ISSN 0308 - 5589

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD
DIRECTORATE OF FISHERIES RESEARCH

FISHERIES RESEARCH TECHNICAL REPORT No. 53

Culture of algae for larval fish and shellfish rearing

Part 1 The development of a 200 l algal culture vessel at Conwy
M.M. HELM, I. LAING and E. JONES

Part 2 Recommended procedures for the culture of *Chaetoceros calcitrans*
I. LAING

Part 3 Production of algae for use in the rearing of larval fish
S.M. BAYNES, L. EMERSON and A.P. SCOTT

LOWESTOFT, 1979

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD
DIRECTORATE OF FISHERIES RESEARCH

FISHERIES RESEARCH TECHNICAL REPORT
NUMBER 53

Culture of algae for larval fish and shellfish rearing.

LOWESTOFT 1979

The authors:

M M Helm BSc is a Senior Scientific Officer; I Laing BSc a Scientific Officer and E Jones a Professional and Technology Officer in Fish Stock Management Division of the Directorate of Fisheries Research. They are stationed at The Fisheries Experiment Station, Conwy.

S M Baynes MSc is a Scientific Officer and A P Scott BSc PhD is a Senior Scientific Officer in The Fish Cultivation Section of the Directorate of Fisheries Research. They are stationed atThe Fisheries Laboratory, Lowestoft.

During the period covered by the report, L Emerson was a Scientific Officer in The Fish Cultivation Section of The Directorate of Fisheries Research; he has recently left the Ministry.

Fish. Res. Tech. Rep., MAFF Direct. Fish. Res., Lowestoft (53) 18pp.

Contents

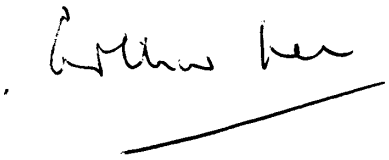
FOREWORD

Provision of food is fundamental to the rearing of animals. For the early life stages of both fish and invertebrate marine species it is absolutely essential to provide the right food species in the right quantities at the right time. So, in the fish and shellfish culture programmes of the Directorate's laboratories at Lowestoft and Conwy respectively, attention has been paid to finding out what are the best food species for the larvae and to culturing these species in ways which maintain regular adequate supplies.

The requirements for algal food (phytoplankton) for fish, crustacean and molluscs are different and the methods of culture developed reflect these differences.

For oysters and clams which are dependent on phytoplankton throughout their life histories a relatively high technology, low running cost, semi-continuous culture system has been developed at Conwy. This provides large volumes of algae cheaply and with great reliability, and is described in this Technical Report by Helm, Laing and Jones. Laing also describes an effective way of culturing one particular species of diatom of considerable value in bivalve culture.

In the case of fish and crustaceans where algae are required only during the larval stages a lower technology culture system may be more appropriate. In this Technical Report Baynes, Emerson and Scott describe the Lowestoft technique for rearing algae especially as foods for rotifers which are themselves used as fish food.



A.J. LEE
Director of Fisheries Research

FISHERIES RESEARCH TECHNICAL REPORT NUMBER 53

Part 1. THE DEVELOPMENT OF A 200 l ALGAL CULTURE VESSEL AT CONWY

by

M M Helm, I Laing and E Jones

1. Introduction

As part of the work on bivalve cultivation at Conwy a pilot-scale hatchery is operated to provide seed oysters for growth trials in the sea. Recently the requirement for seed has increased and, as a consequence, so has the demand for algae of good food value to feed them during the hatchery stages. This has led to the need to develop large-scale algal culture vessels, primarily for the culture of *Tetraselmis suecica* (Kylin) Butch, which can be operated with the minimum of maintenance and yet provide algae as efficiently as the established 20 l scale culture system described by Walne (1966).

Based on experience gained in previous years with various large-scale culture systems, a prototype 200 l vessel was designed and constructed. This has proved successful in a year of continuous operation and a series of units have now been brought into use. This paper describes the design and construction of the vessel and procedures for its operation together with some preliminary results.

2. Design and construction

Experience suggested that the most suitable vessel configuration, on the grounds of production efficiency and simplicity of construction, was a tall, narrow cylinder fitted with an internal fluorescent light source. Further considerations in design were vessel capacity and the duration of the culture period, the aim being to reduce labour input per unit volume of algae produced. A culture of 200 l capable of being operated on a semi-continuous harvesting regime for about two months, was considered optimal. The design based on these requirements is shown in Figures 1 and 2.

Figure 1 The 200 l internally illuminated algal vessels. Note the configuration of the cooling element.

The outer jacket of the vessel is constructed of white pigmented glass-fibre and is slightly tapered to permit easy removal from the mould. It is 150 cm high and is 45 cm diameter at the base and 40 cm diameter at the top. A substantial flange is moulded on at each end to fit the top and base plates which are secured with 12 mm nylon nuts and bolts.

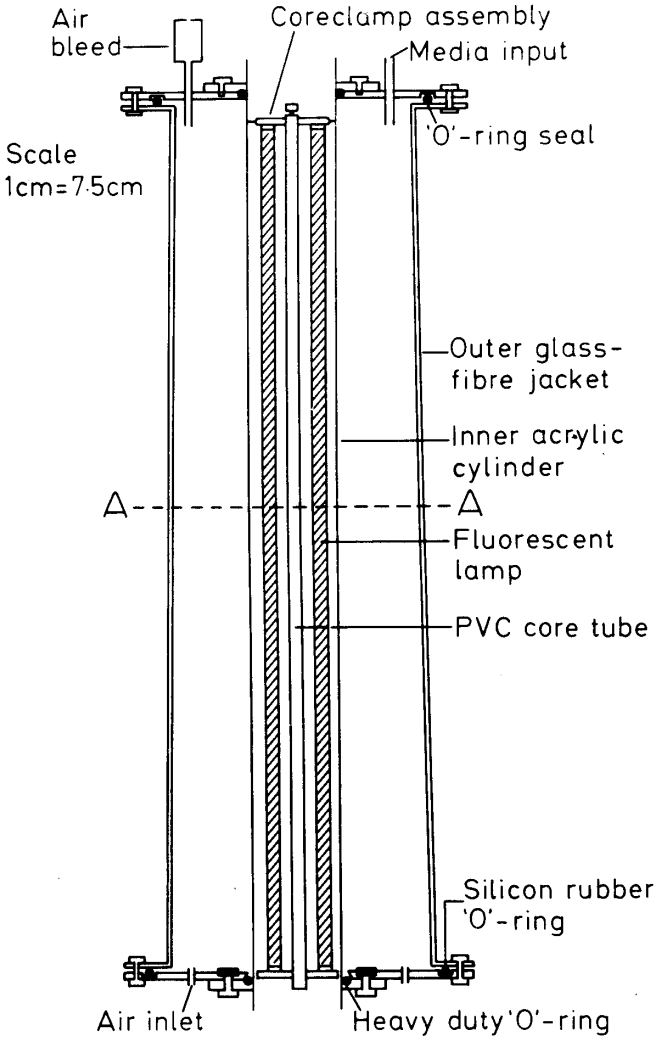


Figure 2 Longitudinal and transverse sections through a vessel; (see text for explanation).

A glass-fibre cooling element of semi-circular cross section, arranged as a series of six evenly-spaced vertical sections alternately inter-connected at top and bottom, is fixed to the outer surface of the outer jacket. These sections are moulded on the outside of longitudinally-cut, 19 mm nominal bore, rigid polyvinyl chloride pipe. Hose adaptors are fitted at each end of the element. Cooling water is circulated through the element to maintain the culture temperature at about 18°C.

