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The effect of seawater quality on the laboratory culture of *Ostrea edulis* L. larvae

by

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INTRODUCTION

For many years it has been known that natural sea water can vary in its ability to support the growth and development of marine invertebrate larvae. *Echinus* larvae develop more rapidly in sea water from certain localities than from others (Wilson 1951, Wilson and Armstrong 1958). Davis (1953) found that sea water would on occasions either kill laboratory cultures of oyster larvae or retard their growth.

By comparing the growth of *Ostrea edulis* larvae in natural sea water from the River Clyde and in an artificial medium, Millar and Scott (1968) were able to assess the extent of changes which occurred in the quality of the natural sea water in late summer and autumn. Sea water of reduced quality could sometimes be improved by pre-treatment with Fuller's earth and magnesium trisilicate, suggesting that dissolved matter was responsible on these occasions for impaired larval growth.

At Conway, Walne (1970) found that the average daily growth increment of native oyster larvae cultured in natural sea water following a standard procedure varied considerably during a 31-week period commencing in January 1969. Suspended particulate material at densities greater than 10^5 per millilitre in pre-filtered sea water was associated with the reduced growth of larvae during certain periods, but there were times when poor growth could not be explained. Without a standard culture environment provided by artificial sea water, it could not be determined whether variations either in the vigour of larvae, or in the food value of their diet or in the quality of sea water were responsible for the observed differences.

This paper reports the results of a series of trials, made at Conway in 1970 and 1971 during the hatchery culture season, in which *Ostrea edulis* larvae cultured in an artificial environment were used as a control to follow variations in the quality of natural sea water.

METHODS

A series of standard trials were carried out in duplicate 1 litre, hard-glass beakers to compare the growth of oyster larvae in sea water from the River Conway with the growth in Lyman and Fleming artificial sea water (from Svedrup et al. 1949). The artificial medium was prepared by dissolving analytical grade reagents in de-ionized water, the salinity being adjusted to the average at Conway during 1969 of 31 parts per thousand. Trials were made whenever newly liberated or young larvae were available.

Approximately 400 larvae were cultured in each beaker. All cultures were fed with a mixture of 50 cells of Isochrysis galbana Parke and 5 cells of Tetraselmis suecica (Kylin) Butch. per microlitre (Walne and Spencer 1968), and treated with 50 i.u. of Penicillin G and 50 mg of Streptomycin sulphate per millilitre to control bacterial growth (Walne 1966). The Conway sea water was finely filtered and ultra-violet sterilized prior to use. Each trial was of 4 days' duration and the water, food and antibiotics were changed after 48 hours. All beakers were aerated at the same rate and kept in a temperature-controlled hatchery maintained at $22 \pm 2^{\circ}C$.

At the conclusion of each trial a random sample of 100 larvae from each culture was measured, and the growth increment during the 4-day period calculated. An index of the quality of natural sea water was obtained by dividing the increment of growth in natural sea water by that in the artificial medium.

Five of the standard trials included experiments which compared the effect of natural and artificial sea water on the nutritional value of the algal foods. On these occasions Isochrysis and Tetraselmis were cultured in both media in Roux flasks of 1 litre capacity. Further trials determined the value of magnesium trisilicate as an agent to improve the quality of natural sea water for larval culture; the method described by Millar and Scott (1968) was used.

RESULTS

Two preliminary trials compared the growth of two different broods of larvae under the experimental conditions. The growth index calculated for each brood within a trial compared favourably, demonstrating the value of this estimate as a method for assessing the condition of sea water for the growth of oyster larvae (Table 1).

Table 1 A comparison of the growth index of sea water calculated for two different broods of larvae cultured simultaneously in each of two trials

Trial	Larval brood	Initial size (µm)	4-day growth increment (µm)		
			Natural sea water	Artificial sea water	Growth index
1	A	205	52.3	45.1	1.16
	B	185	51.2	46.5	1.10
2	C	169	35.7	39.7	0.90
	D	175	37.0	38.8	0.95

In those trials which also compared the food value of the algal diet when the constituent species were cultured either in natural sea water or in the artificial medium, it was evident that changes in the quality of sea water which had a direct effect on larvae had relatively little influence on the nutritional value of the diet. The results of these trials are shown in Table 2.

