased.
5. 96 hour growth and prodissoconch size are suggested as parameters being predictive of spat yield and spat growth respectively.

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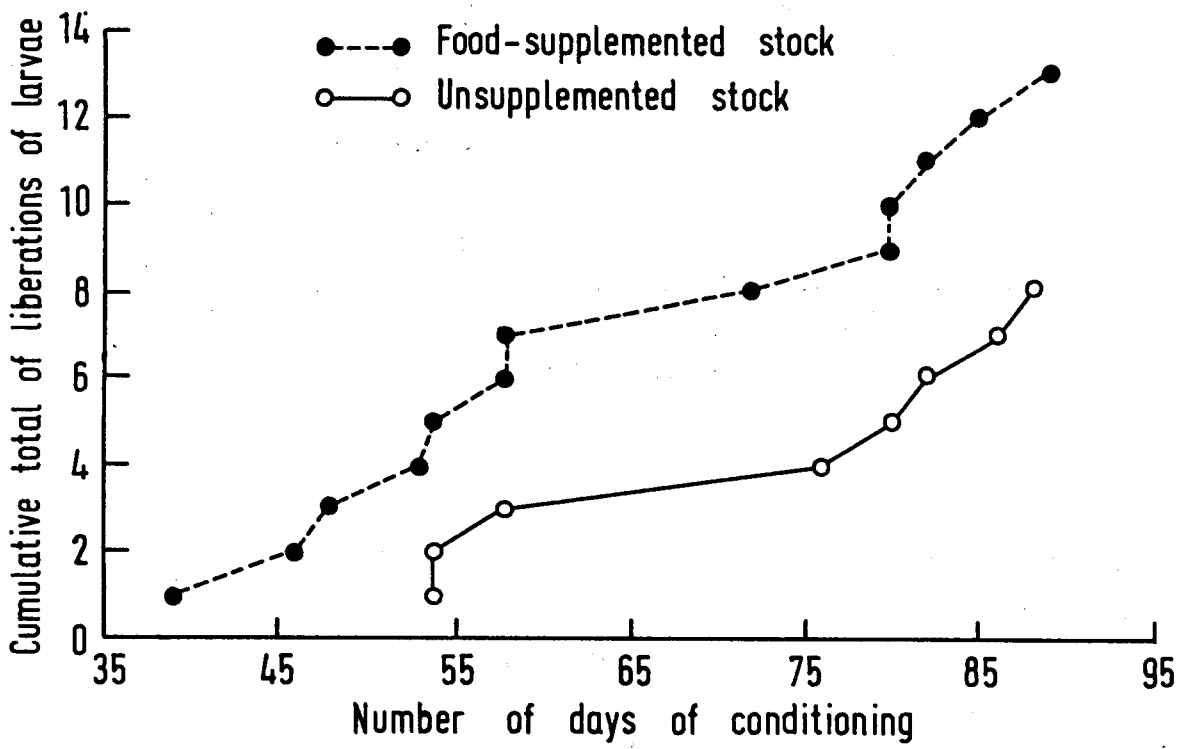

Figure 1 A comparison of the times of production of larval broods from the food-supplemented and the unsupplemented stock oysters in Experiment 2.