Illumination is provided by four 80 W, 150 cm length, 'Daylight' fluorescent lamps. These are mounted around a hollow core of 19 mm diameter bore polyvinyl chloride pipe through which the electrical cables to the bottom lamp-holders are run. The core is fitted, top and bottom, with a clamping arrangement which positions the lamps within the inner cylinder. The inner cylinder, around the lamps, is of 15 cm diameter transparent, extruded acrylic tube. Lamp-holders are fitted to the core clamp assemblies. The average distance from the lamps to the inner surface of the outer cylinder is 13.75 cm. Together with the inner white reflective surface of the outer cylinder, which permits the maximum concentration of light within the vessel, this distance ensures the efficient exposure of the algal culture to the light source.

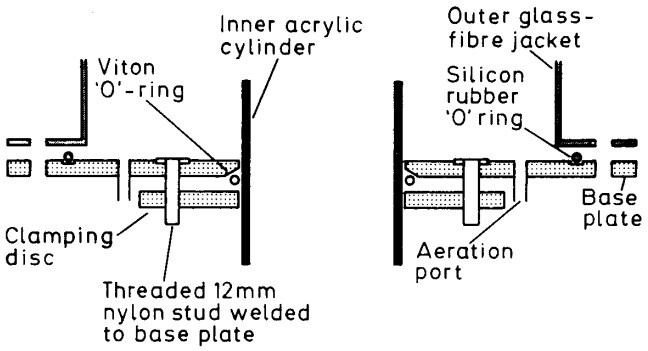


Figure 3 Cross section of the base plate.

The top and base plates (Figure 3) are 60 cm diameter and are made from 12 mm thick white 'Darvic' rigid polyvinyl chloride sheet. Each plate is drilled, towards the perimeter, for twelve 12 mm diameter nylon bolts which are used to secure them to the flanges of the outer jacket. The joins between plates and flanges are sealed with a 7 mm thick silicon rubber 'O'-ring set in a 3 mm deep groove machined around each plate, 2 cm from the perimeter. The inner acrylic cylinder projects about 10 cm beyond both the top and base plates to prevent water splashing the lamp-holders. It is sealed to the plates with a 7 mm thick, 15 cm diameter, 'Viton' 'O'-ring; the central hole in both plates through which the acrylic cylinder passes is machined on the outward facing surface to 45° and a 12 mm thick, polyvinyl chloride clamping disc is tightened on the 'O'-ring to effect the seal.

The base plate is drilled to take two 5 mm bore aeration ports. These provide efficient agitation of the culture at a combined air flow rate of 15 l min^{-1}. A 19 mm bore polyvinyl chloride ball valve is also fitted to the base plate. This is the harvesting outlet and also acts as a drain in the cleaning of the vessel between culture runs.

The top plate is provided with a media input pipe which is closed to the atmosphere when not in use. An air bleed is also fitted. This is packed with cotton-wool which is replaced periodically. A removable inspection port provides access to the top of the vessel for cleaning purposes.

The assembled vessel is mounted on a sturdy, plastic-coated, mild steel frame.

3. Vessel operation

Vessels have been mainly used for the culture of *Tetraselmis*, which is a hardy species capable of being grown successfully in a wide range of conditions. It is likely that for more delicate species some changes in details of the culture operation will be necessary.

3.1 Cleaning and sterilization

New vessels are thoroughly cleaned with a hot detergent solution. After rinsing, they are filled with fresh water and allowed to stand for a week at room temperature. This procedure ensures the removal of readily – leachable, potentially – toxic substances from the materials used in construction.

Sterilization is effected by filling the vessel with a solution of sodium hypochlorite (50 parts/10^6 free-chlorine concentration). This is allowed to stand for 24 h. After draining, the vessel is purged with sterile air for 24 h to drive off residual chlorine. The vessel is then ready for filling with sea water medium. These sterilization procedures are repeated at the end of each culture run.

3.2 Media preparation and filtration

The medium used for growing *Tetraselmis* is the same as that described by Walne (1966) for the culture of *Isochrysis galbana* Parke. It is possible, once the culture is growing strongly, to substitute a simplified recipe of additional nutrients, alternating at successive harvests with the full formulation of nutrient salts, trace elements and vitamins (Table 1).

For *Tetraselmis*, the vessel is filled (200 l comes to about 15 cm from the top) with sea water medium reduced to 25°/oo with fresh water which is then filtered through a sterilizing grade of filter mat or cartridge. All pipework, which is of borosilicate glass or steam-sterilizable, flexible polyvinyl chloride tubing, leading from the filter to the vessel, is steam sterilized for 15 min. at a pressure of 0.35 kg cm^{-2} prior to medium filtration.

Table 1 Stock enrichment solutions for the culture of *Tetraselmis* (from Walne, 1966).

a.

$FeCl_3.6H_2O$	2.60 g
$MnCl_2.4H_2O$	0.72 g
H_3BO_3	67.20 g
EDTA (Na salt)	90.00 g
*$NaH_2PO_4.2H_2O$	40.00 g
*$NaNO_3$	200.00 g
Trace metal solution	2.0 ml
Distilled water	to 2 l

1 ml is added to each litre of sea water

b. Composition of trace metal solution

$ZnCl_2$	2.1 g
$CoCl_2.6H_2O$	2.0 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.9 g
$CuSO_4.5H_2O$	2.0 g
Distilled water	to 100 ml

It is necessary to acidify this solution with HCl to obtain a clear liquid

c. Composition of vitamin stock solution

B_{12}	10 mg
B_1 (Thiamine)	200 mg
Distilled water	to 200 ml

10 ml is added to each 100 l of sea water

* These salts are used alone in the simplified enrichment medium

Prior to fine filtration, pre-filtration of the sea water used in the preparation of the medium is advisable to remove much of the larger particulate material (> 2 µm diameter) generally present in estuarine and coastal waters. There is a wide range of suitable equipment commercially available for this purpose, including cartridge filters and filter assemblies employing diatomaceous earth as an aid to efficient filtration. Using three 23.6 cm diameter Carlson-Ford grade EKS filter mats mounted in a filter press and an inlet pressure of 0.25–0.3 kg cm^{-2}, 200 l of enriched sea water can be filtered in 4–5 h. As an alternative to EKS mats, several companies market cartridge membrane filters of 0.22 or 0.45 µm nominal pore size which can either be steamed *in situ* or autoclaved.

3.3 Inoculation of vessels

To ensure a rapidly-growing culture in large vessels it had been found necessary in previous years to provide a level of inoculation equivalent to an initial concentration of 50 cells of *Tetraselmis* μl^{-1} of culture (5 x 10^4 ml^{-1}). In a 200 l vessel this level is attained by adding 10 l of a vigorous culture at a density of 1,000 cells μl^{-1}. Although culture

runs have tended to be started at higher initial cell concentrations, recent data suggest that 2 l inocula of Erdschreiber cultures are equally as satisfactory. In these cases, initial density is closer to 25 cells μl^{-1}. Cultures used as inocula are generally provided by the semi-continuously operated 20 l flask system described by Walne (1966).

