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Cultivation of marine, unicellular algae

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FOREWORD

This leaflet sets out requirements for the cultivation and maintenance of marine, unicellular algae, drawing on experience with various systems developed at MAFF's Fisheries Laboratory at Conwy, North Wales.

A general introductory section on marine algae and their uses is followed by an explanation of factors affecting their growth, general maintenance, types of culture and examples of the small-scale batch culture methods and large-scale continuous and semi-continuous methods developed at Conwy.

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1. INTRODUCTION

Unicellular, marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish. Oysters and clams feed by filtering them from sea water. Rotifers and brine shrimps also ingest algae, and are then themselves used as food for larval fish and prawns. In some systems, algae are added to the water containing fish or prawns to improve the 'quality', but the way in which this works is not yet fully understood.

Marine algae are simple-celled plants and, like all plants, contain chlorophyll, which traps the energy from light and uses it to convert nutrients and carbon dioxide dissolved in the sea water into organic growth. In the laboratory or hatchery, a collection of algal cells, which are growing and dividing, is known as a culture.

Of the very many types of algae which live in the sea, only a few can be cultured, and only certain types will give good growth when fed to oysters, clams, rotifers, brine shrimps, etc. Some of the types commonly used as foods are listed in Table 1. They comprise two groups — the flagellates, which can swim by the action of one or more flagellae, and the diatoms, which have an outer shell composed of silica, but can nevertheless remain buoyant.

Individual species of algae can be used as food, but feeding mixed diets, consisting of at least one type of diatom and one type of flagellate, nearly always gives much better growth. Examples of some of these algae are shown in Figure 1. *Chaetoceros calcitrans* is one of the more useful diatoms which, because of its small size, is especially suitable for feeding to larval stages of marine animals. However, it is not very easy to grow in large quantities. *Skeletonema costatum* and *Thalassiosira pseudonana* are easier to culture.

Table 1. Types of algae commonly used as food. Those marked with an asterisk are most often fed to oysters and clams

Algae	Cell diameter (microns)
Diatoms	
**Skeletonema costatum*	6
**Chaetoceros calcitrans*	2.5
**Chaetoceros gracilis*	6
Phaeodactylum tricornutum	5
**Thalassiosira pseudonana* or 3H	5.5
Flagellates	
Chlamydomonas coccoides	7
Nannochloris occulata	2
Dunaliella tertiolecta	6.5
Chroomonas salina	7
**Tetraselmis suecica*	8.5
Pyramimonas virginica	4
**Isochrysis galbana*, or T. ISO	5
**Monochrysis lutheri*	4
**Pseudoisochrysis paradoxa*	5

Stock cultures of all of these algae are kept at the Fisheries Laboratory, Conwy. Samples can usually be supplied for a small handling charge.

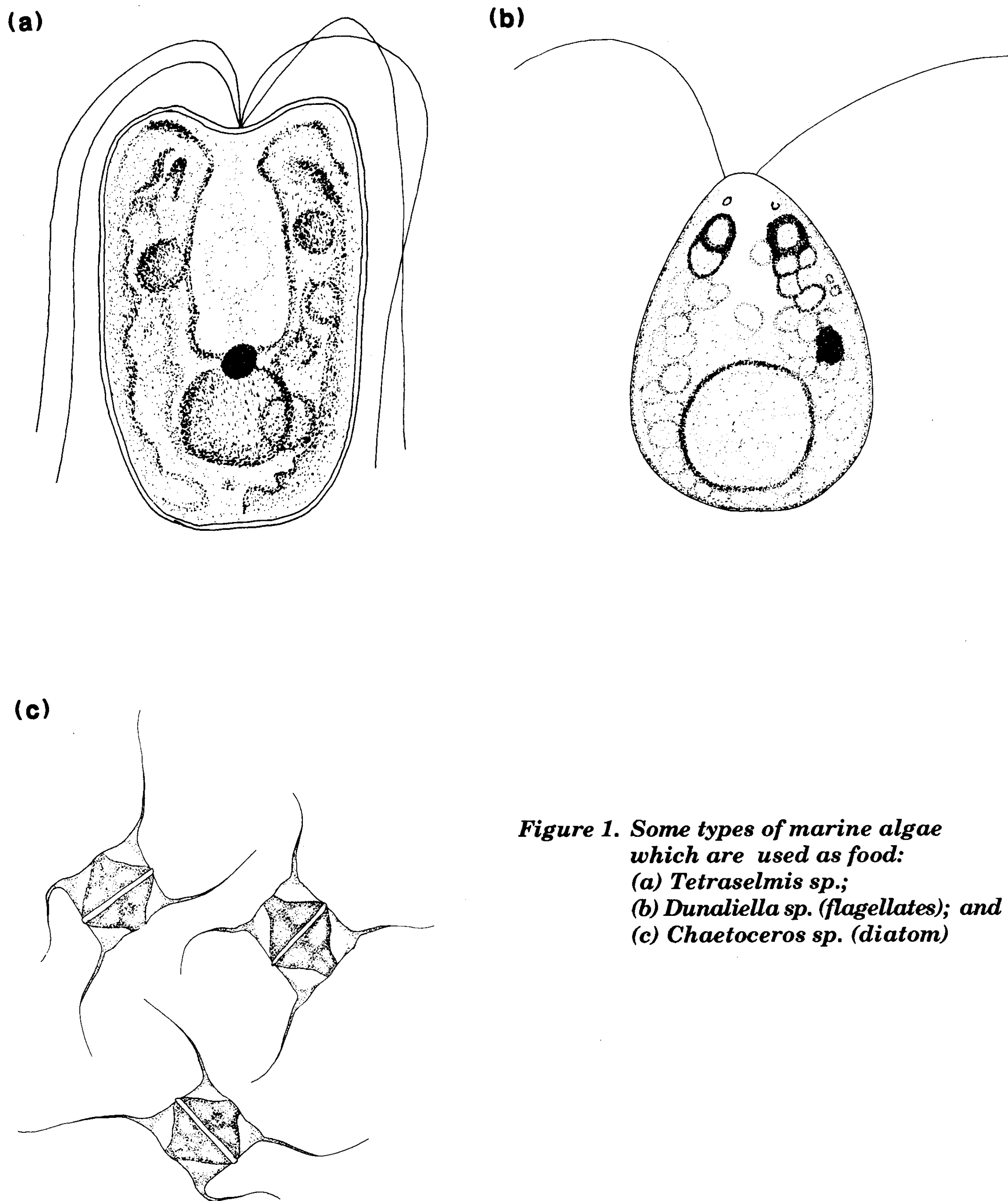

Figure 1. Some types of marine algae which are used as food:
(a) Tetraselmis sp.;
(b) Dunaliella sp. (flagellates); and
(c) Chaetoceros sp. (diatom)

Similarly, with the flagellates, the small *Isochrysis galbana* and *Pseudoisochrysis paradoxa* are very good foods but difficult to culture. *Tetraselmis suecica* is much easier to grow and is often used to feed larger animals.

Marine algae can be observed using a microscope with a magnification of x100 to x400. When feeding algae to animals, it is important to know the concentration of cells in the culture, and this can be determined using an haemocytometer (Appendix 1).

2. CULTURE CONDITIONS NECESSARY FOR GROWTH OF MARINE ALGAE

2.1 Light

This is normally provided by fluorescent lamps. The most commonly used types are 'cool-white' or 'daylight'. Increasing the light intensity usually means better growth and faster division of algal cells and, therefore, the production of more food. It is important to make the most efficient use of the artificial light as lamps also generate heat and may make the culture too hot. The indoor culture systems described in Section 5 of this leaflet are all designed to use light efficiently. Cultures of algae can also be grown outdoors, using natural daylight, and examples of this method are also given in Section 5.

2.2 Heat

Most types of algae grow well at temperatures from 17°C to 22°C. Lower temperatures will not usually kill the algae, but will reduce their growth rate. Above 27°C, most types of algae will die. If necessary, cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air conditioning units.

