ALGAL MASS-CULTURE UNIT FOR FEEDING MARINE LARVAE

DIVISION OF FISHERIES AND OCEANOGRAPHY TECHNICAL PAPER NO. 12

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION, AUSTRALIA 1961

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By B. Wisely and C. Purday

Division of Fisheries and Oceanography
Technical Paper No. 12

Commonwealth Scientific and Industrial
Research Organization, Australia
Melbourne 1961

AN ALGAL MASS-CULTURE UNIT FOR FEEDING MARINE INVERTEBRATE LARVAE

By B. WISELY* and C. PURDAY*

[*Manuscript received February* 10, 1961]

Summary

A unit is described which produces unialgal but not bacteria-free continuous or batch (400 l.) cultures. In three experiments at 19–20°C the flagellate *Isochrysis galbana* Parke (Chrysophyceae) reached maximum densities of 2·0, 0·8, and $2·2 \times 10^6$ cells/ml in 37, 39, and 30 days of culture respectively. In three experiments at 19–20°C the flagellate *Dunaliella tertiolecta* Butcher (Chlorophyceae) gave densities of 1·7, 1·9, and $2·2 \times 10^5$ cells/ml in 13, 63, and 27 days. In five experiments at various temperatures the diatom *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae) gave densities of 1·5, 2·3, 3·2, 3·2, and $6·0 \times 10^6$ cells/ml in 21 (27°C), 11 (27°C), 22 (23°C), 22 (22°C), and 26 (17°C) days. In all experiments densities suitable for feeding larvae were obtained within 10 days.

I. INTRODUCTION

The unit was designed to provide large amounts (up to 400 l.) of several genera of algae of a size suitable (diameter $<7\ \mu$) for rearing marine invertebrate larvae. Pasteurization facilities were included to prevent zooplankton contamination (Loosanoff, Hanks, and Ganaros 1957), and to maintain unialgal cultures of the food species preferred by certain larvae (Loosanoff and Davis 1950; Loosanoff, Davis, and Chanley 1954; Walne 1956; Moyse 1960). Bacteria-free cultures were not attempted because it was considered that the number of bacteria present would not prove excessive for rearing larvae.

Essentially, the unit (Plate 1 and Fig. 1) consists of four fuel drums which can be filled with sea-water, pasteurized, enriched, and aerated. After inoculation, fluorescent tubes are inserted in the lower pair of drums. When a suitable density is reached most of the culture is pumped to an elevated reservoir for subsequent use; the remainder serves as an inoculum for fresh medium siphoned from the upper drums. This allows continuous or batch culture of one or two species of algae over long periods.

II. DRUM UNITS

The drums (Fig. 2; Plates 1 and 2) are 44-gal, galvanized, open-head, fuel drums coated internally with a $\frac{1}{8}$ in. layer of white fiberglass (Fiberglass A/asia, Sydney) and externally with a polyurethane-type paint ("Forminex", Forminex Pty. Ltd., Sydney). The fiberglass provides a non-corrosive, non-toxic reflecting surface and both it and the "Forminex" have withstood the 70°C temperature used periodically for pasteurization during a year's testing. The lids are two circular

* Division of Fisheries and Oceanography, C.S.I.R.O., Cronulla, N.S.W.

pieces of $\frac{1}{2}$ in. waterproof plywood screwed together, with the lower piece fitting inside the drum rim. The space between the drum and lid rims is filled by a gasket made from a length of 16 mm O.D. soft red rubber tubing split along its length and rolled onto the drum rim. It seals when the lid is screwed down by means of

