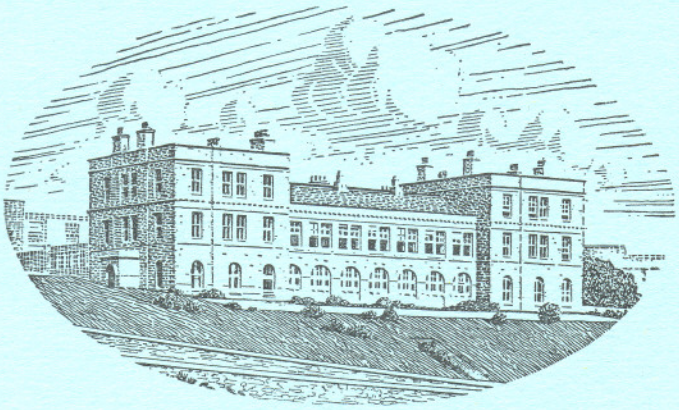
JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM



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R.V. *SARSIA*

A BRIEF DESCRIPTION OF THE MARINE BIOLOGICAL ASSOCIATION'S NEW RESEARCH VESSEL

By G. A. Steven, C. A. Hoodless, R. S. Harrison and F. J. Warren

The Plymouth Laboratory

(With Plates I–IV and Text-figs. 1–5)

From 1922 until the outbreak of the 1939–45 war, the Association's Laboratory at Plymouth was well served by the research vessel *Salpa*—a converted Lowestoft steam drifter having an overall length of about 95 ft. In 1939 *Salpa* was taken over by the Admiralty for naval duties and was not put back into service by the Association on her release after hostilities ended. Her place was taken by a 90 ft. motor fishing vessel on charter from the Admiralty and named *Sabella*. This vessel was only partially converted for research work and was handed back to her owners when *Sarsia* came into service at the end of 1953.

In consultation with the Laboratory staff, *Sarsia* was designed by Messrs Graham and Woolnough, Liverpool, and built by Messrs Philip and Son, Ltd., Dartmouth, to Lloyds 100 A1 classification 'Motor Trawler'. Much thought went into the planning of a ship that would be able to cope successfully and economically both with short trips to near-by grounds to collect and bring back living animals and with extended cruises to more distant and deeper waters. To meet the former requirements the vessel must not be too large and unhandy for inshore dredging and trawling: for the deeper work it was essential that she be large enough to stand up to heavy weather and provide a comfortable 'platform' in all reasonable conditions.

It early became obvious that the restriction on size ruled out steam—in spite of its many virtues—as a medium of propulsion. The cost of maintaining banked up fires at night while the vessel is employed in daily collecting was also a consideration. In spite of its various shortcomings, diesel propulsion was finally decided upon.

General Plan of the Ship

Hull. The hull is of riveted steel construction. Principal dimensions are:

Length overall 128 ft.	Tonnage gross 319
Breadth moulded 28 ft.	Tonnage net 95
Depth moulded 13 ft.	Designed speed 10 knots
Mean load draft 9 ft. 6 in.	

The general appearance of the vessel is clearly shown in Plate I. She has a soft-nosed raked stem and a cruiser stern. The main deck is continuous fore and aft carrying a forecastle forward and a large steel deck-house, the after part of which provides a spacious boat deck. At the forward end are the wheel-house, chart-room and master's accommodation. The wheel-house is raised 3 ft. above the level of the boat deck to provide a good view forward over the forecastle head.

Forecastle. An electric windlass is fitted on the forecastle deck. This supplies power for working the two bower anchors (about 7 cwt. each) and 105 fm. of $1\frac{1}{16}$ in. chain cable—60 fm. on the starboard anchor and 45 fm. on the port one. This cable is stowed in a locker below the main deck. In the forecastle are situated the paint locker, lamp-room, scientific store and main hydrographic winch which is completely enclosed. When this winch is in use a door in the forecastle bulkhead is opened and gives the winch operator a clear view of the hydrographic davit. The scientific store[1] is fitted with shelves for stowage of collecting jars, rails for hanging up small collecting nets, and has ample deck space for storing dredges, Agassiz trawls, and similar deck gear. It also contains a work bench and vice. A large opening gives access to the store from the main deck. This opening is fitted with pound boards which may be used completely to close the opening or to make a 'water step' of any desired height.

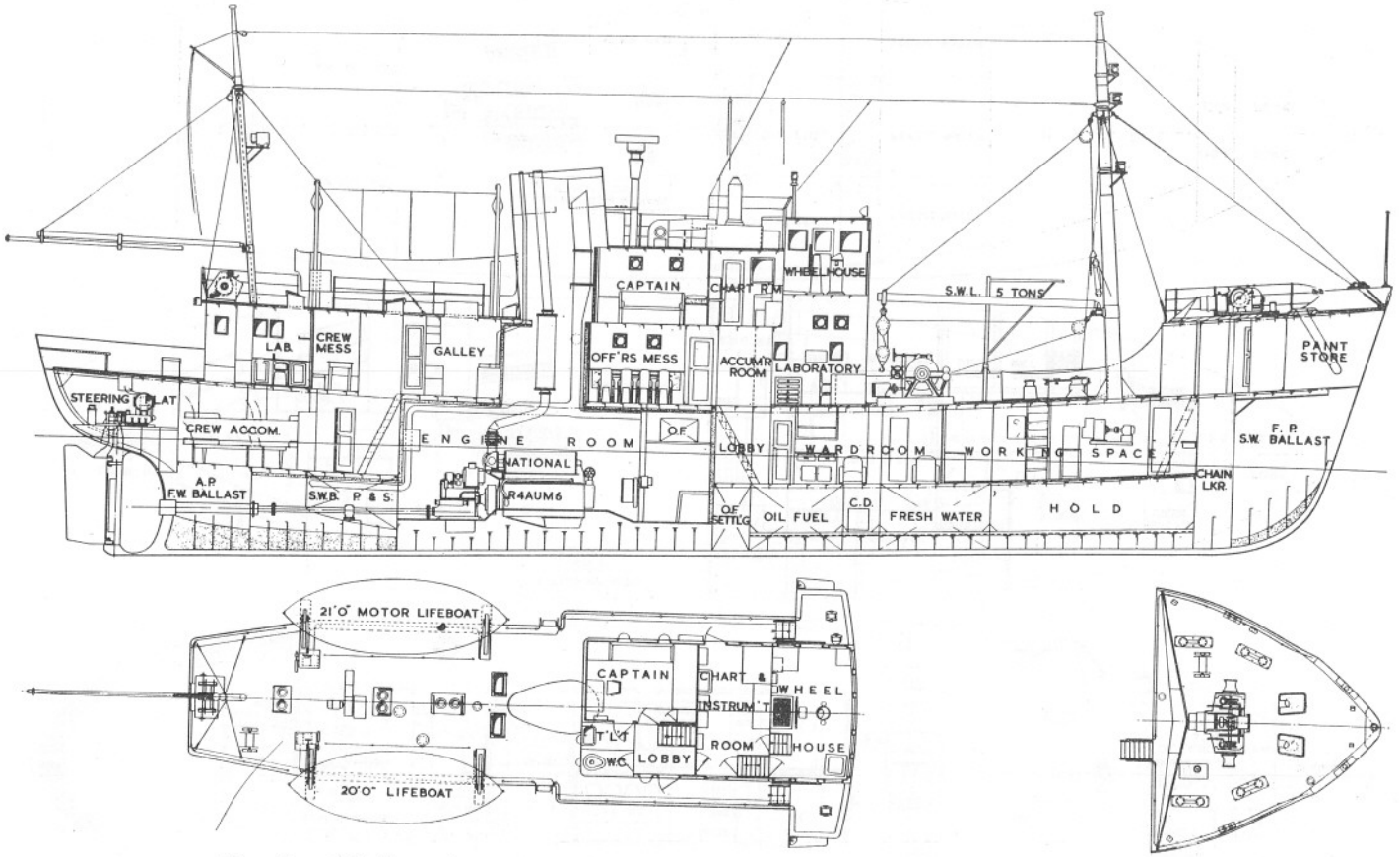
Main deck. The space between forecastle and deck-house is mostly occupied by trawl fitments. The winch is normally sited, immediately forward of the deck-house. It is fitted with three drums, the centre and starboard one each carrying 500 fm. of $2\frac{1}{2}$ in. wire, while the port drum carries 1800 fm. of $1\frac{3}{8}$ in. wire. The trawling fair-leads are so arranged that any wire from the winch can be led to either gallows or to the main derrick on the foremast. Two of the fair-leads are fitted with mechanisms for recording length of wire out in both fathoms and metres—one for the after trawl warp and one for the long small general-purpose wire on the port drum. On the port side of the foredeck a 'Discovery' type hydrographic davit is fitted, and a small hatch with a steel cover provides access to the deck below. The foremast is stepped on this deck and carries a derrick tested to 5 tons safe working load.

The deck on the starboard side of the main deck-house is kept clear for trawl handling. On the port side are two fixed tanks with hinged lids and provided with a continuous supply of salt water. Portable tanks are similarly supplied with running sea water from a supply pipe fitted along the bulwarks.

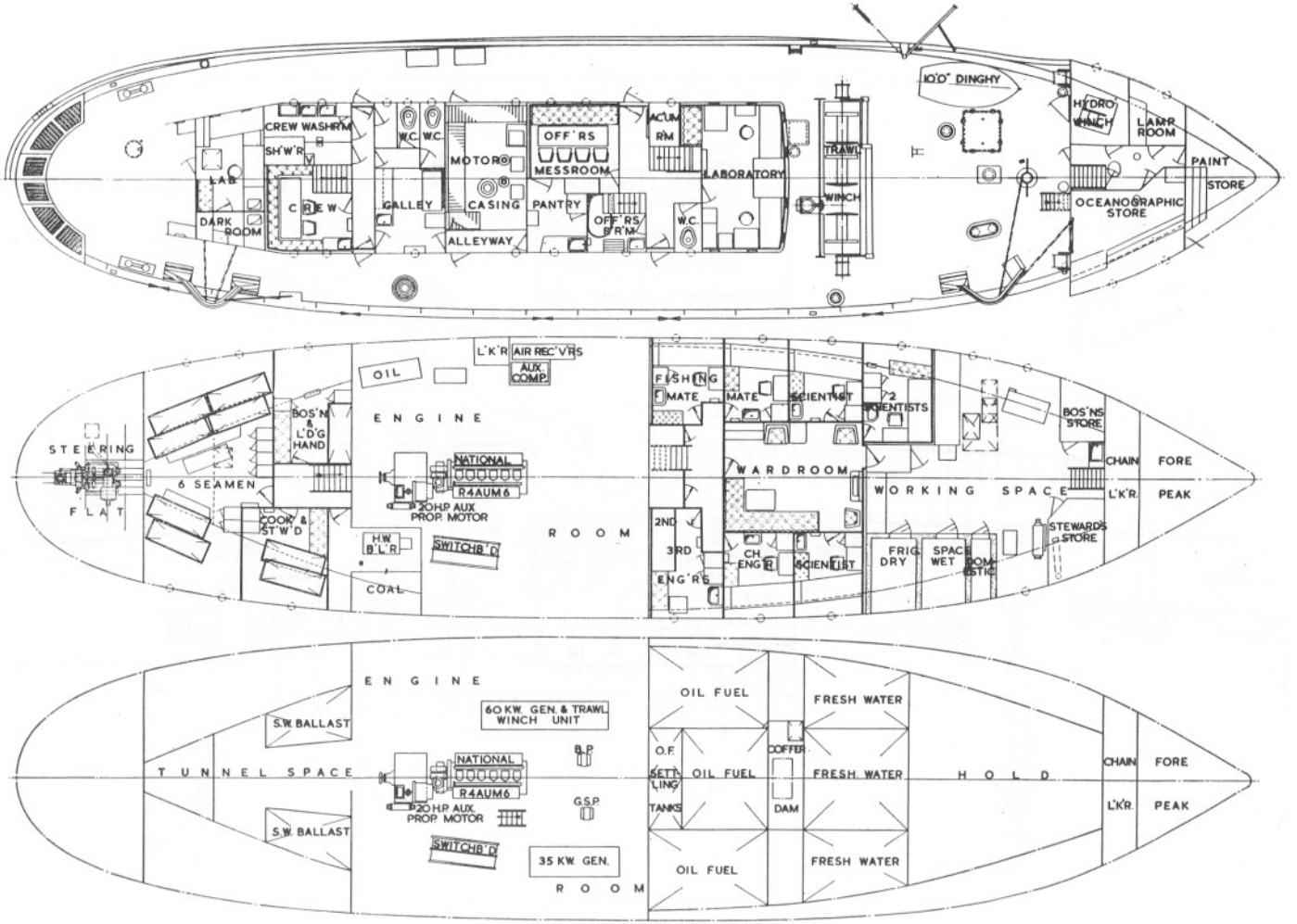
For a distance of 18 ft., between the after end of the deck-housing and the stern, the deck is clear, providing a most useful working space when gear is being used over the stern or quarter.

Main Deck-house. This structure, 60 ft. long and 15 ft. wide, extends from immediately abaft the trawl winch to within 18 ft. of the stern. The main laboratory (mainly hydrographic) is located in the forward end. From this laboratory a door opens aft into a passage-way which extends across the house

[1] Incorrectly named 'oceanographic store' in the builders' drawing in text-fig. 2.



Text-fig. 1. Profile section and general arrangement plan of bridge, boat deck and forecastle deck.



Text-fig. 2. General arrangement plans of main deck, lower deck and hold space.

to doors opening on to the main deck (port and starboard). From this passageway direct access is obtained to the officers' and scientists' messroom, bathroom and W.C.; and to ladders leading up to the wheel-house and down to the deck below.

The messroom, comfortably fitted and provided with the usual appointments, connects directly with a steward's pantry containing a domestic refrigerator, hot cupboard and other usual fitments.

Abaft the messroom and pantry is the engine-room casing, along the starboard side of which an alleyway extends aft to the galley. Its cooking range is of the old-fashioned coal-fired type. Despite objections to carrying coal as an additional fuel, this type of range is welcomed by all as providing the only warm and comfortable spot on board when all the ship's machinery is 'dead', and no shore electrical supply is available.

An athwartships alleyway abaft the galley gives access to the deck, port and starboard. On the port side, forward of this alleyway and opening off it, are crew's toilets and an oilskin locker. Abaft the alleyway, also on the port side, is the crew's wash-place—containing two ordinary hand basins, a trough, and a shower—and a drying room. From the centre of the alleyway a ladder leads down to the crew's sleeping quarters. Abaft the alleyway, on the starboard side —i.e. directly opposite the galley—is the crew's messroom.

In the after end of the deck-housing with a door opening aft, is a second small laboratory—mainly biological—part of which is partitioned off to form a fully fitted dark-room. Abaft this laboratory a small section of the open deck is sheltered by an extension overhead of the boat deck and a corresponding continuation of the casing on each side.

Bridge Arrangements. These arrangements are rather unusual and contribute greatly to easy working. The wheel-house, being raised 3 ft. above the level of the chart-room, provides easy access to the flying bridge which is sited directly above the chart-room. The flying bridge carries the standard compass, radar scanner, ventilating fans and gravity tanks. A wood screen around the fore-end provides adequate shelter.

The wheel-house (Pl. III A), in addition to basic fitments, contains a Decca Radar Unit Type 12, a Marconi 'Graphette' echo-sounder, a Walkers 'Trident' electric log recorder, a telephone, an electric rudder indicator, and an engine-revolution indicator. The port forward window is fitted with a Kent's clear-view screen. Sliding doors, port and starboard, give access to open bridge wings which are carried on sponsons. An engine-room telegraph is fitted on both wings and a revolution telegraph on the port wing. A portable searchlight can be mounted and worked on either wing.

From the starboard side of the wheel-house a few steps lead down into the chart-room (Pl. III B). Across the forward end of this space is a chart table and a long writing table with drawers and cupboards under. The forward bulkhead carries two Kelvin and Hughes echo-sounders—one Type MS 26B

(0–720 ft. or fm.) and one MS 26E (0–2250 fm.). A Decca Navigator decometer unit is fitted to the bulkhead just above the table level. In the cupboards under the table converter units for the echo-sounders and radio-telephone are housed.

On the after bulkhead, the Decca Navigator receiver panel, Redifon radio-telephone and Decca radar receiver panel are fitted. A door opening aft on the starboard side leads into a lobby giving access to the Master's quarters. From this lobby a ladder leads down to the main deck.

Boat Deck. The funnel is oval (Pl. II B) in cross-section and merges into the after part of the Master's accommodation. Abaft the funnel there is, on the starboard side, a 20 ft. standard life-boat and on the port side a 21 ft. motor life-boat fitted with an 8 h.p. Stewart Turner petrol engine. Near the after end of the boat deck the mainmast is stepped. This carries a light derrick which has a sliding boom fitted along its underside. This boom can be slid outboard to obtain up to 8 ft. clearance for light gear worked over the ship's stern or quarter. Abaft the mainmast is a small two-drum hydrographic winch, one drum carrying 500 m of 4 mm (diam.) wire and the other carrying 300 m of 7 mm (diam.) wire.

Lower Deck. Abaft the chain locker on this deck is a large compartment used as a general workroom and store. On the starboard side it contains three cold rooms—one for domestic use and the two others for scientific purposes. There is also a work bench. The after bulkhead, port side, carries a battery of racks for storage of collecting boxes of special design (see p. 396). A small hatch in the port side of this deck opens into the hold space below.

From the working compartment a door leads aft into a comfortably furnished 'wardroom'. Three scientists' cabins open off the wardroom—two single-berth cabins and one double-berth cabin, all with fitted basins supplied with hot and cold running water. Abaft the wardroom is a lobby giving access to three single-berth cabins for ship's officers (mate, fishing mate and chief engineer) and one two-berth cabin shared by engineers II and III. From this lobby, too, a ladder leads up to the deck above, emerging into the alleyway abaft the main laboratory.

Crew's Quarters. The space abaft the engine-room is taken up by the crew's sleeping accommodation. This consists of a double-berth cabin for the cook and steward on the starboard side; a similar cabin for the bosun and leading hand on the port side, and a large central six-berth cabin in the after end of the ship.

The crew numbers sixteen—master, mate, fishing mate, chief engineer and two other engineers, bosun, leading hand, cook, steward and six seamen.

Machinery

Main engine. The main propulsion unit consists of a vertical 4-stroke cycle, single acting diesel engine (National R4 AUM6) of 290 b.h.p. coupled through S.L.M. reverse-reduction gearing to a propeller having anti-clockwise rotation,

viewed from aft to forward. Maximum engine revolutions are 486 per minute. Two lubricating oil pumps, one salt-water and one freshwater pump are incorporated in the main engine. The S.L.M. oil-operated marine gearing (Modern Wheel Drive, Ltd., type M2 WR, size 4) has a gear-ratio of 3·02–1. The blower is of Napier Turbo make, Type T.S. 100. The intermediate shaft runs in a self-lubricated plummer block and the stern tube has U.S. packing glands at the inboard and outboard ends. The propeller is of manganese-bronze, 4-bladed, left hand, of 7·75 ft. diam., and of 7·05 ft. mean pitch.

Fuel consumption at 125 shaft-r.p.m., giving an average (economical) speed of 8½ knots, is 1 ton in 24 h; the steaming range is 22 days, or just over 4000 miles.

Aspinal low-pressure indicators and alarms are fitted for lubricating-oil and cooling-water systems. A 'wrong way' alarm is also provided which operates if the propeller is moved contrary to telegraph orders.

All overboard discharges from engine-room and bilges are at the starboard side as far aft as practicable. By this arrangement the risk of contaminating water samples by ship wastes is reduced to a minimum, all water samples being collected over the port side in a forward position.

Auxiliary Propulsion. The slow speeds necessary in much scientific work cannot be provided by the main engine which has a lower limit of 3–4 knots. After much consideration of various possibilities, a separate low-speed drive was provided quite independent of the main engine. A 20 h.p. variable speed electric motor is geared to the propeller shaft through the main gear-box and so arranged that it cannot be engaged nor remain engaged except when the main engine is disengaged from the reverse-reduction gear. This arrangement works very well in calm weather, but a stronger electric motor would have given greatly improved performance in less favourable conditions.

Auxiliary Service Units. Two diesel-driven D.C. generators are installed—one 60 kW, 220 V driven by a National M4 A6 engine at 1000 r.p.m.; and one 35 kW, 220 V driven by a National M4 A3 engine at 850 r.p.m. Also driven off the M4 A6 engine is the V.S.G. power unit (V.S.G. type K, size 12) for operating the main (trawl) winch. This unit is activated through an S.L.M. clutch and reduction gear-box, ratio 1·54–1 (Modern Wheel Drive, size 1). A Reavall 11·5 cu.ft./min. 2-stage air compressor is driven off the M4 A3 engine. Aspinal alarms are fitted to both auxiliary diesels, as is also an 'Arkon' visual flow indicator for the freshwater systems. The main and auxiliary diesel engines all have freshwater circulation in a closed circuit with a common expansion tank. Heat exchangers are incorporated in the cooling systems.

Other auxiliary machinery in the engine-room comprises:

(1) A Russell Newbury diesel (7·5 b.h.p., 850 r.p.m.) driving a Reavall air compressor, 11·5 cu.ft./min., 2-stage; pressure 350 lb. sq.in.

(2) A Megator T 100 electrically driven general service pump.

(3) A Megator T 100 electrically driven bilge pump.

(4) Alfa Laval fuel and lubricating-oil centrifugal separators.

(5) A Megator M. 8 electrically driven domestic freshwater pump.

(6) A 3·45 single-phase, 50-cycle alternator. The alternator is controlled by an automatic carbon pile voltage regulator giving a stabilized voltage of 220 V. a.c. By hand regulation the unit can be made to provide any other voltage from 100 to 220, thus enabling continental and American types of a.c. mains apparatus to be used on board.

(7) A motor-driven V.S.G. 'A' end-unit for the aft hydrographic winch (boat deck).

The main engines and all auxiliaries are mounted on resilient pads of dexine to reduce vibration. All pipes leading from the engines are fitted with flexible pipe joints to prevent vibration transference from engines to pipes. The underside of the main deck over the engine-room, bulkheads and ship's sides are insulated against conduction of machinery noises to living quarters. Underneath the engine-room flooring, at the forward end, three echo-sounder oscillator tanks are fitted. Ventilation is provided by two Aerofoil dual duty fans situated in the funnel. One fan can be used for either supply or exhaust. Fuel oil is stored in three main bunker tanks having a total capacity of 30 tons.

Steering Gear. Brown's electro-hydraulic steering gear is fitted. The power unit in the steering flat consists of a motor-driven V.S.G. variable delivery pump (mark III, size I, type K). Normal telemotor control is provided from the bridge steering pedestal. The steering unit in the wheel-house remains operative even if the electric steering engine fails, a solenoid-operated valve in the oil system instituting automatic change-over to direct hydraulic control. If this also fails provision is made for emergency steering by hand control from the steering flat.

Windlass. The Reid electric windlass on the forecastle deck has a McClure motor of 11 b.h.p. at 800/1600 r.p.m.—half-hour rating.

Trawl Winch. The trawl winch is an Elliott and Garrood type VS 3–6 hydraulically operated three-drum model made to special order. The drive consists of one size 12, mark III V.S.G. 'A' end of 75 b.h.p. at 650 r.p.m. fitted with single acting auto-control; and one size 12, mark III V.S.G. 'B' end with speed 0–575 r.p.m. A total pull of $3\frac{1}{4}$ tons at an average hauling speed of 150 ft./min is provided.

Main Hydrographic Winch. This winch is hydraulically operated and incorporates type 'K' V.S.G. units, mark III, size III. The motor, V.S.G. 'A' end and starter are in the forward working compartment on the lower deck. The electric motor (by Laurence Scott) is of 25 h.p. at 720 r.p.m. The single drum carries 5000 m of 4 mm (diam.) wire. Average hauling speed is 200 m/min with a 300 lb. load. There is an automatic wire-spreader which can quickly and easily be disconnected if not required.

Aft Hydrographic Winch. This winch is also hydraulically operated and incorporates type 'K' V.S.G. units, mark III, size I. The motor, V.S.G. 'A' end

and starter are all in the engine-room. The electric motor (by Laurence Scott) is of 75 h.p. at 720 r.p.m. There are two drums, one carrying 500 m of 4 mm (diam.) wire and the other carrying 300 m of 7 mm (diam.) wire. Average hauling speed is 200 ft./min with a 600 lb. load.

Heating System. A non-pressure boiler fitted with an 'electromatic' fully automatic oil burner supplies hot running water to bathrooms, living quarters and galley, and also provides central heating throughout the ship. The heating system can be shut off when not required while the hot-water supply is still in use.

Lighting. The vessel is fitted throughout with electric light and power of 220 V d.c. A shore connexion is provided for the purpose of drawing upon shore supply when berthed at a quay with machinery stopped. A rotary converter is incorporated in the lay-out and a change-over switch isolates the necessary circuits from the ship's internal supply system.

Secondary (emergency) lighting is provided from a set of 12 accumulators (see p. 398) housed in a special battery room, as is also the necessary current for bells, the Marconi 'Graphette' echo-sounder and alternative power for the radio-telephone.

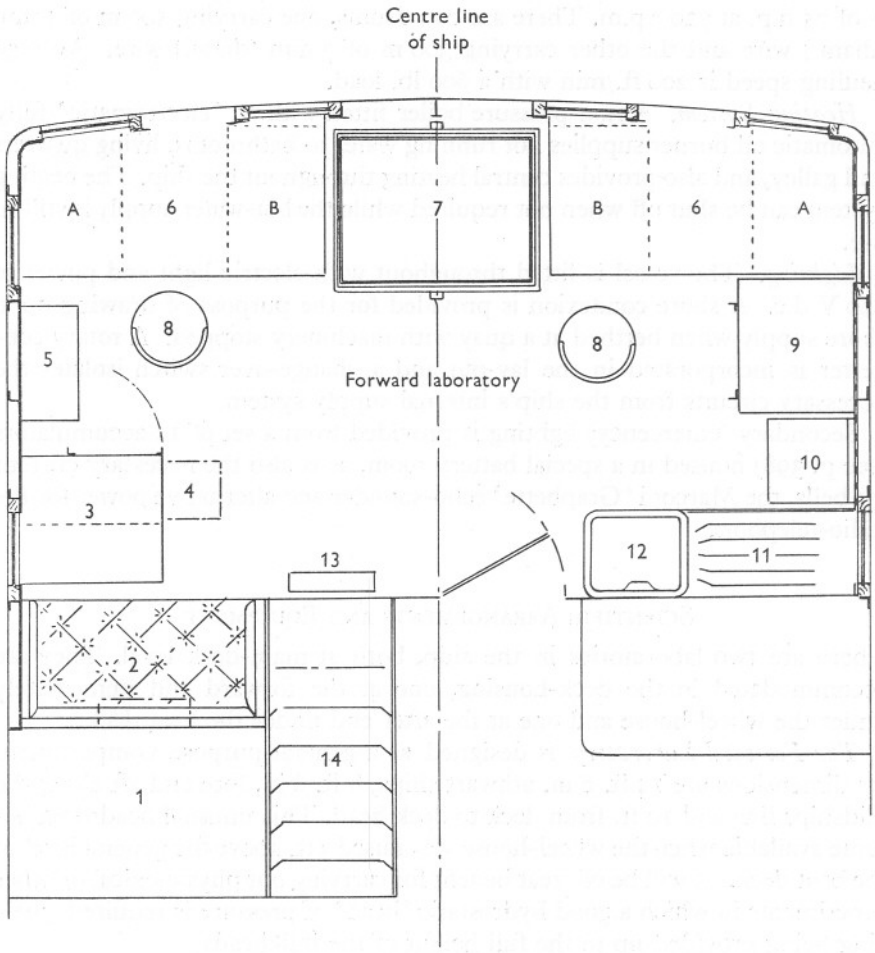
Scientific Arrangements and Equipment

There are two laboratories in the ship, both at main-deck level. They are accommodated in the deck-housing, one at the forward end immediately under the wheel-house and one at the after end under the boat deck.

The Forward Laboratory is designed as a general-purpose compartment. Its dimensions are 14 ft. 6 in. athwart ships, 8 ft. 6 in. fore and aft along the midships line and 10 ft. from deck to deck head. This unusual headroom became available when the wheel-house was raised 3 ft. above the general level of the boat deck. It will be of great benefit for carrying out physiological or other experiments in which a good hydrostatic 'head' of pressure is required, shelving being provided up to the full height of the bulkheads.

In a central position against the forward bulkhead a 'Discovery' type gimbal table is fitted, having surface dimensions of 3 ft. 4 in. by 2 ft. 6 in. On either side of this table, teak-topped laboratory benches extend to the full width of the laboratory; each is 5 ft. 3 in. long and approximately 2 ft. 6 in. in mean width.

Under each bench, in addition to ordinary drawers and cupboards, is a removable drawer unit fitted with cast aluminium-alloy drawer runners. Into these runners standard collecting-boxes are placed which then are used as ordinary drawers until the jars or bottles they contain are all filled. The full box is then lifted right out of its runner and sent below for storage in the racks provided in the working compartment (see p. 392) and a similar box of empty vessels brought up to replace it. By this arrangement the laboratory floor is



Text-fig. 3. Plan of forward laboratory.

kept completely clear of collecting-boxes which normally are awkward to accommodate and to handle in the restricted space available.

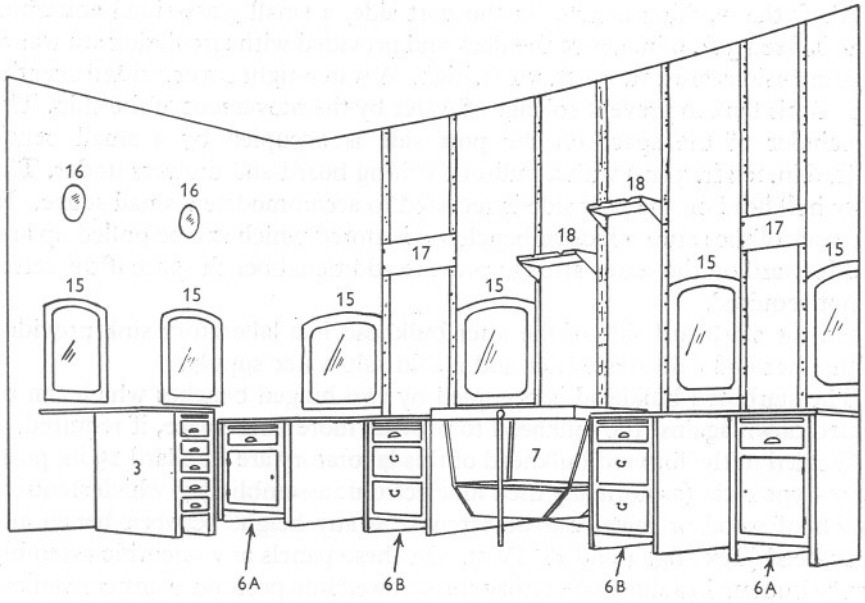
Four kinds of drawer units are provided which take different-sized boxes equipped with different collecting vessels.

Unit 'A' carries two boxes each holding 40 standard salinity bottles.

Unit 'B' takes two boxes each holding 6 full-sized breffits.

Unit 'C' takes two boxes each holding 15 half breffits.

Unit 'D' takes three boxes, one holding 15 half breffits, one holding 24 one-pound honey jars and one holding 28 half-pound honey jars.



Text-fig. 4. Perspective drawing of forward laboratory looking forward and to port.

Key to Text-figs. 3 *and* 4

1. Accumulator room.
2. Settee: terminal board over.
3. Bench with portable top: slide and drawers under.
4. Slide in pulled out position.
5. Aquarium tank with circulating sea water.
6. Laboratory bench with portable top: A, drawer and cupboards under; B, withdrawable drawer unit under. Knee-hole between A and B.
7. 'Discovery' type gimbal table.
8. Chairs.
9. } Hinged tables.
10. }
11. Draining board.
12. Sink.
13. Radiator.
14. Stairs down.
15. Windows fitted with armoured glass and storm screens.
16. Circular scuttles (openable).
17. Standard panels mounted on 19 in. standard post-office-type racks. Other standard racks are shown thus ⌊ ⌋ beside benches 3 and 9.
18. Special shelves mounted on the standard racks. Other specially fitted shelves shown in Pl. IV A. These can be moved or removed with the greatest of ease and others speedily fitted.

The spare space in each unit is taken up with a partitioned drawer and a pull-out writing board. Before the ship puts to sea the laboratory is provided with the drawer units appropriate to the type of work in hand. The spare units are stored in the working compartment near the racks holding the spare boxes.

Abaft the working bench, on the port side, a small glass-sided aquarium tank is fixed 3 ft. 9 in. above the deck and provided with circulating sea water. Its dimensions are 2 ft. × 1 ft. × 1 ft. high. A water-tight cover is fitted over the top of this tank to prevent spillage of water by the movement of the ship. The remainder of the space on the port side is occupied by a small bench (2 ft. 6 in. × 2 ft. 3 in.) with a pull-out writing board and drawers under. The after bulkhead on the port side is recessed to accommodate a small settee. In the back of the settee a hidden bench top is stored which can be pulled up and slid forward on the settee arms to provide additional bench space if the settee is not required.

On the starboard side of the after bulkhead is a laboratory sink provided with a hot and cold freshwater and a cold salt-water supply.

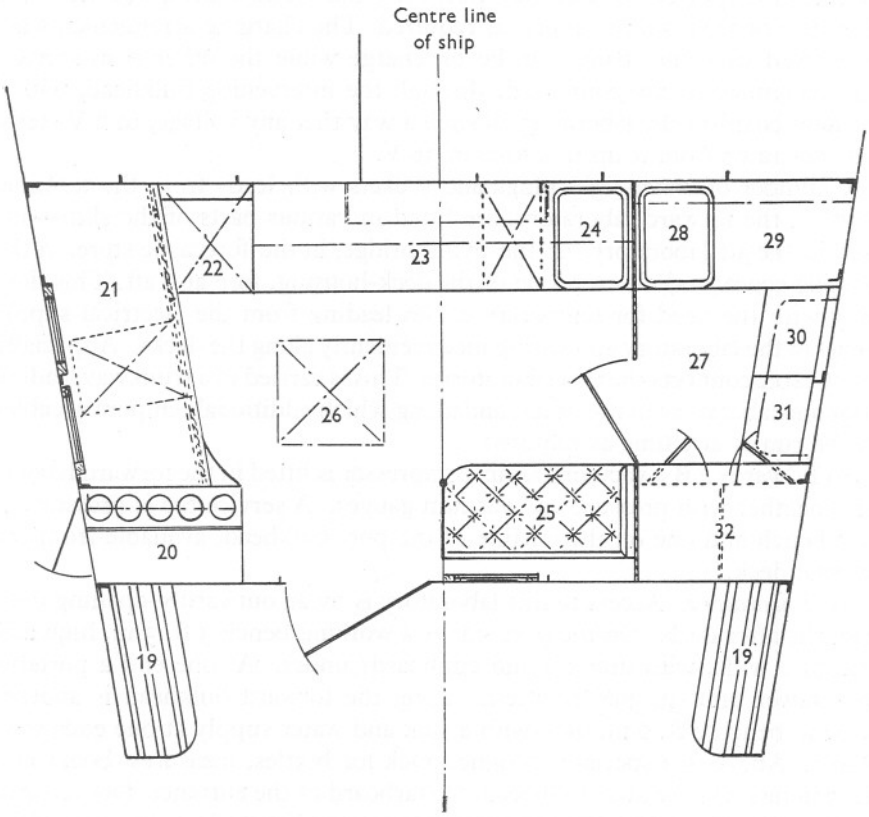
The starboard bulkhead is occupied by two hinged benches which can be folded down against the bulkhead to provide more floor space, if required.

Welded to the forward bulkhead of this laboratory are standard 19 in. post-office-type racks (as normally used for electronic assemblies) to which standard panels of wood or metal can be screwed at any height between bench and deck-head (Text-fig. 4 and Pl. IVB). On these panels any scientific assembly can be built up in a shore laboratory and screwed into position at any convenient place on the bulkhead racking and at any required height. To provide for apparatus assemblies that project on both sides of a panel, or which require access to both sides, other standard racks are provided extending from deck to deck-head, one on the port and one on the starboard side of the laboratory, in front of the side benches. These racks are so constructed that any scientific assembly erected on them can be swung outwards for easy access to any part of the assembly.

A number of panels have been made with special fitments to carry aspirator bottles, books and other standard apparatus (Pl. IVB). These can be accommodated in any position on the racking on the bulkhead, and can be moved to new positions at any time in a few minutes. Other special fittings can be erected on standard panels by any worker at any time, without visiting the ship, and with certainty of their being immediately accommodated on the standard racking on board whenever required.

Each working bench is provided with an additional (loose) whitewood top kept in position, when required, by a fiddle along the outer edge of the fixed top. These additional tops are provided so that any kind of apparatus of any shape or size can be screwed down upon them in any desired position without damage to the expensive permanent top underneath. The additional tops are cheap, expendable and easily renewed when unfit for further service. They can be stored away out of sight when not in use.

Abaft the main laboratory and abutting on its after bulkhead on the port side is a special 'accumulator room' carrying two banks of six accumulators, each bank producing 36 V and having a capacity of 100 amp/h. A selenium type



Text-fig. 5. Plan of aft laboratory and dark-room.

Key

19. Sparred seat.
20. Twine locker with access from outside deck; withdrawable unit under.
21. Bench, 3 ft. 3 in. high with drawers and cupboard under.
22. Bench, 2 ft. 6 in. high, hinged to bulkhead.
23. Laboratory bench, 2 ft. 6 in. high with shelf and bottle-racks over.
24. Sink.
25. Settee.
26. Escape hatch from crew's quarters (in floor).
27. Dark-room.
28. Dark-room sink.
29. Lead-covered bench: shelves over.
30. Hinged table: shelf over.
31. Hinged table.
32. Bench, 2 ft. 6 in. high with two equal cupboards under.

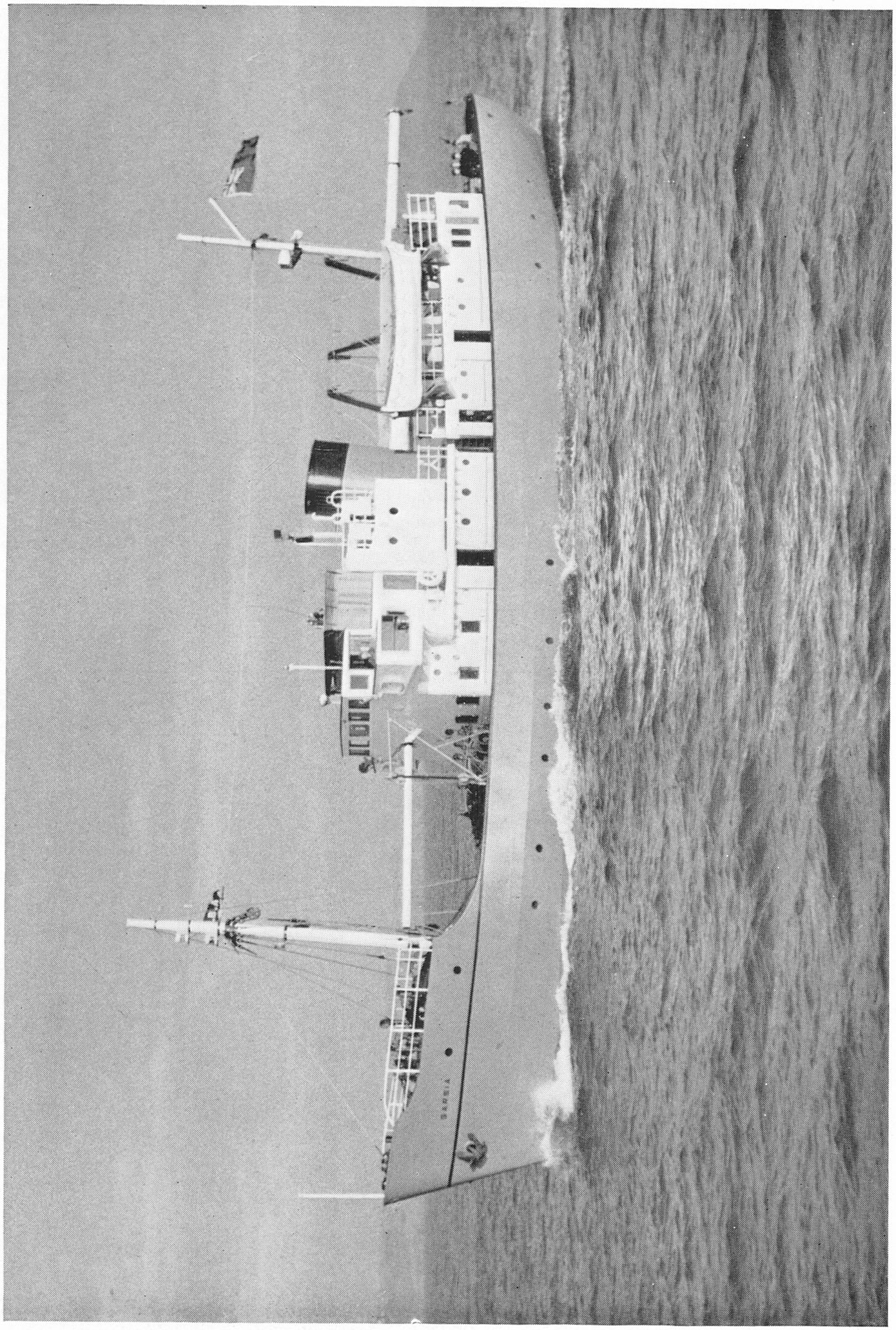
rectifier is employed for charging purposes, the rectifier being fed from the alternator or from shore supply, as required. The charging arrangements are so devised that one 'bank' can be on charge while the other is in service. The accumulators are connected, through the intervening bulkhead, with a terminal board in the laboratory in such a way that any voltage, in 2 V steps, may be drawn from it up to a total of 36 V.

A number of water-tight plugs and sockets with leads from the terminal board in the forward laboratory, are fitted in various parts of the ship—one each in the aft laboratory, on the flying bridge, in the forecastle store, in the working space, and on each side of the deck-housing, fore and aft. This does away with the need for temporary cables leading from the electrical supply points in the laboratory and strung inconveniently along the decks. A specially heavy cable connects the two laboratories. This is carried in a trunkway leading from one laboratory to the other, and along which additional temporary cables can be run at any time as required.

An Edwards RB 4 vacuum pump-compressor is fitted in the forward laboratory together with pressure and vacuum gauges. A service tap is provided at each bench and one on the oustide of the port bulkhead, available from the external deck.

Aft Laboratory. Access to this laboratory is by an outwardly-opening door from the after deck. On the port side is a working bench 3 ft. 3 in. high and 2 ft. 3 in. wide, with drawers and cupboards under. At one end a portable box/drawer unit (p. 396) is fitted. Along the forward bulkhead is another working bench 2 ft. 6 in. high with a sink and water supply at one end (starboard). Above is a specially designed rack for bottles, measures, books and other items. On the after bulkhead, to starboard of the entrance door, a small settee is fitted with a disappearing bench top stowed in the back like that of the settee in the forward laboratory (p. 398). The after bulkhead on the port side of the doorway is occupied by a twine locker with a door opening into it through the deck-house casing from the external deck. In this locker stowage is also provided for winchesters of formalin, spirit and other chemicals required from time to time to be readily available on deck.

Dark-room. Cut off from the aft laboratory by a wood partition and light-proof door is a small dark-room with sink, hot and cold fresh water, benches, cupboards, safelights and all necessary fittings.



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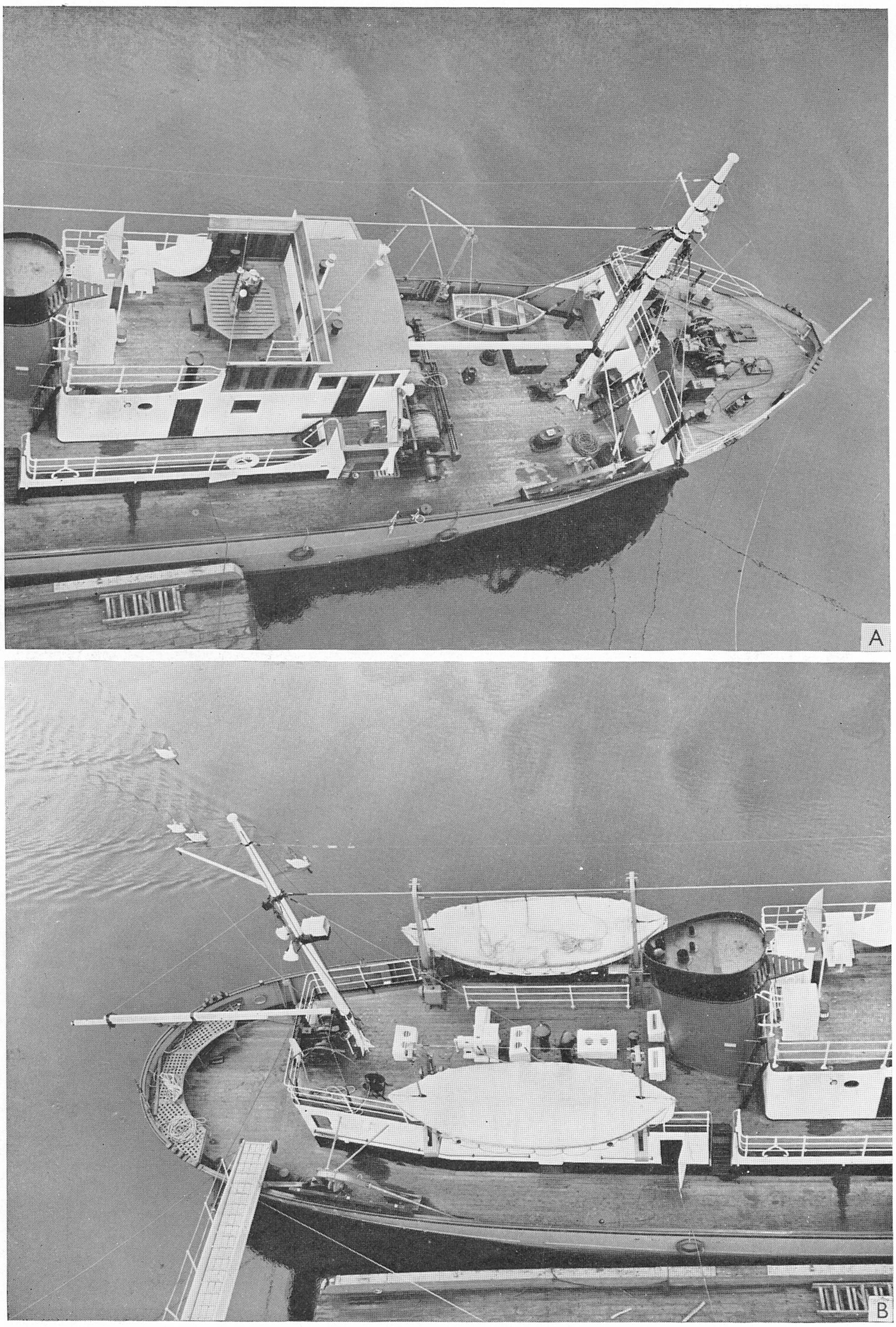
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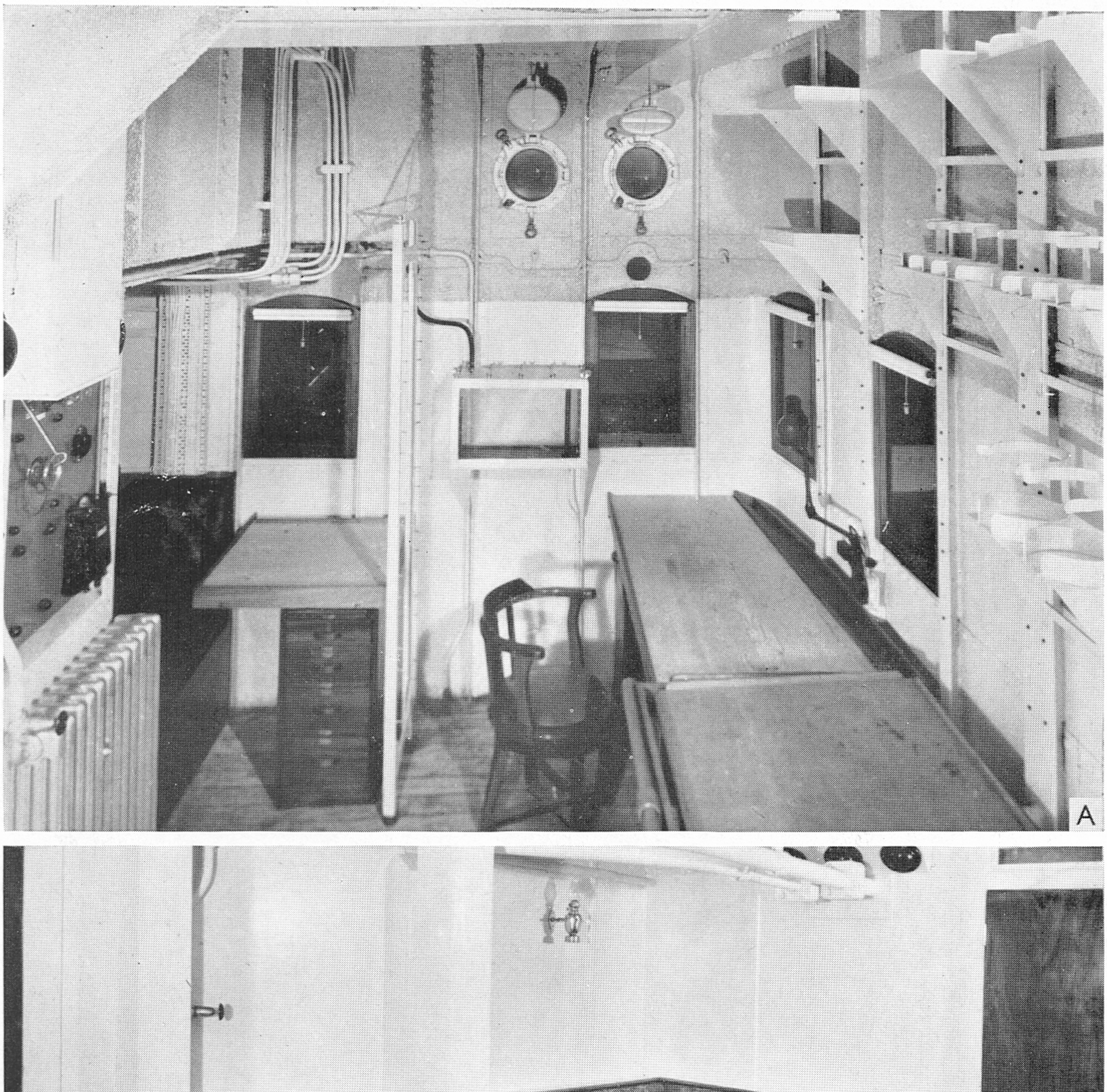

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EXPLANATION OF PLATES

PLATE I

Research Vessel *Sarsia* on arrival off Plymouth from the builders' yard—5 November 1953. Photo D. P. Wilson

PLATE II

A. General view of flying bridge, foredeck and forecastle deck.
B. General view of boat deck and after deck.

PLATE III

A. Wheel-house looking to starboard.
B. Chart-room, looking to port.

PLATE IV

A. Forward laboratory looking to port. Gimbal table in right foreground. Benches shown with removable tops in position.
B. Wardroom.

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ON THE BEHAVIOUR OF BARNACLES

I. THE RELATION OF CIRRAL AND OTHER ACTIVITIES TO TEMPERATURE

By A. J. Southward

From the Plymouth Laboratory and The Marine Biological Station, Port Erin

(Text-figs. 1–6)

Introduction

In previous papers (Southward, 1950, 1951; Southward & Crisp, 1952, 1954*a*, *b*: see also Kitching, 1950; Moore & Kitching, 1939) it has been shown that the geographical distribution of several common intertidal animals in Britain is related to the temperatures prevailing in the different regions. Species of generally southern distribution in Europe are commonest on, or restricted to, the south and west coasts of Britain, where temperatures are higher, while those species of essentially northern character are commoner in the north and east where the temperatures are lower. Where species of northern and southern distribution occur side by side in the same habitat, and are in competition, it seems important to know the range of temperatures over which processes such as feeding and respiration can be carried out, as well as the often narrower range of temperatures within which breeding can take place (cf. Orton, 1920). For example, the two common intertidal barnacles of the European coasts, *Chthamalus stellatus* (Poli) and *Balanus balanoides* (L.), occur side by side only in Britain and Northern France, where they reach the northern and southern limits respectively of their distributions. From the distributions it can be inferred that the vital activities of the two species are keyed to different temperature ranges. This implies that for each species there is an optimum range of temperature, above or below which the species becomes less efficient at feeding, respiring or reproducing. Where the two species occur side by side, there may be periods of the year when one of them is living outside its optimum range, and is at a disadvantage compared with the other species. Therefore, at any given place, the proportion of the annual temperature cycle which lies within the optimum range of temperature for a particular species' vital activities may then afford a measure or index of the relative success of the species there.

The most obvious activity of a barnacle, and one that may play an important part in both feeding and respiration, is the rhythmic beating of the cirri and the associated movements of the terga and scuta. It is likely that this activity reflects the general metabolic level of the barnacle. The frequency of beating

of the cirri is simple to measure, but the experimental conditions necessary for uninterrupted beating are exacting. A barnacle is very sensitive to changes of temperature and illumination and to mechanical shocks; any of these stimuli may cause it to withdraw the cirri and close the terga and scuta. Specimens may also keep the terga and scuta closed in perfectly still water. If, however, a current of water is made to flow over the shell, sooner or later the valves part slightly, and a single cirral ramus may be cautiously put out; eventually rhythmic beating may start. Up to a certain current speed, which differs from species to species, the stronger the current, the less is the barnacle's response to other stimuli. A current of water thus assists the maintenance of regular beating. At high current speeds many species stop beating, and hold the cirri outstretched in the current for periods of up to several minutes (cf. Crisp, 1950), with occasional twisting movements from side to side. This reaction to strong currents can be termed the extension response. At even higher speeds, the terga and scuta may close again (closing response).

A further difficulty in assessing the cirral activity of barnacles is that at least two different types of beat may be shown by different specimens, or even the same specimen, under apparently identical conditions. The type of beat shown at times by all species and specimens can be termed the normal beat: the cirri are almost fully withdrawn into the shell between each extended phase, and the effect of increased temperature is to shorten the extended phase. Another type of beat has so far been observed only in *Balanus balanoides*, *B. crenatus* and *Elminius modestus*: in this beat, which can be termed the fast type, the closed phase is reduced or absent, and the extended phases follow so closely upon each other as sometimes to give the impression of a mere waving to and fro.

Experimental Conditions

Barnacles were brought back to the laboratory attached to small pieces of rock, and were kept continuously immersed in running water. Some specimens had to be kept for several days before examination, and there was thus some risk of acclimatization to laboratory temperatures. In the later experiments specimens were examined within 48 h of collection, being meanwhile kept out of water in a cool damp place.

The cirral activity was observed in a shallow glass trough containing about 750 ml. of water, partially divided down the middle by a Perspex partition (Fig. 1).[1] Sea water from the aquarium supply was filtered through coarse silk mesh, passed through a large coil of glass tubing immersed in a large, thermostatically controlled water-bath, and supplied to one end of the trough through a flow-meter. Excess water overflowed at a constant level at the other end of the trough. The temperature of the running water was varied by heating

[1] A slightly different apparatus was used for observations at Port Erin.

or cooling the water bath, and its rate of flow controlled between 0 and 500 ml. per minute by a screw-clamp.

The barnacles were placed on the near side of the partition and viewed through a lens or dissecting microscope. On the far side of the trough was a paddle-wheel which could be driven, when required, by an electric motor controlled by a variable resistance. For some species the rate of flow of the water supplied through the heat exchange coil was sufficient to stimulate the cirri, but other species needed a stronger current, set up by means of the paddle-wheel, before regular beating could be obtained.

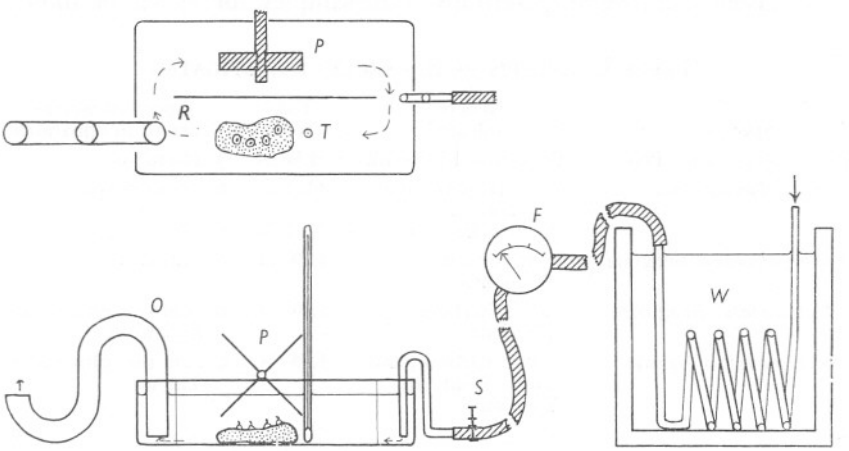

Fig. 1. Diagram of apparatus used for investigating the cirral activity of barnacles. Above: trough in plan; below: side view of whole apparatus. *F*, flowmeter; *O*, constant level outflow; *P*, paddle wheel; *R*, Perspex partition; *S*, screw-clamp on inlet tube; *T*, thermometer, scale about $\frac{1}{8}$th natural size; *W*, thermostatic water-bath and heat exchange coil, not on the same scale.

The frequency of beating was measured by recording with a stop-watch the time in seconds for ten complete openings and closings of the valves accompanied by protrusion of the cirri. The results were converted to the number of beats per 10 sec as this was the most convenient unit for later calculation of the standard deviation. All the results are quoted in these units. About ten specimens were tested at each temperature, and to ensure that so many were active it was necessary to have from two to five times this number in the trough (cf. Cole & Allison, 1937). The mean frequencies of beating given below therefore represent *the average activity of those barnacles active*, and not the activity of the whole sample. All temperatures are expressed in degrees Centigrade.

The water was heated or cooled slowly (about 5° per hour), and the temperature readings were taken close to the barnacles. The apparatus fluctuated

in temperature by up to 0·5° during each experiment, but more rigorous control was not considered worth while, since in comparison with the intrinsic variation between individual barnacles the effects of such fluctuation were negligible. Results have therefore been grouped at the mean temperature of each experiment.

It will be noted that most species showed a large standard deviation, which at some temperatures approached the range of the observations. Each barnacle has its own frequency of beat which is only generally related to that of its fellows. A further probable cause of the great deviation from the mean may be the existence of several age-groups in the samples, for, as will be shown in

TABLE I. SPECIES OF BARNACLES INVESTIGATED

Species	Where collected	Tide-level	Speed of water-current in apparatus
Chthamalus stellatus (Poli)	Rum Bay, Plymouth	H.W.N.	5–10 cm/sec
Balanus balanoides (L.)	(*a*) Brixham, outer harbour	M.T.L.	0·1–0·2 cm/sec
	(*b*) Port Erin bay	M.T.L.	0·1–0·2 cm/sec
Balanus perforatus Bruguière	Wembury, nr. Plymouth	L.W.N.	0·5 cm/sec
Balanus crenatus Bruguière	Sutton harbour, Plymouth	L.W.N.	0·5 cm/sec, plus bursts of 5 cm/sec
Elminius modestus Darwin	Sutton harbour, and Henn Point, nr. Plymouth	L.W.N.	0·5 cm/sec, plus bursts of 5 cm/sec

a later paper, older barnacles beat more slowly than younger specimens. As far as possible the observations were confined to the predominant age-group in the population, excluding recently settled individuals. For most species this meant that specimens of approximately 1–2 years of age were investigated, but it was difficult to separate the age-groups perfectly by size and appearance (see Southward, 1955). In each species, also, the samples were taken from the optimum tide-level (Table I), and it is possible that different frequencies of beating would be shown by specimens from other tide-levels.

RANGE AND FREQUENCY OF CIRRAL BEAT

The two common barnacles, *Chthamalus stellatus* and *Balanus balanoides*, showed quite different cirral behaviour even when compared at their optimum temperatures. The majority of individuals of both species remained closed in still water. But, while *B. balanoides* would set up rhythmic movements with a water current of only 0·1 cm/sec (≡ to a flow of 100 ml./min), *Chthamalus* sometimes needed currents up to 10 cm/sec (≡to 10,000 ml./min) to induce beating. At the latter speed *Balanus balanoides* tended to close the valves, while *Chthamalus*, especially the younger individuals, frequently showed the extension response.

Chthamalus stellatus

Even high current velocities would not induce *Chthamalus* to beat the cirri below 5°. Above this temperature the frequency of beating increased almost linearly with temperature to a maximum of 10 beats/10 sec at 30° (Table II; Fig. 2*c*). The frequency declined at 33° and fell off sharply at 36°, while all beating ceased at 37·5°.

As the normal temperature range experienced by this barnacle in Britain when beating the cirri (i.e. sea temperature) ranges from a mean of 6° in February to a mean of 16° in August, the species is quite clearly operating at the lower limit of its optimum range.[1] In winter, when the inshore water can drop below 6°, it may experience difficulty in feeding and respiring.

Balanus balanoides

The northern barnacle *B. balanoides* continued to beat the cirri down to 1·8°, and would probably remain active below this temperature. At temperatures above 2° the frequency of beating increased linearly to maxima of 6·2 (Port Erin) and 5·6 (Brixham) beats/10 sec at 17° (Table III; Fig. 2*a*, *b*). Above 17° the beating became more variable, although the mean frequency declined fairly steadily down to 30°. All beating ceased beyond 31–31·5°. The slight difference in mean frequency and standard deviation between the Port Erin and Brixham material was probably due to the inclusion of more age-groups in the former sample.

The widest range of monthly mean sea temperatures in Britain is 4–17°, found on the east coast. While *B. balanoides* is thus operating very near its upper optimum limit in Britain, there is no evidence that high temperatures have any detrimental effect on the species. It is, however, possible that the superiority of *Elminius modestus* in south-east England, is related to the latter species' tolerance of a wider range of temperatures than *Balanus balanoides*, as well as to its greater fecundity (Crisp & Chipperfield, 1948).

Balanus perforatus

Specimens of *B. perforatus* from Wembury were examined on two occasions, in November and June. The differences between the two sets of measurements are greater than was found with *B. balanoides* from different localities, but are in some part due also to a difference in age and size of the earlier sample.

It was not possible to induce this species to beat the cirri below 6°. Above 6° the mean frequency of beating increased fairly linearly to 25° (Table IV;

[1] Note: the sea temperatures discussed on this page, and on subsequent pages are derived from the following sources: Admiralty, 1946; Air Ministry, 1949; Deutschen Seewarte, Hamburg, 1927; International Council, 1933; Lumby, 1935; Proudman, Lewis & Dennis, 1937.

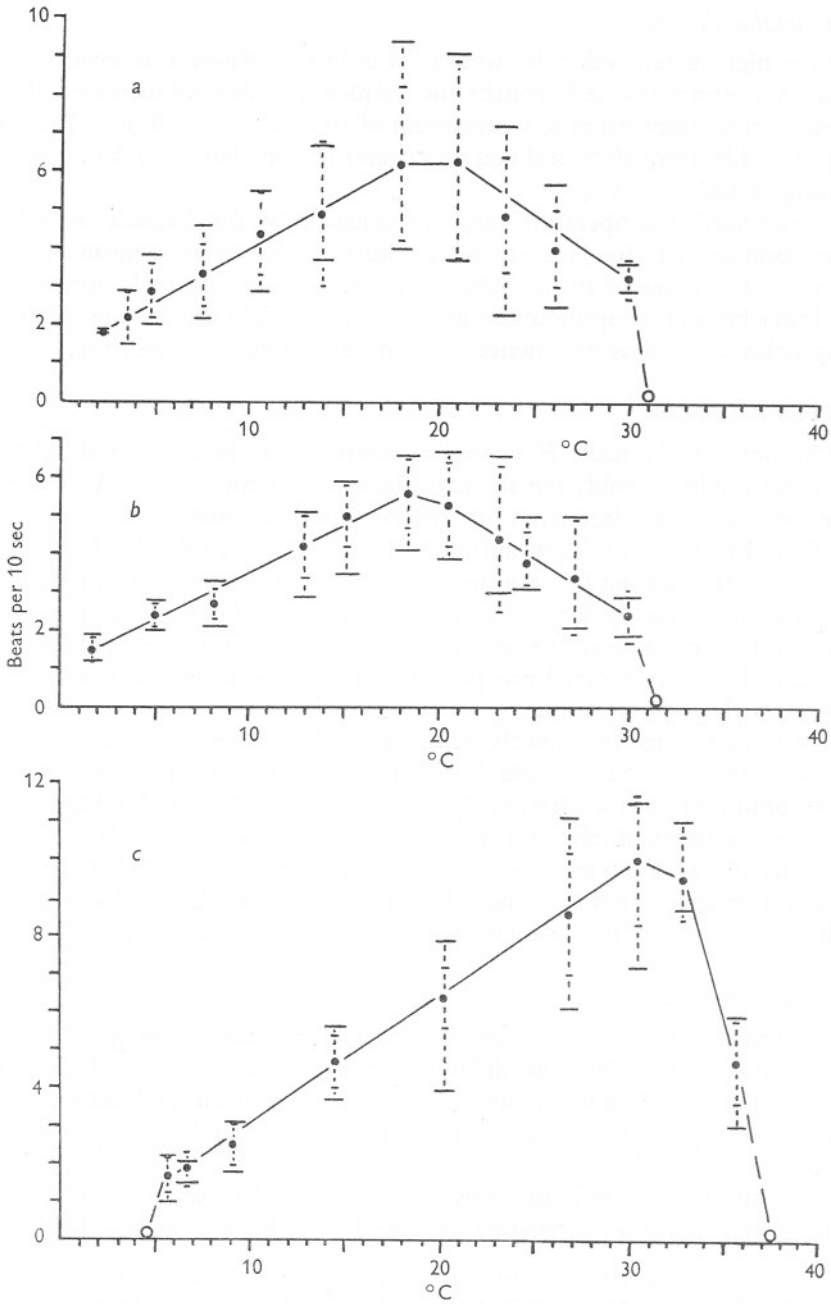

Fig. 2. The frequency and range of beating of the cirri of (*a*) *Balanus balanoides* from Port Erin, (*b*) *B. balanoides* from Brixham, and (*c*) *Chthamalus stellatus* from Plymouth. In this, and subsequent figures, the large open circles denote absence of cirral beat; small open circles refer to single observations only; the small dots mark the mean frequency of beat at each temperature; and the range of observations and the standard deviation at each temperature are shown by large and small cross-lines respectively on either side of the mean.

Fig. 3). Above 25° the beating varied considerably in both sets of measurements, generally tending to decline, although the secondary peaks of activity at 30 and 33° should be noted. Both series declined sharply at 35°, and beating ceased at 35·5–36°. The slightly higher mean frequencies shown by the June series may represent some acclimatization to summer conditions, but the variability at these temperatures prevents any certain correlation.

Like *Chthamalus*, *Balanus perforatus* is at the lower end of its optimum range in Britain, where it normally experiences mean sea temperatures of from 7 to 16°, and may also have difficulty in feeding and respiring during the winter.

Balanus crenatus

For *B. crenatus*, and for *Elminius* also, the most regular cirral movement was obtained by occasional stimulation with currents of 5 cm/sec in addition to the regular current of 0·5 cm/sec (obtained with water flow of 500 ml./min). The actual counts of frequency, however, were made with the regular flow only.

Balanus crenatus showed a comparatively rapid beat at low temperatures, and there was every sign that it would continue beating below the minimum temperature of 4° obtainable at the time. The mean frequency of beating increased more or less linearly with temperature from 4° to a maximum of over 10 beats/10 sec at 21° (Table V; Fig. 4). Above 21° the beating slowed down, and all cirral movement ceased at 25·5°. The great range of frequency of beat at the higher temperatures is noteworthy; to some extent it was due to the ability of some specimens to show the two types of beat already mentioned.

This barnacle is operating quite near its upper optimum limit in Britain, for temperatures above 20° may be experienced during the summer in the sheltered waters favoured by the species. Yet the same species is reported from tropical and subtropical localities by Darwin (1854) and Gruvel (1905). The cirral behaviour of the British specimens suggests that the species is here near the southern limit of its distribution, and supports previous doubts of the validity of the warm-water records (Pilsbry, 1916). Either the tropical records refer to another species, or *B. crenatus* shows a number of varieties adapted to different temperatures, such as are found in *Aurelia aurita* (Mayer, 1914). It is possibly significant in this connexion that in Britain the species is generally restricted to M.L.W.S. or below, and that above this level it is found only beneath stones or in the shade of thick growths of seaweed. These are all measures that would reduce the effect of the high temperatures that may be found on the shore in sunny weather.

Elminius modestus

Specimens of *E. modestus* were examined in February and in June. The February samples were obtained from two localities (see Table I), but the results have had to be combined to cover the whole range of temperature. Beating of the cirri could not be obtained below 2°; above this level the mean

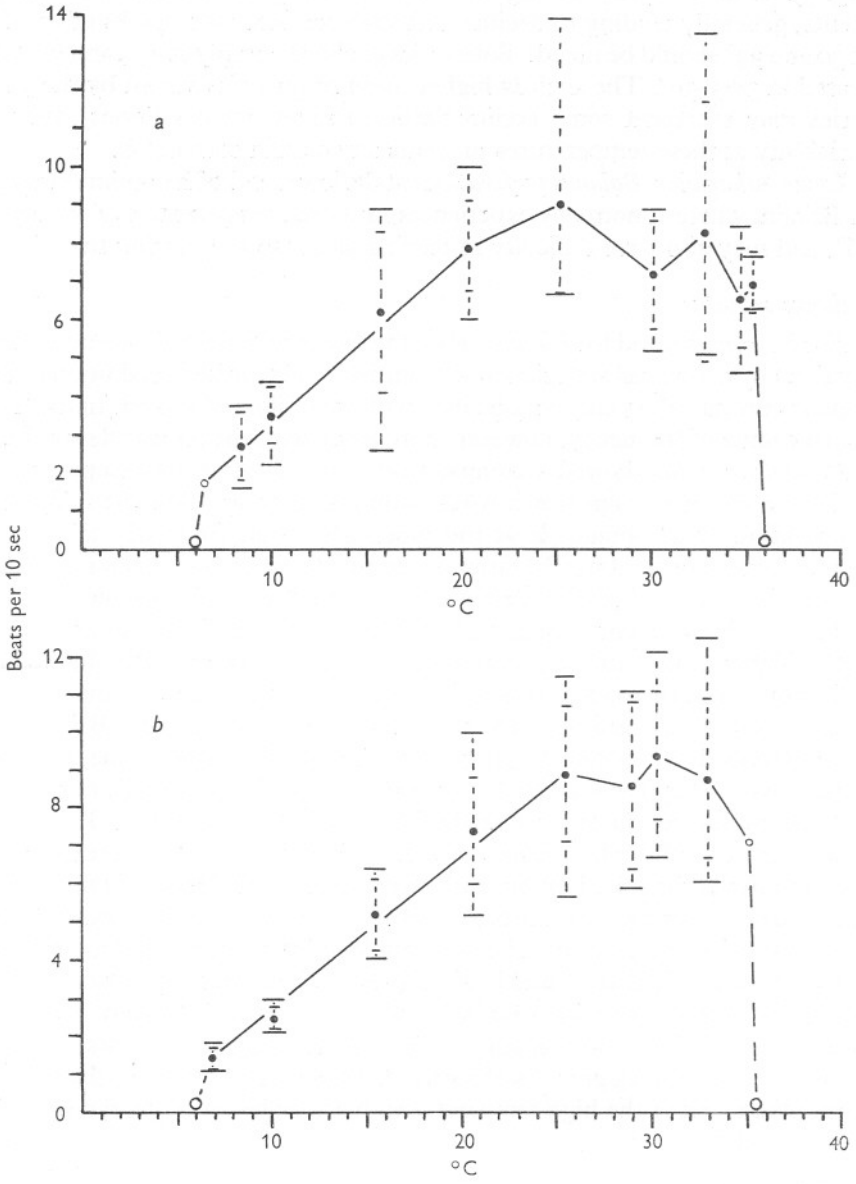

Fig. 3. The frequency and range of cirral beat of *Balanus perforatus*, (*a*) in November, (*b*) in June. For explanation of symbols refer to Fig. 2.

frequency of beating rose very rapidly to maxima of 22 beats/10 sec at 24° in February, and 18·5 beats/10 sec at 16·5° in June (Figs. 5 and 6). Beating then slowed down, declined sharply at 30° and ceased altogether at 33–35°. The difference in position of the maximum in each series is due to the two types of beat shown under apparently identical conditions. The June specimens showed a decline in the proportion of fast beat above 16°, while the February material

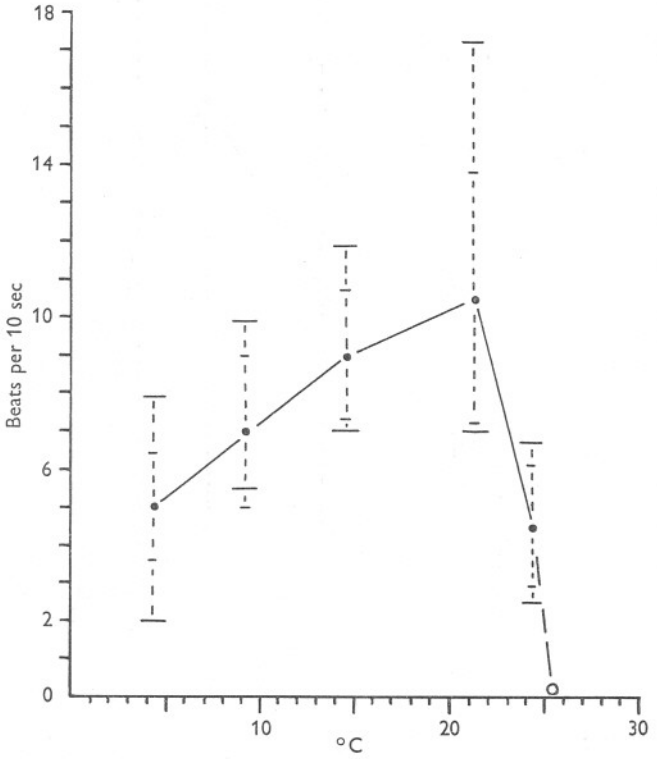

Fig. 4. The frequency and range of cirral beat of *Balanus crenatus*. For explanation of symbols refer to Fig. 2.

showed a quite high proportion of fast beat up to 24°. Although the fast and normal beats can be distinguished by appearance, numerically their frequencies overlap and are difficult to separate. The wide deviation is probably also ascribable to the occurrence of the two types of beat.

On the east coast of England *Elminius* may at times experience temperatures at the lower limit of its optimum range, and may sometimes be at a disadvantage compared with *Balanus balanoides*.

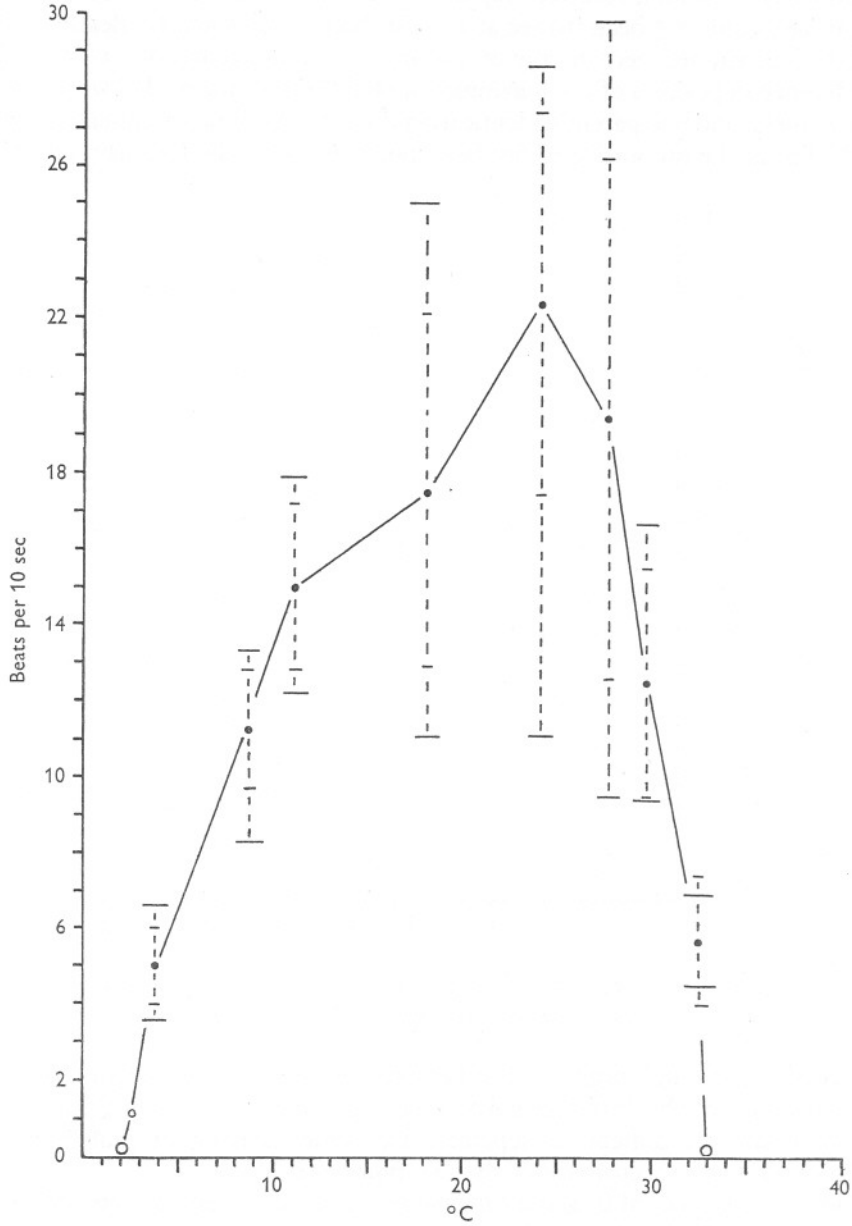

Fig. 5. The frequency and range of beat of *Elminius modestus* in February. For explanation of symbols refer to Fig. 2.