2.3 Nutrients

Nutrients are the inorganic salts required for plant growth. For algae culture, it is convenient to make up strong standard solutions which, in appropriate dilutions in sea water, provide the culture medium. Recipes for the nutrient salt solutions, used for culture of algae at MAFF's Fisheries Laboratory at Conwy are given in Table 2.

2.4 Mixing

By mixing, the necessary light and nutrients become available to all of the cells. Algae cultures are usually mixed by bubbling air through them. This can be supplied from a compressor or via an air blower, and will also act as a carrier gas for carbon dioxide. Cultures may also be mixed by mechanical means using, for example, stirrers.

2.5 Carbon dioxide

Providing the algae with extra carbon, in the form of the gas carbon dioxide (CO_2), will give much faster growth. CO_2 is supplied from compressed gas cylinders, and only a very little is needed (about half of one percent) in the air supplied to the culture. The CO_2 should be passed through a flow meter to ensure that the amount used will keep the pH of the culture between 7.8 and 8.0. The pH can be checked with indicator papers, which change colour with a change in pH, or with a pH meter. Both the air and the CO_2 should be filtered through an in-line filter unit of 0.3 microns to 0.5 microns before entering the culture, as this helps to prevent other, possibly contaminating, organisms from getting into the cultures.

2.6 Salinity

Salinities of between 25 and 30 psu (practical salinity units) (UNESCO, 1981)* are generally best for the culture of flagellates, and between 20 and 25 psu for the culture of diatoms. These salinities can be obtained by diluting sea water with tap water. Salinity can be measured with an hydrometer or a refractometer.

UNESCO, 1981. The practical salinity scale 1978 and the international equation of state of sea water 1980. Tenth Report of the Joint Panel on Oceanographic Tables and Standards. UNESCO, Paris, Tech. Pap. in Mar. Sci., (36**), Annex 1: 13-21.*

Table 2. Nutrient salt solutions

Constituents	Quantities
Solution A	
Ferric chloride ($FeCl_3$)	0.8 g[a]
Manganous chloride ($MnCl_2.4H_2O$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA[b], di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate ($NaH_2PO_4.2H_2O$)	20.0 g
Sodium nitrate ($NaNO_3$)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water[c]	Heat to dissolve
Solution B	
Zinc chloride ($ZnCl_2$)	2.1 g
Cobaltous chloride ($CoCl_2.6H_2O$)	2.0 g
Ammonium molybdate ($(NH_4)_6MO_7O_{24}.4H_2O$)	0.9 g
Cupric sulphate ($CuSO_4.5H_2O$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml with fresh water[c]	Heat to dissolve
Solution C	
Vitamin B_1	0.2g
Solution E	25.0 ml
Make up to 200 ml with fresh water[c]	
Solution D (for culture of all diatoms — used in addition to solutions A and C)	
Sodium metasilicate ($Na_2SiO_3.5H_2O$)	40.0 g
Make up to 1 litre with fresh water[c]	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1 g
Make up to 250 ml with fresh water[c]	
Solution F (for culture of *Chroomonas salina* — used in addition to solutions A and C)	
Sodium nitrate ($NaNO_3$)	200.0 g
Make up to 1 litre with fresh water[c]	(add 1 ml per litre of culture)

(a) Use 2.0 g for culture of Chaetoceros calcitrans in ***filtered*** *sea water.*

(b) Ethylene diamine tetraacetic acid.

(c) Use distilled water if possible.

Note: Chemicals should be obtainable through a supplier of laboratory equipment or through larger chemists.

2.7 Cleanliness

The sea water containing the algae must be clean or unwanted types of algae and other contaminants, which may feed on or compete with the algae, will grow in the cultures.

Small amounts (of up to about 10 litres) of sea water can be autoclaved (sterilised by steam at a high pressure — a pressure cooker is a small autoclave), or pasteurised (heated to 80°C for 1-2 hours, cooling to room temperature for at least 18 hours, then reheated to 80°C for a further 1-2 hours) or boiled. Using these methods, a container of suitable material (e.g. borosilicate glass (Pyrex)), can be treated at the same time.

Larger volumes of sea water can be cleansed by filtration. There is a wide range of suitable equipment commercially available for this purpose, which includes cartridge filters, filter assemblies using diatomaceous earth as an aid to efficient filtration, and 'swimming pool type' filters. The main consideration is that the equipment chosen can cope with the volume of sea water that is needed. Filtration to remove particles greater than 2 microns is essential, and the removal of particles larger than half a micron is desirable. A large quantity of sea water can also be cleansed by passing it slowly through an ultra-violet light sterilising unit following filtration to remove particles greater than 2 microns in diameter.

3. GENERAL MAINTENANCE OF ALGAE CULTURES

3.1 Stock culture

All algae culture systems require a set of 'stock' cultures, usually of about 250 ml in volume, to provide the reservoir of algal cells from which to start the larger-scale cultures which will be used for feeding.

Stock cultures are kept in small flasks, such as 500 ml borosilicate glass, flat-bottomed boiling flasks fitted with cotton-wool bungs. Two types of culture medium can be used:

(a) Erdschreiber culture medium, which is difficult to prepare (Table 3(a)), but very reliable; and
(b) a simpler, but less reliable culture medium, the preparation of which is described in Table 3(b).

3.2 Sub-culture

Stock cultures must be sub-cultured frequently (preferably weekly). Sub-culturing involves inoculating some cells from an old stock culture into fresh culture medium, so that the cells can continue to grow and divide and remain healthy. If sub-culturing is not carried out, the algal cells in the stock culture will eventually die. It is important to take precautions to prevent contaminants from the air entering the stock cultures when sub-culturing.

To start a new stock culture, about 20 ml of algae are taken from a stock culture which has been growing for 6 to 7 days and poured into a flask containing 250 ml of fresh culture medium. After removing the cotton-wool bungs, and before and after pouring, the necks of both flasks should be passed through a gas flame, such as that from a Bunsen burner or spirit lamp, to kill any surface and airborne contaminants, such as bacteria, that might enter the culture. To grow, the new stock culture should be put about 20 cm from a fluorescent lamp that is lit continually. The air temperature around the culture should be less than 25°C. Note that stock cultures do not require an air/CO_2 supply.

After sub-culturing, the remainder of the old stock culture can be used to start a batch culture of up to 10 litres. This method is described more fully in Section 5. If the stock culture is not required immediately, it may be kept for up to 3 weeks on a shelf in a north-facing window (away from direct sunlight), but after this time it should be discarded.

Table 3(a). The preparation of Erdschreiber medium

Constituents			Quantities
(a)	Unfiltered sea water		2 litres
(b)	Soil (woodland or pasture — no insecticides or fertilisers)		1 kg
	(i)	Mix with freshwater	1 litre
	(ii)	Autoclave	60 min at 1.06 kg per square cm (15 psi)
	(iii)	Decant	—
	(iv)	Filter	Whatman no. 1 paper, *then* glass-fibre (GF/C) paper
	(v)	Store in deep freeze	
(c)	Nitrate/phosphate solution, obtained by dissolving:		
	(i)	Sodium nitrate ($NaNo_3$) *and*	40 g
	(ii)	Di-sodium hydrogen phosphate ($Na_2 HPO_4$) *in*	4 g
	(iii)	Distilled water	200 ml
(d)	Silicate stock solution, obtained by dissolving:		
	(i)	Sodium metasilicate ($Na_2SiO_3.5H_2O$) *in*	8 g
	(ii)	Distilled water	200 ml

Procedure	
Take unfiltered sea water	2 litres
plus	
Soil extract	100 ml
plus	
Nitrate/phosphate solution	2 ml
plus ***(for diatoms only)***	
Silicate solution	2 ml
Decant *into*	250 ml
8 clean flasks with cotton-wool plugs	500 ml (each)
Autoclave	35 min (as above)
Stand	for 2 days

Table 3(b). The preparation of a simple medium

Procedure	Quantities
Put sea water into glass or plastic container	2 l
plus	
Solution A (see Table 2)	3 ml
plus	
Solution C (see Table 2)	0.3 ml
plus ***(for diatoms only)***	
Solution D (see Table 2)	3 ml
Mix	—
Into each of 8 flasks	250 ml
either	
Heat	To boiling
or	
Autoclave	35 min at 1.06 kg per square cm (15 psi)
Cool*	Overnight

**Note: Some precipitation may occur, but it will gradually re-dissolve.*

4. SOME TYPES OF CULTURE

There are many different ways of culturing algae. These range from closely-controlled methods on the laboratory bench top, with a few litres of algae, to less predictable methods in outdoor tanks, containing thousands of litres, in which production relies on natural conditions. Several methods have been developed at Conwy, for the production of algae for use as food for various marine animals, and some of these methods are described in Section 5.