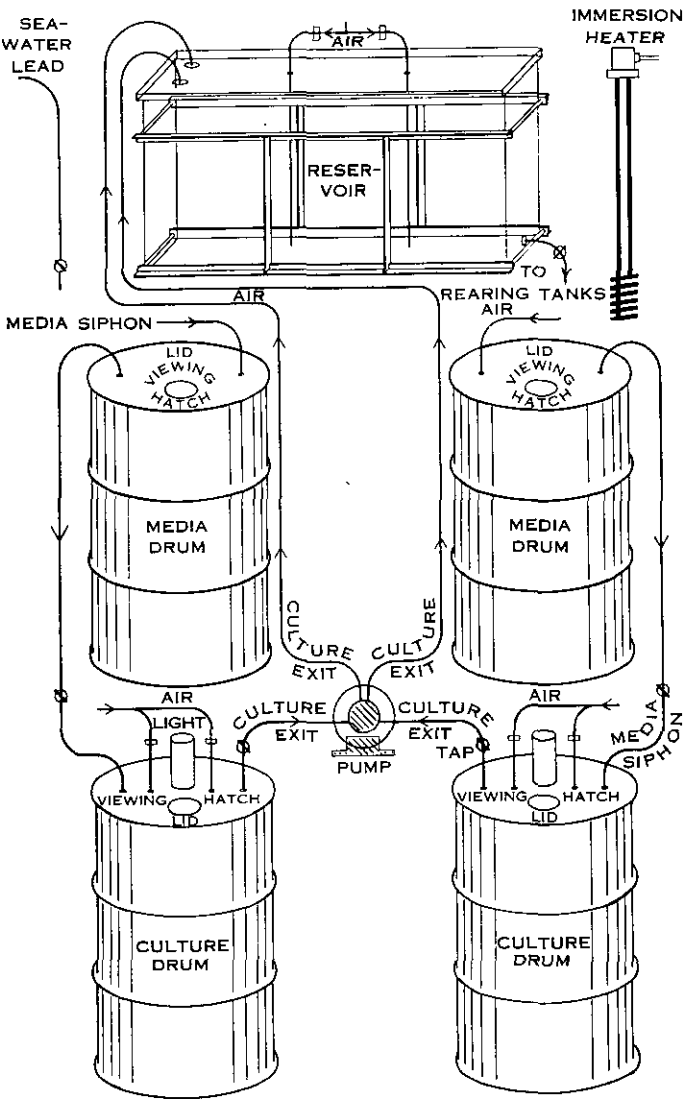

Fig. 1.—Schematic diagram of mass culture unit (see text).

the lugs and wing nuts shown in Plate 2. Figures 2(*a*) and 2(*b*) show the relative positions of the various openings in the lower (culture) and upper (media) drum lids. Of these the air, culture, and breather openings are similar in that each is made from a $\frac{13}{16}$ in. O.D., $\frac{9}{16}$ in. I.D., and a $1\frac{1}{8}$ in. O.D., $\frac{11}{16}$ in. I.D., brass washer, silver-soldered together, chromium-plated, and enclosing a No. 12 neoprene O ring

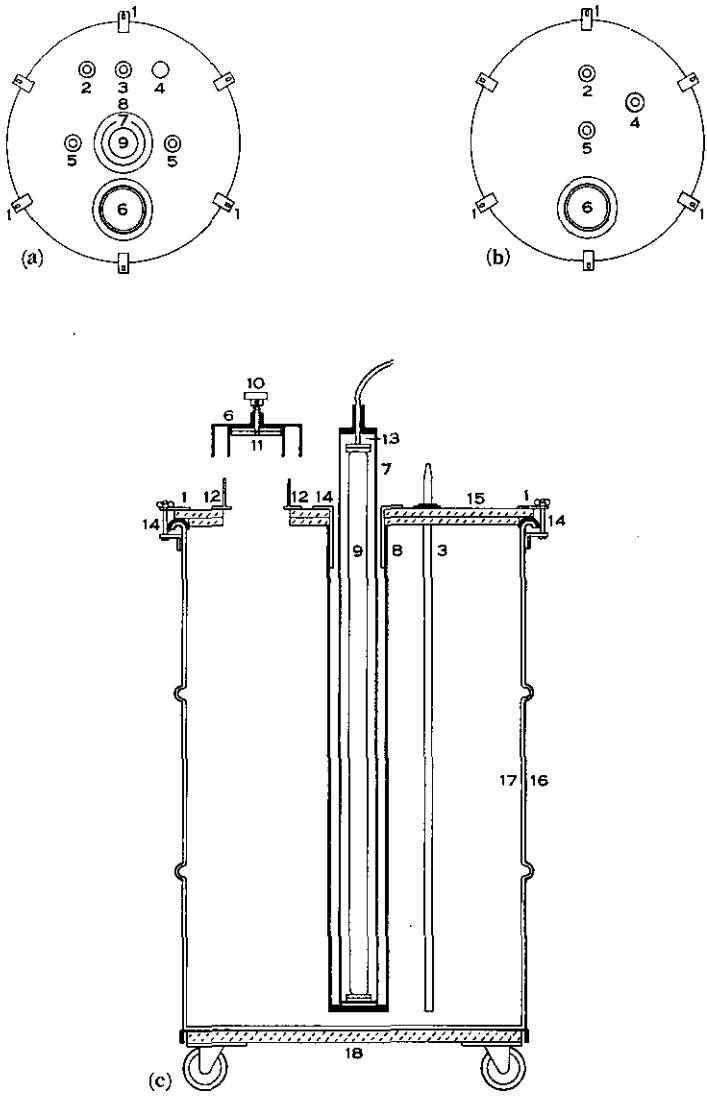

Fig. 2.—(*a*) Plan view of culture drum lid. (*b*) Plan view of media drum lid. (*c*) Vertical section near middle of culture drum. 1, Lug; 2, medium siphon; 3, culture exit tube; 4, opening for thermometer and sampling (kept closed); 5, aeration lead; 6, viewing hatch covering immersion heater opening; 7, 8, inner and outer "Perspex" jackets; 9, fluorescent tube; 10, knob, spring, and shaft of viewing hatch; 11, viewing hatch wiper; 12, immersion heater opening flange; 13, end support of fluorescent tube; 14, screw clamp and gasket; 15, drum lid; 16, drum wall; 17, fiberglass lining; 18, trolley.