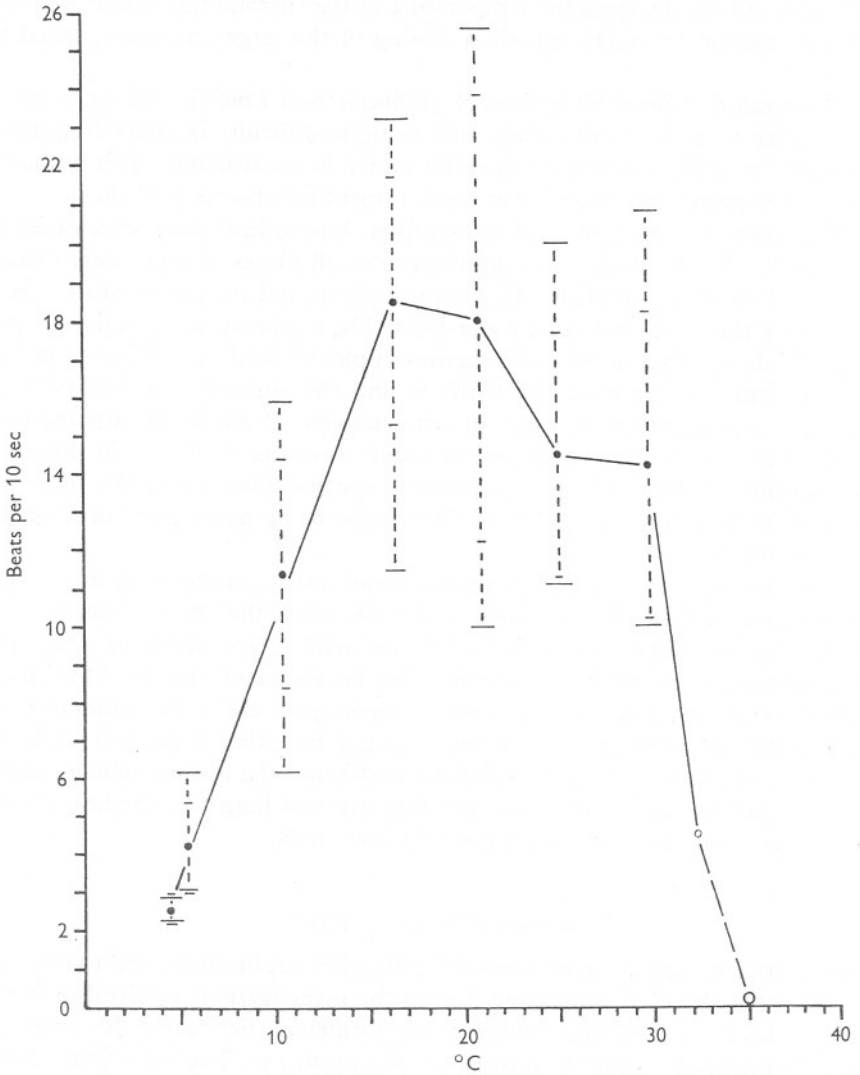

Fig. 6. The frequency and range of beat of *Elminius modestus* in June. For explanation of symbols refer to Fig. 2.

Extreme Range of Activity

A further indication of an animal's adaptation to the temperature range of its environment can sometimes be obtained by determining the upper and lower temperatures at which it loses irritability. When the point of heat coma or chill coma is reached it is reasonable to assume some temporary damage to other vital activities. These points were determined for the barnacles under investi-

gation by slowly changing the temperature of the surrounding water until the normal reaction to touch, namely a closing of the terga and scuta, failed to occur.

Chthamalus, *Balanus balanoides*, *B. perforatus* and *Elminius* did not react to touch after an hour or more at 0°, but owing to difficulty in controlling temperatures at this level more precise information is not available. It is probable that some species can resist lower temperatures for shorter periods.

The points at which 50% of the samples showed heat coma were easier to investigate. The barnacles, still attached to small pieces of rock, were placed in 500 ml. of water, aerated with compressed air, and the temperature raised by heating the vessel in a small water-bath. The temperature was raised 1° per min, and direct observations with thermocouples showed that there was no lag greater than 0·1° between the barnacle and the surrounding medium. As might be expected from its range of cirral activity, *B. balanoides* was the first of the four species investigated to succumb to coma, at 35–37° in different experiments (Table VII). The remaining species followed in the order of their upper limit for cirral activity, *Chthamalus* being noteworthy in resisting coma above 40°.

These temperatures were experienced under water; on the shore they could be experienced only while exposed to the air, when the effect of desiccation would also be present. Towards high-water mark temperatures of up to 38° have been registered by thermocouples placed in the mantle cavity of barnacles during warm weather, although lower temperatures are more common over most of the British summer. It is interesting to note that of the two southern species *Chthamalus stellatus* and *Balanus perforatus*, the former, which occurs much higher up the shore and is therefore exposed longer to the heat of the sun out of water, has the higher point of heat coma.

Reproductive Activity

Many barnacles appear to be cross-fertilizing hermaphrodites, and two of the species were observed copulating during the investigations of cirral activity. Both *Balanus crenatus* and *Elminius*, the former in February, the latter in June, continued to copulate down to 4° and up to 15°. The behaviour of the species was not affected in the least by the change in temperature, or by the temperature level within this range. Moreover, Dr Crisp informs me (personal communications) that he has observed copulation in *Balanus balanoides* at 17°, when the sea temperature in the normal habitat was about 8–10°. It appears probable, therefore, that the temperature limits for copulation, and possibly fertilization, are the same as those for cirral activity. Any limiting effect on reproduction by temperatures within these limits must operate on some other stage.

The process of copulation seems closely connected with cirral activity.

Both rhythmic beating of the cirri and penis extension are stimulated by water movement, and beating seems to be a necessary preliminary to penis extension. As observed in the course of the present work, the cirri first undergo a few rhythmic movements after stimulation, becoming more and more outstretched and stiffly-held as the penis unrolls; then both the unrolled penis and the cirri bend over towards the barnacle in the female phase and the penis is inserted into the mantle cavity of the latter. Usually the cirri are held flat, facing upwards, so that what is morphologically the ventral side of the animal is uppermost. As the penis is withdrawn and begins rolling up again, rhythmic beating recommences, although the valves may close after a few moments.

Discussion

The Range of Activity

Some of the barnacles studied show a relation between the range of temperatures over which the cirri are active and the geographical distribution of the species. For example, the two southern species *Chthamalus stellatus* and *Balanus perforatus* were active from 5° and 6° to 38° and 35° respectively, and did not show any adverse effect of heat on the frequency of beating until nearly 30°. Neither occur in places where the mean sea temperature is lower than 5° or 6° in the winter months, but both venture into the tropics to places where the summer sea temperature may exceed 25°. The northern barnacle *B. balanoides*, on the other hand, was active from below 2° up to only 31° and the frequency of beating was affected by heat at 17°. This species ranges well within the arctic circle to places where subzero sea temperatures may be experienced in winter, but in Europe it does not occur where the monthly mean sea temperature exceeds 18° in the summer.

In addition to the higher minimum temperature for cirral activity in *Chthamalus* and *Balanus perforatus*, the rate of beating (and probably of feeding) of these species was markedly lower than that of *B. balanoides* at and below 10°. In Britain *B. perforatus* is absent from, and *Chthamalus* is scarce in, those areas where the mean sea temperature is much below 10° for several months in the year (i.e. those places where the accumulated temperature deficit below 10° is more than 10 month-degrees). The evidence suggests that where these barnacles occur together and are in competition, the temperature of the water is an important factor governing the success or otherwise of the species.

The activity of the above three species, then, is related to their distribution: the same cannot at present be said for *Balanus crenatus*. The British specimens of this species, judging from their behaviour in the laboratory, are more fitted to a distribution like that of *B. balanoides*, and the supposed tropical specimens must be either a different species or a physiologically adapted race. That the behaviour of a species may vary in different geographical localities seems at

first sight affirmed by the differences shown between the British specimens of *B. balanoides* examined in the present work and some North American specimens examined by Cole, 1929 (see Table VIII). Although both the British and Maine barnacles were active below 2°, the former showed the first signs of the adverse effect of high temperature at 17°, while the Maine specimens were not affected until 23°. But, while the Maine barnacles ceased beating at 27° the British specimens carried on cirral activity to 31°. The range of monthly mean sea temperatures in Maine is about 4–17°, not different from that experienced in some parts of Britain. If the difference in range of activity of these two sets of barnacles is due to physiological adaptation, such adaptation cannot have any relation to present climatic conditions in either locality. The matter is dealt with further below.

It would be interesting to know the range of cirral activity of *Elminius modestus* in its original locality, Australasia. Although the British-bred specimens began to show the first signs of the adverse effect of heat between 20 and 25°, which agrees well with the highest monthly sea temperatures experienced in the native habitat (*ca.* 24°), they were able to carry on beating to 2°. This ability to withstand relatively low temperatures, while explaining the species success in establishing itself in eastern England and the Low countries (Hartog, 1953), does not appear to have any relation to the monthly mean sea temperatures experienced in Australasia in the winter (lowest, 7°). It is possible, therefore, that the species has acclimatized itself to European conditions.

The Rate of Activity

It is interesting that such similar animals should show so great a variation in the frequency of beating of the cirri. To some extent, as noted above, the differences are related to the geographical ranges of the species. A further part of the difference may well be an adaptation to colder or warmer temperatures than the animal experiences elsewhere in its distribution (cf. Fox, 1939), but until observations are made at other localities this cannot be determined.

Such an adaptation, however, would not seem to explain the differing frequencies of beat of the British and Maine specimens of *Balanus balanoides*. As already noted, present sea temperatures in Maine differ little from those in some parts of Britain: yet the frequency of beating of the Maine specimens was some two or three times faster, at a given temperature, than the British specimens examined in the present work (cf. Tables III and VIII). The differences in both range and frequency suggest that the two sets of barnacles are distinct varieties if not different species. It is intriguing to note that the whole pattern of behaviour of the *B. balanoides* from Maine is much more like that of the British *B. crenatus*.

In spite of the differences in frequency of beat, all the barnacles indigenous

to Britain showed a temperature coefficient (Q_{10}) of between 2 and 3 up to their points of maximum activity. The immigrant *Elminius modestus*, however, showed a coefficient as high as 5 at some temperatures. This appears to have been due to a changeover from the normal to the fast beat with increasing temperature, i.e. a behaviour coefficient superimposed on the usual coefficient of a metabolism-coupled process. Unfortunately not enough specimens were examined at the time to show whether this was definitely the case. The phenomena of two different types of beat under identical conditions is dealt with in a later paper (Southward, 1955).

Summary

The range of temperatures over which the cirri were active, and the frequency of beating of the cirri at different temperatures, were measured in five species of barnacles from the British intertidal zone. The range of temperatures at which copulation took place, and the extreme range of temperatures over which the animal remained irritable, were also investigated in some of the species.

The observed temperature range and frequencies of cirral beat differed in each species, but in the three barnacles common on the open coast were related to the geographical distribution of the particular species. The species of southern distribution, *Balanus perforatus* and *Chthamalus stellatus*, had a more rapid cirral beat at high temperatures, and continued active to much higher temperatures, than the northern species *Balanus balanoides*. Conversely, at low temperatures the latter had a greater frequency of cirral beat, and continued active to much lower temperatures, than the southern species.

The range and frequency of cirral beat of the British *B. balanoides* differed greatly from those observed by Cole (1929) for the species in North America. It is possible that the specimens belong to physiological races or even different species.

Of those barnacles more common intertidally in sheltered waters, *B. crenatus* showed an even narrower range of temperatures over which the cirri were active than did *B. balanoides*. It is suggested that the supposed tropical specimens of this species belong to a physiologically adapted race, or else previous doubts cast on the validity of the tropical records are well-founded.

The immigrant *Elminius modestus*, also more common in sheltered waters, showed a wider range of temperatures for cirral activity than *Balanus crenatus*. It was active at lower temperatures than were the native southern species, and at higher temperatures than were the native northern species, and over the whole of its range showed a much greater frequency of cirral beat than any of the native species examined. This behaviour, taken together with its great fecundity, could explain the success of *Elminius* in competition with the native barnacles.

Most of the investigations were carried out while I was holding a D.S.I.R. Senior Research Award at Plymouth; some observations at Port Erin were made while University of Liverpool Research Fellow there.

I am indebted to Dr D. J. Crisp for invaluable criticisms of the typescript, and for stimulating discussions on this work.

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TABLE II. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *CHTHAMALUS STELLATUS*

From H.W.N., Rum Bay, Plymouth, November 1953.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
4·6		Nil	
5·7	1·660	1·0– 2·2	±0·50
6·8	1·90	1·5– 2·2	±0·538
9·2	2·470	1·8– 3·1	±0·544
14·5	4·70	3·7– 5·6	±0·653
20·3	6·355	3·9– 7·9	±0·815
26·9	8·633	6·1–11·1	±1·571
30·5	10·188	7·2–12·5	±1·747
32·9	9·475	8·7–11·0	±1·10
35·7	4·750	3·0– 5·9	±1·091
37·5		Nil	

TABLE III. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *BALANUS BALANOIDES*

(*a*) From M.T.L., Port Erin, Isle of Man, 15 to 22. iv. 53. (*b*) From M.T.L., Brixham, 26. i. to 3. ii. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
(*a*) 2·3	1·833	1·8–1·9	±0·065
3·6	2·20	1·5–2·9	±0·729
4·8	2·890	2·0–3·8	±0·717
7·5	3·350	2·2–4·6	±0·779
10·7	4·433	2·9–5·5	±1·089
13·9	4·944	3·7–6·7	±1·918
18·1	6·183	4·0–9·4	±2·069
21·0	6·30	3·7–9·1	±2·608
23·5	4·933	2·3–7·2	±1·563
26·1	4·043	2·5–5·7	±1·016
30·0	3·250	2·9–3·6	±0·495
31·0		Nil	
(*b*) 1·8	1·480	1·2–1·9	±0·254
5·0	2·360	2·0–2·8	±0·324
8·2	2·666	2·1–3·3	±0·427
13·0	4·181	2·9–5·1	±0·766
15·2	5·040	3·5–5·9	±0·820
18·4	5·550	4·1–6·6	±0·950
20·5	5·290	3·9–6·7	±1·162
23·1	4·40	3·0–6·6	±1·929
24·6	3·780	3·1–5·0	±0·834
27·2	3·433	2·1–5·0	±1·470
30·0	2·40	1·9–2·9	±0·70
31·5		Nil	

TABLE IV. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *BALANUS PERFORATUS*

From L.W.N., Wembury: (*a*) 19. xi. 53; (*b*) 17. vi. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
(*a*) 6·0		Nil	
6·5	One only–1·7		
8·4	2·70	1·6– 3·8	±0·912
10·0	3·542	2·2– 4·4	±0·748
15·8	6·189	2·6– 8·9	±2·196
20·4	7·930	6·0–10·0	±1·237
25·2	9·030	6·7–13·9	±2·345
30·1	7·177	5·2– 8·9	±1·387
32·8	8·30	5·1–13·5	±3·427
34·7	6·625	4·6– 8·5	±1·307
35·4	7·014	6·3– 7·7	±0·835
36·0		Nil	
(*b*) 6·0		Nil	
6·9	1·350	1·1– 1·8	±0·309
10·1	2·450	2·1– 3·0	±0·301
15·5	5·150	4·1– 6·6	±0·892
20·6	7·425	5·2–10·0	±1·383
25·5	8·916	5·7–11·5	±1·798
29·0	8·611	6·0–11·1	±2·170
30·3	9·436	6·7–11·9	±1·664
33·2	8·850	6·0–12·5	±2·132
35·2	One only–7·1		
35·5		Nil	

TABLE V. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *BALANUS CRENATUS*

From M.L.W.N., Sutton Harbour, Plymouth, 16. iii. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
4·3	5·025	2·0– 6·9	±1·442
9·2	7·021	5·5– 9·9	±2·049
14·5	9·010	7·0–11·9	±1·729
21·3	10·530	7·0–17·2	±3·365
24·3	4·460	2·5– 6·7	±1·650
25·5		Nil	

TABLE VI. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *ELMINIUS MODESTUS*

(*a*) From M.L.W.N., Sutton Harbour and Hen Point, Plymouth 15–22. ii. 54. (*b*) From Hen Point only 22–24. vi. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
(*a*) 2·0		Nil	
2·5	One only–1·1		
3·8	5·037	3·6– 6·6	±0·973
8·7	11·166	8·3–13·3	±1·646
11·2	15·027	12·2–17·9	±2·247
18·2	17·518	11·1–25·0	±4·635
24·2	22·423	11·1–28·6	±5·020
27·8	19·431	9·5–29·8	±6·824
29·8	12·528	9·4–16·7	±3·005
32·5	5·70	4·5– 6·9	±1·697
33·0		Nil	
(*b*) 4·5	2·60	2·3– 2·9	±0·424
5·4	4·166	3·1– 6·2	±1·199
10·5	11·413	6·2–15·9	±2·993
16·5	18·472	11·5–23·2	±3·276
21·0	18·011	10·0–25·6	±5·789
25·1	14·446	11·1–20·0	±3·211
29·9	14·166	10·0–20·8	±3·960
32·4	One only–4·5		
35·0		Nil	

TABLE VII. TEMPERATURES AT WHICH IRRITABILITY WAS LOST BY 50% OF SAMPLE

Species of barnacle and tide-level	Chill coma*	Heat coma†
Chthamalus stellatus from M.H.W.N.	0°	43°
Balanus balanoides from M.T.L.	0°	35 to 37°
B. perforatus from M.L.W.N.	0°	38 to 40°
Elminius modestus from M.L.W.N.	0°	36 to 38°

* After more than an hour; owing to the difficulty in controlling temperatures at this level shorter periods could not be observed; it is possible that some species can withstand lower temperatures.

† Instantaneous, at a rate of heating of 1° per min; direct measurement with thermocouples in the mantle cavity showed no lag greater than 0·1° between the water and the barnacle.

TABLE VIII. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF SPECIMENS OF *BALANUS BALANOIDES* AT MT DESERT ISLAND, MAINE, SUMMER 1929

Recalculated from Cole, 1929.*

Temperature (° C)	Range of beat
Some activity below 2°	
5	5·6– 7·9
9	9·3–12·0
13	12·9–17·8
17	15·1–20·9
21	16·6–23·4
26	19·0–24·5
27	beating ceased

* Cole did not give any tables: these figures are derived from his graphs which were fitted to the Arrhenius equation. The abscissae are given as 'log 100 × rate' which has been assumed, on the basis of a later paper (Cole, 1932) in which some raw data is given for the same barnacles, to mean really the log of the reciprocal of the time in seconds for ten beats multiplied by a hundred.

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ON THE BEHAVIOUR OF BARNACLES

II. THE INFLUENCE OF HABITAT AND TIDE-LEVEL ON CIRRAL ACTIVITY

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During a study of the relation between temperature and cirral activity of barnacles, reported in Part I of this series (Southward, 1955), specimens for investigation were collected from those habitats and tide-levels where the particular species was most abundant. However, it is possible that some variation in behaviour may arise from local environmental factors such as tide-level and degree of exposure to wave action, and experiments were carried out to test this possibility.

The experimental conditions under which the barnacles were observed, and the methods of measuring cirral activity, were similar to those described in Part I. In all experiments the material was dealt with within 24 h of collection from the shore. Only one or two temperature levels were used to obtain temperature-frequency relations, as this was sufficient to enable a comparison of behaviour to be made between samples from different localities or tide-levels. Most frequently the temperature of the laboratory sea water supply was used since it required no regulation and remained at uniform temperatures (within 0·5 to 1·0°) for long periods.

The investigations were carried out during the first half of 1954, during the tenure of a D.S.I.R. Senior Research Award. I am grateful to Dr D. J. Crisp for help and advice in this work.

Investigations at Plymouth

Chthamalus stellatus

The work at Plymouth was confined largely to the commonest barnacle in the neighbourhood, *C. stellatus*. Specimens were first taken from two tide-levels at Tinside, below the laboratory, and examined at two temperatures. At both temperatures the two age-groups present in the sample from low water showed a 40–50% greater frequency of cirral beat than that of specimens of corresponding age from high water (Table I). This difference, when tested by the *t* distribution, was found to be significant in comparison with the individual variation (*P* less than 0·1% that the samples from the two tide-levels came from the same population).

Subsequently, however, specimens were taken from high and low tide levels at Wembury, and similarly compared. In this case there was very little difference in the frequency of cirral beat, and the slight differences that were observed were not statistically significant (Table II). The experiments were

TABLE I. FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *CHTHAMALUS STELLATUS*

From two different tide-levels at Tinside, Plymouth, 15. xii. 53.

Temperature (° C)	Year-group	Tide-level	No. in sample	Frequency			
				Mean	Range	S.D.	*t*
13·8	1+	M.H.W.S.	6	3·516	2·2–4·5	0·893	3·33
		M.L.W.N.	12	5·466	3·8–8·4	1·205	
	2+	M.H.W.S.	9	3·188	2·3–3·7	0·459	6·36
		M.L.W.N.	10	5·810	4·1–8·1	1·184	
20·4	1+	M.H.W.S.	8	5·962	4·5–7·4	0·976	7·65
		M.L.W.N.	20	9·725	7·8–12·1	1·240	
	2+	M.H.W.S.	9	4·822	3·7–6·1	0·882	4·78
		M.L.W.N.	12	8·350	6·4–10·2	1·389	

TABLE II. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *CHTHAMALUS STELLATUS*, APPROXIMATELY 1½ YEARS OLD

From two different tide-levels at Wembury, near Plymouth.

Date	Temperature (° C)	Tide-level	No. in sample	Frequency			
				Mean	Range	S.D.	*t*
22. i. 54	20·4	M.H.W.S.	9	5·220	3·8–6·6	0·788	1·07
		M.L.W.N.	10	5·630	4·3–7·2	0·869	
19. ii. 54	19·6	M.H.W.S.	7	5·185	4·2–7·3	1·083	1·21
		M.L.W.N.	8	5·837	4·4–7·1	0·985	

TABLE III. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *CHTHAMALUS STELLATUS*, APPROXIMATELY 1 YEAR OLD

From two different tide-levels at two places in Plymouth Sound, 1–2. vi. 54, temperature 14·9° C.

Place	Tide-level	No. in sample	Frequency			
			Mean	Range	S.D.	*t*
Tinside	M.H.W.S.	9	6·355	5·3–7·7	0·705	3·03
	M.L.W.N.	10	7·780	6·3–9·5	1·014	
Rum Bay	M.H.W.S.	17	6·576	4·9–8·9	1·368	0·45
	M.L.W.N.	10	6·370	5·6–7·2	0·566	

therefore repeated on some more specimens from Tinside, and simultaneously observations were made on barnacles from Rum Bay, a nearby site in Plymouth Sound (Table III). Again the specimens from L.W.N. at Tinside had a significantly faster cirral beat than those from high water, while there was no significant difference between the two sets of barnacles from Rum Bay.

The matter rests there at the moment. It can only be suggested that the *Chthamalus* at low water at Tinside are anomalous compared with those from all other levels and localities, but the reason for this anomaly is not clear.

Elminius modestus

Further tests were made on another barnacle, *E. modestus*, which at Plymouth is common only in the estuaries. Specimens under 1 year old showed a significant difference in cirral beat between individuals from different tide-levels (Table IV). Those from low water had a mean frequency of beat some 20% greater than those living at high water. The older age groups of this species were not tested, as the older specimens among the high water sample could not be induced to beat regularly under laboratory conditions.

TABLE IV. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *ELMINIUS MODESTUS*, UNDER 1 YEAR OLD

From two different tide-levels at Hen Point, near Plymouth, 22. ii. 54, temperature 12·4° C.

Tide-level	No. in sample	Frequency			
		Mean	Range	S.D.	*t*
M.H.W.N.	6	15·350	13·3–16·7	1·126	3·10
M.L.W.N.	9	18·200	15·6–21·8	1·857	

TABLE V. OBSERVATIONS ON *CHTHAMALUS STELLATUS* FROM M.H.W.N., HEN POINT (TAMAR ESTUARY), 23. vi. 54, TEMPERATURE 16·6°

Age group, in years	Percentage of total sample showing rhythmic beating of cirri	Mean frequency of beating, as beats/10 sec
1	50	8·066
2	30	6·433
3 and over	12	5·200

Effect of Age

The difficulty of obtaining regular beating from the older barnacles was frequently encountered during the work described in Part I. The irregular behaviour of the older barnacles seems to be linked with their generally lower mean frequency of cirral beat (see Table I). Some comparative observations showed a decrease, with increasing age, of both the frequency of beat and the proportions of the sample regularly beating (Table V). The two phenomena are probably characteristic of the ageing process in barnacles.

Unfortunately it is not possible to determine critically the effect of age on cirral activity without more exact information of the age of the barnacles examined. The age-groups given here were separated only by estimation, based on experience of the barnacle in question, its size at settlement and its known rate of growth. Although this was sufficient to show that the younger

barnacles have more lasting and frequent cirral activity, further investigation with barnacles of definite age is clearly desirable.

The work at Plymouth indicated that habitat variations might have some influence on the cirral activity of barnacles. It was therefore arranged to continue the work in the Anglesey area (from the Marine Biology Station at Menai Bridge), where a greater variety of habitats were within easy reach, and where the other common British intertidal barnacle, *Balanus balanoides*, was plentiful.

Investigations at Menai Bridge

The experimental conditions at Menai Bridge were similar to those at Plymouth. The water currents used in the apparatus, in addition to a regular flow of water through it, were set up by a jet of compressed air instead of a paddle wheel.

Table VI. The Frequency of Beating of the Cirri, as Beats/10 sec, of two Species of Barnacles

From two different tide-levels at Criccieth, 26. iv. 54, temperature 10·4° C.

Species	Approximate age (years)	Tide-level	No.	Frequency			
				Mean	Range	S.D.	*t*
Chthamalus stellatus	1½	M.H.W.N.	10	2·970	2·1–4·2	0·602	0·72
		M.L.W.N.	10	2·950	1·9–3·7	0·632	
Balanus balanoides	1	M.H.W.N.	20	3·155	2·1–4·1	0·584	12·53
		M.L.W.N.	20	4·705	3·9–6·0	0·664	

Effect of Tide-Level

Specimens of *Chthamalus stellatus* and *Balanus balanoides* were collected from two tide-levels at the north end of Cardigan Bay, and examined the same day. As can be seen from Table VI, there was no significant difference in the frequency of beat of the *Chthamalus* from the two tide-levels. In the case of *Balanus balanoides*, however, the specimens from low water had a markedly faster cirral beat than those from higher up the shore. This difference in behaviour of two species living side by side under identical conditions is surprising, in view of the great similarity, at the temperature used, of the frequency of beat of the high water individuals of both barnacles. It is interesting to note that, again at this temperature, the low water *B. balanoides* would probably be more successful in obtaining food than the *Chthamalus* at the same level.

Further experiments on the same barnacles during subsequent days showed a change in behaviour. After the specimens had been kept for 24 h in running sea water from the laboratory supply the former difference between the high and low water *Balanus balanoides* disappeared, the mean frequencies being, at 18°: high water, 7·45; low water, 7·72. The corresponding frequencies for *Chthamalus* at 17° were: high water, 5·17; low water, 4·85. The similarity

between the two sets of *Balanus balanoides* was found again the following day, when the mean frequencies at 10·4° were: high water, 5·62; low water, 5·56, Comparison with Table VI shows that both the high-water and the low-water samples of *B. balanoides* had increased their frequency of beat after only 3 days in the laboratory, but that the increase was greater among the individuals from the higher tide-level.

These experiments serve to show the extent of acclimatization that may occur. It is not known whether the change in the frequency of beating was a reaction to some quality of the laboratory water (temperature, salinity, pH, silt, etc.), or merely to the continued immersion. Cole (1929, 1932) found no change in the frequency of beat of continuously immersed *B. balanoides* at Mt Desert Island, Maine, but, as noted in Part I, there are important differences in the behaviour of the British and American specimens of this species.

Effect of Habitat

Some preliminary experiments suggested that the fast type of beat (see Part I) was more commonly shown by individuals from sheltered habitats, than by those living on the open coast. A full investigation was therefore undertaken with *B. balanoides*, by collecting a series of samples from four different habitats on the same day. This was possible since the variation in the time of low water around Anglesey permits several localities to be visited on one tide. To avoid acclimatization changes the samples were kept out of water at the laboratory, in a cool place, and examined within 24 h. The measurements of the cirral beat were made at approximately the time of high water the following day, to minimize any inherent tidal rhythm of the animals. Before counting the frequency of beating each sample of barnacles was allowed 30 min in water to eject the air that is often present in the mantle cavity, and to settle down to regular beating. The numbers showing fast beat, normal beat, and those closed or irregular were then noted (Table VII).

It was found that there was invariably a larger proportion of closed or non-beating individuals among the specimens from high water compared with those from lower down on the shore. Furthermore, a much lower percentage of rhythmically beating individuals occurred among the barnacles from the open coast site exposed to wave action than was found at the three sheltered localities. This was evident in samples from both tide-levels. This difference may be habitual, for those barnacles living at high water may be used to rhythmic beating only for short periods when covered by the tide. Individuals from a wave beaten place may also be unused to beating continuously for a different reason. If the continuous water movement resulting from wave action brought an abundant supply of food and oxygen to the barnacles, only limited periods of cirral activity might be necessary for normal growth and respiration.

TABLE VII. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, AND THE PROPORTION OF INDIVIDUALS ACTIVE, OF SAMPLES OF APPROXIMATELY 1 YEAR OLD *BALANUS BALANOIDES*

From different tide-levels and habitats, Anglesey, 30. iv. 54

Locality, and type of habitat	Temperature °C	Tide-level	Fast beat					Ordinary beat					% closed or not beating
			% of sample	No. examined	Frequency			% of sample	No. examined	Frequency			
					Mean	Range	S.D.			Mean	Range	S.D.	
Aberffraw, open coast exposed to wave-action	13·0	M.H.W.N.	0	—	—	—	—	55	8	3·975	2·6–5·9	1·149	45
		M.L.W.N.	0	—	—	—	—	64	10	5·010	3·9–6·9	0·898	36
Aberffraw, open coast sheltered from wave-action	12·2	M.H.W.N.	0	—	—	—	—	81	11	3·127	2·5–4·1	0·735	19
		M.L.W.N.	2	—	One only 13·3			87	12	5·950	3·1–9·8	2·153	11
Menai straits, exposed to currents	12·6	M.H.W.N.	3	—	One only 10·2			82	10	2·930	1·9–4·4	0·707	15
		M.L.W.N.	3	—	One only 10·8			86	10	6·010	4·2–7·4	1·275	11
Menai straits, sheltered from currents	11·6	M.H.W.N.	68	10	15·560	13·9–16·7	0·938	12	3	9·400	7·7–10·5	1·493	20
		M.L.W.N.	0	—	—	—	—	89	10	5·640	4·5–8·3	1·153	11

Note: the number examined is not the number on which the percentages are based, except in one case.

Turning to the mean frequency of the normal cirral beat shown by these samples (Table VII, col. 11) it can be seen that, except for the specimens from the sheltered site without currents, the low-water samples had a mean 30–100% greater than that of the high-water individuals. The least difference was shown by the barnacles from the wave beaten locality at Aberffraw, and the greatest by those from the site in the Menai Straits exposed to currents.

The fast type of beat was not shown by any individuals from the wave-beaten site, and only one fast-beating specimen occurred in the sample from the sheltered open coast site, and two among those from the site with currents. However, among those individuals from the site without wave action or currents, a remarkably high proportion of fast-beating specimens was found in the high-water group. At low water in the same place not a single fast-beating specimen was obtained. On ecological grounds it seems possible that the fast type of beat could, in this case, be an adaptation to life in comparatively still water, a replacement of the natural movement of water over the cirri found in the other habitats. It might only be found regularly among barnacles from high water, since these would have the greatest need of such an adaptation on account of the short time available for feeding and respiratory exchange.

Effect of Oxygen Content

If respiratory need were the driving factor in stimulating the fast type of cirral beat, it should be possible to investigate the relation by varying the oxygen content of the medium, and recording the effect on the type and frequency of beat. Specimens of *B. balanoides* together with *Chthamalus stellatus* for comparison were observed in a small dish holding about 200 ml. water with a lid ground on and sealed with vaseline. Gases were led in through calibrated flowmeters of the manometer type, and bubbled through the water in a manner that caused currents to be set up. Observations were made at regular intervals, the flow of gas being turned off momentarily to arrest the water currents. The temperature was kept fairly constant by immersing the dish in a trough of running tap water.

The effects of lowering the oxygen content by bubbling nitrogen through the water, and of increasing the oxygen content by bubbling that gas through the water, are given in Table VIII, for individual specimens of *Balanus balanoides* and *Chthamalus*. It should be noted that, as the supply of nitrogen and oxygen did not contain any carbon dioxide, the latter may have been driven off from the water. The loss of some of the carbon dioxide, and the resulting decrease in the hydrogen-ion concentration, may have had some influence on the behaviour of the barnacles. The results can only be accepted as indications of responses to variations in the oxygen content on the assumption that the possible changes of carbon dioxide content and pH had a negligible effect. Previous work (Roaf, 1912; Fox & Johnson, 1934) suggests that

the effects of a decrease in the carbon dioxide content and hydrogen-ion concentration are indeed negligible, though an *increase* may influence the cirral beat.

From the table it can be seen that lowering the oxygen content of the water caused a reduction in the cirral activity of both fast and normally beating specimens of *Balanus balanoides*, and had a similar effect on *Chthamalus*. In

TABLE VIII. THE EFFECT OF VARYING THE OXYGEN CONTENT OF THE WATER ON THE FREQUENCY AND TYPE OF CIRRAL BEAT OF INDIVIDUAL BARNACLES, 28–29 iv. 54

A. Oxygen decreased by passing nitrogen through the water at 6 ml./min; temperature 13° C.

Time in min since start of N_2 bubbling	Frequency of individual specimens*											
	Balanus balanoides								*Chthamalus stellatus*			
	From Menai Straits				From Criccieth				From Criccieth			
	a	*b*	*c*†	*d*†	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
0	4·3	3·6	14·1	13·0	3·7	5·0	3·1	4·2	2·6	3·7	3·1	2·9
15	—	—	4·8	4·9	4·1	4·5	—	3·2	2·9	3·0	1·6	4·1
30	G	G	G	G	2·7	2·3	G	G	G	G	0·5	G

B. Oxygen increased by passing the gas through the water at 6 ml./min, temperature 13° C.

Time in min since start	Frequency of individual specimens*												
	Balanus balanoides								*Chthamalus stellatus*				
	From Menai Straits				From Criccieth				From Criccieth				
	w†	*x*†	*y*†	*z*†	*w*	*x*	*y*	*z*	*v*	*w*	*x*	*y*	*z*
0	17·9	11·9	13·9	14·1	3·8	3·5	3·8	3·5	2·1	1·7	2·8	1·3	1·8
15	18·5	C	14·3	9·9	2·3	2·7	2·2	1·8	1·7	1·7	2·6	E	3·2
30	19·3	C	13·7	C	2·3	3·1	2·4	1·1	2·8	4·7	3·3	3·8	4·1
45	18·5	C	C	C	2·3	2·9	2·6	C	2·7	3·7	4·0	E	4·2
	Only one out of 17 of this group open												

Notes. C, terga and scuta tightly closed; G, terga and scuta open, cirri inactive, and in some, protruding; E, cirri remaining extended for longer than usual during open phase of beat.

* As beats per 10 sec.

† Specimens showing the fast type of beat.

each case, the first response was a slowing down of the frequency of beating, followed by a cessation of beating altogether. At the end of the experiments practically all specimens had the terga and scuta slightly open, often with the cirri protruding. This reaction can be termed the gaping response, and probably indicates acute respiratory difficulties (cf. Fox & Johnson, 1934). It will be noted that before the fast-beating specimens became inactive they changed over to the normal type and frequency of beat. From this it might be inferred that the fast type of beat is not related to respiratory need, but it must be remembered that the fast beat in itself may utilize more oxygen than the

normal type, and may thus be the first to be inhibited by severe oxygen lack.

The two species of barnacles reacted differently to an increase in the oxygen content of the water. In five specimens of *Chthamalus* the mean frequency of beating increased from 1·94 to 3·75 beats/10 sec, a gain of nearly 90%. The frequency of *Balanus balanoides*, on the other hand, slowed down, the mean of the normal beat decreasing from 3·6 to 2·6 beats/10 sec, while all but one of the specimens showing the fast type of beat gave the closing response.

The more rapid beat of *Chthamalus* in oxygen-enriched water suggests that the species is adapted to an oxygen content higher than that of the laboratory sea water supply, which may not have been fully saturated with air. In Britain this barnacle is most abundant in wave-beaten places, where the water will nearly always be fully saturated. The cessation or slowing down of both types of beat in *Balanus balanoides* suggests that in these specimens oxygen lack was the main driving factor behind cirral activity. From this assumption it could be argued that the fast type of beat was in fact respiratory in function.

The problem of the fast type of beat therefore remains unsolved by these experiments. It is hoped that it will be dealt with again in a later paper in this series.

Discussion

The studies described here and in Part I take no account of possible inherent diurnal rhythms in the behaviour of barnacles. Diurnal rhythms, generally related to the tidal cycle, have been shown to occur in the rate of feeding, respiration, and other processes in many intertidal (and sublittoral) animals, and may persist even after periods of continuous immersion in the laboratory (literature in Rao, 1954). Although during the present work no signs of diurnal variations in behaviour were noticed, the possibility of their existence in barnacles cannot be discounted. However, in the investigations into the effects of tide-level and habitat on cirral activity, any diurnal rhythms would not have affected the comparisons of different samples of barnacles, as the latter were taken practically simultaneously, treated identically in the laboratory, and examined at about the same time of day and period of tidal cycle.

Segal, Rao & James (1953) have shown differences in the rate of activity of limpets and mussels from different tide-levels in California. They suggest that the differences are related to similar differences found in examples of the species from different latitudes, and that the greater activity, at a given temperature, of the forms from low water is comparable to the greater activity of cold-adapted forms from higher latitudes. This is, of course, possible, since animals living at low water in relatively warm latitudes, and exposed to the air for only a short time, will have a more uniform and cooler environment than those at high water that are exposed to the heat of the sun and air for longer periods. But it must be noted that, except for activities such as heart

beat, most of the other activities of an intertidal animal (feeding, movement of feeding or respiratory appendages, gaseous exchange) take place mainly under water, when the conditions governing the high- and low-water individuals will be almost identical.

However, at first sight the suggestion of Segal *et al.* might seem to explain the greater cirral activity of the British specimens of *B. balanoides* from low water. As was shown in Part I, this barnacle is at its southern limits in Britain, and its behaviour here must represent its extreme limit of adaptation to higher temperatures, i.e. there is probably ample scope for cold-adaptation by those forms living at low water. At the same time the absence, except in one instance, of any difference in the behaviour of *Chthamalus* from different tide-levels could be due to this species having already, at high water, reached its limit of adaptation to low temperature.

Attractive as this explanation might seem, since it would link up with the restriction of *Chthamalus* to high water and of *Balanus balanoides* to lower down the shore at their respective extreme limits of distribution, it does not fit all the observed facts. The differences in the behaviour of barnacles from different habitats, and the acclimatization that can occur in the laboratory, suggest that, in these animals, the tidal differences are not entirely a matter of temperature. It is well known that the rate of growth of intertidal animals is slower at high water than further down the shore, and that correspondingly their life is longer at high water. It seems possible that the whole life and vital activities of the animal living at high water might be keyed to the slower tempo, and that not only growth, but rate of feeding, respiration, and other processes would therefore be slower than at low water.

Finally, it seems worthwhile pointing out the need for caution in comparing the behaviour or activity of intertidal animals from different tide-levels or latitudes until the effect of other habitat variations have been thoroughly investigated.

Summary

The frequency of beating, and other aspects of cirral activity, were observed in samples of three species of intertidal barnacles taken from various habitats and from different tide-levels.

At Plymouth a more rapid beat was found among individuals of *Chthamalus stellatus* from low water at one locality than among those from high water at the same place. At two other localities, however, there was no significant difference in behaviour between samples from high and low water. A difference was found in samples of *Elminius modestus*, those from low water having a more rapid cirral beat than those from high water.

There was again no difference in the frequency of beating of the cirri of *Chthamalus* collected near Anglesey. Samples of *Balanus balanoides* from the same district, however, with one exception, showed a greater frequency of beat

among those individuals from low water, and had more regular cirral activity, than individuals from high water. Among the specimens from the exceptional locality, which was sheltered from wave action and currents, nearly all the high-water individuals showed the fast type of beat, while the low-water individuals showed only the normal beat.

It was suggested that the fast type of beat was an adaptation to assist feeding or respiration in still water. Experiments with varying oxygen content of the water failed to show whether or not the adaptation was respiratory.

The work suggests that the older a barnacle becomes, the slower is its cirral beat, and the less often it shows cirral activity.

The difference in activity of animals from high- and low-tide levels may be related as much to the difference in rate of growth, and possibly of general metabolism, as to any adaptation to temperature differences between the two levels.

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A SUGGESTED METHOD FOR THE ASSAY OF VITAMIN B_{12} IN SEA WATER

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(Text-figs. 1–3)

One of the difficulties encountered in the use of fresh water organisms for the assay of vitamin B_{12} of marine origin is the elimination of salt from the extracts. This has suggested that a marine organism might be used with advantage. Indeed on several occasions marine or supra-littoral species have been proposed as possible assay subjects, but acceptable techniques await development (Lewin, 1954; Droop, 1954; Sweeney, 1954). *Monochrysis lutheri*, a supra-littoral euryhaline species, has advantages over others in being small, robust and giving heavy yields in synthetic media. The method employed in some *Monochrysis* assays carried out in the early spring of 1954 will be described.

Principle

If a sample of sea water to be assayed, containing Z mμg B_{12}/l., is divided into portions A and B and A is diluted r times with a B_{12}-less synthetic seawater substitute, the difference in B_{12} content of the two preparations then is

$$Z\frac{r-1}{r}. \tag{1}$$

If, now, each of these preparations is given the same graded doses of B_{12} the regressions of the responses so obtained can be compared and the value of Z ascertained. In Fig. 1

$$Z=\frac{rM}{r-1}. \tag{2}$$

For a parallel line method, such as this, the doses require to lie on that region of the response curve where linear increase of dose causes linear increase of yield measurements. When the optical measurement used is per cent absorption, this region is between doses of 3 and 18 mμg B_{12}/l. for *Monochrysis*.

The choice of three dose levels, say 3, 6, 9 mμg B_{12}/l., with replications, gives a symmetrical six-point layout which can be treated statistically in the manner described by Finney (1952) for parallel line assays. That is, tests can be made for 'regression', 'difference between preparations', 'linearity' and

'divergence from parallelism', and fiducal limits of the estimate can be calculated.

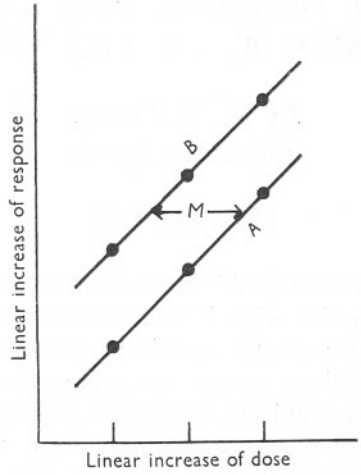

Fig. 1. Diagram of linear parallel line assay. Linear increase of response (vertical) against dose (horizontal) for preparations *A* and *B*. *M* (measured horizontally), difference between regressions in terms of dose.

Preliminary Assay

When the expected value of Z is entirely unknown it is necessary to perform a preliminary assay in order to ensure a convenient value for M. (M can be reduced by diluting the whole sample.) A very rough idea of Z can be obtained by adding to a pilot sample the ingredients (less the water) of the nutrient solution given on p. 437, and comparing the growth obtained with typical *Monochrysis* dose response curves (Fig. 3; Droop, 1954). For expected values of Z up to 10 mμg/l. no dilution of the original sample is necessary other than the twofold one performed by the addition of the nutrient solution (see below). But for larger expected values of Z a proportional further dilution of the original sample should be made.

A value of r of less than 3 is too small for convenience and one much greater tends to cause a lack of parallelism between the two regressions. This is occasioned by the fact that autoclaved enriched natural sea water is not as favourable a medium as the enriched synthetic sea-water.

Experimental

For the detailed assay a sample of 200 ml. is large enough. The predetermined initial dilution is made if necessary and the sample is divided in the

ratio 3:1. The smaller portion is then diluted threefold. Dilutions are effected with the following freshly prepared artificial sea water:

NaCl	30 g
$MgCl_26H_2O$	5 g
KCl	0·75 g
$CaSO_4$	1 g
H_2O (glass distilled) to	1 l.

The two portions are then mixed with their own volumes of the following freshly prepared nutrient solution and the pH adjusted to 7·5:

KNO_3	200 mg
K_2HPO_4	20 mg
Thiamin	1 mg
'T.M.2'[1]	20 ml.
'S.W.2'[1]	10 ml.

Tris-(hydroxymethyl)-aminomethane 1 g.
Glass distilled water to 1 l.

There are now two solutions, one (*A*) containing one-third as much of the original sample as the other (*B*). These solutions are then decanted into test-tubes in 6 ml. aliquots and vitamin B_{12} added as follows:

18 $\mu\mu$g	to each of	four	tubes	containing	solution *A*
18 $\mu\mu$g	,,	,,	,,	,,	*B*
36 $\mu\mu$g	,,	,,	,,	,,	*A*
36 $\mu\mu$g	,,	,,	,,	,,	*B*
54 $\mu\mu$g	,,	,,	,,	,,	*A*
54 $\mu\mu$g	,,	,,	,,	,,	*B*

The tubes are plugged and autoclaved for a few moments at 15 lb. and inoculated with 0·02 ml. of a stock culture of *M. lutheri* and incubated at room temperature (16–22° C.) in continuous 'warm-white' fluorescent light of 100 ft. candles until growth is completed.

Example Assay

All assays of samples of surface water taken in February and March 1954 from Keppel Pier, Millport, on incoming tides, gave results lying between 5 and 10 mμg B_{12}/l. A single example will, therefore, be sufficient to illustrate the method.

Preliminary experiments had suggested that the water was unlikely to yield more than 10 mμg B_{12}/l., so preliminary overall dilutions were not necessary.

The sample, taken from Keppel on 5 April 1954, was assayed immediately.

[1] For compositions of these metal solutions see Droop (1955).

The results are shown in Table I and Fig. 2. Inspection suggests that there is a difference of $M=2{\cdot}4$ mμg/l. between the two regressions.

TABLE 1. ASSAY OF VITAMIN B_{12} IN SEA WATER

The sample is attenuated by a factor of 6 in preparation *A* and one of 2 in preparation *B*.

	Dose 3 mμg/l. B_{12}				Dose 6 mμg/l. B_{12}				Dose 9 mμg/l. B_{12}			
A	30	33	26	36	43	48	42	36	63	54	50	57
B	42	44	34	41	59	54	48	52	70	61	62	67

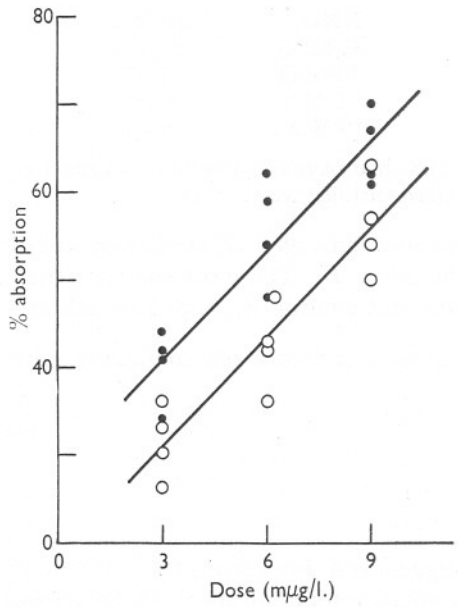

Fig. 2. Assay of Keppel Water, 5 April 1954. Dose of vitamin B_{12} in mμg/l.

$$Z=\frac{rM}{r-1}d, \tag{3}$$

where d represents total overall dilutions, including that effected on addition of the nutrients. Substituting,

$$Z=\frac{3\times 2{\cdot}4}{3-1}\times 2$$

$$=7{\cdot}2.$$

The calculated value of M is obtained from the equation

$$M=\frac{\bar{y}_B-\bar{y}_A}{b}-(\bar{x}_B-\bar{x}_A),$$

where $b=\frac{\Sigma S_{xy}}{\Sigma S_{xx}}$ (Finney, 1952), which when evaluated from the results set out in Table I, gives $M=2{\cdot}344$.

In the symmetrical assay both the calculation of M and the arithmetic for the analysis of variance are much simplified by making use of orthogonal contrasts afforded by the layout as described by Finney (1952).

The analysis of variance of the example, set out in Table II, shows that the assay is satisfactory as far as 'difference between preparations', 'regression' and 'linearity' are concerned, but the variance due to 'deviations from parallelism' is too small.

TABLE II. ASSAY OF VITAMIN B_{12} IN SEA WATER. ANALYSIS OF VARIANCE.

Source	D.F.	Sums of squares	Mean square
Preparation	1	560·7	560·7
Regression	1	2450·3	2450·3
Parallelism	1	0·0	0·0
Quadratic	1	0·75	0·75
Difference of quadratics	1	5·3	5·3
Between doses	5	3017·1	603·4
Error	18	290·9	16·2
Total	23	3308	

The 95% fiducal limits to M are obtained by evaluating

$$\left[M-\bar{x}_A+\bar{x}_B\pm\frac{ts}{b}\left\{(1-g)\left(\frac{1}{N_A}+\frac{1}{N_B}\right)+\frac{(M-\bar{x}_A+\bar{x}_B)^2}{\Sigma S_{xx}}\right\}^{\frac{1}{2}}\right]\div(1-g),$$

where $g=\frac{t^2s^2}{b^2\Sigma S_{xx}}$ and the value of t for a 0·05 probability and 18 degrees of freedom is taken as 2·101 (Finney, 1952).

The limits to M are thus 3·357 and 1·471. Substituting in (3) we get for the B_{12} estimate of the sample: 7·03 mμg/l. with limits 10·07 and 4·41.

DISCUSSION

Monochrysis responds to pseudo-B_{12}, and factor A but not to factor B (Fig. 3) so that its specificity can be compared to that of *Lactobacillus leichmannii*.

The assay method described here has some advantages and some obvious disadvantages. It is reasonably sensitive and admits of statistical treatment, and its design is such as to eliminate all carry over effects, but its greatest advantage is that it is direct in the sense that it involves no extraction procedures which require much time and large samples and, moreover, are possibly subject to errors due to loss of the vitamin. Desalting methods (Provasoli & Pintner, 1953) are at present subject to similar errors. However, the advantages of the *Monochrysis* method are offset by the length of time taken for the assay to complete. *Monochrysis* is an obligate phototroph with a generation time of about 24 h, with the consequence that cultures take about 3 weeks to

mature. It is, therefore, certain that the *Monochrysis* method would never be used with advantage for routine purposes, though the possibility of its being useful in some instances is envisaged.

Monochrysis is one of many B_{12}-requiring marine phytoflagellates which can be handled in a similar manner. A number of such species with differing specificity patterns employed concurrently with the different forms of the vitamin could provisionally yield useful information of the B_{12} content of sea water. *Prymnesium parvum* or *Syracosphaera elongata*, for instance, are as specific as *Ochromonas* in their response.

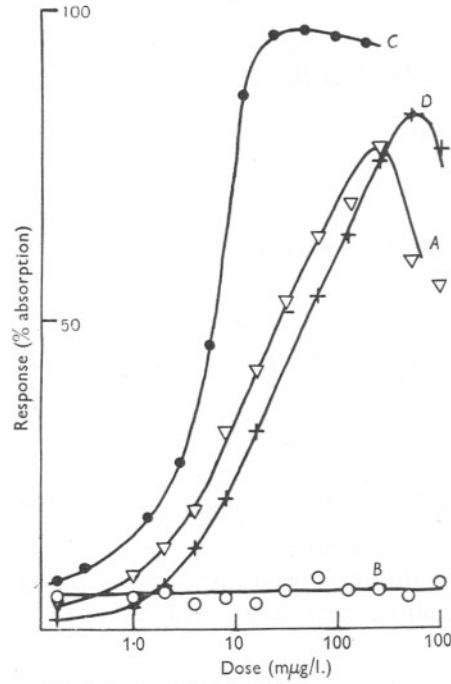

Fig. 3. Response of *M. lutheri* to vitamin B_{12} (curve *c*), factor *A*, pseudo-vitamin B_{12} (curve *D*), and factor *B* in presence of adenine.

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THE CYPHONAUTES LARVAE OF THE PLYMOUTH AREA AND THE METAMORPHOSIS OF *MEMBRANIPORA MEMBRANACEA* (L.)

By D. Atkins, D.Sc.
From the Plymouth Laboratory

(Text-figs. 1–4)

Several cyphonautes larvae (Polyzoa Ectoprocta) have been distinguished and described (Lohmann, 1911; Marcus, 1940; Thorson, 1946), but few have been seen to metamorphose, thus linking the late larva with the adult. Three, belonging to the suborder Cheilostomata, which have been observed to do so, are *Cyphonautes compressus* into *Electra pilosa* L. (Schneider, 1869; Barrois, 1877; Kupelwieser, 1905–6), *Cyphonautes occidentalis* into *Membranipora villosa*, (Robertson, 1908; O'Donoghue, 1927) and an unnamed cyphonautes into *Nichtina* (= *Membranipora*) *tuberculata* (Hastings, 1929, pp. 706–7).[1]

Prouho (1892) found *Alcyonidium albidum* and *Hypophorella expansa* (Ctenostomata) to have cyphonautes larvae, but was unable to rear them to the stage when pyriform and adhesive organs were present, so that the appearance of the late larvae of these two species is unknown. He also reared the cyphonautes of *Electra pilosa* to a young stage, and took later larvae from the plankton (pl. 30, fig. 92). *Alcyonidium albidum* has not been recorded in the Plymouth area; *Hypophorella expansa* has been recorded (Marine Biological Association, 1931, p. 289), but the cyphonautes has not yet been recognized.

During work on the ciliary feeding mechanism of the cyphonautes larva (Atkins, 1955), two species were taken from the plankton in the Plymouth area, one large and one small, and both seen to metamorphose and give rise to colonies.

Inside Plymouth breakwater the common form in the tow-nets throughout 1953 was a small, rather opaque, cyphonautes, which developed into *Electra pilosa* L. and is evidently that known as *Cyphonautes compressus*.[2] It is about 440 μ broad at the base and 360 μ high when ready to metamorphose;

[1] For discussions on the validity of *Membranipora* as the name for the genus see Borg (1931) and Osburn (1950).

[2] In the autumn of 1953 a small number of the larvae of *Electra pilosa* were seen to be parasitized by young stages of a species of *Endodictyon*, probably close to *E. infestans* Gran, which is known to be endozoic in species of *Alcyonidium* (Newton, 1931, p. 128). It would seem probable that the colonies of *Electra pilosa* are parasitized and that the larvae carry the infection. The identification was kindly made by Dr M. Parke.

metamorphosis may, however, occur when the shell is only about 390 μ broad. Young forms with inhalant and exhalant chambers fully formed are no more than 160 μ broad and 130 μ high. The valves of the late larva are of a brownish tint, but are without ornamentation along their basal edges. Fig. 1 shows change in shape of the larva of *Electra pilosa* with growth. These stages have been taken from the plankton and not reared, but as there is considerable difference in size and transparency between the large cyphonautes and the larva of *E. pilosa* the young stages of the two are unlikely to be confounded and the late stages never (cf. Figs. 1 and 3 which are reproduced at the same magnification).

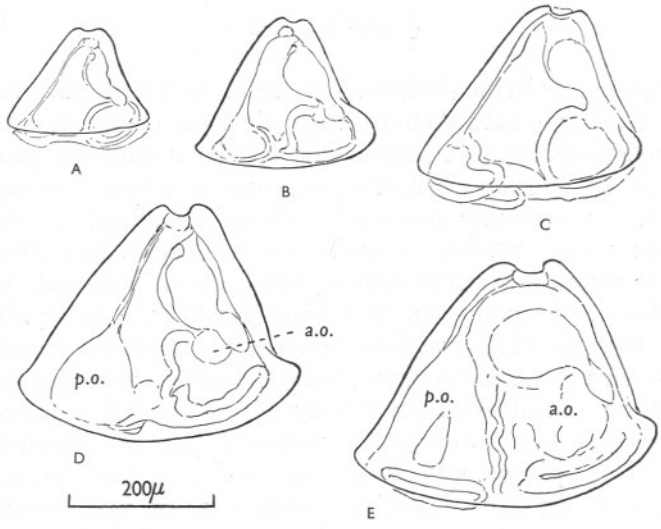

Fig. 1. *Electra pilosa* L. Cyphonautes larva (*Cyphonautes compressus*), to show change in the shape of the shell with growth, and the development of the pyriform (*p.o.*) and adhesive (*a.o.*) organs.

The first cyphonautes to be described, the *Cyphonautes compressus* of Ehrenberg (1838), was apparently lacking in ornamentation even in the late stage, for none is shown in his figure (pl. 44, fig. 2) of a late larva with well-developed pyriform and adhesive organs. He gave the size of this larva as '$\frac{1}{9}$ linie', which as the German line equals 2·18 mm, is about 242 μ. Schneider (1869) was quite possibly right in identifying his larva, which he found to develop into *Electra pilosa*, with *Cyphonautes compressus* Ehrenberg.

The settlement of *C. compressus* has been watched several times at Plymouth, for late larvae when taken from tow-nettings and placed in glass dishes tend to settle rapidly. A larva when about to settle glides over the substratum with the pyriform organ foremost, the vibratile plume clawing at the surface. The corona is extended, the cilia at first beating metachronally, although slowly:

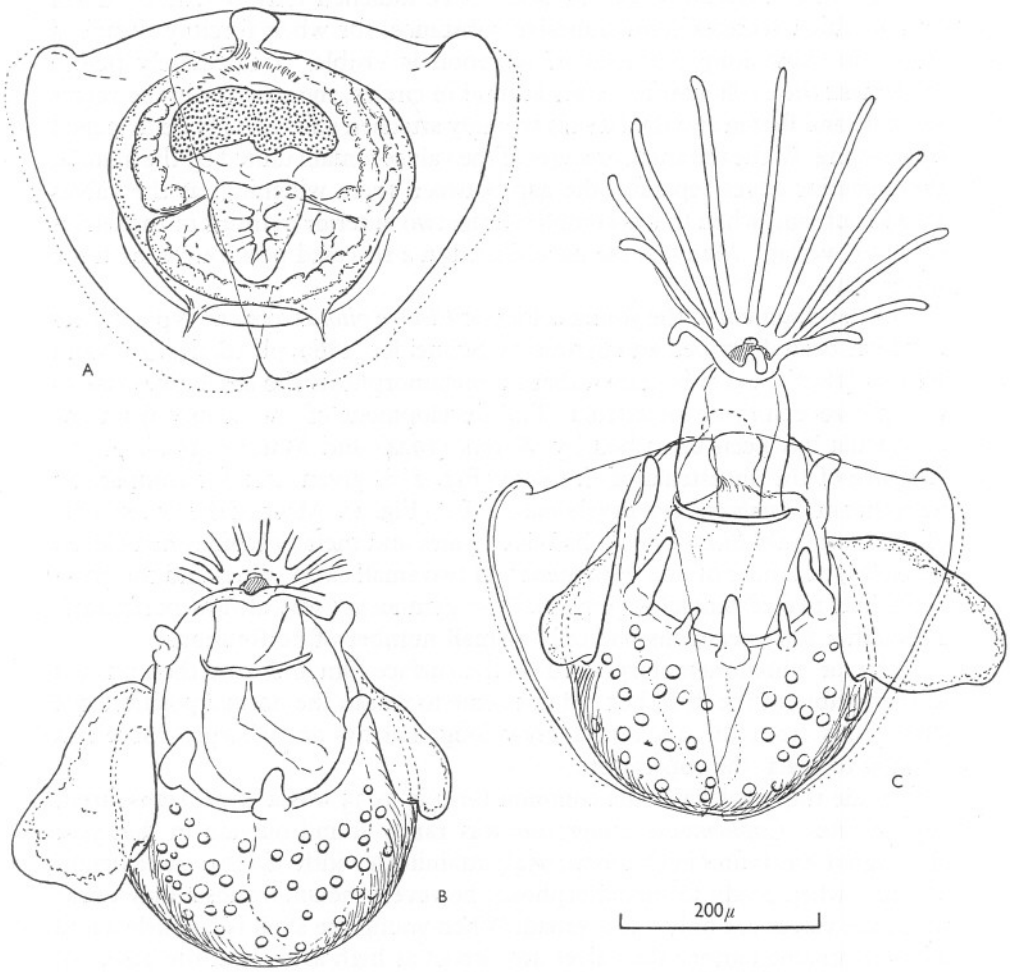

Fig. 2. *Electra pilosa* L. A, metamorphosing larva; B, ancestrula with five spines and the polypid with eleven tentacles; C, ancestrula with seven spines and the polypid with twelve tentacles. The shell is shown in position in A and C; it was removed from B for the figure to be drawn. Both ancestrulae have given rise to three buds, two being lateral and one distal (the latter is indicated by a broken line).

later the cilia become quiescent, extended almost horizontally. When gliding, if gently squirted at with a pipette, it heels over, but is not dislodged: when touched with a needle it can be seen to be attached only by the pyriform organ, which secretes some adhesive substance, for when forcibly detached from the substratum a thread of secretion is visible. Immediately before settlement the cyphonautes moves around in circles, then suddenly the valves separate and flatten and the larva is strongly attached and can only be detached by scraping. While the anterior edges of the valves remain united by the mantle, the posterior edges separate, the gap between them widening, as the valves spread out, and when this is completed the two anterior margins of the valves come to overlap. Many larvae have settled in a reversed position beneath the water film.

The development of the young colony of *Electra pilosa* from the cyphonautes larva has been described and figured by Schneider (1869, pl. 16, figs. 2–7) and Barrois (1877), and it is known that on metamorphosis the larva gives rise to a single zooecium or ancestrula. The development of the colony from the ancestrula has been described by Waters (1924) and Marcus (1926, p. 31). A figure of the ancestrula of *E. pilosa* (Fig. 2) is given here for comparison with that of *Membranipora membranacea* (L.) (Fig. 4). Most of the ancestrulae of the Plymouth *Electra pilosa* had five spines and their polypides had eleven tentacles, but those of one settlement had two small extra spines and the polypides had twelve tentacles (Fig. 2). The gymnocyst was always perforated, although a few specimens had only a small number of perforations.

In larvae which metamorphosed on the surface film and were then pushed to the bottom of the dish, but did not come to rest in the normal position, the three buds from the ancestrula grew long, narrow and sharply curved in seeking to reach the substratum.

Outside the breakwater the common form in 1953 was a large, transparent cyphonautes (*Cyphonautes compressus* was rarely found outside in that year although it was found in October 1954), attaining a width of 840 μ and a height of 640 μ when ready to metamorphose; however, metamorphosis may occur when the valves are only 750 μ broad. When young the shell is colourless and without ornamentation; the valves are almost as high as wide at the base. At about 710 μ basal width the ventral edges of the valves become brownish, but there is still little or no ornamentation. With development of the pyriform organ (Fig. 3, *p.o.*), which is formed before the adhesive organ (*a.o.*), the anterior edge becomes increasingly longer than the posterior, and the greatest width considerably more than the height of the shell. With age also the basal edges of the valves take on a brown tint, and become progressively ornamented with refringent spots which in places appear to coalesce, forming ridges. The shell ornamentation is heavier in some metamorphosed larvae than in others. The posterior margin of the shell usually is almost invisible. Fig. 3 shows change in shell shape with age. These stages have been taken from the plank-

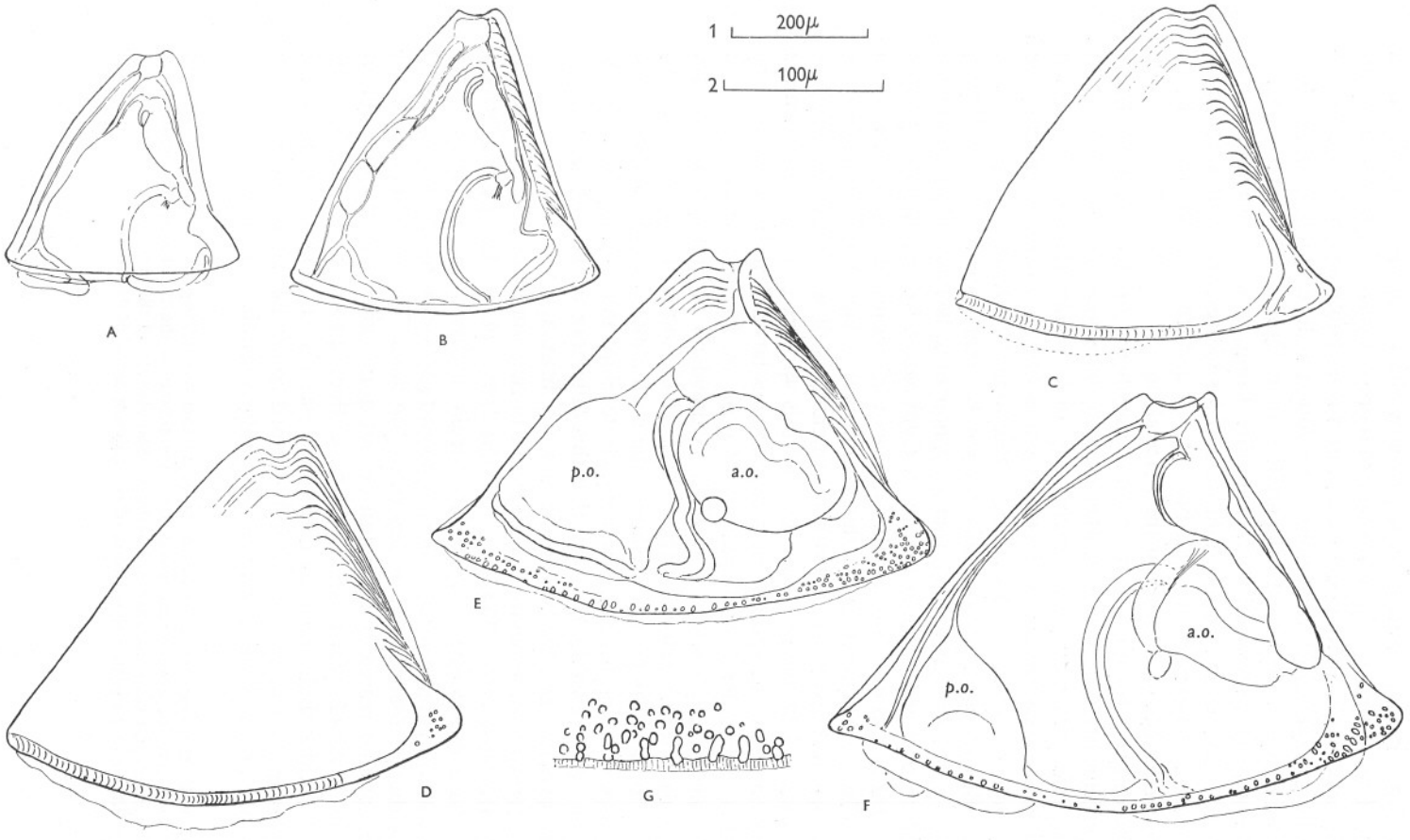

Fig. 3. *Membranipora membranacea* (L.). Cyphonautes larvae, to show change in shell shape with growth and the development of the pyriform (*p.o.*) and adhesive (*a.o.*) organs, and also the appearance of ornamentation along the basal edge of the shell with age. Both the larvae shown in E and F are about ready to metamorphose, although one is smaller than the other. G, small area of the basal edge of the shell enlarged to show the ornamentation and the almost transparent, faintly striated, extension of the shell edge. The valve only is shown in C and D, so that the growth lines are clearly visible. A–F to scale 1; G to scale 2.

ton and not reared.[1] This larva is probably the *C. compressus* of Claparède (1863, pl. xviii, figs. 15–18), if his figures represent stages in the development of one larva, which they appear to do, but it is certainly not the *C. compressus* Ehrenberg (1838) which Schneider (1869) found to develop into *Electra pilosa*. Lohmann (1911) separated Claparède's four figures into three species, *Cyphonautes compressus* Eh., *C. schneideri* Lohm. and *C. borealis* Lohm. Claparède did not give the magnification of his figures, but if all were drawn to the same magnification, then his figs. 15 and 16 are unlikely to be of *C. compressus* Eh. on account of their size alone.

According to Harmer (1926, p. 200) statements in the literature that a cyphonautes has been found in *Membranipora membranacea* (L.) (= *Nichtina telacea*) refer principally, if not exclusively, to *M. mülleri* Bidenkap (= *Electra pilosa* forma *membranacea*, and now known as *Electra crustulenta* (Pallas), see Borg, 1931, p. 11), and he had been unable to find evidence of its occurrence in the British species commonly known by that name. Thorson (1946, pp. 158–9, fig. 87B) mentioned that a cyphonautes, lacking ornamentation in the oldest stage, and which he referred to *Cyphonautes balticus*, metamorphosed in his dishes and was 'presumably the larva of *Membranipora membranacea* (L.)'. The present work shows this assumption to have been wrong, for the large Plymouth cyphonautes with ornamented shell was observed to metamorphose into *M. membranacea* (L.) and this appears to be the first definite observation that this species has a cyphonautes larva. The larva seems to be closest to Schneider's third species (1869, pl. 16, fig. 11), which Lohmann (1911) named *Cyphonautes schneideri*. Schneider's second species (pl. 16, fig. 10), also with an ornamented basal edge, which Lohmann named *C. borealis*, appears to have the posterior edge of the shell almost at right angles to the base, thus differing from the larva which develops into *Membranipora membranacea*. It is possible, however, that this difference in the angle is an individual variation, and the two species are not distinct.

The larva of *M. membranacea* gives rise to twin zooecia while still covered by the flattened valves (Fig. 4A). Nitsche (1871, pl. 38, fig. 3), and Waters (1925) have shown that 'a double ancestrula' occurs in this species, and the latter author figured a young colony of about twenty-two zooids (pl. 21, fig. 1), but neither of them found or described the larva. The twin ancestrulae of young colonies reared at Plymouth do not quite agree with the description given by Waters in that there may be fewer than '4 stout protuberances', but they also differ slightly among themselves (see Fig. 4). The paired proximal tubercles in the colony of seven fully-formed zooids have been pushed on to the undersurface of the colony and one only is visible (Fig. 4F).

[1] Dr A. B. Hastings has informed me by letter that in July 1930 she kept a few larvae of this species alive for a week and noted the development of the tuberculations (which were absent at first) and their increase in numbers. She also noted the increase in length of the anterior edge and associated sharpening of the anterior angle of the shell.

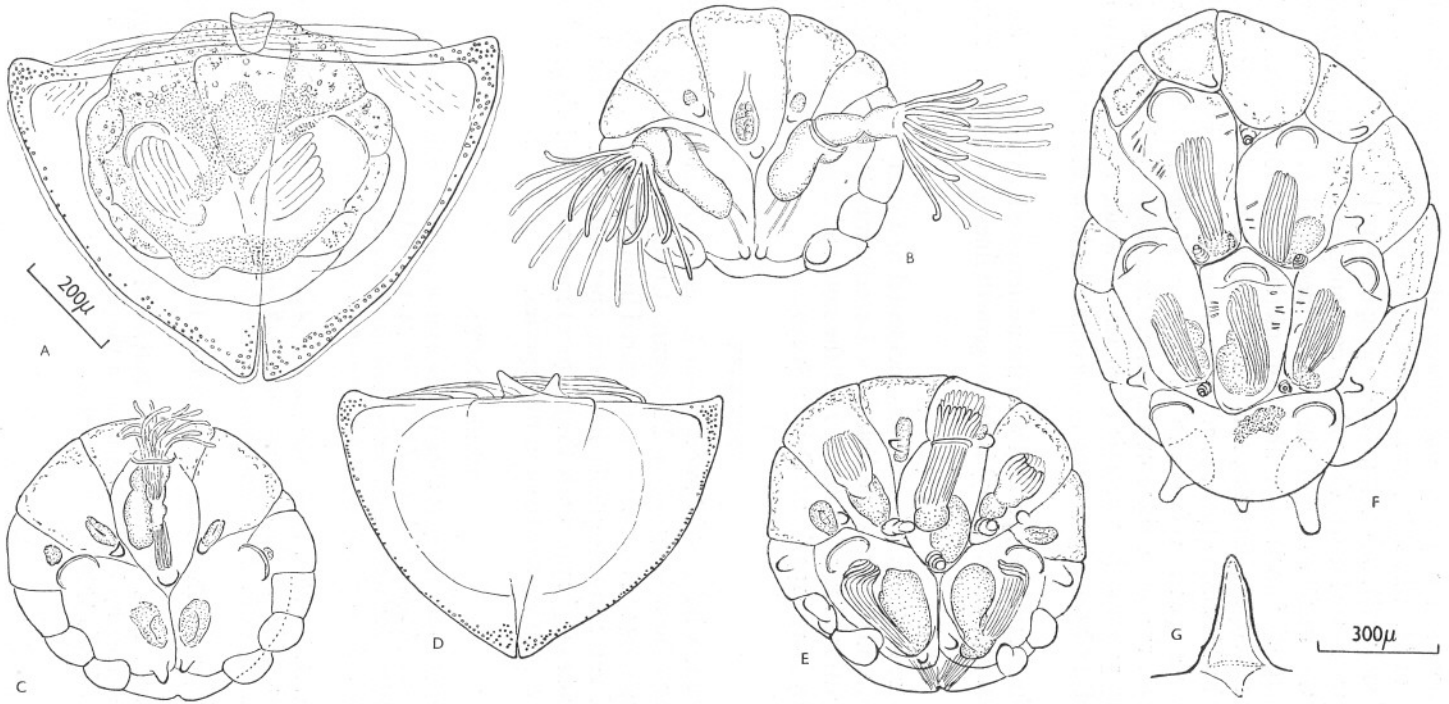

Fig. 4. *Membranipora membranacea* (L.). A, metamorphosing larva; B, C, E, F, development of the colony. G, spine in side view, enlarged. Shells were removed from colonies B and E before drawing; that from colony E is shown at D. The slight asymmetry of the colony shown in F is due to injury in removing it from the bowl in which it grew. B–F to scale in right-hand lower corner.

The development of the young colonies of *M. membranacea* closely resembles that of *M. villosa* Hincks as figured by Robertson (1908) and O'Donoghue (1927) and of *M. tuberculata* (Bosc) as figured by Hastings (1929). These two latter species are known also to have cyphonautes larvae with ornamentation along the basal edges of the valves, described variously as 'teeth' (Robertson, 1908), 'small spine-like denticulations' (O'Donoghue, 1927) and a 'tuberculated edge' (Hastings, 1929), and give rise to twin ancestrulae. As pointed out by Hastings (1929, p. 707) 'the occurrence of twin ancestrulae in three of the six species referred to *Nichtina* (= *Membranipora*) by Harmer (1926, p. 208), the ancestrulae of the other three being unknown, suggests that this may prove to be a generic character'; it would also seem that ornamentation of the basal edges of the valves of the cyphonautes may be a generic character.

The ribbing of the shell parallel with the posterior edge referred to by some authors (Hastings, 1929, p. 707) is due to growth lines and these are shown in Fig. 3C–E.

The work recorded in this note was incidental to a study of ciliary feeding mechanisms, undertaken while holding a Leverhulme Research Fellowship. Dr A. B. Hastings has most kindly read the note and drawn my attention to the papers by Borg (1931) and Osburn (1950).

Summary

The cyphonautes larvae of *Electra pilosa* L. and of *Membranipora membranacea* (L.) have been identified at Plymouth. Change in shell shape of both larvae during growth is described and figured from specimens taken from the plankton. Their metamorphosis has been observed, that of the larva of *M. membranacea* (L.) apparently for the first time, thus definitely proving the presence of a cyphonautes larva in that species.

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THE CILIARY FEEDING MECHANISM OF THE CYPHONAUTES LARVA [POLYZOA ECTOPROCTA]

By D. Atkins, D.Sc.

From the Plymouth Laboratory

(Text-figs. 1–7)

The cyphonautes larva found in certain ectoproct Polyzoa is considered to be most probably a primitive larval form, which has been lost in viviparous species, the larvae of which are degenerate in varying degree and settle within a short time. The cyphonautes is truly planktonic and has a free swimming life of some length—of 2 months according to Marcus (1940, p. 334)—and unlike the other known larvae of the group, has a functional alimentary canal. It obtains its food by maintaining a continuous current of water through the mantle cavity or vestibule, its ciliary mechanism being organized on a plan somewhat resembling that of bivalve molluscs, with an inhalant and an exhalant chamber with interposed ciliary ridges bearing current-producing and food-conveying cilia. As in lamellibranchs the organ creating the water and food currents is separated by a considerable interval from the mouth. In lamellibranchs this interval is bridged by the palps and the oral grooves; in the cyphonautes by the ciliated tract of the funnel. The cyphonautes being a larval form of probably archaic character its method of feeding is of particular interest.

For the study of the ciliary feeding mechanism the larva of *Membranipora membranacea* (L.) (=*Nichtina telacea* (Lamarck)) was mostly used, rather than that of *Electra pilosa* L., because of its greater size and transparency. The former larva attains a basal width of 840 μ, while the latter reaches only 440 μ (see Atkins, 1955). For the indication of currents *Chlorella stigmatophora*, *Peridinium trochoideum* and *Phaeodactylum tricornutum* Bohlin were used. They were most kindly supplied by Dr M. Parke.

Larvae for sectioning were narcotized with magnesium chloride, a few drops of 7% in tap water being added at intervals to a watch-glass of sea water. The narcotized larvae were first dropped into 10% formalin for about a minute, and then transferred to a modification of Bouin's fixative (saturated picric acid in 90% alcohol, 2 parts; saturated corrosive sublimate (water), 3 parts; 40% formalin, 1 part; glacial acetic acid, 2 parts). In this way larvae were fixed extended. If dropped directly into the second fixative they were found to contract strongly even after being well narcotized. Sections were cut 4, 6 and 8 μ in thickness.

It was not until my paper had been written that I had an opportunity of seeing the beautifully detailed and illustrated paper by Kupelwieser (1905–6) on the histology and metamorphosis of the larva of *Electra pilosa*.

The Structure of the Cyphonautes Larva with special Reference to the Mantle Cavity or Vestibule

The general structure of the cyphonautes larva is well known (Fig. 1). The body is enclosed in a triangular bivalve shell, chitinoid and transparent, and is strongly compressed laterally. The valves are approximated by an adductor muscle, composed of smooth fibres, which before the adhesive organ develops has the form of an arc; a muscular loop slung round the adductor attaches it to the body wall over the gut.

The apex of each valve is truncated and grooved for the reception of the apical organ, which bears a tuft of long cilia, sensory in function.

The pallial lobes enclose a large mantle cavity or vestibule. The external epithelium between the anterior and posterior edges of the valves in life is minutely papillate, with protoplasmic protrusions. It bears ciliary tufts and long cilia, which are probably sensory in function, for they have a slow and irregular beat.

Basally the valves gape and the pallial lobes are here thickened and heavily ciliated, forming a complete ring or corona in very young larvae according to Prouho (1892, p. 616). In older larvae the cells of the middle region are reduced in height and bear short, inconspicuous cilia, as first noted by Claparède (1863, p. 108). Thus the corona—the swimming organ of the larva —is divided into separate anterior and posterior regions.