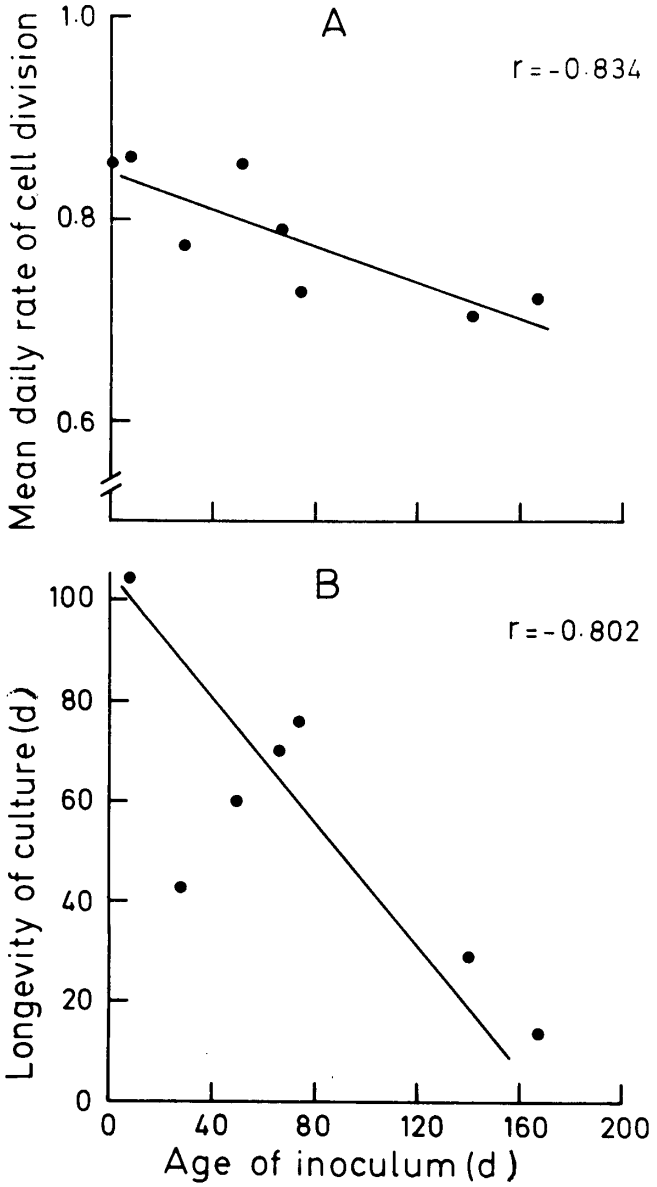


Figure 4 Relationships between the age of inocula and A, the mean daily rate of cell division from inoculation to first harvest; B, the duration of a culture run. The signficance of the correlations are $p < 0.02$ and $p < 0.05$ respectively.

Data in Figure 4 show a significant relationship between the age of the inoculum and both the division rate per day to the first harvest and the longevity of a culture. A younger inoculum is more likely to provide a more rapidly-growing culture initially and one which lasts longer. This suggests some deterioration in the condition of algae provided by 20 l flasks with age even though they are operated with a 50% harvest 3 or 4 d/week. The division rate per day (K) is calculated from the equation

$$K = \frac{1.443}{t} \times \log_e \frac{N_t}{N_0}$$

where N_t and N_0 are the number of cells μl^{-1} at the first harvest and at inoculation respectively, t is the time in days to the first harvest, and 1.443 is the factor transforming $\log_e$ to $\log_2$ in the calculation of cell divisions per unit time. In Figure 4A the culture shown as being started with an inoculum age of 0 days was started from an Erdschreiber culture.

3.4 Control of pH

Carbon dioxide is metered to the cultures from pressurised gas cylinders. Sub-micron filtered, compressed air acts as the carrier gas. To provide sufficient turbulence, cultures are aerated at 15 l min^{-1}. At this rate of airflow it is necessary to bleed CO_2 into the compressed air delivery line at 37.5 ml min^{-1} (0.25% of the total gas flow). This will maintain the pH of the culture at 7.8-8.2 which supports the maximum rate of cell division.

3.5 Observations on cultures

It is a wise precaution to check the pH of cultures twice weekly and more frequently if foaming is noted. Similarly, culture temperature should be routinely recorded. Daily optical determinations of cell density provide a useful guide to the state of cultures.

4. Results

Since at this time only three vessels have been constructed, comparative trials of different operational procedures have not yet been possible. Rather, progress has been made by on-going development as culture runs were proceeding. As more experience has been gained, the longevity of cultures has improved from 14 d in an early trial to greater than 70 d in recent runs. Problems occurred in test runs with the prototype vessel and arose from instability of pH through the lack of precise metering valves and flowmeters for the CO_2 supply. In more recent trials, with improved control gear, the pH has remained fairly stable for the duration of the cultures. Temperatures within the range 15–24°C have had little apparent effect on productivity. Individual cultures have tended to operate within a narrower temperature range. The discussion of results will concentrate on the more recent trials.

In each of the six trials shown in Table 2, the growth of cultures was rapid, enabling the first harvest to be made 5 or 6 d after inoculation. Initial cell concentrations ranged between 68 and 135 cells μl^{-1}, cultures being first harvested at counts ranging between 1450 and 2730 cells μl^{-1}. The rate of harvesting and the volume of culture per harvest has been largely determined by the demand for *Tetraselmis* for feeding to juvenile oysters. As an example of what has been achieved, the progress of trial 3 (Table 2) is shown in Figure 5. Early in the run harvesting was generally carried out three times weekly. The volume taken

Table 2 Data on the culture of *Tetraselmis* in 200 l vessels

Parameter	Culture trial					
	1	2	3	4	5	6
Cell density at inoculation (no./µl)	99	79	86	78	68	135
Age of inoculum (d)	28	73	7	49	67	140
Time to first harvest (d)	5	6	5	6	6	5
Cell density at first harvest (no./µl)	1,450	1,630	1,680	2,730	1,800	1,560
Total volume of culture harvested (l)	1,250	2,740	3,970	2,010	1,920	930
Average volume of culture harvested (l)	78.1	80.6	72.2	74.4	87.3	62.0
Volume harvested at a density of 1000 cells μl^{-1} (l/d)	48.6	49.7	58.1	51.6	52.0	50.6
Volume harvested at a density of 1000 cells μl^{-1} kW^{-1} (l/d)	151.9	155.3	181.6	161.2	162.5	158.1
Longevity of culture (d)	43	76	104	60	70	29

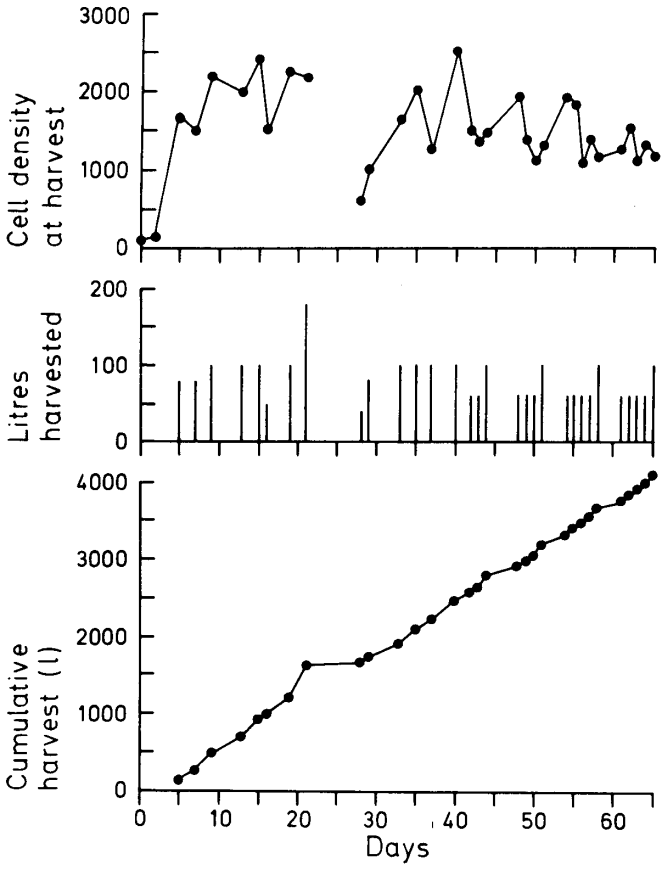


Figure 5 Data of cell density μl^{-1} at harvest, litres of *Tetraselmis* harvested and the cumulative harvest in terms of litres at a standard count of 1,000 cells μl^{-1} during the first 65 days of Trial 3.

off at each harvest was, to some extent, determined by the state of the culture. For example, the harvest volume was as low as 40 l when the cell density was below 1000 cells μl^{-1}, yet three harvests of 100 l each during a week were possible when the culture was growing vigorously. The data of Figure 5 are for the first 65 d of the culture run. This run continued for a further 39 d providing a total of 6045 l of *Tetraselmis* at a standardised cell density of 1000 cells μl^{-1}. As demonstrated towards the end of this run and in more recent trials, cultures can be successfully operated with five harvests per week, these being taken on successive days followed by a two day rest period; four harvests are of 60 l and the fifth of 100 l volume. Production in terms of litres harvested per week at 1000 cells μl^{-1} is similar to a harvesting regime where greater volumes are taken less frequently.