which seals against the 17 mm O.D. glass tubing used in the leads running through these openings. The remaining openings are for the fluorescent tube and immersion heater. The former (Fig. 2(*c*)) is a $3\frac{3}{4}$ in. O.D. brass tube 4 in. long with a circular flange projecting $1\frac{1}{2}$ in. from the lower end, which is securely bolted to the drum lid. The immersion heater opening is a $4\frac{1}{4}$ in. O.D. brass tube 2 in. long with a $\frac{5}{8}$ in. circular flange projecting out at lid level. This flange also projects inwards $\frac{1}{8}$ in. forming a lip which supports the immersion heater when it is in use. These, and all other metal fittings, are chromium-plated. Illumination is provided by an "Atlas" 30 W double life 4500K "Daylight" fluorescent tube, which is totally enclosed (except for a small breather hole at the top) in a waterproof 38 by 3 in. "Perspex" tubular jacket. This unit is lowered, after pasteurization and cooling of the drum, into a second jacket measuring 33 by 4 in. which is held in position jointly by the lid flange and the pressure of the culture medium of the drum. The viewing hatch fits over the immersion heater opening during culture periods and is fitted with a wiper for clear viewing of the culture. The hatch consists of an outer 6 in. O.D. and an inner 4 in. O.D. "Perspex" tube 2 in. long cemented to a flat 6 in. diameter "Perspex" lid which forms the viewing surface. A $\frac{3}{4}$ by $\frac{3}{4}$ in. "Perspex" rod is mounted centrally on the window and has a $\frac{1}{4}$ in. hole drilled vertically through it; this rod forms a housing for a 2 by $\frac{1}{4}$ in. stainless steel shaft which has a knob and spring at the upper end, a sealing neoprene O ring set in a circular groove (not figured) recessed into the rod, and a $3\frac{3}{4}$ in. length of car windscreen wiper bolted transversely to the lower end. Each culture drum is mounted on a circular plywood trolley bearing four swivelling wheels.

III. Accessory Equipment

(*a*) The reservoir (Plate 1) is made of $\frac{1}{2}$ in. "Perspex" and is 4 by 2 by 1 ft with a capacity of 50 gal. It is partly supported by a $\frac{3}{4}$ in. angle brass frame.

(*b*) The electrical supply is from 240 V mains to five three-pin sockets; Nos. 1–4 are at the rear accessible by a door near the waterproof switchboard and No. 5 is on the switchboard. Nos. 1 and 2 supply the fluorescent tubes, No. 3 is a 30 A outlet for the heater, No. 4 is a spare, and No. 5 is for the pump.

(*c*) The pump is a $\frac{3}{8}$ in. "Bakelite" model 3010–01 (Jabsco Pump Co., Burbank, California) with the metal shaft replaced by $\frac{1}{2}$ in. diameter inert plastic ("Teflon") rod flexibly coupled to the shaft of a $\frac{1}{4}$ h.p. 1425 r.p.m. electric motor. All the connecting tubes are heavy plastic tubing (18 mm O.D., 12 mm I.D.).

(*d*) The immersion heater is made of $\frac{3}{8}$ in. O.D., stainless steel tubing with the lower part coiled and containing nickel chrome resistance wire in fused magnesium oxide. Its height is 33 in. and it operates on 4000 W. It is coated with a mica colloid anticorrosive paint ("Micanab" Mica & Insulating Co. Ltd., Sydney). The lead length is 20 ft.

During the early experiments the temperature of the laboratory rose above 30°C on certain days; air conditioning was installed during later experiments to maintain the temperature at 20 ± 3°C.

IV. Culture Procedure

(*a*) *Preparation of Medium*

The Erdschreiber medium used for all cultures is prepared by autoclaving 2 l. of rich garden soil and 2 l. of tap water at 15 lb/sq. in. for 3 hr, filtering overnight with an Eaton-Dikeman No. 541 filter paper, and diluting one part of the resulting earth extract with two parts of tap water. Following the addition of 0·2 g sodium nitrate (B.P.) and 0·03 g sodium dihydrogen phosphate ($NaH_2PO_4 \cdot 12H_2O$)(B.P.) per 50 ml fluid, the resulting enriched earth extract is autoclaved at 5 lb/sq. in. for 1 hr and stored in stoppered containers until required. The working medium is prepared by adding 50 ml of enriched earth extract per litre of pasteurized or sterilized sea-water before inoculation.