Table 2 The growth of larvae in five trials which compared natural and artificial sea water as culture media for the larvae and their algal diet

Culture medium for		Trial				
Larvae	Algae	A	B	C	D	E
		4-day growth increment of larvae (µm)				
Artificial	Artificial	41	70	41	39	35
Artificial	Natural	42	70.	42	42	37
Natural	Artificial	54	73	51	32	37
Natural	Natural	55	71	54	31	38
Initial size of larvae (µm)		176	180	181	173	157

Because there was little advantage in feeding larvae with foods cultured in artificial sea water, it has been practice in subsequent trials to use foods cultured in the large-scale system described by Walne (1966).

Twenty-four trials were completed in 1970 and a further 21 up to the end of July this year, and the calculated growth indices are shown in Figure 1. A trend apparent in 1970, and again this year, suggests that the quality of sea water declines from a peak at the beginning of the culture season towards low values in late May or early June. The mean monthly growth index calculated from the combined results of both the 1970 and 1971 trials for the first six months of the culture season, shown in Figure 2, clarifies the trend. A decreasing proportion of trials in which natural sea water supported greater growth than the control medium is apparent from February to May, with an increase in June and again in July.

The routine culture of oyster larvae is generally interrupted late in May and in June by a bloom of the colonial alga Phaeocystis pouchetti Hariot Lagerh. In 1970 the bloom developed later than is usual in the River Conway and routine larval culture was not resumed until mid-July. In 1969 broods of larvae continued to be liberated by the breeding stock throughout the bloom, although the growth potential of the larvae was progressively depressed. The lowest growth indices were obtained following the peak of the bloom when the colonies were disintegrating. The results of a series of standard trials made during the 1969 Phaeocystis bloom have been reported by Walne (1970). In the past two seasons there were no liberations of larvae from breeding stock exposed to water containing Phaeocystis colonies.

Few results are available for the latter part of the culture season and they are not discussed in this paper.

Data prepared by my colleague, Mr B. E. Spencer, from the 1970 series of standard 75 litre experiments each culturing 100 000 larvae in filtered, ultra-violet sterilized Conway sea water have been compared with the seawater quality results for 1970. The larvae in these large-scale experiments were fed with the same foods and treated with the same antibiotics as the standard trials. Table 3 shows, for the period February to June 1970: the monthly mean of the average daily growth of larvae during the first eight days of culture in the 75 litre experiments; the monthly mean spat yield expressed as a percentage of the initial number of larvae; and the monthly mean percentage of spat surviving after two weeks' growth in Conway sea water under standard conditions in the laboratory. Since the time taken to complete each experiment was approximately five weeks, each of the values shown in Table 3 for a particular month includes only the results of those parts of the experiments which were made predominantly in that month. Also shown in Table 3 are the mean monthly growth indices calculated from the standard 4-day trials.

Table 3 A comparison of the mean monthly growth index for 1970 calculated from the standard trial results, with the monthly averages of various parameters measured in the routine large-scale oyster culture experiments made during the same time period

Month	75 litre experiments			Growth index
	Daily larval growth (μm)	% spat yield	% survival of spat	
February	12.3	34	93	1.32
March	10.9	36.5	70	0.93
April	11.8	35	68	0.92
May	10.5	12	33	0.74
June	No experiments	20	15	1.09

During February, when the mean growth index suggested that the Conway water was above average for culture, high spat yields were obtained in the large-scale experiments and those spat survived well. In March, April and May decreasing growth indices were associated with increasing spat mortalities. The growth of larvae in the 75 litre experiments varied little during this period and spat yields were not affected until May. Poor yields of spat and their poor survival during June may be explained by the fact that the growth and development of larvae in these experiments was completed during the period of May when growth indices indicated that the quality of natural sea water was particularly poor.

These data suggest a correlation between seawater quality as assessed by the standard trials and the success of oyster larval culture in terms of spat production and survival. The results of standard trials have been analysed in an attempt to determine the factors involved in decreasing water quality during spring. In Figure 3A the growth index is plotted against the salinity of natural sea water during each trial, and in Figure 3B against the particle count of unfiltered sea water at the time of each trial where complete data are available. The scatter of points on the graphs indicates that variations in growth index are not primarily related to either of these factors. On two occasions, however, one in March and the other in April 1970, the relatively poor growth of larvae in Conway sea water was directly attributable to salinities below 25 parts per thousand.