There are certain requirements for all methods. A culture must be inoculated, and the algae left to grow and divide. The rate of growth and division varies with different types of algae and also depends on how well the various culture conditions necessary for growth have been met. When there are sufficient algal cells in the container for feeding, one of the three culture methods below may be followed:

4.1 Batch culture

Batch culture is a system where the total culture is harvested and used as food. If required, another culture can be set up to replace it.

4.2 Semi-continuous culture

Semi-continuous culture is a system where part of the culture is harvested and used as food, and the amount taken is replaced with fresh culture medium (clean sea water and nutrient salts). After allowing 2-3 days for the remaining cells to grow and divide, the process is repeated. Semi-continuous cultures may be operated for up to 7 to 8 weeks.

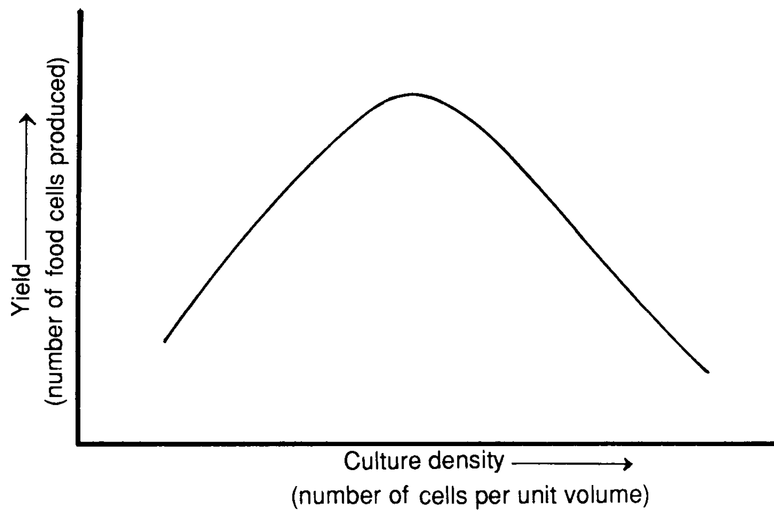

Figure 2. Variation in yield with the density of the algae culture

4.3 *Continuous culture*

This falls into two categories:

(i) *turbidostat culture*, in which the number of algal cells in the culture is monitored and, as the cells divide and grow, an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium; and

(ii) *chemostat culture*, in which a flow of fresh medium is introduced into the culture at a steady, pre-determined rate.

With both types, the surplus culture overflows into a collecting container, from which it can be taken and used as food.

With semi-continuous and continuous culture methods, the number of food cells produced (the yield) varies with the density of the culture as shown in Figure 2. For each type of algae, the greatest yield is obtained by maintaining the culture at an optimum density. This optimum density can be determined experimentally and is given for each of the culture systems described in the following section.

5. SOME CULTURE METHODS

5.1 Batch culture (small volumes of up to 10 litres)

Where small volumes of algae culture are required, for example, of 2 litres to 10 litres per day, production is most conveniently achieved in flasks (Figure 3). An example of a batch culture system for producing 3 litres of food per day from a culture of *Chaetoceros calcitrans* is given below and is described as daily procedures in Table 4.

A set of three, 250 ml stock cultures is started by inoculating from one existing 250 ml stock culture on each of 3 successive days. The new stock cultures are grown at a temperature of about 21°C, and at a distance of 15 to 20 cm from a 65 watt fluorescent tube. After 3 days, and then daily, each of these

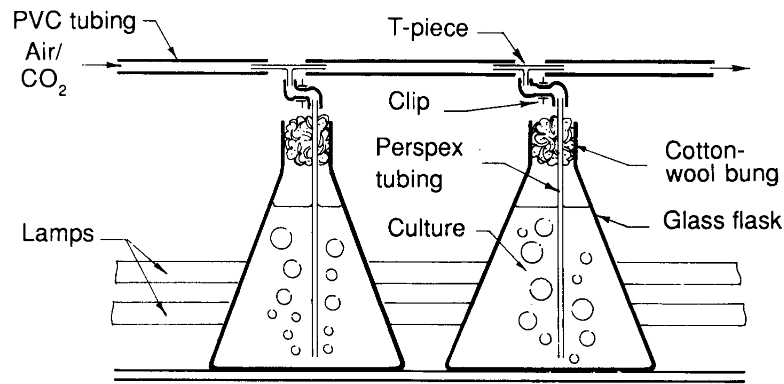

Figure 3. Flask culture

Table 4. Schedule for a batch culture system, producing Chaetoceros calcitrans at a rate of 3 litres per day

Day	Procedure	Inoculum
1	Inoculate new (250 ml) stock culture from existing culture	20 ml
	Grow in light	
2	As Day 1	20 ml
3	As Day 1	20 ml
4	Inoculate new (250 ml) stock culture from Day 1 culture	20 ml
	Remainder starts (3 litre) batch culture	250 ml
	Grow in light	
5	As Day 4, with Day 2 culture	20 ml + 250 ml
6	As Day 4, with Day 3 culture	20 ml + 250 ml
7	As Day 4, with Day 4 culture	20 ml + 250 ml
8	As Day 4, with Day 5 culture. Use 3 litre batch culture started on Day 4 for feeding	20 ml + 250 ml
9 (and onwards)	As Day 4, with stock culture started 3 days previously. Use 3 litre batch culture started 4 days previously for feeding	20 ml + 250 ml

stock cultures is used in turn to inoculate a new 250 ml stock culture and the remainder is added to the 3 litre sea-water culture medium in the flasks, which have been prepared as follows:

> Three-litre borosilicate glass flasks with cotton wool plugs are filled with sea water. The contents are either autoclaved at 1.06 kg per square centimetre (15 psi) for 20 minutes, boiled for 30-45 minutes, or pasteurised. Whichever method is used, the sea water in the flasks should be allowed to cool before adding nutrient salts. Alternatively, sea water that has been filtered through a half micron filter may be used. To the 3 litres of sea water in the flask,
> 6 ml of solution A, 0.6 ml of solution C and 6 ml of solution D (see Table 2) are added.

A fresh 3 litre culture is started daily from a 3-day-old stock culture and aerated with a mixture of air and CO_2 at about 2 to 3 litres per minute. The gas mixture is filtered through an in-line cartridge unit containing a 0.3-0.45 micron filter to reduce the risk of airborne contamination. When grown at about 21°C next to a continually lit, double fluorescent lamp unit, a density of
45 000 to 60 000 cells per microlitre is reached in 3 to 4 days in culture medium prepared from heat-treated sea water. In medium prepared from filtered sea water, growth of *Chaetoceros* is not as rapid and the density will only reach 20 000 to 30 000 cells per microlitre in this time. The culture should immediately be used for feeding, as if kept it will enter a declining phase, collapse, and become unsuitable.

Algae other than *Chaetoceros* usually take longer to grow under similar culture conditions. For these algae, stock cultures should be routinely sub-cultured every 6 to 7 days. The batch cultures, which are started from 6- or 7-day-old stock cultures, will take 7 to 8 days to grow to a density suitable for feeding. Larger containers, of up to 10 litres, may be used for this method of culture. Nutrient salt solutions (see Table 2) are used as follows:

> solution A — 1 ml per litre;
> solution C — 0.1 ml per litre; and
> solution D (diatoms only) — 2 ml per litre.

The batch cultures may be used directly as food or as inocula to start larger volume batch, semi-continuous or continuous food cultures.

5.2 Semi-continuous culture

5.2.1 Two-hundred-litre vessels

A method for large-scale production (mass culture) in 200 litre, internally illuminated, glass reinforced plastic (grp) vessels (Figure 4), using semi-continuous culture is described below.