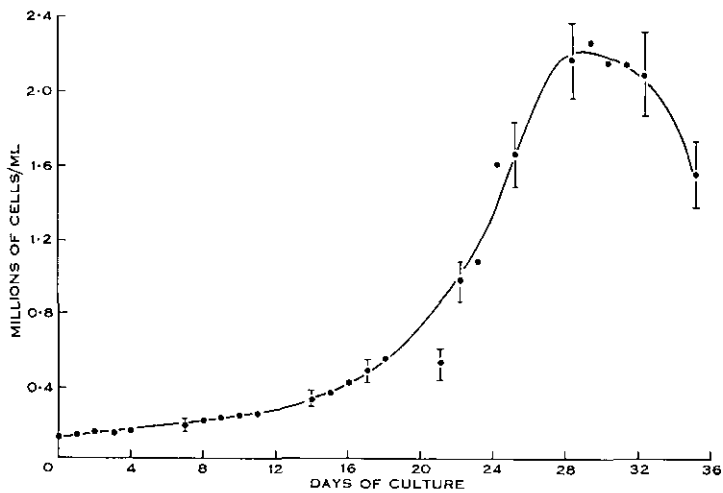

Fig. 3.—Growth of *Isochrysis galbana* Parke at 20°C. Each point is the mean of 10 counts from a daily sample; the vertical lines represent one standard error of the mean. Culture volume 200 l.

(*b*) *Subculturing Stock Algal Cultures*

A series of unialgal 200 ml cultures is maintained at 20±2°C in 250 ml "Pyrex" flasks under constant illumination of 200–300 lumen/ft² from 40 W "Osram White" fluorescent tubes. Subculturing is weekly, but each parent culture is kept a further 3 weeks to provide inocula for the 4 l. cultures described later and to prevent accidental loss of a strain. In subculturing, enriched earth extract and sea-water are put into each flask in the proportions already given and the flask is then plugged with cotton wool and autoclaved at 5 lb/sq. in. for 1 hr before cooling to about 20°C. An inoculum of about 10 ml from the previous week's culture is then poured into the flask.

(*c*) *Preparation of Drum Inocula*

The 4 l. drum inocula are grown in 5 l. aerated "Pyrex" flasks illuminated by *c.* 100 lumen/ft². Each flask, which contains 4 l. of medium, is plugged with

cotton wool containing a central glass tube (the latter is subsequently used for aeration), heated to boiling point, and then cooled to 20°C. A 200 ml inoculum (from a 2-week-old stock culture) is then added. Aeration is maintained at *c.* 25 l./hr. Ten days' growth is usually sufficient to produce a suitable density for inoculating a drum (see Tables 1 and 2).

(*d*) *Mass Culture*

Each drum is prepared by scrubbing and rinsing with sea-water, replacing the lid, and filling to the required level with sea-water. The immersion heater is

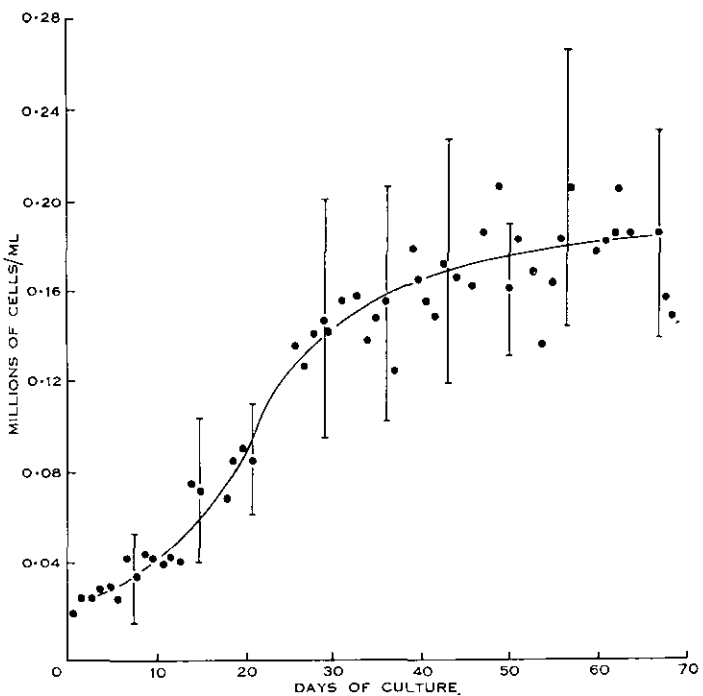

Fig. 4.—Growth of *Dunaliella tertiolecta* Butcher at an average temperature of 23°C for the first 28 days, thereafter at 20°C. Each point is the mean of 10 counts from a daily sample; the vertical lines represent one standard error of the mean. Culture volume 200 l.