The anterior portion of the corona borders the entrance to the pyriform organ, or, in young larvae, the invagination which is the precursor of that organ. At each end posteriorly it is sharply recurved, running inwards and anteriorly toward the glandular region of the pyriform organ.

The posterior portion of the corona borders the exhalant aperture and the strong beat of its cilia, from the apex toward the base of the shell, contributes to the exhalant current.

Reduction of the cilia in the middle region, or inhalant aperture, is no doubt to prevent their action from interfering with the inhalant current. If present in full development, the current produced by them would be in direct opposition to the inhalant current. In young larvae of *Electra pilosa*, with basal width of shell about 160 μ, the inhalant aperture is large compared with the anterior region of the corona.

Two ciliated lobes projecting toward each other across the mantle cavity, separate the middle or inhalant aperture from the exhalant aperture. They are directed slightly downward, beyond the ciliated corona, and posteriorly. Each lobe is continued as a ciliated ridge, ascending in an arc across the

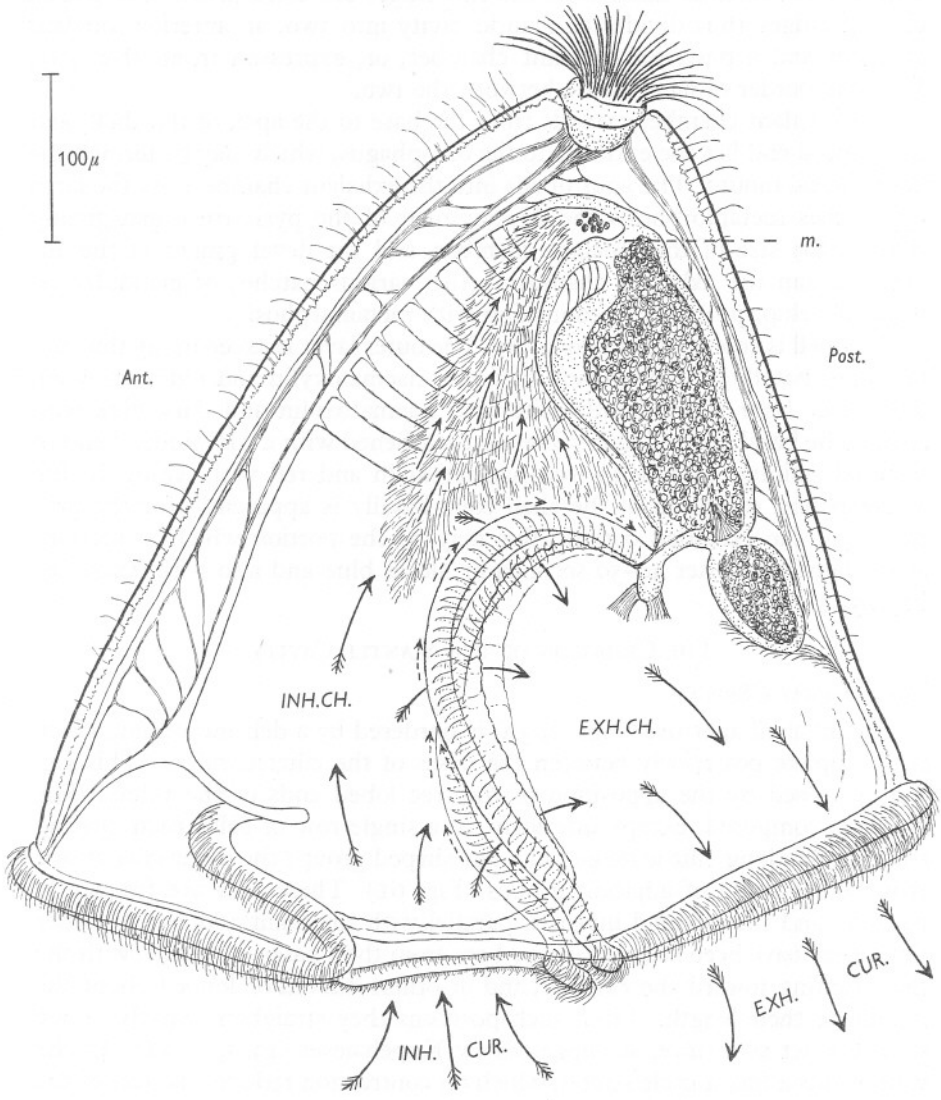

Fig. 1. *Membranipora membranacea* (L.): young cyphonautes larva, to show the direction of the main water current (barbed arrows), of the food currents of the frontal surfaces of the ciliated ridges (small broken arrows) and of the ciliated funnel (small unbroken arrows). The metachronal waves of the lateral cilia of the ridges are indicated. Certain muscles are shown. *INH.CH.* and *EXH.CH.*, inhalant and exhalant chambers. *INH.CUR.* and *EXH.CUR.*, inhalant and exhalant currents; *m*, position of oesophageal mouth.

mantle wall: at their inner ends the two ridges are continuous. The paired ciliated ridges thus divide the mantle cavity into two, an anterior inhalant chamber and a posterior exhalant chamber, or, expressed in another way, form the border of an aperture between the two.

The inhalant chamber extends from the base to the apex of the shell, and at its apical end lies the entrance to the oesophagus, which may be termed the oesophageal mouth. The anus opens into the exhalant chamber. As the larva approaches metamorphosis the development of the pyriform organ greatly reduces the size of the inhalant chamber, and the development of the adhesive organ the exhalant chamber. Also various patches of glandular (?) tissue develop, probably in connexion with metamorphosis.

The shell is composed of two layers. The outer layer is exceedingly thin and fades out basally; it stains black with iron haematoxylin and red with Azan. The inner layer stains faintly with iron haematoxylin and dark blue with Azan. The basal edge of each valve is strengthened with a continuous band of material staining black with iron haematoxylin and red with Azan. In life a transparent extension of the shell edge basally is apparently for the protection of the extended corona; this may be the portion which in sections stains light blue after 40–50 seconds in alcian blue and also with Azan (see Figs. 3, 4).

The Ciliation of the Mantle Cavity

The Inhalant Chamber

The inhalant aperture (Figs. 2, 3A) is bordered by a delicate velum, which is incomplete posteriorly between the bases of the ciliated ridges. This gap can be closed by the apposition of the free lobed ends of the ridges. The velum is composed, except anteriorly, of a single row of cells, each bearing about fifteen long cilia arranged in a fan-shaped group; the outer cilia of one group cross those of adjacent groups (Fig. 6E). These cilia are frequently inactive, and then extend inwards, parallel with the mantle lobes. At other times they have been seen stationary bent from their bases outwards, with the tips pointing toward the exterior, and at others curved or looped about the middle of their length. From such positions they straighten rapidly. They probably act as a sieve, as suggested by Kupelwieser (1905, p. 23). In the velum runs a fine muscle strand, which on contraction reduces the size of the inhalant aperture, the large ciliated cells becoming corrugated. The velar muscles are continuous posteriorly with the longitudinal muscles of the suspensory membranes of the ciliated ridges, while it would appear that anteriorly fibres are given off which are inserted on the pyriform organ. The appearance of the velum where it adjoins the pyriform organ is shown in Fig. 3B, part of a section passing through the extreme posterior region of the anterior portion of the corona, where the ciliated bands turn inward to run across the ventral face of the larva. Kupelwieser (1905–6, p. 23, text-fig. 7)

considered it probable that the velum of *Cyphonautes compressus* inclined outward when relaxed. In the larva of *Membranipora membranacea* it is reflected inward when relaxed, as may be seen in life, and in this position would be likely to interfere less with the ingoing current than in the position suggested by Kupelwieser.

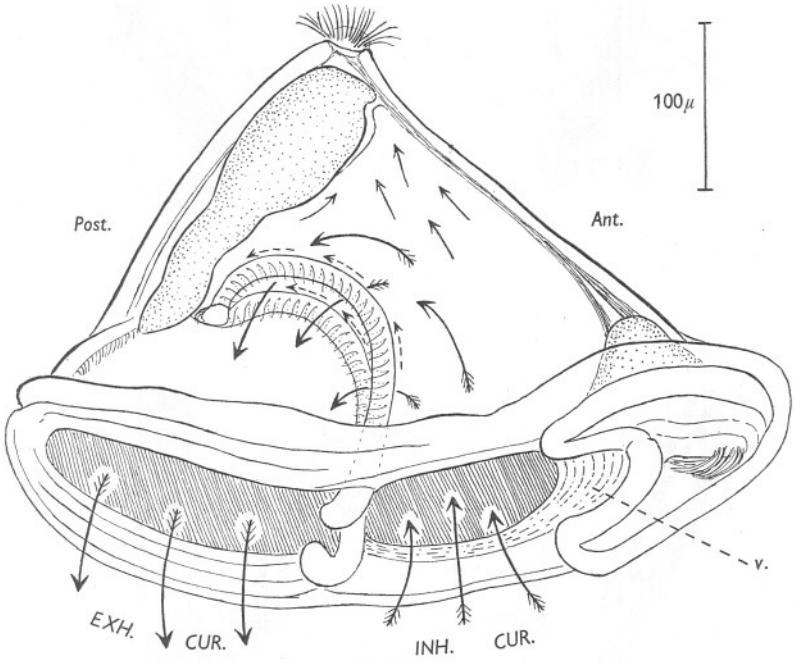

Fig. 2. *Electra pilosa* L.: cyphonautes larva (with pyriform organ well developed, but before the development of the adhesive organ), to show the inhalant and exhalant apertures. *INH.CUR.* and *EXH.CUR.*, inhalant and exhalant currents; *v.*, velum. Arrows as in Fig. 1. Cilia mostly not shown.

At the base of the velum is a ridge of a single row of cells bearing long, coarse cilia (Fig. 3, *c*). These cilia appear active only when stimulated. When at rest they lie with their tips directed anteriorly; their effective stroke is toward the base of the ciliated ridges and they probably function in directing food organisms toward these.

Externally the inhalant aperture is bordered by the much reduced corona (Fig. 3A, *r.c.*), the short cilia of which partake of the activity of the fully developed anterior and posterior portions of the corona and have the same type of metachronal wave, the wave passing uninterruptedly over this region.

The upper part of the inhalant chamber has the form of a highly muscular and ciliated funnel leading to the oesophageal mouth (Fig. 1). Circular muscles are present in its walls and occasionally when the lowest ring only is contracted,

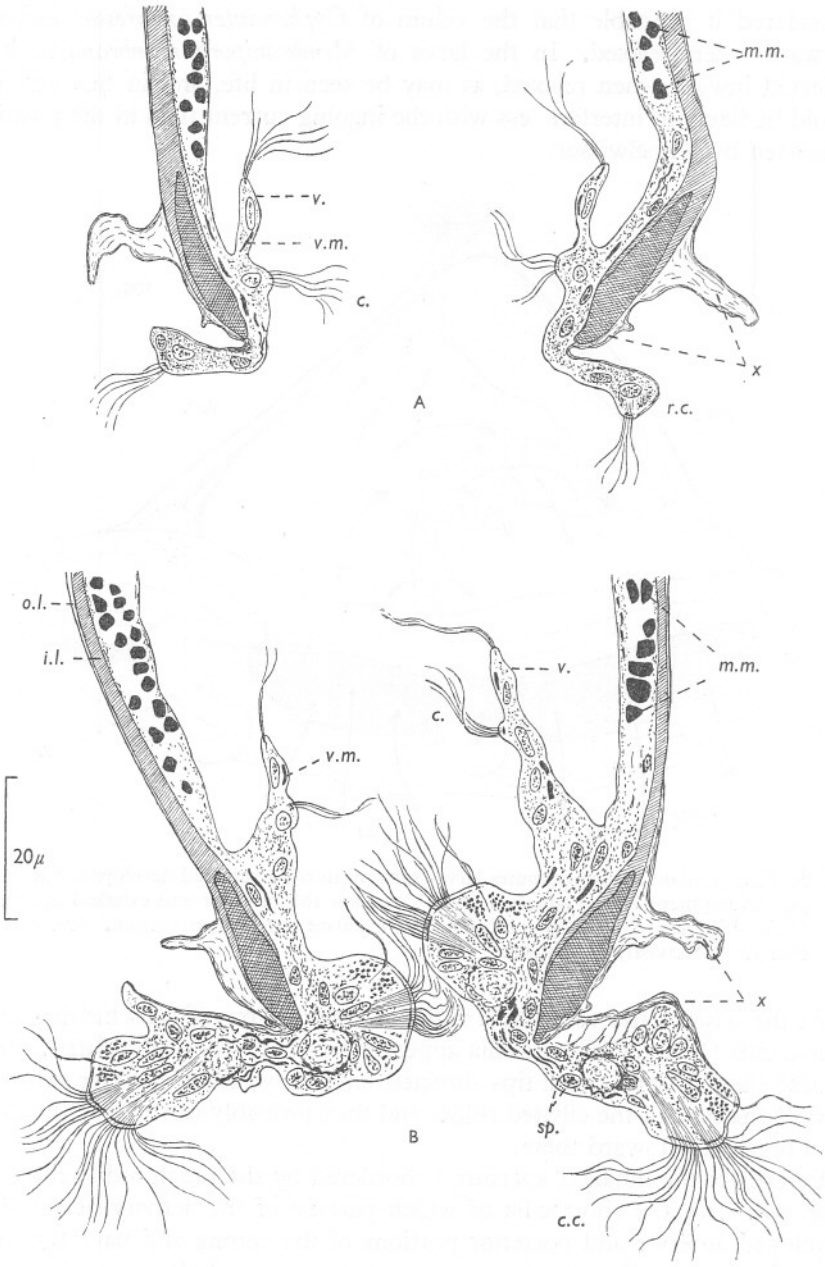

Fig. 3. *Membranipora membranacea* (L.): cyphonautes larva. A: section passing through the inhalant aperture to show the velum (*v.*) and the reduced coronal cells (*r.c.*). The velum is in the relaxed position. B: section passing through the anterior region of the velum (*v.*) near the extreme posterior part of the pyriform organ. The recurving of the corona makes it appear double on each side. *c.*, cilia of the ridge at the base of the velum; *c.c.*, cilia of the corona; *i.l.*, inner layer of shell; *m.m.*, mantle muscles; *o.l.*, outer layer of shell; *r.c.*, reduced coronal cells; *sp.*, spaces due to shrinkage; *v.*, velum; *v.m.*, velar muscle; *x*, region of shell staining light blue with alcian blue and with Azan. The inner layer of the shell is shown hatched and the strengthening band cross-hatched. Heidenhain's iron haematoxylin and alcian blue.

acting as a sphincter, the funnel momentarily has the appearance of an oesophageal or pharyngeal funnel, as remarked by Claparède (1863, p. 107, and pl. 18, fig. 15). In life when fully expanded the 'oesophagus' or 'pharynx' passes indistinguishably into the main part of the inhalant chamber. Contractions of the circular muscles of the funnel appear to occur only when the animal is irritated by the entry into the inhalant chamber of large food organisms, for instance peridinians.

In the cyphonautes of *Electra pilosa* long coarse cilia entirely clothe the funnel and extend well down toward the inhalant aperture. In the cyphonautes of *Membranipora membranacea* the posterior half only of the funnel is ciliated and the ciliation extends as a gradually narrowing tract to a point about half-way down the inhalant chamber (Fig. 1).

The cilia of the funnel may be divided into three regions according to their activity: (1) an outer area over which the cilia are generally inactive unless the larva is feeding, (2) a middle area over which cilia are active at short intervals, and (3) a small inner area over which cilia appear generally active. Immediately in front of the sphincter guarding the entrance to the oesophagus is an unciliated area where food organisms collect before being swallowed or rejected.

The Exhalant Chamber

In the larva of *Electra pilosa* some scattered ciliation is present at the apex of the exhalant chamber and along the base of the suspensory membrane of the ciliated ridges. These cilia are not continuously active. The mantle cilia of the exhalant chamber are not as long as those of the inhalant chamber. In this larva a broad tract of cilia leads from the anus to the mantle edge: the cilia are generally active.

In the larva of *Membranipora membranacea* the exhalant chamber appears to be unciliated, except for cilia clothing the outer surface of the rectum, near the anus. These cilia are fully active only when a faecal pellet is being expelled. Their position of rest is at the beginning of the effective stroke.

The exhalant aperture is bordered by the posterior portion of the corona (Fig. 4), the cilia of which beat from the direction of the apex of the shell toward its base.

Glandular cells of the corona described and figured by Kupelwieser (1905–6, p. 5; pl. IV, figs. 17, 18) for the larva of *Electra pilosa* fixed in Flemming's and Hermann's solutions, were absent in my sections of the larva of *Membranipora membranacea*. It may be that such glandular cells are only present in late larvae ready to metamorphose, or it may be a specific difference, or perhaps the fixative used (see p. 451) may have dissolved the contents.

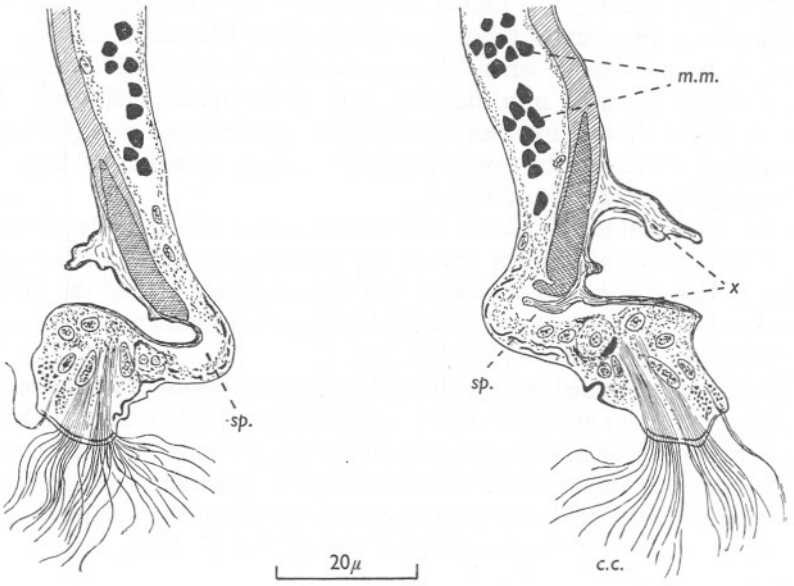

Fig. 4. *Membranipora membranacea* (L.): cyphonautes larva. Section passing through the exhalant aperture. *c.c.*, cilia of the corona; *m.m.*, mantle muscles; *sp.*, space due to shrinkage; *x.*, region of shell staining light blue with alcian blue and with Azan. The inner layer of the shell is shown hatched and the strengthening band cross-hatched. Heidenhain's iron haematoxylin and alcian blue.

The Structure of the Ciliated Ridges separating the Inhalant and Exhalant Chambers

The ciliated region of the ridges is suspended from the pallial lobes by a narrow, delicate, mobile membrane, which becomes contracted on fixation. Along the base of the suspensory membrane runs a muscle strand, which distally is continuous with the velar muscle. Proximally it is inserted on the body wall near the stomach (Fig. 1). At some little distance from its insertion a short branch is given off, which loops round the shell adductor muscle (Fig. 5B). From the longitudinal muscle of the suspensory membrane fine fibres run transversely into the ridges (Fig. 1). In each ciliated ridge is a fine longitudinal muscle fibre (Fig. 6A, *l.m.r.*). The muscular systems of the ciliated ridges, velum, pyriform organ and the adductor muscle are closely connected. The relation of the inner ends of the ciliated ridges to one another and to the shell adductor, before the development of the adhesive organ, is shown in Fig. 5B.

Seen in the living larva, the two curved ciliated ridges have much the appearance of narrow gill filaments, but lateral, current-producing cilia are present on the inner surface only.

On the ridges the ciliated cells are in three tracts, a frontal, a latero-frontal and a lateral (Fig. 6A). This ciliation is continued on to the free, lobed extensions of the ridges and these have a tract of latero-frontal and of lateral cilia on their outer as well as on their inner surfaces. No mucous glands could be distinguished in connexion with the ciliated tracts on the ridges: a group of large clear cells is present in the free lobed ends of the ridges, but these did not become blue when the larvae were placed in water tinted with methylene blue. In sections, spherules staining heavily with iron haematoxylin are arranged peripherally in these cells.

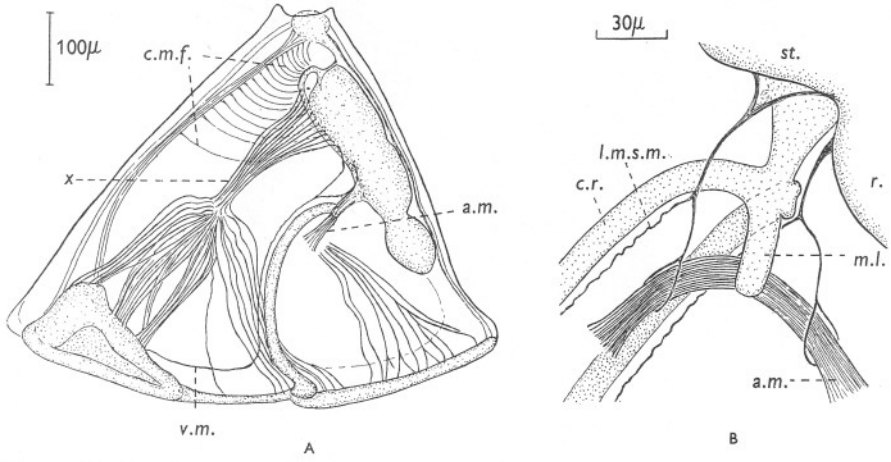

Fig. 5. *Membranipora membranacea* (L.): cyphonautes larva. A: the chief muscles of one mantle lobe as seen immediately after fixation in the fixative given on p. 451. B: sketch to show the relation of the ciliated ridges and their muscles to the shell adductor muscle, as seen in life. *a.m.*, adductor muscle of the shell; *c.m.f.*, circular muscles of the ciliated funnel; *c.r.*, ciliated ridge; *l.m.s.m.*, longitudinal muscle of the suspensory membrane; *m.l.*, muscular loop connecting the adductor muscle to the body wall over the stomach; *r.*, rectum; *st.*, stomach; *v.m.*, velar muscle, which continues as the longitudinal muscle of the suspensory membrane; *x*, muscle, which on contraction drags the mouth region down toward the ciliated ridges.

(1) *Frontal cilia.* The frontal cilia are long and coarse, and are borne on a single row of large cells. They occur in a group across the inner or apical end of each cell (Fig. 6B). These cilia appear active only when stimulated by the presence, in the inhalant current, of food organisms, which they send toward the inner ends of the ridges and the mouth. When at rest they lie with the tips directed toward the outer ends of the ridges, their position of rest being at the beginning of the effective stroke. When not actively beating they yet sometimes have a slight vibratory movement.

(2) *Latero-frontal cilia.* These long and rather stout cilia are latero-frontal in position and, although differing from those of bivalves in the direction of their beat, for convenience will here be termed latero-frontal cilia. They

occur to the inner side of the frontal cilia and separated from the lateral cilia by an unciliated space. When at rest they are almost straight. They are about 20–30 μ long and 3·5 μ apart in the larva of *M. membranacea*. Only the tips appear to bend during the beat, which is slow, with the effective stroke away from the frontal and toward the lateral cilia. This direction was unexpected, but careful focusing at a magnification of 1360 diameters confirmed it. These

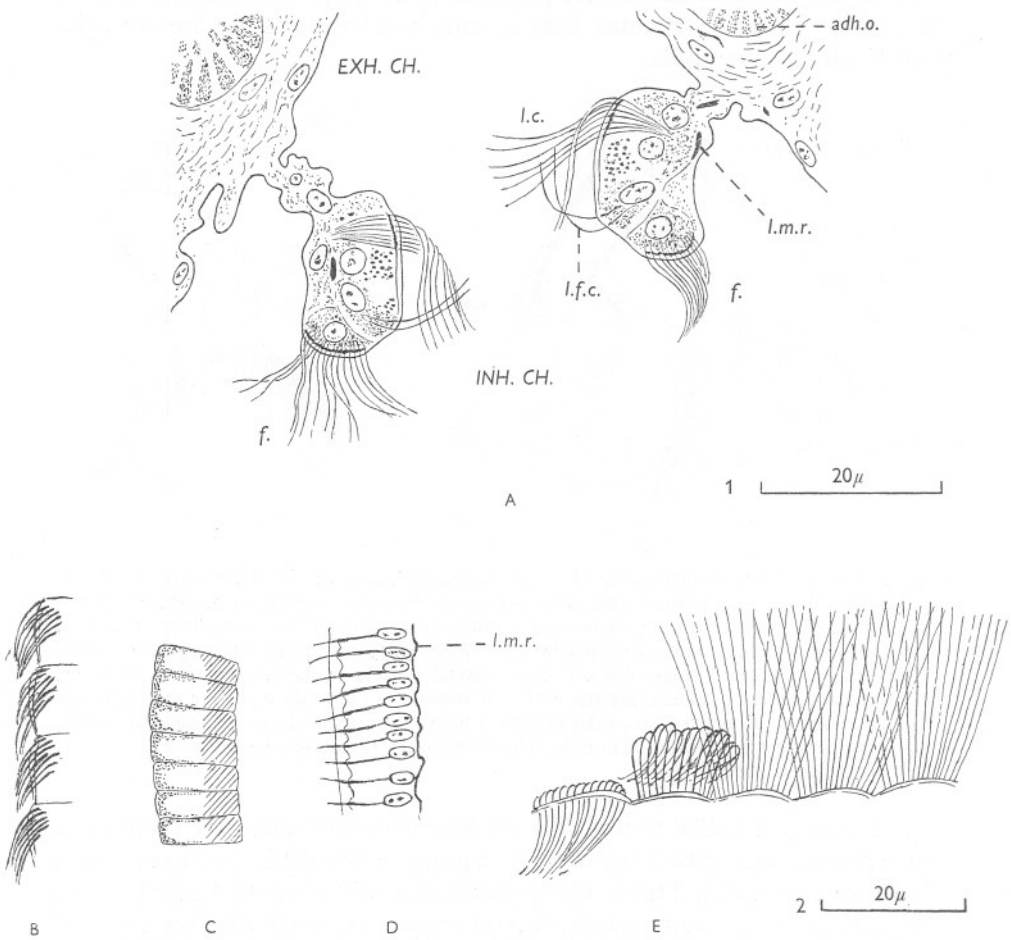

Fig. 6. *Membranipora membranacea* (L.): cyphonautes larva. A: transverse section through the ciliated ridges near their inner ends, in a specimen with well-developed adhesive organ (*adh.o.*). B: frontal cells in side view (living). C: lateral cells in surface view (living). The oblique rows of basal granules are indicated. D: latero-frontal cells in a section in a plane perpendicular to that of the beat. E: fan-shaped groups of cilia on the velum (living). *f.*, frontal cilia; *l.c.*, lateral cilia; *l.f.c.*, latero-frontal cilia; *l.m.r.*, longitudinal muscle of the ciliated ridge. *INH.CH.* and *EXH.CH.*, inhalant and exhalant chambers. A and D, Heidenhain's iron haematoxylin. A and D to scale 1; B, C and E to scale 2.

cilia probably form, to some extent, a grid between the inhalant and exhalant chambers, and, depending on movements of the suspensory membranes of the ridges, may project into the inhalant chamber, or across the aperture between the two. They are borne on a row of small, narrow, rectangular cells with their length across the length of the ridge. There is probably one cilium to a cell (Fig. 6D). The cilia appear to be arranged alternately, one being inclined at a lesser angle to the lateral surface than the next. In sections two diverging ciliary rootlets are very evident (Fig. 6A), but the fibres which proceed from these appear to form one cilium in life. Kupelwieser (1905, p. 23), however, considered that there were two cilia to a cell and that their function was sensory. He compared these ciliated cells with similar, but less developed, ciliated cells of the corona.

(3) *Lateral cilia.* The lateral ciliated cells occur in a single row on the inner surfaces of the ridges. The cells are rectangular with their length transverse to that of the ciliated ridges. The cilia are borne on the abfrontal half of the surface of the cell. The basal granules are arranged in oblique rows (seen living at a magnification of 1360 diameters) (Fig. 6C), as are those of the lateral cilia of lamellibranchs (Atkins, 1938, p. 332).

The cilia are long and beat across the length of the ridge, from the inhalant toward the exhalant chamber. They produce the main water current. The metachronal wave is that characteristic of lateral, current-producing cilia: it runs at right angles to the direction of beat, and passes up the left ridge and down the right one. The waves are laeoplectic, according to the nomenclature employed by Knight-Jones (1954, p. 504).

When at rest the cilia are wrapped across the ridge with the tips projecting beyond the frontal edge: their position of rest is at the beginning of the effective stroke.

The lateral cilia suffer intermissions which are independent of stoppage of the coronal cilia. When two or three drops of 7% magnesium chloride in tap water are added to a watch-glass in which the larvae are swimming, intermissions of the lateral cilia cease. Magnesium chloride does not appear to affect the action of the frontal, latero-frontal or mantle cilia.

The Mode of Feeding

Water, with contained food particles, is drawn into the inhalant chamber by the action of the lateral cilia on the ciliated ridges, and passes between the ridges into the exhalant chamber, leaving by the exhalant aperture (Figs. 1, 2).

When the larva is not feeding the funnel cilia are mostly inactive, as are also the frontal cilia of the ridges, and many food organisms pass through into the exhalant chamber and out through the aperture. The ciliated ridges can be separated, allowing this to occur. It is doubtful to what extent the latero-frontal cilia act as strainers, since the direction of their beat is toward the exhalant chamber, although it appears that little more than their tips bend.

When the larva is feeding the frontal cilia are active and food organisms travel up the ridges. From the inner ends of these they are conveyed over the ciliated tract of the funnel to the unciliated area in front of the oesophageal mouth. The cilia of this tract are long and powerful and some organisms in the anterior stream of the inhalant current pass directly on to this tract, without travelling up the ridges.

Organisms arriving at the oesophageal mouth are either swallowed singly or in small collections according to size, for instance *Peridinium troichoideum*, 20–30 μ in diameter, is swallowed individually, while *Chlorella stigmatophora*, 2·5–4·5 μ in diameter, is taken in small collections. These organisms were used merely to demonstrate the currents and it is not known whether the cyphonautes larva is able to digest them.

The inhalant and exhalant currents are occasionally reversed, food organisms, and at times a faecal pellet, passing from exhalant to inhalant chamber and out through the aperture. This reversal is possibly due to contraction of the shell adductor, and of muscles of the upper part of the mantle, approximating the valves and forcing water from the exhalant to the inhalant chamber. It has been observed to occur during stoppage of the lateral cilia. Reversal of the beat of the lateral cilia was not seen.

Methods resorted to by the cyphonautes whereby unwanted material is rejected are as follows. When the gut is full and no more food can be accepted, or when distasteful material arrives at the mouth, the oesophageal valve remains closed. Rejection is effected by the funnel cilia ceasing to beat—except for those over a small area in front of the mouth, which are generally active—then organisms already collected before the mouth, stream downwards, caught in the water current set up by the lateral cilia, and pass between the apical ends of the ridges into the exhalant chamber. Rejection may be assisted by the mouth region being dragged downward toward the ciliated ridges by contraction of muscles running from the body wall, over the anterior region of the stomach, to the mantle lobe about the middle of the inhalant chamber. These muscles are shown in Fig. 5A at *x*.

Ehrenberg (1838, p. 395 and pl. 44, fig. 2), who first described the cyphonautes, demonstrated, by means of indigo in the surrounding water, the passage of material through the vestibule to the mouth and then through the gut and out at the exhalant aperture. Claparède (1863, p. 108) also observed this current, but apparently neither of them noticed one passing between the ciliated ridges, nor did Robertson (1908). Robertson (p. 273) ascribed to the action of the cilia of the oral atrium (funnel) the current setting toward the mouth. Kupelwieser (1905, pp. 17, 26) attributed to the pyriform organ with its ciliated groove and plume the function of conveying particles into the median atrium (inhalant chamber). He (p. 26) mentioned the occasional passage of small particles from one atrium to the other, between the ciliated ridges.

The Digestive System

The digestive system is simple. The gut appears to be entirely ciliated, except for the valve at the entrance to the oesophagus (Fig. 7A). This valve is closed by a sphincter of striated muscle fibres. Muscle strands run between the valve and the stomach (Fig. 7B), and also between it and the mantle. The oesophagus is short: it is surrounded by a layer of strong circular muscle fibres (Fig. 7C). There appear to be no muscle fibres surrounding the stomach, intestine or rectum, but muscles pass between the gut and the mantle (Fig. 1). The stomach gradually decreases in diameter and passes imperceptibly into the intestine. There is little or no difference in the appearance of the epithelial lining of the two regions. A ventral tract of glandular or secretory cells, the contents of which stain darkly with iron haematoxylin, stretches from the anterior region of the stomach almost to the posterior end of the intestine. The tract is widest near the entrance of the oesophagus. The cells of the side walls of the stomach are particularly deep and contain many inclusions, which in life are yellow and brownish in colour. Similar inclusions are present in the less deep intestinal cells. In sections stained with iron haematoxylin the contents of the cells appear vacuolated, many of the vacuoles having faintly staining inclusions; both vacuoles and inclusions increase in size toward the bases of the cells. These cells are probably digestive in function (Fig. 7D, E).

The cilia on both sides of the constriction separating intestine from rectum are long, particularly a ring of intestinal cilia which form a long tuft or plume projecting into the intestine (Fig. 7H). From their appearance in cross-section (Fig. 7F) it would appear that the latter have a spiral or rotatory movement. They possibly function in forming faecal matter into a cord, or, together with the adjacent long rectal cilia, in passing it into the rectum. Especially common in the anterior region of the rectum are gland cells with darkly staining contents, which presumably form the transparent coat surrounding the faecal pellets. The greater length of the rectum is lined with an epithelium, the cells of which bear a coat of dense short cilia, and contain many vacuoles showing inclusions, which are possibly excretory (Fig. 7G). In sections an anal sphincter could be distinguished, but not one at the constriction between intestine and rectum, although one probably exists.

Alcian blue, after about 40 sec., does not stain the free border of the gut cells, including those of the oesophageal valve, but does stain brightly the free border of the cells of the mantle cavity or vestibule, including those of the region immediately in front of the oesophageal valve (Fig. 7A).

The exact manner in which food, which has collected in the unciliated region in front of the oesophageal valve, is taken into the oesophagus could not be followed, the movement being very rapid. There appeared to be a momentary protrusion of the valve region and sometimes contraction of muscles of the ciliated funnel. Although the gut is entirely ciliated, movement

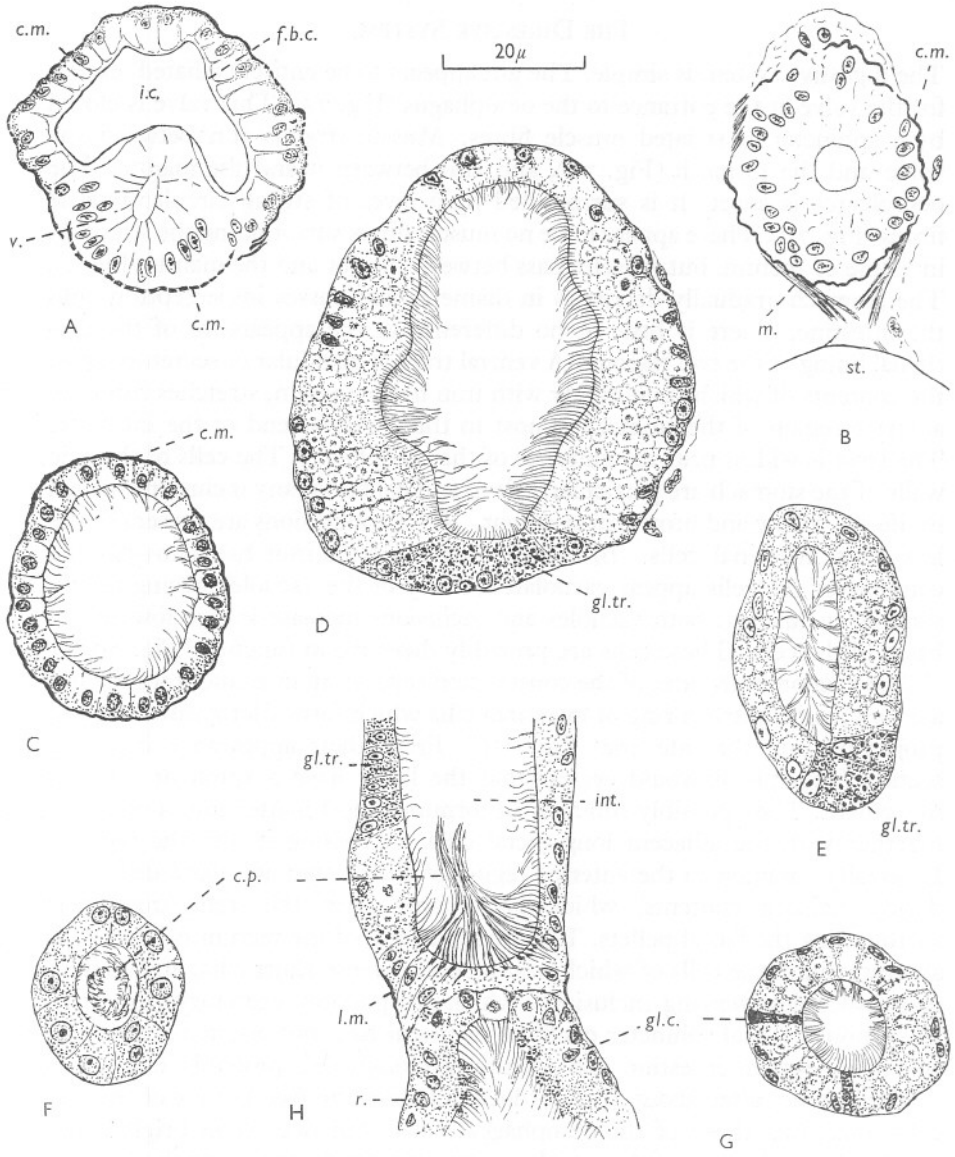

Fig. 7. *Membranipora membranacea* (L.): cyphonautes larva. A: section through the unciliated oral end of the inhalant chamber and obliquely through the valve (*v.*) leading to the oesophagus. Transverse sections of: B, the oesophageal valve; C, the oesophagus; D, the stomach; E, the upper region of the intestine; F, the lower region of the intestine, showing the ciliary plume (*c.p.*) cut across; G, the middle region of the rectum, with short cilia; H, longitudinal section through the constriction between the intestine (*int.*) and the rectum (*r.*), showing the long ciliary plume of the intestine, and the upper part of the rectum with gland cells and long cilia; *c.m.*, circular muscle fibres; *c.p.*, ciliary plume; *f.b.c.*, free border of cells which stains bright blue with alcian blue; *gl.c.*, gland cells; *gl.tr.*, glandular tract of stomach and intestine; *i.c.*, inhalant chamber; *int.*, intestine; *l.m.*, longitudinal muscle fibres; *m.*, muscle fibres passing between oesophageal valve and stomach wall; *r.*, rectum; *st.*, stomach; *v.*, oesophageal valve. Heidenhain's iron haematoxylin and alcian blue.

of food within it was rarely seen. Long cilia were visible, but appeared motionless. Exceptionally, in one larva of *Electra pilosa*, an active stirring was seen of many *Chlorella* forming the contents of the stomach.

When a cyphonautes larva is placed in water containing many *Peridinium trochoideum*, the gut becomes tightly packed with them, and faecal pellets are expelled with the peridinians almost unchanged, but bound together by mucus. It would appear that under these conditions, the peridinians are forced through the gut so rapidly that the digestive enzymes have not sufficient time in which to act. Or it may be that the cyphonautes is unable to digest peridinians, which are heavily armoured with plates, thought to consist mainly of cellulose. The gut may become so filled with *Peridinium* and *Chlorella* that no division is visible between intestine and rectum, then just before defaecation a constriction appears between the two regions. Devèze (1953) has recorded the feeding of the cyphonautes of *Electra pilosa* on micro-organisms less than 0·9 μ in diameter, but the larvae of both *E. pilosa* and *Membranipora membranacea* can certainly swallow organisms up to at least 30 μ in diameter.

The Swimming of Cyphonautes

The ciliated corona is the locomotive organ of the larva. The metachronal wave of these cilia passes round the corona in a clockwise direction when the larva is viewed from above, travelling without a break over the much reduced cilia of the middle region or inhalent aperture. The larva swims with the apical organ foremost: frequently it swims in a vertical position. Sometimes, while in an upright position, it will maintain itself at the same level, going around very slowly in a circle, clockwise, that is in the same direction as the metachronal wave, and swaying exceedingly slightly from side to side, the coronal cilia, meanwhile, beating with a perfect metachronal rhythm. On occasions the level was maintained so closely that the larva remained in focus for minutes on end when viewed at a magnification of 115 diameters. Suddenly the valves would contract, the coronal cilia stop beating and the larva drop: it may do this without apparent cause or when touched by another organism.

The larva probably changes direction when swimming by protruding or withdrawing part of the corona, or possibly by protruding the pyriform organ with its vibratile plume.

The work recorded in this paper forms part of a study of ciliary feeding mechanisms undertaken while holding a Leverhulme Research Fellowship.

Summary

The cyphonautes larva obtains its food by maintaining a continuous current of water through the mantle cavity, which is divided into inhalant and exhalant chambers, separated by the paired ciliated ridges. The water current is

produced by lateral cilia on the ridges, on which there are also frontal, food-conveying, cilia. Food organisms travel from the inner ends of the ciliated ridges over the ciliated tract of the funnel of the inhalant chamber to the mouth.

Rejection of unwanted material is effected by the mouth remaining closed, while material in front of it is carried off in the current passing into the exhalant chamber, most of the funnel cilia meanwhile remaining inactive. Rejection is assisted on occasions by the mouth region being dragged downward toward the ciliated ridges by contraction of certain muscles in the mantle.

The alimentary system is simple. The mouth, closed by a sphincter, leads into a short oesophagus and this in turn into the stomach, which passes imperceptibly into the intestine. A constriction generally separates the intestine from the rectum. Notes are given on the histology of the gut.

Observations on swimming of the larva are given.

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THE COMPARATIVE EXTERNAL MORPHOLOGY AND REVISED TAXONOMY OF THE BRITISH SPECIES OF *IDOTEA*

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(Text-figs. 1–11)

This paper aims at describing the external morphology of seven British species of *Idotea* from the functional and developmental aspects, thus revising earlier descriptions, such as those of Collinge (1917), which were based largely on adult specimens. Limitations of earlier descriptions are apparent, but some characters can be sifted from the older literature and combined with characters emphasized in this paper to give a schematic description of the seven species.

I am grateful to Mr J. S. Colman for criticism of this work, to Dr I. Gordon for facilities at the British Museum (Natural History), and for examining the Linnaean specimens of *Oniscus marinus*; and to Mr G. M. Spooner for help in the construction of the Key.

Material and Methods

The crustacean genus *Idotea* belongs to the suborder Valvifera of the order Isopoda, and the seven species of the genus which are dealt with in this investigation are *I. linearis* (Pennant), *I. viridis* (Slabber),[1] *I. baltica* (Pallas),[1] *I. neglecta* G. O. Sars, *I. emarginata* (Fabricius), *I. granulosa* Rathke, and *I. pelagica* Leach.

All these species, and no others, are recorded from the Isle of Man (Moore, 1937, etc.), though the records of *I. viridis* are open to some doubt; the finding of *I. viridis* in this investigation seems to constitute the first certain record of the species in the Isle of Man (Naylor, 1955*a*). Collinge (1917), in his review of the Idoteidae, records two other species from Britain: *I. metallica* Bosc. and *I. sarsi* Collinge. *I. metallica* does not seem to be a British resident, since only single specimens have been recorded, often from amongst *Lepas* on driftwood (Tattersall, 1911), whilst *I. sarsi* is synonymous with *I. baltica*. I have examined the type specimens of *I. sarsi* in the British Museum and they cannot be distinguished from *I. baltica*.

[1] Holthuis (1949) maintains that *I. viridis* Slabber should now be called *I. chelipes* (Pallas), and that *I. baltica* (Pallas) should now be called *I. marina* (L.). Dr Gordon has examined the Linnaean specimens of '*Oniscus marinus*', which resemble *Idotea granulosa* more than they do any other species. Neither of Holthuis's suggestions is adopted in the present paper.

I have collected all seven species, the shore species *I. granulosa* and *I. pelagica* in particular, from various localities around the Isle of Man, and all but *I. linearis* were taken in some quantity. In addition, for morphological comparison with Manx specimens, material has been obtained from other localities in the British Isles.

Material was preserved in Baker's calcium formol solution, 5% sea-water formalin, or 70% alcohol. For microscopical examination appendages were mounted directly into glycerine from freshly killed specimens; semi-permanent preparations were made in Berlèse solution of glycerine jelly.

The staining and mounting methods given by Collinge (1917, 1918) proved impracticable. They are laborious and they entail the use of Canada balsam, the high refractive index of which makes accurate study of arthropod exoskeletons very difficult.

All measurements of body length were taken down the mid-dorsal line, irrespective of the shape of the telson. The larger specimens were measured against a non-parallax scale under a low-power dissecting microscope, and the smaller specimens were measured under a monocular microscope with a micrometer eyepiece.

The terminology adopted for the major divisions of the body is that used by Hansen (1925) and Howes (1939), namely:

Cephalon: the head and the first thoracic segment fused together.

Thorax: the last seven thoracic segments.

Abdomen: strictly speaking composed of six segments, but dorsally it appears to consist of three segments, the last of which is fused to the *telson*.

General description of *Idotea*

Size and shape

The length of a juvenile newly emerged from the brood-pouch varies from 1 to 3 mm, according to the species. Males of the shortest species, *I. pelagica*, are recognizable at about 4 mm, and they range to about 12 mm. On the other hand, males of a larger species, *I. emarginata*, are identifiable at about 7 mm in some cases, and they range to about 30 mm in length. Within any one species, the size range of females is less than that of the males, and the female of a copulating pair is always smaller than its mate.

Shape varies from the long, filiform *I. linearis*, to the small, robust form of *I. pelagica*, and in all cases the ovigerous female is relatively broader than the male of the same species.

The Front Cephalic Appendages

Antennules (Fig. 1)

The antennules are the most anterior of the appendages. Each has a three-jointed basal part and a flagellum consisting of one joint, the latter bearing on its antero-medial surface a number of aesthetascs. Each aesthetasc consists of

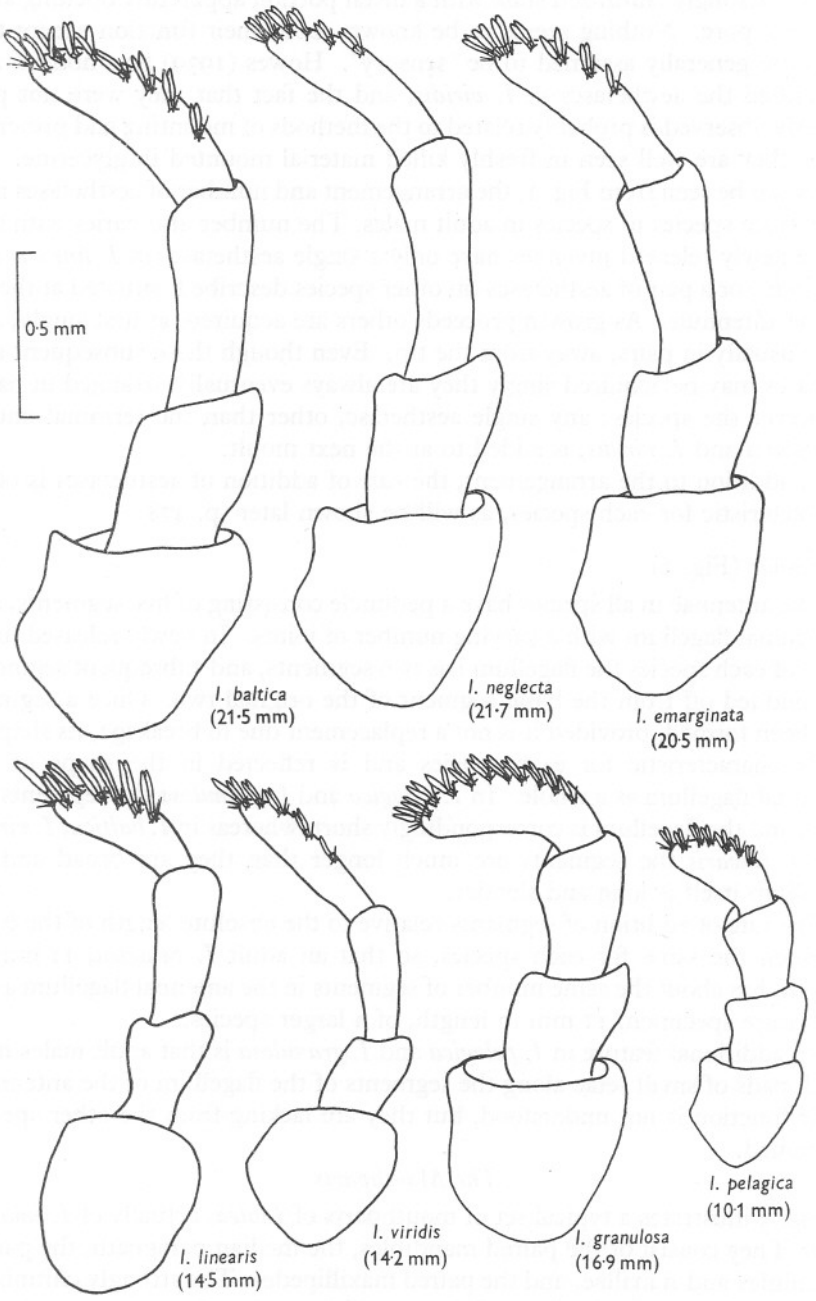

Fig. 1. The right antennule of adult *Idotea*, seen from below.

a fairly strongly chitinized stalk with a distal portion apparently opening at its tip by a pore. Nothing seems to be known about their function except that they are generally assumed to be 'sensory'. Howes (1939) first noticed and described the aesthetascs in *I. viridis*, and the fact that they were not previously observed is probably related to the methods of mounting and preservation; they are well seen in freshly killed material mounted in glycerine.

As can be seen from Fig. 1, the arrangement and number of aesthetascs may vary from species to species in adult males. The number also varies with age, since newly released juveniles have only a single aesthetasc (in *I. linearis* and *I. viridis*) or a pair of aesthetascs (in other species described) situated at the tip of the antennule. As growth proceeds others are acquired, at first singly, and later usually in pairs, away from the tip. Even though these subsequent aesthetascs may be acquired singly they are always eventually arranged in pairs, whatever the species; any single aesthetasc, other than the terminal one of *I. linearis* and *I. viridis*, is added to at the next moult.

In addition to the arrangement, the *rate* of addition of aesthetascs is often characteristic for each species, as will be shown later (p. 478).

Antennae (Fig. 2)

The antennae in all species have a peduncle consisting of five segments, and a terminal flagellum with a varying number of joints. In newly released juveniles of each species the flagellum has two segments, and subsequent segments are budded off from the basal segment of the original two. Once a segment has been formed, provided it is not a replacement due to breakage, its shape is fairly characteristic for each species and is reflected in the shape of the antennal flagellum as a whole. In *I. pelagica* and *I. granulosa* the segments are stout and the flagellum is correspondingly short, whereas in *I. baltica*, *I. viridis* and *I. linearis* the segments are much longer than they are broad and the flagellum itself is long and slender.

The rate of addition of segments relative to the absolute length of the body is much the same for each species, so that an adult *I. pelagica*, 11 mm in length, has about the same number of segments in the antennal flagellum as an immature specimen, 11 mm in length, of a larger species.

An additional feature in *I. pelagica* and *I. granulosa* is that adult males have thick pads of small setae along the segments of the flagellum of the antennae; their function is not understood, but they are lacking from the other species examined.

The Mouthparts

Fig. 3 illustrates a typical set of mouthparts of *Idotea*, actually of *I. emarginata*. They consist of the paired mandibles, the median paragnath, the paired maxillules and maxillae, and the paired maxillipedes. The strongly chitinized, plate-like labrum fits in front of the mandibles and projects downwards and backwards; it is not figured.

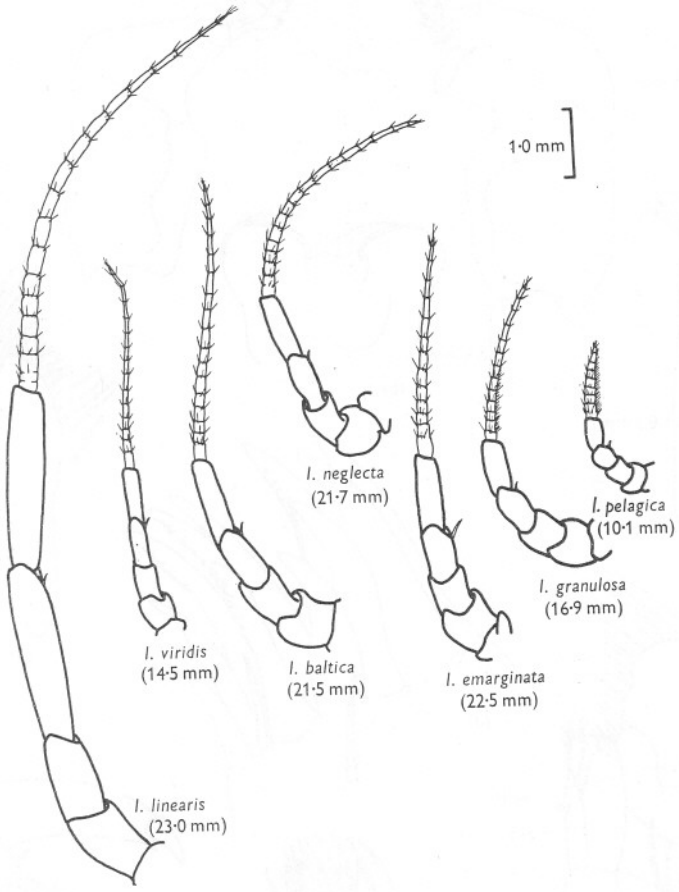

Fig. 2. The right antenna of adult *Idotea*, seen from above.

Mandibles (Fig. 3*a*)

It has not previously been noted that the mandibles of *Idotea* are asymmetrical, though the feature is probably common in the Malacostraca as a whole (Calman, 1909). Each mandible is L-shaped; one arm has strong muscular attachments and lies horizontally, parallel to the sides of the head, whilst the other arm points downwards and inwards, meeting its fellow medially on the ventral side of the head. Ventrally placed on each mandible is a strongly chitinized incisor process, and above this is the lacinia mobilis, which is more strongly chitinized on the left mandible than on the right. When the mandibles are closely apposed, the incisor process of the right appendage fits between the lacinia and the incisor process of the left side.

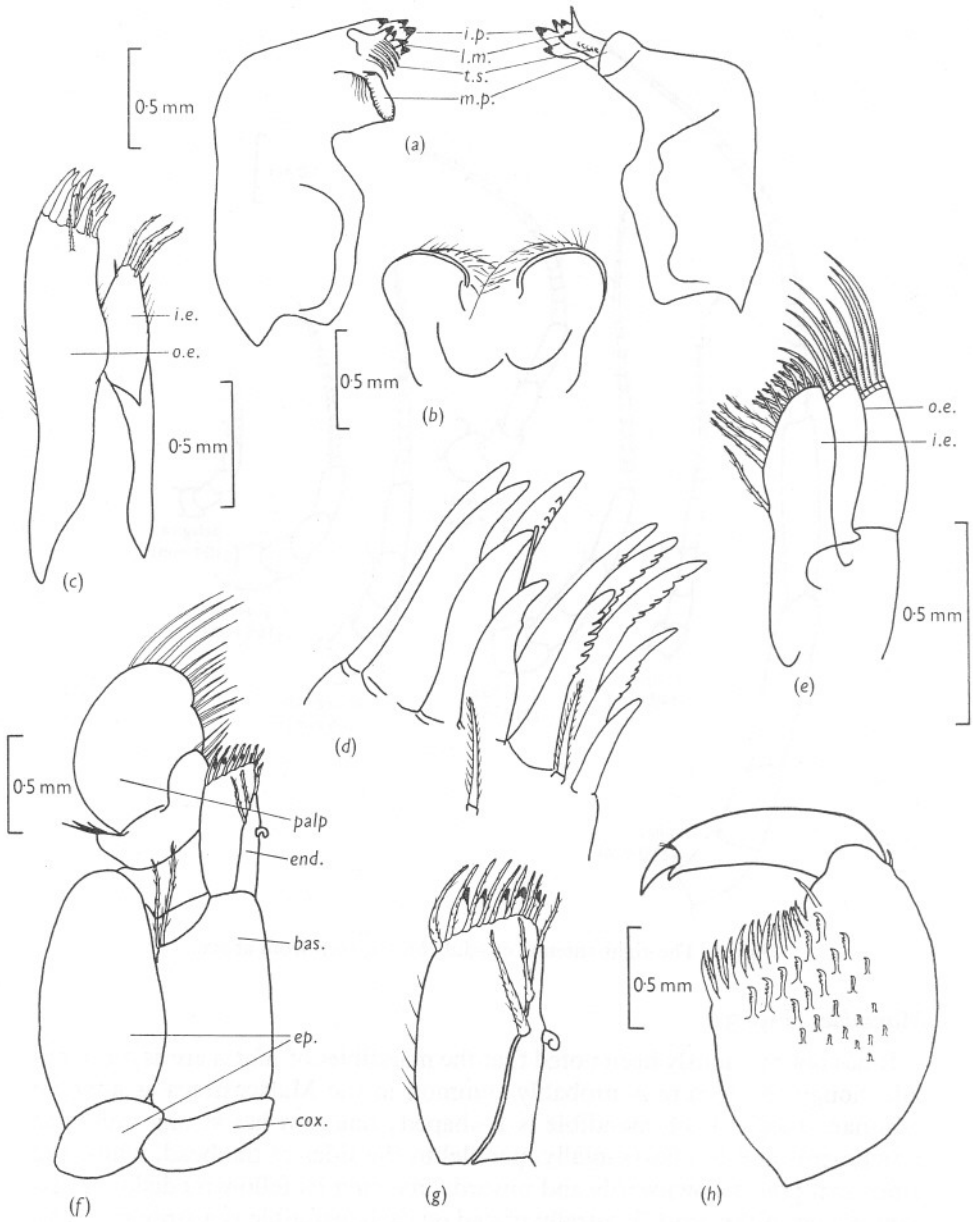

Fig. 3. The mouthparts of an adult *I. emarginata*, 20·5 mm in length: (*a*) *mandibles*, from above (*i.p.*, incisor process; *l.m.*, lacinia mobilis; *t.s.*, toothed spines; *m.p.*, molar process); (*b*) paragnath; (*c*) right *maxillule*, from below (*i.e.*, inner endite; *o.e.*, outer endite); (*d*) tip of outer endite of maxillule; (*e*) right *maxilla*, from above (*o.e.*, outer endite; *i.e.*, inner endite); (*f*) left *maxillipede*, from above (*end.*, endite; *bas.*, basipodite; *ep.*, epipodites; *cox.*, coxopodite); (*g*) endopodite of maxillipede; (*h*) terminal joint of *1st leg*.

Above the lacinia is a row of upwardly curving toothed spines, and above the spines is the molar process. The molar processes of the two mandibles are arranged differently; the left molar process faces upwards, whilst that of the right side faces downwards.

The mandibles lack any trace of a palp.

Paragnath (Fig. 3*b*)

The ventral mouth opening, bordered anteriorly by the labrum and laterally by the mandibles, is bordered posteriorly by the median, bilobed paragnath. Between the left and right lobes of the paragnath is a median groove into which project brush setae of the other appendages (see below). The median surfaces of the paragnath are covered with curved spines, which project forwards and upwards into the oral cavity.

Maxillules (Fig. 3*c*)

Each maxillule has two endites, the large outer endite and the smaller inner endite, each having fairly strong muscular attachments. The outer endite has at its tip twelve chitinized spines and two flexible flattened setae fringed with small stiff bristles (Fig. 3*d*). *This arrangement of spines and blades is the same for each species, throughout the whole of the life cycle.*

The maxillules, like the maxillae and the maxillipedes, are attached posteriorly and project forwards below the paragnath. The ventral spines of the outer endite, which are used in scraping the food material, appear spatulate and are mostly without teeth; one of this group is a rod-like structure. The upper (inner) spines, except for the small median one, are usually strongly toothed; these spines serve to push food into the mouth (Naylor, 1955*b*).

The inner endite again has a constant number of processes at its tip. They consist of four brush setae (three long and one short), with a small spine at the bases of the setae.

Maxillae (Fig. 3*e*)

These are more fragile, flattened structures, much less chitinized than the maxillules. Each again has two endites, the outer endite itself being bilobed. Both lobes of the outer endite have a row of combing setae along their anterior edges and, as can be seen in Fig. 3*e*, the outer setae show an increase in size.

The inner endite lies above and medial to the outer endite. It has two rows (upper and lower) of small brush setae, merging to smoother spines laterally, and there are, placed medially, two to four large brush setae analogous to those on the inner lobe of the maxillule.

Maxillipedes (Fig. 3*f*)

The maxillipedes are broad, flattened appendages covering the other mouthparts ventrally. Arising laterally from the coxa are two epipodites, and arising

medially is the basis. Articulating in front of the basis are the medial endopodite and the distal four-jointed palp, the outer lobe of which bears long smooth spines.

The endopodite (Fig. 3*g*) is turned vertically upwards along its medial edge and is thus L-shaped in cross-section. The appendage of each side has a single coupling hook situated on the vertical flange, and these interlock to make the maxillipedes, functionally, a single structure. The L-shape of the endopodite was first described by Howes (1939) for *I. viridis*, but it is common to all the seven species.

Along the upper edge of the endopodite, and extending forwards and laterally across the anterior border, is a row of brush setae, again comparable with those of the preceding two pairs of appendages. Beneath the brush setae is a row of heavily chitinized, smooth, scraping spines which have a curious double chisel edge at their tip (Naylor, 1955*b*).

A feature not mentioned by Collinge (1917) is to be found on the maxillipede of the adult female. The appendage as described so far is typical of all specimens, but in females with a brood pouch there is, in addition, on the inner posterior border of the maxillipede, a small lappet fringed with setae (Fig. 4*e*). This projects into the brood pouch, since the first oostegite covers the base of the maxillipede, and it appears that movements of the maxillipede cause the lappet to create a current of water over the developing eggs in the brood pouch. Ovigerous females, otherwise at rest on weed, and certainly not feeding, are often seen to raise and lower their maxillipedes with some regularity.

Calman (1909), not specifically referring to *Idotea*, states that 'in ovigerous females of...certain genera of the Valvifera the coxopodite bears a small lappet, fringed with setae, projecting backwards into the marsupium'. By virtue of the position of this structure, and its occurrence in the adult female, Calman suggests that the lappet may be homologous with the oostegites of the first five thoracic segments.

The Appendages of the Thorax

Legs

The thorax is composed of seven segments, and in adult specimens each segment bears a pair of walking legs. Newly released young have only six pairs of legs, the last segment being small and devoid of appendages. After two moults, in *I. emarginata* and probably in other species as well, the seventh segment acquires the first signs of a pair of legs in the form of a pair of small rudiments projecting forwards. Only after a third moult does the animal acquire its full complement of legs.

The first pair of limbs are short and each has a palmate distal joint (dactylopodite), which bears rows of comb spines on its inner surface (Fig. 3*h*). This surface is held beneath the mouthparts during feeding, and it aids in keeping them free of debris.

On the more posterior segments the legs become longer and more slender, being particularly so on the last three thoracic segments. None of the last six pairs of legs are used in feeding, but some are modified in the adult male and

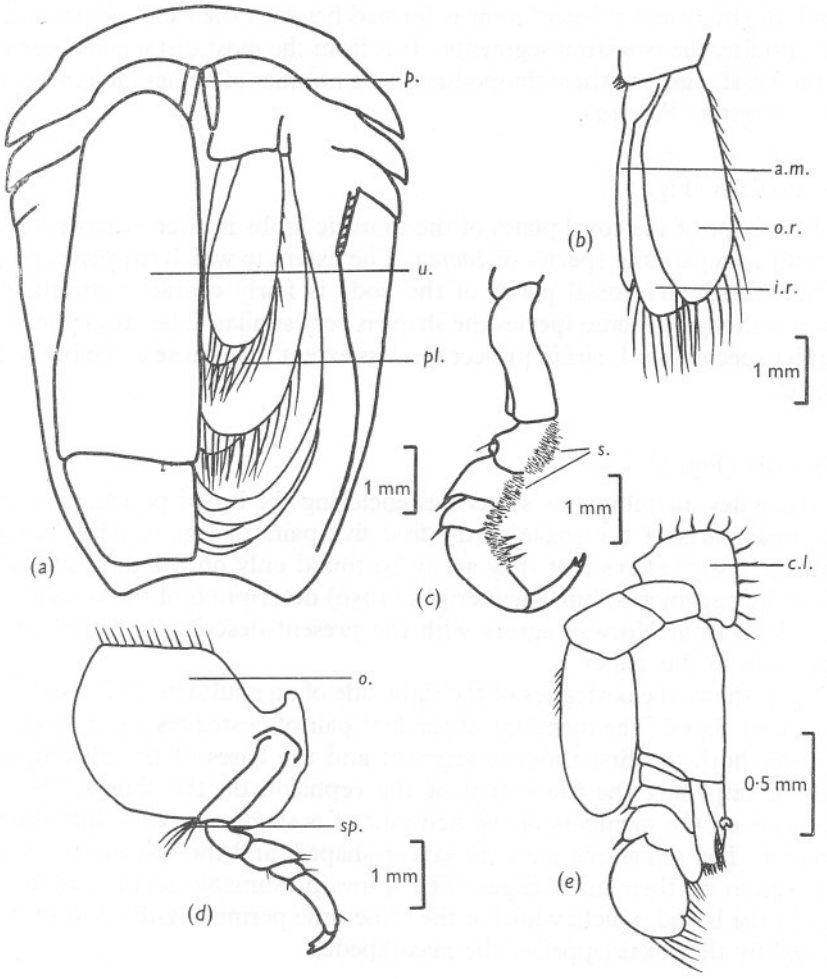

Fig. 4. Secondary sexual characters of *Idotea emarginata*: (*a*) *abdomen of male*, from below, showing penes (*p.*) (*u.*, uropod; *pl.*, pleopod); (*b*) *appendix masculinus* (*a.m.*) on the second pleopod of the male (*o.r.*, outer ramus of pleopod; *i.r.*, inner ramus); (*c*) *2nd leg of adult male*, showing pads of finesetae (*s.*); (*d*) *4th leg of adult female*, with spines (*sp.*) and oostegite (*o.*); (*e*) *maxillipede* of adult female, showing lappet (*c.l.*).

female. The second pair of legs of males of all species (and all succeeding legs in *I. linearis* and *I. pelagica*) bear thick pads of fine setae on their inner surfaces (Fig. 4*c*). This pair of legs is used by the male in clasping the female in the

periods of pairing prior to copulation, when it would appear that the pads of setae give a good grip on the smooth surface of the female.

In adult females the first five or six legs acquire spines which support the oostegites in their task of forming a brood pouch. The legs flex inwards beneath the body and a 'knee' joint is formed between the ischiopodite and the meropodite, the two basal segments. It is from the most distal and inner edge of the basal segment, the ischiopodite, that a number of spines fan out beneath the oostegites (Fig. 4*d*).

Coxal Plates (Fig. 11)

The shape of the coxal plates of the thoracic limbs is often regarded as important in separating species of *Idotea*. The extent to which the plates project from beneath the dorsal plates of the body is fairly characteristic for each species, though in some species the shape is very similar. The coxal plates of a slender species like *I. viridis* project to a less extent than those of a more robust species.

Oostegites (Fig. 5)

Oostegites, membranous structures enclosing the brood pouch, arise from the inner parts of the coxae of the first five pairs of legs of adult females. Collinge (1917) states that they are to be found only on the third and three succeeding segments, but Kjennerud's (1950) description of the oostegites of *I. neglecta* from Norway agrees with the present description for all species dealt with in this paper.

Fig. 5 shows the oostegites of the right side of an adult female *I. emarginata* seen from above. The members of the first pair of oostegites are so shaped as to cover both the first thoracic segment and the bases of the maxillipedes, without impeding the movement of the cephalon on the thorax. Hooked processes on the members of the first pair of oostegites serve to link the two together. The remaining ones are saucer-shaped, and the last two pairs bear long spines on their inner edges. The spines presumably serve to retain the eggs in the brood pouch, whilst at the same time permitting the flow of water created by the coxal lappet of the maxillipede.

The External Genitalia

The male genital ducts open at the tips of the paired penes, the latter arising from the first abdominal segment and projecting back towards the bases of the pleopods (Fig. 4*a*).

The female genital ducts open on the fifth thoracic segment.

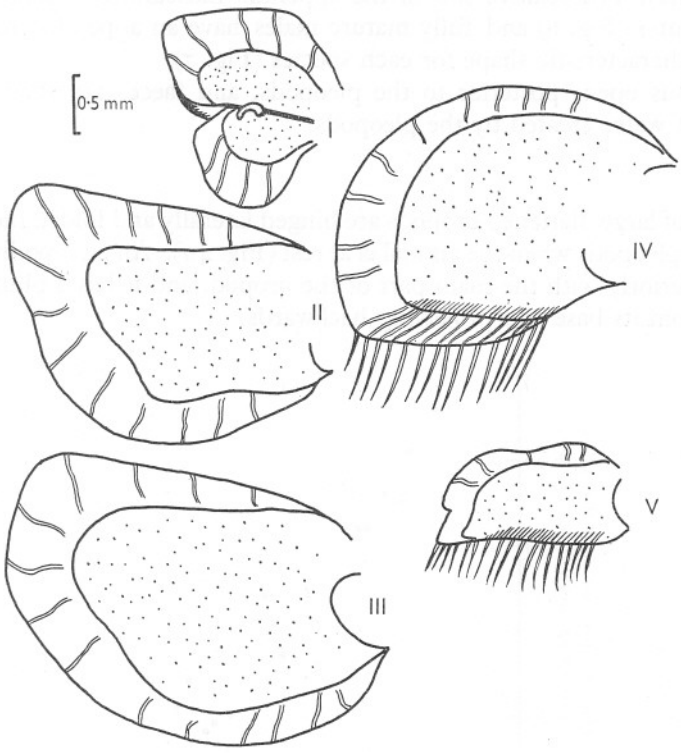

Fig. 5. The right oostegites of an adult female *I. emarginata*, from legs I–V, seen from above.

The Appendages of the Abdomen

Idotea illustrates the typical malacostracan arrangement of six pairs of abdominal limbs, namely, five pairs of pleopods and one pair of uropods. Fusion of the segments themselves, however, is such that only three are distinguishable, the last of these being incompletely fused with the telson.

Pleopods

The pleopods consist of five pairs of bilobed, lamellar structures, arising close together behind the last thoracic segment and projecting backwards beneath the abdomen (Fig. 4*a*). The current of water created by movement of these appendages serves for both respiration and swimming.

The first two pairs of pleopods possess long setae fringed with fine hairs, but such setae are lacking from the other pleopods. The inner ramus of the second pleopod of adult males bears a stylet, or appendix masculinus (Fig. 4*b*), a paired structure which serves to transfer sperm from the penes to

the oviducts. The relative size of the appendix masculinus decreases as the male matures (Fig. 6) and fully mature males have an appendix masculinus of fairly characteristic shape for each species (Fig. 7).

The anus opens posterior to the pleopods, and faeces are shed into the current of water created by the pleopods.

Uropods

A pair of large, flattened uropods are hinged laterally and folded medially to cover the pleopods when the animal is at rest (Fig. 4*a*). An endopodite articulates posteriorly with the main part of the uropod, and it has a plumose seta arising from its base and projecting backwards.

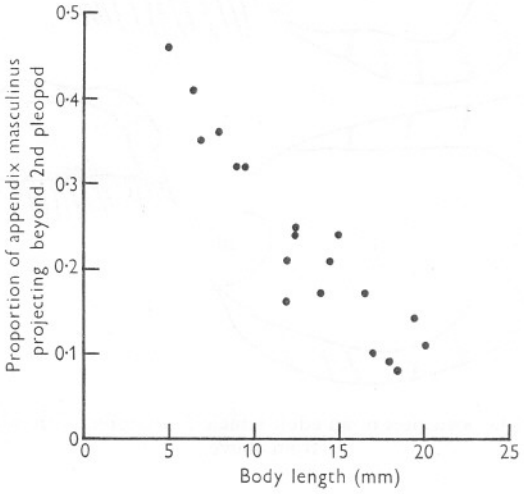

Fig. 6. The proportion of the appendix masculinus which projects beyond the second pleopod, plotted against the body length in mm, for *I. granulosa*.

The Telson

All species begin life with a similar, uniformly rounded telson, but as growth proceeds each species acquires a characteristic telson shape. Fig. 8 illustrates the shape of the telson of specimens of various sizes of all species studied; it shows the close similarity of telson shape in young forms, even of such species as *I. baltica* and *I. emarginata*.

The Taxonomic Value of Various Structures

Numbers of Aesthetascs and Antennal Segments

Howes (1939) noted that the number of aesthetascs and antennal segments in *I. viridis* increased as the animal increased in length. With sufficient data he was able to divide the growth of *I. viridis* into a series of clear-cut growth

stages (instars), each growth stage having a characteristic number of antennal segments and aesthetascs, and having a characteristic size-range. The characters of one stage changed to those of the succeeding one after a moult.

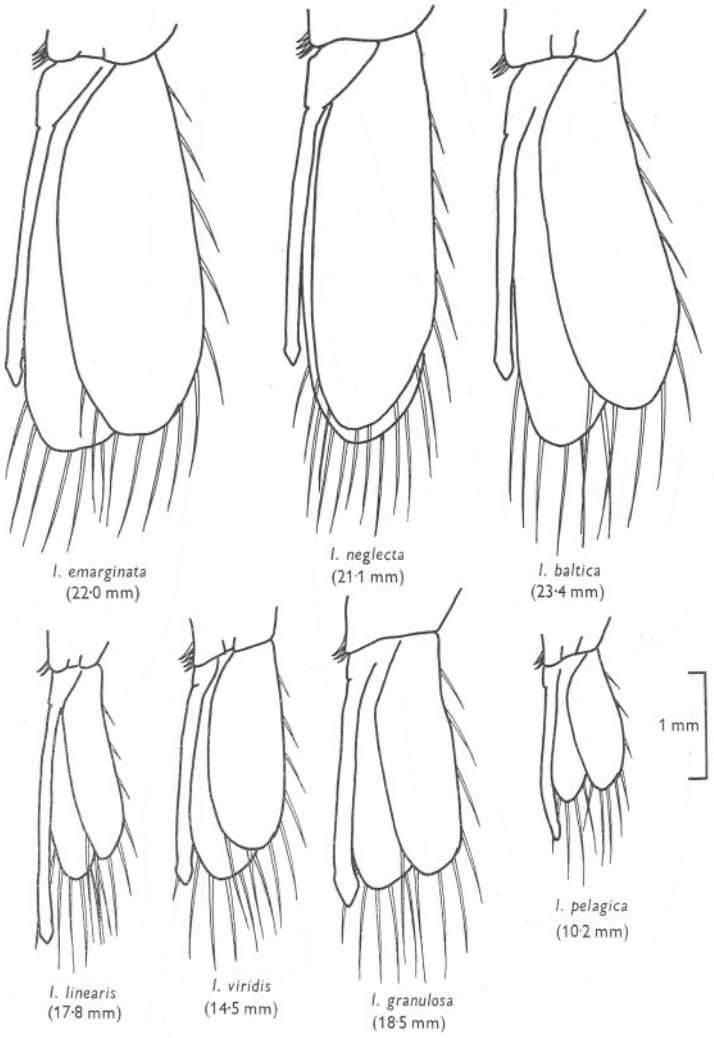

Fig. 7. The second pleopod of male *Idotea*, showing the appendix masculinus in each case.

Kjennerud (1950) treated *I. neglecta* in the same way and obtained a similar series of instars. Obvious differences between similar stages of the two species led Kjennerud (p. 15) to express the view that 'very little is known of the first stages of development in *Idotea* spp., although it is possible that the relation between the size of the animal, the joints on the flagellum of the antenna

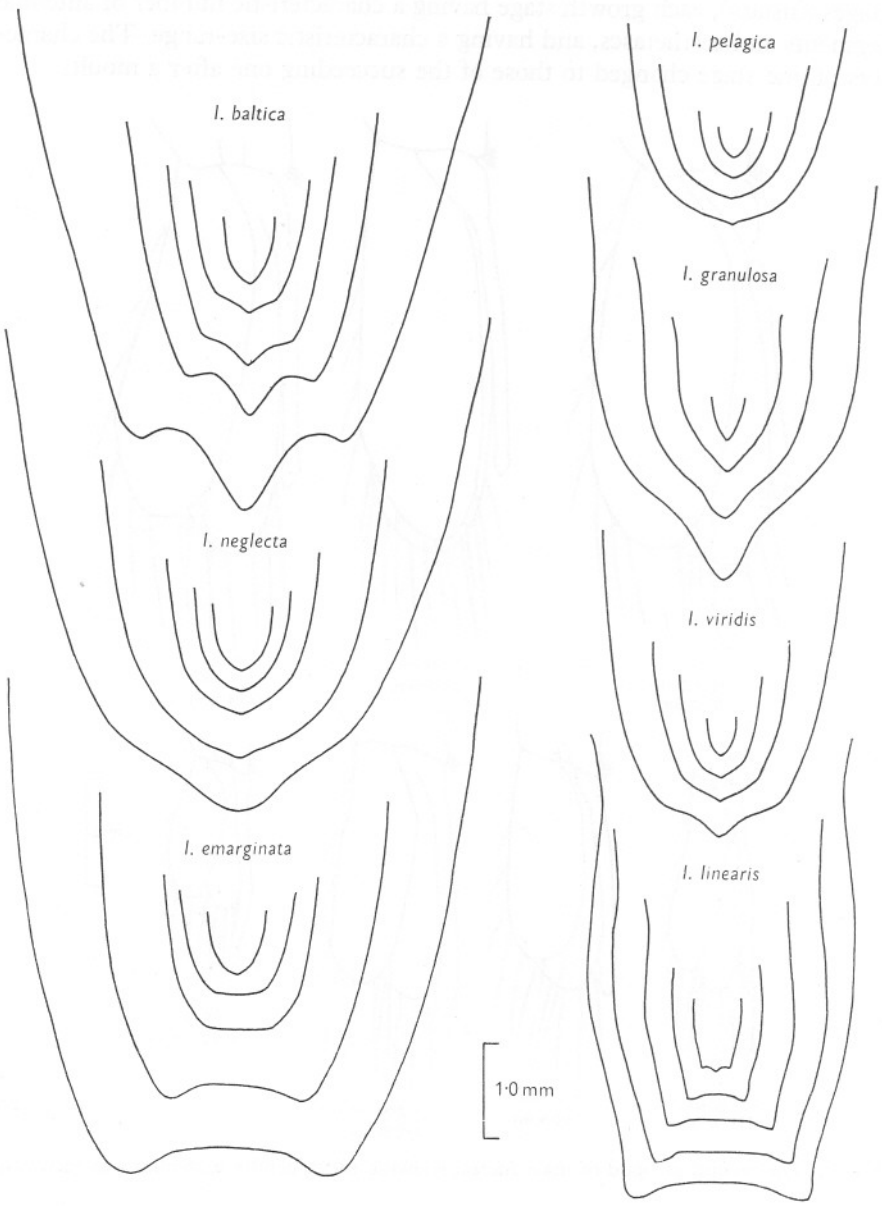

Fig. 8. The development of the telson of seven species of *Idotea*. The body lengths of the specimens under each species were as follows: *I. baltica* (2·2, 5·4, 8·5, 12·0 and 20·0 mm); *I. neglecta* (2·7, 4·3, 6·6, 13·8, and 19·0 mm); *I. emarginata* (2·2, 4·6, 6·8, 12·8 and 19·5 mm); *I. pelagica* (1·7, 3·7, 7·2 and 10·2 mm); *I. granulosa* (1·9, 6·3, 10·8, and 16·2 mm); *I. viridis* (1·7, 4·6, 8·9 and 14·0 mm); *I. linearis* (3·5, 6·3, 11·0, 16·2 and 22·9 mm).

and the number of sense rods on the distal joint of the antennula will prove to be so characteristic for each species that one can distinguish not only between various *Idotea* species, but also between the different instars within each species'.