Further analyses have compared the growth indices of trials made during periods of spring and neap tides. The mean growth index with the calculated 95 per cent confidence limits for spring tides was 0.94 ± 0.14 and for neap tides 0.93 ± 0.09.

Another possibility was that larvae in successive months exhibited decreasing vigour and became more susceptible to small environmental changes which had a negligible effect on larvae early in the season. This hypothesis was tested by comparing the mean monthly 4-day growth increment of larvae in artificial sea water and in Conway water calculated from the combined 1970 and 1971 trials (Table 4).

Table 4 The mean monthly 4-day growth increment of larvae in artificial and natural sea water calculated from the results of the combined 1970 and 1971 trials

Month	4-day growth increment (µm)	
	Artificial sea water	Natural sea water
February	40.4	49.8
March	49.5	48.5
April	44.3	38.5
May	41.2	33.1
June	44.0	38.4
July	43.1	44.4

These data indicate that the hypothesis is invalid since the potential of larvae indicated by their growth in artificial sea water varies relatively little throughout this time period compared with their growth in natural sea water.

The effect of magnesium trisilicate treatment of natural sea water on its quality for larval culture has been tested on seven occasions. In 1969 and 1971 six trials were made during the course of the *Phaeocystis* bloom in May and June and immediately following its decline in early July. On these occasions the treatment was unsuccessful. On the seventh occasion, during September, larval growth was improved in sea water which had been treated but at this time natural sea water was above average in quality (growth index 1.10).

DISCUSSION

The results presented demonstrate the variability in the quality of Conway sea water for the culture of *Ostrea edulis* larvae both in the short term and seasonally. Reduced spat yields and increased mortalities of the juveniles, which were experienced towards the summer in 1970, are probably related to the decreasing quality of sea water during this period. Although the large-scale experiments of the current season have not as yet been fully analysed, it is likely that a similar trend will be apparent.

As far as commercial hatcheries are concerned, April, May and June should be among the more profitable months of the season. Juveniles produced then can be transferred from the comparatively expensive indoor spat maintenance systems to the sea in the minimum period of time. Sea-water temperatures are rising and there is an abundance of suitable food for good spat growth and survival. Any loss of production during this period could have a severe effect on hatchery economy.

Analyses of results to determine the cause of the progressive decline in seawater quality in the early part of the season proved negative. It may be that changes in the composition of sea water are responsible. One clue is provided by the depression of growth during Phaeocystis blooms. It is possible that oyster larvae are sensitive to the metabolites produced by successive blooms of phytoplankton. Chlorophyll a records for North Wales sea water published by Jones and Spencer (1970) show an increase from mid-February to peaks in late April and May. Further high values in June were associated with the annual Phaeocystis bloom but from late June the values decreased rapidly to the levels observed in February. Davis and Guillard (1958) suggested that the poor growth of oyster and clam larvae in laboratory feeding experiments was due to metabolites produced by certain flagellates. Impaired growth of larvae during dinoflagellate blooms had previously been reported by Davis and Chanley (1956).

It is probable that a similar pattern of variations in seawater quality will occur in other biologically productive estuaries. This factor will have to be taken into consideration in siting future hatchery units. Experiments have shown that oyster larvae can be cultured successfully in an artificial environment and the spat produced grow and survive well in the artificial medium (Stephenson and Helm 1971). The cost of preparing artificial sea water for larval culture during periods of reduced water quality would probably be offset by the increased spat productivity which can be expected.

SUMMARY

1 A technique is described which has been used to assess the quality of sea water for the culture of oyster larvae.

2 The results of a series of trials made in 1970 and 1971 are presented.

3 Conway sea water varies considerably in quality both in the short term and seasonally.

4 Reduced spat yields and decreasing spat survival in routine large-scale oyster culture are apparently related to a general decline in seawater quality during spring.

5 It is suggested that oyster larvae are sensitive to metabolites produced by phytoplankton blooms.

REFERENCES

DAVIS, H. C., 1953. On food and feeding of larvae of the American oyster C. virginica. Biol. Bull., Woods Hole, 104, 334.

DAVIS, H. C. and CHANLEY, P. E., 1956. Effects of some dissolved substances on bivalve larvae. Proc. natn Shellfish Ass., 46, 59-74.

DAVIS, H. C. and GUILLARD, R. R., 1958. The relative value of ten genera of micro-organisms as foods for oyster and clam larvae. Fishery Bull. Fish Wildl. Serv. U.S., 58, 293-304.