then used to raise the temperature to 70°C (about 4 hr) and vigorous aeration (500 l./hr) is maintained to ensure thorough mixing. If the aeration leads do not reach the bottom of the drum a cool layer (<30°C) persists there from which ciliate contamination can result about 10 days later, particularly in flagellate cultures. The drum is reheated to 70°C the following day, the immersion heater replaced by the viewing hatch, and the enriched earth extract added. When the drum has cooled to 20°C (24 hr later), the aeration rate is lowered to about 200 l./hr and at this stage

the inocula and fluorescent tubes are inserted in the culture drums. The illuminance at the centre of the drum is *c.* 400 lumen/ft^2, decreasing to 50 lumen/ft^2 at the periphery. When the culture has reached a suitable density it may be pumped out completely from the drum to the reservoir and a new culture commenced. Alternatively, most of it may be removed and the remainder left as the inoculum for new, pasteurized medium, siphoned from an upper drum. After each use the pump and connecting tubes are flushed with 70% alcohol and the reservoir cleaned by filling with fresh water for 2 days and then heating to 50°C with the immersion heater. Although higher temperatures would be more desirable, the "Perspex" of the

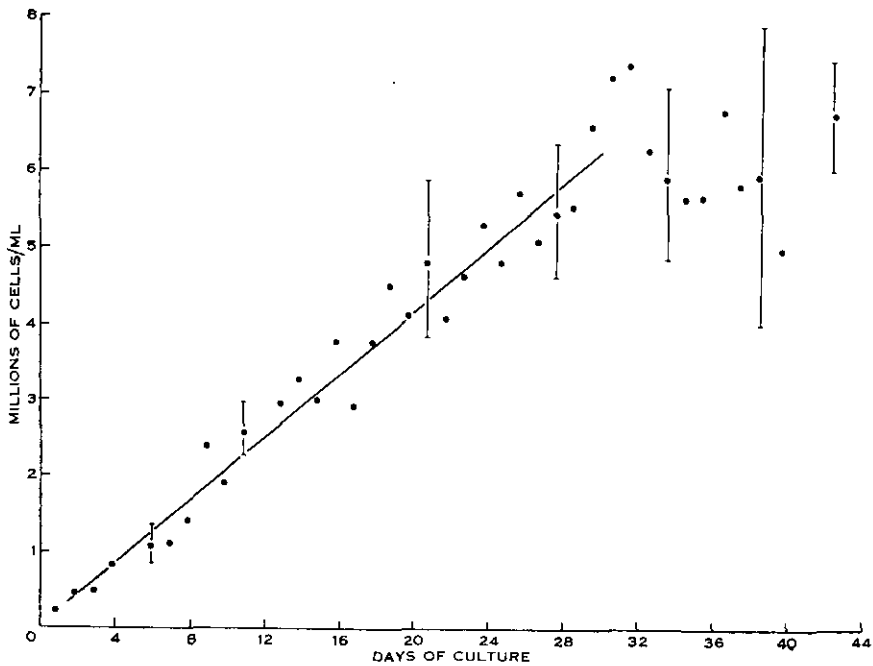

Fig. 5.—Growth of *Phaeodactylum tricornutum* Bohlin with the average temperature rising gradually from 16 to 20°C over the growth period. Each point is the mean of 10 counts from a daily sample; the vertical lines represent one standard error of the mean. Culture volume 200 l.

reservoir becomes thermoplastic at such temperatures; in practice the above method has been found satisfactory for the type of work described here.

(*e*) *Density Counts*

In the present experiments a 20 ml sample was withdrawn daily between 1600 and 1700 hr and the temperature at that time noted. The algae in the sample were killed by adding three drops of Bouin's Fixative to prevent movement during counting. The sample was agitated before each of the 10 subsamples was withdrawn, and counts were made with a Nebauer haemocytometer (*Isochrysis*, *Dunaliella*) or a Petrov Hauser bacteria counter (*Phaeodactylum*).

V. Results

The algae used in these experiments were *Isochrysis galbana* Parke (Chrysophyceae (Parke 1949; Kain and Fogg 1958)); *Dunaliella tertiolecta* Butcher (Chlorophyceae (Butcher 1959; McLachlan 1960)); and *Phaeodactylum tricornutum* Bohlin (Bacillariophyceae (Spencer 1954)). Figures 3, 4, and 5 illustrate results obtained for *Isochrysis*, *Dunaliella*, and *Phaeodactylum* cultures respectively, and Tables 1 and 2 summarize conditions and results obtained for these and replicate cultures.