The principle has been applied to Manx specimens of *I. emarginata*, but it was found difficult to divide the growth of this species into discrete instars (Naylor, 1955*c*). Greater variation than was found by Kjennerud, both in the number of aesthetascs and in the number of joints in the antennal flagellum,

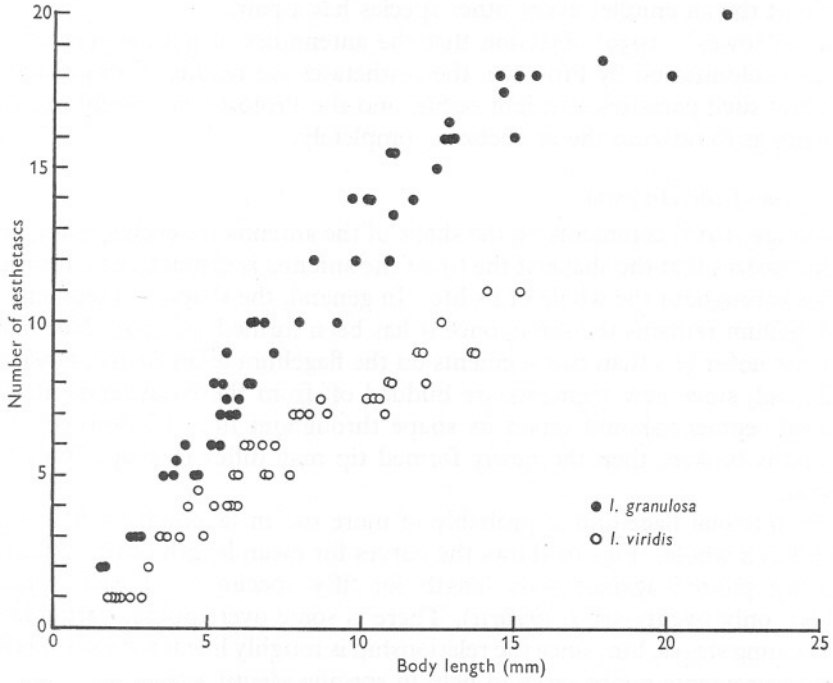

Fig. 9. Individual plots of the number of aesthetascs against body length, for fifty specimens each of *I. viridis* and *I. granulosa*.

limits the general application of the method; this applies not only to *I. emarginata* but to Manx and other British specimens of *I. neglecta*, *I. viridis*, and other species as well. In addition, as has been stated earlier (p. 470), the rate of addition of antennal segments with increasing body length is much the same for each species, except *I. viridis*.

Even, however, if division into instars and its use in taxonomy is subject to limitation, there is some taxonomic value in plotting the number of aesthetascs against the length of the body of the idoteid, irrespective of instars; the two species *I. viridis* and *I. granulosa*, which are often confused, may be completely separated by this character. Fig. 9 shows individual plottings of the number of aesthetascs against body length, for specimens of *I. viridis* and *I. granulosa*.

It will be seen that the two species hardly overlap, and they may be separated at all sizes; except in large specimens *I. granulosa* has more aesthetascs than its body length expressed in millimetres, whilst *I. viridis* generally has fewer.

The Form of the Antennule

The arrangement of the aesthetascs, rather than their number, is probably of more value in taxonomy. Reference to Fig. 1 shows that *I. viridis* and *I. linearis* may be separated from all other species by their single aesthetasc at the tip of the antennule; every other species has a pair.

As to Howes's (1939) objection that the antennules of marine species are liable to colonization by Protozoa, the aesthetascs are readily distinguishable from any such parasites as might occur, and the Protozoa are rarely in such numbers as to obscure the aesthetascs completely.

The Form of the Antenna

Collinge (1917) comments on the shape of the antenna for each species, and he emphasizes that the shape of the tip of the antenna is characteristic for each species throughout the whole of its life. In general, the shape of a segment of the flagellum remains the same, once it has been formed (p. 470). Normally there are never less than two segments on the flagellum of an *Idotea*, however small, and, since new segments are budded off from the basal segment, the terminal segment should retain its shape throughout life. If, however, the antenna is broken, then the newly formed tip may differ in shape from the original.

The antennal flagellum is probably of more use in taxonomy if it is considered as a whole. Fig. 10 shows the curves for mean length of the antennal flagellum plotted against body length for fifty specimens of each species studied (only twenty-six *I. linearis*). There is some overlapping, particularly in the young stages, but, since the relationship is roughly linear for each species the measurements might serve to help in specific identification.

The variation in the shape of the antennal flagellum, from species to species, is best understood when the mode of life of each species is taken into consideration. Species living in situations exposed to wave action have short, stout appendages, whilst those living in sheltered situations have more fragile appendages (Naylor, 1955*a*).

The Maxillule

Collinge (1917) figures and describes the tip of the outer endite of the maxillule for each species, and he states that there is a characteristic number of maxillulary spines for each species. Using these data in a key, Nierstrasz & Steckhoven (1930) separate five species of *Idotea* on the number and degree of toothedness of the spines on the maxillule. More recently, Howes's (1939) description of the spines in *I. viridis* and Kjennerud's (1950) description of those for

I. neglecta both differ from the data given by Collinge for the same species.

The present descriptions again differ, in that every species has the same number of spines on the maxillule, namely, twelve (p. 473). This has been verified in specimens of all species from many British localities, and Kjennerud's description for *I. neglecta* from Norway agrees with British specimens except that it does not include the two blade-like structures fringed with setae (Fig. 3*d*). Howes's description for *I. viridis* omits the most slender of the spines, as well as the blade-like structures.

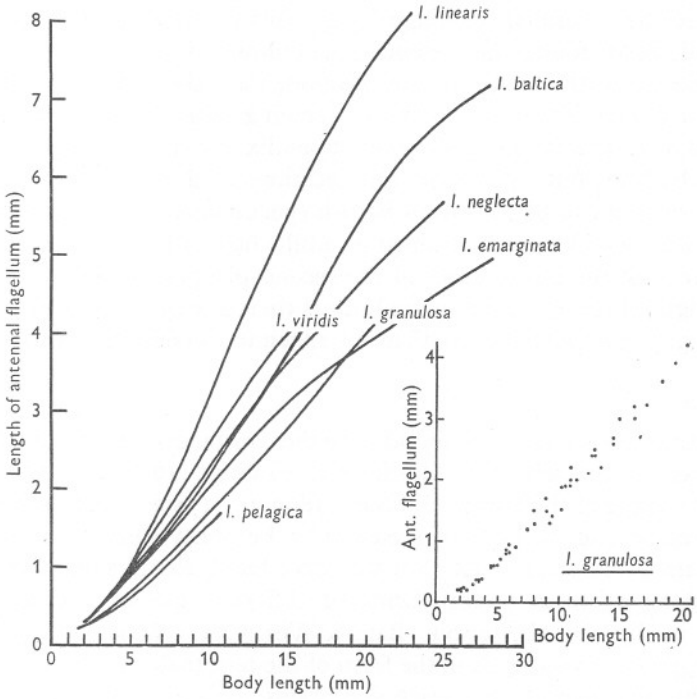

Fig. 10. Mean curves, showing the length of antennal flagellum plotted against body length, for seven species of *Idotea*. Inset shows individual plots for fifty specimens of *I. granulosa*.

The degree of toothedness of the spines varies from species to species, and sometimes throughout the life of each species. Broadly speaking though, the ventral spines, used in scraping, are less toothed than the dorsal ones, which are used in pushing food masses upwards between the mandibles (Naylor, 1955*b*).

In conclusion, it can be said that observations on the maxillules of Manx and other British specimens of *Idotea* do not conform with descriptions given by Collinge (1917), and the key for identification of *Idotea* given by Nierstrasz & Steckhoven (1930) appears to be partly invalidated.

The Coxal Plates

A taxonomic feature often emphasized, and particularly so by Dollfus (1894–5), was the shape of the coxal plates. There is some variation in the form of the plates as growth proceeds, as has been described for *I. viridis* by Howes (1939), but the feature still seems to be of some importance in separating the species (p. 476).

The Appendix Masculinus of the Second Pleopod

Though Sars (1899) regarded this structure as important, and figured it for each species he described, Collinge (1917) fails to mention it in his review. Kjennerud (1950) found the appendix masculinus of *I. neglecta* to be much longer, relative to the length of the pleopod, than allowed for by Sars, and Kjennerud quotes Tattersall (1906) as agreeing with this objection. Manx specimens of *I. neglecta* again show an appendix masculinus longer than that described by Sars, but still further modifications of all Sars's descriptions are necessary since it can be shown, in British specimens of all species, that once the appendix masculinus has attained its adult shape it increases in length at a slower rate than the inner ramus of the second pleopod (p. 478).

The length of the appendix masculinus is therefore of use in separating the species (Fig. 7), provided the length of the specimen is taken into consideration.

The Telson

Conflicting reports are to be found as to the constancy of shape of the telson of each species, throughout its life-history. Collinge (1917) found few variations in the shape of the telson, and Sars (1899, p. 81) states that he found the terminal segment of the body of *I. baltica* 'to be pretty constant even in very young forms'. Tattersall (1906), on the other hand, found some difficulty in distinguishing between young forms of different species because of the similarity in shape of the telson. Bate & Westwood (1868, p. 381) describe for *I. tricuspidata*, 'variations in the form of the terminal segment of the body, with length and size of respective specimens', but it should be noted, as Kjennerud points out, that the telson shapes illustrated for *I. tricuspidata* by Bate & Westwood probably belong to three or four different species.

Howes (1939) discussed these points of view in the light of his own work on *I. viridis*, in which changes in shape of the telson did take place as the animal aged, and he found (p. 307) 'reason to believe that changes in shape of the abdomen occur during the growth of *I. baltica* as they do in *I. viridis*'.

The present investigation reveals the fact that changes take place in the shape of the telson of all species, each beginning life with a more or less uniformly rounded telson and only acquiring the typical adult shape later in life (Fig. 8). Thus, as with many features, though the adult telson shape is charac-

teristic for each species, the juvenile telson shapes are by no means distinct from one another. Sars's figures of adult telsons are very good for the species which he deals with, but the figures given by Collinge are sometimes inadequate.

An additional factor: Habitat

The ecology of *Idotea* is discussed elsewhere (Naylor, 1955*a*), and it is shown that each species has a fairly characteristic habitat. *I. pelagica* and *I. granulosa* are resident intertidal species, *I. viridis* is a brackish water form, and *I. emarginata*, *I. neglecta*, *I. baltica* and *I. linearis* are chiefly sublittoral. If therefore the habitat of a specimen is known it could well aid in identifying that specimen.

Discussion

Any attempt to classify organisms must seek differences which are readily measured, and which are constant for similar stages of the organisms concerned. Thus, in the case of *Idotea*, which attains adult form only gradually, rather than by a distinct metamorphosis, any differences must ideally persist over the whole development of the animal. This factor appears to limit many of the earlier descriptions; the full extent of the life-cycle seems not to have been covered in the descriptions such as those by Collinge (1917), and some cases of apparent misidentification are to be found in subsequent literature.

It is with this in mind that Howes (1939, p. 307) ends his discussion of the growth of *I. viridis* with the statement that 'only the breadth/length ratio of the cephalon and the number of spines on the outer lobe of the maxillule emerge as constant throughout the whole period (of growth)'. With regard to these characters, it has been shown above (pp. 473 and 483) that the number of spines on the maxillule is useless as a taxonomic character because all species have the same number; and reference to the species-by-species descriptions given below will show the close similarity of the cephalon measurements. Therefore, of the characters studied by Howes, none would appear to be of certain value in taxonomy.

The general study of growth of all species does, however, reveal some differences of value in separating species. What is more, in the light of Huxley's (1932) emphasis of the importance of allometry in taxonomy, rates of change, such as the rate of addition of aesthetascs on the antennule, might well be used in separating some species.

The problem, therefore, in giving adequate taxonomic descriptions of species, is to fit the recent principles of experimental taxonomy with their element of 'change', into the older concept of the more 'static' nature of the species (see Gilmour, 1952). This applies even more if local differences are found within an individual species; *I. viridis*, for example, may mature at different instars in different localities (Howes, 1939), and this may well be

true of other species as well. More work is clearly needed to determine any geographical variation within any one species.

An attempt has been made to embody the characters of later developmental stages, as well as those of adults, in the following key.

KEY TO BRITISH *IDOTEA*

(The smallest specimens, up to about 6 mm, are disregarded.)

1. Telson with apical border straight or concave.
   (*a*) Body elongate, narrow: mesosome segments 2–7 less than twice as wide as long. Antenna $> \frac{1}{2}$ body length. Coxal plates very small, not reaching posterior border of any segment (Fig. 11). Telson with lateral corners acutely angled, and with a smaller median spine in youngest specimens. Colour brown-green, with white or darker markings. Mainly sublittoral, in fully marine conditions. **I. linearis** (L.)
   (*b*) Body less elongate: mesosome segments 2–7 twice as wide as long, or more. Antenna $< \frac{1}{2}$ body length. Coxal plates 3–7, at least, reaching posterior border of segment.
      (i) Telson with apical border concave and angulate at sides (adult) or straight with rounded corners (juvenile), the sides somewhat rounded (Fig. 8). Coxal plates 5 and 6 not sharply angled (i.e. angle $> 45°$). In shallow coastal waters; mainly sublittoral. **I. emarginata** (Fab.)
      (ii) Telson with apical border straight, with rounded corners (adult) or widely rounded corners (juvenile); the sides straight. Coxal plates 5–7 very sharply produced, making angles of less than 45°. An infrequent, offshore species. **I. metallica** Bosc.
2. Telson with apical border produced and more or less angulate in centre.
   (*a*) Telson apical border tridentate or nearly so; median process acute and well protruding. Green or brown, often with pattern of spots and lines. Marine, chiefly sublittoral, in shallow water. **I. baltica** (Pallas)

   (N.B. Large specimens of *I. viridis* may have angled shoulders to the telson, but this brackish water species differs structurally from *I. baltica* by having a single aesthetasc at the tip of the antennule, instead of a pair, and coxal plates 2, 3 and 4 do not reach the posterior border of the segment as they do in *I. baltica*.)
   (*b*) Telson apical border not tridentate, almost invariably rounded laterally.
      (i) Antenna shorter: segment 3 of peduncle, viewed from below, not longer than its maximum width: flagellum usually $< \frac{1}{5}$ body length (Fig. 11). Antennule stouter (Fig. 1), the number of aesthetascs exceeding the length of the body in millimetres. ♂, adult appendix masculinus protrudes beyond 2nd pleopod (Fig. 7). Intertidal, amongst algae or barnacles.
         (*aa*) Antenna very short: only segment 5 of peduncle longer than wide: flagellum markedly shorter than peduncle (Fig. 2). Telson broadly rounded; in adults with a small median point which is obtuse (Fig. 8). Colour mostly purplish with white patterning. Frequents exposed barnacle-covered rocks, sheltering in algal tufts, dead barnacles, etc. **I. pelagica** Leach

(*bb*) Antenna less short: peduncle with segments 4 and 5 longer than wide, and flagellum about as long as peduncle (Fig. 2). Telson narrower, with shallow concavity along its sides, its apical margin typically with a distinct median process which is acute (but rounded at the tip (Fig. 8)). Colour mostly red, brown or green. Large specimens frequent fucoids in the mid-tidal zone; smaller specimens amongst *Cladophora* etc. **I. granulosa** Rathke

(ii) Antenna longer: segment 3 of peduncle viewed from below longer than maximum width, flagellum equal to or more than $\frac{1}{5}$ body length (Fig. 2). [If the flagellum is about equal to $\frac{1}{5}$ body length, but the sides of the telson are slightly concave, turn to (i, *bb*) above.] Antennule more slender (Fig. 1), the number of aesthetascs equal to or less than body length in millimetres. ♂, appendix masculinus does not project beyond the 2nd pleopod in the marine species.

(*aa*) Body more slender, width = $\frac{1}{4}$ length, or less. Head narrow, only $1\frac{1}{2}$ times as wide as its median length. Coxal plates 3 and 4 not reaching posterior margin of segment. Antennule tip with single aesthetasc, longer than the rest (Fig. 1). Adult ♂ appendix masculinus extending to or just beyond tip of 2nd pleopod (Fig. 7). Colour mostly uniformly green or brown. Confined to brackish water, sometimes in pools above H.W. **I. viridis** Slabber

(*bb*) Body more robust, width > $\frac{1}{4}$ length. Head wider, at least $1\frac{3}{4}$ times as wide as long. Coxal plates 3 and 4 reaching posterior border of segment. Antennule with paired aesthetascs at tip (Fig. 1). ♂, adult appendix masculinus not reaching tip of 2nd pleopod (Fig. 7). Marine, from L.W.S. downwards, amongst weed and sometimes under stones. **I. neglecta** Sars

(N.B. The telson of this species is broader than in *I. viridis* and *I. granulosa*, but more pointed at the apex than in *I. pelagica*.)

Specific Descriptions

Idotea pelagica (Fig. 11)

Body short and stout; 3–$3\frac{1}{2}$ times as long as broad. *Cephalon* rather more than $1\frac{1}{2}$ times as broad as long in the mid-dorsal line; anterior and posterior borders slightly concave. *Antennule* extending well beyond the second, but hardly beyond the third, segment of the antennal peduncle; first segment not greatly expanded in relation to the other segments, which are all robust. *Aesthetascs* in pairs, numbering up to 16 in males and up to 12 in females; aesthetascs greater in number than the body length in millimetres. *Antenna* very robust; flagellum by no means as long as peduncle and less than $\frac{1}{6}$ body length; most segments of flagellum broader than long, and with pads of fine hairs in adult males; segments numbering up to about 9 in both sexes, with terminal joint very stout and about $\frac{1}{4}$ the length of the subterminal segment. *Coxal plates* fairly broad, widening posteriorly and reaching posterior border of segments 4–7. *Legs* all very robust, the terminal claw being relatively larger than that

of any other species; all legs after the first have pads of fine setae on their inner surfaces, in adult males. *Abdomen* with broadly rounded *telson* having a short median, obtuse tooth; not keeled. *Appendix masculinus* extending beyond the tip of the 2nd pleopod for nearly $\frac{1}{2}$ its own length in small males (4–5 mm) and for $\frac{1}{4}$ its length in large males (10–11 mm).

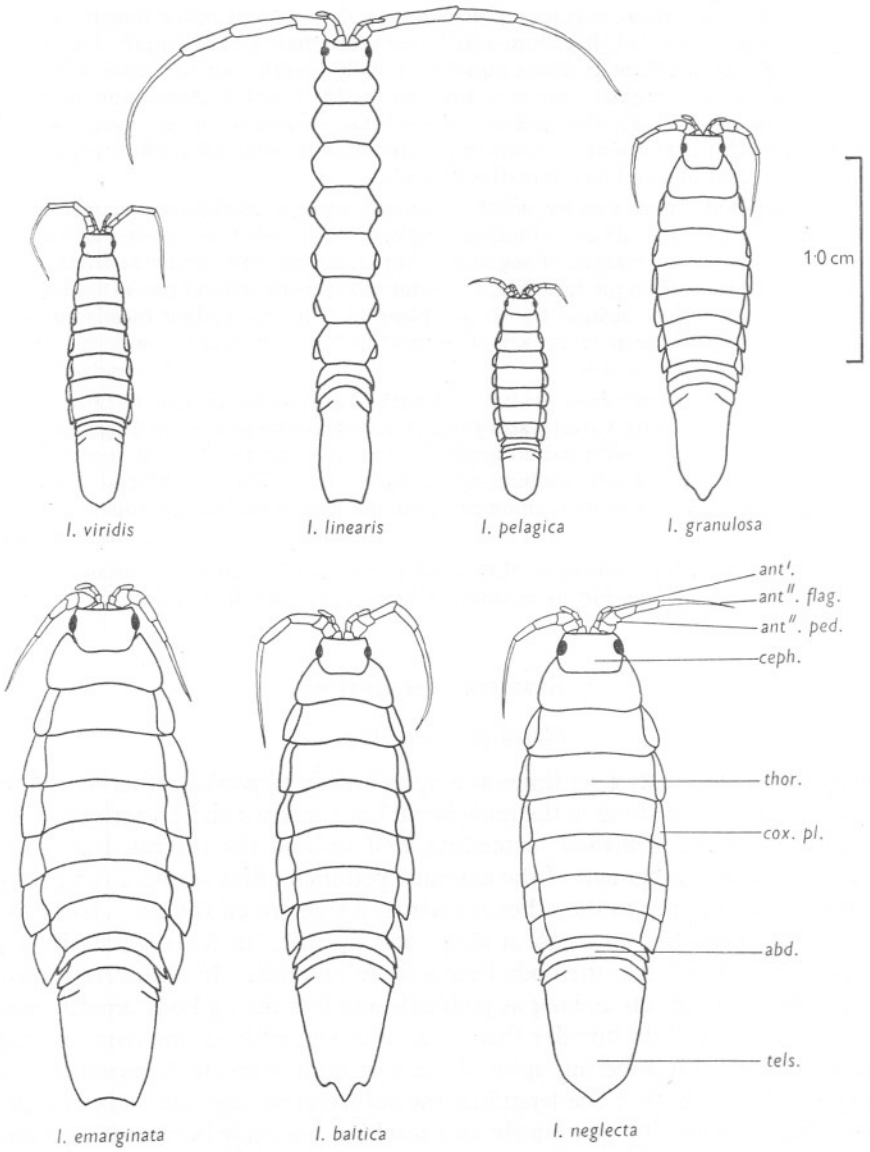

Fig. 11. Adult males of seven species of *Idotea*, seen from above.

Length at release from brood pouch 1–2 mm; males recognizable at about 4 mm, ranging to 11 mm; females 7–10 mm.

Colour: mostly dark purple-brown, with white diamond-shaped patches or elongated stripes down the mid-dorsal line, and with white markings along the edges of the dorsal side; females often darker than males.

Habitat: typically on shores exposed to wave action, where weeds are stunted and barnacles abundant. The colouring merges well with the background of barnacles.

Idotea granulosa (Fig. 11)

Body oval, narrowing rather sharply posteriorly; length often rather more than $3\frac{1}{2}$ times the width. *Cephalon* about $1\frac{1}{2}$ times as broad as long; anterior border almost straight, posterior border somewhat concave. *Antennule* extending beyond the second, but not beyond the third, segment of the antennal peduncle; first segment somewhat expanded, the second rather less so, and the others robust. *Aesthetascs* arranged in pairs, numbering up to 22 or more in males, and up to 16 or so in females; aesthetascs greater in number than the body length in millimetres. *Antenna* robust, flagellum not reaching length of peduncle (except possibly in largest males), and usually less than $\frac{1}{5}$ body length; segments of the flagellum numbering up to 16 in males, and 13 in females; largest males have tufts of setae on the flagellum; terminal segment pointed, and about $\frac{1}{3}$ the length of the subterminal one. *Coxal plates* fairly narrow, broadening posteriorly, and not extending to the posterior border until segment 4 and posterior ones. *Legs* robust; the second leg of adult males having pads of fine setae on the inner surfaces of the 4 most distal joints. *Abdomen* narrowing sharply at first, with rather concave lateral sides; *telson* with long, acute, median spine and very obtusely rounded lateral shoulders; not keeled. *Appendix masculinus* in smaller males projecting by about $\frac{2}{5}$ its own length, but only for about $\frac{1}{10}$ its own length in larger specimens (15–20 mm).

Length at release from the brood pouch 2 mm; some males recognizable at 5 mm, ranging to 20 mm or a little more; adult females 6–13 mm.

Colour: mostly uniformly brown, red or green, depending on the nature of the weed inhabited; occasionally with longitudinal white markings.

Habitat: predominantly a shore form, particularly abundant on *Ascophyllum*, and fucoids; smaller individuals prefer small weeds such as *Cladophora* and *Polysiphonia*. Occasionally on drift weed.

Idotea viridis (Fig. 11)

Body slender; length 4–5 times the width, though sometimes less, particularly in adult females. *Cephalon* about $1\frac{1}{2}$ times as broad as long; hardly concave, either anteriorly or posteriorly. *Antennule* extending well beyond the third peduncular joint of the antenna; first joint expanded, others slender.

Aesthetascs usually arranged in pairs except for a single one, slightly larger than the rest, situated at the extreme tip of the antennule (any other single ones are added to at the next moult); aesthetascs number up to 11 in males, and up to 9 in females. *Antenna* long and slender; flagellum longer than the peduncle except in very small forms, and about $\frac{1}{4}$ the body length; segments of the flagellum numbering up to 18 in males, and up to 13 in females; terminal style roughly cylindrical, about $\frac{1}{2}$ the length of the subterminal segment. *Coxal plates* narrow, increasing in size posteriorly, and only those of segments 5, 6 and 7 reaching the posterior border of the segment. *Legs* long and very slender, second limb of adult males bearing pads of fine hairs. *Abdomen* with sides subparallel, slightly keeled posteriorly in the mid-dorsal line; *telson* with a single, median tooth, hardly acute, and with obtuse lateral corners. *Appendix masculinus* of most males projecting for about $\frac{1}{10}$ of its own length beyond the second pleopod, though hardly projecting at all in the largest males.

Length when first released from the brood pouch 1–2 mm; males recognizable often from about 5 mm, ranging to 15 mm; adult females range from 6 to 10 mm.

Colour: mostly uniformly green or brown, sometimes with white markings; females often darker than males, as with all species.

Habitat: prefers brackish water; often in brackish pools above H.W.N., which are not continuously reached by the sea. It is also recorded from the tidal region in estuarine conditions.

Idotea linearis (Fig. 11)

Body very slender; length 4–7 times the width, but mostly between 5–6 times. *Cephalon* nearly twice as broad as long; deeply concave anteriorly, and slightly so posteriorly. *Antennule* extending just beyond the second peduncular joint of the antenna; first joint very much expanded and rounded, others slender. *Aesthetascs* long and slender, arranged in pairs except for a terminal one separate from the rest; numbering up to 19 or more in adult males and 17 or more in adult females. *Antenna* very long and slender, peduncle rather longer than the flagellum; flagellum about $\frac{1}{3}$ body length, having up to about 20 segments in males and rather less in females; terminal style pointed, less than $\frac{1}{2}$ the length of the subterminal segment. *Coxal plates* very small, never extending to the posterior border of any segment. *Legs* long and slender, becoming somewhat palmate distally: in addition to the usual armature of spines, the second and succeeding legs of adult males have pads of fine setae on their inner borders. *Abdomen* with sides slightly incurved: *telson* with concave posterior border, and with a small median spine in young specimens. *Appendix masculinus* extending well beyond the tip of the second pleopod, by about $\frac{1}{3}$ its own length, or rather less, in adult males.

Length at release from the brood pouch between 2 and 3 mm; males often recognizable from about 15 mm, ranging to over 20 mm in length; females rather less in size.

Colour: green or brown, with darker or lighter longitudinal stripes; adult female often darker than male, frequently with paler markings around the edges.

Habitat: usually sublittoral, associated with drift weed or bottom living algae, though occasionally found intertidally.

Idotea baltica (Fig. 11)

Body oblong oval; length usually more than 3 times the width. *Cephalon* width rather more than $1\frac{1}{2}$ times length; somewhat concave anteriorly and posteriorly. *Antennule* extending to or just beyond the distal end of the 3rd segment of the antennal peduncle; 1st and 2nd joints expanded, others elongated and fairly robust. *Aesthetascs* in pairs, numbering up to about 20 in males and about 16 in females. *Antenna* slender, with flagellum longer than peduncle and about $\frac{1}{4}$ the body length; flagellum segments numbering up to 20 in males and 15 or so in females; terminal style pointed, and about $\frac{1}{3}$ the length of the subterminal segment. *Coxal plates* large in adults, extending from anterior to posterior borders of all segments from the 2nd or 3rd backwards, becoming wider posteriorly. *Legs* fairly robust; 2nd leg of adult males having pads of fine setae. *Abdomen* dorsally keeled, with more or less straight sides, tapering to a tridentate *telson*; median process long and acute, lateral processes smaller (obtuse and less pronounced in young specimens). *Appendix masculinus* reaching just about the end of the 2nd pleopod in smaller males, but failing to do so by about $\frac{1}{10}$ of its own length in larger males.

Length about 2 mm in newly released juveniles; males first recognizable at about 10 mm, ranging to 30 mm; adult females 10–18 mm.

Colour: sometimes uniformly green or brown, but often with white marbling in the form of spots, or pale longitudinal lines, particularly along the edges; female often darker than male.

Habitat: generally sublittoral, but not infrequently found on the shore, e.g. on *Halidrys* at L.W.M.; often on drift weed.

Idotea neglecta (Fig. 11)

Body oblong oval; length a little more than 3 times the width. *Cephalon* rather more than $1\frac{1}{2}$ times as broad as long: concave anteriorly and posteriorly. *Antennule* extending to (or nearly to) the distal end of the 3rd joint of the antennal peduncle; 1st and 2nd joints expanded, others elongated and fairly robust. *Aesthetascs* in pairs, numbering up to 12–14 in males, and 10–12 in females. *Antenna* fairly slender, flagellum longer than peduncle and $\frac{1}{5}$–$\frac{1}{4}$ the

body length; flagellum segments numbering up to 20 in males and up to about 14 in females; terminal style pointed and averaging $\frac{1}{3}$ the length of the subterminal segment. *Coxal plates* wide, extending the whole length of the segment from the 2nd or 3rd to the last. *Legs* robust, the 2nd legs of adult males having pads of fine setae. *Abdomen* with sides fairly straight and converging posteriorly, with hind border of *telson* characterized by a median obtuse tooth, and obtusely rounded lateral shoulders; keeled. *Appendix masculinus* fails to reach the tip of the second pleopod by up to $\frac{1}{3}$ of its own length.

Length on release from the brood pouch about 2 mm; males recognizable from about 8 mm, ranging to nearly 30 mm; adult females range from 10 to 16 mm.

Colour: often uniformly brownish, sometimes with white longitudinal lateral markings, and occasionally with white marbling over the whole dorsal surface; adult females mostly darker than males.

Habitat: generally sublittoral, often on accumulations of organic matter rather than on attached weed; occasionally on surface drift weed.

Idotea emarginata (Fig. 11)

Body oblong oval; length hardly more than 3 times the width. *Cephalon* about $1\frac{3}{4}$ times as broad as long; both anterior and posterior borders concave. *Antennule* extending beyond the 3rd joint of the antennal peduncle; first and second joints expanded, others fairly robust. *Aesthetascs* in pairs; numbering up to 16 in males, and up to about 14 in females. *Antenna* somewhat robust, flagellum longer than peduncle and about $\frac{1}{6}$–$\frac{1}{5}$ the length of the body; flagellum segments numbering up to about 18 in males and 14 in females; terminal style pointed, and about $\frac{1}{3}$–$\frac{1}{2}$ the length of the subterminal segment. *Coxal plates* broad, extending over the whole length of the segment in segments 2 or 3 and posterior ones; becoming wider posteriorly. *Legs* robust; second leg of adult males having setose pads. *Abdomen* with sides curving slightly outwards, and with an emarginate *telson*, the lateral shoulders forming two obtuse points which are recognizable from a size of about 5 mm onwards; telson at first bluntly rounded, then cut straight across, then showing the emarginate character as growth proceeds. *Appendix masculinus* almost reaching the tip of the second pleopod in small males, though it fails to do so by up to $\frac{1}{4}$ of its own length in adult males.

Length when first released from the brood pouch about 2 mm; males first recognizable from 7–9 mm, depending on the season of release, and ranging to about 30 mm; adult females from 9 to 18 mm.

Colour: males, particularly, often uniformly brown in colour, though sometimes with white markings; females generally darker in colour, often with

longitudinal lateral white bands, or alternating white and darker transverse bands.

Habitat: generally sublittoral on accumulations of algae, though occasionally on drift weed.

SUMMARY

Detailed descriptions of the external morphology, from the functional and developmental aspects, are given for seven species of *Idotea*. The taxonomic value of various structures is discussed in relation to previous work on the genus, and a key to the identification of *Idotea* is given. Specific descriptions are revised.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

VIII. FOOD UPTAKE, ASSIMILATION AND EXCRETION IN ADULT AND STAGE V *CALANUS*

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(Text-figs. 1–4)

Introduction

Apart from a few scattered observations (Hensen, 1887; Gran, 1902) the study of the food of *Calanus* began with Dakin (1908). He examined the remains present in the gut, and this was the method used also by Esterly (1916), Lebour (1922) and Marshall (1924). They all found that these remains consisted of a greenish mush containing the skeletons of numerous planktonic organisms, chiefly diatoms and dinoflagellates. Naked flagellates were, however, occasionally seen, and it was realized that the food might in reality consist largely of organisms which had no skeleton and could leave no recognizable remains.

The feeding of *Calanus* can also be studied in the laboratory by keeping the copepods in a suspension of food organisms and seeing if the organism is taken in, and if so whether or not the animal thrives on it. Such experiments have been made by numerous workers (Crawshay, 1915; Clarke & Gellis, 1935; Fuller & Clarke, 1936; Fuller, 1937; Harvey, 1937; and Clarke & Bonnet, 1939). Their criterion for assessing the value of an organism as food was usually either survival time or successful moulting. In female *Calanus* the food value of different organisms can also be tested by their effect on egg production (Marshall & Orr, 1952).

A simple method of finding out whether the food has been taken in or not is by observing whether faecal pellets are produced. This method was first used by Raymont & Gross (1942), and some data were also given by Harvey (1942).

In *Calanus* digestion takes place in the wide anterior part of the gut, and as the food mass passes into the narrow posterior part it is gradually compacted into a cylindrical pellet. When this is ejected, it is surrounded by a delicate pellicle which is sometimes produced into a 'tail' at one end. The pellicle may correspond to the chitinous one described by Forster (1953) on the faecal pellets of decapods.

Descriptions of the variation in size and shape of *Calanus* faecal pellets on several different foods are given by Raymont & Gross (1942) and Marshall & Orr (1955), together with some information on the numbers produced and the differences between male, female and Stage V. The size and shape of faecal pellets show great individual variation; indeed in an experiment in which two *Calanus* had been introduced by mistake into one dish, the two sets of pellets were easily distinguishable. The shape and size also depend on the food from which they are produced. With *Dunaliella* they are short and dark with an average length of about 500 μ for females and Stage V, and about half this for males. In general those produced with flagellates are dark but with *Dicrateria* and *Hemiselmis* the pellets tend to be smaller and paler than those with *Dunaliella*. With *Chromulina* the pellets are very sticky and difficult to deal with. With *Chlorella* they tend to fluff up and disappear. With diatoms the pellets are long and pale in colour; for example, with *Skeletonema* they average in females 950 μ in length. When *Calanus* is fed on thecate dinoflagellates such as *Prorocentrum* and small *Peridinium* spp. the presence of unbroken shells in the pellets gives them a characteristic granular appearance.

The size of the pellets is unaffected by the concentration of the food unless this is low, e.g. with a concentration of *Dunaliella* of 8 cells per mm^3, they measured about 300 μ instead of the usual 500 μ (Fig. 1 and Table V). When food is very scarce indeed a few pellets are still produced but these are 'ghosts' being very small and almost transparent.

In one experiment (Table I) 13 *Calanus* were kept individually in a food culture and examined at frequent intervals. The different females had different rates of faecal pellet production but these remained fairly constant over the 24 h.

Methods

Two methods were adopted to study the food uptake of *Calanus*. The first was to count the number of faecal pellets produced under standard conditions. The individual variations can be very great, and to avoid this source of error experiments were made with duplicate or triplicate samples of 20 *Calanus*, each in a bowl containing about 400 ml. of the culture to be tested. The number of faecal pellets in aliquot samples was counted after a period of about 16–24 h. The volume per *Calanus*, 20 ml., was later found to be too low for optimum results (see p. 502).

Since the amount of food taken varies with the developmental stage and the season of the year, *Dunaliella* was used as the standard food and other cultures compared with it. When different species were compared as food an attempt was made to provide equal volumes of cell substance in the cultures used. This method, however, was superseded by the second, and results obtained by its means are used only to confirm or supplement later observations.

The second method was to use radioactive phosphorus (^{32}P) as a tracer

element. Cultures of many phytoplankton organisms can be grown using ^{32}P as part source of the phosphorus necessary and these can in turn be fed to *Calanus*. The estimation of ^{32}P is rapid and extremely sensitive, and by this means the uptake, assimilation and excretion of *Calanus* can be accurately measured. Carrier-free orthophosphate in dilute hydrochloric acid was used as supplied by the A.E.R.E., Harwell, and from 0·25–2·0 mc was added to each litre of culture solution. When growth was good this gave a reasonably high activity by the time the cultures had reached a stage suitable for use. Many of the cultures were grown using only dilute modified Miquel solution (Ketchum & Redfield, 1938), but to others soil-extract or other growth-promoting substances were added. The cultures were considered suitable for experiment when most of the ^{32}P had been taken up by the cells. Phosphorus is an important limiting element for plant growth in the sea, and is also important in animal nutrition. It is present in all the major food constituents and should therefore give a good idea of the assimilation but some caution is necessary in interpreting the results.

It has been shown by various workers (Kamen & Spiegelman, 1948; Gest & Kamen, 1948; Goldberg, Walker & Whisenand, 1951; Rice, 1953) that when algae are grown in phosphate-rich media, some of the phosphorus in the culture organisms may be labile or very loosely bound. Rice has shown that in cultures with low P concentrations, only about 2% is exchangeable after they are a week old. In our cultures phosphorus was a limiting factor for growth, and as a rule they were not used until the ^{32}P in solution had dropped below 10% and sometimes below 5% of the total. On a few occasions when there was a considerable proportion of the ^{32}P in the filtrate, the organisms were filtered off or centrifuged, washed and re-suspended in membrane-filtered natural sea water.

Since it has been stated that the loosely bound phosphorus can be removed by washing, an experiment was made to find whether the percentage assimilated of the phosphorus in the culture was significantly lower after repeated washing. A culture of *Lauderia* was centrifuged (10 min at 2500 rev/min), the supernatant liquid siphoned off and the cells re-suspended in filtered sea water. This was done four times and the resulting suspension compared as food with the original, uncentrifuged culture, using the method described below. Since two-thirds of the cells had been lost, the original culture was diluted to give approximately the same cell content. The results are shown in Table II. In the centrifuged culture the percentage utilization varied from 87·2 to 93·5, in the untreated from 86·9 to 91·3, an insignificant difference.

After a considerable amount of trial and error, the following method was adopted.

Calanus, usually adult females, were picked out from a tow-netting and kept starved for several days in a cool room in the dark. It was found that they fed better and behaved more uniformly under such conditions than if they were

taken from the sea and used for an experiment the same day. The number of cells in the radioactive culture was estimated, and dilutions made by adding sea water which had been filtered through a 'Gradocol' membrane of average pore diameter 0·9 μ. The usual dilutions were in the ratio 100:10:1. Five bottles of about 70 ml. capacity were used for each dilution and a single *Calanus* introduced into each. In addition, two bottles were filled with filtrate from the strongest by filtering it through a double layer of Whatman no. 42 paper. This was usually sufficiently fine to remove all the organisms. A *Calanus* was also added to each of the bottles with filtrate. When *Calanus* are kept in a solution containing inorganic ^{32}P, some of this is taken up in the body. The results from the two control *Calanus* gave a measure of this, enabling a correction to be made if necessary.

Each bottle was then tied in a dark cloth bag and attached to a wheel which was rotated slowly in a vertical plane (about one revolution in 2–3 min) to avoid settling out of the food organisms. The slow rotation is not likely to have any effect on the behaviour of the *Calanus*. Samples of the culture dilutions and of the filtrate were taken in triplicate or quadruplicate with a 0·2 ml. delivery pipette and dried on a metal disc (planchette) for measurement of the activity.

After a period, usually of about 16–24 h, the experiment was stopped. The *Calanus* was removed from each bottle, washed in three changes of sea water, transferred to a disc in a small drop of water, roughly torn up to avoid self-absorption of the ^{32}P and dried slowly on a warm plate. The radioactivity was then measured. On many occasions a sample of the final wash water was also tested.

The contents of each bottle in turn were then transferred to a rectangular Perspex dish with the inside angles bevelled to facilitate examination of the whole of the bottom. The dish was placed on a Perspex sheet marked off in squares and the faecal pellets and eggs (if present) removed under a binocular microscope. These also were washed three times, transferred to discs and dried for measurement of the activity. A sample of the final wash water was tested in each case.

In some experiments many of the faecal pellets were found broken or crushed at one end. It seems as if the *Calanus* must encounter a considerable number of them when swimming and must damage them. Any appreciable loss of ^{32}P after the pellicle is broken might cause a serious error in the results. To find if this was important nine sets of faecal pellets (20–60 in each) some whole and some broken, were left overnight on coverslips in a drop of water. In the morning the water was withdrawn and tested as well as the faecal pellets. The radioactivity of the water was found to be only just over 1% of that of the pellets and the loss is therefore negligible. When faecal pellets are broken, small fragments may be lost during washing, and although this loss was never serious, it will tend to make digestion results appear higher.

A description of the method of measuring the radioactivity is given in this *Journal* by Spooner (1949). The discs were exposed in a lead castle at a fixed distance from the aluminium end-window of a Geiger-Müller counter and the impulses counted on a scaling unit. Corrections were made for quenching time, distance from the end-window, background and, when the experiment lasted for more than a day, for decay as well. The results can be expressed either as number of pulses per minute, or, since the activity of the culture and the number of cells are known, as cell equivalents.

In Table III are shown the results of a typical experiment with *Skeletonema costatum* culture. It shows a number of characteristic features. In the first place there is a considerable variation in the behaviour of the individual *Calanus*. Some fed well, some not so well and one not at all. Secondly, the volume filtered was much lower at the highest concentration. The number of faecal pellets was approximately the same at the two higher concentrations and considerably lower in the lowest. The cell equivalents per faecal pellet were approximately the same throughout, although this was not often the case in other experiments. The percentage of the phosphorus-containing portion of the diatom utilized showed no significant difference at the different dilutions.

The rate of filtration, F, of the *Calanus* can be calculated (Gauld, 1951) according to the formula

$$F = v \frac{\log_{10} C_0 - \log_{10} C_t}{t \log_{10} e},$$

where v is the volume of the bottle in ml., t the duration of the experiment in days, C_0 the original and C_t the final concentration of the food organisms. The initial concentration C_0 was obtained by measuring the activity in aliquot samples of the culture used and the final concentration C_t was obtained by subtracting from this figure the activity in the body, eggs and faecal pellets of the *Calanus*.

It should theoretically be possible to obtain the same results by measuring the activity in an aliquot sample from the bottle at the end of the experiment or by counting the cells in the bottle at the beginning and end of the experiment. The last method is less accurate because the error of counting cells is relatively high and the count has to be multiplied by a large figure owing to the smallness of the sample. Similarly, by counting the pulses in the culture at the end by the usual method an error within the normal variation of the count may sometimes cause a large difference in the calculated volume filtered. Since the substance of the cells removed from the culture is concentrated in the body, eggs and faecal pellets of the *Calanus*, this figure is not subject to the same error.

The results of an experiment with *Skeletonema costatum*, in which all three methods were used, is shown in Table IV. The cells were pale and the counts were not very satisfactory; the ten initial counts (each of 3·2 mm^3) varied from

24 to 53, although the final counts were more uniform. Using the final cell counts of the three samples in which filtration was highest, the figures are of the same order of size as by the normal method. For counting the activity of the culture at the end a Veall liquid counter (1948) was used with 2 ml. diluted to 10 ml. in each case. The three sets of figures (ml. filtered in 24 h) show a rough agreement but that based on the activity of the *Calanus* body and faecal pellets is likely to be the most accurate.

In other balance experiments, using *Skeletonema costatum*, *Chaetoceros decipiens* and *Syracosphaera elongata*, the agreement was even less satisfactory.

Effect of Different Factors on Food Uptake

Concentration of food

If *Calanus* is purely a filter feeder the number of faecal pellets produced should rise with increasing concentration of food. This has been noted in the sea for copepod pellets in general (Harvey, Cooper, Lebour & Russell, 1935).

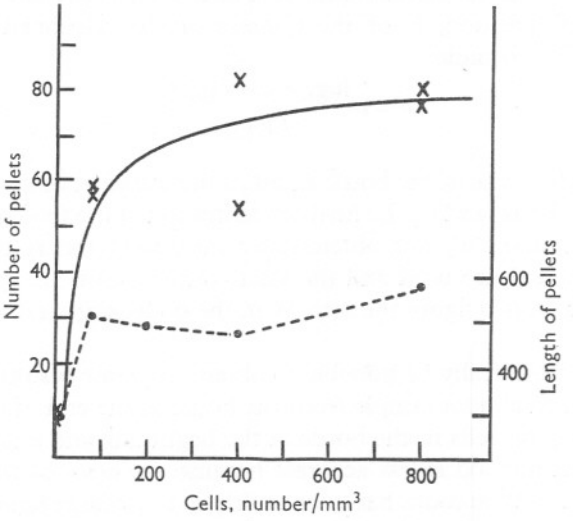

Fig. 1. Effect of concentration of *Dunaliella* on faecal pellet production. ——, number of pellets produced in 24 h; - - -, length of faecal pellet in μ.

Many experiments were made by keeping *Calanus* in bowls containing varying concentrations of the same culture, the number of faecal pellets produced being counted at the end of the experiment. The number usually did increase with increasing concentration but only up to a certain point varying with the organism used. Above this point the number did not increase regularly. The result of one experiment with *Dunaliella* is shown in Fig. 1 and Table V.

It appears that if *Calanus* is a filter feeder, it is not an automatic one but is able to slow down or stop feeding in high concentrations. This confirms observations made by Gauld (1953) that *Calanus* can remain for a long time in a thick suspension of cells, even swimming normally, without feeding.

In most of these experiments there was a tendency for the organisms to settle out or, in the case of some flagellates, to aggregate. The *Calanus* too, especially the females, have a habit of sinking to the bottom of the vessel and rummaging about there, and in dishes where there has been settling out they will get more than the calculated amount. This source of error was avoided in the later experiments with labelled phosphorus by putting the *Calanus* in closed bottles on a rotating wheel.

In a poor concentration of food where few cells are available they will often ingest threads from filter-paper or other such objects and the number of faecal pellets will be higher than one would expect.

When different concentrations were used in an experiment with labelled cultures the highest was much in excess of what is ever found in the sea; the next strength, 10% of this, was equal to or greater than what would be a rich concentration in the sea. Finally it was hoped that the third strength (usually 1%) would represent a moderate concentration in the sea.

In the highest concentration the volume filtered was often lowest. The number of faecal pellets produced in the 10% was always more than a tenth of that in the 100% and was often nearly as high. In the 1% the number produced was usually more in proportion to the concentration of cells, but it was often very low and with a very small number of faecal pellets the loss of one can make a considerable difference to the results. Figures for the weakest concentration may therefore be less accurate; the percentage digested will appear higher and the volume filtered lower than they should be.

To study the feeding of *Calanus* in very low concentrations of cells, such as are found in the sea in winter, a culture of *Chaetoceros decipiens* was grown with a quantity of ^{32}P much larger than usual (1 mc in 200 ml.). With this culture much greater dilutions could be used and reliable counts of pulses still obtained. Even in concentrations as low as 1 cell per ml. and in bottles of 150 ml. capacity the volume filtered in 24 h remained low (under 10 ml.). Similar results were obtained with a much larger diatom, *Ditylum brightwellii* (Table XIV).

Temperature

Most of the experiments were done at room temperature which varied from 10 to 20° C and was always somewhat above sea temperature at the same time. By counting the number of faecal pellets produced by *Calanus* kept at temperatures varying from 5 to 15° C it was found that there was usually a rapid rise in numbers between 5° and 10° C and a lesser rise between 10° and 15° C but the results were variable.

Volume of Container

It was found that the volume of the vessel in which the *Calanus* were kept had an effect on their feeding. In one experiment 15 females were kept individually in dishes of three different volumes (15, 50 and 100 ml.) containing the same culture of *Skeletonema*. These were not stirred during the 16½ h of the experiment. The *Calanus* showed considerable individual variation, but the volumes filtered in the 15 ml. dishes were decidedly lower (average 1·3 ml./24 h) than in the 50 or 100 ml. dishes (average 7·8 and 7·9 ml./24 h). Later experiments were therefore done in bottles of 70 ml. capacity or, in a few cases, 35–40 ml.

Light

It is well known that sunshine or even diffuse light out of doors is lethal to *Calanus* (Huntsman, 1924; Klugh, 1929, 1930); it also affects the respiration (Marshall, Nicholls & Orr, 1935). The harmful effect of bright light reduced the uptake of food in *Calanus* exposed to it (as judged by faecal pellet production), but those of the first generation of the year, which in the Clyde sea area live at the surface, are more resistant to its effects than those of the overwintering generation.

Experiments with radioactive cultures of *Syracosphaera* and of *Lauderia* showed that even in diffuse light indoors the volume of culture filtered was less than with *Calanus* kept completely in the dark. The results with *Syracosphaera* are shown in Table VI. These experiments were done in February in a well-lit room with windows on both sides and overhead, whereas before this all the radioactive feeding experiments had been done well away from the window in a north-facing room. The rather dim diffuse light there is not likely to have affected the *Calanus* appreciably, but subsequently all the experimental bottles were tied in black cloth bags. Since *Calanus* feeds more in the dark than in the light it appears that they do not catch their food organisms by sight.

Age of Culture

It is known that the chemical composition of the cells in a culture may vary with age and with the nutrients present. It is only during the exponential stage of growth that the composition remains approximately constant (Fogg, 1953). Our cultures were almost all grown in sea water enriched with a modified Miquel solution, but it was not possible always to use them at the same age and the amount digested may vary with the growth of the culture.

A number of experiments were made with a single culture of the diatom *Lauderia borealis* at different stages of its growth, and Table VII shows that the percentage utilized decreased with age. The dilutions used in the experiment varied but this did not affect the utilization appreciably. Finally, the old culture was compared with a new young culture of approximately the same

cell concentration and the utilization of the latter was found to be a good deal higher.

In a young diatom culture during the phase of rapid multiplication the cells remain suspended, and it is during this period that the utilization by *Calanus* is high. With the *Lauderia* used this period was about 5 days. The cells then sank to the bottom and became smaller, paler in colour and were united in shorter chains than in a young culture. The change in the amount assimilated indicates that there is a corresponding change in the phosphorus-containing substances built up by the cell.

A later series of experiments with a culture of *Chaetoceros decipiens* up to 18 days old showed only a very slight effect. Similar experiments on a culture of a coccolithophore *Syracosphaera elongata* 16 and 33 days old showed no fall in digestibility, although by the thirty-third day the cells were depositing coccoliths in quantities.

Size of Food Cells

In discussions on the question of the food organisms available and suitable for *Calanus* in the sea much stress has been laid in recent years on the minute flagellates which form an important food of pelagic organisms such as larval molluscs. It was found by Ussing (1938) that the minimum distance between the finest setules on the maxillule in female *Calanus* was 5·7 μ. He suggested that the smallest organisms *Calanus* could filter off must have their greatest diameter more than this. Raymont & Gross (1942), however, kept *Calanus* alive and healthy on a diet of minute flagellates. The possible importance of bacteria also was suggested by Clarke & Gellis (1935), though later Fuller & Clarke (1936) concluded that bacteria were not important as food. The concentrations used were higher than would be expected in the sea.

Various organisms of different sizes were used in feeding experiments on *Calanus* (see Tables XIV–XVI), and the volumes filtered in 24 h are shown in Fig. 2. It is apparent that for organisms below a size of about 10 μ there is a very much lower filtration rate than for organisms of greater size. In the experiments with very small flagellates it was noted that the volume filtered was often greatest in the highest concentration instead of in the lowest as is more usual. This is what might be expected if the cells in a very high concentration gradually clogged up the filtering setae. In calculations of the volume filtered a complete filtration of the food organism is assumed and if it can to any extent pass the filter the calculation is unreliable.

A possible explanation of the failure to take small flagellates might be that they were all unpalatable to *Calanus*, and a search was made for some inert particle of uniform size which could be used instead. Indian ink and carmine are both ingested to a certain extent but the size of the particles is very variable. Dr D. W. Henderson of the Experimental Station, Porton, kindly

supplied us with a suspension of radioactive spores of *Bacillus globigii*, 0·7 μ^3 in volume, and Table VIII shows the results of an experiment made with them. Before use the suspension was washed twice by centrifuging and then resuspending in membrane-filtered sea water. It will be seen that the volume

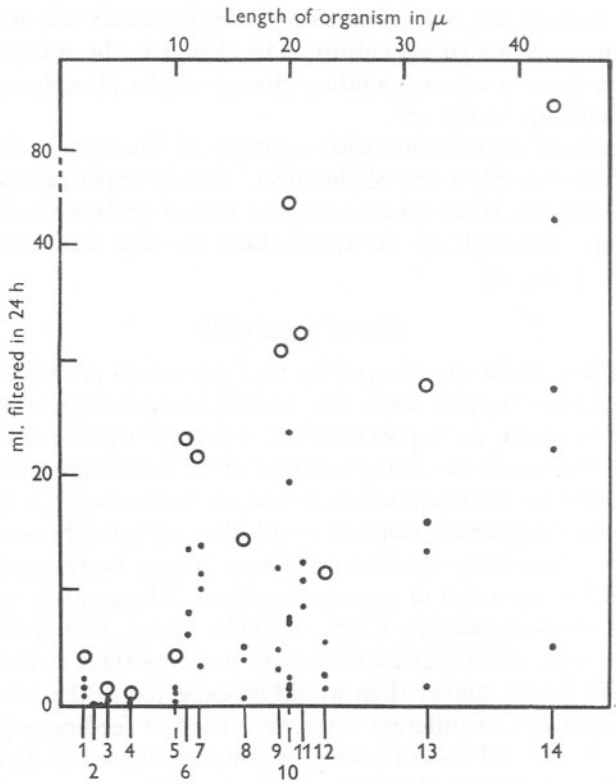

Fig. 2. Relation of size of food cell to volume filtered. •, average with each concentration used in feeding experiments; ○, maximum volume filtered by a single *Calanus*. (1) *Chromulina pusilla*, (2) *Nannochloris oculata*, (3) *Dicrateria inornata*, (4) *Monochrysis lutheri*, (5) *Prymnesium parvum*, (6) *Gymnodinium vitiligo*, (7) *Prorocentrum triestinum*, (8) *Gymnodinium veneficum*, (9) *Chlamydomonas pulsatilla*, (10) *Syracosphaera elongata*, (11) *Cryptomonas* sp., (12) *Platymonas carteriaformis*, (13) *Oxyrrhis marina*, (14) *Prorocentrum micans*.

filtered is extremely low. The small amount apparently digested is probably due to the spores and faecal pellets still in the gut of the *Calanus* at the time it was killed. It is clear that *Calanus* is unable to filter such a small particle efficiently.

Dr M. R. Droop suggested that although the small flagellates might not be taken directly, they might be eaten by larger flagellates which in turn can be eaten by *Calanus*. A culture of the small alga *Nannochloris* (2–4 μ in diameter)

which was itself very little used by *Calanus* was inoculated with a larger flagellate *Oxyrrhis*, which rapidly increased in numbers by feeding on the *Nannochloris* and was then used in a feeding experiment on *Calanus* (Table XVI). The volume filtered and the percentage assimilated showed that the *Nannochloris* could thus be utilized indirectly.

Harvey suggested in 1937 that in a mixture of large and small cells *Calanus* would select the large, and some experiments were made with labelled cultures to test this. The *Calanus* were given a mixture of a very small flagellate (*Dicrateria inornata* 3–5·5 μ) and a larger organism. Previous experiments had shown that in a unialgal culture of *Dicrateria*, *Calanus* produced only a few faecal pellets and filtered only a small volume of water. Feeding was compared in Dicrateria alone, in a mixture of *Dicrateria* of about the same concentration with a large diatom *Lauderia*, and in *Lauderia* alone. Since only the *Dicrateria* was radioactive, feeding in *Lauderia* could be measured only by the number of faecal pellets produced.

The results are shown in Table IX. When *Lauderia* was present nearly five times as many faecal pellets were produced as with *Dicrateria* alone, the amount of *Dicrateria* assimilated was nearly four times as much and over five times the volume of water was filtered. This could be explained by supposing that the *Calanus* in its preference for *Lauderia* was unable to reject the *Dicrateria* which was with it. An alternative explanation is that *Dicrateria* is too small to be efficiently filtered, but that the mucus exuded in cultures of diatoms entangles the cells so that they are ingested with the diatoms. That the second is the more probable explanation was shown by an experiment using *Prorocentrum micans* instead of *Lauderia*. This dinoflagellate exudes much less mucus than *Lauderia* and although the quantities used were greater, the difference between the unialgal and the mixed culture was not marked. With these organisms then it seems that *Calanus* is unable to select the larger from a mixture.

Another experiment was made with *Ditylum brightwellii* and *Chaetoceros decipiens*, two of the genera used by Harvey. Parallel sets were used, one having radioactive *Ditylum* and non-radioactive *Chaetoceros*, the other having radioactive *Chaetoceros* and non-radioactive *Ditylum*. The *Ditylum* used was about fifty times the volume of the *Chaetoceros*. If *Calanus* had shown a preference for the larger of the two diatoms, the volume filtered in the set with radioactive *Ditylum* should have been much the greater, but as will be seen in Table X there is no marked difference between the two.

Choice of Food

To find out whether *Calanus* could acquire a preference for one species rather than another, irrespective of size, a series of experiments was made by keeping *Calanus* in two different kinds of food, and after a few days putting

each set in a mixture of the two foods. Organisms were chosen whose skeletons could be easily recognized in the faecal pellets, and the first experiments were done by examining these faecal pellets.

When a *Calanus* is transferred from one kind of culture into another the change over in the skeletons in the faecal pellets may not be complete until the third pellet has been extruded.

In a qualitative experiment using *Calanus* which had been kept in cultures of *Coscinodiscus centralis* and *Prorocentrum micans* it was found that after some hours in a mixture of the two both lots had taken *Prorocentrum* freely, but that *Coscinodiscus* fragments were present in about two-thirds of the faecal pellets of those fed originally on *Coscinodiscus* and in only one-fifth of those fed on *Prorocentrum*. The diatoms sank to the bottom of the dish while the flagellates swam freely and this may have affected the results. After 3 days, however,

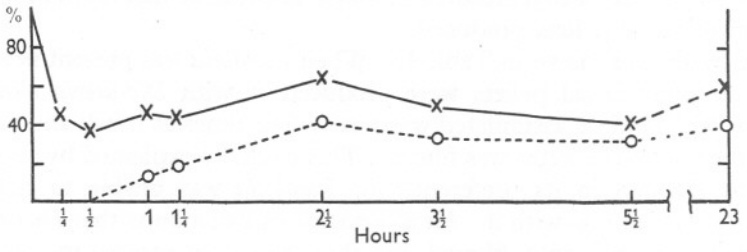

Fig. 3. Choice of food experiment. *Calanus* feeding in a mixture of *Peridinium trochoideum* and *Prorocentrum micans*. ——, % *Prorocentrum* in the faecal pellets of those accustomed to *Prorocentrum*; - - -, % *Prorocentrum* in the faecal pellets of those accustomed to *Peridinium*.

there was no difference between the pellets from the two lots. These experiments suggest that there may be for the first hour or two some power of selection.

Two experiments were then carried out in a similar way comparing Stage V *Calanus* accustomed to *Prorocentrum micans* and *Peridinium trochoideum* respectively. The results were similar but only the second, in which improvements in method were made, will be described (Table XI and Fig. 3). The *Calanus* which had been kept for 10 days in their respective cultures were put singly into dishes containing about 15 ml. of a mixture with about 900 cells of *Prorocentrum micans* and 3900 cells of *Peridinium trochoideum* per ml. This mixture was chosen in an attempt to give approximately equal volumes of each food. The dishes were examined at frequent intervals and the faecal pellets produced by each *Calanus* removed (Table I). These were squashed individually under a coverslip and the skeletons of both organisms counted. The skeletons as a rule remained whole and were easily counted, but occasionally they were so numerous or so many had split into halves that the number was not very accurate. When the number of faecal pellets was small all were

examined but when it was large a sample of three was taken from each *Calanus* and the total number calculated. Individual variation in feeding rate and in the proportions eaten was marked and the average is therefore given. As Fig. 3 shows, there is a considerable difference in the behaviour of the two sets. Those accustomed to *Prorocentrum micans* ate equal numbers of the two species from the first half hour onwards whereas those accustomed to *Peridinium trochoideum* did not begin to eat *Prorocentrum micans* till after half an hour had elapsed and even then the proportion was low. After $2\frac{1}{2}$ h they reached a fairly steady state. The numbers and not volumes are shown in Fig. 3 and Table XI. If we allow for volume there appears to be a preference for *P. micans*, much more marked in those accustomed to *P. micans*.

The number of organisms whose skeletons remain whole and can be counted easily in faecal pellets is very limited, but by growing cultures with ^{32}P one can use foods which cannot be thus recognized. A much more accurate measure of the cells taken up can also be made.

Feeding experiments were done by keeping *Calanus* in two different food cultures and then measuring their uptake in a mixture of the two, only one of which was radioactive. One such experiment compared the utilization of a large diatom (*Lauderia borealis*) with a small (*Skeletonema costatum*). From measurements of the cells in the culture it was calculated that a *Lauderia* had 122 times the volume of a *Skeletonema* cell. Six of each set of *Calanus* were kept in the mixture for $\frac{1}{2}$ h, six for 1 h, six for 2 h, and six for 16 h. A summary of the results is shown in Table XII, and it is clear that there is no significant difference between the two sets.

A similar experiment was made with *Calanus* (fed for a week previously) using *Lauderia* and radioactive *Syracosphaera*, and again there was no significant difference between the two. In a third experiment using radioactive *Lauderia* and *Cryptomonas* (Table XIII) the *Calanus* which had been kept in *Lauderia* did for the first hour eat more *Lauderia* than those kept in *Cryptomonas*, although this difference was not observed in the 2 h samples.

The results of the experiments with ^{32}P do not confirm either Harvey's observations or the results we obtained by counting skeletons in faecal pellets. If selection takes place it does not do so to a marked degree. It is difficult to understand the mechanism of selection since, as has already been mentioned, *Calanus* feeds better in the dark than in the light.

Utilization of Different Species

A variety of different organisms was tested to find their value as food. They were chosen from the main groups found in the phytoplankton and included a variety of size from the 1–3 μ of *Chromulina* to the 100 μ or so of *Coscinodiscus*. Special attention was paid to the very small flagellates since it has often been suggested that they may be of outstanding importance as food for copepods.

Some of these are open sea forms and others were isolated from shore pools.

The following organisms were used:

CHLOROPHYCEAE

Dunaliella sp. (Plymouth strain 81)
Chlamydomonas pulsatilla Wollenweber
Platymonas carteriaformis nom. prov. (M. R. Droop's strain 10)
Chlorella stigmatophora Butcher (Plymouth strain 65)
Nannochloris oculata Droop (M. R. Droop's strain 66)

CHRYSOPHYCEAE

Chromulina pusilla Butcher (Plymouth strain 90)
Monochrysis lutheri Droop (M. R. Droop's strain 60)
Dicrateria inornata Parke (Plymouth strain B)
Pseudopedinella sp. (Plymouth strain 91)
Coccolithus huxleyi (Lohmann) Kamptner (Plymouth strain 92)
Hymenomonas carterae (Braarud & Fagerl.) Braarud (Plymouth strain 17)
Syracosphaera elongata Droop (M. R. Droop's strain 62)
Prymnesium parvum Carter (M. R. Droop's strain 65)

BACILLARIOPHYCEAE

Coscinodiscus centralis Ehrenberg (Plymouth strain 105)
Thalassiosira gravida Cleve (Plymouth strain 112)
Lauderia borealis Gran (Plymouth strain 111)
Skeletonema costatum (Grev.) Cleve (Plymouth strain 106)
Rhizosolenia delicatula Cleve
Chaetoceros decipiens Cleve (Plymouth strain 107)
Ditylum brightwellii (West) Grun. ex Van Heurck (Plymouth strain 110)
Nitzschia seriata Cleve (Plymouth strain 124)

CRYPTOPHYCEAE

Cryptomonas sp. (Plymouth strain 23)
Hemiselmis rufescens Parke (Plymouth strain D)

DINOPHYCEAE

Exuviaella sp. (Plymouth strain 18)
Prorocentrum micans Ehrenberg (Plymouth strain 97)
P. triestinum Schiller (Plymouth strain 98)
Oxyrrhis marina Dujardin (M. R. Droop's strain 18)
Gymnodinium vitiligo nom. prov. Ballantine (Plymouth strain 102)
G. veneficum nom. prov. Ballantine (Plymouth strain 103)
Peridinium trochoideum (Stein) Lemm. (Plymouth strain 104)

Diatoms

Table XIV shows all the experiments made by using cultures grown with ^{32}P. All the diatoms tested were eaten freely and the size of the cell had apparently no effect on the amount taken up. Since diatoms normally occur in chains even those with very small cells will be easily filtered.

With the four species in which assimilation was measured the proportion digested was high, the average being always over 50% and usually over 80%. The percentage did not vary appreciably with the concentration, the apparent slight rise in low concentrations having perhaps been caused by the incomplete recovery of faecal pellets. The percentage is lowest in the spring diatom *Skeletonema* and considerably higher in the three others. Yet *Skeletonema* is undoubtedly the most important diatom food in the Clyde sea area.

As is to be expected the actual quantity of food taken up is greatest when the concentration is highest and this is true also of the number of faecal pellets produced. The quantities are intermediate in the middle concentrations and least in the lowest concentrations, but, as has already been pointed out for faecal pellets, the relationship is not linear. One would therefore expect the volume of water filtered to be lowest in the high concentration and this is usually the case. It must be admitted, however, that in the experiments this volume shows a very great variation, not only among individual *Calanus* but between different experiments on the same species.

Flagellates

Table XV gives the results of the feeding experiments with cultures of flagellates grown with ^{32}P. The relation of volume filtered to size has already been discussed.

The percentage digested is high in most cases, but there are exceptions in *Dicrateria inornata*, *Chromulina pusilla* and possibly *Nannochloris* sp. in which so few faecal pellets were produced and so little taken up in the body that the results are unreliable.

The two very similar forms, *Dunaliella* sp. and *Chlamydomonas pulsatilla* were used. The second is considerably the larger and as can be seen in the table it is a good food. The first is a little smaller and has also been shown by experiments on egg production (Raymont & Gross, 1942; Marshall & Orr, 1952) to be a good food at least when present in high concentrations, but it has the disadvantage that it aggregates readily and is therefore difficult to sample accurately.

Although *Chlorella* can be taken in by *Calanus*, the faecal pellets produced are peculiar in that they seem to consist mainly of a mass of unaltered cells. That some of these at least are viable was proved by Dr M. R. Droop, who grew a culture of *Chlorella* from a faecal pellet. When the pellets are punctured or broken (and they seem to be particularly fragile) a cloud of cells emerges

and the whole pellet soon disappears. *Chlorella* is therefore unsuitable for quantitative work since by the end of an experiment many faecal pellets may have completely disintegrated. In one or two experiments where the *Calanus* were kept in small crystallizing dishes and the faecal pellets removed at frequent intervals, the percentage digested was low, usually below 50% and often only 15 or 20%. The food present in the gut probably accounts for most of the small amount found in the body so that digestion is even lower than it appears to be.

Dinoflagellates

Table XVI shows the results of all experiments with radioactive cultures of dinoflagellates. That with *Peridinium trochoideum* was not done as usual in rotated bottles, but in 50 ml. crystallizing dishes and therefore the figure for volume filtered may not be so reliable.

On the whole dinoflagellates are, with the possible exception of *Prorocentrum triestinum*, digested well. The volume filtered is also high and as one might expect with fairly large cells rises with lowered concentration. Indeed the highest figure we have obtained for volume filtered (84 ml.) was got with one out of the five *Calanus* in a culture of *Prorocentrum micans* containing 5 cells per ml.

The culture of *Oxyrrhis* used (see p. 505) also contained a large number of very small *Nannochloris* cells on which they were feeding. Table XV shows, however, that even in much higher concentrations the amount of *Nannochloris* actually ingested is negligible, so that the results obtained can be considered as due to *Oxyrrhis* alone. This is shown also by the fact that there is an increase in volume filtered with increasing dilution.

Prymnesium parvum is interesting in that, under certain conditions of growth, it is poisonous to fish, both in the sea (Otterstrøm & Steemann Nielsen, 1939) and in fish ponds (Shilo & Aschner, 1953). With *Calanus*, however (Table XV) a culture grown by Dr M. R. Droop showed no evidence of being harmful. *Gymnodinium veneficum*, which has been found to be poisonous to fish by Miss D. Ballantine of the Plymouth laboratory (personal communication), was also tested on *Calanus* (Table XVI). In rich cultures it caused death, but only after one or more days, which is much slower than with fish. That the *Calanus* ate it was shown by the production of faecal pellets. Another culture grown with ^{32}P present was tried in a quantitative feeding experiment, using lower concentrations. Few of the *Calanus* fed freely but in those which did, faecal pellet production was normal and digestion high.

Digestion

When faecal pellets taken in tow-nettings are teased out and examined they sometimes appear to consist largely of unaltered cells of diatoms and other organisms. This, and the rapid production of faecal pellets in cultures in the

laboratory, led observers to suppose that digestion went on only to a limited extent.

For the experimental feeding of *Calanus* it is possible to choose food organisms which give rise to faecal pellets in which the skeletons of the food organisms can be counted and the degree of digestion assessed visually. One such experiment was made by feeding *Calanus* on *Prorocentrum micans*. In the gut the *Prorocentrum* readily breaks up into two halves, and it is possible to see whether these are empty or if they contain an appreciable amount of undigested material. The concentrations ranged from 33 to 1·7 cells/ml. and the dilutions were made with outside sea water which contained at that time a variety of other organisms but no *P. micans*. In addition, the culture contained about 25% of empty shells which were also presumably ingested by the *Calanus*. Five sets each of fifteen *Calanus* were used and the faecal pellets produced were counted. A sample of the faecal pellets from each was crushed, the *Prorocentrum* shells counted and the number fully digested assessed. This varied from 66% in the highest concentration to 100% in the lowest. Estimates of degree of digestion are of course not very reliable. Even when most of the shells were empty, there was still a mass of greenish material sometimes retaining the shape of the original cell body present in the faecal pellet and this was probably only partly digested.

By the use of cultures grown with ^{32}P it was possible to estimate the digestion of a large variety of organisms, and these results are shown in Tables XIV–XVI. Although in most cases digestion appears to be very high, there are exceptions. The percentage digested is usually between 50 and 100% but very low results were obtained with *Dicrateria*, *Chromulina* and *Chlorella*, as well as with the spores of *Bacillus globigii* (p. 504).

From the single experiment on non-radioactive *Prorocentrum micans* it did appear that there was a tendency to digest more in low concentrations. This tendency is not confirmed in the experiments with radioactive cells, for the differences in the percentage digested between the richest and poorest concentration is usually within experimental error.

Faecal pellets are sometimes produced at the rate of one every 5 or 6 min, and it is astonishing that, in these conditions, digestion of ^{32}P is still as high as 80 or 90%. A low content of phosphorus in copepod faecal pellets, as compared with the phytoplankton was also found by Harvey *et al.* (1935), although they thought that the phosphorus was liberated directly into the sea as phosphoproteins and phospholipins.

Nearly all the feeding experiments were made with starved females, and it seemed possible that when first fed after a period of starvation digestion might be more efficient. A number of females was therefore kept for 6 days in a rich culture of *Chaetoceros* before use. They produced a large number of faecal pellets and when used for a feeding experiment the percentage digested was still found to be over 90% and the volume filtered was 24–46 ml. per *Calanus* in 24 h.

Distribution of ^{32}P in the *Calanus* Body

Rough dissections of numerous *Calanus* were made to determine the fate of the ^{32}P taken in. The *Calanus* was fixed in a drop of formalin and then dissected with needles under a binocular microscope on a series of circular coverslips of the same diameter as the planchettes used. These were then dried and their activity measured. The phosphorus begins to leach out immediately after fixation, and up to 12% may be lost overnight so the dissections were done within a few hours of fixation.

The fat was separated off as far as possible on the first coverslip, and then the *Calanus* was transferred to a second and most of the gut taken out and put on a third. The reproductive system was then dissected out. After this the carcass was transferred to a fourth coverslip and the muscles, apart from those inside the appendages, removed to a fifth. As can be well understood the separation of the different tissues was by no means complete. When fat was present (always in Stage V but rarely in females) it was never possible to leave it all on the first coverslip and the whole series contained some. The body fluid came out on the first (fat) or second (gonad) coverslip. It is difficult to disentangle the oviduct from the muscles to which they are attached and some eggs are left with the carcass. Otherwise this consists of the exoskeleton, appendages with their contained muscles, and the nervous system. On several occasions most of the nerve cord was removed and read separately, but the ^{32}P content was very small.

In spite of the crudity of the method, the results were fairly consistent (Table XVII). In females without fat, the carcass and the reproductive system each contained roughly 30–50% of the whole, the muscles 6–16% and the gut usually under 10%. In Stage V and in a newly moulted female the fat contained up to 40% and the gonad was very low. If allowance were made for the small relative volume of the reproductive system the percentage activity would be still higher. In two fat females it was much lower than usual, whereas the fat contained 8 and 24%. In males the gonad was surprisingly low, being not much higher than in Stage V and the muscles were slightly higher, 12–25%. The fat fraction was unexpectedly high. It was never possible to dissect out the thin-walled vas deferens whole and its contents may have been included with the fat.

A number of females were fed for only a short time (5 min up to several hours) to see if it was possible to trace the path of the ^{32}P but in these *Calanus* the gut was usually packed full of the food and its activity was much higher than usual (15–40%). In addition, it was difficult to retain the food within the gut and it contaminated the other coverslips.

The fact that the fat in Stage V and immature females has a high value, whereas the gonad is high in mature females, confirms what had already been suspected, namely that most of the fat as it disappears goes to form the gonad.

Some ripe females were kept in a culture of radioactive *Chaetoceros*; the eggs were removed daily and their activity measured as well as that of each female at the end of the experiment. Unfortunately it was late in the year and egg-laying was poor. Those females which laid 12 to 20 eggs had 17–30% of the ^{32}P present in the eggs besides 30–45% of the ^{32}P in the reproductive system.

In another experiment in April when females were laying actively, ten were kept individually in a culture of radioactive *Dunaliella* for 2 days and on the third were transferred to inactive sea water; the eggs laid (41–158 per female) accounted for 15–50% of the total ^{32}P taken up.

In actively laying females producing perhaps several hundred eggs, the phosphorus contained in the eggs must be many times that retained in the body.

The minimum period of feeding in a radioactive culture before radioactive eggs are laid seems to be about 6–8 h. On several occasions when a female had laid twice during the night the younger eggs were more highly radioactive than the older. Thus a female which was fed for about 18 h in a radioactive culture of *Lauderia* laid two clutches of eggs (69 and 59) during this time, both of which were washed, dried, and measured for activity. The older set which must have been laid after the female had fed for about 6 h had an average of 0·5 counts per egg per min.; the younger which were laid after about 15 h of feeding had about 21 counts per egg per min.

Excretion of ^{32}P in Solution

Apart from the faecal pellets produced *Calanus* must excrete the products of its metabolism. We attempted to measure the rate of this excretion so far as phosphorus was concerned by feeding a number of *Calanus* on a rich radioactive culture for about a week then putting them singly in small dishes of membrane-filtered sea water and measuring the loss of ^{32}P by the *Calanus*. Every 2 or 3 days each *Calanus* was removed, washed in three changes of water and transferred to a planchette. The water was then almost completely removed, the activity measured and the *Calanus* returned to its dish. This handling of the *Calanus* is severe treatment but it lasted only for 1 or 2 min on each occasion and did not apparently lead to any deaths. The reading of activity when the water is removed in this way is only a little below that obtained when the animal is torn up and dried, and in any case the error will remain about the same. The rate of loss is shown in Fig. 4.

It should be possible to measure the increase of ^{32}P in the water in which they are kept, and an attempt was made to do this also. There were, however, in many dishes large and unexplained losses and it is possible that some ^{32}P may have been taken up again by the *Calanus* or adsorbed on the glass or taken up by bacteria adhering to the glass. An experiment to test the last two possibilities gave, however, no definite results.

Two experiments on excretion were made, the first with twenty females fed on *Chaetoceros* when the water in the dishes was changed every time the animal was examined; the second with eight Stage V and seven females fed on *Syracosphaera*, when the water was not changed. All the Stage V which survived had moulted into females by the end of the experiment but the act of moulting had no recognizable effect on the rate of loss of ^{32}P and there was in any case very little difference between Stage V and females.

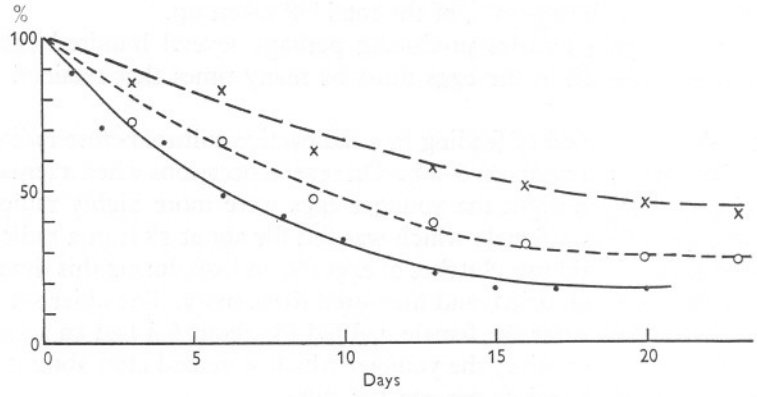

Fig. 4. Excretion of ^{32}P by starved *Calanus*. —●—, *Calanus* ♀ fed on *Chaetoceros*; —○—, *Calanus* ♀ fed on *Syracosphaera*; —×—, Stage V fed on *Syracosphaera*.