While these harvesting procedures provide satisfactory yields, there is evidence that improvement may be possible by employing a more critical harvesting regime. Figure 6 relates calculated post-harvest cell densities to the daily division rate in the ensuing 24 h period. Data from a number of trials have been included. These data are from periods during the culture runs when total weekly harvests of about 300 l at prevailing cell densities were made. The logarithmic relationship (significance $p < 0.001$) shows the effect of self shading on cell division rate at increasing post-harvest cell densities. From the relationship the cell density one day after harvesting can be calculated for a range of post-harvest cell densities. It is then possible, using these values, to determine for each post-harvest density the daily harvesting volume at 1000 cells μl^{-1} which can hypothetically be maintained. These extrapolations are shown in Table 3. The advantage, in terms of an improved yield, of harvesting daily to a density of 700 cells μl^{-1} is evident, although it has not yet been proved in

practical trials. Few data are available on daily division rate at post-harvest cell densities below 700 cells μl^{-1}. The division rates obtained in newly inoculated cultures prior to the first harvest suggest that it may be relatively constant up to 700 cells μl^{-1}.

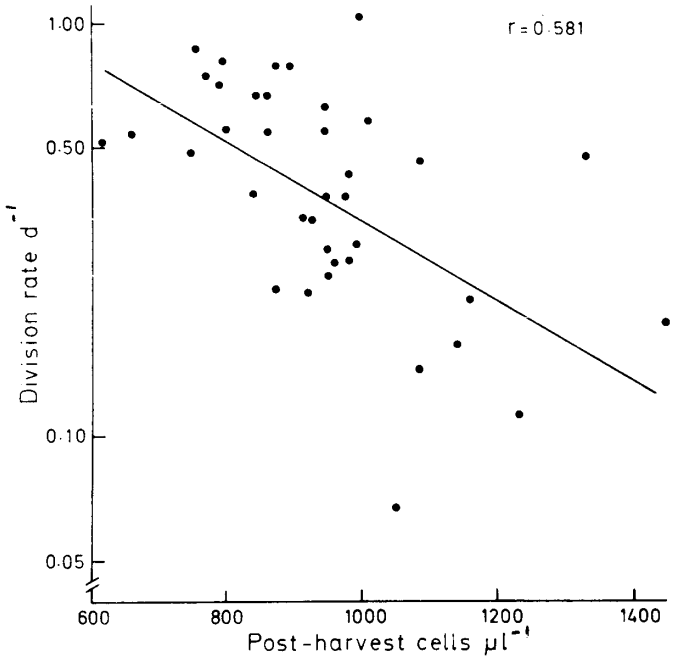


Figure 6 The relationship between the cell density of a culture immediately following a harvest and the daily rate of cell division in the ensuing 24 h period.

Table 3 Data relating to hypothetical daily harvest volumes for a range of post-harvest cell densities. The values are calculated from the relationship shown in Figure 6.

Post-harvest density cells μl^{-1}	Division rate d^{-1}	Cell density μl^{-1} after 24 h	Daily harvest (1) at 1,000 cells μl^{-1} from a 200 l culture
700	0.640	1,091	78.2
800	0.525	1,151	70.2
900	0.413	1,198	59.6
1,000	0.331	1,258	51.6
1,100	0.266	1,323	44.6
1,200	0.213	1,391	38.2

Trial 3 provided an average daily harvest of 291 ml per litre of vessel capacity at 1000 cells μl^{-1}. This compares with an average of 330 ml per litre of vessel capacity at 1000 cells μl^{-1} obtained in 20 l flask culture over the same time period. In terms of the light energy input, average daily yields at a standardised cell density of 182 l kW^{-1} in a 200 l vessel compare with 125 l kW^{-1} in a 20 l flask. On average, cultures have lasted 64 days and have provided a total of 3297 l of *Tetraselmis* at a daily yield of 51.8 l at 1000 cells μl^{-1}.

5. Discussion

The 200 l algal culture vessels have largely achieved the efficiency and reliability of production for which they were designed. Improvements in operational procedures have been made in the light of experience; developments in media composition and light input aimed at greater productivity continue.

One of the most important points is the low labour input in the operation of these vessels. Once set up and running, approximately 30 min on each harvest day is required for the general routine duties associated with the system and 15 min per vessel per day for harvesting and counting the cell density of the cultures. This amounts to 3 h d^{-1} for a ten-vessel system.

Vessels have been successfully cleaned and sterilized *in situ* between runs without the necessity to dismantle them. Recent results suggest that this operation will be an infrequent requirement in view of the longevity of cultures.

At the present stage of development each vessel is capable of providing about 52 l d^{-1} of *Tetraselmis* at a density

Table 4 An estimate of the cost of algal production based on 200 l internally illuminated vessels

Item	Capital £	Depreciation years	Daily cost £
Ten 200 l vessels	2,700	10	0.74
Filter and pump	800	10	0.22
Pipework and ancillary gear	500	10	0.14
Maintenance 5% per year			0.55
			1.65
Electricity – 108 kWh d^{-1} at 2 pence kWh^{-1} (light, pumping, steaming, air compressor)			2.16
Fresh water for cooling (28,800 l d^{-1} at .0124 pence l^{-1})			3.58
Labour – husbandry (3h d^{-1} at £1.75 h^{-1})			5.25
Filter mats, enrichment salts, CO_2			3.00
Total			15.64

Daily production of algae = 520 l
Cost per litre = 3.0 pence.

of 1000 cells μl^{-1}. To operate a moderately-sized oyster hatchery capable of producing one million juveniles per month at a mean length of about 5 mm, a daily production of 500 l of algae, 90% of which can be *Tetraselmis*, is necessary. This level of *Tetraselmis* production could be achieved, with some spare capacity, by operating ten 200 l vessels. An estimate of the cost of algal production based on these vessels as operated at Conwy is given in Table 4. The capital cost of the vessels is based on buying small quantities of the necessary raw materials and on-site workshop labour costs. A cost per litre of algae produced of 3.0 pence compares with approximately 8 pence per litre from the considerably more labour intensive 20 l flask system.

6. References

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.

FISHERIES RESEARCH TECHNICAL REPORT NUMBER 53

Part 2. RECOMMENDED PROCEDURES FOR THE CULTURE OF *Chaetoceros calcitrans*

by

I Laing

1. Introduction

Chaetoceros calcitrans (Paulsen) Takano is a non-chain forming marine centric diatom. Its median cell volume is usually quoted as 50 μm^3 (Walne, 1970) but may be as low as 30 μm^3, depending on culture conditions. Its small size and proven value as a food species makes it invaluable in the culture of bivalve larvae. It is acceptable to straight-hinged veliger larvae of the Pacific oyster, *Crassostrea gigas*, and is also an excellent food for larger larvae and spat (Walne, 1970 ; Helm and Millican, 1977).

Like many other species of diatoms, *Chaetoceros* is difficult to culture semi-continuously on a reasonably large scale. When batch cultured following the procedures described here reliable yields at high cell concentrations can be expected. Batch culture is the total harvesting of a food supply culture after a prescribed period of growth, and inoculation of a new culture to take its place.

This report describes methods and media for the batch culture of *Chaetoceros calcitrans* in stages of 2 l, 20 l and 200 l.

2. Maintenance of Master Cultures

A master, or stock, culture is a unialgal culture of a species kept in a maintenance medium such as Erdschreiber (Fϕyn, 1934) under closely controlled conditions of temperature and illumination. It is available as a pure strain of the species to provide lines of starter cultures. The sterile procedures described here should also be followed for maintenance of the starter cultures, so that the master culture will only be needed to provide a line of starter cultures once a year.