The vessels are 150 cm high, 40-45 cm in diameter and each has a central lighting unit into which are fitted six fluorescent lamps. A glass-fibre cooling pipe is moulded onto the outer jacket (Appendix 2). These vessels are most useful for growing diatoms, but they may also be used for flagellates. The vessels are sterilised by filling with a solution of sodium hypochlorite (50 parts per million (ppm) free-chlorine concentration). Note that domestic bleach contains about 100 000 to 150 000 ppm chlorine, so a dilution of 1 ml per 2-3 litres would give the required concentration. They are allowed to stand for 2-4 hours and then drained, and flushed with filtered air for 24 hours to drive off residual chlorine.

The vessels are filled (200 litres come to about 15 cm from the top) with filtered sea water at
20 psu to 25 psu salinity for diatom cultures or 25 psu to 30 psu for flagellate cultures. For diatom cultures, filtration to 2 microns is usually sufficient, while for culture of flagellates, filtration to half a

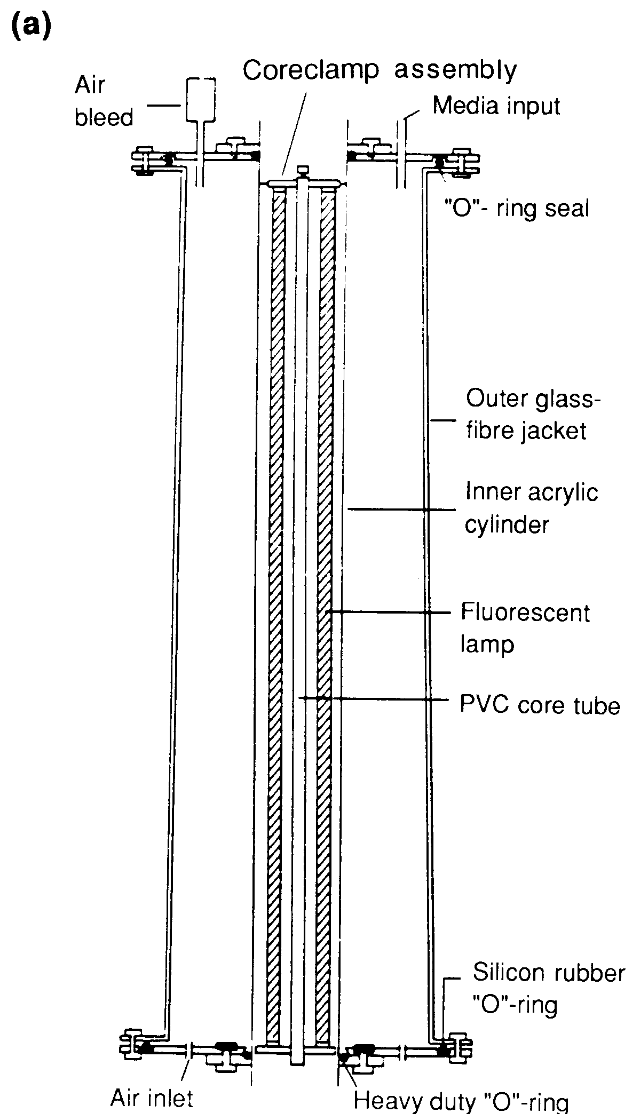

Figure 4. Two-hundred litre, internally-illuminated, glass-reinforced plastic culture vessels: (a) longitudinal section; and (b) photograph of the system (for dimensions, see text)

micron is preferable. Two-hundred millilitres of solution A, 20 ml of solution C and, for diatom cultures, 1 200 ml of solution D (see Table 2) are added to the vessels. The culture is inoculated with 2 to 5 litres of a 4- to 8-day-old batch culture, grown as described in the previous section and aerated with a filtered air/CO_2 supply at about 15 litres per minute.

Cultures should reach densities suitable for harvesting after 4 to 7 days. Those suitable for various types of algae are given in Table 5, which also gives the densities to which the cultures should subsequently be diluted for the maximum yield. The amount of harvest which achieves this yield can be calculated from the following equation:

$$\text{volume to harvest in litres} = 200 - \frac{200 \times \text{density to which culture needs to be diluted}}{\text{actual culture density of algae harvested}}$$

After harvesting, the vessels are topped-up to 200 litres with filtered sea water of the correct salinity. For each litre harvested, 1 ml of solution A, 0.1 ml of solution C and, for diatom cultures, 6 ml of solution D (see Table 2) are added. It is usually more convenient to harvest the culture every 2 to 3 days (e.g. Mondays, Wednesdays and Fridays). That part of the harvest which is required for feeding on the intermediate days can be aerated in a plastic container away from bright light and in a cool place.

Table 5. Semi-continuous culture methods for various types of algae in 200 litre, internally-illuminated vessels

Algae	Culture density (cells per microlitre)			Usual life of culture (weeks)
	Suitable for feeding	To which culture should be diluted for next harvest		
		in 2 days	in 3 days	
Diatoms				
Skeletonema costatum *Thalassiosira pseudonana*	6000	1250	625	6-7
Phaeodactylum tricornutum	10000	1400	700	7-8
Chaetoceros calcitrans	15000-20000	4800	2400	2-3
Flagellates				
Tetraselmis suecica	1000	160	80	7-8
Chroomonas salina	2000	240	120	2-3
Dunaliella tertiolecta	3000	350	175	4-5
Isochrysis galbana *Monochrysis lutheri* *Pseudoisochrysis paradoxa*	10000	1440	720	2-3

The length of time during which the culture is able to produce food will vary with the type of algae, as shown in Table 5. Production of algae from 200 litre vessels should average the equivalent of 60-80 litres per day at the cell densities given in Table 5. When the culture is no longer required, or it has come to the end of its production period, the vessel can be drained and cleaned with a stiff brush, to remove any algae adhering to the sides. The vessel can now be sterilised, as described above, in preparation for a new culture.

5.2.2 Sixty-litre polyethylene bags

A further large-scale production method utilises 25 cm wide polyethylene tubing made into bags and hung from a framework (Figure 5; see also Appendix 2). In the figure, the bag is shown folded in half, but it could also be cut to form two 30 litre bags, with the bottom of each heat-sealed across the end. This simple method for producing either diatoms or flagellates is suitable for use indoors with fluorescent lamps, and outdoors with natural daylight. The heat used in the manufacture of the bags ensures that they are sterile when supplied.

When setting up a system, a small hole is made in the top of each half of the bag and filtered sea water, at 20 psu to 25 psu salinity for diatom culture and 25 psu to 30 psu for flagellates, is pumped-in until the bag is almost filled. To each half, 30 ml of solution A, 3 ml of solution C and (for diatoms) 150 ml of solution D are added (see Table 2), and then about 2 litres of a batch culture that has grown for 4-8 days. An air line of 7 mm diameter Perspex tubing is fitted into each half of the bag and aerated vigorously. The culture is allowed to grow.