Daily estimates of mass culture populations were affected by several sources of error. Despite careful repetition of the sampling and counting procedure, there was variation in the daily counts of the 10 subsamples (Figs. 3, 4, and 5). This variation tended to increase as the culture density increased; it was least in *Isochrysis*, intermediate in *Phaeodactylum*, and most in *Dunaliella*. A settling out process took place in the *Phaeodactylum* and *Dunaliella* cultures and resulted in an increasingly thick ooze forming on the bottom of the drum. Since this was not in circulation it was not available for sampling, and consequently the maximum densities given in Tables 1 and 2 are probably low values. On several occasions fluctuations of the air supply were accompanied by corresponding fluctuations in the sample densities.

The results (Figs. 3, 4, and 5; Tables 1 and 2) show that cultures suitable for feeding larvae were available within 10 days in all experiments. Densities continued to increase rapidly in two of the *Isochrysis* cultures (Experiments 1 and 3), to reach maxima around $2{\cdot}0\times10^6$ cells/ml in 30–35 days, followed by a sharp decline. The relatively slower growth and lower peak density in Experiment 2 ($0{\cdot}8\times10^6$ cells/ml in about 40 days) did not appear to be directly attributable to temperature differences since the temperatures were similar (*c.* 20°C) in Experiments 2 and 3. Rapid growth took place in all three *Dunaliella* cultures, but detailed comparisons are not attempted because of the relatively large standard error encountered in counting this species (Fig. 4), and also because of temperature differences occurring during the early weeks of culture (Table 1). The *Phaeodactylum* experiments were made before temperature control was installed and consequently cover a wide range. The results indicate, as already suggested by Spencer (1954), that high temperatures (25–30°C) are not advisable for this species and that relatively better yields can be obtained below 20°C. The rapid decline noted in Experiment 3 was associated with high temperatures (30, 32, and 32°C) experienced in days 11, 12, and 13 of culture (not detailed in Table 2).

VI. Acknowledgments

Thanks are due to Dr. Mary Parke of the Plymouth Marine Laboratory for kindly supplying cultures of the algae used in these experiments. This work forms part of a program on the biology of fouling organisms carried out in cooperation with the Royal Australian Navy.

VII. References

Butcher, R. W. (1959).—An introductory account of the smaller algae of British coastal waters. Part I. Introduction and Chlorophyceae. *Fish. Invest., Lond.* Ser. IV **1959**: 1–74.

TABLE 1

GROWTH IN CULTURE UNIT

(*a*) *Isochrysis galbana* Parke

		Experiment No.					
		1		2		3	
Started		21.viii.59		14.iv.60		11.vii.60	
Initial density (cells/ml)		$5 \cdot 2 \times 10^4$		$6 \cdot 6 \times 10^4$		$1 \cdot 6 \times 10^4$	
Days grown		44		48		33	
Maximum density (cells/ml)		$2 \cdot 0 \times 10^6$		$0 \cdot 8 \times 10^6$		$2 \cdot 2 \times 10^6$	
	Age (weeks)	Density	Temp.	Density	Temp.	Density	Temp.
Average weekly density (millions of cells/ml) and temperatures (°C)	1	0·083	17	0·104	22	0·167	20
	2	0·313	19	0·202	20	0·252	20
	3	0·659	19	0·373	20	0·475	21
	4	1·398	21	0·550	20	1·510	19
	5	1·508	19	0·703	20	2·070	18
	6	0·967	20	0·755	20		
	7	0·130	19	0·685	20		

(*b*) *Dunaliella tertiolecta* Butcher

		Experiment No.					
		1		2		3	
Started		25.iii.60		28.ix.59		29.vii.60	
Initial density (cells/ml)		$2 \cdot 0 \times 10^2$		$2 \cdot 6 \times 10^4$		$2 \cdot 8 \times 10^2$	
Days grown		68		20		28	
Maximum density (cells/ml)		$2 \cdot 1 \times 10^5$		$1 \cdot 7 \times 10^5$		$3 \cdot 1 \times 10^5$	
	Age (weeks)	Density	Temp.	Density	Temp.	Density	Temp.
Average weekly density (millions of cells/ml) and temperatures (°C)	1	0·032	23	0·063	19	0·072	20
	2	0·060	24	0·166	20	0·106	18
	3	0·090	24	0·144	19	0·157	19
	4	0·144	21			0·232	18
	5	0·160	20				
	6	0·164	20				
	7	0·183	20				
	8	0·180	20				
	9	0·194	20				