At the Fisheries Experiment Station, Conwy a master culture of *Chaetoceros* is kept in 250 ml of Erdschreiber medium in a cotton wool plugged 500 ml borosilicate glass flat-bottomed boiling flask. The composition and preparation of Erdschreiber medium is given in Appendix I. The master culture is kept in a cooled incubator operating at 15°C and is illuminated by two 8 W 'daylight' fluorescent lamps providing 450 lx at the culture surface. To maintain the master culture in a vigorous state it is necessary to subculture at monthly intervals. Following removal of the cotton wool plug and flaming of the neck of the flask with a bunsen burner, an inoculum of 20 ml is decanted into a flamed vessel containing sterile Erdschreiber medium.

3. Starter cultures

Starter cultures are grown to provide inocula to start larger volume cultures for feeding purposes. A line of starter cultures is set up by inoculating one 500 ml flask containing 250 ml of Erdschreiber medium on each of three successive days. For each inoculum 20 ml of the master culture is used. The starter cultures are grown at 21°C ± 1°C at a distance of 15–20 cm from a 65 W 'cool-white' fluorescent tube, giving a level of illumination at the culture surface of 4750–5250 lx. Each culture grows in three days to a sufficient cell density to inoculate a fresh 250 ml Erdschreiber starter (with about 20 ml) and a 2 l nutrient enriched sea water culture (with the remaining volume, about 250 ml).

4. Two-litre cultures

Sea water at ambient salinity is filtered to 2.5 μm–5.0 μm diameter particle size with diatomite ('545' Grade Celite – Johns Manville Ltd) coated Carlson-Ford 'White 400' papers in a British Filter PF 30B plate filter unit. Filtration with glass fibre filter papers or cartridge filters is equally satisfactory. It is then autoclaved in 2 l aliquots in cotton wool plugged, 3 l, flat-bottomed borosilicate glass boiling flasks at 1.06 kg cm^{-2} for 20 minutes and then allowed to stand for two days prior to nutrient enrichment. The salt concentrations used are given in Appendix II. Each day a fresh 2 l culture is inoculated from a starter culture and aerated with a mixture of 1% CO_2 carried in a flow of compressed air at 2.5 l per minute. This proportion of CO_2 maintains the pH of the culture at about 7.8. The gas mixture is filtered through a Whatman 'Gamma 12' cartridge unit containing a 0.3 μm filter to reduce the risk of airborne contamination. When grown at 21°C ± 1°C and at a level of illumination of 25, 000 lx provided by 'cool-white' or 'daylight' lamps, a density of 40,000–50,000 cells μl^{-1} is typically reached in three to four days (Figure 1). The cells will range in mean volume from 35–45 μm^3 and will have a dry weight of 10–15 $\mu g/10^6$ cells. The culture may then be used for feeding purposes. If kept longer it will rapidly enter a declining phase and collapse, at which time it is unsuitable as a food.

5. Twenty-litre cultures

The salinity of the sea water is adjusted to 15‰ by the addition of appropriate quantities of fresh (tap) water and 20 l is then filtered through a British Filter PF 30B plate

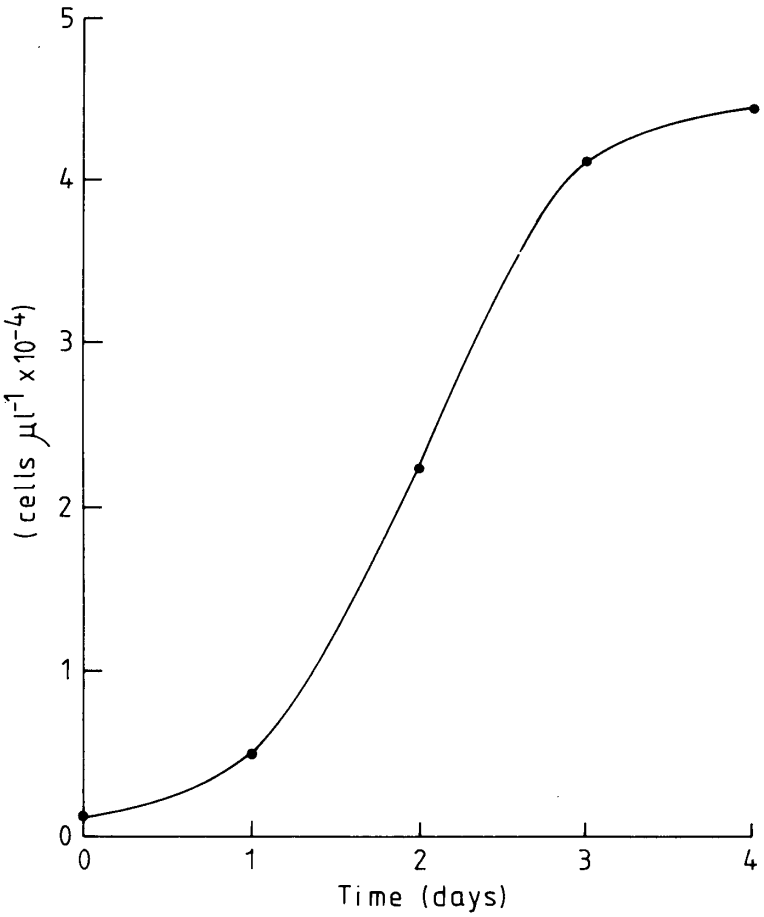


Figure 1. Typical growth curve for *Chaetoceros calcitrans* cultured in autoclaved sea water medium.

filter unit containing Carlson-Ford asbestos fibre mats (Grade EKS) into a steam-sterilised 22 l capacity spherical borosilicate glass vessel. Alternatively, 0.45 µm membrane cartridge filters may be used. Nutrient enrichment is made from stock salt solutions similar to those described in Appendix II, except that 3.25 g of Ferric chloride ($FeCl_3.6H_2O$) is used in Stock A. The appropriate amount of a 2 l culture is used as an inoculum to give a density of 1,000 cells μl^{-1} in the 20 l culture. The volume of inoculum can be calculated from:

$$\text{Inoculum volume (l)} = \frac{\text{Culture volume (l) x required inoculum density (cells/}\mu l^{-1})}{\text{Density of inoculating culture (cells/}\mu l^{-1})}$$

The inoculated culture is grown at 21°C ± 1°C at an illumination intensity of 15,000 lx, measured at the centre of an empty flask, provided by four 125 W 'daylight' fluorescent lamps. The culture is supplied with a filtered air/CO^2 mixture as described for the 2 l culture but at an air flow rate of 5 l min^{-1}. In three or four days a peak cell density of 18,000–24,000 cells μl^{-1} is achieved. Cells are on average 40–50 μm^3 in volume and contain 12–18 µg dry matter/10^6 cells.

The procedures described above may also be used for the 2 l culture stage in medium prepared from filtered sea water.

6. Two hundred-litre cultures

Large-scale cultures in the vessels described by Helm *et al.* (in Part 1 of this report) may be inoculated at 1,000 cells μl^{-1} from the 20 l stage and grown using the techniques described above. In three to four days a culture density of 25,000–30,000 cells μl^{-1} may be attained. The mean cell volume will be in the range 30–40 μm^3 and the dry weight content of the cells 8–14 µ g/10^6 cells.

7. Yield

If three or four vessels for each stage are operated then a 2 l, 20 l and 200 l culture can be harvested daily. Allowing for inocula, this will give a yield equivalent to about 275 l at 20,000 cells μl^{-1} d^{-1}.

8. References

Fϕyn, B., 1934. Labenszyklus, Cytologie und Sexualitat der Chlorophycee *Cladophora suhriana* Kutzing. Arch. Protistenk, *83*: 1–56.

Helm, M.M. and Millican, P.F., 1977. Experiments in the hatchery rearing of Pacific oyster larvae (*Crassostrea gigas* Thunberg). Aquaculture, *11*: 1–12.