There was considerable individual variation, but the curves in Fig. 4 show that excretion is most rapid in the first week of starvation and becomes progressively less in the second and third weeks. The excretion was much higher than was expected. It was observed that unhealthy *Calanus* lost much more than healthy and in the few days before death the ^{32}P content might fall rapidly down to a figure of as little as 2 or 5%. This seems an impossibly low figure for the body of even a moribund *Calanus*, and it is possible that the newly acquired ^{32}P is not in equilibrium throughout the body or is metabolized first. In view of the handling of the *Calanus* involved the results given should be considered as over—rather than under—estimates; these losses will also be accentuated by the starvation of the *Calanus* after a period of feeding on a rich source of phosphorus.

Uptake of ^{32}P from Solution

It has already been mentioned that *Calanus* kept in a cell-free solution of ^{32}P will become radioactive. The means by which they take in the phosphate is unknown. It may be by 'drinking' either by the mouth or by the anus, or it may be taken through the thin parts of the exoskeleton. The amount taken up varies directly with the concentration but there is a great deal of individual

variation. No measurements were made of the non-radioactive P in solution, and it is possible that the uptake of ^{32}P by *Calanus* may depend on the total inorganic P present. In low concentrations (as in the filtrates of the cultures used in feeding experiments) the uptake is very low and is usually negligible when compared with the amount found in feeding *Calanus*. It is interesting, however, that after keeping females some hours in a solution of inorganic ^{32}P the eggs laid are slightly radioactive, indicating that, however the phosphate is taken in, it is rapidly distributed throughout the body.

Comparison of Feeding in Males, Females and Stage V *Calanus*

In our experiments females fed best, especially after being kept in the laboratory some days without food. A number of feeding experiments were also done with male and Stage V *Calanus*, but these were much more erratic in their feeding and very often produced no faecal pellets and took up very little ^{32}P. When they were feeding and could be compared with females it was found that the males produced usually fewer and always smaller faecal pellets and filtered less water. Stage V *Calanus*, on the other hand, on the few occasions when they fed freely, compared well with females in number and size of faecal pellets and volume of water filtered. There was no evidence that the utilization of any food organism differed with these stages of *Calanus*.

Utilization of Iodine

The utilization of phosphorus was so much higher than had been expected that it was thought advisable to test some other element which might occur in limiting concentration in the sea. Other radioactive isotopes besides ^{32}P are available for studies on feeding, and from each new information can be gained. Radioactive iodine (^{131}I) has a long enough half-life (8 days) for phytoplankton cultures to be grown and then used in feeding experiments on *Calanus*. *Lauderia borealis* was grown in Miquel culture solution with ^{131}I added. The culture grew rapidly and, after a week, had passed its period of optimum growth, although only 27% of the ^{131}I had been taken up by the diatom cells. The *Lauderia* was therefore collected on a filter and then re-suspended and used in different dilutions for a feeding experiment. Even after this treatment there was a considerable amount of ^{131}I in solution, and the *Calanus* in the filtrate from the 100% strength took up a substantial amount. Table XVIII shows the results of an experiment with female *Calanus* lasting 18 h. The total removed, faecal pellets produced, and volume filtered was remarkably small in the highest concentration as compared with the next, one-tenth of its strength, but this is not unusual. The percentage digested is very low when compared with that in experiments with ^{32}P and rises with decreasing concentration. As already mentioned, the higher figures in the 1% however may

be partly due to the loss of one or two faecal pellets. From the results we may conclude that iodine in diatoms is retained by *Calanus* only to a slight degree.

A culture of *Syracosphaera* grown in Miquel with added ^{131}P took up so little of the iodine that it could not be used.

Discussion

The two most striking results which emerge from this series of experiments are the low volumes of water filtered daily by *Calanus* and the high percentage of the food digested. Both these results are at variance with generally accepted opinion. The volume of culture filtered varied from less than 1 ml. to about 30–40 ml. per day; one or two individuals exceeded this but they were exceptional. Digestion was usually over 50% and often over 90%.

In the early experiments made to determine the volume of water which a *Calanus* could filter daily (Fuller & Clarke, 1936; Fuller, 1937) volumes of 1 to 5 ml. were found. The *Calanus* were, however, kept in a very small volume of water during the experiment (3 ml. per *Calanus*) and it seemed that Gauld's later work (1951), when he kept individual *Calanus* in 100 ml. beakers, would be more reliable. He found a good deal of individual variation (42–101 ml.), but his average value was about 70 ml. a day. Harvey (1937), in the course of some short experiments on selective feeding, found values much higher even than this, reaching with *Ditylum brightwellii* rates of 168 and 240 ml. per day. It has already been pointed out that a small error in the estimation of the number of food cells may lead to a large error in the final calculation. Thus in the concentrations which Gauld used (mostly 10 to 20 cells per mm^3) an error of 1 or 2 per mm^3 in the final cell count would lead to an error of 10–50 ml. in the calculation of volume filtered. This type of error is avoided in the current experiments by measuring directly the total quantity taken up.

There is another possible source of error in the earlier work. In almost all cultures some settling out of the cells takes place unless the water is stirred (Ryther, 1954), and since *Calanus* has a habit of feeding on the bottom it will tend to get a richer supply of food than the calculated one.

Recently, Ryther (1954) has found that a substance which inhibits the feeding of *Daphnia* is produced in cultures of *Chlorella*, *Scenedesmus* and *Nitzschia*. On increasing the concentration of the food the volume filtered decreased. When a culture was in the exponential growth phase, little of this inhibitory substance was found in the water, and the *Daphnia* was affected mainly by the food ingested. In senescent cultures the inhibitory substance was present in solution also and the effect on feeding was more marked.

In the experiments on feeding *Calanus* with old cultures of *Lauderia* there was little evidence of a decrease in filtration, though the percentage digested decreased markedly. In high concentrations the volume filtered usually fell

off but it seemed likely that the *Calanus* was unable to ingest the large quantity of food involved rather than that an inhibitory substance was present. There was not an inverse correlation in the lower concentrations, such as Ryther found. Although none of our experiments was designed to test for the presence of inhibitory substances, a general view (Tables XIV–XVI) does not indicate their presence in the older cultures.

A striking fact in all the experiments is the great variation between individual *Calanus* even under the same conditions. In any of the experiments some of the *Calanus* might be eating freely and others not at all, even although they were apparently as healthy and active as the rest. In different experiments on the same food species, differences were even more marked and *Calanus* filtered an average of 23·9 ml. of *Skeletonema* culture on one occasion and, on another, in a concentration little richer, only 5 ml. In Table XIX, for example, the results for *Skeletonema* indicate that more water is filtered in the higher concentrations. Yet a reference to Table XIV will show that in the experiments from which these results come, the opposite is usually true. There are, however, many factors which may influence the result which were not taken into account, e.g. age, or rather state of growth, of culture, time of year, and age or state of maturity of females.

Gauld observed that a *Calanus* can live even in a rich food culture without feeding, and this has been confirmed by our own observations. This does indicate that they are not automatic filter feeders in the sense that they must ingest all the time they are moving. Esterly (1916) has described how a food pellet collected on the filtering setae may be scattered again by a flick of the maxillipedes and this may be the usual method of rejection. The very diverse movements which Lowndes (1935) has described for the mouthparts of a *Calanus*, particularly for the second antenna, may also enable them to divert the feeding current.

The low filtration figures found make it difficult to understand how *Calanus* can maintain itself in the sea. If we accept the respiration figures found experimentally for *Calanus* (Marshall *et al.*, 1935; Clarke & Bonnet, 1939) there is apparently still a gap between the food required and the food normally present in the sea. This difficulty cannot be got over by postulating the presence of large numbers of μ-flagellates since *Calanus* seems unable to filter them off.

According to Fuller & Clarke (1936) and Fuller (1937) a *Calanus* needs to filter from 40 to 70 ml. per day to maintain itself when the phytoplankton is moderately rich. Gauld, who found daily filtrations of this order, himself showed that because of vertical migration *Calanus* will spend only part of the day feeding in the phytoplankton-rich waters. Thus the rate of filtration would have to be increased considerably for them to obtain enough.

It has been suggested by Fuller & Clarke (1936) that the fat reserve in *Calanus* might help to tide it over a period of scarcity. In Stage V *Calanus*,

however, in which it is prominent, it often persists till they moult at the end of the winter and then disappears gradually. The dissection results (Table XVII) show that when Stage V moult to females, the fat may be used to build up the ovary. Digby (1954) has made the suggestion that during the winter *Calanus* are cannibals and that this accounts for the large fall in numbers over the winter.

In our experiments females were used in most cases since they fed more regularly than Stage V. Often in winter Stage V fed poorly or not at all even if starved for some days before an experiment. It may be that the metabolism and therefore the food requirement of the over-wintering Stage V *Calanus* is lower than it is during the rest of the year.

When one considers the concentration of food cells usually present in the sea, our low filtration volumes are all the more unexpected. Table XIX shows the maximum number of cells taken up by the best-feeding *Calanus* in all the experiments that were made with concentrations of food cells such as may occur in the sea. The upper limit for this was put at 16,000 per ml. Concentrations of this magnitude certainly occur only for short periods during phytoplankton outbursts, e.g. in the Oslo fjord (Braarud, 1939) and in the Norwegian Sea (Halldal, 1953), but the lowest concentrations used, down to 1 cell per ml. may be found in the sea even in winter. It will be seen that the *Calanus* can ingest up to a maximum of 600 large cells such as *Ditylum brightwellii* per day, up to 12,000 of *Syracosphaera* or *Peridinium trochoideum* and up to 50,000 of small forms such as *Platymonas*, *Cryptomonas* or *Prorocentrum triestinum*. By far the largest number was with the spring diatom *Skeletonema costatum* when nearly 400,000 were ingested. These are also very small cells but they are usually united in chains and so are more easily filtered. With still smaller forms, e.g. *Dicrateria inornata* or *Nannochloris*, the highest number ingested may be quite small (200 to 400), confirming the suggestion that these cells pass through the maxillary filter. *Chromulina* is however an exception to this for, though it is even smaller than *Nannochloris*, up to 18,000 per day were ingested in a concentration of 16,000 per ml. In low concentrations of organisms, such as might be found in the sea in winter, only a few hundred cells at most will be ingested.

While the methods used in the experiments enable us to calculate the volume filtered with some degree of certainty, the estimates of the digestion of the food taken in depend on the phosphorus alone. As is shown in Tables XIV–XVI its digestion in marine phytoplankton forms is unexpectedly high. On the other hand, the experiments with a culture labelled with ^{131}I showed that only a very small fraction of this was retained. The same must be true of other elements such as the silicon in the diatom skeleton.

The possibility that the ^{32}P may have been only loosely bound to the algal cell has already been discussed (p. 497) and Rice (1953) has shown that, under experimental conditions, little of the phosphorus can have been labile.

There were a few species in which digestion was much lower, e.g. *Chromulina*, *Dicrateria*, and possibly *Nannochloris*. In *Chlorella* viable cells were excreted and digestion could not be measured because of the breaking up of the pellets. *Bacillus globigii* spores were presumably completely indigestible, and the apparent digestion gives some measure of the spores present in the gut.

Very high filtrations are not possible in rich food cultures because faecal pellet production beyond a certain rate is not physically possible. The peristaltic movements of the gut are slow and the compacting of the faecal pellets gradual. Two *Calanus* feeding on rich *Chaetoceros* cultures produced 166 and 211 faecal pellets in 15 and 24 h respectively, i.e. a faecal pellet every $5\frac{1}{2}$ or 7 min, yet even at these rates 86% was digested. These are maximum feeding rates, and as a rule faecal pellets were produced much more slowly. In concentrations such as are found in the sea (Table XIX) the highest rates were with *Chaetoceros* and with *Prorocentrum micans*, and amounted to about one every quarter of an hour; with the spring diatom *Skeletonema* the rate was about half this.

The number of cells found in one faecal pellet was counted in the experiment on choice of food already described. The maximum numbers for *Prorocentrum micans* and *Peridinium trochoideum*, which were mixed in the food culture, were 129 and 188, the averages 30 and 40. Many pellets contained 50–70 of each species. This compares well with the average figures calculated from the radioactive cultures, namely 74 and 139.

In a later experiment with *Prorocentrum micans* some of the faecal pellets were taken from three of the *Calanus* and crushed and the skeletons in them counted. The total number of cells in all the faecal pellets was then calculated and compared with the number calculated from the radioactivity of the body and faecal pellets. The number of cells per faecal pellet varied from 5 to 44 but it was not always easy to distinguish the skeletons. The agreement was reasonably good; the number of cells eaten was by radioactive measurement 1304, 1655 and 324, and by visual count 865, 1573 and 252. Although the great majority of the cells in the faecal pellets were completely empty, the percentage digestion was not so high as usual, varying from 42 to 71%.

On the whole then, when a comparison is possible the observations on faecal pellets do confirm the results obtained with radioactive cultures. Reports have often been made of faecal pellets containing many undigested cells, but when we consider the total number which a faecal pellet may represent, e.g. 1000–7000 *Skeletonema*, a few dozen undigested cells would, although apparently important, be only a small fraction of the total. There is, however, always a large amount of unidentifiable debris, and it would be desirable to know the degree of assimilation of other substances besides those containing phosphorus.

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Summary

1. Cultures of diatoms and phytoflagellates were grown with radioactive phosphorus (^{32}P) present and used in feeding experiments on *Calanus*. After feeding the uptake and the volume of culture filtered was estimated by measuring the radioactivity of the body, eggs and faecal pellets.

2. The volume filtered varied from less than 1 ml. to over 40 ml. in 24 h; it is less in high concentrations of food cells but does not increase in very great dilutions.

3. *Calanus* feeds better in the dark than in the light.

4. Digestion of an old diatom culture was in some cases lower than that of a young one.

5. It was found that organisms below a size of about 10 μ could not be readily filtered off, and in such cases the volume filtered decreased with dilution of the culture.

6. Some experiments on the choice of one food rather than another show that if selection does take place it is only to a slight degree.

7. All the diatoms used could be eaten freely and most of the other algae. Digestion was poor in a few of the smallest, e.g. *Dicrateria*, *Chromulina* and *Chlorella*, but in most organisms it was unexpectedly high.

8. A high proportion of the ingested ^{32}P goes to the ovary in females and to the fat in Stage V *Calanus*. In an actively laying female a large proportion of the ^{32}P appears as eggs.

9. The excretion of ^{32}P by females initially well fed and then starved was at first rapid and slowed off after a few days.

10. Radioactive iodine (^{131}I) was used in one feeding experiment, but the utilization was low.

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TABLE I. RATE OF FAECAL PELLET PRODUCTION

Thirteen *Calanus* feeding on a mixture of *Prorocentrum micans* and *Peridinium trochoideum*

Successive time intervals	Rate of faecal pellet production per hour													Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	
15 min	0	0	*	8·0	0	8·0	0	0	0	3·8	7·5	0	4·0	2·6
20–25 min	4·0	3·8	0	0	0	6·7	3·0	3·0	3·0	0	5·7	8·2	2·7	3·1
20–25 min	2·7	2·7	3·0	2·4	2·5	7·2	5·0	2·6	2·9	11·4	5·7	5·0	7·2	4·6
1½ h	2·9	2·9	2·1	1·4	0	5·5	5·3	2·0	3·3	7·1	6·5	6·3	4·2	3·8
1½ h	2·0	4·0	4·1	4·1	0·7	6·1	3·5	5·2	5·8	7·1	7·1	7·2	5·4	4·8
2 h	3·1	4·6	3·6	5·6	0·5	5·8	3·1	6·9	5·8	7·9	7·9	6·4	7·8	5·3
17 hr	3·0	3·7	2·1	4·5	1·8	2·6	0·9	3·5	3·0	7·1	2·6	6·4	5·5	3·6

* Excreted a pellet before the first examination.

TABLE II. COMPARISON OF WASHED AND UNWASHED CELLS

Concentrations	No. *Calanus* used (♀)	Average for 1 *Calanus* in 24 h				
		No. faecal pellets	Counts/min in faecal pellets	Counts/min in body	% used	ml. filtered
460 *Lauderia*/ml. Centrifuged 4 times	4	51	2,450	21,550	89·6	32·9
320 *Lauderia*/ml. Untreated	4	28	2,500	19,090	88·6	29·9

TABLE III. SEPTEMBER 1953. UTILIZATION OF *SKELETONEMA COSTATUM*

0·13 counts/cell. 17 hr

Concentration	*Calanus* (♀)	Eggs		Faecal pellets		Body (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
		No.	(c/min)	No.	(c/min)				
37,100 c/min/ml., 288 cells/mm³	1	11	5,592	125	64,550	74,730	144,872	55·4	4·9
	2	12	4,667	120	45,850	92,510	143,027	67·9	4·8
	3	11	3,311	121	53,830	58,410	115,551	53·4	3·9
	4	0	—	80	43,860	54,620	98,480	55·5	3·3
	5	13	3,108	144	57,770	35,590	96,468	40·1	3·2
9,275 c/min/ml., 72 cells/mm³	1	0	—	*c.* 96	38,900	83,100	122,000	68·1	19·2
	2	0	—	*c.* 110	44,950	81,510	126,460	64·5	20·1
	3	0	—	73	29,060	30,020	59,080	50·8	8·5
	4	0	—	104	78,090	77,840	155,940	49·9	23·1
	5	23	19,070	98	75,360	67,230	161,660	53·4	23·9
1,855 c/min/ml., 14 cells/mm³	1	0	—	20	11,510	12,183	23,693	51·4	17·0
	2	0	—	0	—	28	28	—	—
	3	0	—	28	13,280	24,069	37,349	64·4	28·3
	4	1	215	38	14,150	24,890	39,255	64·0	30·1
	5	0	—	23	10,910	21,531	32,441	66·2	24·1
Filtrate, 1,895 c/min/ml.	A	0	—	0	—	16	—	—	—
	B	0	—	0	—	16	—	—	—

TABLE IV. JANUARY 1955. COMPARISON OF DIFFERENT METHODS OF MEASURING VOLUME FILTERED, USING *SKELETONEMA COSTATUM*

1·0 counts/cell. 23½ h

Initial concentration	*Calanus* (♀)	Bottle (vol. in ml.)	Faecal pellets No.	Faecal pellets (c/min)	Body less filtrate (c/min)	Total removed (c/min)	% assimilated	ml. filtered/ 24 h	Diatom counts: Cells/ml. at end	Diatom counts: ml. filtered/ 24 h (calc. by cells)	Liquid counter: c/min/ml.	Liquid counter: ml. filtered in 24 h
11,410 c/min/ml., 11,200 cells/ml. Liquid counter 4642 counts/min/ml.	1	70	47	40,530	120,246	160,776	74·7	16·1	—	—	3,751	15·24
	2	71	33	44,680	130,646	175,326	74·5	17·7	9,100	15·06	3,575	18·93
	3	71	23	28,460	82,006	110,466	74·2	10·6	—	—	4,225	6·83
	4	71	33	34,410	98,916	133,326	74·2	13·0	—	—	4,163	7·90
	5	72	27	26,323	79,106	105,429	75·1	10·1	—	—	4,710	—
	6	72	46	38,290	103,046	141,336	72·9	13·8	—	—	4,136	8·48
	7	73	80	70,650	176,046	246,696	71·4	26·2	7,200	32·94	3,474	21·61
	8	73	28	42,690	66,376	89,066	74·5	8·4	—	—	4,384	4·26
	9	76	*c.* 40	43,250	175,646	218,896	80·2	22·6	7,000	36·47	3,354	25·23
	10	75	33	40,160	117,946	158,106	74·6	15·6	—	—	4,088	9·72
Filtrate, 435 c/min/ml.	A	38	1	5	65 } 54	—	—	—	—	—	159	—
Liquid counter 185 counts/min/ml.	B	38	0	—	43 }	—	—	—	—	—	135	—

TABLE V. EFFECT OF CONCENTRATION OF *DUNALIELLA* ON FAECAL PELLET PRODUCTION

Cells/mm³	8	80	200	400	800
Faecal pellets/ *C*/24 h	11 } 10 } 8	59 } 58 } 57	69 } 67 } 64	81 } 68 } 55	82 } 80 } 78
Size of faecal pellets in μ	296	510	490	470	576

TABLE VI. FEBRUARY 1954. UTILIZATION OF *SYRACOSPHAERA* IN LIGHT AND DARK

16 counts/cell/min. 7.30 a.m.–5.30 p.m.

Concentration	*Calanus* (♀)	Faecal pellets No.	Faecal pellets (c/min)	Body minus filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
11,335 c/ml./min, 720 cells/ml. Dark	1	13	493	22,731	23,224	97·9	5·0
	2	28	1,423	43,902	45,325	96·9	9·9
	3	15	459	42,112	42,571	98·9	9·3
	4	13	629	31,142	31,771	98·0	6·9
	5	11	199	20,759	20,958	99·1	4·5
11,335 c/ml./min, 720 cells/ml. Light	1	12	506	30,552	31,058	98·4	6·7
	2	4	268	10,401	10,669	97·5	2·3
	3	2	252	10,933	11,185	97·7	2·4
	4	0	—	273	273	—	—
	5	4	138	7,713	7,851	98·2	1·7
Filtrate, 135 c/ml./min	A	1	0	11 } 8 } 6	—	—	—
	B	0	—				

TABLE VII. EFFECT OF AGE OF CULTURE ON ASSIMILATION

Age of culture in days	Number of *Calanus* (♀)	Concentration used, cells/ml.	% used	Volume filtered in 24 h
5	5	155	98·4	18·2
10	8	460 320	88·1	31·4
25	6	174	66·7	14·6
32	6	1,590	58·5	8·5
5	6	1,310	73·0	19·7

TABLE VIII. SEPTEMBER 1954. UTILIZATION OF SPORES OF *BACILLUS GLOBIGII*. 18 h.

Concentration	*Calanus* (♀)	Faecal pellets No.	Faecal pellets (c/min)	Body less filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
1 %, 9520 c/ml./min	1	23	536	55	591	9·3	0·09
	2	32	344	49	393	12·5	0·07
	3	16	401	59	460	12·8	0·07
	4	19	422	51	473	10·8	0·07
	5	19	729	54	783	6·9	0·11
0·5 %	1	12	36	3	39	7·7	0
	2	26	248	24	272	8·8	0·11
	3	21	362	15	377	4·0	0·14
	4	21	188	11	199	5·5	0·05
Filtrate, 60 c/ml./min	A	0	—	2 } 1	—	—	—
	B	1	1	0 }			

TABLE IX. TEST FOR SELECTION BY SIZE, USING RADIOACTIVE *DICRATERIA*

Concentrations in cultures	No. *Calanus* used	Average for 1 ♀ *Calanus* in 24 h: No. faecal pellets	Counts/min in body	Counts/min in faecal pellets	Total counts/min	ml. filtered
54,000 *Dicrateria* and no *Lauderia*/ml.	5	10·7	2,613	2,030	4,657	0·57
43,020 *Dicrateria* and 1,070 *Lauderia*/ml.	5	50·8	9,856	6,046	16,050	2·43
1,070 *Lauderia* and no *Dicrateria*/ml.	5	49·7	—	—	—	—
456,000 *Dicrateria* and no *Prorocentrum micans*/ml.	5	47·4	7,204	12,847	20,051	4·73
456,000 *Dicrateria* and 108 *Prorocentrum micans*/ml.	5	104·1	7,059	18,403	25,462	6·27
108 *P. micans* and no *Dicrateria*/ml.	4	60·0	—	—	—	—

TABLE X. TEST FOR SELECTION BY SIZE, *DITYLUM–CHAETOCEROS*

Concentrations of food cells	*Calanus* (♀)	Eggs No.	Eggs (c/min)	Faecal pellets No.	Faecal pellets (c/min)	Body (less filtrate)	Total removed (c/min)	Total removed Cell vol. $\mu^3 \times 10^3$	% used	ml. filtered/ 24 h
Culture										
122 *Ditylum* (radioactive) cells/ml.	1	12	803	110	7,843	15,120	23,766	23·0	67·0	5·7
	2	0	—	0	—	79	79	0·7	—	—
11,500 *Chaetoceros* cells/ml.	3	24	284	46	1,369	9,367	11,020	10·7	87·6	2·6
	4	18	1,641	85	9,326	25,573	36,540	35·4	74·5	8·8
8,575 c/ml./min	5	11	607	80	6,808	17,128	24,543	23·8	72·3	5·8
	6	25	1,916	89	11,669	28,242	41,827	40·5	72·1	10·1
	7	4	45	15	452	7,686	8,183	7·9	94·5	1·9
Filtrate, 950 c/ml./min	A	0	—	0	—	17 } 18	—	—	—	—
	B	11	4	3	3	19 }				
Culture	1	34	138	1	2	882	1,022	0·7	—	—
343 *Ditylum* cells/ml.	2	25	3,353	105	952	48,965	53,270	38·1	98·2	5·7
	3	15	697	98	2,102	63,345	66,144	48·0	96·8	7·2
10,500 *Chaetoceros* (radioactive) cells/ml.	4	0	—	29	129	13,236	13,365	9·7	99·0	1·5
	5	15	10	68	1,309	58,815	60,134	43·6	97·8	6·5
	6	16	1,256	90	3,994	120,675	125,925	91·3	96·8	13·9
18,945 c/ml./min	7	11	42	70	1,332	38,295	39,669	28·8	96·7	4·2
	8	24	130	77	1,687	67,075	68,892	49·9	97·6	7·4
Filtrate, 840 pulses/ml.	C	0	—	1	0	26 } 25	—	—	—	—
	D	0	—	1	34	24 }				

TABLE XI. CHOICE OF FOOD IN A MIXTURE OF *PERIDINIUM TROCHOIDEUM* AND *PROROCENTRUM MICANS*

Time of examination	7 *Calanus** kept 10 days in *P. trochoideum*						6 *Calanus* kept 10 days in *P. micans*					
	Faecal pellets produced	Faecal pellets examined	Skeletons excreted				Faecal pellets produced	Faecal pellets examined	Skeletons excreted			
			P. trochoideum		*P. micans*				*P. trochoideum*		*P. micans*	
			No.	%	No.	%			No.	%	No.	%
11.46 a.m. to 12.01 p.m. (¼ h)	4	4	205	100	0	0	4	4	162	54·9	133	45·1
12.05–12.16 p.m. (½ h)	3	3	117	100	0	0	5	5	299	63·6	171	36·4
12.17–12.38 p.m. (1 h)	5	5	367	86·1	59	13·9	9	9	431	51·8	401	48·2
12.39–1.03 p.m. (1¼ h)	8	8	471	80·9	111	19·1	13	13	586	53·2	515	46·8
2.02–2.42 p.m. (2½ h)	29	17	790	59·1	548	40·9	50	17	1,774	42·7	2,334	57·3
3.30–4.11 p.m. (3½ h)	37	18	1,493	68·4	835	31·6	54	16	1,879	50·7	1,828	49·3
5.25–6.14 p.m. (5½ h)	52	19	2,861	67·9	1,354	32·1	87	18	3,205	57·7	2,351	42·3
10.35–11.35 a.m. (23 h)	315	21	15,258	61·1	9,725	38·9	484	18	9,671	38·8	15,259	61·2

* 1 ♂ included.

TABLE XII. CHOICE OF FOOD

Radioactive *Lauderia* with non-radioactive *Skeletonema*. 6 *Calanus* used in each set

Time in mixture (h)	*Lauderia*-fed, average/*Calanus*				*Skeletonema*-fed, average/*Calanus*			
	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)
½	3·5	438	4,478	4,916	2·2	313	3,994	4,307
1	8·2	1,270	4,972	6,242	4·5	714	4,580	5,294
2	10·3	1,961	7,580	9,541	12·5	2,236	8,242	10,478
16*	106·7	11,047	32,855	43,790	88·3	11,021	27,594	38,615

* Count of pulses for bodies includes eggs laid.

TABLE XIII. CHOICE OF FOOD

Radioactive *Lauderia* with non-radioactive *Cryptomonas*. 6 *Calanus* used in each set

Time in mixture (h)	*Lauderia*-fed, average/*Calanus*				*Cryptomonas*-fed, average/*Calanus*			
	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)
½	6·8	352	907	1,259	5·0	364	520	884
1	9·7	913	1,540	2,453	7·8	612	916	1,528
2	15·7	1,562	2,456	4,018	14·5	1,507	1,986	3,493

TABLE XIV. UTILIZATION OF DIATOMS

Species	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
Lauderia borealis, 29 μ av. diam., 17–43 μ length	*c.* 14	627	5	77	77–88	80·4	6·6–21·2	11·5
	c. 14	63	5	40	83–90	85·6	12·0–36·4	26·5
	c. 14	6	5	8	77–95	87·8	2·7–26·2	11·3
	c. 15	645	5*	46	68–81	73·1	0·9– 9·4	4·0
	5	155	5	4	—	—	6·4–32·0	18·2
	10	320	4	27	87–91	88·6	24·3–38·0	29·9
	10	460	4	51	87–94	89·1	29·8–38·7	32·9
	25	174	6	10	66–68	66·7	11·2–20·0	14·6
	32	1,590	6	118	49–66	58·5	3–12	8·5
	5	1,310	6	113	67–79	73·0	10–33	19·7
Skeletonema costatum, 4 μ in culture	13	288,000	5	183	40–68	54·5	3– 5	4·0
	13	72,000	5	144	50–68	57·4	9–24	19·0
	13	14,400	4	33	51–66	61·5	17–30	23·9
	c. 6	194,000	5	115	78–84	80·7	3– 5	3·8
	c. 6	19,400	4	48	81–87	83·8	3– 6	4·9
	c. 6	970	5	1	—	—	0·1– 1·6	—
	c. 7	770	5	3	79–96	87·0	1– 2	1·3
	11	41,700	6	75	71–89	77·6	2·4–16·8	9·8
	5	36,700	5	16	46–87	72·2	0·5– 2·3	1·2
		3,670	4	3	52–72	62·3	2·2– 2·2	2·2
	7	11,200	10	40	71–80	74·6	8·4–26·2	15·4
Chaetoceros decipiens, 14–21 μ diam., size range 20–78 μ	28	945	4	51	89–94	90·7	2–12	7·2
	28	170	5	2	—	—	0– 7	1·8
	28	17	5	3	—	—	3	2·9
	4	630	5	95	93–97	95·8	3·6–43·3	22·4
	4	63	5	31	91–98	95·3	3·8–24·7	19·9
	4	3	5	28	83–98	93·9	8·3–14·4	10·4
	8	68	5	8	97–100	98·2	1·9–10·3	6·3
	8	7	5	6	98–99	98·5	1·7– 6·2	4·1
	8	1	5	17	90–97	92·7	3·4– 8·4	5·8
	15	490	4	67	87–89	89·1	4·7– 8·1	6·1
	15	110	6	24	93–97	95·1	4·3– 8·9	6·6
	15	22	6	7	98–99	98·2	3·0– 8·6	5·0
	18	78	5	42	82–91	86·1	5·6– 8·9	7·7
	18	78	5†	12	90–98	94·4	0·2– 3·7	1·1
Ditylum brightwellii, 20–60 μ diam.	*c.* 20	63	4	76	85–89	87·2	1–10	7·0
	c. 20	13	5	28	90–95	91·7	2–16	9·4
	c. 20	1	4	6	87–98	93·4	2–18	11·7

* Males. † Stage V.

TABLE XV. UTILIZATION OF FLAGELLATES

Species	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
Chlamydomonas pulsatilla, 19 × 12 μ	3	62,000	5	85	88–90	89·0	3·0 – 5·6	4·57
	3	6,200	5	11	93–98	96·4	1·1 –31·0	11·83
	3	620	5	5	98–99	98·7	1·5 – 6·1	3·21
Platymonas carteriaformis, 20–26 μ × 14–16 μ	15	41,500	6	95	61–93	77·5	2·1 – 3·1	2·59
	15	4,150	5	20	76–95	81·4	0·5 –11·6	5·15
	15	415	4	11	57–83	75·1	0·1 – 3·2	1·4
Nannochloris oculata, 2–4 μ	5–25	2,368,000	5	5	61–87	74·3	0	0
	5–25	119,000	5	1	27·3	—	0	0
	5–25	12,000	5	1	66·7	—	0	0
Chromulina pusilla, *c.* 1–3 μ	28	1,636,000	5	36	27–59	39·4	1·1 – 4·0	2·24
	28	163,600	5	15	46–68	54·2	1·4 – 1·7	1·56
	28	16,360	5	3	47–63	54·9	0·1 – 1·1	0·44
Monochrysis lutheri, 5–7 × 5–7 × 2 μ	7	188,000	4*	34	50–84	71·1	0·7 – 1·2	0·45
	7	18,800	4*	10	60–96	81·8	0·2 – 0·8	0·42
	7	1,880	4*	9	69–97	82·0	0 – 0·7	0·22
Dicrateria inornata, 3–5·5 μ	18	2,180,000	3	59	15–41	25·9	0·2 – 0·8	0·53
	18	106,000	3	10	38–63	48·7	0·1 – 0·2	0·16
	18	10,600	3	2	62–67	64·5	0 – 0·3	0·18
	25	534,000	5	56	33–38	35·1	0·3 – 1·3	0·84
	25	279,000	4*	50	39–59	48·5	0·2 – 0·9	0·51
Syracosphaera elongata, 18–30 × 12 μ	7	3,100	5	32	96–98	97·0	0·7 – 1·4	1·03
	7	200	5	29	98	98·0	3·6 –29·3	19·3
	7	20	5	8	98–100	98·7	3·1 –43·5	23·7
	11	720	5	38	97–99	98·1	4·5 – 9·9	7·1
	16	4,500	6	31	87–94	91·5	1·14– 1·83	1·43
	16	1,125	6	36	91–96	93·9	3·50–11·45	7·70
	16	2,800	5	15	88–99	92·6	0·5 – 3·5	1·62
	33	280	5	9	92–97	94·9	2·2 –25·6	7·35
	33	28	5	3	—	—	0·4 – 6·7	2·16
	13	575	4*	15	97–99	98·3	0·2 – 4·1	1·80
Prymnesium parvum, 9–12 × 5 μ	14	40,000	5	8	92–99	96·6	0·10– 0·47	0·37
	14	4,000	5	8	78–99	90·6	0·10– 4·22	1·77
	14	400	5	6	—	—	0·86– 1·76	1·32
Cryptomonas sp., 19–27 × 6–10 μ, average 22 × 8 μ	16	16,000	5	112	51–84	62·0	4·9 –16·7	8·7
	16	1,600	4*	24	58–89	70·0	15·7 –32·4	22·6
	16	160	5	14	64–86	77·4	5·0 –24·7	10·8

* One omitted because it did not feed.

TABLE XVI. UTILIZATION OF DINOFLAGELLATES

Species and size	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
Prorocentrum micans 43 × 27 μ	*c.* 27	68	5	69	70–86	79·3	15·3–27·6	22·2
	27	540	5	54	86–96	89·6	0·5– 9·4	5·3
	27	54	5	25	89–96	93·4	12·7–37·1	27·7
	27	5	5	17	92–99	97·1	29·8–84·3	42·5
P. triestinum 10–14 μ	*c.* 30	12,200	5	67	43–54	47·3	2·4– 4·2	3·5
	c. 30	1,220	5	31	49–73	61·7	6·2–17·8	13·7
	c. 31	1,020	5	39	53–65	60·2	6·9–15·6	11·3
	c. 31	102	5	12	49–83	72·6	2·2–21·5	10·1
Oxyrrhis marina, 20–45 × 15–30 μ, with *Nannochloris oculata**	14	20,400	5	52	86–92	88·7	0·6– 2·1	1·7
	14	2,040	5	34	92–95	93·9	8·2–21·7	13·4
	14	204	5	8	97–99	97·9	1·5–28·1	16·1
Gymnodinium vitiligo 6–12 μ, 14 μ long	42	7,840	5	33	86–94	88·8	5·1– 8·5	6·2
	42	784	5	15	94–97	95·8	4·8–19·2	13·6
	42	78	4 †	8	97–99	98·0	1·4–23·2	9·7
Gymnodinium veneficum 15 × 13 μ	28	13,800	5	24	75–92	83·5	0·1– 1·3	0·6
	28	1,380	5	23	90–99	94·0	0·5–11·1	5·1
	28	138	5	3	99	98·6	0·3–14·5	3·9
Peridinium trochoideum 25 × 19 μ	32	1,750	10	81	68–85	76·4	0·7– 7·5	4·8

* 56,000 *Nannochloris*/ml. in highest concentration.
† One which did not eat omitted.

TABLE XVII. PARTITION OF ^{32}P IN THE *CALANUS* BODY

Date	Stage of *Calanus*	Fat (%)	Carcass (%)	Reproductive system (%)	Gut (%)	Muscles (%)
23. xii. 53	M.	25·4	34·6	14·0	0·7	25·2
23. xii. 53	M.	23·3	51·9	5·0	1·1	18·7
28. xii. 53	M.	26·2	54·9	1·7	5·5	11·8
28. xii. 53	M.	37·6	42·1	1·0	1·3	17·9
28. xii. 53	M.	31·0	49·5	0·9	1·6	17·0
28. xii. 53	M.	35·1	45·2	0·6	4·6	14·4
28. xii. 53	M.	33·0	50·9	1·0	2·1	13·0
1. xii. 53	F.*	24·4	43·8	17·8	3·1	11·0
1. xii. 53	F.*	8·3	47·5	28·9	4·0	11·4
10. xii. 53	F.†	12·6	55·8	4·2	2·8	7·6
30. xi. 53	F.	—	49·5	25·2	14·4	10·7
30. xi. 53	F.	—	45·7	38·4	8·3	7·5
30. xi. 53	F.	—	41·1	40·8	7·1	11·0
4. xii. 53	F.	—	45·5	32·3	6·0	16·2
4. xii. 53	F.	—	38·5	47·1	5·2	9·2
4. xii. 53	F.	—	45·3	44·4	1·4	8·9
4. xii. 53	F.	—	48·8	44·0	1·1	6·1
10. xii. 53	V	39·8	36·7	8·2	2·3	13·0
10. xii. 53	V	25·4	48·3	11·3	6·7	8·3
18. xii. 53	V	35·3	43·5	4·9	7·6	8·7
18. xii. 53	V	34·1	48·7	2·5	10·2	4·5
18. xii. 53	V	25·7	56·0	0·4	6·6	11·3

* Fat females. † Newly moulted.

TABLE XVIII. UTILIZATION OF *LAUDERIA BOREALIS* WITH ^{131}I

18 h at 15–17° C

Concentrations	*Calanus* (♀)	Faecal pellets: No.	Faecal pellets: c/min	Faecal pellets: c/faecal pellets/min	Body less filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
4775 c/ml./min, 5215 cells/ml. 100 %	1	82	3,404	42	421	3,825	11·0	1·09
	2	55	1,182	21	314	1,508*	21·6	0·46
	3	80	3,763	47	611	4,374	14·0	1·26
	4	54	1,668	31	248	1,916	13·0	0·57
	5	60	1,639	27	224	1,863	12·0	0·57
10 %	1	66	2,496	38	593	3,093†	19·3	9·04
	2	72	3,001	42	788	3,789	20·8	11·20
	3	72	3,383	47	823	4,206	19·6	12·55
	4	73	3,376	46	816	4,192	19·5	12·48
	5	89	2,540	29	628	3,168	19·8	9·38
1 %	1	3	189	63	320	509	62·9	15·46
	2	7	74	11	109	183	59·6	5·23
	3	7	227	32	104	331	31·4	9·69
	4	5	164	33	217	381	57·0	11·27
	5	3	38	13	88	126	69·8	3·66
Filtrate 3070 c/ml./min	A	0	—	—	103 } 122	—	—	—
	B	6	4	1	142 }			

* Including 12 from eggs laid. † Including 4 from eggs laid.

TABLE XIX. MAXIMUM UPTAKE OF ONE *CALANUS* IN SEA-WATER CONCENTRATIONS

Species	No. cells/ml.	Counts/ cell	Time of expt. (h)	No. faecal pellets/24 h	Cell equiv. per faecal pellet	Total for 24 h: No. cells taken up	Total for 24 h: ml. filtered
Skeletonema costatum	14,000	0·15	17	53·7	6,953	373,000	30·1
	11,200	1·02	23½	81·7	3,023	247,000	26·2
	3,670	0·28	16½	8·7	932	8,134	2·2
	970	0·66	18	1·3	1,443	1,924	1·6
	770	0·94	65	2·2	589	1,305	1·7
Lauderia borealis	627	31·0	17	112·9	11	1,198	21·2
	63	31·0	17	38·1	5	192	36·4
	6	31·0	17	7·1	2	15	26·2
	645	25·8	16	91·5	6	575	9·4*
	460	2·6	15½	52·7	290	15,310	38·7
	320	2·6	15½	37·2	275	10,230	38·0
	174	0·5	48½	11·4	279	3,176	20·0
	155	3·9	10	2·5	1,332	3,197	21·8
Chaetoceros decipiens	945	1·35	15½	54·2	211	11,440	11·7
	170	1·35	15½	7·7	143	1,106	6·8
	17	1·35	15½	4·6	11	53	3·2
	630	120·0	16	141·0	159	22,420	43·3
	63	120·0	16	22·5	78	1,752	31·2
	3	120·0	22½	36·7	1	43	14·4
	490	179·0	17	81·9	46	3,784	8·1
	110	179·0	18½	22·1	34	756	8·9
	22	179·0	18½	11·7	13	146	8·6
	78	311·0	17	31·1	21	667	8·9
	68	87·0	16	19·5	33	651	10·0
	7	87·0	16	10·5	4	42	6·3
	1	87·0	21½	0	—	6	8·4
Ditylum brightwellii	63	385·0	18½	84·3	7	622	10·4
	13	385·0	18½	44·1	4	189	15·9
	1	385·0	18½	14·3	1	15	17·8
Chlamydomonas pulsatilla	6,200	0·68	17	15·5	10,710	166,300	31·0
	620	0·68	17	5·6	653	3,685	6·1
Platymonas carteriaformis	4,150	0·14	16	33·0	1,427	47,000	11·6
	415	0·14	16	22·5	60	1,350	3·2
Nannochloris oculata	12,000	0·03	18	2·7	97	258	0
Chromulina pusilla	16,000	0·01	16¼	4·4	4,077	18,050	1·1
Dicrateria inornata	10,900	0·15	16	3·0	133	400	0·3
Syracosphaera elongata	4,500	1·22	16½	42·2	195	8,230	1·8
	1,125	1·22	16½	40·7	298	12,140	11·5
	3,100	16·1	16	16·5	262	4,330	1·4
	200	16·1	16	49·5	104	5,125	29·1
	20	16·1	16	10·5	64	675	43·5
	720	16·0	10	67·2	101	6,800	9·9
	575	23·2	17½	24·7	97	2,324	4·1
Prymnesium parvum	4,000	0·17	15¾	22·9	739	16,900	4·2
	400	0·17	15¾	9·1	78	718	1·8
Cryptomonas sp.	1,600	0·85	15¼	20·5	1,936	39,630	32·4
	160	0·85	15¼	14·2	252	3,567	24·7
Prorocentrum micans	540	16·0	17	83·3	58	4,827	9·4
	54	16·0	17	22·6	74	1,675	37·1
	5	16·0	17	12·7	25	316	84·3
	68	111·0	9	120·0	15	1,746	27·6
P. triestinum	12,200	0·84	17½	71·3	702	50,060	4·2
	1,220	0·84	17½	38·4	519	19,920	17·8
	1,020	0·76	21½	55·8	274	15,310	15·6
	102	0·76	21½	22·3	91	2,030	21·5
Oxyrrhis marina	2,040	0·60	18½	46·7	839	39,190	21·7
	204	0·60	18½	19·5	254	4,938	28·1
Gymnodinium vitiligo	7,840	1·4	17	25·4	2,566	65,220	8·5
	784	1·4	17	79·9	1,409	13,930	19·2
	78	1·4	17	14·1	117	1,658	23·2
Gymnodinium veneficum	13,800	1·54	18½	68·8	243	16,700	1·3
	1,380	1·54	18½	66·2	218	14,440	11·1
	138	1·54	18½	11·7	159	1,850	14·5

* Male *Calanus*.

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THE ROLE OF MICRO-ORGANISMS IN THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY

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During the course of earlier work with larvae of *Ophelia bicornis* Savigny it became increasingly clear that a major factor in stimulating these larvae to settle and metamorphose is the presence, on sand grains of suitable size, of living micro-organisms such as bacteria, in numbers neither too many nor too few (Wilson, 1954). It had been shown that an acid-cleaned sand (which is neutral or almost so) kept in sea water becomes increasingly attractive to these larvae with time. It had been admitted, however (*loc. cit.*, p. 366), that these results were derived from a comparison of experiments not designed to this end, and that further tests, planned for the purpose, were desirable.

Methods were identical with those of 1953 (Wilson, 1954), except that the relative abundance of the unmetamorphosed, metamorphosing and metamorphosed larvae in each sample as expressed in words in the tables was in 1954 based on random partial counts, giving a probable slight increase in accuracy of assessment. Unmetamorphosed larvae were not counted except when sticking to a sand grain, while metamorphosing larvae were recorded as in early, mid or late metamorphosis. These stages are not usually shown in the tables but are referred to in the text wherever the stage of metamorphosis is a matter of interest.

Fertilizations and experiments were mostly in a mixture of sea water from the International Hydrographical Station E1, and from the Clyde (by kind co-operation of the Millport Marine Station). Except when otherwise stated, all sea waters were passed through Berkefeld filter candles before use. The larvae used in the tests were always 5 days old.

The Experiments

In preparation for the experiments some Bullhill Bank sand which had been collected on 10 June 1952 was thoroughly cleaned in hot concentrated sulphuric acid on 21 April 1954, washed, and stored in distilled water. Such acid-cleaned sand is almost white in colour. On various dates subsequently, as noted in the description of each experiment, a quantity was removed from the distilled water, washed with filtered sea water and strewn thinly over the

bottom of one or more covered glass dishes filled with filtered sea water. These were left on a bench shielded from direct light from the sky. The water in some dishes was changed a number of times at intervals of days or weeks; in others it was not changed. To a few dishes a little peptone sea water (Spencer, 1952, p. 98) was added from time to time to encourage bacteria. Further quantities of sand were similarly soaked for varying periods in unfiltered water from the laboratory circulation (tank water), sometimes mixed with mud from the bottoms of the tanks.

Each experiment was divided into two sections. Each section comprised a set of sands tested together in the same free-choice dish and often separately in conical vessels as well. The ability of the larvae to metamorphose was checked for each experiment by a control dish strewn with fresh Bullhill Bank sand. In these control dishes metamorphosis was invariably almost 100%.

Experiment 1 (*Table I*)

Section A. Four samples of acid-cleaned sand soaked in sea water for different lengths of time and one sample soaked in tank water were tested against a sample of the same acid-cleaned sand which had been stored in distilled water throughout the experiment (see Table I for dates and other details). It will be seen from the table that the longer the sand had soaked in sea water the bigger the settlement which was obtained. These soaked sands were observed to have numbers of unidentified organisms on and among their grains. Some of the organisms were almost certainly algal; bacteria would not be seen at the magnification used. All the sands were clean, but that soaked since 11 June 1954 (sample 4 in Table I, section A) had more 'algal' growths than the others. The sand from distilled water was perfectly clean.

Section B. The acid-cleaned sand samples tested here had been soaked in tank water, in one instance with the addition of tank mud, or in sea water to which peptone had been added. Some of these waters and media were changed from time to time. All the sands were discoloured by organic growths, the tank water sands being various shades of brown owing to algal growths, including diatoms. The sand from sea water with peptone medium was grey, even after washing well, and showed fluffy growths on some grains. While all these sands (after rinsing well in sea water) attracted more larvae to settle than did the sand straight from distilled water it does not appear that they were as effective as some of the cleaner sands in section A. A direct comparison with that section cannot, however, be made.

Experiment 2 (*Table II*)

Section A. It is known from earlier work that natural sands after washing and drying, or after sterilization in water at about 100° C, lose any attractive property they may have had in the fresh state, and some of them become

repellent. In this series of tests acid-cleaned sands which had become attractive after soaking in sea water for several weeks (samples 2 and 5) were likewise shown to lose their acquired attractiveness by these treatments, and one of them after treatment (sample 6) seems to have become repellent. This sand from tank water and mud (sample 5), had, before washing and drying, unidentified flocculent growths on the sand grains, which were visibly more overgrown than the grains of the sand which had been soaked in clean filtered sea water (sample 2) and which appeared to be clean, except for a few small growths seen on some grains before treatment. After treatment these had disappeared, although a few dead growths were still to be seen on the grains comprising samples 6.

Section B. These tests showed that washing and drying had little or no effect on acid-cleaned sand from distilled water, so far as larval settlement is concerned, but again demonstrated that acid-cleaned sand which had acquired some degree of attractiveness by soaking in sea water lost this property after washing and drying.

Experiment 3 (*Table III*)

Section A. It was again shown that acid-cleaned sand gains in attractiveness during soaking in sea water but that this soaking has to be prolonged for several weeks to be effective. The gain after only 1 week (sample 2) was very slight, after 3 months (sample 3) very marked. Treatment with alcohol (sample 4) or formalin (sample 5) destroyed the attractiveness (the fixatives were well washed out first with fresh water, then with sea water), but there is no evidence that this particular sand had been made repellent by these treatments.

Section B. Fresh Bullhill Bank sand, collected a few days before the experiment began, was soaked for 4 days, 2 days and 1 day respectively in sea water to which peptone had been added. The peptone induced heavy growths of bacteria, especially so after 4 days, and all these sands had a pronounced smell which disappeared during rinsing in sea water before the tests were made.

From Table III it will be seen that the longer the sand had been in peptone sea water, the less attractive it was. This is even more evident if the state of the metamorphosing larvae in the conical vessels be considered. Most of those in sample 2, which had been 4 days in peptone sea water, were early and mid-metamorphosis stages, but in sample 3, which had been 2 days in peptone sea water, there was a smaller proportion of early stages and a decidedly larger proportion of late stages. In sample 4, exposed in peptone sea water for only 1 day, the metamorphosing larvae were almost without exception late stages.

Table III also shows that the fresh Bullhill Bank sand retained its attractiveness more effectively in sea water than when stored (in a stoppered

jar) in its natural moist state, as collected at low tide. It is known from earlier work that the attractive property gradually diminishes when such sand is so stored.

Experiment 4 (*Table IV*)

Section A. Samples of acid-cleaned sand were soaked in sea water containing peptone, some of them for several weeks. One sample was kept sterile, in all the others bacterial growths took place and were heavy in those immersed for more than a day. Sample 2, which had been soaking longest with changes of the medium, was discoloured dark grey. This sand had also some flocculent brown growths, not present in the other sands which were relatively clean after rinsing before use although small organisms on the grains could be seen here and there.

The settlements obtained with these sands, after rinsing in sea water, are shown in Table IV. All (samples 2–5) attracted more larvae than the sand from sterile concentrated peptone sea water (sample 1), but except for sample 3 which had soaked for 10 days the settlements were not really heavy. Sample 3 produced a good settlement but the sands immersed for longer or shorter periods did not. Moreover, the metamorphosing larvae in sample 3 in the conical vessel experiment were mainly mid and late metamorphosis stages; while in all other samples, including 6, the metamorphosing larvae had a higher proportion of early stages and a smaller proportion of late. From this it would seem that acid-cleaned sand in peptone sea water increases in attractiveness for a time and then decreases (but compare Expt. 5 B). This result should also be compared with that obtained for fresh Bullhill Bank sand (Expt. 3 B) kept in peptone sea water; only a decrease in attractiveness was then recorded.

Another portion of sand from sample 2 was washed in tap water and sterilized by heating almost to boiling-point (sample 6). The attractiveness was less than the sand not so treated, but it did not become repellent. The sand grains were cleaner than the unsterilized grains in sample 2, in particular the flocculent growths were no longer visible. In the conical vessel test of this sand most of the metamorphosing larvae were relatively early stages, there being very few late stages and a smaller proportion than in sample 2.

Section B. The four sand samples tested here comprised two distinct and unrelated tests. In the first test an acid-cleaned sand soaked in sea water for about a month (Table IV B, sample 1) was compared with a similar acid-cleaned sand soaked in sea water plus 'B.B. water' (see Wilson, 1954, p. 363) for over a year (sample 2). The latter sand had been used in the 1953 series of experiments, but had been kept since then with only a single addition of fresh filtered sea water, in March 1954. Both these sands, compared with the control sample 4 from distilled water, were attractive to the larvae but that which had been soaked for a year was much more attractive than that which

had been in sea water for only a month. This year-old sand was seen to have minute diatoms, other algae, a few flagellates, and apparently bacterial slimes on the grains, whereas the other sands were clean. It was not as dirty, however, as sample 2 in section A of this experiment, and was not noticeably discoloured.

The second comparison was between an acid-cleaned sand (sample 4) and similar sand (sample 3) which had been enclosed within a bolting silk envelope and buried in Salthouse Lake (Station II) sand which had previously been heated in tap water near to boiling point to ensure maximum repellence. The test was to determine if any part of the repellent property of a repellent sand can be transferred to a neutral sand in close proximity though not in contact with it. Some earlier experiments had indicated that such transference, if it occurs at all, takes place more readily when physical contact between grains is allowed (Expt. 43, Wilson, 1953*a*) and doubtfully so when the two sands are separated by bolting silk (Wilson, 1953*b*). But in these earlier experiments the sands used were fresh and unsterilized, and the attractive factor would therefore be present as well. In the present test this would not be so. The result (Table IV B) gives no indication that any part of the repellent factor was transferred. In the free-choice dish no significant difference was discernible, while comparison of the conical vessel tests suggests that sample 3 was a little more attractive than the control (sample 4). This result is explicable if it be assumed that the repellent factor consists of organic matter, which after heating is too adherent to the grains to be passed through the meshes of bolting silk. Bacteria would be able to pass through the meshes, and it is possible that the neutral sand within the silk envelope would receive bacteria from those that would multiply on this organic matter after the heated sand, heaped around the silk envelope, had been in sea water for a short time. Thus the neutral sand after receiving adherent living bacteria, and perhaps other organisms, would be slightly attractive.

Experiment 5 (*Table V*)

Section A. This was a repetition of Expt. 3 B after the sands had been kept for another 6 days. The result (see Table V) was much as before, no further marked decrease in attractiveness of the originally fresh Bullhill Bank sand having taken place, perhaps because the grains in the earlier experiment already carried as many micro-organisms as could be accommodated on their surfaces. However, the metamorphosing larvae in sample 4 from the conical vessel were not almost all late stages as they had been in the corresponding sample in Expt. 3 B, and in general there was detectable a further slight decline in attractiveness.

Section B. Some of the acid cleaned sands tested in Expt. 4 A after immersion for varying periods in peptone sea water (medium not changed) were re-tested after a further period had elapsed. The result (Table V B) is much as

before (Table IVA), there not being any definite increase in attractiveness of sample 3, and especially sample 4, such as might have been expected. Sample 2 was, as before, more attractive than either. These results indicate that acid-cleaned sand kept in peptone sea water does not necessarily show a regular increase in attractiveness with time when kept in this medium, as seemed to be implied by Expt. IVA. It is not known if the organisms encouraged by the peptone were the same species for all samples and the observed difference in settlement between the various sand samples could relate to a specific difference between the organisms grown, or to their proportional abundance. It may be noted that all sands looked nearly quite clean, only a few unidentified growths being noted on some grains. The sand of sample 4 definitely had fewer visible growths on the grains than did samples 2 and 3. No such growths were seen on any grains from sample 1.

Discussion

A consideration of the 1954 experiments taken in conjunction with those of previous years makes it clear that the factor most active in inducing settlement of *Ophelia bicornis* larvae is the presence, on the sand grains, of living micro-organisms. The organisms must be neither too few nor too abundant. Presumably for a sand to attain maximum attractiveness they must be of particular species in certain relative abundance. In the experiments in which acid-cleaned sands were soaked in filtered sea water it is most unlikely that the latter requirements were even approximately fulfilled; indeed it seems probable that the species of organisms which grew under those conditions were mostly not the same and were not in the same relative abundance as those present on sand grains freshly gathered from the surface of the Bullhill Bank. None the less, after several weeks of soaking in sea water, they did impart a very considerable degree of attractiveness to acid-cleaned sand. No other explanation of the observed results seems possible.

The period needed to impart a marked attractiveness to acid-cleaned grains was very considerably shortened by adding peptone sea water to the filtered sea water in which they were immersed, but this never made the sand as attractive as did some of the prolonged soakings in filtered sea water without the addition of nutrients. Presumably the grains after immersion in the peptone sea water would become coated with micro-organisms different from those that grew slowly in pure filtered sea water, and they may well have become too abundant to attract very many larvae.

It was observed that fresh Bullhill Bank sand after immersion in peptone sea water became less attractive than before, especially after a heavy growth of bacteria had taken place. These observations support the conclusion that the quantity and quality of the living organic film on the grain surface is of first rate importance to the settling *Ophelia* larva. To the identity of the species concerned and their actual abundance there is at present no clue.

This series of studies has been another demonstration of the influence of the minutest micro-flora and fauna on the distribution and activities of larger microscopic animals and ultimately on the macro-fauna itself. They suggest that more attention could profitably be paid to the most minute living constituents of bottom deposits and their interrelations with the larger organisms subsisting in or on those soils.

The organic films left on natural sand grains after washing and drying are undoubtedly to a large extent responsible for the floatation properties of dried grains, discussed in previous papers (see especially Wilson, 1952). Less than one per cent of acid-cleaned grains stored in distilled water float when sprinkled dry on the surface of water, but during the course of the experiments recorded in this paper it was found that after some days of immersion in sea water a markedly higher percentage floated (after washing and drying). After several weeks or months the percentage of floatable grains was still higher and in one instance was over 90%. The percentage floatability of acid-cleaned sand rose steeply and more quickly during immersion in peptone sea water, and the floatation of fresh Bullhill Bank sand was also raised by immersion in peptone sea water. That flotation can be caused by electrical charges produced on the grains by rubbing is probably also true, but is likely to have little bearing on problems concerned with settlement reactions.

It seems certain that dead organic matter coating sand grains is repellent to *Ophelia bicornis* larvae and renders many sands such as Salthouse Lake sand (Stations I and II), after the silt has been washed away, distasteful to these larvae. The repellent effect is likely to be most marked if there are no living micro-organisms present as well, as when sands have been sterilized in water at 100° C, or after fixation or drying. Sands will thus vary in attractiveness and repellence according to the number and kinds of living micro-organisms, and according to the amount and quality of the non-living organic materials coating the grains. Grain size, and perhaps also shape, is another and lesser factor influencing larvae about to settle, but is not the main factor as was earlier thought (Wilson, 1948). That grade does exert some influence has often been demonstrated (Wilson, 1953*b*).

Summary

Experiments in the summer of 1954 made it clear that the factor most active in inducing metamorphosis and settlement of *Ophelia bicornis* Savigny larvae is the presence on sand grains of living micro-organisms, such as bacteria, and that these should be neither too few nor too abundant. It would appear likely, and for this there is some evidence, that certain species are more effective in promoting settlement than are others, though there is at present no clue to the identities of the species concerned. Dead organisms and non-living organic matter are not attractive and may indeed be repellent, as a consideration of some of the results suggests.

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TABLE I. EXPERIMENT 1

Begun 5. vii. 54 with larvae 5 days old. Results on 7. vii. 54

Section A

	Acid-cleaned Bullhill Bank sand soaked in					
	(1) Distilled water since 21. iv. 54	(2) Filtered sea water, unchanged since 23. iv. 54	(3) Filtered sea water, changed 4 times since 23. iv. 54	(4) Filtered sea water, changed twice since 11. vi. 54	(5) Filtered sea water, unchanged, since 26. vi. 54	(6) Unfiltered tank water unchanged since 26. vi. 54
	Settlements after 2 days in free-choice dish					
Metd	None	Many	Many	Fair number	Very few	Very few
Meting	1 or 2	Many	Fair number	Many	Very few	Few
Unmet.	Very few	Fair number	None	Good number	Several	Fair number
	Settlements after 2 days in conical vessels					
Metd	Few	Very many	Very many	Fair number	—	—
Meting	Several	Many	Good number	Very many	—	—
Unmet.	Fair number	Several	Several	Good number	—	—

Section B

	Acid-cleaned Bullhill Bank sand soaked in				
	(1) Distilled water since 21. iv. 54	(2) Unfiltered tank water, changed 4 times since 23. iv. 54 (sand dark brown)	(3) Unfiltered tank water, unchanged since 23. iv. 54 (sand pale brown)	(4) Unfiltered tank water and mud, changed 4 times since 23. iv. 54 (sand brown)	(5) Filtered sea water containing peptone, changed 3 times since 23. iv. 54 (sand grey)
	Settlements after 2 days in free-choice dish				
Metd	None	Few	Fair number	Fair number	Several
Meting	Very few	Several	Several	Few	Several
Unmet.	Several	Good number	Good number	Several	Fair number
	Settlements after 2 days in conical vessels				
Metd	See A 1 above	Very few	Several	Good number	Many
Meting	—	Few	Fair number	Fair number	Many
Unmet.	—	Several	Many	Several	Few

Metd = metamorphosed; meting = metamorphosing; unmet. = unmetamorphosed.
Scale of words used to express number in Tables I–V:

Very few (sometimes also expressed in numbers 1–4)
Few
Several
Fair number
Good number
Many
Very many
Multitude
Great multitude

TABLE II. EXPERIMENT 2

Begun 10. vii. 54 with larvae 5 days old. Results on 12. vii. 54

Section A

	Acid-cleaned Bullhill Bank sand soaked in					
	(1) Distilled water since 21. iv. 54	(2) Filtered sea water, unchanged since 23. iv. 54 (sand almost white)	(3) Sand as (2) washed and dried	(4) Sand as (2), sterilized at about 100° C	(5) Unfiltered tank water and tank mud, changed 4 times since 23. iv. 54 (sand dark brown)	(6) Sand as (5), washed and dried
	Settlements after 2 days in free-choice dish					
Metd	None	Many	None	None	Good number	None
Meting	None	Many	None	Very few	Good number	None
Unmet.	Fair number	Several	Fair number	Good number	Fair number	Very few
	Settlements after 2 days in conical vessels					
Metd	Very few	Many	Very few	Few	Many	None
Meting	Few	Many	Few	Very few	Fair number	None
Unmet.	Fair number	Fair number	Fair number	Fair number	Fair number	Very few

Section B

	(1) Acid-cleaned Bullhill Bank sand in distilled water since 21. iv. 54	(2) Sand as (1), washed and dried	(3) Sand as (1) in filtered sea water, unchanged since 26. vi. 54	(4) Sand as (3), washed and dried
	Settlement after 2 days in free-choice dish			
Metd	None	One	Several	None
Meting	None	One	Several	None
Unmet.	Fair number	Fair number	Fair number	Several
	Settlements after 2 days in conical vessels			
Metd	See A 1 above	None	Several	None
Meting	—	Several	Several (early to late)	Several (all early)
Unmet.	—	Fair number	Fair number	Fair number

TABLE III. EXPERIMENT 3

Begun 21. vii. 54 with larvae 5 days old. Results on 23. vii. 54

Section A

Acid-cleaned Bullhill Bank sand soaked in

	(1) Distilled water since 21. iv. 54	(2) Filtered sea water, unchanged since 14. vii. 54	(3) Filtered sea water, changed 4 times since 23. iv. 54	(4) Sand as (3) fixed in alcohol for 1 h	(5) Sand as (3) fixed in neutral formol for 1 h
	Settlements after 2 days in free-choice dish				
Metd	None	None	Many	Very few	None
Meting	None	Very few	Many	Very few	Very few
Unmet.	Fair number	Good number	Fair number	Good number	Several
	Settlements after 2 days in conical vessels				
Metd	Very few	Several	Very many	None	Few
Meting	Fair number	Several	Good number	Fair number	Many
Unmet.	Many	Many	Several	Many	Fair number

Section B

Fresh Bullhill Bank sand (collected 15. vii. 54) soaked in

	(1) Filtered sea water since 17. vii. 54	(2) Filtered sea water containing peptone, unchanged since 17. vii. 54	(3) Filtered sea water containing peptone, unchanged since 19. vii. 54	(4) Filtered sea water containing peptone, unchanged since 20. vii. 54	(5) Fresh Bullhill Bank sand kept in natural moist state since 15. vii. 54
	Settlements after 2 days in free-choice dish				
Metd	Multitude	Few	Very few	Several	Very many
Meting	Good number	Few	Fair number	Many	Fair number
Unmet.	Several	Few	Several	Fair number	Few
	Settlements after 2 days in conical vessels				
Metd	Great multitude	Several	Good number	Multitude	Great multitude
Meting	Very few	Multitude	Great multitude	Multitude	Few
Unmet.	None	Good number	Very few	None	None

TABLE IV. EXPERIMENT 4

Begun 24. vii. 54 with larvae 5 days old. Results on 26. vii. 54

Section A

Acid-cleaned Bullhill Bank sand soaked in

	(1) Sterile concentrated sea water peptone since 1. v. 54	(2) Filtered sea water containing peptone, changed 5 times since 23. iv. 54	(3) Filtered sea water containing peptone, unchanged since 14. vii. 54	(4) Filtered sea water containing peptone, unchanged since 21. vii. 54	(5) Filtered sea water containing peptone, unchanged since 23. vii. 54	(6) Sand as (2) washed in tap water and sterilized at about 100° C
	Settlements after 2 days in free-choice dish					
Metd	None	Very few	Fair number	Very few	Very few	Very few
Meting	Several	Fair number	Good number	Few	Several	Few
Unmet.	Fair number	Good number	Fair number	Fair number	Fair number	Fair number
	Settlements after 2 days in conical vessels					
Metd	Very few	Very few	Many	None	Few	Very few
Meting	Fair number	Very many	Very many	Very many	Very many	Good number
Unmet	Several	Many	Fair number	Very many	Many	Fair number

Section B

Acid-cleaned Bullhill Bank sand

	(1) Soaked in filtered sea water, unchanged since 26. vi. 54	(2) Soaked in 'B.B. water' plus an equal volume of filtered sea water since 10. vii. 53. Some filtered sea water added in March 1954	(3) In bolting silk envelope in filtered sea water, amid Salthouse Lake (St. II) sand sterilized at about 100° C, since 16. vii. 54	(4) Soaked in distilled water since 21. vi. 54
	Settlements after 2 days in free-choice dish			
Metd	Fair number	Many	Very few	Very few
Meting	Fair number	Good number	Very few	Very few
Unmet.	Fair number	Many	Several	Fair number
	Settlements after 2 days in conical vessels			
Metd	Fair number	Multitude	Fair number	None
Meting	Good number	Multitude	Fair number	Fair number
Unmet.	Very many	Many	Fair number	Fair number

TABLE V. EXPERIMENT 5

Begun 27. vii. 54 with larvae 5 days old. Results on 29. vii. 54

Section A

	Fresh Bullhill Bank sand (collected 15. vii. 54) soaked in				
	(1) Filtered sea water unchanged since 17. vii. 54	(2) Filtered sea water containing peptone, unchanged since 17. vii. 54	(3) Filtered sea water containing peptone, unchanged since 19. vii. 54	(4) Filtered sea water containing peptone, unchanged since 20. vii. 54	(5) Fresh Bullhill Bank sand kept in natural moist state since 15. vii. 54
	Settlements after 2 days in free-choice dish				
Metd	Very many	Several	Few	Fair number	Very many
Meting	Fair number	Several	Few	Good number	Many
Unmet.	Very few	Few	Several	Fair number	Very few
	Settlements after 2 days in conical vessels				
Metd	Great multitude	Fair number	Fair number	Very many	Great multitude
Meting	Few	Many	Very many	Very many	Fair number
Unmet.	None	Very few	Very few	Very few	None

Section B

	Acid-cleaned Bullhill Bank sand soaked in			
	(1) Distilled water since 21. vi. 54	(2) Filtered sea water containing peptone, unchanged since 14. vii. 54	(3) Filtered sea water containing peptone, unchanged since 21. vii. 54	(4) Filtered sea water containing peptone, unchanged since 23. vii. 54
	Settlements after 2 days in free-choice dish			
Metd	Few	Fair number	Very few	None
Meting	Few	Fair number	Several	Few
Unmet.	Fair number	Good number	Good number	Fair number
	Settlements after 2 days in conical vessels			
Metd	Several	Multitude	Very many	Several
Meting	Several	Multitude	Many	Multitude
Unmet.	Very many	Few	Good number	Very many

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AN IMPROVED 'VACUUM' GRAB FOR SAMPLING THE SEA-FLOOR

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(Text-fig. 1)

The sampler to be described takes a small unstratified sample of the sea-bed, the sample being subject to a minimum of loss while being brought up. Such samples are of use in survey work, or for studies on grade size, mineralogical composition, chemical properties, or microfauna and flora of the deposits.

Although core-samples, in which the original stratification is retained, can be taken in soft muddy sediments without difficulty, it is not at all easy to take cores of sand or gravel. On such deposits an unstratified sample may be the best that can be obtained. Coarse sediments may be sampled by a dredge fitted with canvas bag, but when a small sample is required quickly, a light instrument which can be lowered vertically from the ship on a rope or hydrographic wire is needed. 'Snapper' samplers do not always take a satisfactory sample as the jaws may be prevented from closing by a pebble or shell wedged between them, much of the sample being lost while hauling up (cf. Stetson, 1938, p. 7). Stetson describes an instrument which takes a small semicircular core of sediment, the sample being sealed in the coring tube while being brought up, so preventing any loss of material. While no doubt taking good samples, the instrument is rather heavy (57 kg) and complicated in construction.

When large numbers of samples are to be taken, an 'underway' sampler as described by Pratje (1952) may be used with advantage. This requires, however, a suitably positioned winch to run out the wire as fast as the sampler descends, whereas the sampler described here can be worked either by hand or from any small winch.

The new sampler was constructed in the first place to take samples for mineralogical analysis. It is a modification of the original 'Vacuum' grab described by Hunt (1926), which is still used at Plymouth from time to time. Hunt's sampler consists of a metal cylinder containing air at atmospheric pressure, with an opening sealed by a glass plate. On reaching the sea-bottom the glass is broken by a bayonet-striker, and the resulting inflow of water to compress the air in the chamber is used to draw in a sample of sediment. Although an efficient instrument under favourable sea conditions, it was considered that the original sampler was open to considerable improvement. The most serious disadvantage is that broken glass gets mixed in with

the soil sample, and the removal of the remaining fragments at the mouth of the chamber is an unpleasant operation, made more difficult by the presence of grease which is smeared round the glass to give a watertight joint. In rough weather it is difficult to obtain a satisfactory sample, probably because the sampler does not hit the bottom in an upright position. To remove the sample it is necessary to unscrew the base-plate of the chamber through a number of turns, and a quicker method of emptying out the sample was clearly desirable.

The new sampler, while retaining many of the features of the original 'Vacuum' grab, differs in having the chamber sealed by a metal plug instead of a glass plate, the shape is rather more streamlined to aid vertical descent, and the chamber can be opened more quickly to remove the sample.

Description of the Sampler

The sampler (Fig. 1) consists essentially of a cylindrical pressure chamber (*C*) with detachable lid (*L*) having a central opening or 'gullet' (*G*) in the bottom connected to the exterior by a downwardly directed sampling tube, consisting of two components, an upper fixed 'gullet-tube' (*T*) and a lower sliding funnel-shaped 'mouth-tube' (*M*). An extension of the gullet-tube upwards into the chamber serves as a trap-tube (*A*), which enables the sample to be retained in the chamber. The gullet is sealed by a plug (*P*) which is held in position by three retaining hooks (*H*), while the sampler is being lowered. On reaching the sea-bed, the mouth-tube slides upwards over the gullet-tube, so releasing the plug, which flies up through the trap-tube into the chamber, followed by an inrush of water and sediment collected from around the entrance to the mouth-tube.

The pressure chamber is a cylinder of 4 in. bore brass-tube, $\frac{1}{4}$ in. thick and 13 in. long, closed at the bottom by a heavy-gauge circle of metal. The lid has a rubber ring set in a lip to ensure a watertight joint, and is kept in position by a 'samson' (*S*) which can swivel sideways on bolts attached to the pressure chamber. A set screw bears down on the centre of the lid. The trap-tube (*A*) is a length of 1 in. bore pipe, 10 in. long. To the base of the pressure chamber is bolted the gullet-tube (*T*) bearing attachments for the three

Legend to Fig. 1.

Fig. 1. Vertical section through the sampler. *A*, trap-tube; *C*, pressure chamber; *E*, stop; *G*, 'gullet'; *H*, retaining hooks (three in all, set at 120° to one another round the gullet-tube. They are pivoted between pairs of lugs attached to the gullet-tube); *L*, lid of pressure chamber; *M*, mouth-tube (stippled); *O*, collar on mouth-tube; *P*, plug; *R*, rubber washer; *S*, 'samson' securing lid; *T*, gullet-tube; *W*, brass washer; *X*—*X*, the trap-tube, washers and gullet-tube are attached to the pressure chamber by three bolts (not shown) along this axis, and opposite the retaining hooks; Y_1, slot in mouth-tube which allows limited vertical movement relative to the gullet-tube; Y_2, slot in mouth-tube to allow retaining hook to disengage completely.

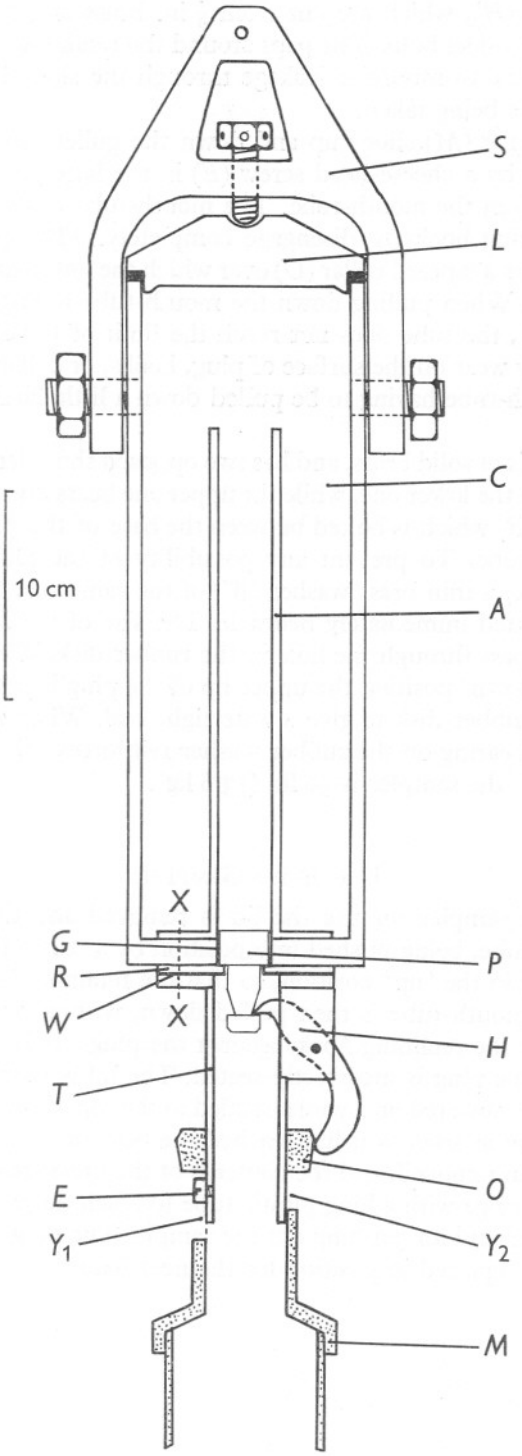

Fig. 1.

retaining hooks (*H*), which are cut from $\frac{1}{4}$ in. brass and pivoted on $\frac{3}{16}$ in. diameter stainless steel bolts. The gaps around the retaining hooks are kept as narrow as possible to minimize leakage through the side of the gullet-tube while a sample is being taken.

The mouth-tube (*M*) slides up and down the gullet-tube, its movement being restricted by a cheese-head screw (*E*) in the latter, sliding in a longitudinal slot (Y_1) in the mouth-tube. The mouth-tube is also slotted (Y_2) to allow the retaining hooks to disengage completely. The upper part of the mouth-tube bears a tapered collar (*O*) over which the lower ends of the retaining hooks slide. When pulling down the mouth-tube to engage these hooks against the plug, the tube does not reach the limit of travel defined by the screw. Thus any wear on the surface of plug, hooks, or collar is compensated for by the mouth-tube having to be pulled down a little farther to retain the plug.

The plug (*P*) is of solid brass, and has two opposed shoulders. The retaining hooks engage on the lower one, while the upper one bears down on a $\frac{1}{8}$ in. thick rubber washer (*R*) which is bolted between the base of the pressure chamber and the gullet-tube. To prevent any possibility of the plug being pushed through the hole, a thin brass washer (*W*) of the same size and shape as the rubber one is fitted immediately below it. The size of the *lower* shoulder is such as just to pass through the hole in the rubber disk. When the sampling tube is in the 'down' position the upper lip of the plug is pressed against the surface of the rubber disk to give a watertight seal. When under water, the water pressure bearing on the rubber washer reinforces this seal.

The weight of the sampler is 30 lb. (13·6 kg).

Use of the Sampler

To prepare the sampler for use the lid is removed and the plug dropped down the trap-tube, being pushed into position by a stick. This is done with the mouth-tube in the 'up' position, so that the retaining hooks are clear of the plug. The mouth-tube is then pulled down, without exerting excessive force, to engage the retaining hooks against the plug. It is at once apparent at this stage if the plug is incorrectly seated. The lid is then replaced.

The sampler is lowered on a wire shackled to the top of the 'samson', and is brought up again as soon as it has reached the bottom.

The lid is then removed, and the contents of the pressure chamber washed into a jar. A syringe with a long plastic tube to reach down to the bottom of the chamber is useful for washing out the sample. The plug is retrieved from the sample and replaced in position for the next haul.

The samples obtained consist of about ½ l. of sediment and 1¼ l. of water. If allowed to settle in a jar the excess water can be poured off, but for some purposes it would be sufficient to strain off the water by pouring the sample into a fine mesh sieve.

Efficiency of the Sampler

In shallow water, the volume of sediment taken increases with depth. Experiments in Cawsand Bay on a bottom of muddy sand gave the following approximate volumes of soil at different depths:

2·7 m	10 ml.	11·9 m	35 ml.
7·3 m	20, 20 ml. (2 samples)	12·8 m	100, 90, 90 ml. (3 samples)
11·0 m	100 ml.	16·5 m	250 ml. (shell gravel)

In deeper water off Plymouth (50–70 m) the sample volume is usually over 500 ml., occasionally much more. The sampler has been tested in fairly rough weather, and samples have been successfully obtained, although these are sometimes of a smaller volume than those taken under favourable conditions. Under normal sea conditions it is exceptional for the instrument not to bring up a sample.