TABLE 2

GROWTH IN CULTURE UNIT

Phaeodactylum tricornutum Bohlin

		Experiment No.									
		1		2		3		4		5	
Started		3.viii.59		13.x.59		15.i.60		15.ii.60		22.ii.60	
Initial density (cells/ml)		$6{\cdot}7\times10^{4}$		$1{\cdot}1\times10^{3}$		$1{\cdot}6\times10^{3}$		$1{\cdot}0\times10^{3}$		$1{\cdot}8\times10^{3}$	
Days grown		43		30		14		30		31	
Maximum density (cells/ml)		$7{\cdot}3\times10^{6}$		$1{\cdot}8\times10^{6}$		$2{\cdot}2\times10^{6}$		$4{\cdot}5\times10^{6}$		$3{\cdot}5\times10^{6}$	
	Age (weeks)	Density	Temp.	Density	Temp.	Density	Temp.	Density	Temp.	Density	Temp.
Average weekly density (millions of cells/ml) and temperatures (°C)	1	0·651	16	0·503	25	0·276	25	0·330	25	0·683	24
	2	2·328	16	1·660	28	1·854	28	1·040	26	1·844	22
	3	3·739	17	1·508	27			3·008	22	2·767	22
	4	4·871	18	0·955	29			3·658	22	3·243	23
	5	6·224	19					4·362	22	3·420	21
	6	5·803	20								

KAIN, J. M., and FOGG, G. E. (1958).—Studies on the growth of marine phytoplankton. II. *Isochrysis galbana* Parke. *J. Mar. Biol. Ass. U.K.* **37**: 781–8.

LOOSANOFF, V. L., and DAVIS, H. C. (1950).—Conditioning *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. *Biol. Bull., Wood's Hole* **98**: 60–5.

LOOSANOFF, V. L., DAVIS, H. C., and CHANLEY, P. E. (1954).—Food requirements of some bivalve larvae. *Proc. Nat. Shell Ass.* **45**: 66–83.

LOOSANOFF, V. L., HANKS, J. E., and GANAROS, A. E. (1957).—Control of certain forms of zooplankton in mass algal cultures. *Science* **125**: 1092–3.

MCLACHLAN, J. (1960).—The culture of *Dunaliella tertiolecta* Butcher—a euryhaline organism. *Canad. J. Microbiol.* **6**: 367–79.

MOYSE, J. (1960).—Mass rearing of barnacle cyprids in the laboratory. *Nature* **185**: 120.

PARKE, MARY (1949).—Studies on marine flagellates. *J. Mar. Biol. Ass. U.K.* **28**: 255–86.

SPENCER, C. P. (1954).—Studies on the culture of a marine diatom. *J. Mar. Biol. Ass. U.K.* **33**: 265–90.

WALNE, P. R. (1956).—Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. *Fish. Invest., Lond.* Ser. II **20** (9): 1–23.