Walne, P.R., 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus*. Fishery Invest., Lond., Ser. 2, *26* (5), 62 pp.

Appendix I. The composition and preparation of Erdschreiber medium (Fϕyn, 1934)

Constituents

1. Sea Water. Autoclave 2 l in a 3 l borosilicate glass flat-bottomed boiling flask with cotton wool plug at 1.06 kg cm^{-2} for 20 minutes. Stand for 2 days.

2. Soil extract: prepared as follows:

   (a) mix 1 kg soil from a woodland or pasture area untreated with artificial fertilizers, insecticides etc. with 1 litre fresh water;

   (b) autoclave at 1.06 kg cm^{-2} for 60 minutes;

   (c) decant;

   (d) filter through Whatman No. 1 paper and then through glass fibre (GF/C) paper;

   (e) autoclave in 1 litre aliquots in polypropylene bottles at 1.06 kg cm^{-2} for 20 minutes;

   (f) store in deep freeze until required;

   (g) autoclave 100 ml in 500 ml borosilicate glass flat-bottomed boiling flask with cottom wool plug at 1.06 kg cm^{-2} for 20 minutes.

3. Nitrate/phosphate stock solution. Dissolve 40 g $NaNO_3$ and 4 g No_2HPO_4 in 200 ml distilled water. Autoclave in 500 ml flask at 1.06 kg cm^{-2} for 20 minutes.

4. Silicate stock solution. Dissolve 8 g $Na_2Si0_3.5H_20$ in 200 ml distilled water. Autoclave in 500 ml flask at 1.06 kg cm^{-2} for 20 minutes.

Procedure

Add 100 ml soil extract (2) to 2 l sea water (1). With sterile pipette add 2 ml nitrate/phosphate stock (3) and 2 ml silicate stock (4). Decant 250 ml into 8 empty autoclaved 500 ml flasks with cotton wool plugs. Use bunsen burner to flame necks of flasks immediately before and after decanting/pipetting.

Appendix II. Nutrient salt stock solutions for the enrichment of autoclaved sea water.

Stock A

$FeCl_3.6H_2O$		1.3	g +
$MnCl_2.4H_2O$		0.36	g
H_3BO_3		33.6	g
EDTA		45.0	g
$NaH_2PO_4.2H_2O$		20.0	g
$NaNO_3$		100.0	g
Trace metal solution *		1.0	ml
Distilled water	to	1.0	l

Add 2 ml Stock A per l of sea water

***Trace metal solution**

$ZnCl_2$		2.1	g
$CoCl_2.6H_2O$		2.0	g
$(NH_4)_6Mo_7O_{24}.4H_2O$		0.9	g
$CuSO_4.5H_2O$		2.0	g
Distilled water	to	100.0	ml

Acidify with sufficient conc. HCl to obtain a clear solution

+ Amount for enrichment of autoclaved sea water; for filtered sea water use 3.25 g.

Stock B

Vitamin B_{12} (Cyanocobalamin)		10.0	mg
Vitamin B_1 (Aneurine hydrochloride)		200.0	mg
Distilled water	to	200.0	ml

Add 0.2 ml Stock B per l of sea water

Stock C

$Na_2SiO_3.5H_2O$		4.0	g
Distilled water	to	100.0	ml

Add 2 ml Stock C per l of sea water

FISHERIES RESEARCH TECHNICAL REPORT NUMBER 53

Part 3. PRODUCTION OF ALGAE FOR USE IN THE REARING OF LARVAL FISH

by

S M Baynes, L Emerson and A P Scott

1. Introduction

The Fish Food Production Unit at the Fisheries Laboratory, Lowestoft, is concerned with the culture of algae, rotifers and *Artemia* for larval fish rearing. The culture techniques used here in 1975 were described by Reynolds (1975) and since that time many of the systems have been altered. This report describes the techniques currently in use.

2. Choice of algae

Reynolds (1975) described the culture of only two algal species: *Phaeodactylum tricornutum* Bohlin and *Dunaliela tertiolecta* Butcher. These are still cultured in the Unit as they are generally reliable and fast growing. However, recent research (Howell, 1977; Scott and Baynes, in press) has shown that they are not suitable for turbot larvae rearing. Other algae, such as *Isochrysis galbana* Parke, or *Pavlova lutheri* (Droop) Green, must be added to the larvae rearing tanks to ensure good survival of the larvae. The reason for this requirement has still be be established.

Many of the changes and additions to the procedures described by Reynolds have resulted from the need to grow algae other than *Dunaliella* and *Phaeodactylum.* For instance, *Isochrysis* and *Pavlova* require different culture techniques because :

i. they will not grow in chemically sterilized water;

ii. they require vitamins;

iii. they require more stable temperatures;

iv they require greater hygiene.

Furthermore, the system described by Reynolds was unable to provide sufficient algae for the larvae rearing programme; a more economical and productive mass-culture system was necessary.

3. Water supply

Many of the changes were only possible as a result of an improved sea water supply. Before 1975 the sea water available for algal culture was from a supply pumped directly from the surface water just off the beach. Despite being allowed to settle for up to a week, suspended material in the water rapidly clogged filters in the supply line and algal growth, in the water, was not reliable. To overcome this variability, Reynolds used a 3% solution of household cooking salt as a substitute for sea water. Now sea water is obtained from a "well" sunk in the beach at low-water mark (Scholes, in press). The initial filtration by the beach sand means that no settlement is required and the water can be passed through a fibre cartridge-filter (Balston Ltd., Maidstone) to remove particles of size down to 5 μm. After this, the water may be used for culture work. Water drawn through the beach well contains less than 6% of the bacteria present in the surface water (Table 1). These bacteria and other viable organisms must be removed before the water can be used successfully for algal culture. The sterilization procedures used are summarized in Appendix 1.

Table 1 Total number of bacterial colonies developing after 48 h incubation at 18°C, from 25 ml water samples*

Sample	Number of bacterial colonies	
	Duplicate	
	A	B
Surface sea water	>1000	>1000
Beach well water	51	77
Autoclaved sea water	0	0

*[0.45 μm membrane filters, through which 25ml water samples had been filtered, were incubated on sea water nutrient agar plates]

4. Culture medium

Sea water is used as the basis of our culture medium. Many recipes for nutrient enrichment are detailed in the relevant literature. The one used by the Fish Food Production Unit for the last five years, with very good results, has been adapted from that described by Walne (1966); its composition is detailed in Appendix 2. The concentrated salt solutions are made up in bulk and dispensed into screw-top bottles before autoclaving. The required volume of concentrate is added to the sea water after it has been sterilized. If the sea water has been autoclaved it is first allowed to cool as adding the concentrate whilst the water is still warm causes precipitation.

5. Main algal production

Polythene bag culture vessels of 480 1 capacity are used for the main algal production. Although large, they have

proved to be very easy to manage and the capital outlay required is small. The bags are supported vertically by a cylindrical framework of galvanised 'Weldmesh' (B.R.C. Engineering Co. Ltd. Stafford). Each frame is 1.8m high and 0.6m in diameter. The Weldmesh is clamped around a 70mm thick disc of concrete for stability (Figures 1 and 2). The polythene bags are cut lengths of clear "lay-flat" tubing (Transatlantic Plastics Ltd., London) heat-sealed top and bottom. This material is available in several widths : that used at Lowestoft is 0.9m, 10000 extra heavy gauge. The inside of a new bag is sufficiently free of potential contaminants to make sterilization unnecessary. This design of vessel is based on that used by Seasalter Shellfish Co. Ltd., Whitstable (Farrar, 1975).