Mr G. R. Forster has made underwater observations on the sampler in a depth of *c.* 10 m, which explain the small samples obtained in shallow water and in rough weather. On a sandy bottom the sampler drew in a crater of sand about 5 cm deep and a little wider than the mouth of the sampling tube (7·5 cm). When lowered in the normal way the sampler fell over on its side almost at once, so that the volume of sand taken should depend on the amount sucked in during the brief period when it is still upright. In very shallow water the rate of suction is such that only a small amount of sand is collected before the sampler falls on its side. In deeper water of say 30 m or more the rate of suction should be sufficient to fill the chamber before the sampler falls over, so that the sample volume should not vary greatly with depth (cf. Hunt, 1926, p. 533). When the sampler was held upright on the bottom while taking the sample a much larger volume than usual was taken, the sampling-tube penetrating for about its full length.

When the instrument was dropped free through the water from a vertical position it tended to fall with a spiral motion. If dropped from a horizontal position it did not right itself completely during the descent. Under normal working conditions the drag exerted by the wire or rope on which it is lowered would keep the sampler upright, but in rough weather the strain would not be constant and might cause the sampler to veer about during the descent, and so take a small sample or none at all.

Both the above defects might be remedied by fitting light fins or a form of drogue to the top of the instrument, and by lowering the centre of gravity of the sampler. When filled with air the 'centre of gravity' of the sampler under

water is at about the mid-point of its length, so that it has no natural tendency to fall the right way up.

These modifications have not been attempted, as the sampler has so far obtained samples whenever required, and any additions would make the sampler clumsy to handle. Where samples of a quantitative nature are required, some such stabilizing attachment would be desirable.

The maximum size of particle taken is limited by the diameter of the gullet, in this instance 18 mm, so that the sampler would be ineffective on bottoms of stones or large shells, as would most other instruments except a dredge. To increase the size of the opening would necessitate strengthening the retaining hooks as the total force exerted by the water would increase with the size of the plug.

Similarly, the sampler would have to be strengthened if it were to be used at a great depth. The present sampler has been tested to a depth of 448 m with success, 1200 ml. of sand being collected in a single haul.

There may be a small loss of material via the trap-tube while the sampler is being brought up. In depths over about 45 m the air in the pressure chamber is compressed to such an extent that when upright the water-level in the chamber is above the top of the trap-tube. While hauling up, the air expands and expels water, with sediment in suspension, down to the level of the top of the trap-tube. If a flap valve were fitted to the top of the trap-tube to prevent this loss some alternative exit for the expanding air would have to be provided. Loss of fine material is considered to be small, however, and very much less than that from most other bottom samplers.

Conclusions

The new sampler has been found to be an improvement on Hunt's 'Vacuum' grab, both in ease of removal of the sample, and in the substitution of a plug for the original glass plate. On the other hand, increasing the length of the sampler has made it less stable so that it falls over almost as soon as it reaches the bottom with the result that samples in shallow water of less than about 15 m depth are not very large.

Although Hunt considered that the 'Vacuum' grab was capable of taking quantitative samples, the author considers that there must be considerable variation in the area and depth of sediment excavated, as the sampler must seldom hit the bottom truly vertically. It is therefore not suitable in its present form for making quantitative estimations related to the area of the sea-floor sampled.

The large volume of water relative to that of sediment in the sample indicates that the diameter of mouth-tube might be increased so as to obtain a larger sample.

In spite of these limitations it is considered that the sampler meets the needs for which it was designed.

I am indebted to Mr F. G. C. Ryder for his skill in making the apparatus from rough drawings, to the crew of M.L. *Gammarus* and the Master and crew of R.V. *Sarsia* for carrying out tests at sea, and to Mr G. R. Forster for underwater observations on the behaviour of the sampler. I am grateful to Mr O. D. Hunt and Dr G. A. Steven for helpful comments on the manuscript.

Summary

A modified form of Hunt's 'Vacuum' grab is described for taking small samples of the bottom. The sample is subject to the minimum of loss while being hauled up, and was designed to take samples for mineralogical analysis.

The new instrument is simpler to use than the original grab, and is capable of taking samples in moderately rough weather, and in depths of 10 m to at least 448 m.

Since the area and depth sampled varies under different conditions, some modifications would be necessary for the instrument to take quantitative samples of the sea-floor.

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HEMIMYCALE COLUMELLA (BOWERBANK): A SHORT DESCRIPTION AND HISTORY OF THE SPECIES

By G. R. Forster
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(Text-figs. 1–3)

These notes arise from the difficulty met with in identifying a pinkish encrusting sponge which has been frequently collected by hand from rocks at 5–10 fathoms. The sponge was at first identified, by a process of elimination, with the deep water genus *Inflatella*. Fortunately some preparations were shown to Dr Lévi at Roscoff to whom the species was at once familiar as *Stylotella columella* (Bowerbank). It was then found that this species had been described originally by Bowerbank from an Exmouth specimen, and subsequently by Topsent from both south and west coasts of France. Topsent found the unusual, if not unique, character of this sponge in possessing, besides siliceous spicules, a great abundance of calcareous granules. These granules are almost certainly the 'gemmules' described and figured by Bowerbank. De Laubenfels in 1928 collected one specimen from Plymouth Sound which he identified as *Stylotella columella*; but proposed (1932) that the species should be included in the genus *Prianos* Gray. Burton in 1934 renamed the species *Hemimycale columella*, figuring the spicules as styles. Arndt (1935) returned to *Stylotella* and retained Burton's figure of the spicules. As the styles have been found, so far, only as a small minority amongst the spicules, it has been thought worth while to provide a further description; and to discuss the differing views on the systematic position of the species.

The sponge consists of a crust, normally several millimetres thick, the surface of which is rather slimy and bears numerous shallow circular pits. These pits are very conspicuous while the sponge is alive and growing on a rock, and each consists of a group of pores. The oscules are rather infrequent, occurring on small conical papillae; they contract and close within 15 sec of a slight disturbance. The oscules are usually indistinguishable in preserved specimens. There is a distinct dermal layer or membrane which contains only scattered spicules except around the projecting rim of each pit. This rim, also slightly contractile, is supported all the way round by a single layer of numerous radially placed spicules. The main skeleton is composed of vertical fibres or bundles of spicules. The fibres often break up into smaller branches

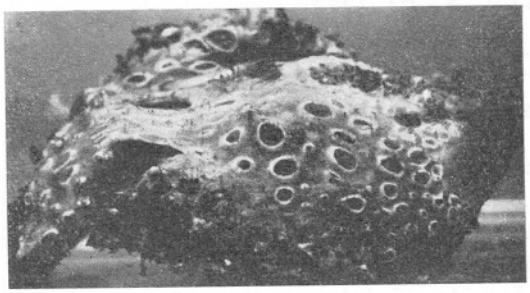

Fig. 1.

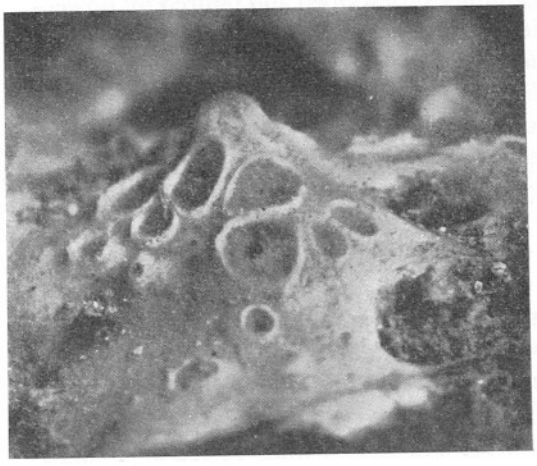

Fig. 2.

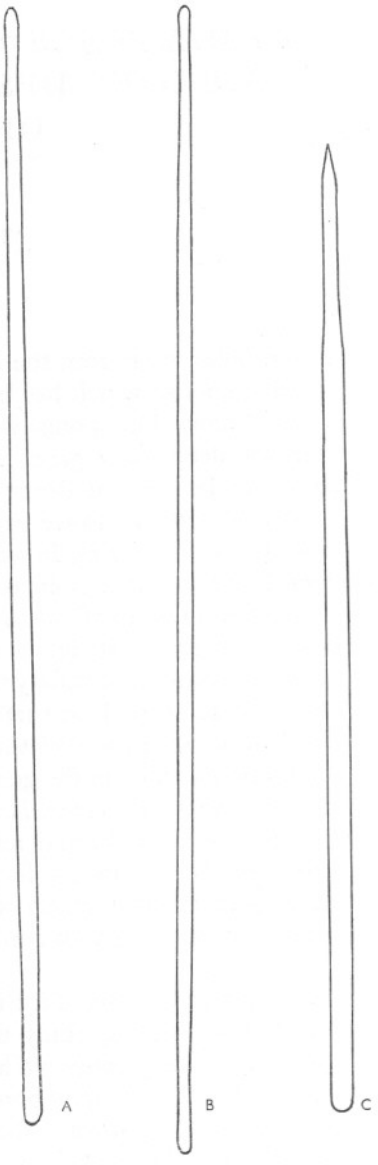

Fig 3.

Fig. 1. Living *Hemimycale columella*. Approx. natural size.

Fig. 2. Side view of the same colony, enlarged, showing an oscule.

Fig. 3. A, strongyl, 355 by 6 μ; B, strongyl, 385 by 5 μ; C, style, 325 by 8 μ.

or give off side branches which interlock with those of neighbouring fibres. The spicules consist of strongyles with a very slight constriction near one or both ends (Fig. 3), of the following dimensions: twenty spicules measured at random ranged from 330 to 420μ with a mean length of 373μ; ten spicules had a mean breadth of 5·85μ, ranging from 5 to 6μ. Styles were also present though definitely rare: with a sample of fifteen, the mean length was 340μ and the range from 290 to 380μ. The mean breadth of five styles was 7·6μ and the range from 6·5 to 9μ.

With specimens from Naples, Topsent (1925) found the spicules sometimes entirely subtylostrongyles, but showing all gradations from subtylostrongyles to subtylostyles.

The pointed ends of the styles were often (Fig. 3) constricted rather sharply at a short distance from the point instead of tapering evenly. Topsent (1925) described the styles of three different species of *Stylotella* as having similar constrictions towards the pointed end but with a series of two or three steps in contrast with the single step of *Hemimycale columella*.

The species was first described as *Desmacidon columella* by Bowerbank (1874, p. 243); although the form of the sponge was termed massive, the arrangement and shape of the spicules agreed fairly well with that of the Plymouth specimens. The chief character which Bowerbank stressed was the gemmules. These bodies varied greatly in size and were 'exceedingly abundant, in some parts as to completely obscure the tissues beneath them'. This describes exactly the appearance of the calcareous granules in my own preparations. Although the gemmules which Bowerbank figured were considerably larger than any which I have seen, they are identical in appearance with the largest granules in a preparation of de Laubenfels' specimen. There seems no reason to doubt that Bowerbank's gemmules were also calcareous granules. Of the eleven species of *Desmacidon* described by Bowerbank only *D. fruticosum* remains in the genus.

D. columella was changed in 1891 by Topsent to *Stylinos columella*. *S. jullienni* was designated in 1892 as the type species of this genus; as this species had a skeleton composed solely of styli, the inclusion of the species *Desmacidon columella* seems at first sight hardly justifiable. Topsent, however, considered that the strongyles showed an intergrading series with the styles; this does not occur in preparations from Plymouth specimens, and, whether it takes place or not, *D. columella* seems sufficiently different from *Stylinos jullienni* and the other species of *Stylinos* for it to be included in a separate genus. Burton regards *S. jullienni* as a species of *Mycale* (verbal communication), and, therefore, since *Desmacidon columella* clearly cannot be absorbed into the genus *Mycale* as well, though, as will be shown later, it possesses some mycalid affinities, another name must be found for it.

Later, in 1894, Topsent rejected *Stylinos* as synonymous with *Stylotella* Lendenfeld (1885), whose definition was: sponges of soft texture, the spicules

being styli only, singly or in bundles. *Stylotella* was revised in 1914 by Hallman who designated *S. agminata* as the type species. This species was very doubtfully assigned to the Suberitidae; while the other species described by Lendenfeld and those of Topsent were excluded from the genus, with no suggestion as to their true position. Topsent, however, continued to use *Stylotella* as an axinellid genus, noting in 1934 that Hallman's work 'leaves no doubt at all on this subject'! Moreover, Burton considers *S. agminata* to be a species of *Hymeniacidon* (verbal communication), and therefore as far as *Desmacidon columella* is concerned, he is in agreement with Hallman in rejecting *Stylotella* as the generic name. Meanwhile, in 1932 de Laubenfels had proposed to change *Stylotella columella* to *Prianos columella*, a genus which he had resurrected for Californian species. *Prianos* was introduced by Gray (1867) for *Reniera amorpha* O. Schmidt (1864). Its validity was doubted by Vosmaer 1885 and later (apparently unknown to de Laubenfels) Topsent, 1925, p. 710, found *R. amorpha* to be a true *Reniera* but synonymous with *R. cratera* O. Schmidt (1862). It is thus impossible to agree with de Laubenfels in accepting *Prianos* as a valid genus; nor in the similarity, which he suggests, between Schmidt's *Reniera* species and the species *Desmacidon columella* in having spicules 'in confusion'. For with *Reniera amorpha* Schmidt described the spicules as linked to form an irregular network by spongin, and in *Desmacidon columella* the spicules are grouped to a very large extent into branching polyspicular fibres without spongin.

Burton (1934) introduced the name *Hemimycale* for Bowerbank's *Desmacidon columella* but described the spicules as styles with occasional strongyles. Dr Burton has been kind enough to examine my preparations and now agrees as to the relative rarity of the styles. *Hemimycale* is therefore the first valid name for the species *Desmacidon columella*; but it has not been generally accepted. Arndt (1935), though using Burton's description, returned to *Stylotella* which was placed in the *Biemnidae*. Lévi (1950) notes *Stylotella columella* as fairly common in the vicinity of Roscoff, and following Topsent retains the species in the Axinellidae. Dr Lévi has since found that larvae of *Desmacidon columella* resemble those of *Mycale* species; thus as a generic for the former species, *Hemimycale* is both acceptable and appropriate.

Summary

Hemimycale columella, a siliceous sponge with a simple skeleton composed chiefly of strongyles, also possesses numerous calcareous granules. The history of the sponge since its discovery by Bowerbank is reviewed and reasons given for rejecting the earlier genera in which it was placed: *Stylinos* Topsent, *Stylotella* Lendenfeld, Hallman and *Prianos* Gray. *Hemimycale* Burton is re-established.

I am much indebted to Dr Lévi for his assistance and guidance, and for permission to publish his observations on the larvae. I am also grateful to Dr M. Burton for his help, especially on the synonyms of various early genera.

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LOCAL VARIATIONS IN THE COLOUR PATTERN OF THE PRAWN *LEANDER SERRATUS* (PENNANT)

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(Text-figs. 1–2)

During investigations into the endocrinology of *Leander* (=*Palaemon*) *serratus* (Pennant) several differences in physiology (which will be reported in this *Journal* at a later date) have been found between the prawns from Plymouth and those from Roscoff on the north coast of France. These experiments involved the repeated handling and the close examination of about a thousand prawns from Roscoff and many more from Plymouth. Increasing familiarity with these animals led to the realization that the two samples could readily be distinguished by eye. It was not always possible to determine the provenance of a single individual in this way, but any group of five or six could be told at a glance. A detailed examination of the external morphology showed that the characteristic differences by which the provenance of these animals could be determined lay not in any anatomical feature but in the disposition and arrangement of the chromatophores which form the pattern on the dorsal side of the cephalothoracic shield and the anterior part of the abdomen. Great individual variations were to be seen, but differing trends of development of the patterns were found in the two populations. There was some degree of overlap between the populations, but only about 15% of the prawns lay in this region of overlap and thus failed to bear the marks of their locality; the remaining 85% could be assigned to either Roscoff or Plymouth with little difficulty. Recently I have been able to examine about forty prawns from Concarneau, on the Atlantic coast of France, and these again are mostly different from either of the other two samples.

The colour pattern of *Leander* is formed by an arrangement of varicoloured chromatophores of different types. Most of the body is covered by a groundwork of scattered, small, red and yellow, bichromatic chromatophores. Bands of deeper colour are formed by the closer massing of larger, physiologically different, bichromatic, red and yellow chromatophores which appear to contain the same pigments, but react differently to hormones. Placed singly are large, white, light-reflecting chromatophores, which also are bichromatic, possessing a relatively inconspicuous red component. Colour photographs illustrating some of these chromatophore types will be found in

Knowles (1955) and Knowles, Carlisle & Dupont-Raabe (1955). Many of the chromatophores on the legs, and some on the uropods, are monochromatic and in these parts patterns exist which are differently compounded from those on the body, but with these regions we are not now concerned. The marked differences between individuals and populations are to be found most noticeably in the dispositions of the bands of colour and the single white chromatophores on the anterior part of the body.

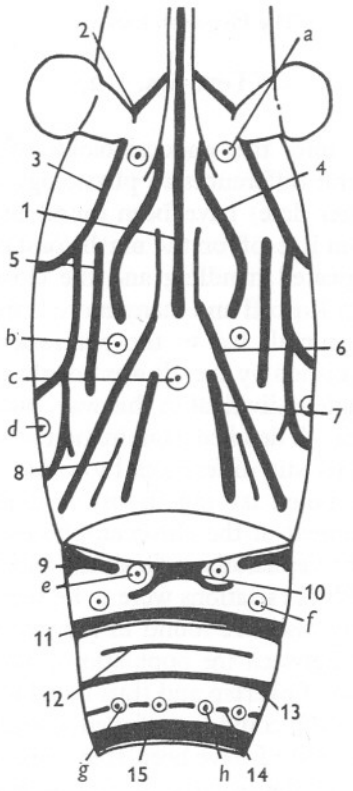

Fig. 1. Diagram of the arrangement of the bands of large chromatophores and the single white chromatophores on the dorsal side of the cephalothorax and the first two abdominal segments. 1–15, the bands of large chromatophores; *a–h*, the white chromatophores.

An arrangement of the bands and the white chromatophores which can be considered a basic pattern from which the variants diverge is represented diagrammatically in dorsal view in Fig. 1. The heavy black lines represent the bands of large, red and yellow bichromatic chromatophores, the circles the white, reflecting chromatophores, with the red component as a dot in the centre. This pattern, or something very like it, can be seen in many individuals from all three localities which have been investigated, particularly in the

younger prawns. In the smaller animals it may be simplified by the omission of white chromatophores *e* and bands 9 and 10.

The main variations on this pattern take the form of reduction or suppression of certain parts or the anastomosis of bands. Asymmetry is very common: indeed, marked asymmetry seems to be more common than near symmetry.

In prawns from Plymouth one of the most constant features is the duplication, as in Fig. 2A–C, of white chromatophore *c*. This occurs in about 80% of individuals. Bands 7 from left and right frequently anastomose in front of chromatophore *c*, rarely behind. Band 1 is usually joined with either bands 6 or bands 7. The lateral horns of band 10 on the first abdominal tergite often continue round chromatophores *e* and fuse with bands 9. Band 14 and the associated chromatophores *g* and *h* may be reduced or absent. Plymouth prawns have a much greater tendency than any of the French animals to complicate the pattern by arabesques, such as are illustrated in Fig. 2B, C.

Among prawns from Roscoff only a small proportion, about 20%, shows the duplication of chromatophore *c* which is so common in prawns from Plymouth. The lateral horns of band 10 do not continue round chromatophores *e* to fuse with band 9. The main variants from the standard pattern are the suppression or great reduction in most individuals of bands 8; the frequent anastomosis of bands 7 behind chromatophore *c*, never in front of it; the reduction or interruption of bands 12 and 14, with often an increase in number of chromatophores *g* and *h* (Fig. 2E); and occasionally anastomosis of band 1 with either or both of bands 6.

In prawns from Concarneau there was a great tendency for the bands to be broken up into dotted lines, especially the narrower bands (e.g. Fig. 2G). Bands 7 usually anastomosed both before and behind chromatophore *c*, so that it came to lie in a ring. Band 1 never fused with bands 6, but did so sometimes with bands 7. The lateral horns of band 10 were often prolonged to run towards chromatophores *f* and frequently a small transverse band was developed in the mid-line between bands 10 and 11 (Fig. 2G–J). This is quite different in appearance from the slight longitudinal bar which may be present in the same position in prawns from Plymouth (Fig. 2C).

Many other individual variations may occur, but these listed above seem to be most constant in the three populations and serve best to distinguish them. Individual variations which occur in all three populations are shifts in position of chromatophores *d* so that they may not be visible in a strictly dorsal view; the appearance of an extra white chromatophore somewhere on the cephalothorax, particularly in the mid-line towards the posterior edge (Fig. 2E); or the absence of one or more of these (e.g. *b* on the right side in Fig. 2H).

It must be emphasized that these differences are described on the basis of relatively small samples of several hundred animals only (many less in the Concarneau sample). Moreover, there is the possibility that the general aspect at each locality may shift year by year. The descriptions above, and all

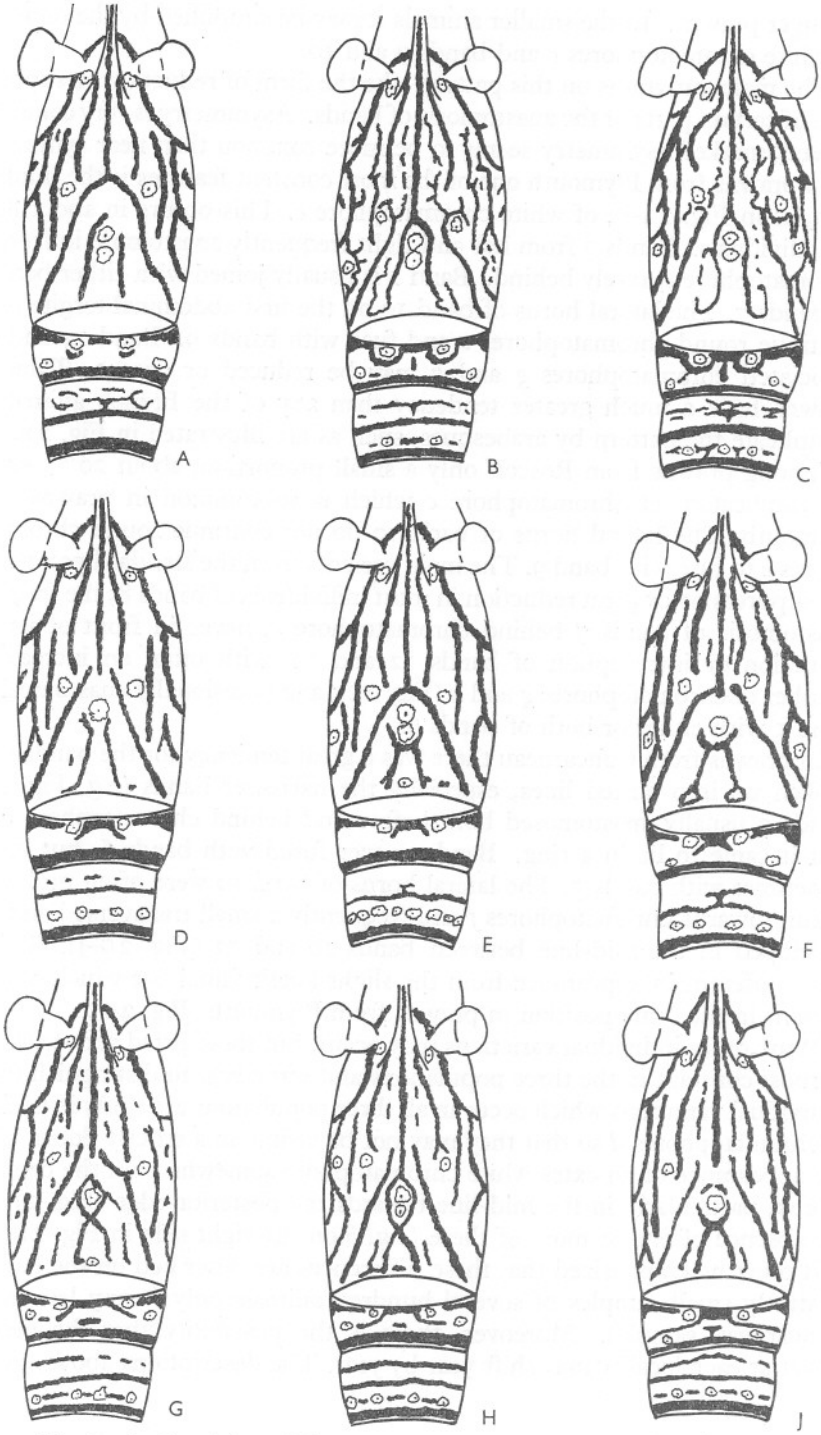

Fig. 2. Sketches in dorsal view of the patterns of colour on the cephalothorax and first two abdominal segments of female prawns of 50–55 mm overall length (rostrum to telson). A, B, C, prawns from Plymouth; D, E, F, prawns from Roscoff; G, H, J, prawns from Concarneau.

the drawings, refer particularly to females between 50 and 55 mm in length (rostrum to telson), and in smaller animals the pattern is often simplified or nearer to the standard. But even in the smallest post-larva at Plymouth the main characteristic variations can be distinguished in a proportion of individuals.

There appears to be no reason for regarding these differences as subspecific variations; it is more probable that every locality which is sufficiently isolated will show characteristic variations. The barriers which separate the three localities which I have studied are well pronounced and may form almost complete blocks in the interchange of population. They are, of course, the English Channel, and Cape Finistère and Ushant. In cross-breeding experiments with the populations from Roscoff and Plymouth there has been no depression in the fertilization rate, in the speed at which eggs are laid or in the viability of the eggs. I have reared the F_1 generation from reciprocal crosses as far as the first post-larval stage, but the numbers so far have been too few to draw any conclusions about the genetical basis of the pattern variations.

Summary

Characteristic differences in the colour patterns of prawns from Roscoff, Concarneau and Plymouth are described and figured. There is no sign of any sterility between the populations from Roscoff and Plymouth.

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THE DICHOTOMOUS SPECIES OF *CODIUM* IN BRITAIN

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(Plate I and Text-figs. 1–5)

The name *Codium tomentosum* (or its nomenclatural synonym *C. dichotomum*) has been applied to dichotomous representatives of the genus in the British Isles ever since the genus was established by Stackhouse in 1797. The purpose of this paper is twofold: to show that three distinct species are involved; and to clarify the nomenclature.

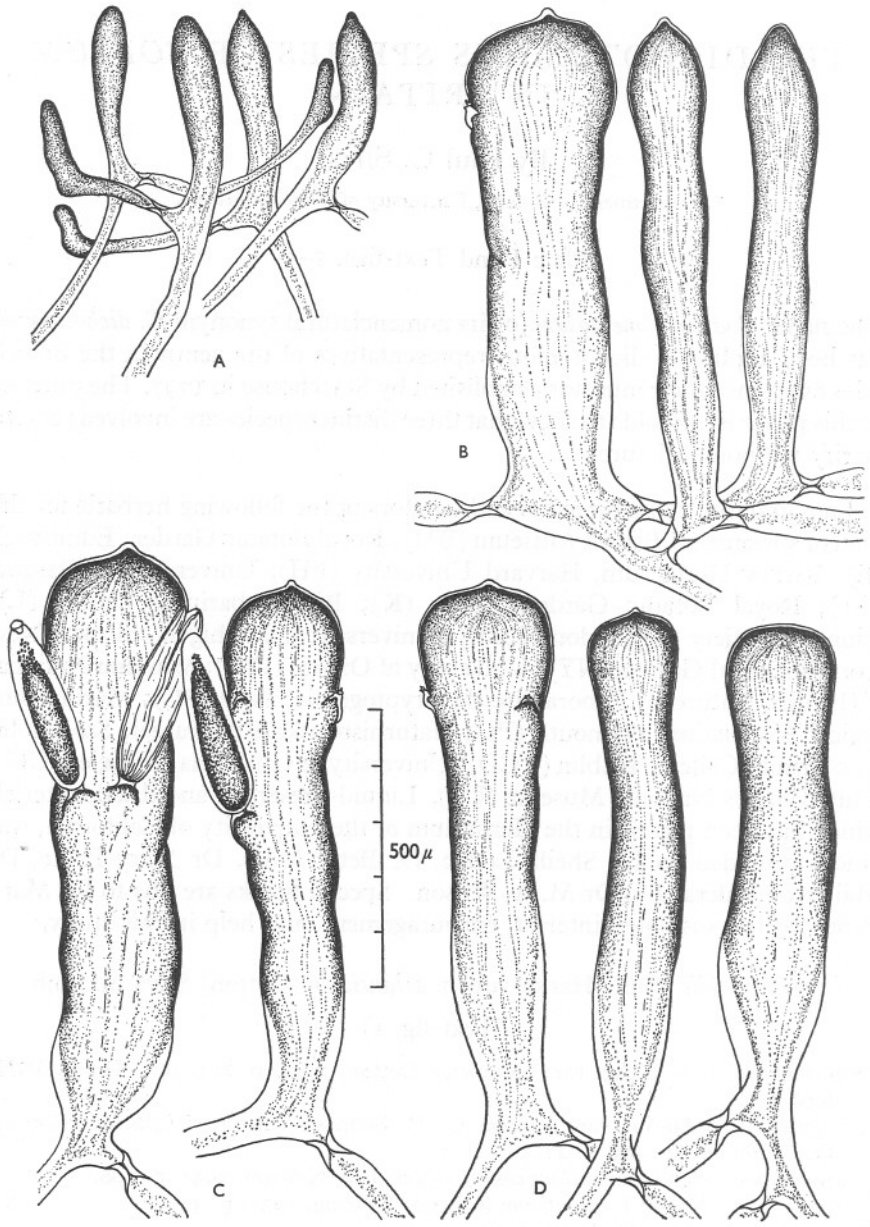
I am grateful to the directors and curators of the following herbaria for the loan of specimens: British Museum (BM); Royal Botanic Garden, Edinburgh (E); Farlow Herbarium, Harvard University (FH); University of Glasgow (GL); Royal Botanic Gardens, Kew (K); Rijksherbarium, Leiden (L); Linnean Society of London (LINN); University of Michigan (MICH); New York Botanical Garden (NY); University of Oxford (OXF); Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris (PC); Marine Biological Association, Plymouth (PL); Naturhistoriska Riksmuseet, Stockholm (S); Trinity College, Dublin (TCD); University of California, Berkeley (UC); United States National Museum (US). Liquid-preserved and dried material, which has been placed in the Herbarium of the University of California, was kindly provided by Dr Sheila Lodge, Dr Betty Moss, Dr Mary Parke, Dr Máirín de Valéra, and Dr M. A. Wilson. Special thanks are due to Dr Mary Parke for her sustained interest, encouragement, and help in this study.

Codium fragile (Sur.) Hariot subsp. *atlanticum* (Cotton) Silva, n.comb.

(Text-fig. 1)

Codium mucronatum J. Ag. var. *atlanticum* Cotton, 1912, p. 114, pl. VII; pl. VIII, figs. 3–5.
C. fragile (Sur.) Hariot var. *typicum* O. C. Schmidt subvar. *atlanticum* (Cotton) O. C. Schmidt, 1923, p. 47.
C. tomentosum Stackh. var. *atlanticum* (Cotton) Lily Newton, 1931, p. 106.
C. fragile (Sur.) Hariot f. *atlanticum* (Cotton) Levring, 1937, p. 34.
C. atlanticum (Cotton) De Valéra, 1939, p. 12.

Thallus composed of one to several erect robust fronds arising from broad, spongy, basal disk; fronds 15–25 (−50) cm high, dichotomously branched (to 9 orders), dichotomies at times telescoped to give fastigiate appearance;



Text-fig. 1. *Codium fragile* subsp. *atlanticum*. A, utricles from tip; B, utricles from base; C, D, utricles from middle of frond. A, B, D, Carna, *de Valéra*; C, Port St Mary, *Lodge*.

branches terete (at times flattened at base), tapering from base (*c.* 10 mm diam.) to apex (2·5–4 mm diam.). Utricles irregularly cylindrical or clavate, (130–) 170–330 μ max. diam., 780–1100 (–1200) μ long, usually 3–5 × long as broad; apices slightly rounded, often terminating in a blunt (or occasionally sharp) point or umbo; wall of utricle *c.* 1·5 μ thick, thicker at apex (–12 μ) and often prolonging point into mucron 10–14 μ long. Hairs (or hair scars) common, one or two per utricle, borne 130–200 μ below apex. Gametangia ovoid, oblong, or fusiform, 80–130 μ diam., 260–400 μ long, one or two per utricle, each borne on protuberance near middle of utricle. Medullary filaments mostly 28–68 μ diam.

Type. Rock pool, Roonah Point (opposite Clare Island), Co. Mayo, Eire, June 1910, *A. D. Cotton* (K).

Geographic distribution. Western Ireland northward to western Scotland, Orkney Is., Isle of Man, Northumberland, Dorset, Norway.

Representative specimens examined. **Ireland.** CLARE: Kilkee, Oct. 1897, *E. George* (BM). GALWAY: Muigh Inis, Carna, Oct. 1943, *M. de Valéra.* MAYO: Kinnacorra, Clare I., Aug. 1911, *A. D. Cotton* (BM); Roonah Point, June 1910, *A. D. Cotton* (K) (type). SLIGO: Sligo Bay, July 1850, *Hennedy* (GL). DONEGAL: Bundoran, July 1850, *W. H. Harvey* (TCD). ANTRIM: Portrush, Oct. 1858, *Hennedy* (GL); Giants Causeway, May 1910, *A. D. Cotton* (K); Larne, Aug. 1865, *Mrs C. A. Johnson* (BM). DOWN: Tara Point, Ards Peninsula, Aug. 1953, *C. I. Dickinson* (K). **Isle of Man.** Bradda Head, Port Erin, April 1912, *R. J. Harvey-Gibson* (K); Port St Mary, May 1950, *S. Lodge.* **Scotland.** BUTE: Eilans, Cumbrae, Aug. 1891, *E. Batters* (BM); Keppel, Cumbrae, Apr. 1955, *E. Conway* (GL). ARGYLL: Iona, June 1826, *R. K. Greville* (E). ORKNEY: South Ronaldshay, Aug. 1891, *G. W. Traill* (BM); Pharay, Aug. 1880, *Boswell* (E); Westray, Aug. 1845 (TCD). INVERNESS: Mallaig, Aug. 1949, *E. Conway* (GL). **England.** NORTHUMBERLAND: Berwick-upon-Tweed, Oct. 1949, *B. Moss*; Farne Is., Nov. 1952, *B. Moss.* DORSET: Swanage, Aug. 1894, *E. Batters* (BM).

Codium fragile (Sur.) Hariot subsp. *tomentosoides* (van Goor) Silva, n.comb.

(Text-figs. 2, 3)

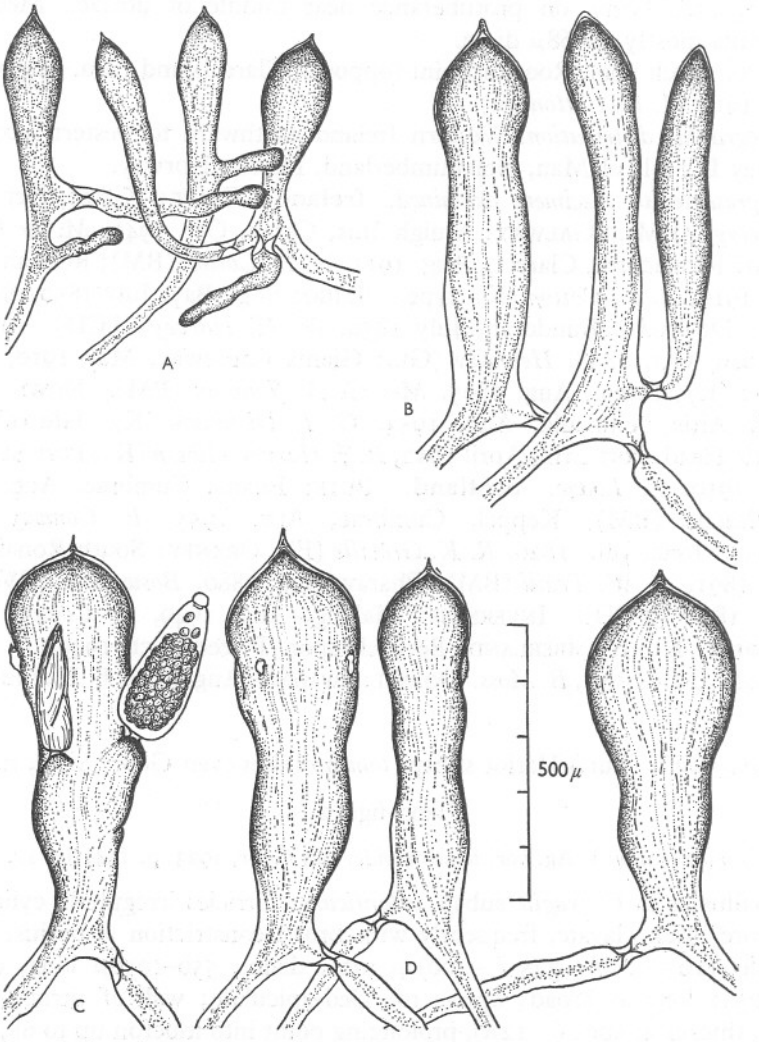
Codium mucronatum J. Ag. var. *tomentosoides* van Goor, 1923, p. 134, fig. 1*c*.

Thallus as in *C. fragile* subsp. *atlanticum.* Utricles irregularly cylindrical or more often clavate, frequently with broad constriction at or just below middle, (105–) 165–325 (–400) μ max. diam., 550–1050 μ long, usually 2·5–5·5 × long as broad; apices rounded-apiculate; wall of utricle *c.* 1·5 μ thick, thicker at apex (–12 μ), prolonging point into mucron up to 68 μ long. Hairs (or hair scars) common, one or two per utricle, borne 160–260 μ below apex. Gametangia ovoid, oblong, or fusiform, 72–92 μ diam., 260–330 μ long,

one or two per utricle, each borne on protuberance near middle of utricle (410–560 μ below apex.) Medullary filaments mostly 26–68 μ diam.

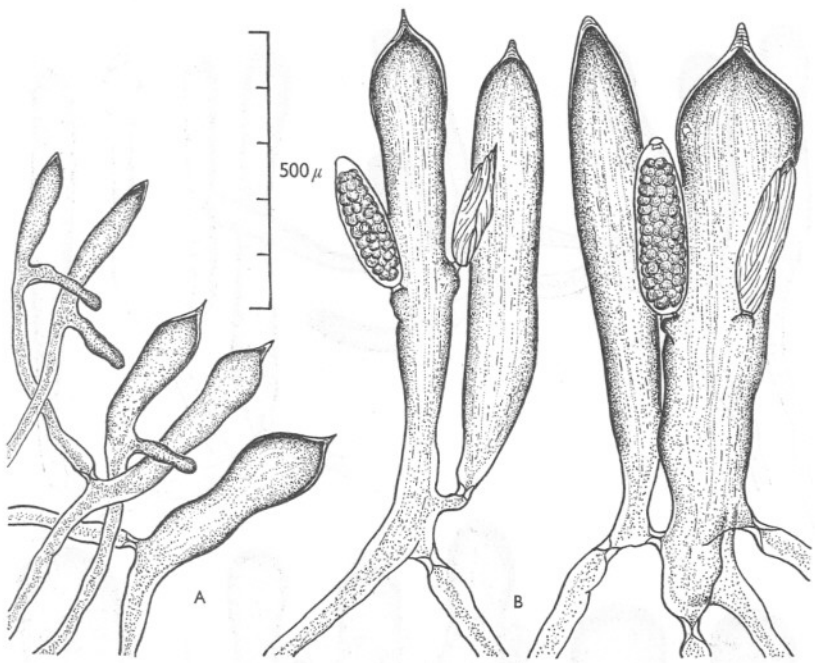
Type. Huisduinen, Netherlands, 1900, *Mrs J. L. Redeke-Hoek* (specimen not located). In the absence of the holotype, I have selected as neotype a specimen from Helder, Netherlands, annotated by van Goor, in the possession of Prof. J. Heimans, Hugo de Vries Laboratorium, Amsterdam.

Geographic distribution. Southern England, Scotland (Argyll), Ireland (Cork) France, Netherlands, Scandinavia.



Text-fig. 2. *Codium fragile* subsp. *tomentosoides.* A, utricles from tip; B, utricles from base; C, D, utricles from middle of frond. A, D, Steer Point, *Hunt*; B, C, Schouwen, Holland, Aug. 1938, *N. J. N. Kamp* (L).

Representative specimens examined. **England.** CORNWALL: Trevone, Aug. 1953, open pool in current, mid-littoral, *M. A. Wilson.* DEVON: Bantham, July 1953, edge of large deep pool, *M. A. Wilson*; Yealm Estuary, near Steer Point, Nov. 1939, on oyster, *O. D. Hunt* (BM). DORSET: Kimmeridge Ledges, Apr. 1955, *C. I. Dickinson* (K). SUSSEX: Elmer Sands, 3 miles E. of Bognor, Aug. 1954, cast up, *R. W. J. Keag* (K). **Scotland.** ARGYLL: Loch Sween, June 1953, *E. Conway* (GL). **Ireland.** CORK: Lough Ine, July 1950, *S. Lodge.*



Text-fig. 3. *Codium fragile* subsp. *tomentosoides.* Lough Ine, *Lodge.* A, utricles from tip; B, utricles from middle of frond.

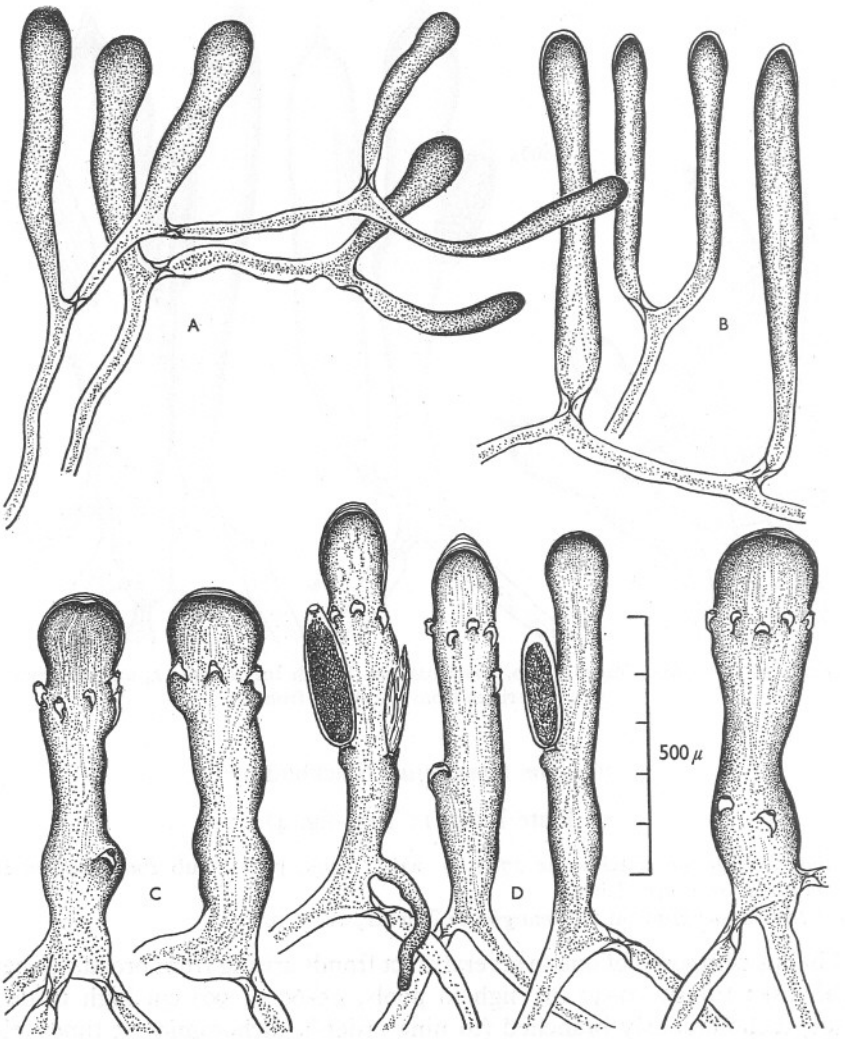
Codium tomentosum Stackhouse

(Plate I, fig. 1; Text-fig. 4)

Codium tomentosum Stackhouse 1797, p. xxiv. 1795, p. 21 (sub *Fucus tomentosus* Hudson excl. syn. Linn.).
Fucus tomentosus Hudson var. *marginifer* Turner, 1811, p. 1.

Thallus composed of one to several erect fronds arising from broad, spongy, basal disk; fronds 20–40 cm high in pools, 45–60 (–90) cm high in deep water, dichotomously branched (to nine orders), dichotomies at times telescoped to give subfastigiate appearance, at other times distant (internodes

7–8 cm long), rarely proliferous; branches mostly terete but frequently flattened at dichotomies, 3–5 mm diam. in lower parts, to 8 mm at dichotomies, attenuating to 1·5–2 mm at apices, terminal segments often long (5–11 cm). Utricles cylindrical or slightly clavate, often with hip-like enlargement near base, (65 –) 100–200 (– 275) μ max. diam., (420 –) 500–800 (– 1050) μ long, (3 –) 4–7 (– 9) × long as broad; apices rounded or tending to be pointed; apical wall usually moderately thickened (– 44 μ), lamellate, at times introrsely umbonate and/or extrorsely bluntly pointed. Hairs (or hair



Text-fig. 4. *Codium tomentosum*. A, utricles from tip; B, utricles from base; C, D, utricles from middle of frond. A–C, Carna, *de Valéra*; D, Wembury, *Ryder*.

scars) common, numerous, borne at conspicuous distance below apex (130–235μ), forming collar below broadest part of utricle. Gametangia ovoid, oblong, or fusiform, 50–110μ diam., 165–340μ long, one to four per utricle, each borne on prominent protuberance one-half to two-thirds distance below apex. Medullary filaments mostly 20–50μ diam.

Type. 'On the Devonshire and Cornwall Coasts: on the Long Rock between Marazion and Pensance[1] plentiful. Hon. Mr. Wenman' (specimen not located). In the absence of the holotype, I have selected as neotype a specimen in the Linnean Society collected at Acton Castle, presumably by Stackhouse.

Geographic distribution. Western Ireland, Orkney Islands, southern England, Channel Islands, Netherlands southward to Morocco, Azores Islands, Algeria.

Representative specimens examined. **Ireland.** CORK: Bantry Bay, July 1808, *Miss Hutchins* (K) (type of *Fucus tomentosus* var. *marginifer* Turner). CLARE: Kilkee, July 1833, *W. H. Harvey* (TCD, K, NY). GALWAY: Muigh Inis, Carna, Oct. 1943, *M. de Valéra*; Renvyle Point, Jan. 1831, *R. J. Shuttleworth* (BM). MAYO: Clare Island, Aug. 1911, *A. D. Cotton* (BM, K, NY). SLIGO: Sligo Bay, July 1850, *Hennedy* (GL). DONEGAL: Malin Head, July 1897, *M. R. Clarke* (FH, NY). ANTRIM: Ballycastle, 1797–98, *R. Brown* (BM). **Scotland.** ORKNEY ISLANDS, *J. H. Pollexfen* (BM). **England.** CORNWALL: St Mary's, Scilly Islands, Sept. 1953, *E. Conway* (GL); Acton Castle, *Stackhouse* (LINN) (neotype); King's Cove, June 1799, *D. Turner* (BM, K); Mullion Cove, Jan. 1932, *Lyle* (BM); Gerrans Bay, *E. M. Holmes* (BM); West Looe, Nov. 1952, *M. Parke* (PL). DEVON: Wembury, Oct. 1953, *F. G. C. Ryder* (PL, UC); Salcombe, May 1953, *D. Ballantine* (PL); Torquay, Wyatt's *Algae Danmonienses* no. 35 (BM, E, K, L, NY, TCD, UC); Exmouth, 1864, *Mrs C. A. Johnson* (BM). DORSET: Weymouth, Sept. 1892, *E. Batters* (BM); Swanage, Aug. 1894, *E. Batters* (BM). SUSSEX: Brighton, July 1889, *Miss Palmer* (BM). **Channel Islands.** Guernsey, Aug. 1840, *Lady Mansell* (BM).

A plant of *C. tomentosum* in which the dichotomous branches bear numerous simple and forked proliferations was described by Turner as a variety (*marginifer*) of *Fucus tomentosus*. This proliferous condition is rare in *Codium tomentosum* compared to *C. vermilara*.

Codium vermilara (Olivi) Delle Chiaje

(Plate I, fig. 2; Text-fig. 5)

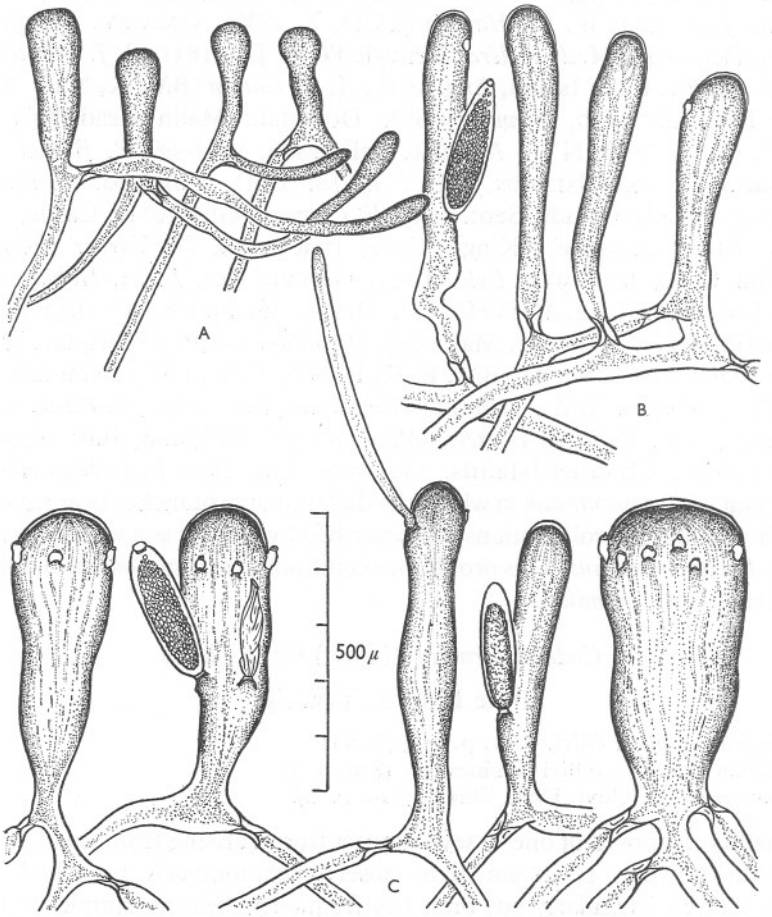
Lamarckia vermilara Olivi, 1792, p. 258, pl. VII.
Myrsidrum vermilara (Olivi) Rafinesque, 1810, p. 98.
Codium vermilara (Olivi) Delle Chiaje, 1829, p. 14.

Thallus composed of one to several erect fronds arising from small, spongy, basal disk; fronds 10–45 cm high, closely dichotomously branched (to 10 orders), often irregularly so, branches frequently bearing simple or forked proliferations; branches terete, tapering from *c.* 4 mm at base to 1·5–2 mm

[1] Old spelling of Penzance (ED.).

at tips, terminal segments usually short. Utricles clavate to pyriform, (65–) 90–240 (–340) μ diam., (330–) 400–650 (–1000) μ long, 1·5–4·5 (–7) × long as broad; apices rounded; apical wall slightly to moderately thickened, 5 to 15 (–50) μ, lamellate. Hairs (or hair scars) common, numerous, borne on shoulder of utricle 60–110 μ below apex. Gametangia oblong to fusiform, (52–) 60–80 (–96) μ diam., 200–290 μ long, one to four per utricle, each borne on protuberance one-half to two-thirds distance below apex. Medullary filaments mostly 18–45 μ diam.

Type. No specimens mentioned in original description. I have selected as neotype a specimen in the Herbarium of the University of California at Berkeley distributed as Flora Exsiccata Austro-Hungarica no. 794 under the name *C. tomentosum*, collected by Zay at Portoré, Croatia.



Text-fig. 5. *Codium vermilara*. Wembury, *Ryder*. A, utricles from tip; B, utricles from base; C, utricles from middle of frond.

Geographic distribution. Ireland (Galway, Antrim), Scotland (Argyll), southern England, Channel Islands, Norway, France southward to Morocco, Mediterranean, including Adriatic.

Representative specimens examined. **Ireland.** GALWAY: Galway, *McCalla* (TCD). ANTRIM: Portrush, 1835, *D. Moore* (K, TCD); Cushendall, *W. H. Harvey* (BM). **Scotland.** ARGYLL: Portnahaven, Islay, 1826, *J. Chalmers* (BM, GL, K). **England.** DEVON (north coast): Ilfracombe, Oct. 1807, *R. Hare* (BM). CORNWALL: Penzance, *M. P. Merrifield* (BM); St Michael's Mount, June 1799, *D. Turner* (K); Acton Castle, June 1798, *Stackhouse* (LINN); Falmouth, *F. W. Smith* (BM); Gerrans Bay, *E. M. Holmes* (BM). DEVON (south coast): Wembury, Oct. 1953, *F. G. C. Ryder* (PL, UC), Salcombe, July 1941, *M. Parke* (PL); Harbrick, Torbay, March 1833, *Mrs Griffiths* (BM); Torquay, *Lyon* (BM); Exmouth, *Miss Baker* (K); Sidmouth, Nov. 1829 (TCD). DORSET: Weymouth, Dec. 1911, *A. D. Cotton* (K); Swanage, *E. M. Holmes* (US). HANTS: West Cowes, Isle of Wight, *Count Pouliatine* (BM). **Channel Islands**: Jersey, *F. P. Girdlestone* (MICH).

DISCUSSION

The presence in the British Isles of a *Codium* with mucronate utricles was first demonstrated by Cotton (1912), who described *C. mucronatum* var. *atlanticum*. This entity is herein referred to *C. fragile* in accordance with the general agreement that the latter is conspecific with *C. mucronatum*. Taxonomic treatment of *C. fragile* is complicated by the following factors: (i) the species in the broadest sense may be considered a complex assemblage of populations and series of populations, some of which are morphologically homogeneous and others heterogeneous; (ii) evidence is present for hybridization or gene flow between plateaus of morphological uniformity; (iii) the species has by far the most extensive geographic distribution of any *Codium*, occurring on all continents; (iv) unusual physiological adaptability is implied by the variety of habitats, from the sublittoral to the midlittoral, from surf-swept rocks to quiet shell-mud flats; (v) the species has the vigour and adaptability of a weed and is spreading rapidly in such places as Scandinavia, France and Spain. A complete consideration of *C. fragile* will be presented in a forthcoming monograph of the genus. For the present account it is sufficient to state that in the British Isles there are two series of populations of mucronate *Codium*, each relatively uniform morphologically, which I consider best treated as subspecies of *C. fragile*.

The present centres of distribution of *C. fragile* are the Pacific and Subantarctic oceans. By far the greatest morphological heterogeneity occurs in Japan. In fact, almost all subspecies (geographically discrete series of morphologically uniform populations) can be matched by one or more Japanese collections. It is possible that *C. fragile* subsp. *atlanticum* was introduced

from the Pacific within historical times. According to Cotton (1912, p. 116) it was collected at Bantry Bay by Miss Hutchins (*c.* 1808?). Another early collection is one made by Greville on the island of Iona in 1826. Chalmers (*Algae Scoticae Exsicc.* no. 13, 1826) stated that *Codium* was unknown in Scotland before 1825, when it was discovered on Iona by Berkeley. This subspecies seems to be spreading: Cotton (p. 119) noted that it probably was established on the Isle of Man in 1900, and the presently cited Northumberland and Swanage collections are the first published English records. (The Swanage record seems out of range and some doubt is thus raised as to the correctness of the locality.) The earliest known collection from Norway is dated 1918 (Hussay near Molde, *N. Wille*, S).

Codium fragile subsp. *tomentosoides* seems definitely of very recent occurrence in Europe. It was first collected in Holland in 1900 (van Goor, 1923, p. 133), Denmark in 1920 (Rosenvinge, 1920, p. 131), Sweden in 1938 (Brandholmen near Långö, Bohuslän, *C. Engblad*, S), Norway in 1952 (Bratvaer near Kristiansund, *H. Printz*, UC), Atlantic France in 1946 (Iles de Glénan and Concarneau, *R. Lami*, PC), and England apparently in 1939 (Yealm Estuary, at Steer Point, S. DEVON, *O. D. Hunt*, BM). The place of origin seems most likely Japan.

These two subspecies of *C. fragile* are easily distinguishable anatomically. The apices of the clavate-cylindrical utricles of subsp. *atlanticum* are rounded, extrorsely umbonate, or provided with a very short, usually blunt mucron. The utricles of subsp. *tomentosoides* tend to be shorter and stouter, have a pronounced median or submedian constriction, and are sharply mucronate.

Subsequent to Cotton's demonstration of *C. mucronatum* (*C. fragile*) in the British Isles, the name *C. tomentosum* remained to encompass the non-mucronate dichotomous forms. That two distinct species are involved has not been recognized heretofore.

Codium tomentosum and *C. vermilara* differ sufficiently in habit to be distinguishable in the field with a little practice. In *C. tomentosum* branching is usually regularly dichotomous and the branches are often flattened at the dichotomies; the base of the thallus is thick and terminal segments are often long. In *C. vermilara* branching is usually somewhat irregular, often proliferous, at times divaricate, and the branches are strictly terete; the base of the thallus is not noticeably thick and terminal segments are usually short. *C. tomentosum* is often more robust and more distantly branched than *C. vermilara*. Herbarium specimens are not so readily distinguishable on the basis of habit.

Anatomically the two species are unmistakable. The position of hair scars is significant: in *C. tomentosum* they are well below the apex and at a constriction, so that the utricle expands above them; in *C. vermilara* they are usually high on the shoulders of the utricle, or if lower, never at a constriction, so that the utricle tapers rather than expands immediately above them. The average

length/breadth ratio is greater in *C. tomentosum* than in *C. vermilara*. Thickened apices are common in both species: in *C. tomentosum* a blunt point is frequently formed while in *C. vermilara* the thickening is more uniform. The cylindrical or narrowly clavate utricles of *C. tomentosum* are in contrast to the clavate or pyriform utricles common in *C. vermilara*.

C. vermilara is common throughout the Mediterranean (including the Adriatic). In the Atlantic it extends from Morocco to France and the British Isles. Aalesund and Kristiansund in Norway constitute the most northerly records. *C. tomentosum*, on the other hand, is primarily an Atlantic species, occurring from the British Isles south along France, Spain, and Portugal to Morocco. It occurs in the Mediterranean only along the Algerian coast. Heretofore, *C. tomentosum* has been credited with cosmopolitan distribution, but careful study reveals that more than thirty distinct species have been confused under this name.

Separating the two non-mucronate species in the British Isles was much easier than deciding their correct names. Application of the type method was made difficult by the absence of holotypes. The earliest name applicable to a dichotomous *Codium* would seem to be Hudson's *Spongia dichotoma* (1762, p. 489) or its isonym *Fucus tomentosus* (1778, p. 584). However, as Linda Newton (1953, p. 403) has explained, Hudson's binomials must be considered illegitimate synonyms of *F. elongatus* L. (the type of which is referable to *Himanthalia*), which Hudson cited as a synonym. This unfortunate circumstance resulted from the erroneous citation by Linnaeus of Morison's *Fucus spongiosus teres ramosior viridis erectis* (1699, p. 647, sect. 15, pl. 8, fig. 7), which misled Hudson to cite the Linnaean binomial. Miss Newton concluded, as I have concluded, that *Codium tomentosum* Stackhouse (1797) may be treated as a new name (rather than a transfer of *Fucus tomentosus* Hudson) in accordance with Art. 81 of the International Code of Botanical Nomenclature, inasmuch as Stackhouse explicitly excluded *Fucus elongatus* L. from his concept of *Codium tomentosum*.

In determining the application of the name *Codium tomentosum* Stackhouse, one is faced with the problem that Stackhouse had both non-mucronate species in hand, as shown by specimens in the Linnean Society. His description fits either species; the plant figured on Plate VII of his 'Nereis' is very likely *C. vermilara*. On the basis of the following considerations, however, it seems advisable to fix the application of *C. tomentosum* as delimited herein: (i) *C. tomentosum*, rather than *C. vermilara*, was the basis of treatments given by Turner (1811, p. 1, pl. 135), Harvey (1846, pl. XCIII), and Cotton (1912, p. 113, pl. VIII, figs. 1, 2); (ii) shifting the name *C. tomentosum* to *C. vermilara* would leave *C. tomentosum* as circumscribed herein without a name.

It is of historical interest to determine the identity of Morison's, Ray's and Hudson's plants. The two specimens in Herb. Morison (OXF), representing *Fucus spongiosus teres ramosior*...and *Fucus spongiosus teres viridis*...(1699

p. 647, nos. 7, 8), are both referable to *C. tomentosum*. The *Spongia dichotomos compressa, ex viridi splendens* (Ray, 1724, p. 29, no. 4) in Herb. Sherard (OXF) is also representative of *C. tomentosum*. Hudson had both species in hand, judging from specimens at Kew, the British Museum, and Edinburgh.

Codium vermilara, occurring as it does in the Mediterranean, might be expected to have been described early. Although no authentic specimens have been located, the identity of Olivi's *Lamarckia vermilara* (1792), to judge from the description and figure, seems certain. While the lack of any reference to Olivi raises doubt that *Codium vermilara* Delle Chiaje (1829) should be considered a new combination, closer study of the rare Delle Chiaje work answers the question. It is clear from Delle Chiaje's choice of epithets in *Codium* that he was following Rafinesque (1810, p. 97), who in turn cited Olivi. Thus it seems proper to use the binomial *C. vermilara* (Olivi) Delle Chiaje.

SUMMARY

The dichotomous species of *Codium* in the British Isles are described, compared, and illustrated. These include *C. fragile* subsp. *atlanticum*, *C. fragile* subsp. *tomentosoides*, *C. tomentosum* and *C. vermilara*.

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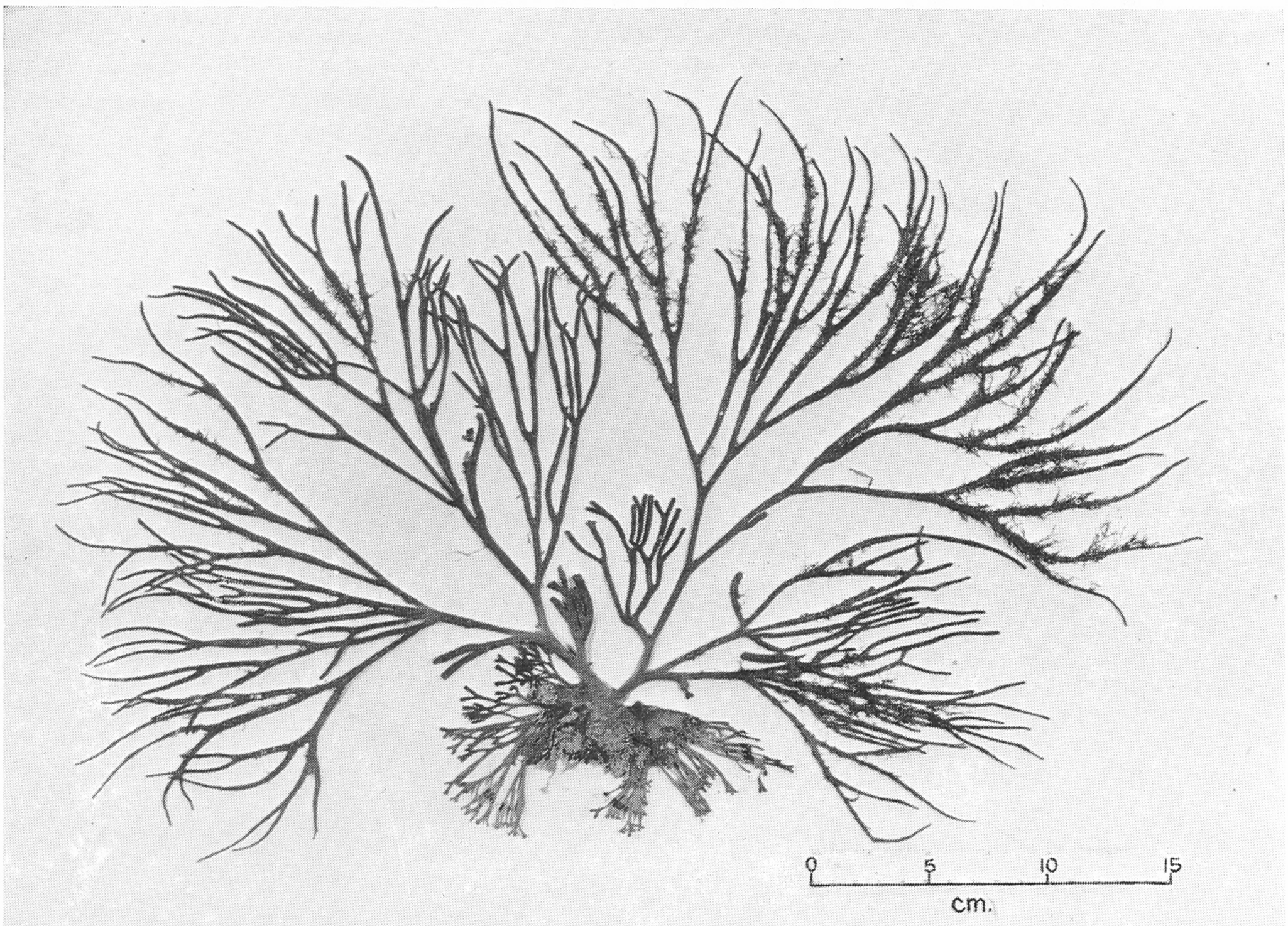

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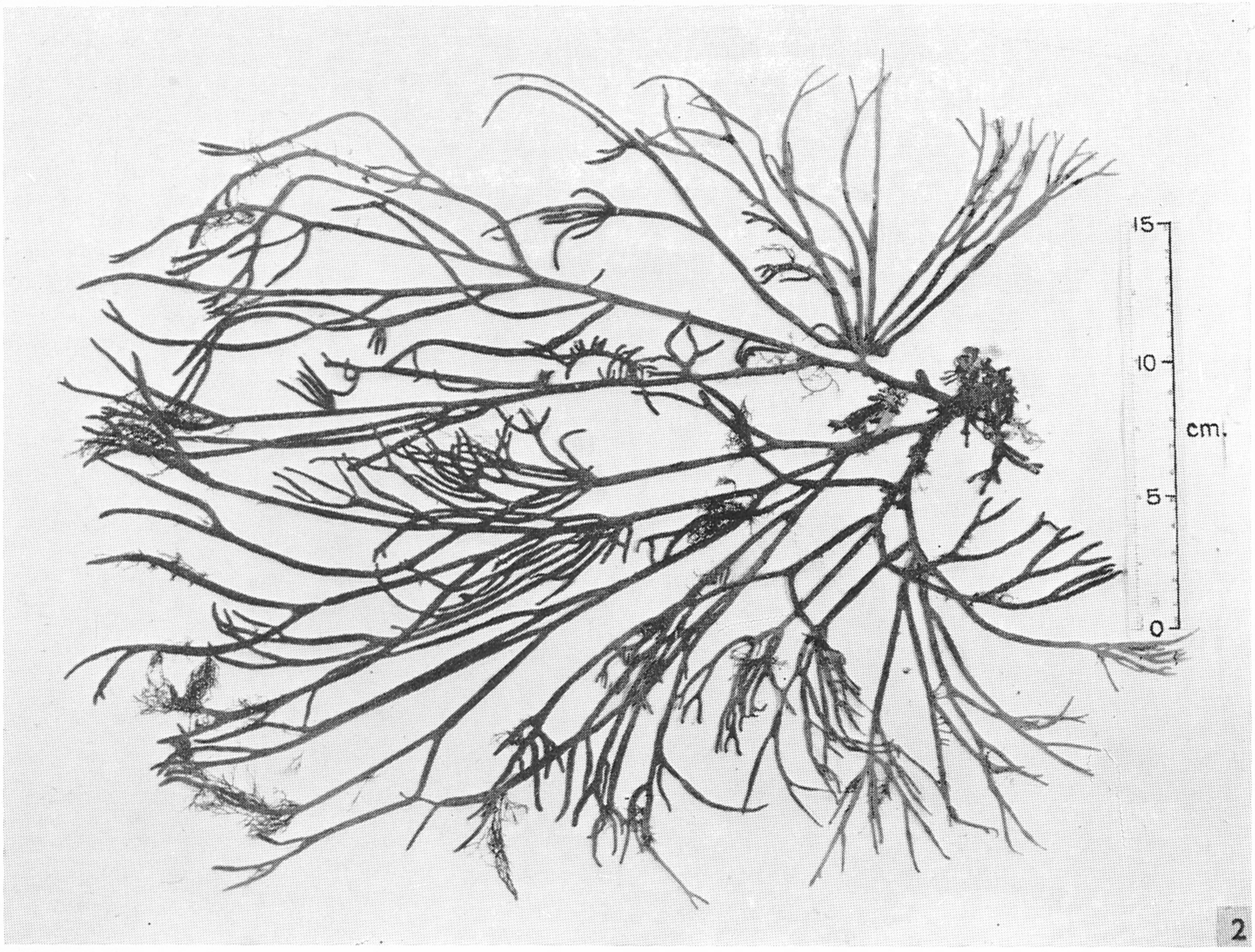

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EXPLANATION OF PLATE I

Fig. 1. *Codium tomentosum*. Small deep pool, Church Reef, Wembury, Devon, Oct. 1953, *F. G. C. Ryder* (UC).
Fig. 2. *Codium vermilara*. Large pool, mid-littoral, Church Reef, Wembury, Devon, Oct. 1953, *F. G. C. Ryder* (UC).

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STUDIES ON MARINE FLAGELLATES

II. THREE NEW SPECIES OF *CHRYSOCHROMULINA*

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The Plymouth Laboratory
Irene Manton and B. Clarke
Botany Department, Leeds University

(With total of 81 Figures in text and on Plates I–IX)

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INTRODUCTION

The three new species to be described below are the first of a considerable series of related forms, recently isolated from marine plankton off the British coasts and possessing in common a number of rather unusual characters which make their classification difficult. They are all very small and extremely fragile unicellular flagellates, with the characteristic golden brown plastid colour and metabolic products found in the Chrysophyceae to which they undoubtedly belong. Within this group their placing is more difficult and it cannot even be adequately discussed until the whole range of new forms is available for comparison. The most that we can do on this occasion is to make some provisional generic comparisons in order to establish a system of nomenclature. Unfortunately, existing descriptions of relevant genera are both scanty and incomplete, a defect which is easily explained by the nature of the organisms and the need for special facilities for their adequate study. The generic identification which we propose to adopt is therefore provisional to the extent that the little known genus *Chrysochromulina*, into which we believe our organisms can be placed, is founded on one freshwater species which is described in terms which will need substantial revision if this identification is to be accepted. Generic revision must, however, wait until the whole range

of forms has been described, and we must therefore limit the present discussion to our reasons for the choice of the name.

These reasons are essentially that our forms and *Chrysochromulina* agree in possessing three rather long filiform appendages, two of which are flagella. In the only other Chrysophycean genera for which three appendages have been recorded, namely *Prymnesium* and *Platychrysis*, the third appendage is so short as scarcely to justify the epithet filiform, and it differs in other ways (to be discussed on a later occasion) which do not suggest identity. The third appendage in the only species of *Chrysochromulina* (*C. parva* Lackey), on the other hand, was described as a third flagellum (Lackey, 1939). This attribution is not borne out either by the description of its behaviour or by the figure which accompanies the description. Both resemble those of our organism sufficiently to make it virtually certain that the third organ in *C. parva*, as in our organism, is not a flagellum but an organ of very different properties and use.

In our organisms the third appendage is a retractile organ with an adhesive tip, in some species (though not in those to be described here) extensible to many times the length of the flagella and in all used for temporary anchorage of the cell to a solid surface, though this may perhaps not be its sole function. This power of anchorage is apparently correlated (though in ways which are not yet fully understood) with the practice of phagotrophic feeding, floating particles of various sizes being engulfed through the end of the cell away from the attachment of the appendages. This notwithstanding, the organisms are capable of photosynthesis and under normal conditions require light; their dependence on solid food for certain nutrients, however, explains the difficulty encountered in making unialgal cultures bacteria-free.

In addition to the various characteristics associated with the third appendage, all our species show a covering of scales when seen under the electron microscope; such scales being apparently of an organic nature since we can find no sign of mineral impregnation either calcareous or siliceous. They are of very varied sizes and shapes and in themselves they would provide most excellent diagnostic characters not only for the delimitation of species but also in all probability for the separation of several genera each with several species in our range of material alone. Unfortunately their small size, which makes the scales invisible with the light microscope in almost every species, would lead to great difficulties of identification of such genera by the ordinary student of plankton. While therefore we cannot avoid mentioning such characters—even if this introduces some doubt about specific identifications by a light microscopist—we do not propose to found genera on characters which cannot be seen with the light microscope. We therefore propose to describe most of our species under the one genus *Chrysochromulina*, and to amend the generic diagnosis to incorporate the new knowledge at the end of the series.

Since, however, it is obvious that an essential part of any generic diagnosis must involve a description of the third filiform appendage formerly referred to

as a third flagellum, it will be convenient if we give it at once some name less misleading than flagellum and less cumbersome than 'third filiform appendage'. We therefore propose to call it a *haptonema* (Gr. ἅπτω, to attach, and νῆμα, pl. νῆματα, a thread) to give it a term of its own without reference to other morphological categories. The full description of its structure and properties will then emerge as the species are themselves described and further investigated.

Materials and Methods

The establishment of unialgal cultures has been an essential pre-requisite to the effective study of the structure and behaviour of these organisms. The methods of isolation have been in no way peculiar and the cultures so established have been successfully maintained for several years at Plymouth in Erdschreiber culture solution in a culture room kept at about 15° C. and illuminated with fluorescent lighting to supplement limited daylight from a north window.

For studying the behaviour of the living cells, especially the motion of their various appendages, the use of the modified dark ground condenser of Cooke, Troughton and Simms, illuminated by a Pointolite lamp, has been of the very greatest help. Unfortunately photography at high magnifications is not possible with this system without a more powerful source of light than has been available to us. It is, however, the best means of demonstrating the haptonema to a visual observer.

Without dark ground it is possible, if the cells are not moving rapidly, to see the appendages quite well under an oil immersion objective or a good dry lens without special lighting, but recording them photographically by this means is extremely difficult (see, however, Fig. 24, Pl. I; Fig. 55, Pl. V; and Fig. 77, Pl. VIII). They can also be picked out by phase-contrast or ultraviolet photography, but in the smallest organisms such as those to be described here the optical interference caused by the body may be a serious difficulty. It was therefore found by experiment that the best way of recording these structures is to dry the cells down on to a glass slide after preliminary fixation with either osmic vapour or other simple killing agent such as iodine in KI. It is sometimes an advantage to precede killing by narcotizing with magnesium chloride to prevent retraction of the haptonema. After the preparation has been dried, it must be freed from the salt crystals by immersion in distilled water, followed by a second drying. This part of the process does not appear to disturb the attached cells which, after removal of the salt which otherwise will deliquesce, can be kept indefinitely in the dry condition. The appendages can then be seen even with a relatively low-power dry lens and for photographic purposes the fact that they are spread on a flat surface is a great advantage. They must, however, be examined dry, and so the use of high resolution lenses is precluded.

Formalin fixation, as commonly used for plankton studies, is quite inappropriate for these organisms. The appendages are invariably lost, the cell bodies become bloated, collapsed or otherwise misshapen and the chromatophores are indistinguishable. The organisms are then unrecognizable.

For investigating the feeding habits, and in particular for demonstrating the ingestion of solid food, it is sufficient to supply the cultures with suitable coloured particles and to leave them overnight undisturbed. By next morning the ingested particles can be detected inside the cells and recorded photographically. Various pigments such as carmine, or cultures of small coloured micro-organisms, have been used for this purpose successfully, but the most satisfactory both for its apparent palatability to almost all the organisms and also for purposes of photographic record have been the densely black particles of fine graphite sold under the name of 'Aquadag'. It is desirable to wash this several times in culture fluid before use to avoid risk of chemical contamination, after which it is readily ingested and very clearly visible.

The application of intravitam stains, notably cresyl blue, is a help for demonstrating the presence, though not the details, of the surface covering of scales and of the ejectile organelles which will be mentioned in the descriptions. The scales appear as a pale rose-violet line which could not be distinguished optically from a simple membrane.

For the electron microscopy the methods used have been those previously applied to other types of motile cells (see Manton & Clarke, 1951, *et seq.*) with the sole modification that the present material is considerably more sensitive than is usual and fixation times have to be shorter. It is sufficient to expose the live cells to the vapour from 2% osmium tetroxide solution for 5 sec to cause death and this is sufficient treatment for direct preparations killed on a formvar-coated carrier. After killing they are dried, and eventually shadowcast with gold-palladium.

Direct preparations of this kind are usually to be preferred for revealing the details of scales, but for the general form of the cell with its various appendages suitably spread it is often better to strip from glass as described in Manton, Clarke, Greenwood & Flint, 1952. This procedure makes possible a more extensive examination of alternative specimens before selecting a favourable cell for detailed study. Each of these methods will be represented in the plates.

In assembling our observations for publication we have attempted to obtain pictorial demonstration of all features which lend themselves to this treatment using photography where possible. For each species we have included photographs and drawings at a standard magnification with the light microscope, since such views are indispensable for the ordinary purposes of identification. For the relative lengths of the appendages with respect to the body size some verification is possible on the photographs, more especially of undried material,

though the figures quoted were actually obtained from living material and from cells recently killed with osmic vapour, in each species more than 100 individuals having been measured. For the type of detail which can only be seen with the electron microscope some adjustment of magnifications is necessary in relation to the actual size of the organisms in order to fit the micrographs on to a limited page area. This may obscure the relative differences of size which exist between the species, though these can at once be apprehended from the visual and low-power views assembled on the first plate of each species. It should also perhaps be pointed out that at high magnifications the performance of the electron microscope is liable to slight fluctuations and the values given on the remaining plates are therefore only approximate.

The electron microscope used has been the Philips instrument in the Leeds University Botany Department, except for Fig. 61, Pl. VI, which was taken on a similar instrument in the research laboratory of Messrs Tootal Broadhurst Lee Co. Ltd of Manchester, whom we wish to thank for giving one of us temporary facilities for working there as a guest.

In the descriptive part of the text, for convenience, the detailed account of each species is preceded by a formal diagnosis in English and Latin. The subsequent descriptions can then be abbreviated to comparative notes to incorporate the Figure and Plate references. The first species will be described the most fully and reference to it must be made for certain details common to all three species.