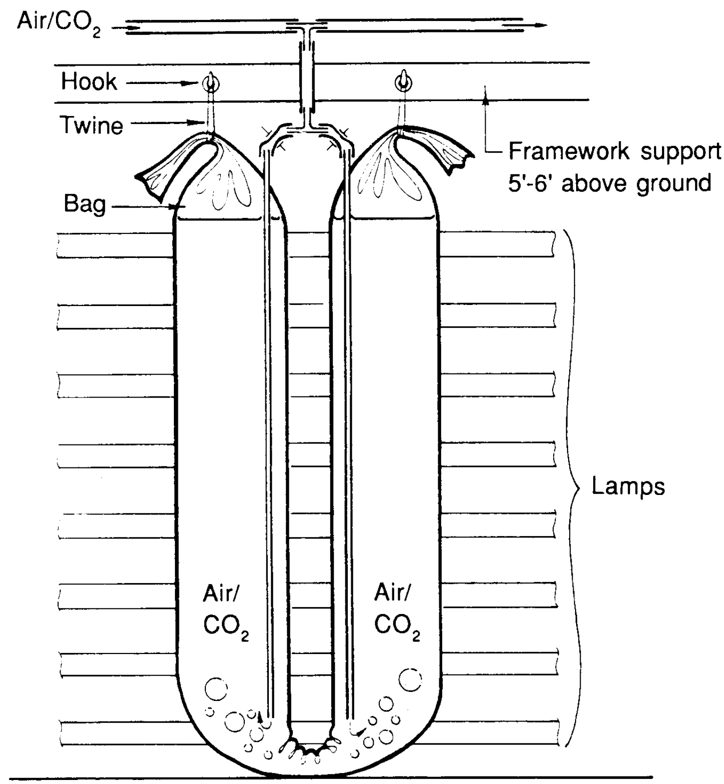

Figure 5. Sixty-litre polyethylene bag culture

From an indoor bag system, continually illuminated diatom cultures may be harvested after 2-3 days, and flagellates after 4-5 days. Harvests can then be taken three times per week (e.g. on Mondays, Wednesdays and Fridays) by either pumping or siphoning-out the algae cultures, removing 50 litres from diatom cultures and 30 litres from flagellate cultures. (For siphoning, the framework must be raised or a sunken area provided to give a sufficient head of water.) That part of the harvested culture required for feeding on the intermediate days (Tuesdays, Thursdays, Saturdays and Sundays) can be aerated in a plastic container away from bright light.

The culture in the bag is topped-up with filtered sea water at the correct salinity, added to both sides of the bag to ensure that the culture remains evenly mixed. For flagellates, 15 ml of solution A and 1.5 ml of solution C or (for diatoms) 25 ml of solution A, 2.5 ml of solution C and 125 ml of solution D, should be added to each side of the bag (see Table 2). After harvesting for three weeks, production from the culture becomes unreliable, so it is wise to use all of the culture for feeding and discard the bag. A fresh culture can now be started in a new bag.

To ensure a continual supply of algae, one-third of the required number of culture bags should be started at a time in rotation (i.e. for every three bags of a particular species of algae, start one the first week, another the second week and the third the following week).

Production in outdoor bag cultures is very variable, depending upon the amount of light available and also on the temperature. Direct sunlight should be avoided, as this causes the cultures to become too hot and to collapse. The framework for the bags should therefore face north or (if impracticable) suitable shading should be provided. As the rate of growth will be variable, an estimate of culture density, using an haemocytometer, will be required (see Appendix 1). Cell densities suitable for harvesting and feeding are given in the first column of Table 5.
When these densities have been reached or exceeded, the above procedures should be followed for harvesting and topping-up the bags with fresh medium. After about ten semi-continuous harvests, the culture and bag should be replaced.

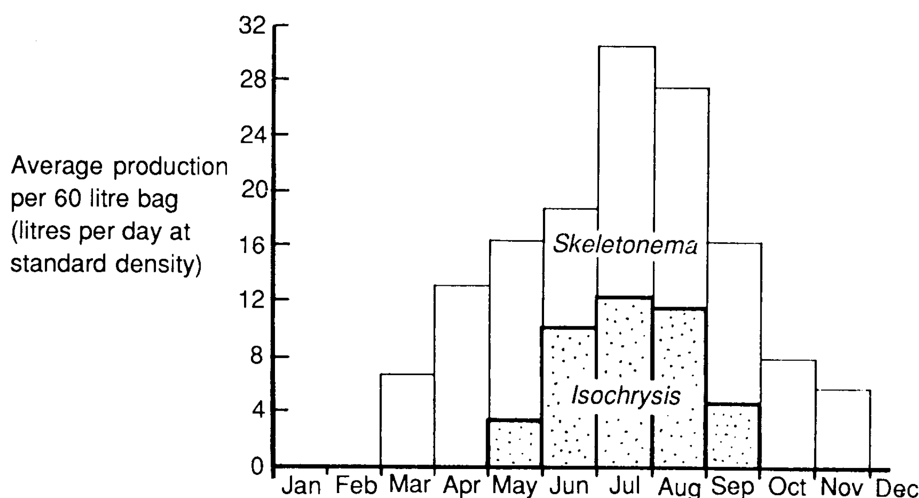

Figure 6. Monthly production of Skeletonema (a diatom) and Isochrysis (a flagellate) from 60 litre polyethylene bag cultures grown outdoors and supplied with CO_2. Production adjusted to standard density for comparison: Skeletonema — litres at 6 000 cells per microlitre; and Isochrysis — litres at 10 000 cells per microlitre

Figure 6 shows the production from outdoor cultures of *Skeletonema* (a diatom) and *Isochrysis* (a flagellate) throughout the year. At Conwy, only in July and August does an outdoor unit give algae production rates as great as those achieved indoors throughout the year.

Bags made from tubing wider than 25 cm, and thus holding greater volumes of culture, do not support greater productivity than the 25 cm bags, because the cell densities reached at harvesting are correspondingly lower due to self-shading of the cells. As costs increase with wider bags, it is more economic to use 25 cm tubing.

The addition of CO_2 is important, as it increases yields five-fold in indoor cultures and by two and a half times in outdoor cultures. Outdoor cultures are less affected, as the CO_2 produced in cell respiration is not used during the night, allowing the natural CO_2 levels to build-up slightly.

5.3 Continuous culture (40 litre vessels)

This method is suitable for the culture of flagellates. The internally-illuminated, continuous culture vessels are made from polyethylene tubing supported by a metal framework (Figures 7 and 8; see also Appendix 2). They consist of 160 cm lengths cut from 71 cm wide polyethylene, 'layflat' tubing. The tubing is free of potential contaminants due to the heat used in the manufacturing process and no further sterilisation is necessary. The cut length is heat-sealed across the width of one end and positioned around the acrylic cylinder containing the lamps. The six nuts and bolts securing the outer supporting mesh jacket are fastened and the outer, reflective, sheet of white, corrugated plastic is held in place by 12.7 mm nylon power belting (nylon strapping), which also supports the sensor housing unit against the outer surface of the culture.

The polyethylene tubing is filled (38 litres) with sea water at 25 psu to 30 psu salinity that has been filtered through a sterile, 0.45 micron, particle retention cartridge filter. If the water has a high silt load, it should first be passed through a 2 micron filter. Solution A (100 ml) and solution C (10 ml) (see Table 2) are added to the sea water in the vessel. This is 2.5 times the usual amount, and is added to ensure that nutrient levels do not become limiting at the high cell

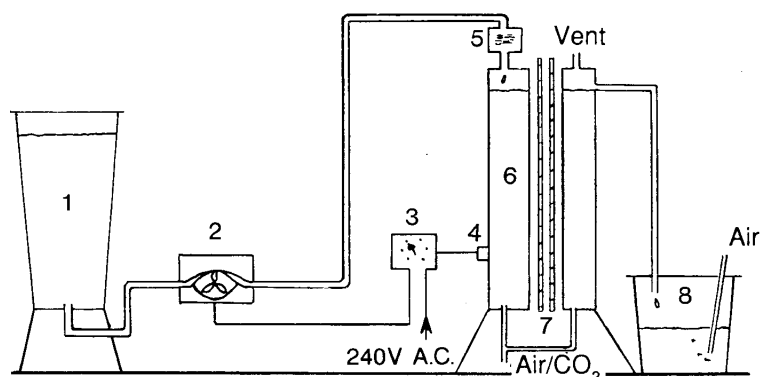

Figure 7. Diagram of continuous culture apparatus (not drawn to scale): (1) sea-water medium reservoir (200 litres); (2) peristaltic pump; (3) resistance sensing relay (50-5 000 ohm); (4) light-dependent resistor (ORP 12); (5) cartridge filter (0.45 micron); (6) culture vessel (40 litres); (7) six 80 W fluorescent lamps; and (8) collecting reservoir (125 litres)

densities at which the cultures are maintained. A 2.5 cm diameter circle is cut from the tubing, with its centre about 7 cm above the water level. Into this is fitted a 1.9 cm rigid, PVC tank connector, from which a 150 cm length of 1.5 cm bore flexible PVC tubing is run into a 125 litre collecting vessel. The overflow allows for automatic harvesting of the culture into the reservoir.