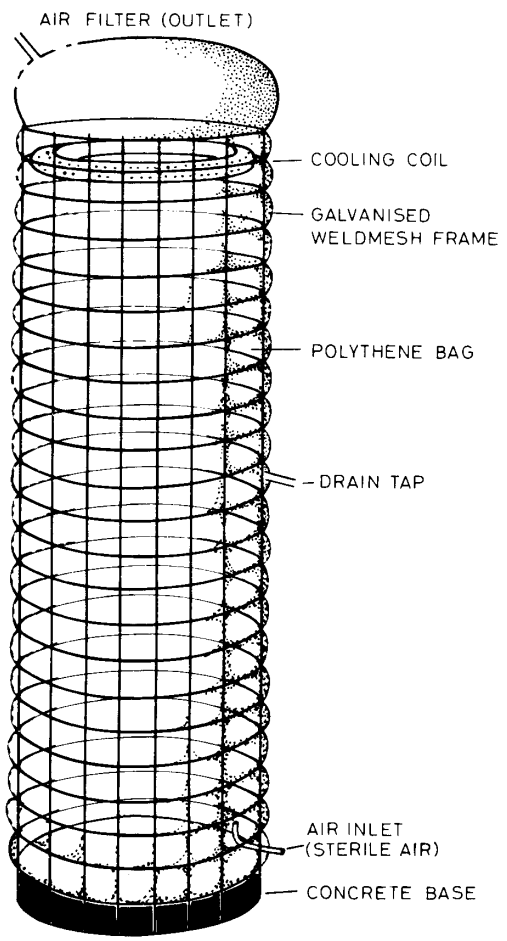


Figure 1 Diagram of the construction of polythene bag vessel.

The bags have been used with both natural and artificial lights. The culture system is very suitable for use out of doors. It takes up a relatively small ground area ($0.3m^2$) for the volume contained yet gives a large surface area ($3.2m^2$) for light penetration. The culture is completely enclosed and therefore less prone to contamination than are open tanks and ponds. This system has been used in the open air with no additional lighting over the last three summers (May-October) and production has been in excess of needs. The cell densities achieved in these large culture vessels are less than those achieved with the more conventional culture techniques, such as flask culture in artificial light (Table 2). The amount of light available to these cultures is the major factor limiting cell density. Light is rapidly attenuated within the culture by the self-shading of the cells. In general, the greater the diameter of the culture vessel, the lower the maximum cell density possible with a fixed light intensity. In order to maintain cell division rates, part of the culture is removed periodically.

Table 2 Cell densities obtained regularly with different algal species in culture at Lowestoft.

Species	Cell density (Thousands of cells/µl)		
	Outdoor bag* culture 480 l (600 mm dia)	Indoor bag+ culture 480 l (600 mm dia)	Indoor flask+ culture 20 l (300 mm dia)
Pavlova lutheri	8	5	16
Isochrysis galbana	8	5	16
Phaeodactylum tricornutum	25	12	30
Dunaliella tertiolecta	2	1	3

Incident light intensity

* 200–2000 µEm^{-2} s^{-1} natural photoperiod

\+ 200 µEm^{-2} s^{-1} continuous light

Up to twelve plastic bag vessels at a time have been used out of doors. Groups of three cultures have one quarter of their volume removed every fourth day, allowing the cultures to replace that amount themselves during the three intervening days. This means that the cell density is near its maximum and the rate of cell division is low, but there is enough time between croppings for the yield to be maintained even if the weather is particularly dull.

The cultures remain productive for 6–8 weeks after which contaminants begin to influence the growth. The cultures are then discarded and replacements are set up in new bags.

6. Setting up a culture

To start a culture the bag is first fully inflated with sterile air. The air pressure gives the bag rigidity within the framework whilst it is filled with sterilized sea water, which is

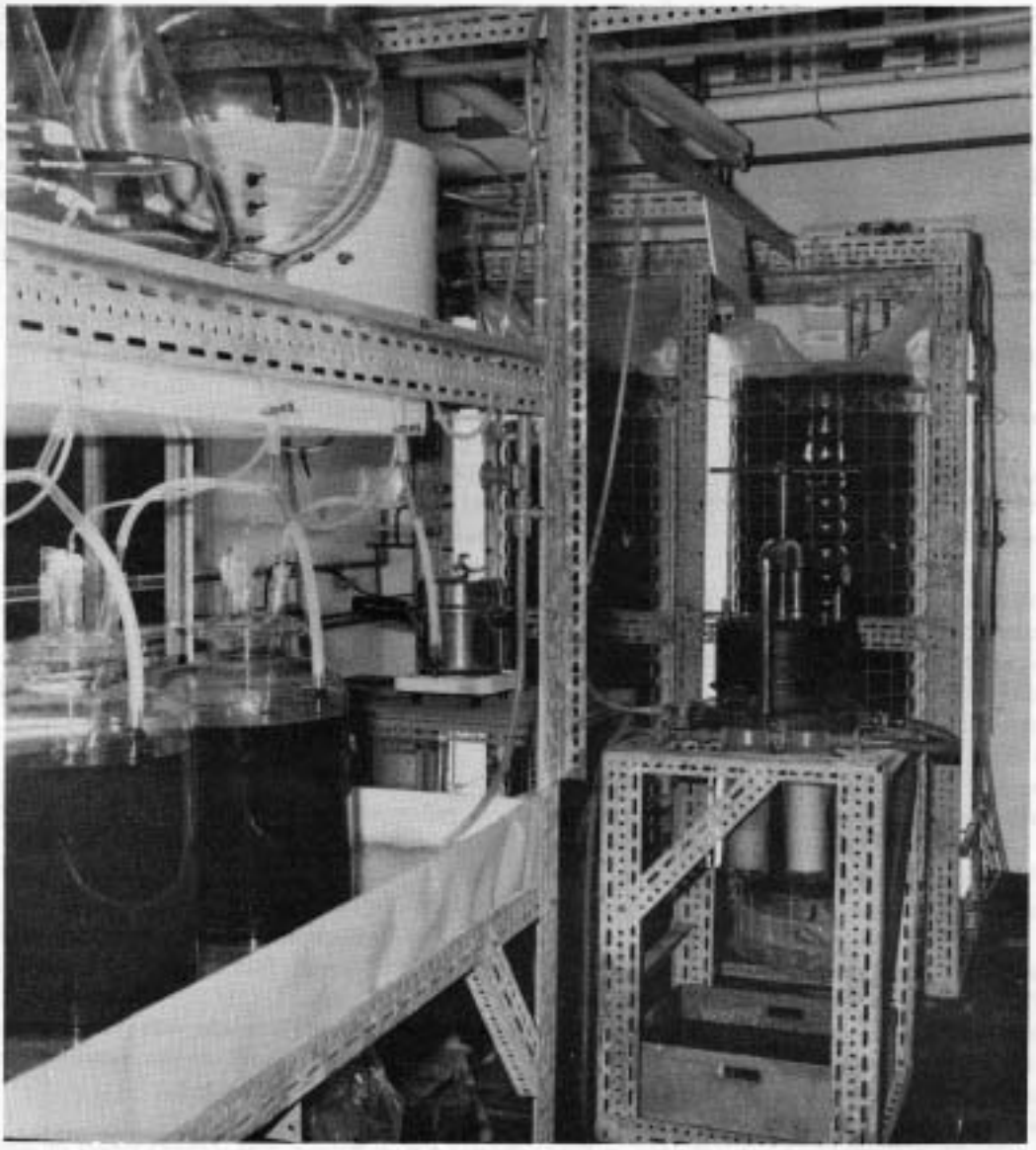


Figure 2 Part of the algal culture facility at Lowestoft.
Two polythene bag vessels are set up at the back of the laboratory. On the left are the 20 litre, high-density cultures, grown in glass jars. The filter press, and steam generator for steaming the seawater pipes are visible behind the jars.

normally done directly from a sterilizing filter-press (British Filters Ltd., Marlow). Both operations can be carried out through a small hole cut in one corner at the top of the bag. An outlet tap in the side of the bag and an air-line at the bottom are inserted by first making the holes with glass tubing of exactly the required diameter that has been drawn to a point; the flanges of stretched polythene that this creates are sufficient to hold and to seal the straight tubes in place. Anything inserted into the bag is first sterilized and the polythene, at the point of insertion, swabbed with acidified alcohol. The nutrient concentrate and the algal inoculum (20 l) are introduced through the hole at the top of the bag, which can then be closed with an air filter. Aeration is provided by compressed air, enriched with carbon dioxide and filter sterilized (Gamma 12 units, Whatman Ltd., Maidstone). Approximately 5 l air min^{-1} is bubbled through the culture, introducing 1% or 2% CO_2. The amount is adjusted using flowmeters (G A Platon Ltd., Basingstoke) so that the medium has pH of 7.5.