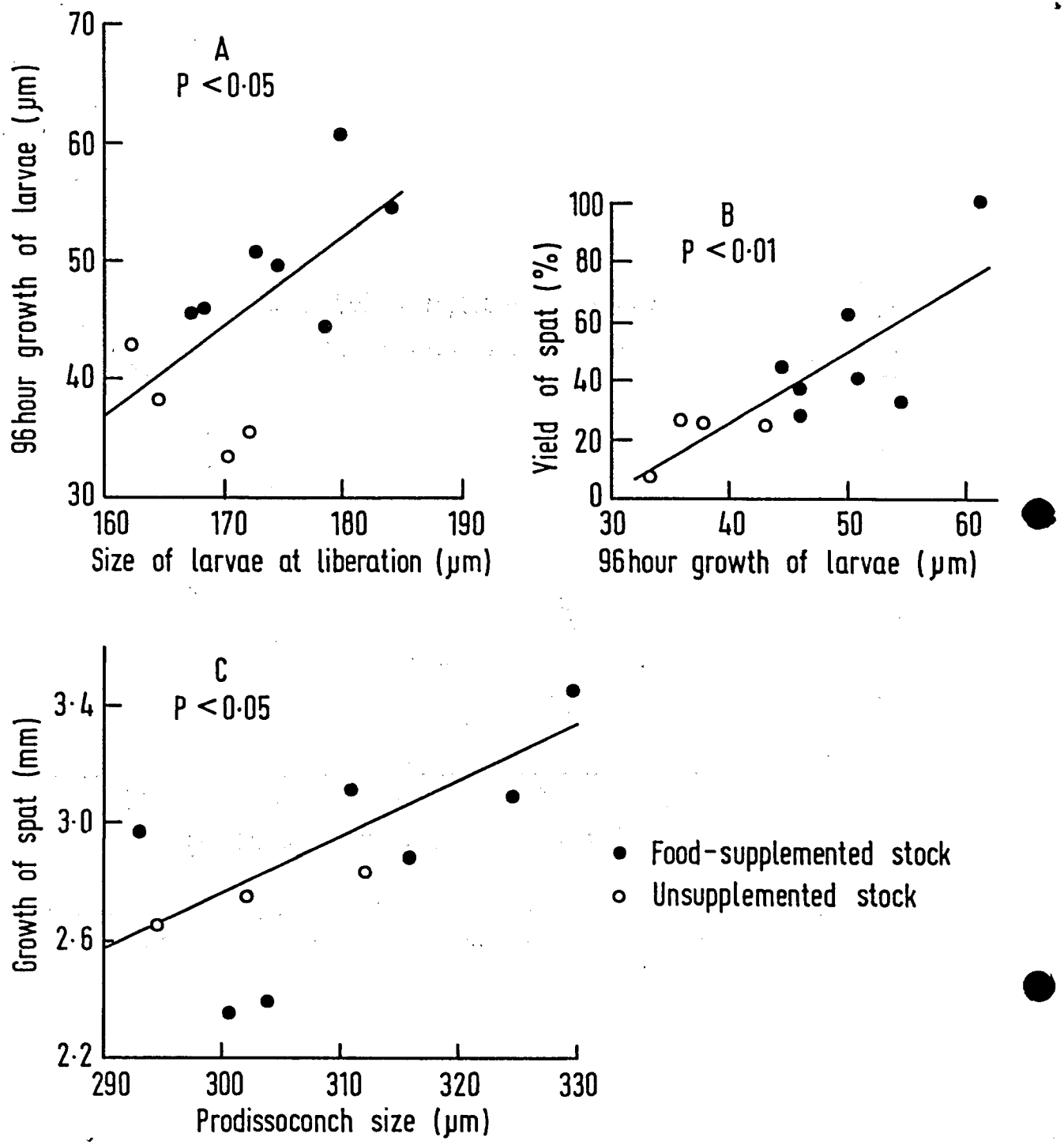

Figure 2 The inter-relations of some of the parameters of larval and spat viability measured in Experiment 2. The calculated regression lines are shown.

A The relationship between the mean size of larvae on liberation and their growth during the following 96 hours.

B The relationship between the growth of larvae in the 96 hour period following liberation and the percentage yield of spat obtained from them.

C The relationship between the prodissoconch size of settled larvae and their growth as spat in the first 15 days after settlement.