JONES, M. and SPENCER, C. P., 1970. The phytoplankton of the Menai Straits. J. Cons. perm. int. Explor. Mer, 33 (2), 169-180.

MILLAR, R. H. and SCOTT, J. M., 1968. An effect of water quality on the growth of cultured larvae of the oyster Ostrea edulis L. J. Cons. perm. int. Explor. Mer, 32 (1), 123-130.

STEPHENSON, R. R. and HELM, M. M., 1971. Supplementary algal feeding of a laboratory breeding stock of Ostrea edulis L. and its effect on the potential of larvae. Submitted to ICES 1971, K:23.

SVEDRUP, H. U., JOHNSON, M. W. and FLEMING, R. H., 1949. The Oceans. Prentice-Hall, New York, 1087 pp.

WALNE, P. R., 1966. Experiments in the large-scale culture of the larvae of Ostrea edulis L. Fishery Invest. Lond., Ser. 2 25 (4), 53 pp.

WALNE, P. R., 1970. Present problems in the culture of the larvae of Ostrea edulis. Helgolander wiss. Meeresunters, 20, 514-525.

WALNE, P. R. and SPENCER, B. E., 1968. Some factors affecting the growth of the larvae of Ostrea edulis L. ICES C.M. 1968/K:15, 7 pp. (mimeo).

WILSON, D. P., 1951. A biological difference between natural sea waters. J. mar. biol. Ass. U.K., 30, 1-19.

WILSON, D. P. and ARMSTRONG, F. A. J., 1958. Biological differences between sea waters: experiments in 1954 and 1955. J. mar. biol. Ass. U.K., 37, 331-348.