We wish to thank the following for special help: Dr J. E. Morton of Queen Mary College for translating the English diagnoses into Latin, Dr L. H. N. Cooper of the Plymouth Laboratory and Messrs D. Vaux and A. C. Burd of the Ministry of Agriculture and Fisheries Laboratory, Lowestoft, for bringing in the samples of sea water from which these organisms have been isolated, and Miss D. Ballantine for testing their possible toxicity to fish.

Specific Descriptions

Chrysochromulina kappa n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal, showing some metaboly, 4·5–6·5 (exceptionally 4–10·5) μ in diameter. Two flagella and one haptonema[1] arising close together at one pole; the flagella equal, homodynamic, $1\frac{1}{2}$–$2\frac{1}{2}$ times the cell diameter in length, smooth, shortly mucronate at the tip under the electron microscope; the haptonema a little longer than the flagella when fully extended, with a basal swelling, a club-shaped tip and a translucent sheath visible only with the electron microscope. The periplast covered by very thin transparent sculptured scales visible only with the electron microscope, on any one cell ranging

[1] For derivation and use of this term see p. 581.

in shape from oval to polygonal and in size from $0{\cdot}3 \times 0{\cdot}4\mu$ to $0{\cdot}5 \times 0{\cdot}8\mu$ with, at the flagellar pole, a few scales bearing a soft central spine.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, sometimes 1 or none, golden brown; in cells of the motile phase usually saucer-shaped, parietal, with a single globular body (pyrenoid?) placed centrally on the internal face of each; in cells of the non-motile phase deeply lobed or stellate. Oil and leucosin produced. Ejectile muciferous bodies localized in the cytoplasm mainly at the non-flagellar pole. Nutrition holophytic and/or phagotrophic. Not toxic to fish.

In the motile phase asexual reproduction of pigmented and non-pigmented cells by fission into 2, 3 or 4 daughter-cells. In the non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce 4 daughter-cells with walls; motile phase probably liberated from walled daughter-cells through a pore.

Habitat: the sea off Port Erin, Isle of Man (15 June 1939, type culture) and off the south coast of England at position Lat. N. 49° 51′, Long. W. 04° 00′ (8 April 1950) at 10 m. and Lat. N. 49° 21′, Long. W. 04° 54′ (9 May 1950) at surface. Type culture (Plymouth collection 'K') deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili sphaeroidali, paululum formam mutanti, $4{\cdot}5\mu$ ad $6{\cdot}5\mu$ (rare 4 ad $10{\cdot}5\mu$) latitudine; duobus flagellis et unico haptonemate (vide p. 581) conjunctim exorientibus altero axi; flagellis similibus, homodynamicis, longioribus $1\frac{1}{2}$ ad $2\frac{1}{2}$

Legends to Text-figs. 1–12

Chrysochromulina kappa n.sp. (× 5000)

Fig. 1. Individual swimming with flagella and haptonema behind body in the position characteristic for the species during rapid swimming. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scales; *ss*, spined scales.

Fig. 2. Individual anchored by haptonema which is scarcely extended; cell containing recently ingested graphite particles (*g*).

Fig. 3. Anchored cell with haptonema partly extended; cell ejecting large mass of graphite.

Fig. 4. Swimming cell containing large mass of graphite.

Fig. 5. Individual swimming with flagella and haptonema in front of body, haptonema fully extended; graphite particles being ingested.

Fig. 6. Swimming individual lacking chromatophores; an ingested bacterium at non-flagellar pole.

Fig. 7. Early fission stage: 4 flagella, 1 haptonema, 4 pyrenoid-like bodies, chromatophores dividing.

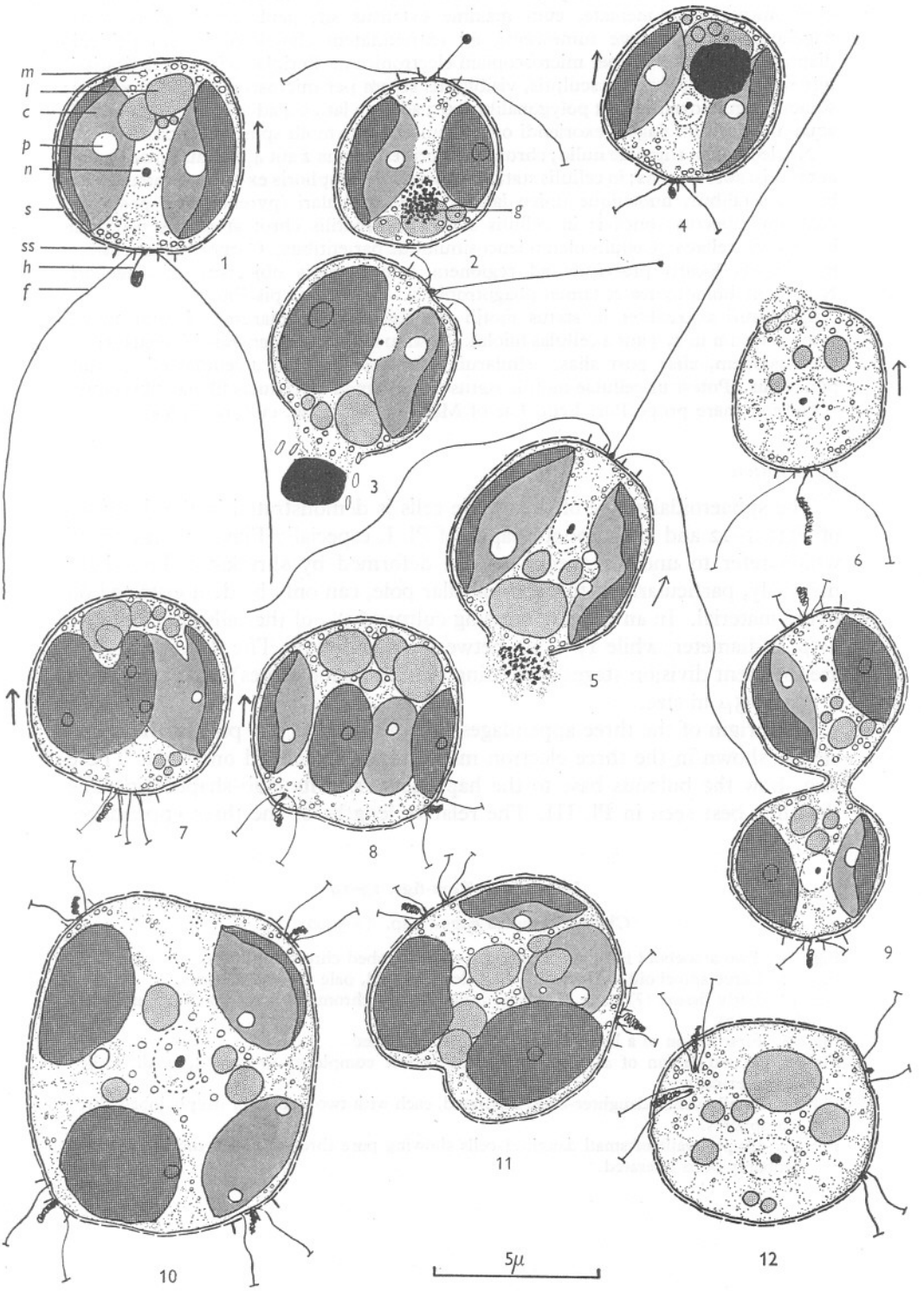
Fig. 8. Fission stage: flagella separating, 2nd haptonema formed, 4 chromatophores.

Fig. 9. Late fission stage just before separation of daughter-cells.

Fig. 10. Stage with 4 pairs of flagella and 4 haptonemata before double-fission to give 4 daughter-cells.

Fig. 11. Early fission stage to give 3 daughter-cells.

Fig. 12. Colourless individual with 4 pairs of flagella and 4 haptonemata prior to double-fission.



Text-figs. 1–12.

cellulae latitudine, teretibus, breve mucronatis ut videantur per microscopiam electronicam; haptonemate, cum maxime extensus sit, paululum longiore quam flagellum, basaliter leve tumescenti, ad extremitatem clavuloso, induto pelliculo diaphano visibili solum per microscopiam electronicam. Periplasto induto delicatissimis squamis diaphanis et sculptis, visibilibus solum per microscopiam electronicam; squamis figura ovalibus aut polygonalibus, 0·3 ad 0·5 μ lat., 0·4 ad 0·8 μ long.; nonnullis squamis ad situm flagelli exoriendi ornatis unaecuique molli spinulo centrali.

Nucleo unico, stigmate nullo; chromatophoris ex norma 2 aut 4, nonnunquam unicis aut nullis, aureo-fulvis; in cellulis status motilis chromatophoris ex norma crateriformibis, parietalibus, unocuique unico largi corpore globulari (pyrenoidali?) posito ad centrum aspectus concavi; in cellulis status non-motilis chromatophoris profunde lobatis vel stellatis. Cellulis oleum leucosinumque parientibus. Corporibus ejectilibus muciferosis locatis praecipue ad regionem cytoplasmatis objectam axi flagellari. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in status motili per fissionem cellularum coloratarum vel incoloratarum in 2, 3 aut 4 cellulas filiolas. In statu non-motili generanti (asexualiter ?) per fissionem, aliae post alias, cellularum amoeboidalium ad 4 cellulas filiolas cum parietibus. Potest ut cellulae motilis status per foramen ex cellulas filiolas tiberentur.

Habitat mare prope Port Erin, Isle of Man (15 Jun. 1939, cultura typica).

Description

The sphaeroidal shape of the motile cells is demonstrated in the drawings of Figs. 1–12 and in the photographs of Pl. I, especially Figs. 22, and 24–26 which refer to undried specimens not deformed by shrinkage. The slight metaboly, particularly at the non-flagellar pole, can only be demonstrated on living material. In an actively growing culture 65% of the cells are from 5 to 6·5μ in diameter, while 17% are between 4·0 and 5·0μ. The remaining 18% are incipient division stages (including double-fission stages) and range from 6·5 to 10·5μ in size.

The origin of the three appendages near together at one pole of the cell is clearly shown in the three electron micrographs assembled on Pl. II. These also show the bulbous base to the haptonema and its club-shaped tip. The sheath is best seen in Pl. III. The relative lengths of the three appendages

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Legends to Text-figs. 13–19

Chrysochromulina kappa n.sp. (× 5000)

Fig. 13. Two amoeboid individuals showing slightly lobed chromatophores.

Fig. 14. Large amoeboid individual with 4 deeply lobed, pale chromatophores.

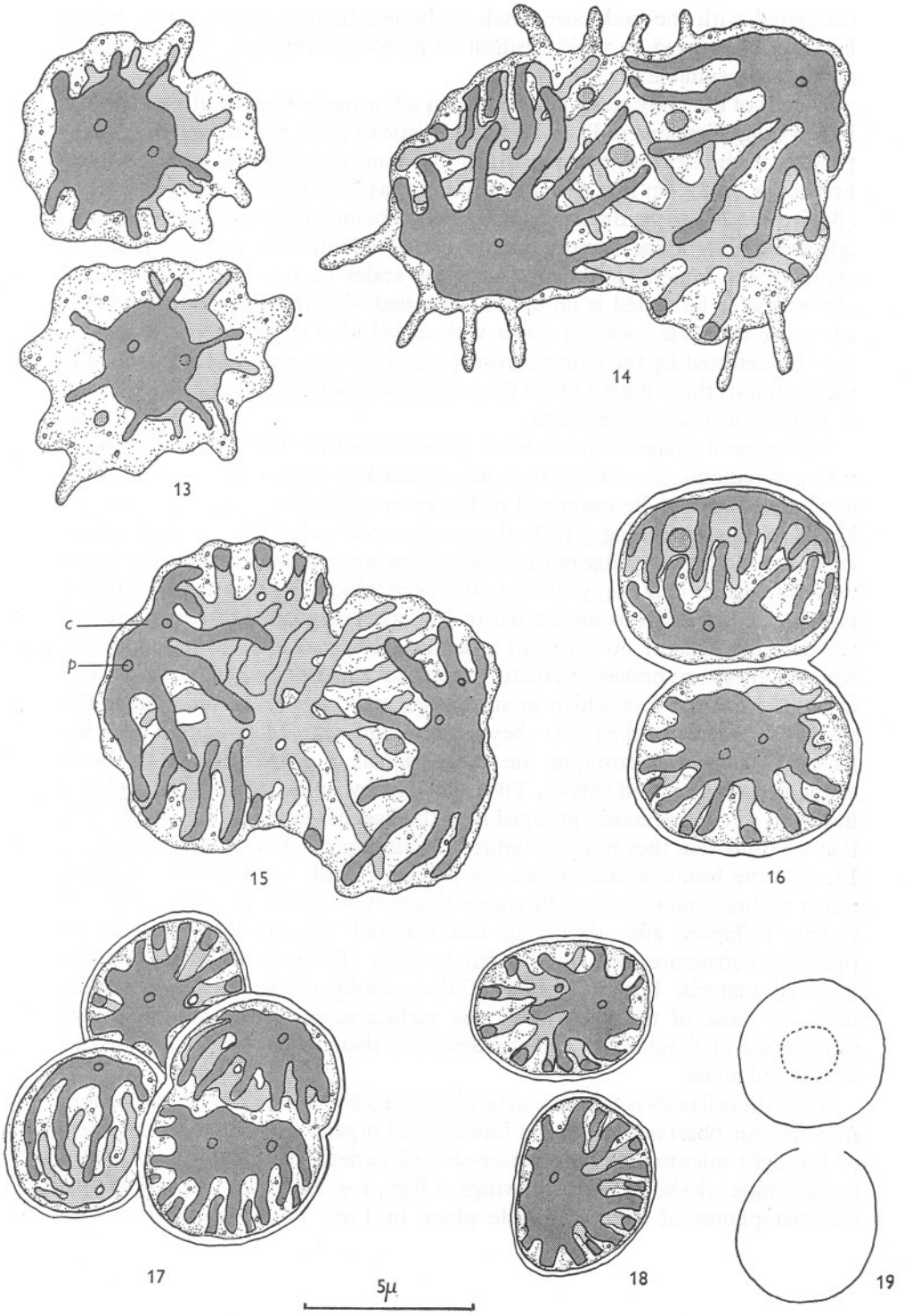
Fig. 15. Early fission (?) stage of a large individual: 4 chromatophores (*c*), 8 pyrenoid-like bodies (*p*).

Fig. 16. First fission of a large, walled cell just completed.

Fig. 17. Second fission of a large, walled cell almost completed to give 4, small, walled daughter-cells.

Fig. 18. Small, walled daughter-cells, separated, each with two stellate or deeply lobed chromatophores.

Fig. 19. Empty walls of small daughter-cells showing pore through which motile stage has probably been liberated.



Text-figs. 13–19.

compared with the body size needs to be ascertained on specimens which have not been dried, to avoid falsification by body shrinkage. Figs. 22 and 24 on Pl. I are suitable for this purpose.

Details of the body scales can be seen at a low magnification in Pl. II and at higher magnifications in Pl. IV. Characteristic of them is their extreme transparency to the electron beam in which they contrast markedly with the silicified scales of a form like *Synura* (Manton, 1955) or with calcified coccoliths (Braarud *et. al.*, 1952; Braarud, 1954), which are intensely opaque to electrons, appearing black in the electron microscope. Our failure to detect any chemical evidence for mineral impregnation of the scales in any species of *Chrysochromulina* so far tested is no doubt correlated with these optical properties, and since the scale covering stains with cresyl blue sufficiently strongly for it to be detected by the light microscope as a distinct rose-violet line outside the periplast, there must clearly be an organic basis to the scales which may in fact be their sole component.

The general shape of the scales is plate-like with a delicate surface sculpturing of radiating lines which become enhanced by shadow-casting and which may therefore best be examined in the reversed prints of Figs. 34 and 35, Pl. IV (compare with Fig. 33). In the smallest oval scales (Fig. 35) these ridges extend to the extreme edge of the scale producing a crenate margin; the larger polygonal scales (Fig. 34) possess a raised rim which obscures the ends of the ridges. Occasionally, as on the left of Fig. 34, polygonal rims can be found surrounding little or no traces of a central disk; it is not, however, known whether these are perhaps immature scales or whether the rim is in fact detachable from the disk which in such cases may have fallen out. Since the scales can only be studied after they have become detached from the body we have no means of determining the precise details of distribution on the body of the various sizes and shapes. The relatively few spined scales (Fig. 35) are, however, so conspicuously grouped near the flagellar pole (cf. Fig. 33, Pl. IV) that we think that they must be limited to this region. As may best be seen in Fig. 35, the heads of such scales are polygonal with a raised rim and faint radiating lines, though the four coarse ridges subtending the spine (which is usually collapsed after drying in this material) are possibly not entirely preformed structures but could partly be folds. They are invariably present in dried material but the details of their configuration are less constant than are those of the pattern of true surface sculpturing. The biological significance of these scales and the details of their modes of production are wholly unknown.

Since the cell body is too large to be penetrated by the electron beam in whole mounts, our observations on the internal cell organs are at present confined to the light microscope. The saucer-shaped parietal chromatophores of the motile phase are shown in the drawings of Figs. 1–5, and 7–11, with the stellate chromatophores of the non-motile phase in Figs. 13–18. Sometimes the

chromatophores of the motile stage appear striated, their number is usually two and we have never found more than four, the latter being in most cases early division stages. Cells with one chromatophore and division stages with three chromatophores are occasionally seen. Individuals which lack chromatophores are also occasionally seen, though they are always of small size, 4–4·5μ in diameter (Fig. 6).

A conspicuous refringent body, perhaps equivalent to the so-called pyrenoids of some other authors (see below), is situated near the centre of the inner face of each chromatophore (Figs. 1–5, 7–11). These bodies vary in size and sometimes appear to be surrounded by non-refringent material. They appear to divide at the same time as the chromatophores (Figs. 5, 7), but it is not known whether they are actually attached to the inner face of the chromatophore since they have sometimes been observed apparently lying away from it. They do not stain with acid fuchsin, iodine, neutral red or cresyl blue, but with Millon's reagent they become gold, pink or rose-red. The 'pyrenoids' of certain naviculoid diatoms were found to become orange-gold after treatment with Millon's reagent (Tschermak-Woess, 1953), and those of the coccolithophorid *Pontosphaera roscoffensis* showed a comparable negative reaction with other common reagents (Chadefaud & Feldmann, 1949). After osmic fixation these bodies in *Chrysochromulina kappa* become very conspicuous, sometimes appearing a vivid green. A similar behaviour has been found (Dr J. W. G. Lund personal communication) in the 'pyrenoids' of the chrysophycean *Apistonema pyrenigerum*.

The nucleus is sometimes visible in the living cells; it is of medium size and is placed centrally in the body, usually slightly nearer to the flagellar pole (Fig. 1). Vesicles of leucosin, varying greatly in number and size in different individuals, are present, usually towards the non-flagellar pole, but occasionally small leucosin vesicles are also present near the flagellar pole (Fig. 7). Small oil globules are distributed throughout the cytoplasm.

Very small refringent organelles, possibly corresponding to the muciferous bodies of some workers (Chadefaud, 1936), can be seen in the peripheral cytoplasm. No great number are present in cells of this species (Figs. 1–12), and they seem to be localized mainly near the non-flagellar pole, but small groups are also present round the flagellar pole (Figs. 1–6). Under bright illumination, or when very dilute cresyl blue is added to the live cells, these organelles shoot out their contents as small rods or batons if extruded quickly (Fig. 3) or, alternatively, their contents may ooze out as small balloons which can enlarge to equal the cell in diameter. Immediately before the rods are shot out, very small disks appear to be shot off in a manner reminiscent of the behaviour described by Hovasse (1949) for the discobolocysts of *Cyclonexis annularis*. In *Chrysochromulina*, however, it seems probable that these disks are surface scales which are removed as the organelles discharge their contents. The biological significance of these discharges is unknown.

Explanation of Plates I–IV

Chrysochromulina kappa n.sp.

I

Fig. 20. A cell killed with osmic vapour and dried on a glass slide. Photographed dry, without a coverslip. Magnification × 1000.

Fig. 21. Another cell of the same.

Fig. 22. A cell killed with osmic vapour and photographed in liquid culture medium under a sealed coverslip; the two flagella only but not the haptonema visible in the plane of focus chosen. Visual light, oil-immersion objective, magnification × 1000.

Fig. 23. A cell treated as for Fig. 20 but killed and dried on quartz before mounting in water with a trace of iodine in KI under a sealed quartz coverslip. High power U.V. photograph, glycerine-immersion lens, magnification × 3000.

Fig. 24. Parts of two undried cells from the preparation of Fig. 22 photographed with visual light 24 h after the preparation was set up. The protoplasm has darkened slightly in the dilute osmic solution resulting from vapour killing. The coiled haptonema and two flagella are visible for both cells though the body of the left-hand cell has broken away. Visual light, oil-immersion objective, magnification × 2000.

Fig. 25. An undried cell showing the effects of graphite feeding, otherwise like Fig. 22. A pellet of ingested graphite is in focus between the two plastids at the pole away from the flagella. Visual light, oil-immersion objective, magnification × 2000.

Fig. 26. Another cell of the same showing a larger pellet of ingested graphite.

Fig. 27. Low-power electron micrograph of a cell killed with osmic vapour and dried directly on the formvar film. Electron micrograph M. 113, 27 gold palladium shadowing, 60 kV, magnification × 3000.

II

Fig. 28. A cell killed with osmic vapour directly on the formvar film. The body, scales, coiled haptonema and the two flagella, are visible, with a bacterium and a *Caulobacter* type of organism touching the tip of the right-hand flagellum. Electron micrograph M 120·26, 60 kV, magnification × *c.* 6000.

Fig. 29. Another cell of the same showing the uncoiled haptonema and its bulbous base. Electron micrograph M 121·1, 60 kV, magnification × *c.* 6000.

Fig. 30. Parts of two other cells of the same: the right-hand cell showing the tightly coiled haptonema breaking away just above its bulbous base, the left-hand cell disrupted, the bulbous base and the two flagella alone remaining. Electron micrograph M. 122·14, 40 kV, magnification × *c.* 6000.

III

Fig. 31. Part of a cell similar in treatment to those on Pl. II, showing the coiled haptonema with its sheath. (For a better view of the scales see Pl. IV.) Electron micrograph M 114·6, 60 kV, magnification × 20,000.

Fig. 32. Part of a similar cell with the haptonema partially extended, and fibrillar components visible in the flagella. Electron micrograph M. 114·4, 60 kV, magnification × 20,000.

IV

Fig. 33. Part of the right-hand cell of Pl. II, Fig. 30, to show the bulbous base of the haptonema and body scales more clearly. Electron micrograph M. 122·18, 40 kV, magnification × 20,000.

Fig. 34. Reversed print of part of the preceding to show sculpturing of scales more clearly Electron micrograph M. 122·18, magnification × 30,000.

Fig. 35. Similar details from another cell to show some small rimless scales and a collapsed spined scale, from the region of the flagellar bases. Electron micrograph M.182·19, 40 kV, magnification × 30,000.

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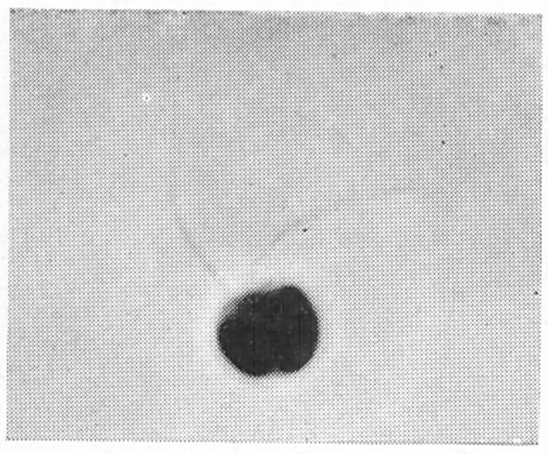

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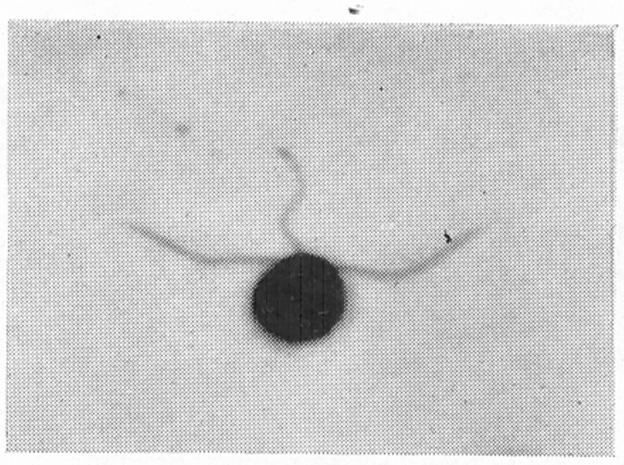

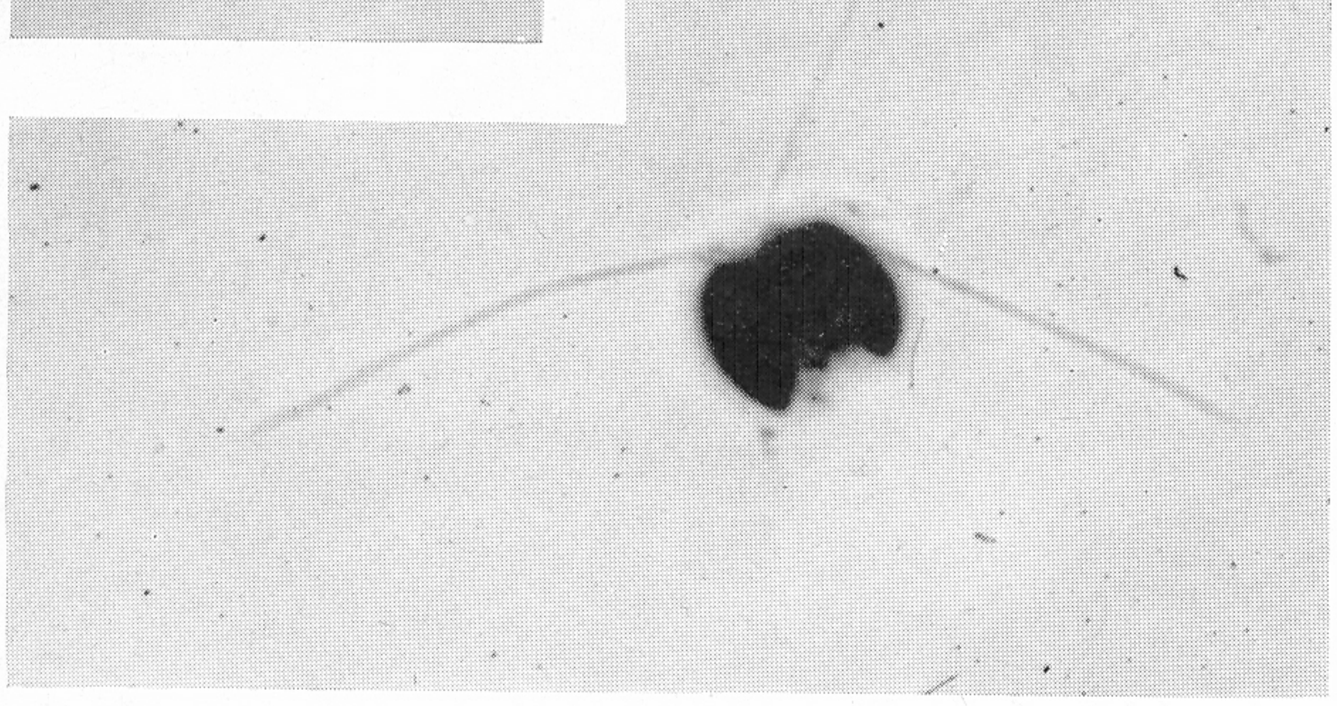

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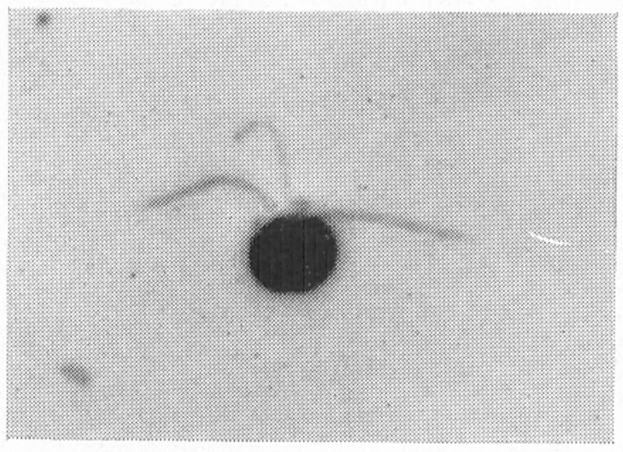

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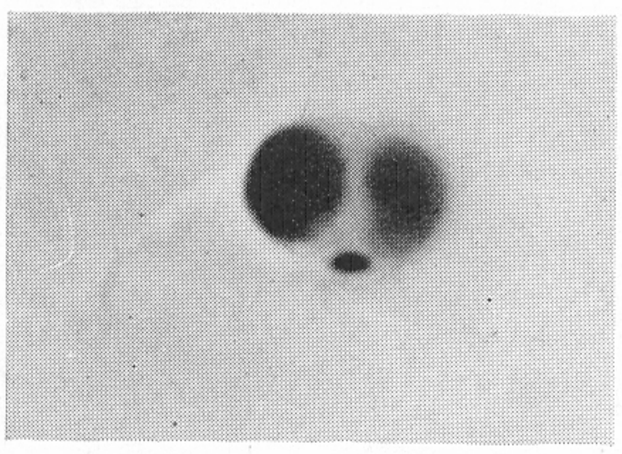

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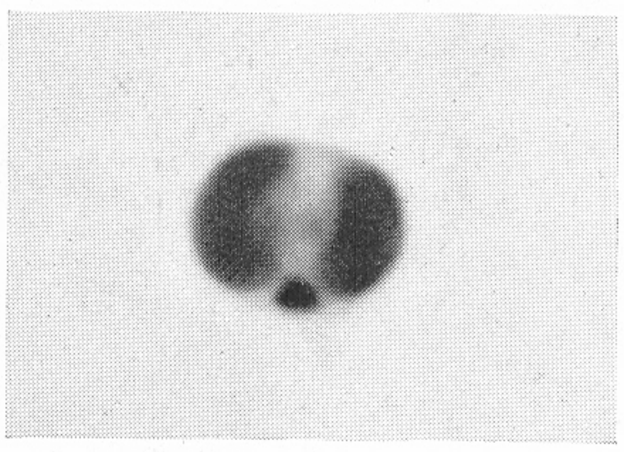

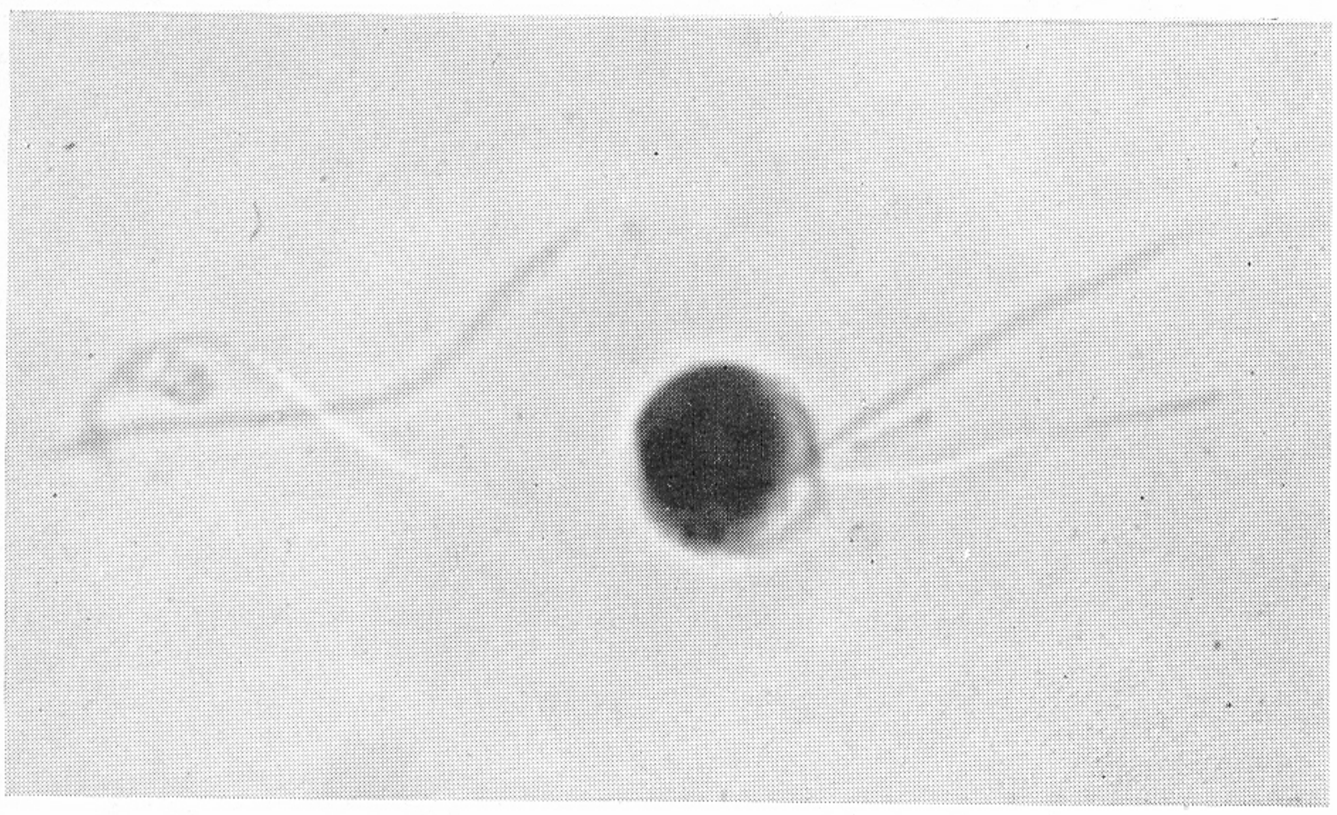

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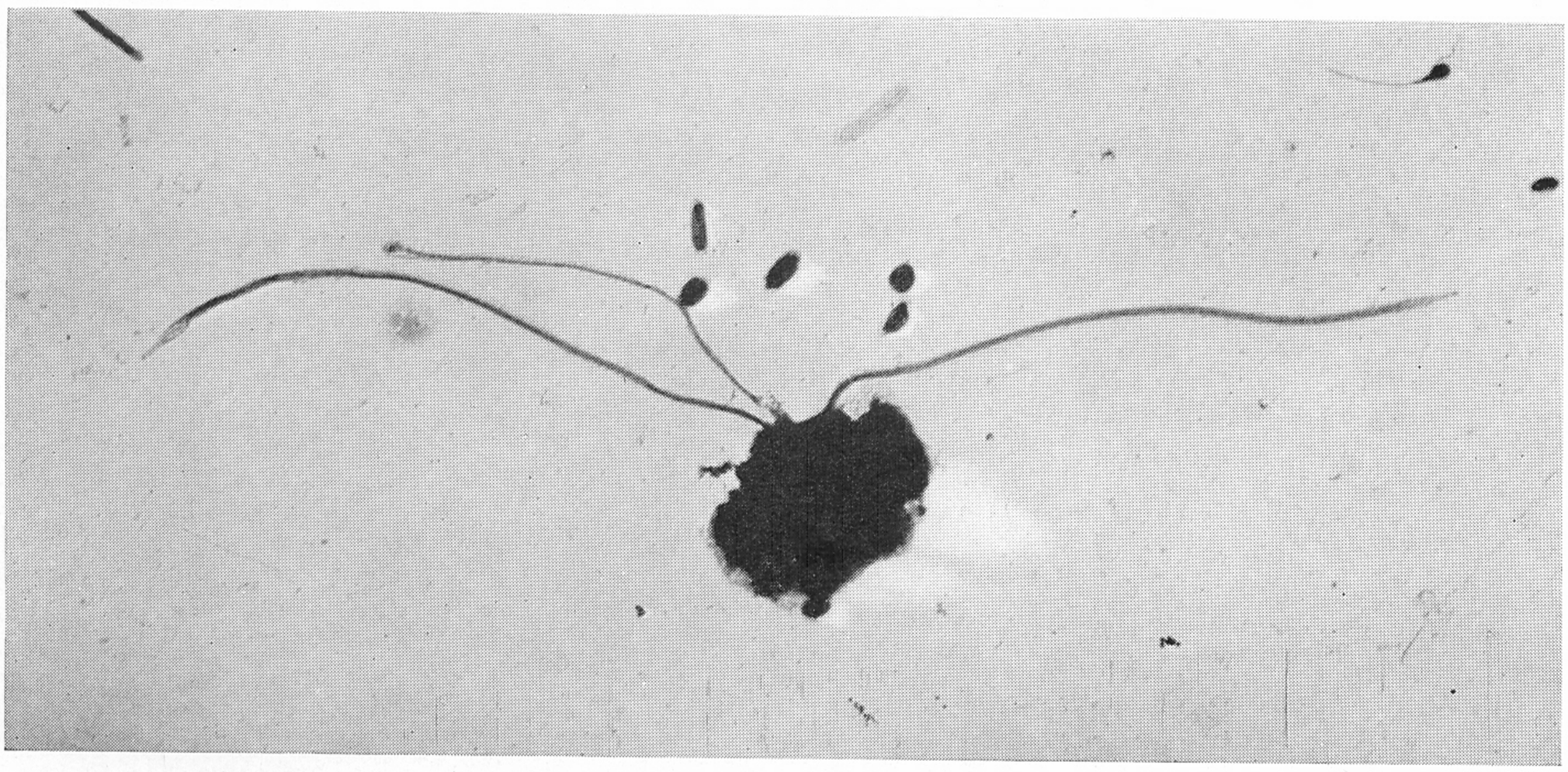

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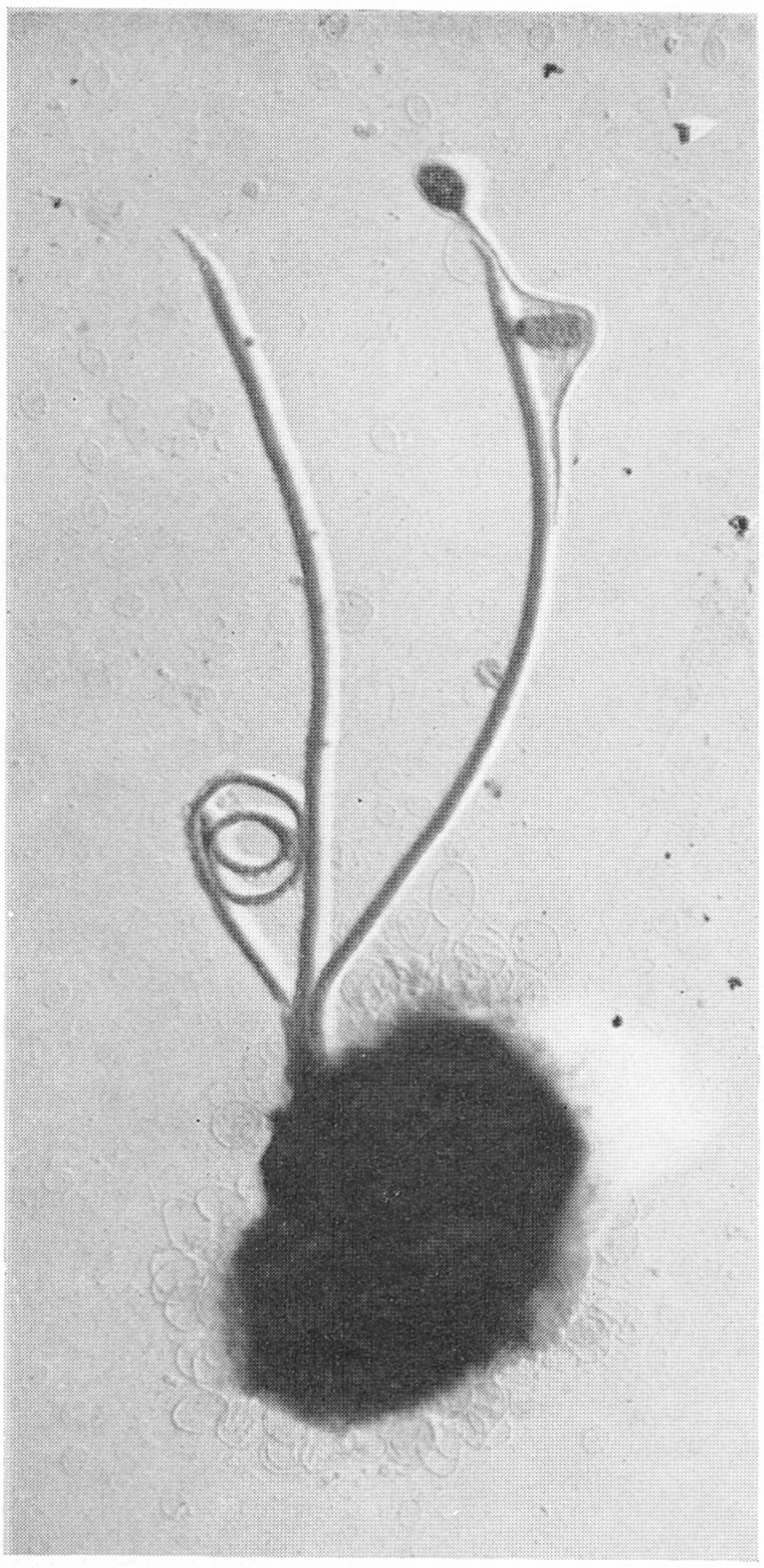

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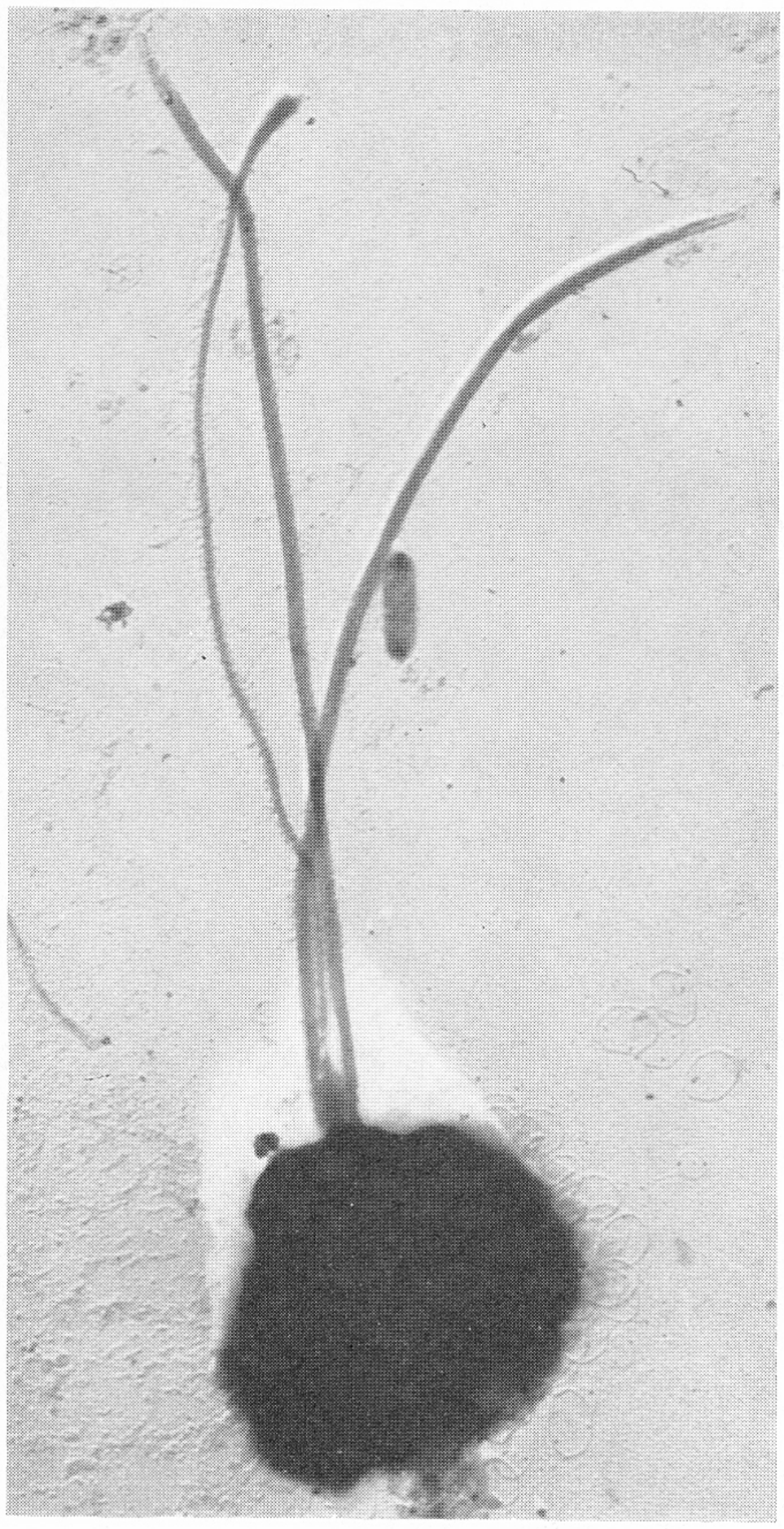

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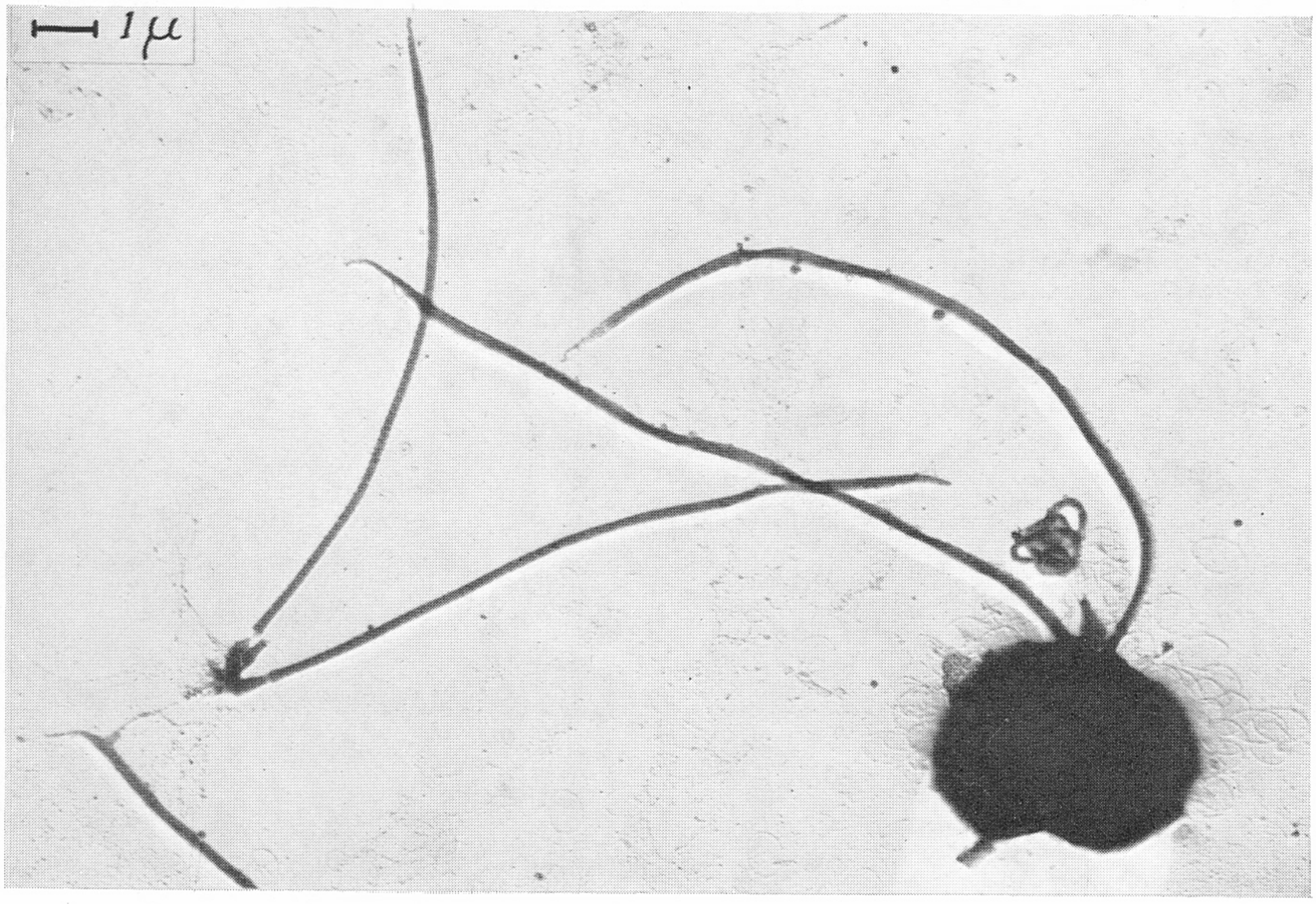

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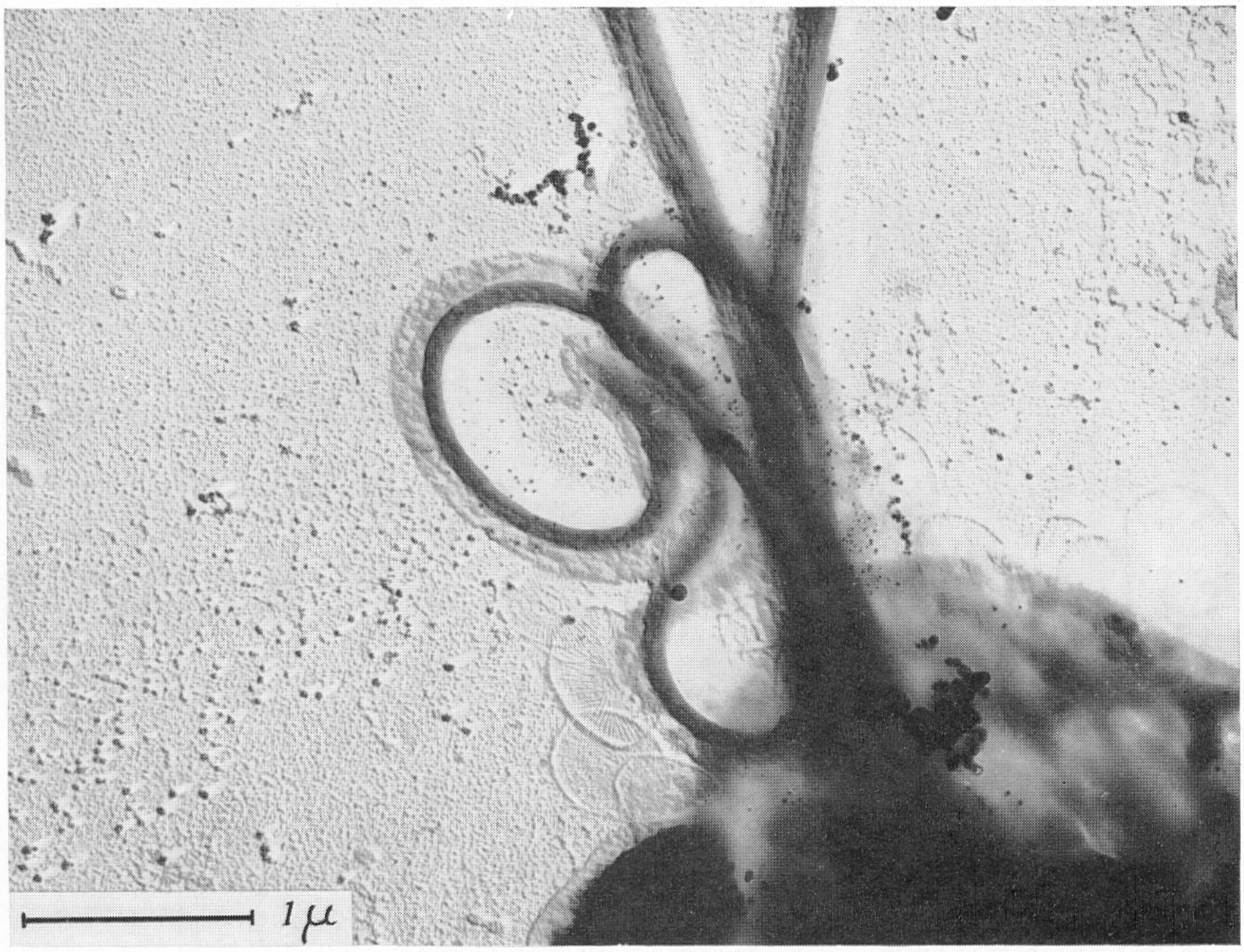

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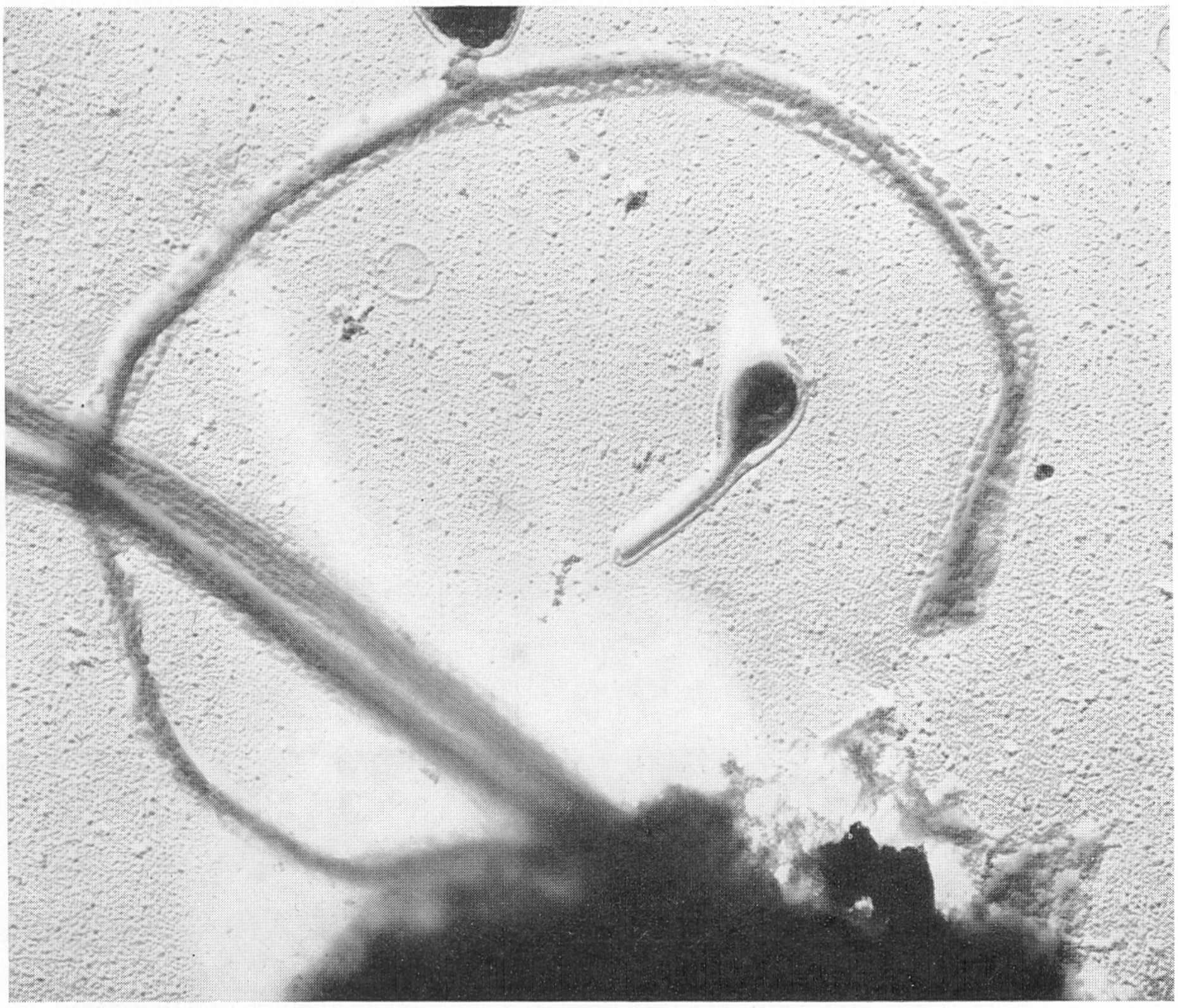

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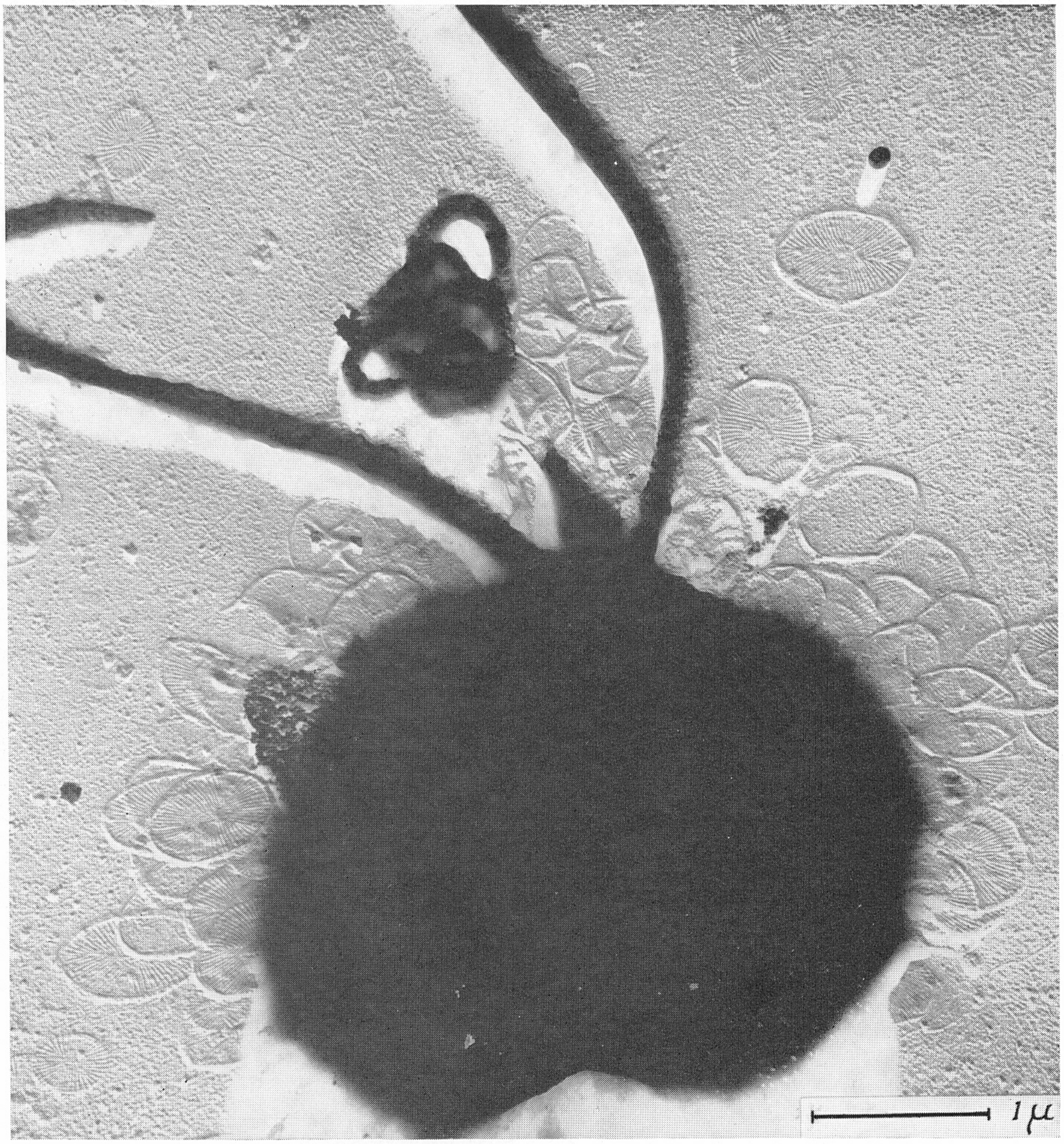

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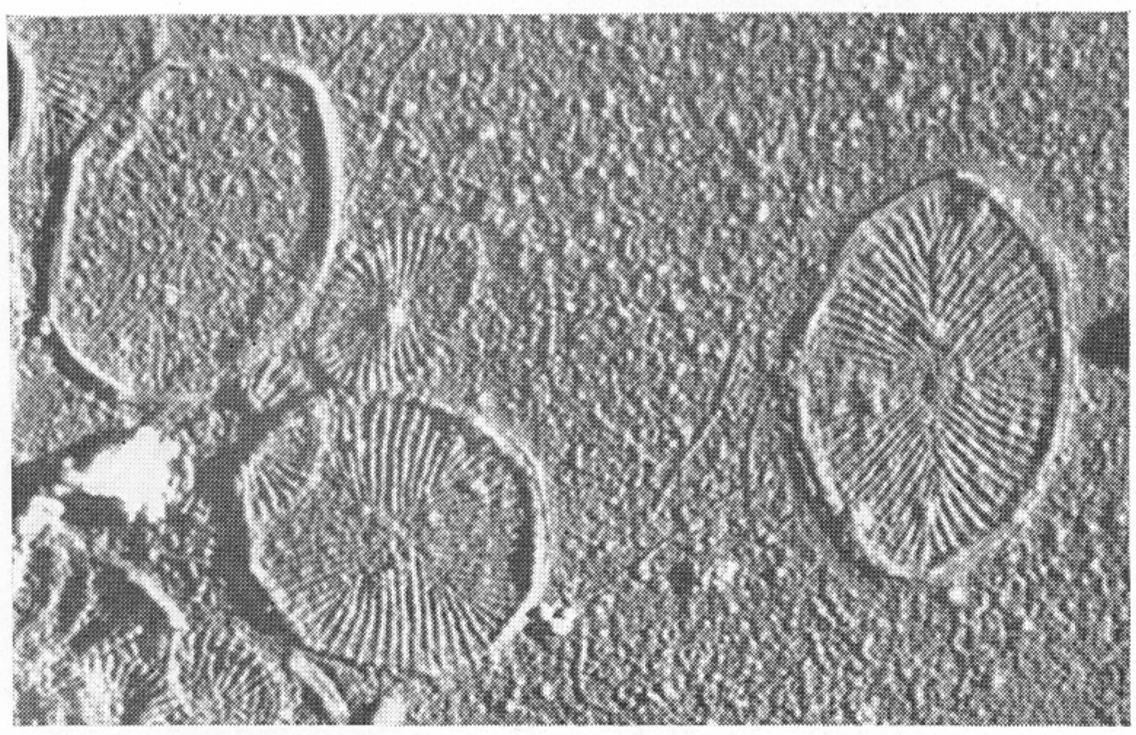

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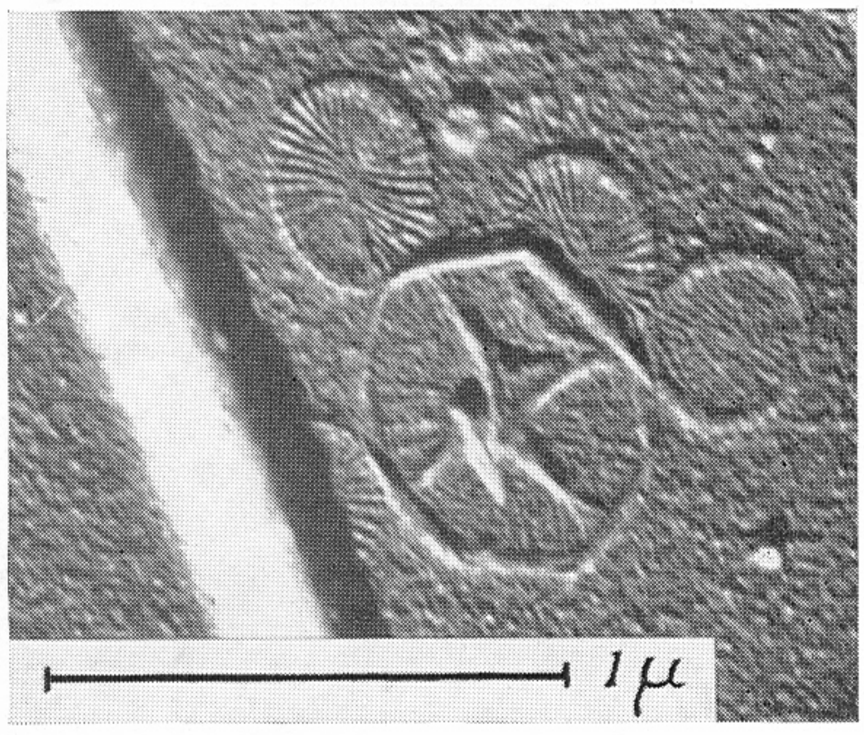

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(*Facing p.* 591)

In the localized peripheral areas in which the muciferous organelles occur, a few granules stain bright blue with cresyl blue and are perhaps mitochondria. Two or three slightly larger masses stain a blue-green with this reagent; these are perhaps physodes (cf. Bourrelly & Magne, 1953; Magne, 1954).

The locomotory movements, which are some of the most significant attributes of these organisms, are unfortunately unsuited to diagrammatic demonstration, and drawings of a few characteristic attitudes to accompany a brief verbal description must suffice. In spite of the absence of a stigma, the motile cells show a marked phototactic reaction when exposed to uneven illumination in a Petri dish, accumulating preferentially towards, or away from, the light according to the intensity. When mounted under the microscope the cells move rapidly in rather large circles for a limited period, after which they slow down and either attach themselves temporarily to the surface of the slide or else move off again in a different direction. The cell body rotates and gyrates during swimming. When viewed under dark ground illumination the appendages are held as in Fig. 1 during periods of most rapid swimming, that is directed *backwards* with the haptonema almost or completely coiled up and the two vibrating flagella diverging at a wide angle so that their free ends are further apart than the width of the cell body. In this position the haptonema has never been seen fully uncoiled, and it is generally coiled so closely to the body that it may be difficult to see. When slightly uncoiled at the base (Fig. 6) the cells appears to move more slowly. Sometimes, however, individuals are seen moving with the flagella and haptonema directed *forwards*, when the haptonema is commonly extended to its full length (slightly longer than the flagella) and is held stiffly out in front of the body while the flagella splay out laterally (Fig. 5) showing an undulating movement.

At any moment after a period of active swimming the cells may slow down and anchor themselves by the tip of the haptonema to any sufficiently firm surface which under the conditions of viewing is commonly the surface of the glass slide. The flagella may continue to vibrate with undiminished vigour and the cell body then carries out peculiar shaking or dancing movements, or it may occasionally move slowly in a circle round the point of anchorage. These movements are characteristic of most of the forms examined by us with short haptonemata and they are an extremely useful character for quickly recognizing these organisms in a mixed sample. When attached by its distal extremity the haptonema can be fully extended, but usually it is partly or even almost wholly coiled up except at the base (Figs. 2, 3). When tightly coiled it is liable to be hidden beneath the body, but the characteristic movements will show that it is attached. When only a short amount is uncoiled at the basal end the remainder is coiled in a flat spiral appearing to the eye as a disk with the point of attachment in the centre. This disk-like appearance, however,

can only be seen under an oil-immersion objective without which such an individual could momentarily be mistaken for a species of *Prymnesium*.

Phagotrophy is of common occurrence (Figs. 2–6), suspended particles, bacteria and small plant forms up to $2{\cdot}5\mu$ in diameter being ingested. The actual process of ingestment is difficult to analyse since the act itself is very rapid. Recently ingested cells of other organisms can sometimes be seen in Brownian movement within a vacuole. By the use of graphite it has been possible to ascertain with certainty that the site of ingestion is at the non-flagellar pole of the cell. A colourless, slightly granular, substance seems to flow out from this region to surround the particles (or cells) being ingested (Figs. 5, 6), which are then quickly drawn within the body (Fig. 2). Ingested particles at first seem to be moved about within the body but they are gradually compacted together, apparently within a vacuole, until they form a rounded pellet (Fig. 4; and Figs. 25 and 26, Pl. I). Material can be ingested, moved about in the cell, rounded off and then thrown out again (Fig. 3) all in the space of 5 min. In a very short time after graphite has been added to a culture individuals can be seen swimming quite normally with large masses of graphite inside them (Fig. 4). A cell 5μ in diameter is able to accommodate a mass as large as $2{\cdot}5 \times 2\mu$. This species tends to keep ingested material in the body fairly close to the pole at which it entered. When the graphite masses are ejected from the body some of the muciferous organelles (see p. 589) are commonly seen to discharge their contents as rods at the same time (Fig. 3). It is probable that all these processes can take place both when the cell is attached and when it is swimming.

Reproduction of the motile stage is by fission to produce usually two equal daughter-cells, each $4–5\mu$ in diameter, but fission can give daughter-cells of unequal size. When fission is unequal the smaller daughter-cell occasionally lacks chromatophores. The two new flagella generally develop as the chromatophores are dividing (Fig. 7). The second haptonema then appears as the cell broadens to separate the two pairs of flagella prior to the actual fission (Fig. 8). But the chromatophores can divide, giving four in an individual, before the new flagella are produced, or the cell can divide without concurrent chromatophore division, in which case one chromatophore passes to each daughter-cell or one has two and the other none.

Occasionally in this species large motile cells, $9–10{\cdot}5 \times 7–9\mu$, are found (Fig. 10) which bear four pairs of flagella and four haptonemata and contain four chromatophores. These cells divide into four daughter-cells, each of which usually contains one chromatophore but occasionally one of the daughter-cells may contain two chromatophores or none. Such cells are probably caused by a delay in the first cleavage until preparations for the next have already begun. Fig. 11 shows an early stage of fission in a cell $8 \times 7\mu$ in which one of the daughter cells has two flagella and a haptonema, while the other has already formed two new flagella ready for the next fission. Such double-

fission stages have been seen infrequently in individuals lacking chromatophores (Fig. 12), though such specimens are always smaller than those containing chromatophores, measuring 6–6·5 × 6·5–7 μ.

A culture of this species when grown in Erdschreiber culture solution produces from 2 to 3 million motile cells per ml. at the peak of growth. After the peak of growth has been passed, for reasons which have not been analysed, the motile cells migrate to the bottom of the flask forming there a dark olive-green skin. The word migrate is used to express the fact that cells collect preferentially on one side of the flask showing a phototactic response. If this skin is scraped from the bottom and examined the non-motile stages illustrated in Figs. 13–18 are found. When the cells lose motility they become amoeboid, measuring about 7 μ in diameter and, as far as can be checked by the use of cresyl blue, appear to discard their scale covering. At the same time small lobes appear at the periphery of the chromatophores (Fig. 13). Large naked (?) amoeboid stages up to 13 × 9 μ are also found in which the chromatophores are so very finely lobed, and so pale in colour, that they are very difficult to distinguish (Figs. 14, 15). The pyrenoid-like bodies could be seen in some of these amoeboid stages, but they are smaller than in the motile phase. A few very small leucosin vesicles are also present in some of the cells. Ingested bacteria could be detected inside these cells and graphite particles were ingested.

The origin of the large amoeboid cells (Figs. 14, 15) has not been traced. They may be either the product of fusion of the smaller amoeboid forms (Figs. 13) or they may be double-fission stages in the non-motile phase. Stages, which are apparently early division stages of these large naked amoeboid forms (Fig. 15), show four chromatophores and eight pyrenoids. From the sizes of the different stages the large amoeboid cells must either contract considerably in size or else divide before they round off and develop a thin smooth wall. No large secondary mucilaginous envelope, as was found in the genera *Prymnesium* by Carter (1937) and Conrad (1941), and in *Isochrysis* and *Dicrateria* by Parke (1949), has been found surrounding the walled stages.

The smooth-walled cells with two or four pale chromatophores, stellate or finely lobed, divide to produce four walled daughter-cells (Figs. 16, 17). These daughter-cells become free from the parent membrane and usually have two chromatophores, but sometimes there appears to be only one; the chromatophores are still stellate or deeply lobed in form (Fig. 18). These daughter-cells could quite easily be mistaken for the free, non-motile, walled cells belonging to either the genus *Phaeaster* Scherffel (1927) or *Sarcinochrysis* Geitler (1930). The genus *Pleurochrysis*, recently described by Pringsheim (1955), differs from the non-motile phase of *Chrysochromulina* in not possessing finely lobed chromatophores, and in showing longitudinal divisions in the cells, to give short filaments which grow out to produce irregularly ramified colonies.

The swarmer of *Pleurochrysis* differs also in lacking a haptonema, and has either fine granules on the external membrane or is surrounded by a solid granulated capsule.

The reconstitution of the motile phase from the small, walled, Phaeaster-like cells (Fig. 18), which are ovoid to slightly bean-shaped and measure from $3{\cdot}5 \times 2{\cdot}5$ to $6 \times 4\mu$, has not yet been observed, but empty walls (Fig. 19) with a circular pore about $1{\cdot}5\mu$ in diameter have been found, suggesting that their contents are so liberated. It would be particularly interesting to watch the behaviour of the chromatophores immediately prior to and immediately after the liberation. As was stated earlier the chromatophores of the motile stage sometimes appear striated, when seen in face view, as if there might be lobes lying flat against the inner face.

In this species, if the olive brown skin on the bottom of a flask is washed well a number of times to remove all free cells and all motile cells and then fresh culture solution added, in 7–10 days the skin has gone from the bottom of the flask and a thick culture of the motile phase is then present in the medium.

Chrysochromulina minor n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal to ovoid with a flattened flagellar pole and the opposite pole slightly tapering, some metaboly, 3–5 (exceptionally $2{\cdot}5$–$7{\cdot}5)\mu$ in diameter. Two equal homodynamic flagella, 2–3 times the cell diameter in length, with a distinct knobbed mucronate tip more than twice the flagellar diameter in length visible with the electron microscope. One haptonema[1] slightly shorter than the flagella when fully extended, arising with them at the flattened cell pole. Periplast covered by very thin sculptured scales, nearly circular to ellipsoid, sometimes hexagonal to octagonal, $0{\cdot}2 \times 0{\cdot}3$ to $0{\cdot}5 \times 0{\cdot}7\mu$ in size.

Cells uninucleate, no stigma. Chromatophores usually 1 or 2, sometimes 4, 8 or none, deep golden-brown, in motile phase saucer-shaped, parietal, with medium sized globular body (pyrenoid ?) on inner face placed near the margin nearest the flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Muciferous bodies localized near the non-flagellar pole. Nutrition holophytic and/or phagotrophic. Non-toxic to fish.

Asexual reproduction of pigmented and non-pigmented forms in motile phase by fission into 2 or 4 daughter-cells; in non-motile phase by fission of amoeboid cells to produce 4 walled daughter-cells which probably liberate motile phase.

Habitat: the sea at position Lat. N. 49° 19′, Long. W. 7° 26′ at 5 m. depth on 14 June 1950. Type culture (Plymouth No. 52) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association;

[1] For derivation and use of this term see p. 581.

preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili sphaeroidali aut ovoidali, aliquanto planato ad axem qua inserta sint flagella, paululum subulata ad axem objectum flagellis, forma mutabili, 3 μ ad 5 μ (rare 2·5 μ ad 7·5 μ) latitudine. Duobus similibus flagellis homodynamicis, longioribus 2 aut 3 cellulae latitudine, utriusque ad extremitatem distincta regione nodosa et mucronata, longa plus bis obesitate flagelli, solum per microscopiam electronicam visibili; unico haptonemate, conjunctim flagellis exorienti, paulum breviore, cum maxime extensus sit, quam longitudo flagelli. Periplasto induto delicatissimis squamis sculptis, prope circularibus aut ellipsoidalibus, nonnunquam hexagonis aut octagonis, 0·2 μ ad 0·5 μ lat., 0·3 μ ad 0·7 μ long., visibilibus solum per microscopiam electronicam.

Nucleo unico, nullo stigmate; chromatophoris ex norma unico aut duobus, nonnunquam 4, 8 vel nullis, profunde aureofulvis, in cellulis status motilis crateriformibus, parietalibus, unocuique corpore globulari medio magnitudine (pyrenoidali?) in aspectu concavo locato ad marginem juxta axem qua oriantur flagella; in cellulis status non-motilis chromatophoris profunde lobatis aut stellatis; cellulis oleum leucosinumque parientibus. Corporibus muciferosis locatis ad regionem objectam axi flagellari. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem cellularum coloratarum vel incoloratarum in 2 aut 4 cellulas filiolas. In statu non-motili generanti per fissionem amoeboidalium cellularum ad 4 cellulas filiolas, parietibus praeditas, generandas, quibus potest ut cellulae status motilis liberentur.

Habitat mare ad locationem Lat. N. 49° 19′, Long. W. 7° 26′, profund. 5 m. (14 Jun. 1950).

Description

This species is smaller and usually slightly more ovoid in shape than *C. kappa* when alive (Fig. 36), though this difference is obscured after fixation. In an actively growing culture 75% of the cells are from 3 to 5 μ in length or diameter, 5% are 2–3 μ and the remaining 20% are early fission stages (including double-fission stages) from 5 to 7·5 μ. The flagella, 2–3 times the length of the cell, are relatively slightly longer than in *C. kappa* and are more easily seen, though they are more readily thrown off when the species is examined under the light microscope. No bulbous swelling can be detected at the base of the haptonema with the light microscope in this species though a comparable structure can be demonstrated with some difficulty with the electron microscope. The length of the haptonema when fully extended is slightly less than that of the flagella, thereby differing from the situation in *C. kappa* in which it is slightly more. The club-shaped extremity is similar in the two species but the translucent haptonemal sheath is slightly less conspicuous in *C. minor* (Fig. 60, Pl. VI).

The scales (Figs. 62–4, Pl. VII) are very similar in the two species, although we have so far failed to find any trace of spined scales in *C. minor*. The chromatophores of the motile phase are very similar to those of *C. kappa*, but are more deeply pigmented and more frequently show striations; they are

usually 1 or 2 but sometimes 4, 8 or none. Individuals lacking chromatophores, seen frequently in this species, measure usually 2·5–3μ (Fig. 40). When one chromatophore is present it lies laterally (Fig. 36); when about to divide it moves to the non-flagellar pole (Fig. 39) and after division the two chromatophores return to the lateral position (Fig. 38).

In this species the pyrenoid-like bodies (p. 589), reacting in the same way to stains and Millon's reagent as in *C. kappa*, are smaller and are excentrically placed near to the periphery of a chromatophore on the side towards the flagellar pole (Figs. 36–38), not centrally on its inner face as in that species. The position of the nucleus, leucosin vesicles, oil globules and muciferous bodies are as in *C. kappa* except that the muciferous bodies are fewer and none seem to be present at the flagellar pole. Their position at the opposite pole changes, as in *C. kappa*, with the metaboly of the body. The behaviour of the muciferous bodies is similar in both species.

Swimming is more rapid than in *C. kappa* and tends to be in straight lines over considerable distances rather than moving in circles. As in *C. kappa* the most usual attitude, and the only one associated with rapid swimming is with the flagellar pole and appendages directed backwards, but the flagella lie closer together so that their ends are separated by less than the width of the cell (Fig. 36). The attitudes of the haptonema are the same as those described for *C. kappa*, both when projecting backwards and when projecting forwards, but in the latter position the flagella lie more nearly in front of the body than in

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Legends to Text-figs. 36–44

Chrysochromulina minor n.sp. (× 5000).