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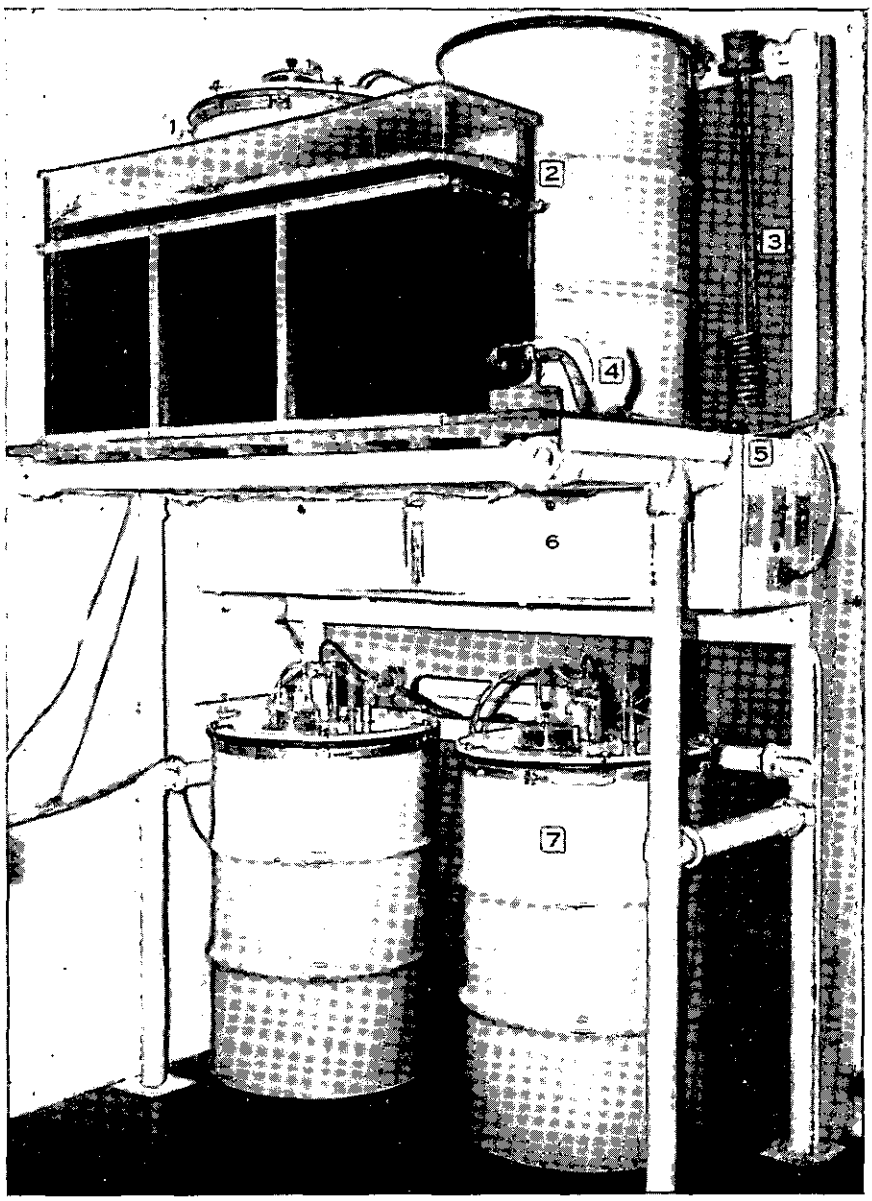
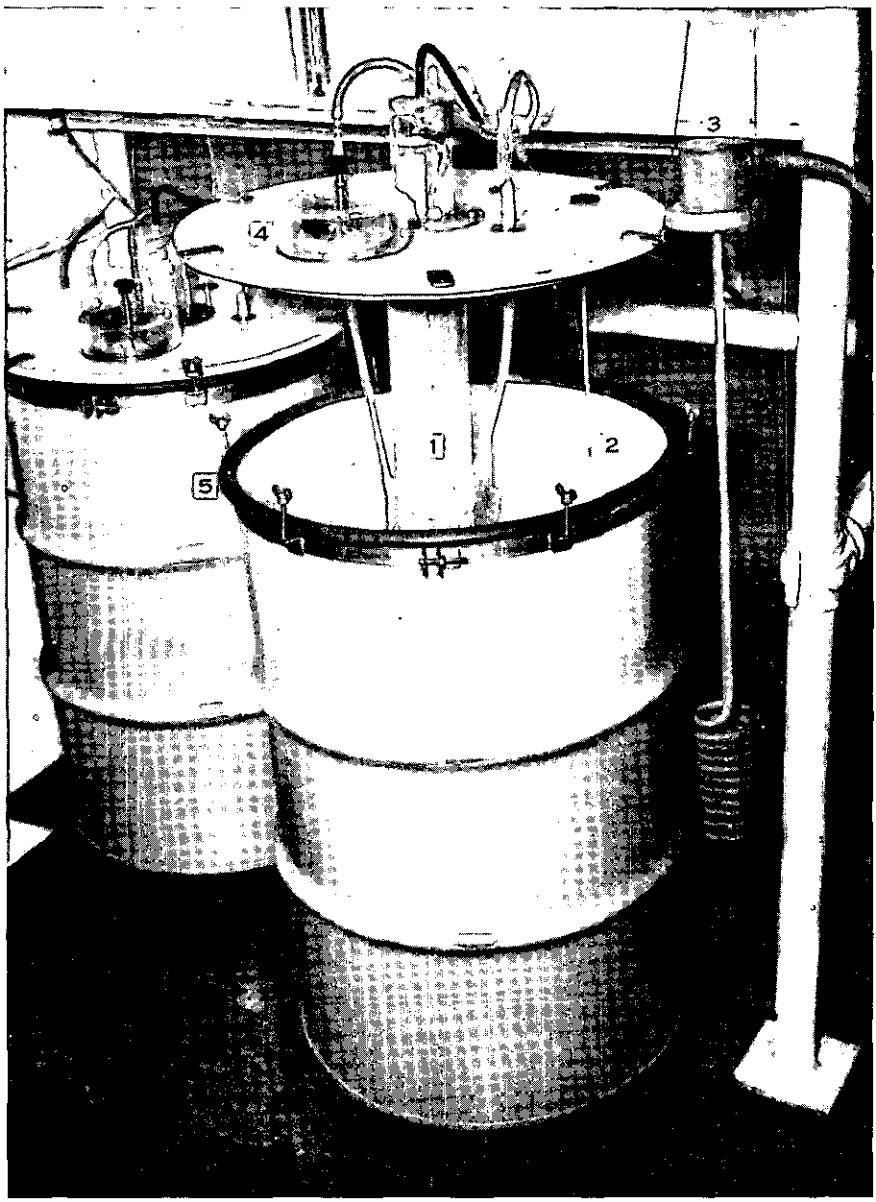


General view of mass culture unit showing 1, media drum; 2, reservoir containing a culture; 3, immersion heater; 4, pump; 5, switchboard; 6, storage cupboard; 7, *culture drum.*



Culture drum showing 1, outer and inner "Perspex" jackets containing fluorescent tube, with aeration leads alongside; 2, thermometer; 3, immersion heater; 4, viewing hatch; 5, clamping wing nut and gasket.