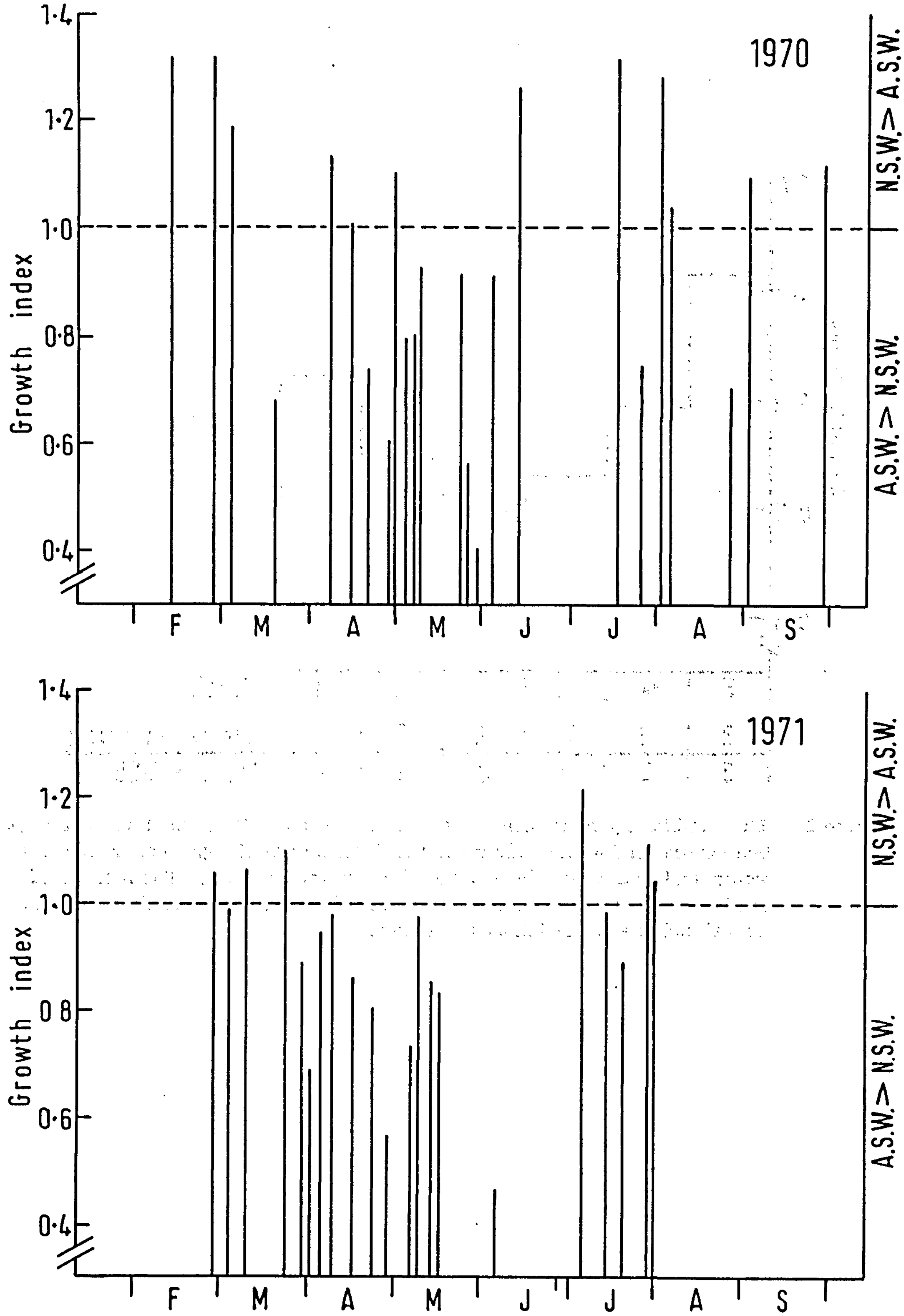


Figure 1 The calculated growth index for each of the standard trials made in 1970 and 1971. Values greater than 1.00 indicate that the growth of oyster larvae was greater in natural sea water than the artificial medium, and vice-versa.

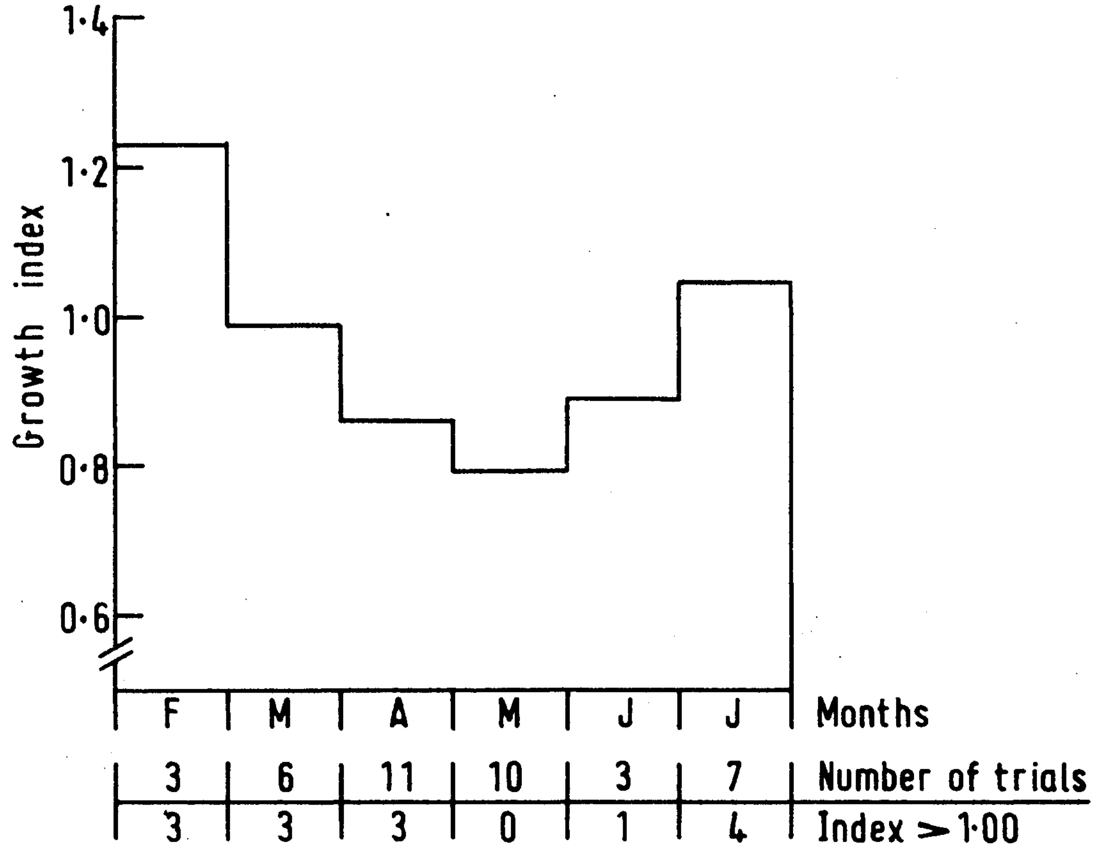


Figure 2 The combined growth indices of both the 1970 and 1971 standard trials have been expressed as monthly means to demonstrate the general trends in sea-water quality between the months of February and July. The number of trials completed appropriate to each month and also the number in which the growth index exceeded 1.00 are shown.