A supply of filtered air, enriched with sufficient carbon dioxide to maintain culture pH at 7.6-7.8 (about 0.25% CO_2 by volume) is introduced through a 0.4 cm bore, 150 cm long, acrylic tube inserted into the top of the culture. A flow rate of about 15 litres per minute ensures efficient mixing of the culture.

Cooling water, at a flow rate of about 0.35 litre per minute, is allowed to run down over the outer culture surface in order to maintain the culture temperature at about 21°C.

The 40 litre culture should be inoculated with a 2 litre batch culture that has gown for 7 to 8 days.

Automatic harvesting of the culture is controlled by the following method. A cadmium sulphide photo-conductive cell is enclosed in a light-proof housing against the outer surface of the culture. The housing is placed about 50 cm from the base of the vessel and positioned so that the stream of air bubbles rising through the culture does not interfere with its operation. The resistance of the photo-conductive cell will increase as the light intensity reaching it from the lamps falls, when density of the culture increases, due to growth and division of the algal cells. A circuit switching the peristaltic pump will be energised when the resistance of the cell becomes greater than a present value on a relay sensitive to input resistance in the range 50-5 000 ohm. The 11-pin relay, connected to the light-dependent resistor and peristaltic pump, is shown in Figure 9. Sea water, at 25 psu to 30 psu and enriched with 2.5 ml of solution A per litre and 0.25 ml of solution C per litre, is then pumped from the culture medium reservoir through the filter into the vessel and the volume is maintained by an overflow. The outflow of algae culture from the vessel is collected in an aerated container. As the culture is diluted, the decrease in resistance of the photo-conductive cell, caused by the higher light intensity now reaching it, is sensed by the relay and the pump circuit is switched off.

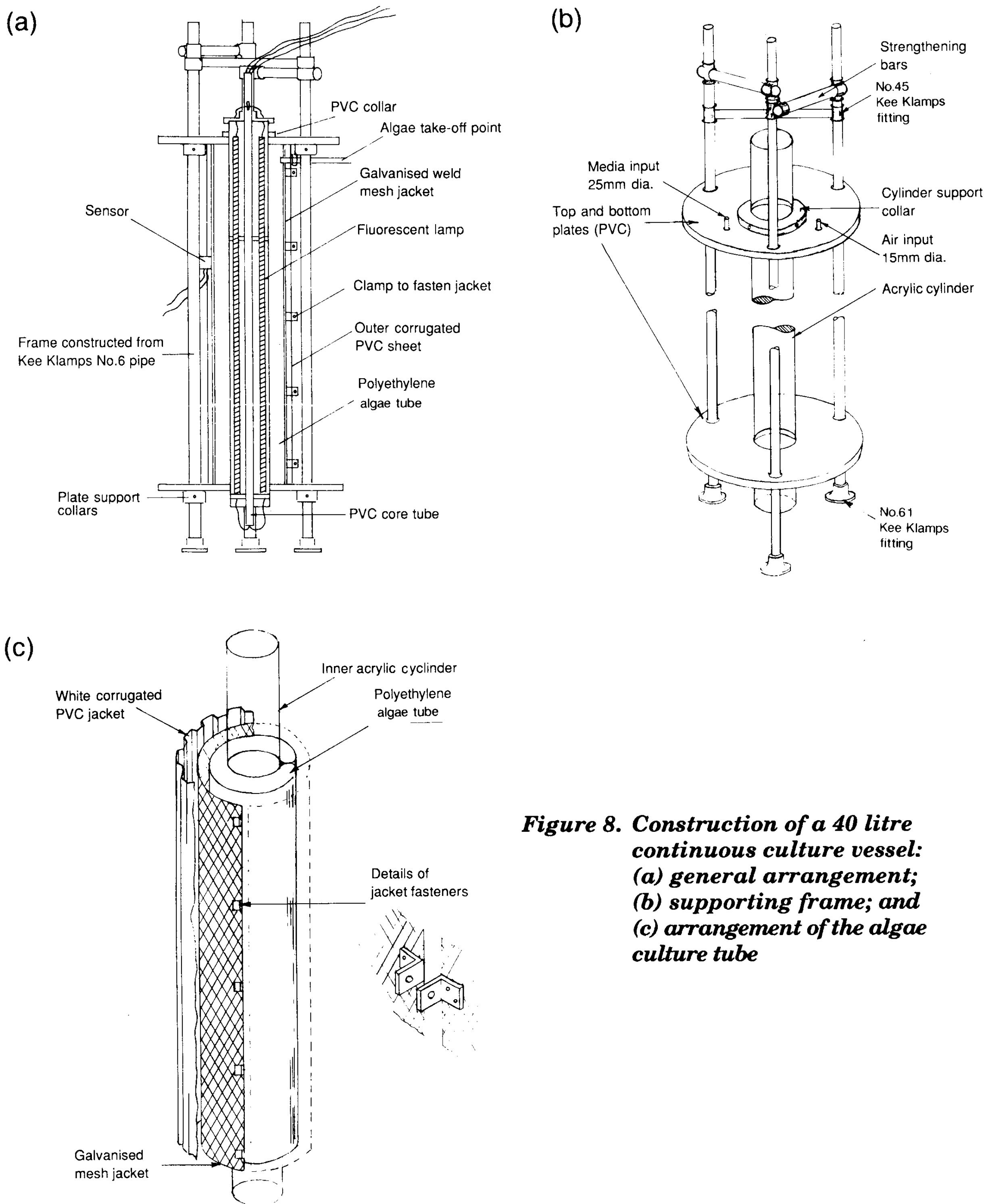

Figure 8. Construction of a 40 litre continuous culture vessel: (a) general arrangement; (b) supporting frame; and (c) arrangement of the algae culture tube

The relay should be set so that automatic harvesting of the culture occurs at the density that gives the most yield. The optimum densities for some types of commonly grown algae are given in Table 6, together with the expected lives of the cultures. Production of 30-40 litres per day, at the cell densities given in Table 6, can be expected from these 40 litre vessels. When the yield begins to fall appreciably, all of the culture should be harvested for feeding and the bag discarded. A new, clean bag should be fitted to the vessel and the above operating procedure repeated.

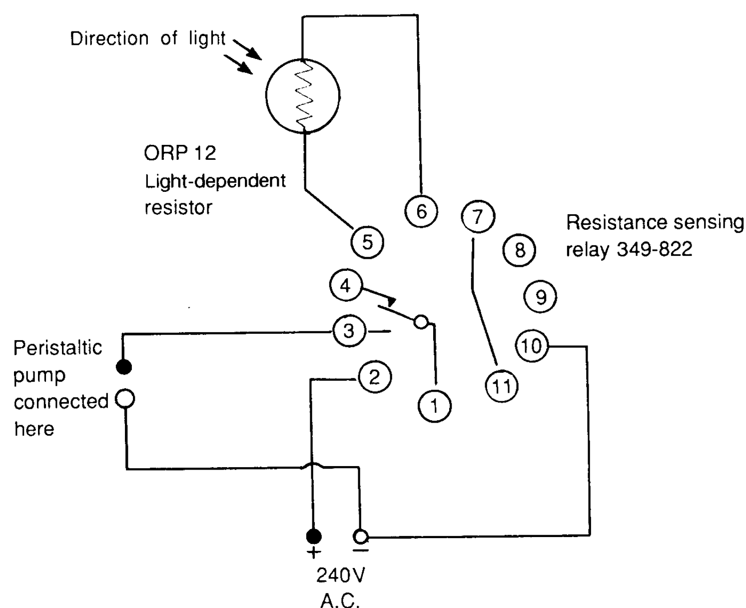

Figure 9. Circuit diagram for resistance sensing relay (349-822, RS Components, London), light-dependent resistor (ORP 12, RS Components) and peristaltic pump

Table 6. Continuous culture methods for various types of algae in 40 litre, internally-illuminated vessels (suitable only for flagellates)

Algae	Culture density for most yield (cells per microlitre)	Usual life of culture (weeks)
Tetraselmis suecica	2000	3-6
Chroomonas salina	3000	2-3
Dunaliella tertiolecta	4000	3-4
Isochrysis galbana		
Monochrysis lutheri	20000	2-3
Pseudoisochrysis paradoxa		

6. OTHER METHODS

The various culture methods described in detail above have all been developed and tested at the Fisheries Laboratory, Conwy. The most suitable method for any application will depend on the resources and facilities available and the amount of algae required.