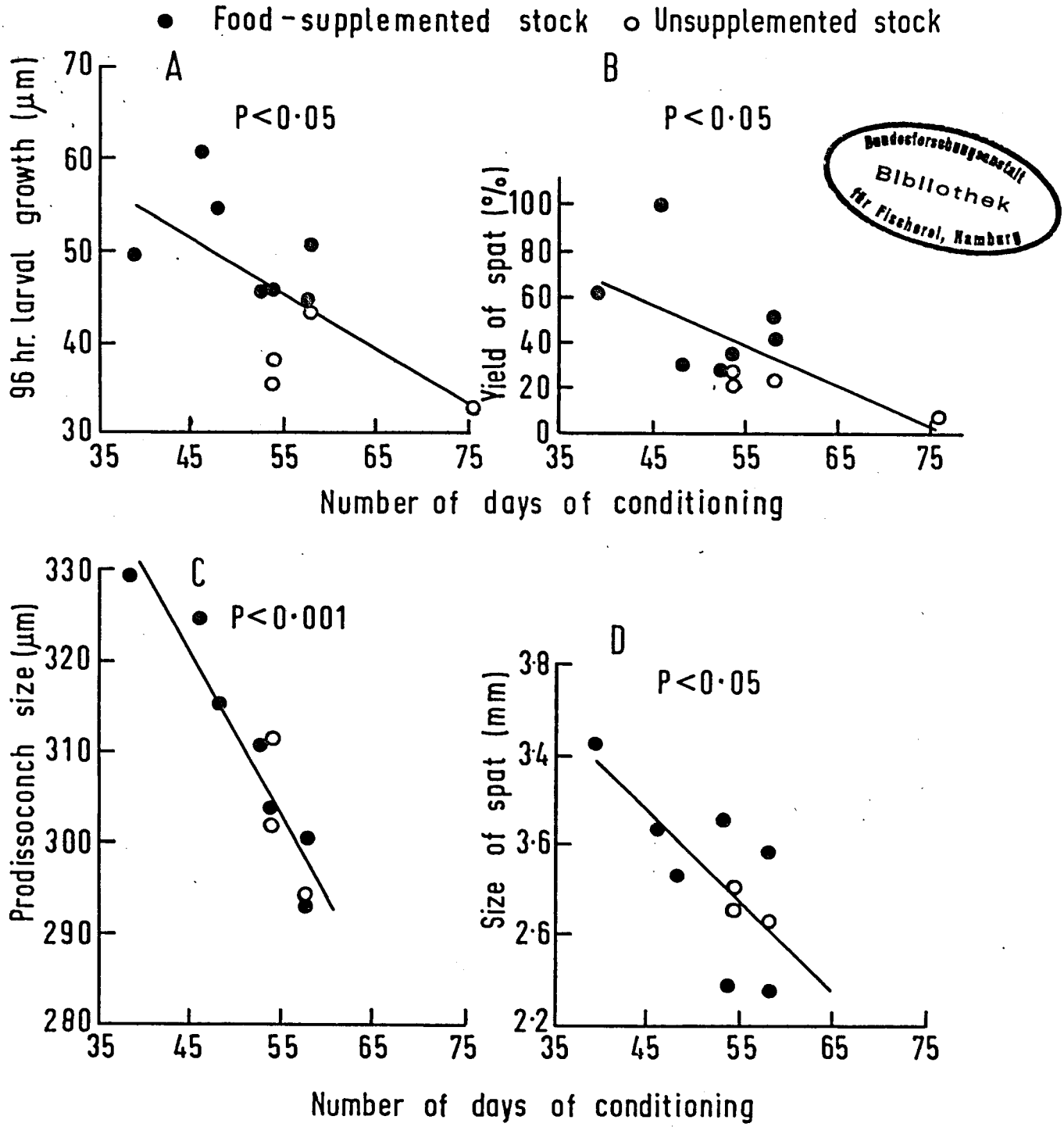

Figure 3 The decline in larval and spat viability as measured in Experiment 2 are related to the period for which the adults had been conditioned when the larval broods were released. The calculated regression lines are shown.

The relationships shown are those between the period for which adults had been conditioned when the larvae were released and

A The 96 hour growth of larvae,

B The percentage yield of spat,

C The size of larvae at settlement (prodissoconch size),

D The growth of spat during the first 15 days after settlement.