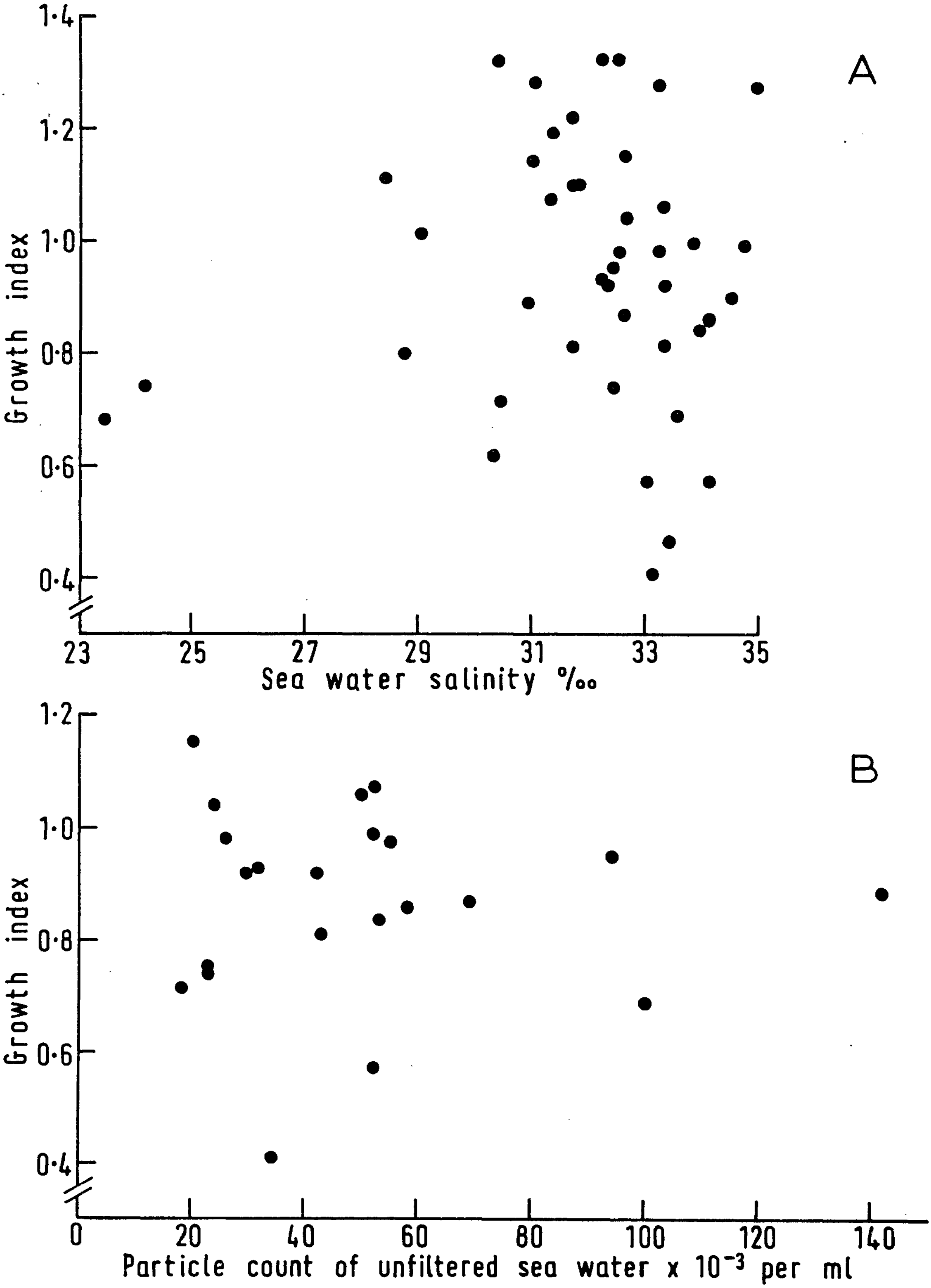
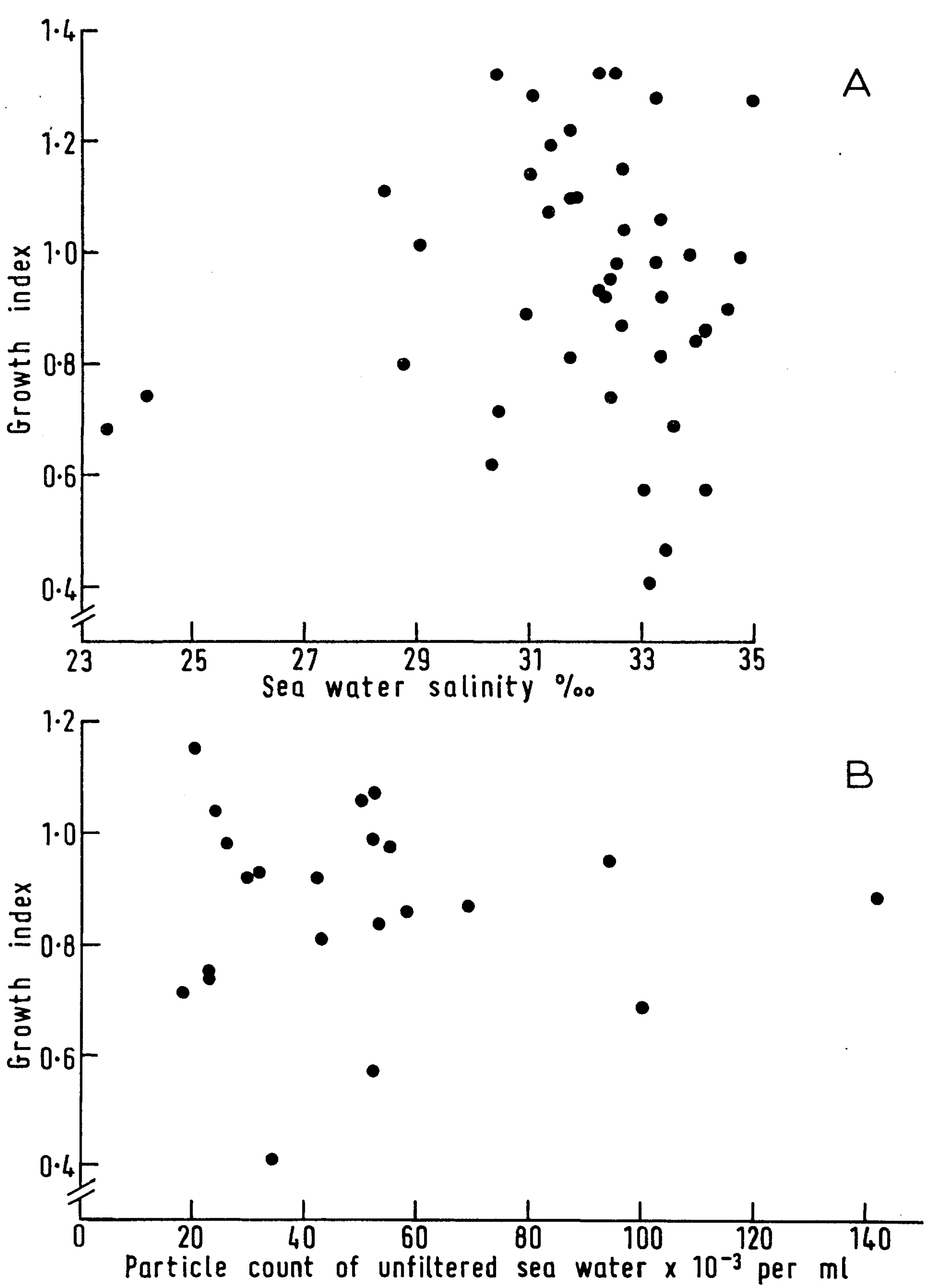


Figure 3 A The relationship between the salinity of natural sea water and the growth index obtained in the 1970 and 1971 standard trials.

B The relationship between the particle count of unfiltered natural sea water and the growth index obtained in the 1970 and 1971 standard trials where complete information was available.



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