There are other, similar, methods for growing marine algae that may be more appropriate in certain circumstances. For example, externally-illuminated 20 litre glass jars can be operated as batch cultures or semi-continuous cultures for a variety of algae. Another, widely used, type of culture container includes a mesh framework to support a 200-400 litre externally-illuminated polyethylene bag. Both of these methods have the disadvantage of being less efficient, as the light energy cannot penetrate the culture as well as the internally-illuminated unit, but they do have the advantage of being easier and cheaper to construct. Polyethylene bag cultures may also be operated as continuous, chemostat, cultures by using small pumps to introduce fresh filtered medium to the culture at a pre-determined rate.

Extensive methods may be employed to culture marine algae. Outdoor tanks are nutrient-enriched with agricultural fertilisers. Adding (per 1 000 litres of sea water) 1.5 g of urea ($NH_2CON H_2$:46% nitrogen), 1.6 g of triple superphosphate (P_2O_5:19.9% phosphorous) and
10.6 g of sodium metasilicate ($Na_2SiO_3.5H_2O$:13% silica) will provide the required amounts of nitrogen, phosphorous and silica to stimulate growth and division of algal cells and, depending on the temperature of the sea water and the amount of sunshine on the tanks, blooms of algae will develop. This system can be operated as a batch culture or managed as a semi-continuous or continuous culture with an inflow of nutrient-enriched sea water to provide fresh medium as an impetus to further algal growth. The method is especially suitable for providing algae for feeding to bivalve mollusc spat held in nursery systems, or for mass 'grow-out' of brine shrimps (*Artemia*).

7. CONCLUSIONS

Whatever method of large-scale algae culture is adopted, it is liable to be expensive and technically difficult to operate. For this reason, a great deal of research is now being directed towards a suitable algae replacement diet for use in commercial aquaculture. Successful development of such a diet will perhaps eventually remove the need for large-scale production of algae for food.

Technical details follow on estimation of algae culture (Appendix 1), vessel construction (Appendix 2), equipment (Appendix 3), and terminology (Appendix 4), together with a short list of review articles (Appendix 5).

APPENDIX 1 How to estimate the density of an algae culture using an haemocytometer

1. Put a sample of about 20 ml of algae culture into a small pot.

2. For flagellate culture, add one or two drops of a 4% formalin solution, to kill the algae (4% formalin = 100 ml of 40% formaldehyde solution per litre of water.)

3. Press the coverslip down over the counting area on the slide.

4. Mix the sample thoroughly, with a Pasteur pipette.

5. Introduce a drop from the Pasteur pipette into the chamber at the edge of the coverslip. Do not force the sample in — allow it to run in by capillary action.

6. For small cells, allow one or two minutes to settle-out onto the bottom of the counting chamber.

7. Clear the Pasteur pipette by rinsing in fresh water.

8. View the slide under a microscope, using x100 or x400 magnification, depending on the size and number of cells.

9. Count the cells. For each individual square marked on the slide, count all of the cells lying within it or overlapping the lines on the right-hand or bottom sides. Count the cells in a known number of squares (e.g. 9 large squares or 40 small squares). This corresponds to a known volume (see instructions on slide or supplied with it). Repeat until a total of at least 200 cells, and preferably 400 cells, have been counted. Take an average count to calculate density of culture (number of cells in a known volume).

10. Remove the coverslip. Wipe the coverslip and counting chamber on slide with a clean tissue. Replace the coverslip (step 3) and repeat steps 4-10 for the next sample.

APPENDIX 2 Vessel construction

A2.1 Construction of 200 litre vessels (see text Figure 4)

The outer jacket of the vessel is constructed of white, pigmented glass-fibre. It is 150 cm high and is 45 cm in diameter at the base and 40 cm in diameter at the top. (The slight taper is merely to aid removal from the mould when making the cylinder.) 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. (Metal bolts may corrode and/or be toxic to the algae.)

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

Illumination is provided by six 80 W, 150 cm long, daylight, fluorescent tubes mounted in the transparent, extruded acrylic cylinder, which projects beyond both the top and bottom plates to prevent water from splashing the lamp holders.

The lamps are mounted around a hollow core, 19 mm diameter bore, polyvinyl chloride pipe, through which electrical cables are fed to the bottom lamp holders. The core tube is fitted with three plates, the bottom plate being made from 6 mm thick, polyvinyl chloride sheet with six lamp holders equally spaced on a 90 mm pitch circle diameter. This plate slides freely on the core tube and can be locked into position by an OBA screw. The centre plate is made from
6 mm-thick aluminium plate, drilled to support and position the fluorescent tubes; this plate is positioned two-thirds of the way up the core tube and is secured by an OBA screw.

The complete light assembly is suspended from the top plate which is made from a 12 mm-thick, 19 cm diameter polyvinyl chloride disc. The disc has three sections removed, to allow the heat to dissipate from the light tubes and to allow access for the electrical cables connected to the top lamp holders. A central boss, 40 mm in diameter by 40 mm long, is attached to the disc and has a 3 mm deep, circular groove, 15 cm in diameter machined for it to locate on the end of the acrylic cylinder. The top plate slides freely on the core tube and may be clamped by an OBA screw to suspend the light unit at the correct position within the acrylic cylinder.

The top and base plates of the vessel itself are 60 cm in 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 joints 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 litres per minute. 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, which is packed with cotton-wool and 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.

New vessels are thoroughly cleaned with a solution of hot detergent. 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.

A2.2 Construction of 60 litre polyethylene bags (see text Figure 5)

Each polyethylene bag is made from 3.4-3.7 m length material cut from a roll of 25 cm wide, extra heavy gauge, clear, 'layflat' tubing. This is folded in half and secured at both ends to hang from a suitable supporting framework. When full of algae culture, each bag weighs over 60 kg, so a sturdy framework is needed. For an indoor culture system, 8 horizontal, 240 cm, 125 W, fluorescent lamps are arranged vertically in the framework. Up to 10-12 bags may be hung close to these lamps — 5-6 on each side. In an outdoor system, the number of bags will be determined according to the size and strength of the framework.

A2.3 Construction of 40 litre vessels (see text Figure 8)

Text Figure 8 shows the arrangement of the vessel. For durability and ease of construction, the framework for supporting the polyethylene algae tube is made from galvanised 'Kee Klamp' fittings, but other forms of scaffold products would be equally suitable. The framework consists of three 2.5 m lengths of 33.7 mm diameter, no. 6 'Kee Klamp' pipe, with feet (no. 61 fittings) located on the end of each upright. Six collars (no. 75 fittings) are also attached to support the top and bottom plates. The assembled frame is strengthened with six 90° connectors (no. 45 fittings) constructed in the manner shown in text Figure 8(b). The top and bottom plates are
46 cm in diameter and made from 12 mm-thick, white, 'Darvic', rigid, polyvinyl chloride sheet. Both plates have the centres removed to accommodate the 15 cm diameter, 165 cm long, transparent acrylic cylinder, which in turn is suspended through the top and bottom plate and supported by a 12 mm-thick, polyvinyl chloride collar attached to the acrylic cylinder by means of three equally-spaced OBA screws.

Three equally-spaced holes (35 mm in diameter) are drilled on a 37 cm pitch circle diameter, to allow the plates to slide freely on the upright pipes. This allows for adjusting the final position of the top and bottom plates with the six no. 75 fittings, three locked under each plate, which support the weight of the assembled vessel. The top plate is also provided with a 25 mm diameter medium input pipe and a 15 mm diameter air inlet pipe.

The polyethylene algae bag is made from a 160 cm length of 71 cm wide heavy or extra-heavy gauge, clear, 'layflat' tubing heat-sealed across the bottom. It is placed around the acrylic cylinder as shown in text Figure 8(c). An outer supporting jacket of 150 cm x 90 cm, made from galvanised weld mesh is placed around the algae bag, and fastened by five clamps along its length. The light sensor housing and a 150 cm x 90 cm sheet of reflective material (white corrugated PVC) is held in position against the mesh by 12.7 mm power belting.