Indoors, artificial lighting is provided by five 85 W, 1.8m fluorescent "Daylight" tubes (Thorn Lighting Ltd., Enfield) between each pair of bags (Figure 2). The lighting control gear and ballast is mounted away from the bags to minimize heat production near the cultures. Even so, the fluorescent tubes themselves produce sufficient heat to raise the culture temperature 5°C above ambient. As most algal species used for mass cultivation have temperature optima around 20°C (Table 3) temperature control is required for the indoor cultures. Where air conditioning is not available, effective

cooling is obtained by pumping cold water (10°C), held in a refrigerated reservoir, through a loop of 70mm lay-flat tubing around the top of each bag (see Figure 1).

Table 3 Temperature range for the commonly cultivated algal species.

Species	°C
Pavlova lutheri	14–25
Isochrysis galbana	14–22
Phaeodactylum tricornutum	8–24
Dunaliella tertiolecta	12–30

7. Maintenance of starter cultures

All starter cultures at Lowestoft are grown in autoclaved sea water under continuous artificial light, at 20°C, with CO_2 enriched aeration. The stock cultures (600 ml) of each species are kept in triplicate. Each is sub-cultured weekly by pouring one third of the volume into 400 ml of sterile medium in a clean, sterile flask. The remaining two thirds is used to inoculate 3.5 l cultures as required, and these cultures provide the inocula for 20 l cultures, after approximately one week's growth. The light intensities used are 25 μEm^{-2} s^{-1} for the 600 ml, 50 μEm^{-2} s^{-1} for the 3.5 l, and 200 μEm^{-2} s^{-1} for the 20 l cultures.

A set of master cultures of the species grown regularly is also kept. These are small volume (60 ml) slow growing cultures, which are sub-cultured every three weeks, and inocula for the starter cultures are produced from these when required. The master cultures are not aerated, are kept at a lower temperature (12°C) than the other cultures, and within each 24 h period are subjected to a light intensity of 15 μEm^{-2} s^{-1} for 16 h and darkness for 8 h. The medium used for them is the same as that detailed in Appendix 2, but with the addition of 25 ml of soil extract to each litre of sea water. This is thought to provide micronutrients that are missing from the basic medium and which might limit the life of the alga in culture. Soil extract is made by autoclaving 1 kg of garden loam in 1 litre of distilled water, and filtering out the sediment. The extract is then re-autoclaved in screw-top bottles.

Although the required species are kept in culture at Lowestoft, they were obtained initially from collections such as that held at the Culture Collection of Algae and Protozoa, 36 Storey's Way, Cambridge.

8. Use of algae in turbot larvae rearing

The vast majority of the algal material produced by the Fish Food Production Unit at Lowestoft is used to feed cultures of the rotifer *Brachionus plicatilis* which is given as the first food for larval turbot. It seems that the species of alga used for rotifer production makes little difference to their rate of reproduction (Scott and Baynes 1978); at Lowestoft the four species mentioned above are actually used in rotation.

It has been shown that algae are an essential addition to turbot larvae rearing tanks during the rotifer feeding stage (Scott and Baynes, in press). Unless algae are added the fish are most unlikely to survive more than 10 days after hatching. Of the species of algae mentioned in this report, *Dunaliella tertiolecta* does not support growth of turbot larvae beyond 7mm (approx. 21 days) whereas additions of any of the other species give good survival.

The large volume, low density algal cultures from the polythene bag vessels have been used for maintaining the bulk rotifer production, but additions to the larvae rearing tanks have been from the higher density, low volume cultures. In this way excess water that has to be removed from the tanks is kept to a minimum.

9. References

Farrar, S. (1975) Low risk oyster culture in Spain. Fish Farm. Int. *2* :29.

Howell, B.R. (1977) Aspects of the development of cultivation techniques for flatfish. Ph D Thesis, University of Liverpool, 105 pp.

Reynolds, N. (1975) The culture of algae and rotifers in the Fish Food Production Unit at Lowestoft. Fish. Res. Tech. Rep., MAFF Direct. Fish. Res., Lowestoft, (13) 6 pp.

Scholes, P. (In press) Seawater well system at the Fisheries Laboratory, Lowestoft and the methods in use for keeping marine fish. J. Mar. Biol. Ass. U.K.

Scott, A.P. & Baynes, S.M. (1978) Effect of algal diet and temperature on the biochemical composition of the rotifer, *Brachionus plicatilis*. Aquaculture *14* :247–260.

Scott, A.P. & Baynes, S.M. (In press) The effect of unicellular algae on the survival and growth of turbot larvae *(Scophthalmus maximus L.)* In: Proc. Symp. Finfish Nutrition and Feed Technology, 20–23 June 1978, Hamburg, Fed. Rep. of Germany.

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.

The reference to proprietary products in this report should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.

Appendix I Methods of sterilizing sea water

	Method	Advantages	Disadvantages
Autoclaving	1.05 kg cm^{-2} (15 lb/in^2) for 20 min. 5% distilled water added before sterilization to reduce precipitation	Very effective. Useful for reliable sterilization of small volumes such as those for stock cultures. Sterilization is possible in the culture vessel	Volume of water sterilized at any one time is limited by the capacity of the autoclave (less than 20 l) and some precipitation always occurs
Filtration	Sterilizing grade of fibre filter sheets fitted in filter-press and sterilized before filtration by autoclaving or steaming	Large volumes can be handled (over 1000 l d^{-1})	Filter sheets have a limited life and must be sterilized daily
Chemical Sterilization	2 ml of hypochlorite solution (11.5% chlorine w/v) added per litre of sea water. Neutralized after 30 min with an equal volume of sterilized $NaHSO_4$ solution (150g l^{-1})	Large volumes may be treated *in situ*	Water sterilized by this method will not support good growth of *I. galbana* or *P. lutheri*, although *D. tertiolecta* and *P. tricornutum* will grow well
Ultra-violet light irradiation	Sea water is passed over a high intensity u.v. light source	Large volumes can be handled (over 1000 l d^{-1})	Operation ceases if power supply is interrupted. Flow rate determines the efficiency of sterilization

Appendix II Sea water nutrient enrichment solutions (after Walne, 1966)

$FeCl_3.6H_2O$		1.30 g
$MnCl_2.4H_2O$		0.36 g
H_3BO_3		33.60 g
EDTA (Na Salt)		45.00 g
$NaH_2PO_4.2H_2O$		20.00 g
$NaNO_3$		100.00 g
* Trace metal solution		1.0 ml
Distilled water	to	1 litre

1 ml of this solution is added to each litre of sea water

Vitamin B_{12} (Cyanocobalamin)		10 mg
Vitamin B_1 (Thiamin)		200 mg
Vitamin H (Biotin)		10 mg
Distilled water	to	100 ml

This solution should be acidified to pH 4.5 before autoclaving. 0.1 ml is added to each litre of sea water.

* Trace metal solution :

$ZnCl_2$		2.1 g
$CoCl_2.6H_2O$		2.0 g
$NH_46Mo_7O_{24}.4H_2O$		0.9 g
$CuSO_4.5H_2O$		2.0 g
Distilled water	to	100 ml

It is necessary to acidify this solution with HCl to obtain a clear liquid.