Illumination is provided by six 80 W, 150 cm length, daylight, fluorescent tubes mounted in a transparent, extruded acrylic cylinder, which projects beyond both the top and bottom plates to prevent water from splashing the lamp holders.

The complete light assembly is constructed and fitted as described for the 200 litre, internally-illuminated, glass-reinforced plastic vessels.

APPENDIX 3 Lists of equipment

A3.1 Basic equipment (stock cultures and flask cultures)

Conical or flat-bottomed boiling flasks (500 ml for stock cultures and small volumes of nutrient solutions).
Non-absorbent cotton wool.
Chemicals (see Text Table 2).
Balance for weighing from 0.1 g to 500 g.
Stainless-steel spatula (or old spoon, for weighing chemicals).
Weighing container (e.g. 500 ml beaker).
Glass measuring cylinders (100 ml, 250 ml, 2 litres).
Glass flasks (3 litres to 10 litres, for keeping stock solutions and for algae culture).
Gas burner (e.g. Bunsen, or spirit lamp).

Air blower or compressor and attendant plumbing.
CO_2 gas cylinders and regulator valves.
'In-line' air filter holders fitted with 0.3 micron disposable cartridges.
Flow meters (CO_2: to 700 ml per minute; air: to 100 litres per minute).
Flexible air-line tubing (PVC, 6 mm bore).
Glass or Perspex tubing (4 mm bore, for air-lines in cultures).
Plastic 'T' pieces.
Tubing clips.

Fluorescent lamps — integral units or wired, with electrical cable, via bi-pin lamp holders to ballasts.

Microscope (x100 and x400 magnification).
Haemocytometer, and coverslips to fit.
Pasteur pipette, with teats.
Tissues, notebook and pen.

Autoclave/pressure cooker or sea-water pump, filters, reservoir and attendant plumbing.

A3.2 Additional equipment (Erdschreiber preparation)

Buchner apparatus (10 litre flask and funnel to fit).
Filter pump, to fit on cold-water tap.
Coarse filter papers (e.g. Whatman no. 1 (23 cm diameter)).
Fine filter papers (e.g. Whatman GF/C (possibly cut to size from large sheets)).
Polypropylene bottles (1 litre, to autoclave and store soil extract).

A3.3 Additional equipment (for larger volume cultures)

Filtered air/CO_2 supply and fluorescent lamps (see A3.1).
Sea-water pumps, filters, reservoir and plumbing.
Plumbing for freshwater cooling supply.
Brushes for cleaning.
Polypropylene containers/reservoirs (10 litres/125 litres) for holding harvested culture.

For 60 litre vessels only

Extra-heavy-gauge, clear, 'layflat' tubing (25 cm wide).
Timber and screws for framework.
Twine and hooks.

For 40 litre and 200 litre vessels

Transparent acrylic cylinder (15 cm in diameter).
PVC pipe (19 mm and 25 mm bore).
PVC sheet (6 mm and 12 mm thick).
Aluminium plate (6 mm thick).
OBA screws.

For 40 litre vessels only

'Kee Klamp' pipe (33.7 cm in diameter) and fittings (nos. 45, 61, 75).
Extra-heavy-gauge, clear, 'layflat' tubing (71 cm wide).
Heat sealer.
Galvanised weld mesh sheet (150 cm x 90 cm) with clamps, nuts and bolts.
White PVC sheet (150 cm x 90 cm).
PVC tank connector (19 mm bore).
Flexible PVC tubing (15 mm bore).
Perspex tubing (4 mm bore).
ORP 12 light-dependent resistor, and housing.
Resistance sensing relay.
Peristaltic pump, fitted with silicon rubber tubing.
Power belting (12.7 mm wide) and clips.

For 200 litre vessels only

White-pigmented, glass-reinforced plastic, and mould.
Nylon nuts and bolts (12 mm in diameter).
Silicon rubber 'O'-rings (7 mm thick, 15 cm and 56 cm in diameter).
PVC ball valve (19 mm bore).
Flexible PVC pipe (19 mm bore) and hose adaptors to fit.
Rigid PVC pipe (5 mm bore).
Mild-steel frame to support vessel.

APPENDIX 4 Some terms explained

Density:	When applied to a culture, density means the number of algal cells in a given volume of culture, which is often expressed as cells per microlitre.
Microlitre:	One millionth of a litre in volume (= 1 µl).
Micron:	One millionth of a metre in length (= 1 µm).
Pasteurisation:	Is a process for heating a solution (e.g. sea water) to the temperature at which it almost boils, then allowing it to cool. This process can be repeated. The effect is to sterilise the culture without altering it chemically, as in the case of boiling. This method is not often used for large volumes, due to the cost of heating and the large reservoir capacity needed for the process.
pH:	Works on a scale of 0 to 14, to describe increasing acidity (from pH 7 to 0) or increasing alkalinity (from pH 7 to 14) of a solution. pH 7 is neutral (i.e. neither acid nor alkaline).
Precipitation:	Occurs when some of the salts that are dissolved in a solution settle out, forming a cloudy appearance on the bottom of the container.
Salinity:	Is the amount of salt in sea water, usually measured as practical salinity units (UNESCO, 1981)*.
Yield:	Is the total number of algal cells produced for feeding.

UNESCO, 1981. The practical salinity scale 1978 and the international equation of state of sea water 1989. Tenth Report of the Joint Panel on Oceanographic Tables and Standards. UNESCO, Paris, Tech. Pap. in Mar. Sci., (36**) Annex 1: 13-21.*

APPENDIX 5 Review articles on the large-scale production (mass culture) of algae

BECKER, E. W. (ED.), 1985. 'Production and Uses of Microalgae'. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, FRG, 189 pp.

DE LA NOVE, J. AND DE PAUW, N., 1988. The potential of microalgal biotechnology: a review of production and uses of microalgae. Biotechnol. Adv., **6**: 725-770.

LAING, I. AND AYALA, F., 1990. Commercial mass culture techniques for producing microalgae. pp.447-477. In: I. Akatsuka (Ed.), 'Introduction to Applied Phycology'. SPB Academic Publishing bv, The Hague, The Netherlands.

RICHMOND, A. (ED.), 1986. 'CRC Handbook of Microalgal Mass Culture'. CRC Press, Boca Raton, USA, 536 pp.

SHELEF, G. AND SOEDER, C. J. (EDS.), 1980. 'Algae Biomass: Production and Use'. Elsevier/North Holland Biomedical Press, Amsterdam, 312 pp.

SOEDER, C. J., 1980. Massive cultivation of microalgae: results and prospects. Hydrobiologia, **72**: 197-209.

SOEDER, C. J. AND BONSACK, R. (EDS.), 1978. Microalgae for food and feed. Ergebn. Limnol., **11**: 1-300.

STEIN, J. R. (ED.), 1973. 'Handbook of Phycological Methods, Culture Methods and Growth Measurements'. University Press, Cambridge, UK, 448 pp.

Recent Laboratory Leaflets

No. 50	Mussel cultivation in England and Wales. 1980.
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No. 52	A review of development of the Solent oyster fishery, 1972-80. 1981.
No. 53	Prospects for fuller utilisation of UK fish meal capacity, 1981.
No. 54	Background to scientific advice on fisheries management. 1982.
No. 55	Rockall and its fishery. 1982.
No. 56	Scad in the North-east Atlantic. 1983.
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No. 59	The bass (*Dicentrarchus labrax*) and management of its fishery in England and Wales. 1987.
No. 60	The scientific essentials of fisheries management and regulations. 1987.
No. 61	The North Sea cod and the English fishery. 1988.
No. 62	Crayfish culture. 1990.
No. 63	Cultivation of Pacific oysters. 1990.
No.64	Stability and the objectives of fisheries management: the scientific background. 1991.
No.65	Cultivation of Manila clams. 1991.
No.66	Storage and care of live lobsters. 1991.