Fig. 36. Individual swimming with flagella and haptonema behind the body, in the position characteristic for the species during rapid swimming. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scales.

Fig. 37. Anchored individual with 2 chromatophores and very small masses of ingested graphite near flagellar pole.

Fig. 38. Anchored individual ingesting minute particles of graphite at non-flagellar pole; cell body swaying round in circles in a clockwise direction from anchoring point.

Fig. 39. Individual swimming with flagella and haptonema in front of the body, haptonema fully extended; ingested graphite (*g*) towards flagellar pole; chromatophore moved from lateral position to pole prior to division.

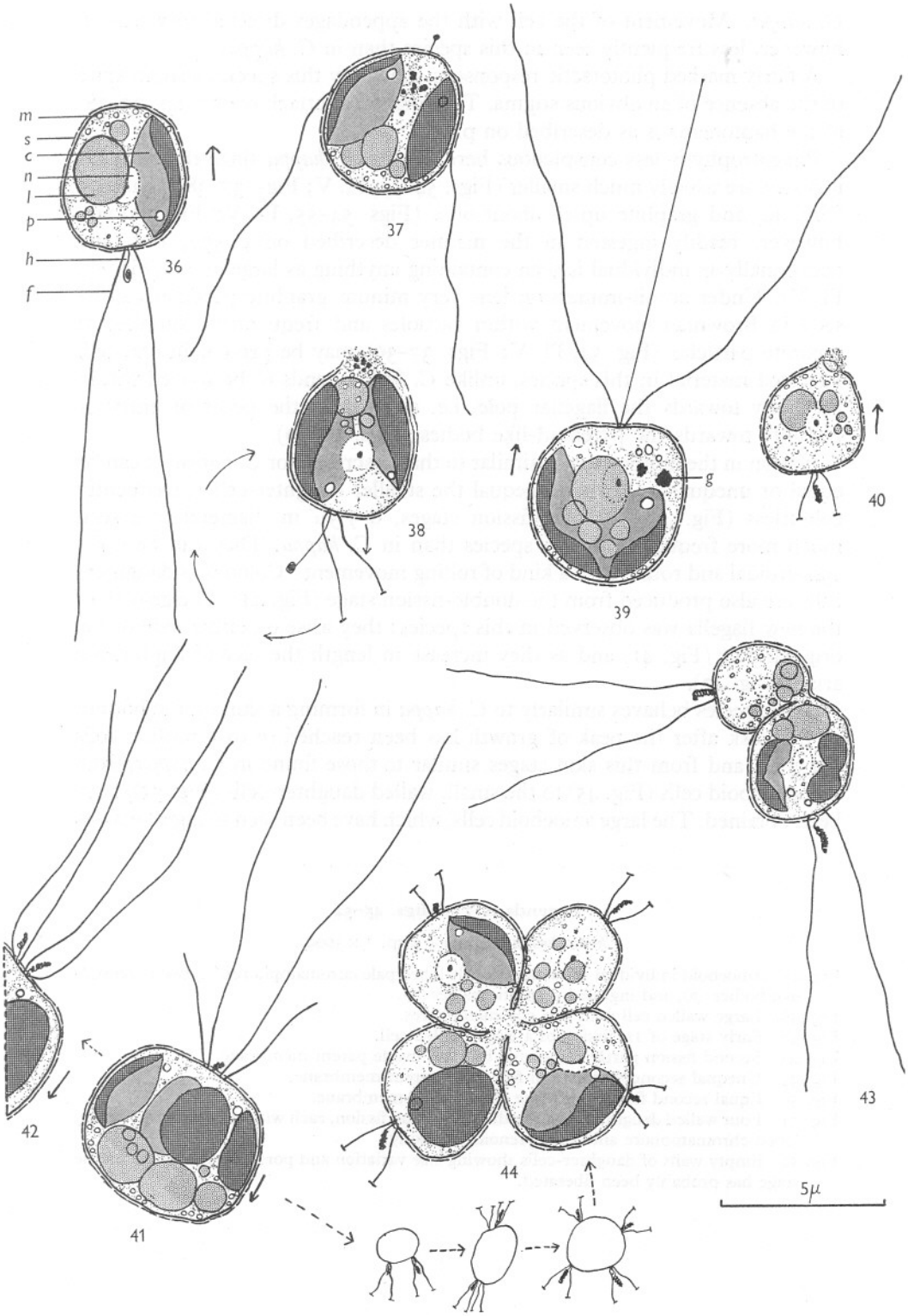
Fig. 40. Swimming individual lacking chromatophores, ingesting a bacterium at non-flagellar pole.

Fig. 41. Early fission stage with 4 chromatophores and two new flagella just developing; second haptonema not yet formed.

Fig. 42. Flagellar pole of individual in early fission stage; two new flagella nearly as long as original flagella and second haptonema developed.

Fig. 43. Two motile daughter-cells just before separation after unequal fission; larger daughter-cell with two dividing chromatophores and smaller without chromatophores.

Fig. 44. Double-fission practically completed giving 4 daughter-cells, one of which lacks a chromatophore; earlier stages shown diagrammatically below.



Text-figs. 36–44.

C. kappa. Movement of the cell with the appendages directed forwards is, however, less frequently seen in this species than in *C. kappa*.

A fairly marked phototactic response is shown by this species also, in spite of the absence of an obvious stigma. The method of attachment (Figs. 37–38) of the haptonema is as described on p. 591.

Phagotrophy is less conspicuous here than in *C. kappa* since the particles ingested are usually much smaller (Figs. 54–55, Pl. V; Figs. 37–38). Bacteria (Fig. 40) and graphite up to about $0{\cdot}5\mu$ (Figs. 54–55, Pl. V; Fig. 39) are, however, readily ingested in the manner described on p. 592, but only occasionally an individual is seen containing anything as large as 1μ (Fig. 53, Pl. V). Under an oil-immersion lens very minute graphite particles can be seen in Brownian movement within vacuoles and frequently a number of separate particles (Fig. 54, Pl. V; Figs. 37–39) may be present in one cell. Ingested material in this species, unlike *C. kappa*, tends to be moved within the body towards the flagellar pole, i.e. away from the point of entry or possibly towards the pyrenoid-like bodies (Figs. 37, 39).

Fission in the motile stage is similar to that described for *C. kappa*; it can be equal or unequal, and when unequal the smaller daughter-cell is frequently colourless (Fig. 43). Double-fission stages, $6–7{\cdot}5\mu$ in diameter, are seen much more frequently in this species than in *C. kappa*. They can be quite sphaeroidal and rotate with a kind of rolling movement. Colourless daughter-cells are also produced from the double-fission stage (Fig. 44). The growth of the new flagella was observed in this species; they arise on either side of the original pair (Fig. 41) and as they increase in length the second haptonema arises (Fig. 42).

This species behaves similarly to *C. kappa* in forming a skin on the bottom of the flask after the peak of growth has been reached (6 to 7 million cells per ml.), and from this skin stages similar to those found in *C. kappa*, from the amoeboid cells (Fig. 45) to the small, walled daughter-cells (Fig. 51), have been obtained. The large amoeboid cells, which have been seen to ingest masses

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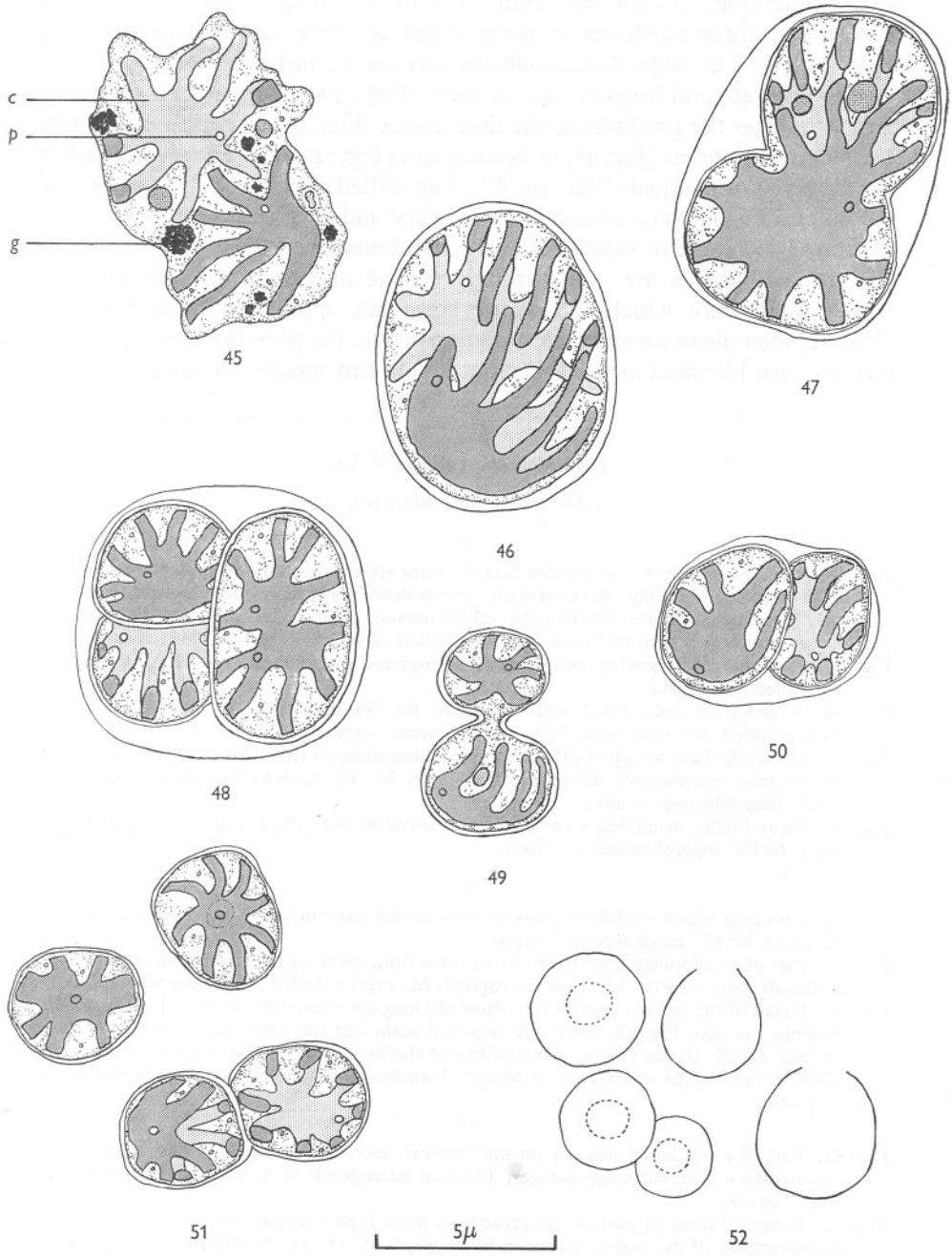
Legends to Text-figs. 45–52

Chrysochromulina minor n.sp. (× 5000).

Fig. 45. Amoeboid individual showing 2 deeply lobed pale chromatophores (*c*), two pyrenoid-like bodies (*p*), and ingested graphite masses (*g*).
Fig. 46. Large walled cell with two chromatophores.
Fig. 47. Early stage of 1st fission of a large, walled cell.
Fig. 48. Second fission partly completed but still inside parent membrane.
Fig. 49. Unequal second fission; cell free from parent membrane.
Fig. 50. Equal second fission; cell free from parent membrane.
Fig. 51. Four walled daughter-cells, product of second fission, each with one stellate or deeply lobed chromatophore and one pyrenoid-like body.
Fig. 52. Empty walls of daughter-cells showing size variation and pore through which motile stage has probably been liberated.



Text-figs. 45–52.

of graphite (Fig. 45), measure from 4×8 to $5{\cdot}5 \times 8{\cdot}5 \mu$. As in the previous species the chromatophores in these stages are very pale and finely lobed (Fig. 45–51). The large, walled cells, usually ovoid, measure from $4{\cdot}5 \times 7{\cdot}5$ to $6 \times 8 \mu$ (Fig. 46) and fission stages of them (Figs. 47–51) have been obtained. In this species the products of the first fission (Fig. 47) can either remain in the parent membrane (Fig. 48) or become free (Figs. 49, 50). The second fission can be equal or unequal (Figs. 49, 50). The walled daughter-cells, product of the second fission, vary considerably in shape and size (Fig. 51); they can be sphaeroidal to ovoid or somewhat angular and measure from $2 \times 2{\cdot}5$ to $3 \times 4 \mu$; the chromatophores are still distinctly stellate or very deeply lobed. The empty walls from which the motile stage has apparently been liberated (Fig. 52) show also a considerable variation in size, the pore through which the cell has been liberated measuring about 1μ. In this species when fresh culture

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Explanation of Plates V–VII

Chrysochromulina minor n.sp.

V

Fig. 53. A cell photographed in a sealed liquid mount after osmic vapour killing, to show the effect of graphite feeding; an abnormally large pellet of ingested graphite is visible between the two chromatophores. Visual light, oil-immersion objective, magnification $\times$ 2000.

Fig. 54. A similar cell with two small graphite pellets of a size more usual in this species.

Fig. 55. A similar cell showing two patches of ingested graphite and also the two flagella and coiled haptonema.

Fig. 56. A group of cells killed with iodine in KI (in sea water) and dried on glass. Photographed dry with visual light, magnification $\times$ 1000.

Fig. 57. Part of the same group of cells as the preceding stripped from glass and remounted for the electron microscope. Electron micrograph M. 160·1, gold palladium shadowing 60 kV, magnification $\times$ 3000.

Fig. 58. More highly magnified view of the lowest cell of Fig. 57. Electron micrograph M. 160·2, 60 kV. magnification $\times$ *c.* 6000.

VI

Fig. 59. Two cells transferred from glass to show coiled haptonemata. Electron micrograph M. 160.7, 60 kV, magnification $\times$ 5000.

Fig. 60. Part of a cell killed directly on the formvar film, showing the coiled haptonema with its sheath more clearly. Electron micrograph M. 174·17, 60 kV, magnification $\times$ 20,000.

Fig. 61. Detail of the tip of a flagellum to show the long attenuated point with slight terminal swelling (see also Fig. 58, Pl. V). A detached scale and the basal part of a *Caulobacter* (cf. Fig. 28, Pl. II) are visible in the middle of the field. Electron micrograph M. 203·26 taken on the Philips microscope at Messrs Tootals of Manchester, 60 kV, magnification $\times$ 15,000.

VII

Fig. 62. Part of a cell killed directly on the formvar, more highly magnified to show scales and the bases of the three appendages. Electron micrograph M. 174·11, 60 kV, magnification $\times$ 20,000.

Fig. 63. Reversed print of part of the preceding, more highly magnified to show the details of sculpturing of the scales. Electron micrograph M. 174·11, magnification $\times$ 30,000.

Fig. 64. Scales from another specimen showing raised rims more clearly. Electron micrograph M. 174·5, 60 kV, magnification $\times$ 30,000.

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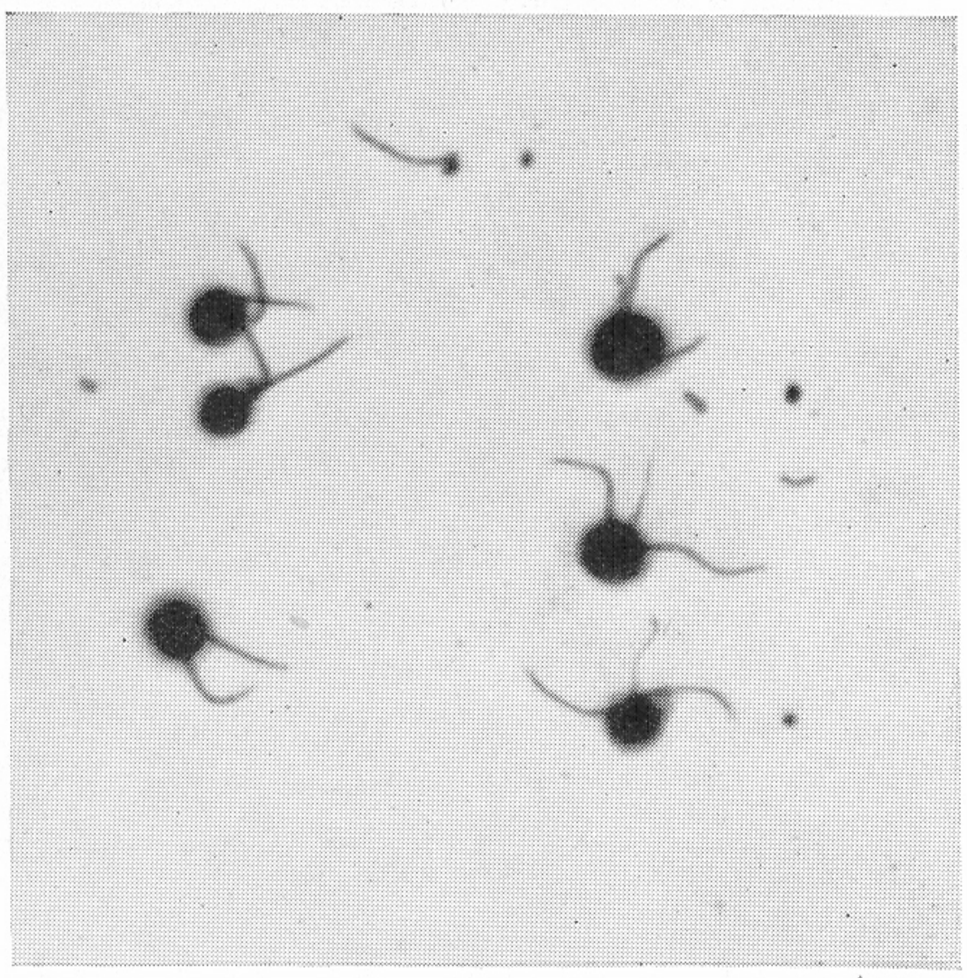

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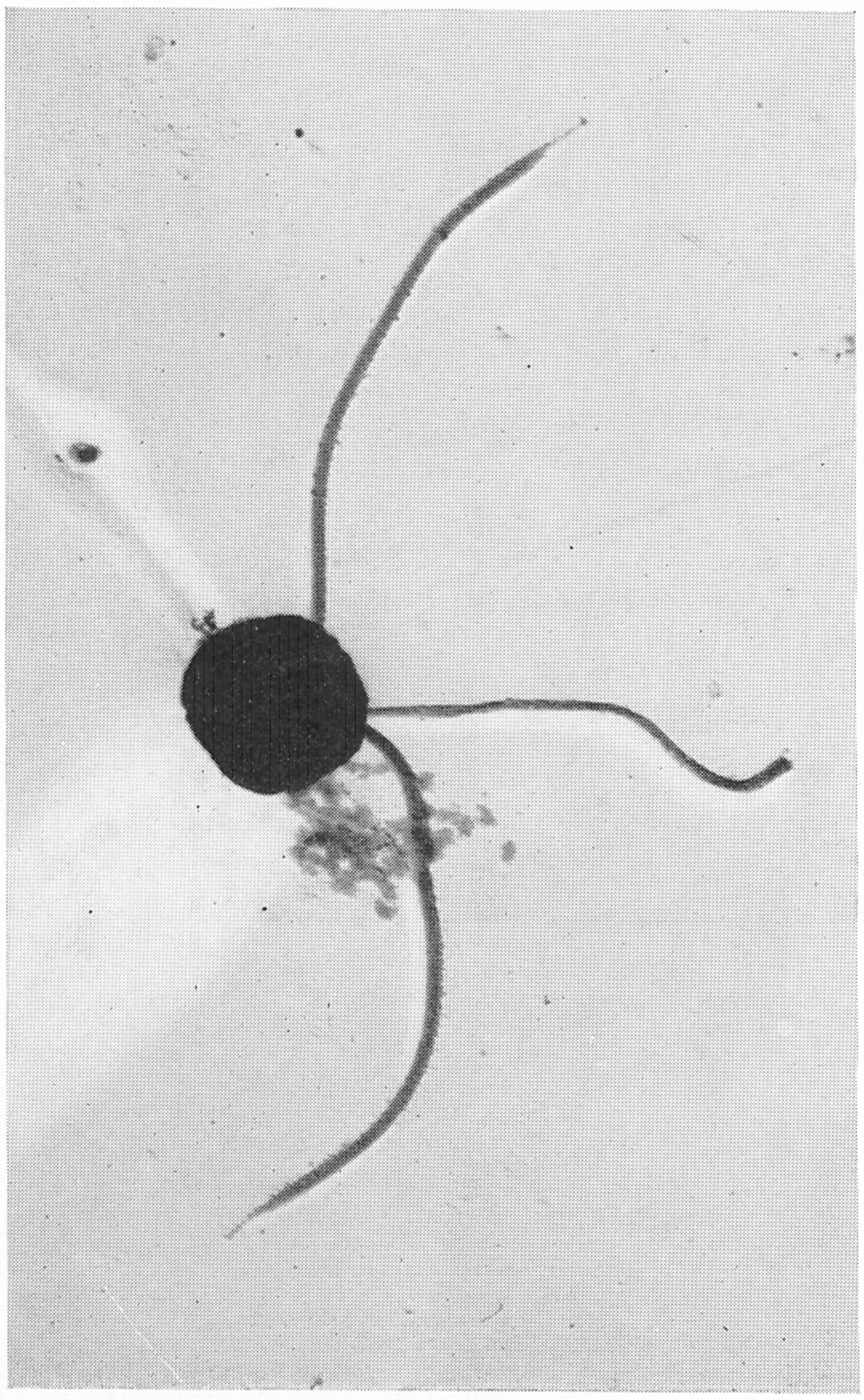

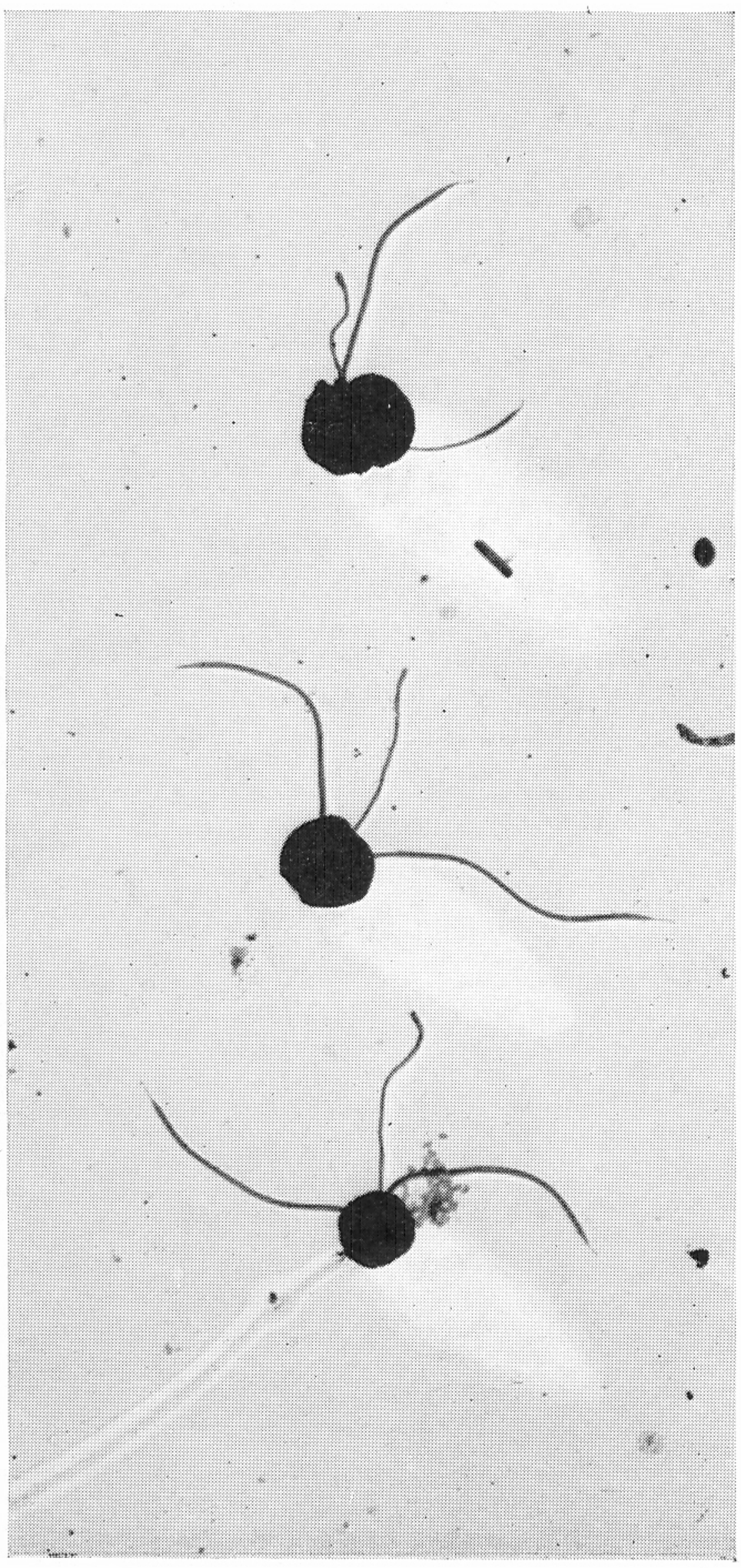

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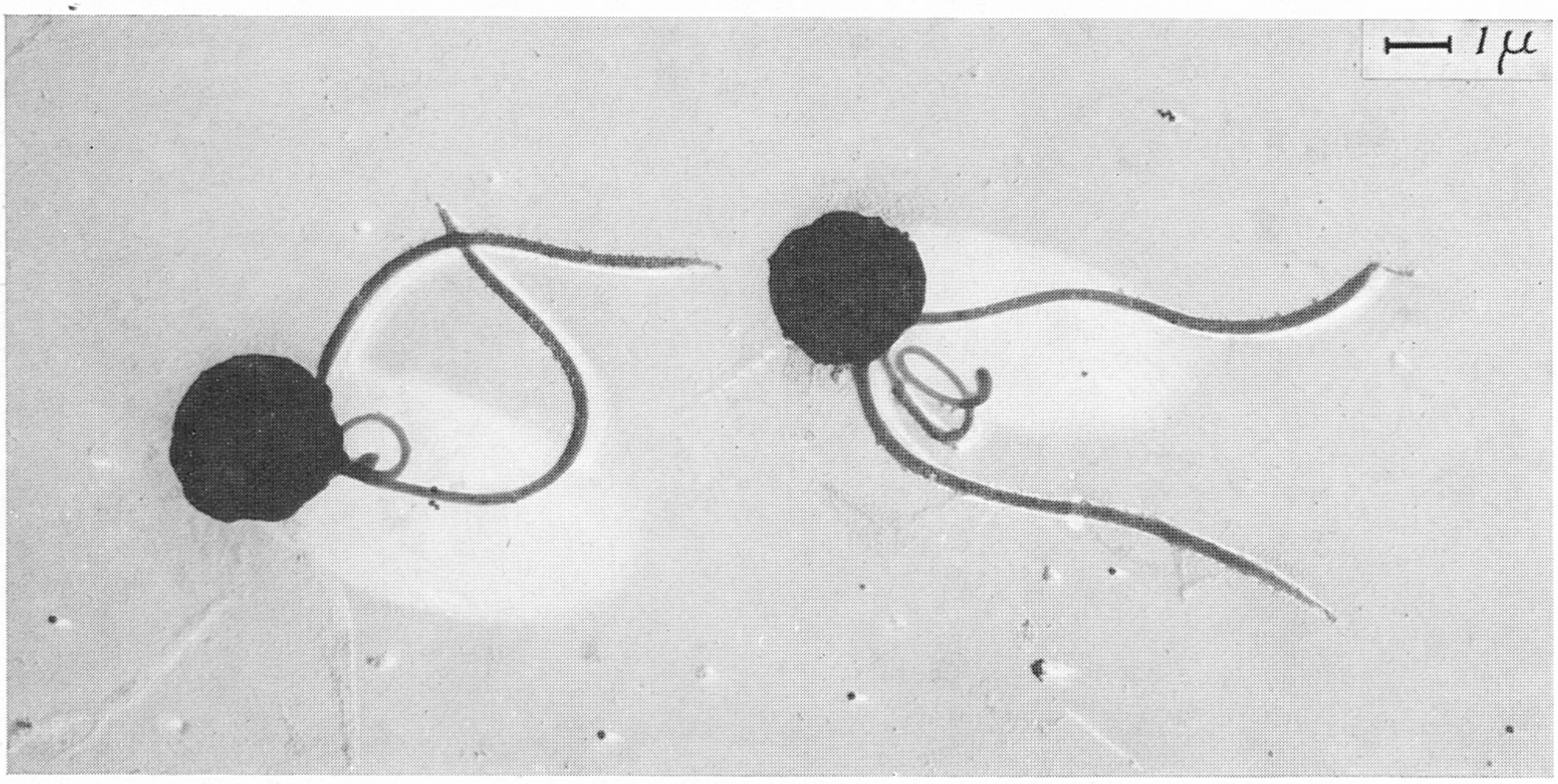
(*Facing p.* 600)



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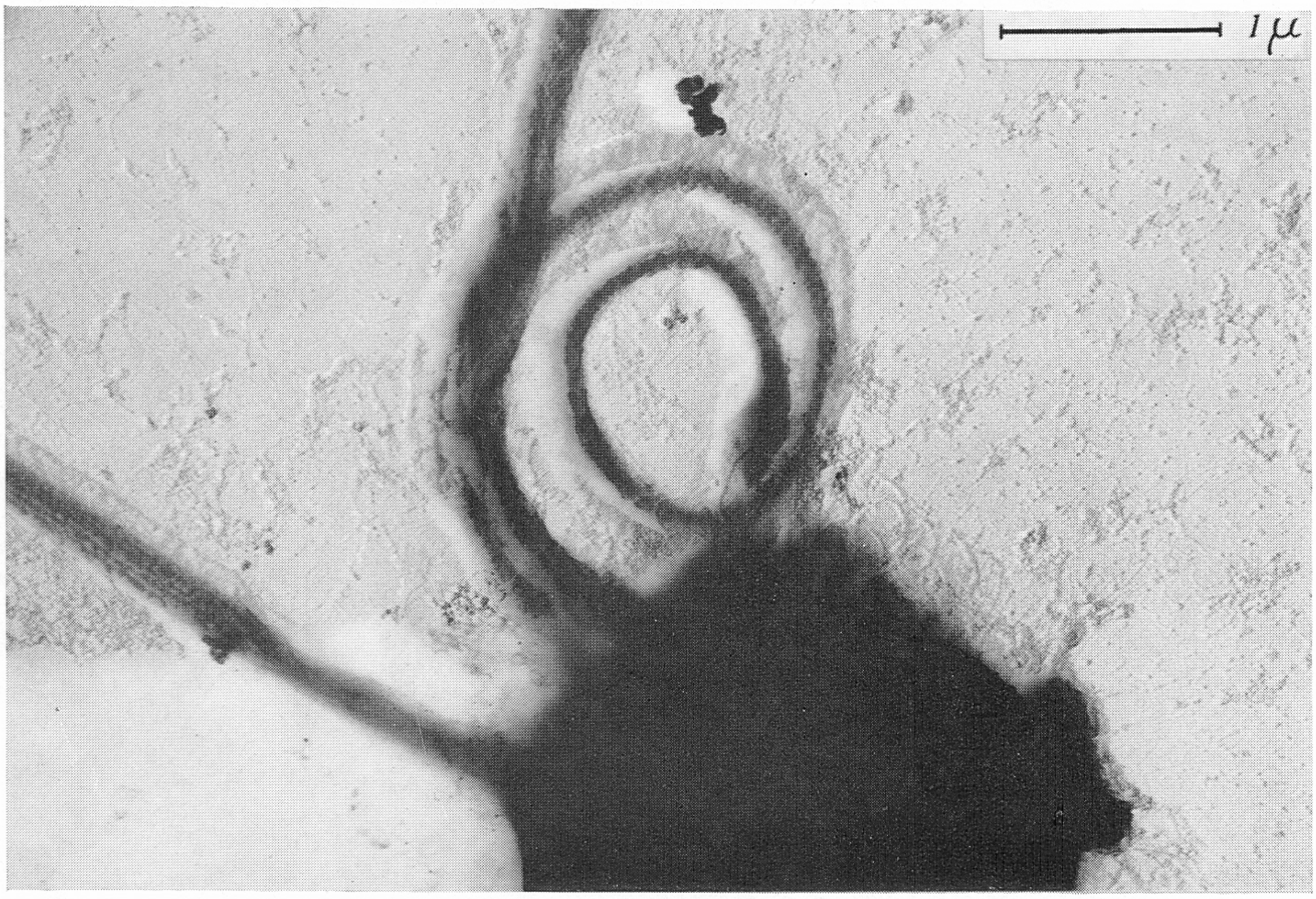

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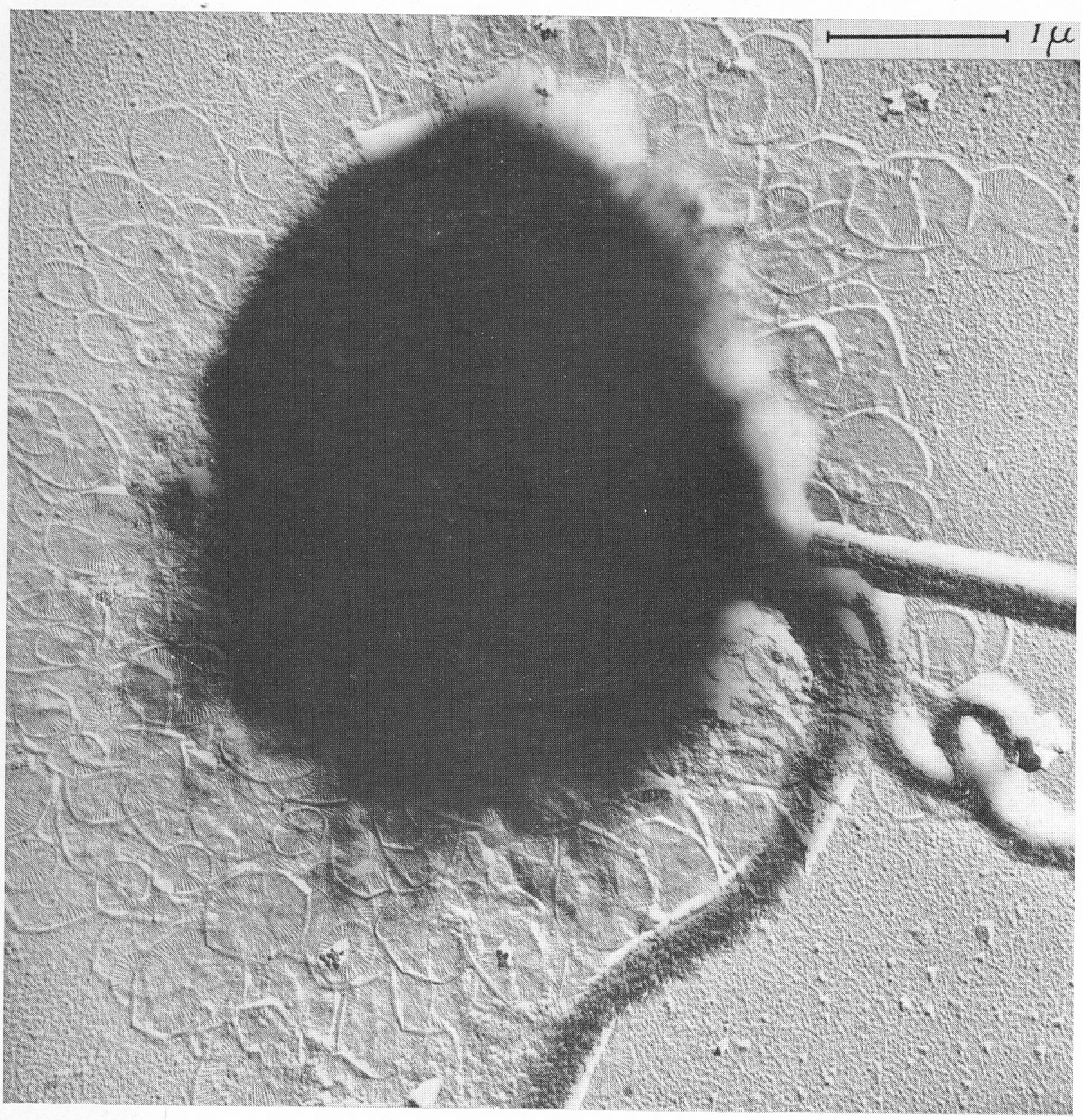

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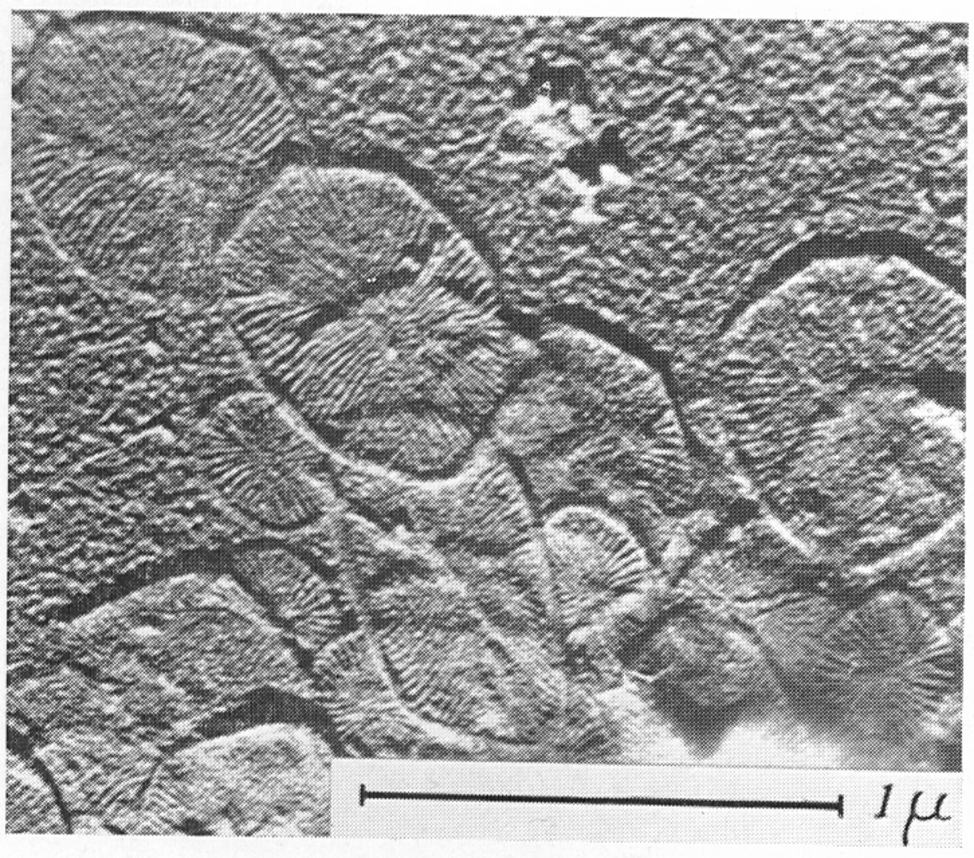

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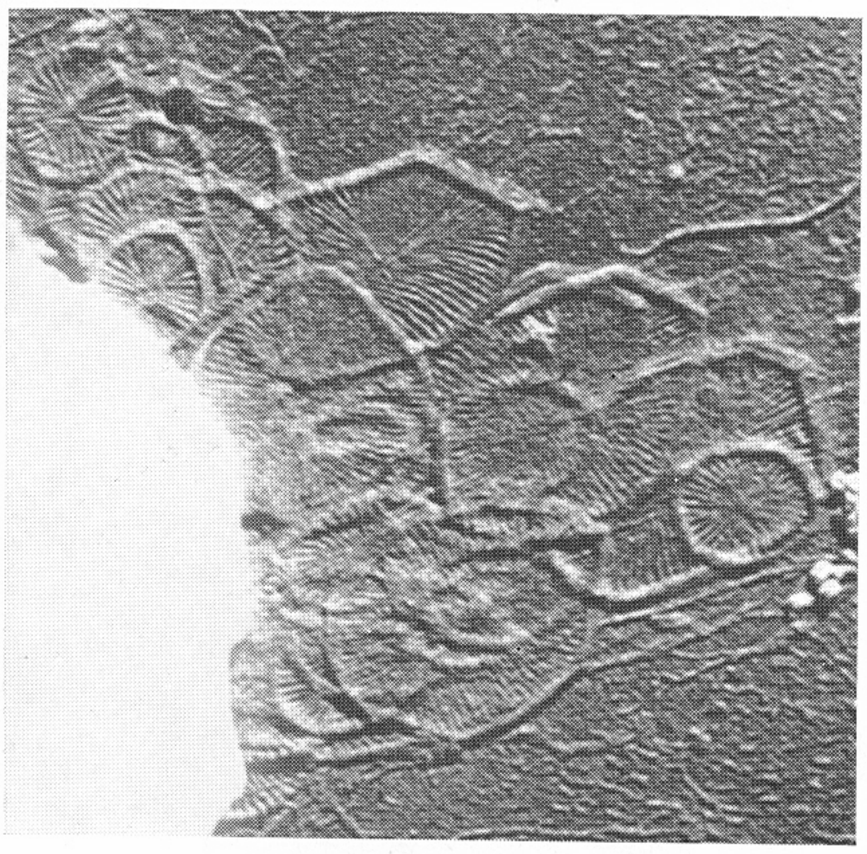

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solution is added to the skin on the bottom of a flask, after it has been washed, the motile phase is quickly produced, much more rapidly than in *C. kappa*, so that in 3–4 days a fairly thick culture of the motile stage is obtained.

Chrysochromulina brevifilum n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal to pyriform with flagellar pole usually flattened; metaboly of body well-marked particularly at non-flagellar pole; cells 4–7μ (exceptionally 3·5–11·5μ) in length or diameter. Two equal, homodynamic, shortly mucronate flagella, $2\frac{1}{2}$–$3\frac{1}{2}$ times cell diameter in length and one haptonema,[1] 2–$2\frac{1}{2}$ times cell diameter in length when fully extended, arising close together at flattened pole. Periplast covered by very thin sculptured ellipsoid scales, approximately 0·7μ across, each with a central spine attached by four decurrent ridges, spine length approximately equal to scale diameter.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, occasionally 1 or 8, golden brown; in motile phase saucer-shaped, ellipsoid or oblong, parietal with small globular body (pyrenoid ?) on inner face of each near the margin towards the non-flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Muciferous bodies conspicuous, usually in rows, but their position changing with the metaboly of the body. Nutrition holophytic and/or phagotrophic. Not toxic to fish.

In motile phase asexual reproduction by fission into 2 or 4 daughter-cells: in non-motile phase by fission of amoeboid cells to produce 4 walled daughter-cells which probably liberate motile phase.

Habitat: the sea at position Lat. N. 49° 21′, Long. W. 04° 54′ at surface on 9 May 1950.

Type culture (Plymouth No. 39) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association: preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, sphaeroidali vel pyriformi, ex norma planata ad axem qua inserta sint flagella; forma manifeste mutabili imprimis ad axem objectum flagellis; cellula 4μ ad 7μ (rare 3·5μ ad 11·5μ) latitudine. Duobus similibus flagellis homodynamicis, breve mucronatis, longioribus $2\frac{1}{2}$ ad $3\frac{1}{2}$ cellulae latitudine; unico haptonemate conjunctim flagellis exorienti, longiore, cum maxime extensus sit, 2–$2\frac{1}{2}$ cellulae latitudine. Periplasto induto delicatissimis squamis sculptis ellipsoidalibus, prope 0·7μ latitudine, unaecuique squamae spinulo centrali unico per quattuor juga decurrentia affixa; spinuli longitudine subaequali squamae latitudini, visibilibus solum per microscopiam electronicam.

Nucleo unico, nullo stigmate; chromatophoris ex norma 2 aut 4, nonnunquam unico aut 8, aureo-fulvis; in cellulis status motilis chromatophoris crateriformibus,

[1] For derivation and use of this term see p. 581.

ellipsoidalibus aut oblongis, parietalibus, unocuique chromatophoro parvo corpore globulari (pyrenoidali?) locato in aspecto interiore ad marginem remotam ab axi qua inserta sint flagella; chromatophoris profunde lobatis aut stellatis in cellulis status non-motilis. Cellulis oleum leucosinumque parientibus. Corporibus muciferosis ex norma conspicuosis, plerumque in ordinibus instructis, sed locatione variosa secundum mutationem formae cellulae. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem in 2 aut 4 cellulas filiolas; in statu non-motili generanti per fissionem cellularum amoeboidalium ad cellulas filiolas, parietibus praeditis, producendas, quibus potest ut cellulae status motilis liberentur.

Habitat mare ad locationem Lat. N. 49° 21′, Long. W. 04° 54′ ad summum mare (9 Mai 1950).

Description

In the motile phase this species is larger than *C. minor* (Figs. 73, 75, 77, Pl. VIII) and shows greater variation in cell-shape caused by the more pronounced metaboly of the body (Figs. 65–69) than either *C. kappa* or *C. minor*. In an actively growing culture, excluding cells in which fission has already started, 77% of the cells are from 4–7μ in diameter or length, 7% are from 3·5 to 4·0μ and the remaining 16% are early fission stages (including double-fission stages) from 7 to 11·5μ.

The flagella, $2\frac{1}{2}$–$3\frac{1}{2}$ times the body diameter in length, are fairly robust and can be seen quite clearly under the light field, although they tend to be thrown off from the body under light and dark ground fields even more rapidly than those of *C. minor*. The haptonema also shows up very clearly under the light

Legends to Text-figs. 65–72

Chrysochromulina brevifilum n.sp. (× 5000).

Fig. 65. Individual swimming with flagella and haptonema behind body, in the position characteristic for the species during rapid swimming; chromatophores dividing. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *ss*, spined scales.

Fig. 66. Anchored cell swaying round in circles on nearly fully extended haptonema; chromatophores broadening preparatory to division, 4 pyrenoid-like bodies; small mass of graphite just ingested at non-flagellar pole.

Fig. 67. Individual anchored by haptonema which is hardly extended; cell elongated by metaboly: large mass of ingested graphite (*g*) at non-flagellar pole.

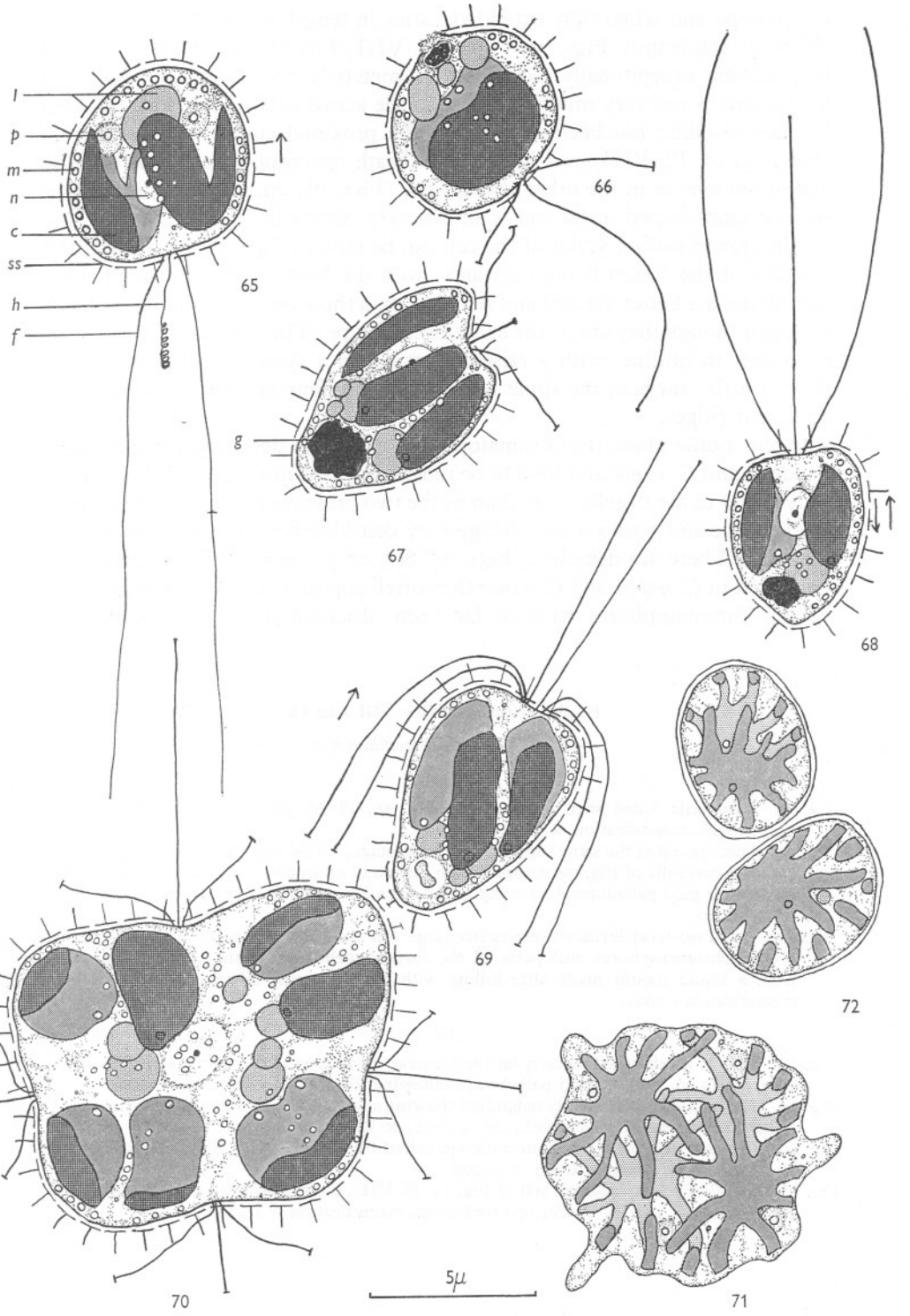
Fig. 68. Individual swimming with flagella and haptonema in front of body, haptonema fully extended; ingested graphite at non-flagellar pole.

Fig. 69. Early fission stage: 4 flagella, 2 haptonemata fully extended, body elongated and chromatophores displaced by metaboly; an ingested bacterium in a vacuole at non-flagellar pole.

Fig. 70. Stage with four pairs of flagella and four fully extended haptonemata developed prior to double-fission.

Fig. 71. Large amoeboid individual with four deeply lobed pale chromatophores and four pyrenoid-like bodies.

Fig. 72. Two walled daughter-cells from which motile stage is probably liberated; each with two pale stellate chromatophores.



Text-figs. 65–72.

microscope and when fully extended varies in length generally from $1\frac{1}{2}$ to 2 times the cell length (Figs. 73, 75, 77, Pl. VIII; Fig. 68); if, however, the cell has become exceptionally elongated by metaboly then the fully extended haptomena is not very much longer than the actual cell length (Fig. 69). No bulbous swelling has been detected at the proximal end of the haptonema (Figs. 73, 75, Pl. VIII) and its delicate sheath covering appears not so wide nor so obvious as in the other two species (Figs. 78, 79, Pl. IX); the slightly swollen club-shaped distal end is shown very clearly in Fig. 78, Pl. IX.

The spined surface scales of the cell can be seen in Figs. 78–81, Pl. IX, the position of the spines being outwards from the body (Figs. 78–79, Pl. IX). The spines are better defined and stronger than those on the few polar scales of *C. kappa* though they are of the same general type. The scale itself is ovoid to polygonal in outline, with a raised margin and a system of fine sculptural ridges on the surface; the spine is subtended by four powerful, buttress-like decurrent ridges.

In the motile phase the chromatophores are not so deeply pigmented as in *C. kappa* and *C. minor* and tend to be more elongated in shape and to lie in the body closer to the flagellar pole than in the two previous species (Figs. 65, 67). Their shape and position can change very considerably with the metaboly of the body. There are usually 2 (Figs. 65, 66), or 4 (Figs. 67, 69), occasionally 1 or 8. As in *C. kappa* and *C. minor* they often appear striated. No individuals lacking chromatophores have so far been observed in *C. brevifilum*. The

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Explanation of Plates VIII and IX

Chrysochromulina brevifilum n.sp.

VIII

Fig. 73. Two cells killed with iodine as in Fig. 56, Pl. V, photographed dry on glass. Visual light, magnification × 1000.

Fig. 74. Another cell of the same showing a division stage. Visual light, magnification × 1000.

Fig. 75. The two cells of Fig. 73 stripped from glass and examined with the electron microscope after gold palladium shadowing. Electron micrograph M. 185·1, 60 kV, magnification × 3000.

Figs. 76, 77. Two focal levels of one rather large cell showing an ingested graphite pellet, several chromatophores and parts of the three appendages. Visual light photograph from a liquid mount made after killing with osmic vapour, oil-immersion objective, magnification × 2000.

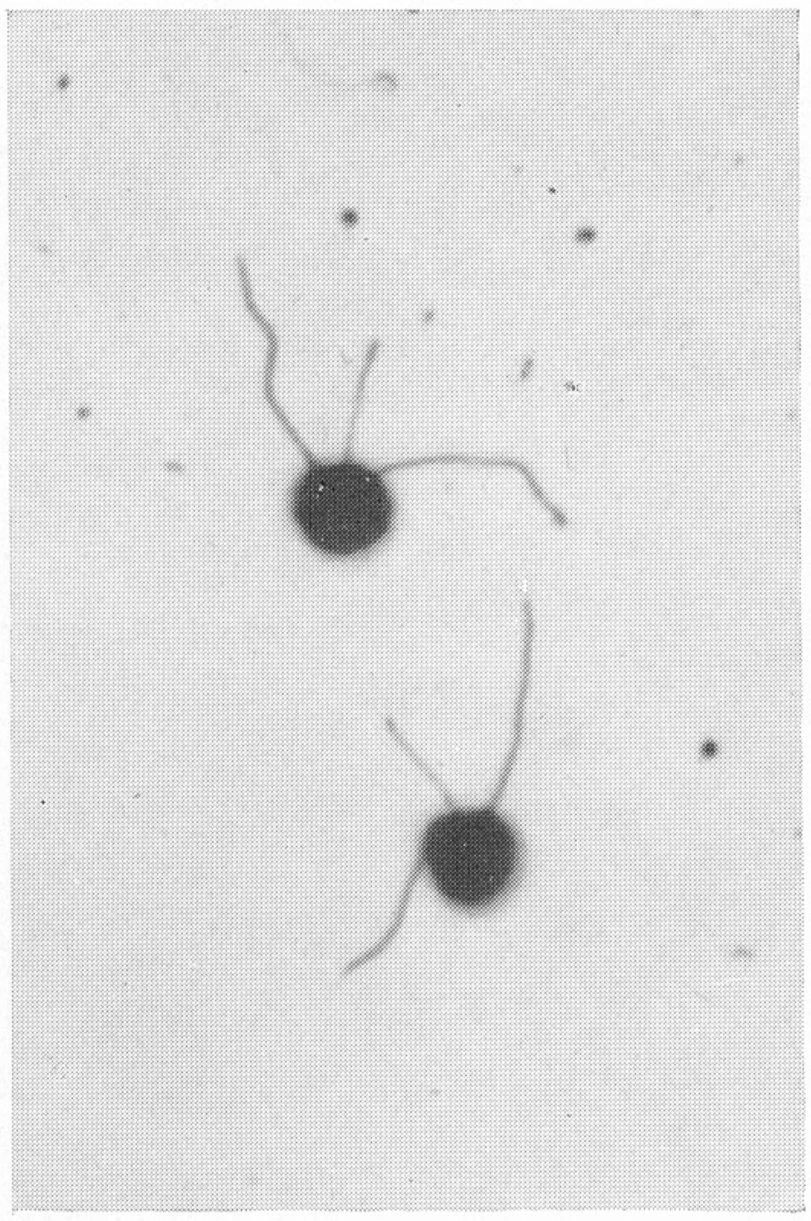
IX

Fig. 78. Part of a cell killed directly on the formvar film showing the haptonema. Electron micrograph M. 150·17, gold palladium shadowing, 60 kV, magnification × 5000.

Fig. 79. A similar cell more highly magnified showing spined scales in position and part of the coiled haptonema with its sheath. M. 150·20, 60 kV, magnification × 10,000.

Fig. 80. Reversed print of scales from the lower cell of Fig. 75, Pl. VIII. Electron micrograph M. 183·4, 60 kV, magnification × 20,000.

Fig. 81. Detail of scales from the cell of Fig. 74, Pl. VIII, after transfer from glass. Electron micrograph M. 183·10, 60 kV, reversed print, magnification × 20,000.



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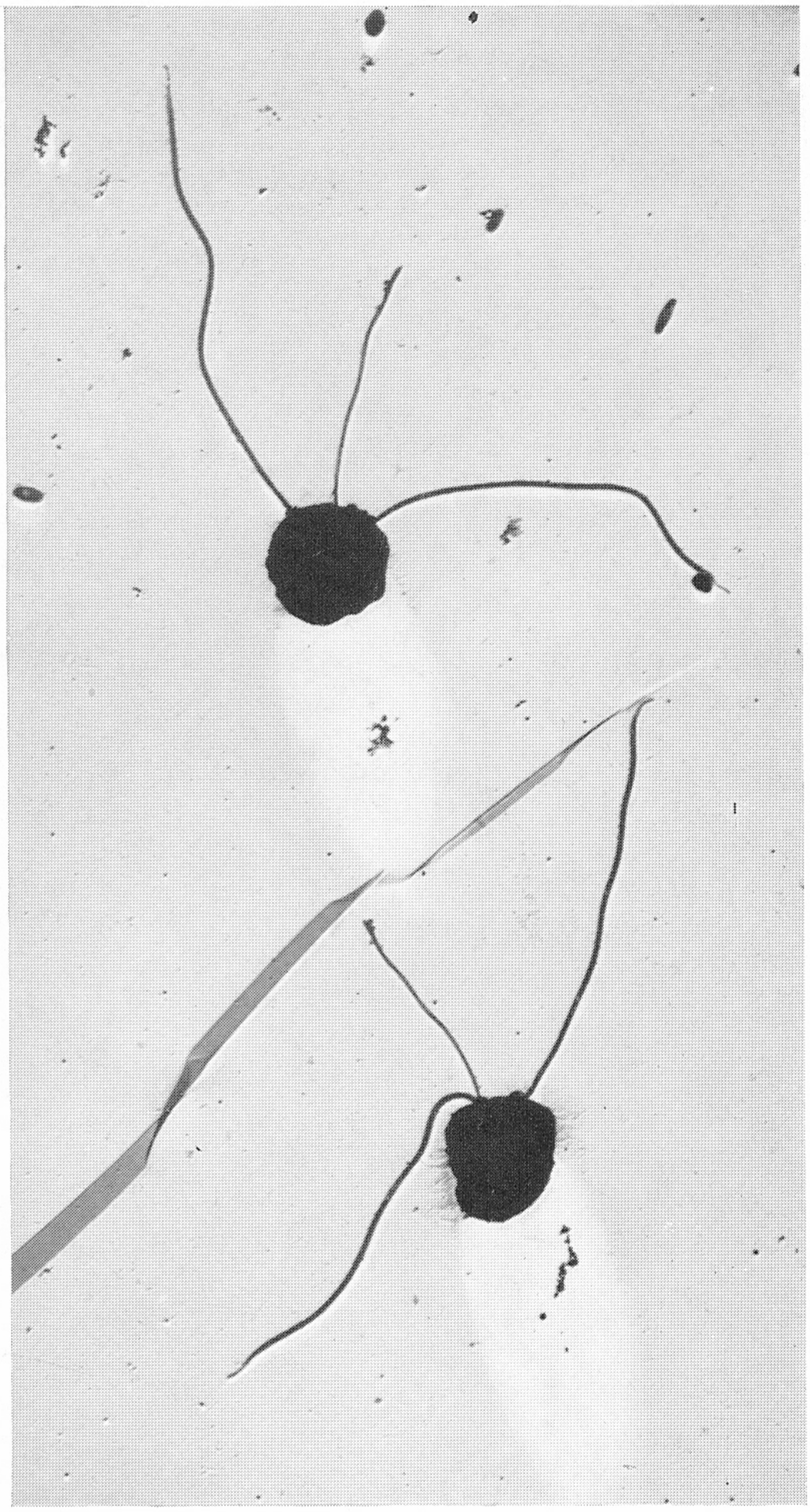

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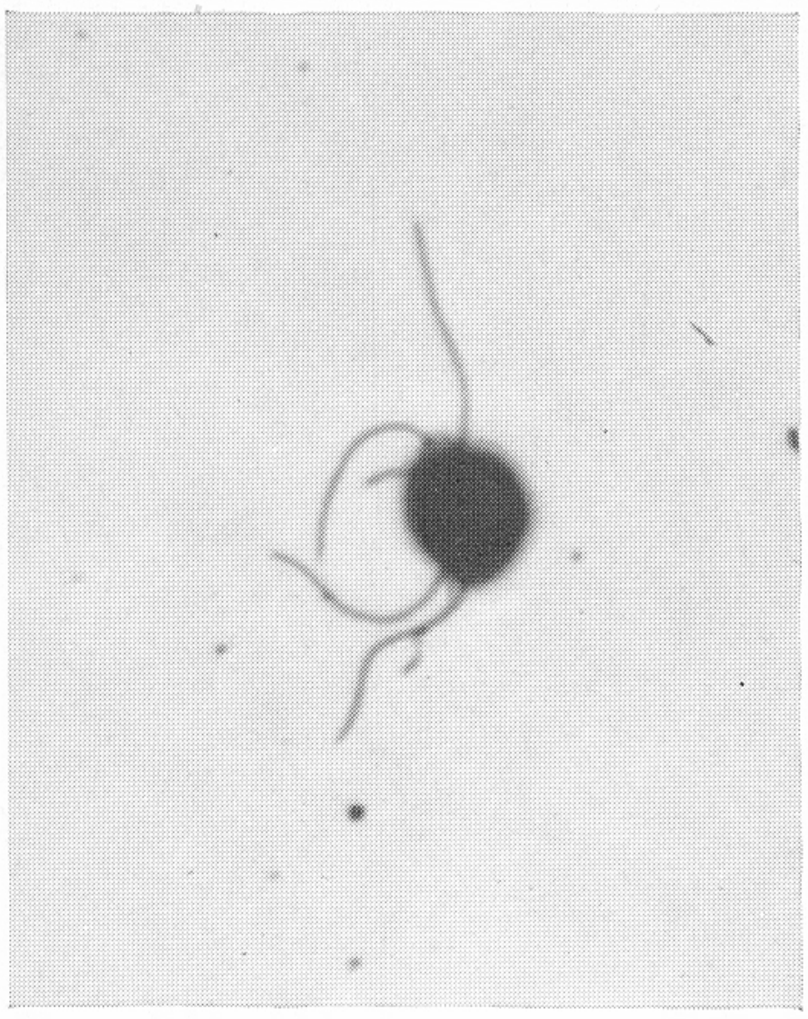

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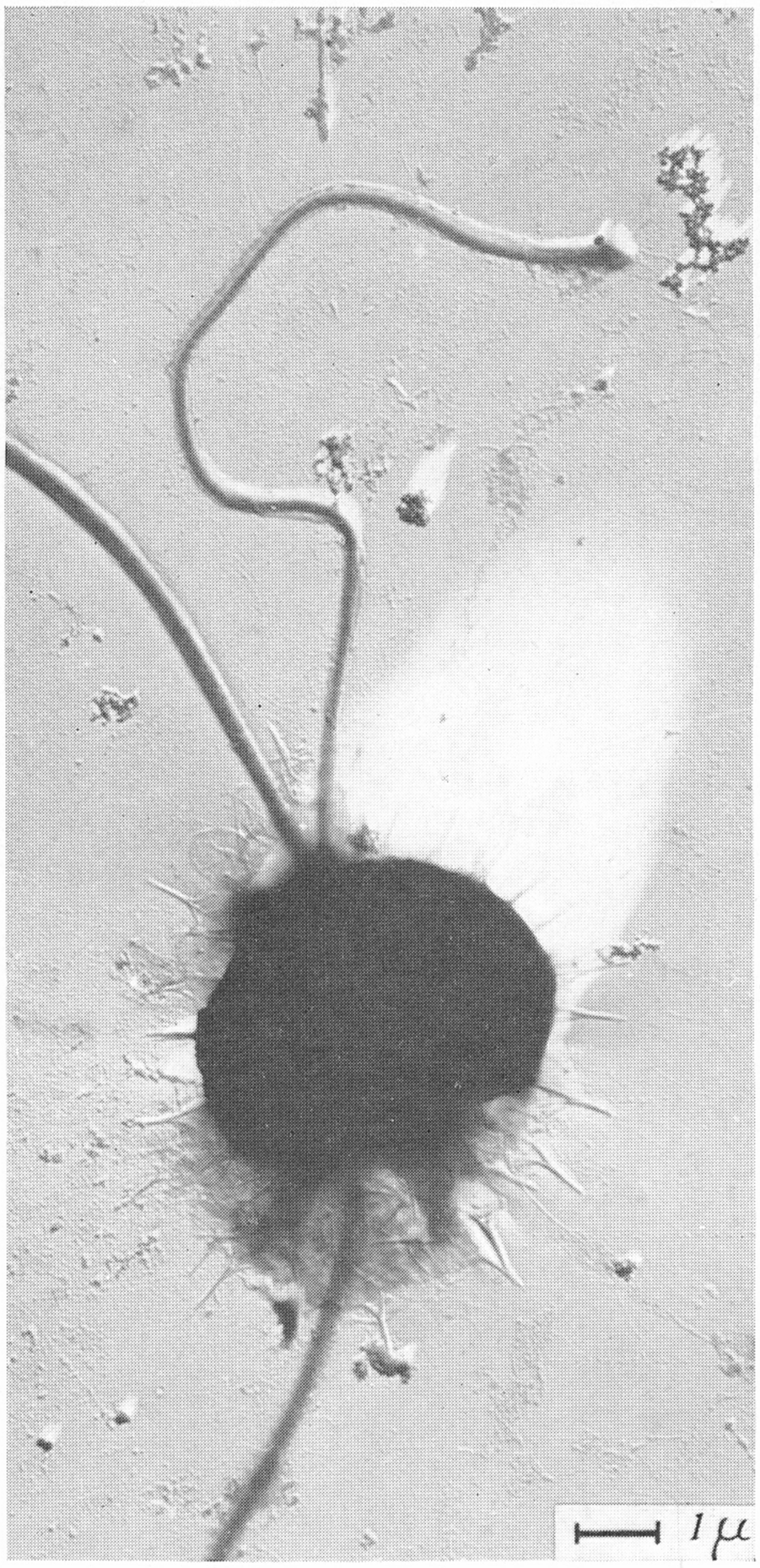
(*Facing p.* 604)



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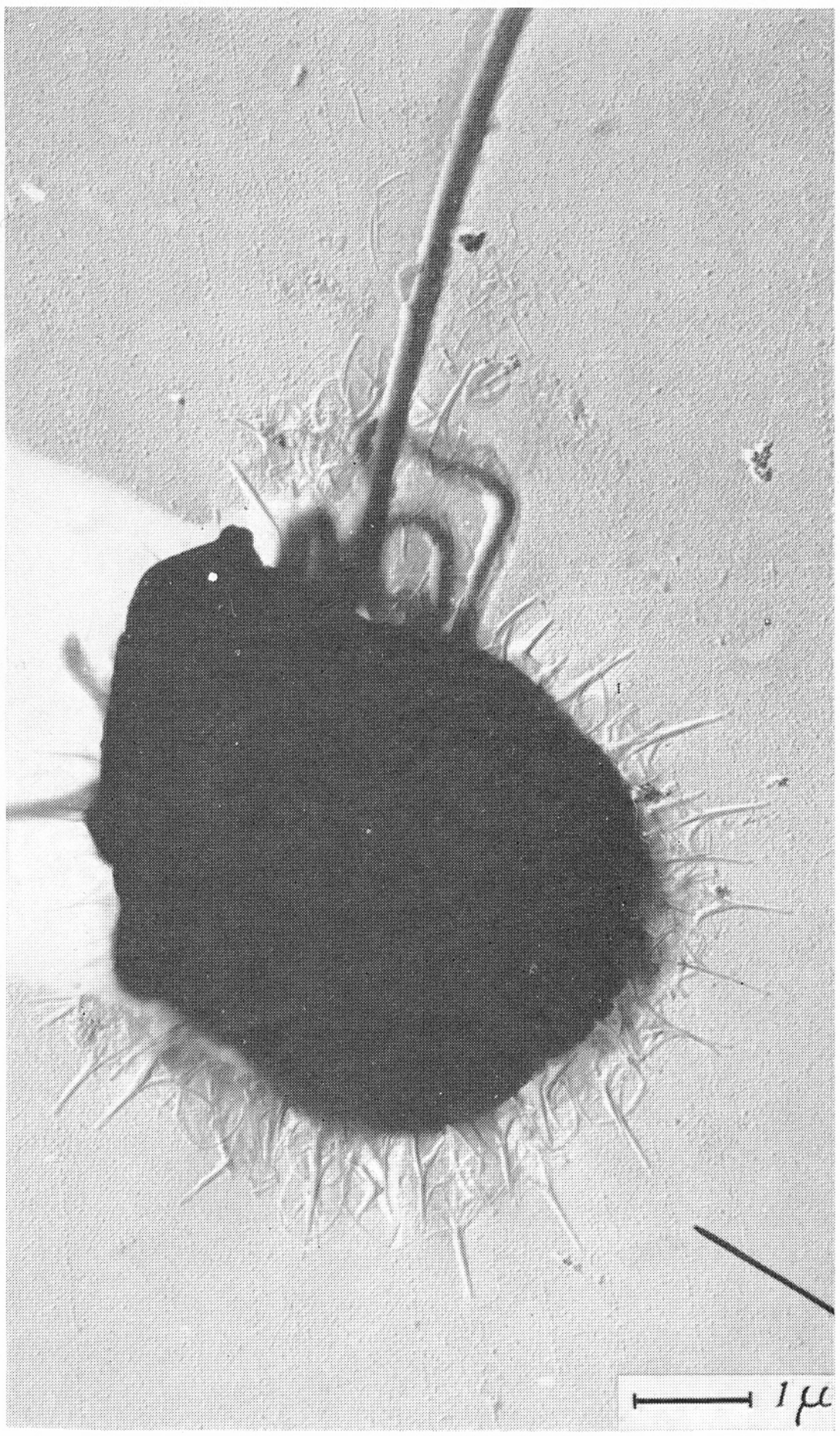

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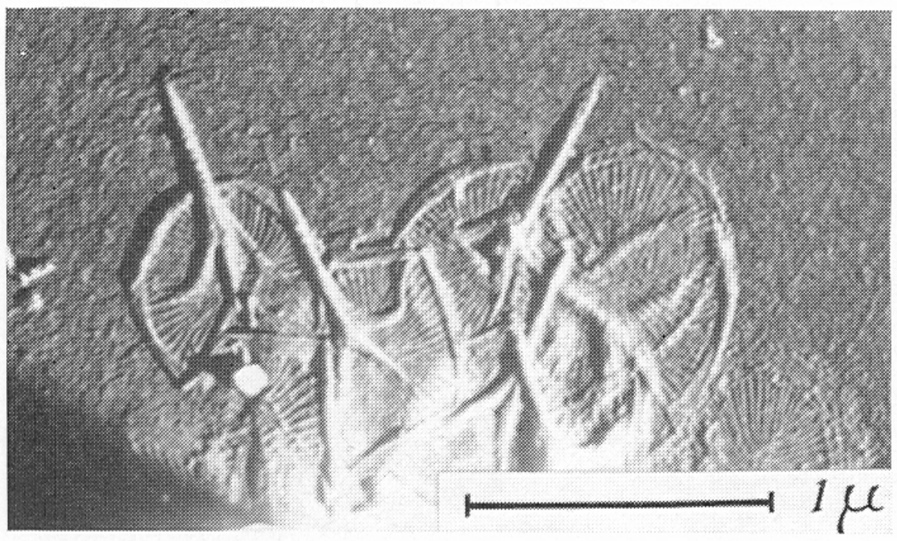

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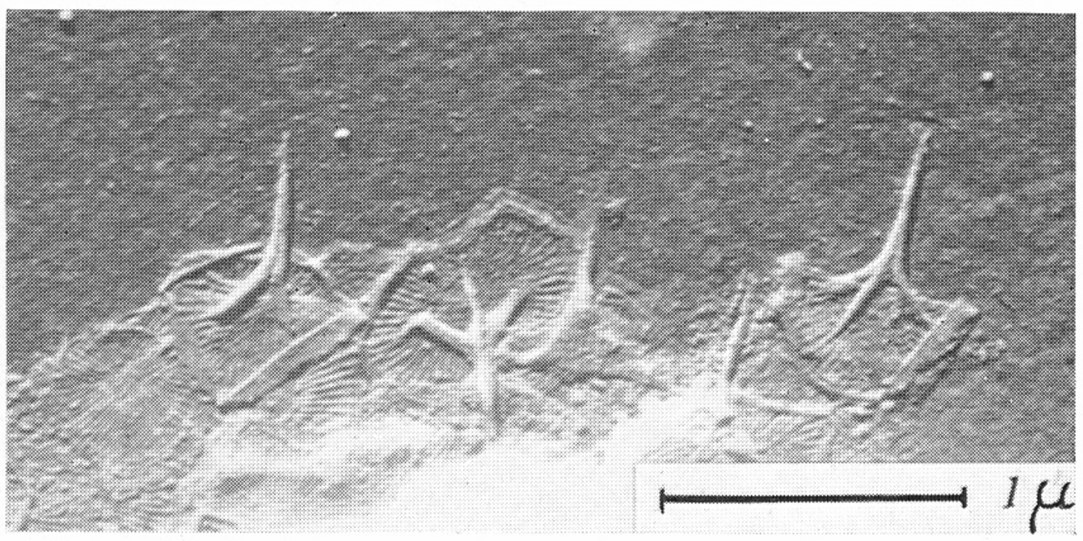

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(*Facing p.* 605)

pyrenoid-like bodies also react in the same way to stains and Millon's reagent as do those of *C. kappa* and *C. minor*. They are small and are excentrically placed near to the periphery of a chromatophore, as in *C. minor*, but in *C. brevifilum* they lie on the side away from the flagellar pole (Figs. 65–69). They are frequently surrounded by non-refringent material (Fig. 65).

The position of the nucleus, leucosin vesicles and oil globules is as in *C. kappa* and *C. minor*. The muciferous bodies are much larger and more conspicuous in *C. brevifilum* than in the two previous species. When the cell body is more or less sphaeroidal they appear to lie in the peripheral cytoplasm in four rows, running from the flagellar pole to the opposite pole, with additional ones at the non-flagellar pole (Fig. 65). Their position, however, does not remain constant but changes with the metaboly of the body (Figs. 66–69). When they discharge their contents rapidly, short rods are ejected, but if discharge is slow the contents ooze out to form a balloon which eventually becomes detached from the cell as a sphere which can remain visible for some considerable time.

In this species granules, possibly mitochondria, lying in the same position as the muciferous bodies, again stain bright blue with cresyl blue.

As in the two previous species, the flagella and haptonema are usually directed backwards when the cells are moving most rapidly; the flagella are not splayed out from the body as in *C. kappa*, but lie rather close together (Fig. 65) as in *C. minor*; the haptonema can be coiled up close to the body, partly extended, or sometimes, when movement slows down, even wholly extended straight out behind the body.

This species does not swim very rapidly. It has a slowish rolling movement rotating and gyrating, moving only for short distances; it then slows down, almost stops and in a very short time shoots off again with almost a jump usually in a different direction. This jumping type of movement is seen frequently: a cell jumps or jerks a short distance, stops, then jumps again and stops and can continue doing this for several minutes. This type of action is probably connected with a sweeping movement of the flagella characteristic of this species. The flagella lying either out from the body, or close to the body (Fig. 69), appear to stiffen suddenly and they are then brought close together into the position shown in Fig. 68 by a quick sweeping movement. This quick movement causes the cell to jump. Swimming with the flagella and haptonema in front of the body (Figs. 68–69) is seen more often than in *C. kappa* and *C. minor*. As in the two previous species, a marked phototactic response is shown although again there is no obvious stigma.

Phagotrophy occurs commonly in *C. brevifilum*. The cells readily ingest bacteria or graphite up to a size of 2μ, occasionally up to $2{\cdot}5\mu$, in the same manner (Figs. 76, 77, Pl. VIII; Figs. 66–69) as described for *C. kappa*; small cells $4{\cdot}5\mu$ in length have been found to contain graphite masses as large as 2μ in diameter. In this species the ingested material always remains very close to

the non-flagellar pole (Figs. 66–69) and apparently is not moved about inside the body as in *C. minor*.

Temporary attachment of the cells by means of the haptonema (Figs. 66, 67) takes place in a similar manner to that described for *C. kappa*. The usual dancing of the body occurs when the attached haptonema is very little extended (Fig. 67) and the cell, as in the previous species, could then be mistaken for a *Prymnesium*. The exact details of configuration of the coiled part of an attached haptonema are more difficult to determine than in the two previous species as the body usually obscures the whole organ; when seen, however, it appears to be coiled in a flat spiral as described on p. 591. When the attached haptonema is fully extended (Fig. 66) the cell can frequently be seen to swing round in a circle from the central anchoring point.

Fission in the motile stage (Fig. 74, Pl. VIII) can be equal or slightly unequal and is similar to that described for the two previous species, except that here the two new flagella and the second haptonema tend to be formed before there is any broadening of the cell preparatory to the actual fission (Fig. 69). Double-fission stages (Fig. 70), $8{\cdot}5 \times 10{\cdot}5$ to $9{\cdot}0 \times 11{\cdot}5 \mu$, are seen much less frequently in this species than in *C. kappa* and *C. minor*. When seen, however, the four haptonemata are sometimes fully extended as shown in Fig. 70.

As in the two previous species the motile phase, after the peak of growth has been reached (1 to 2 million cells per ml.), eventually settles on the bottom of the flask and passes into the amoeboid phase, the cells of which develop finely lobed, very pale chromatophores (Fig. 71). In this species, as in *C. minor*, the products of the first fission can either remain in the parent membrane or become free. The second fission is usually equal, but can be slightly unequal. The walled daughter-cells (Fig. 72), produced by this fission, are usually ovoid with one side rather flattened; they range in size from $2{\cdot}5 \times 3$ to $4 \times 5 \mu$. Empty walls from which the motile stage probably has been liberated have so far not been seen in this species. As in *C. kappa* and *C. minor*, if fresh culture-solution is added to a flask containing only the non-motile stage, the motile stage is produced and the skin disappears at approximately the same rate as in *C. kappa*.

Discussion

It is obvious that many details of structure, behaviour and life history are still uncertain in all of these species, some of which can be clarified only by much further work. A conspicuous omission, which we hope shortly to remedy, is a report on the micro-anatomy of the cells and their appendages by means of thin sections and the electron microscope. Had we delayed the present description until this side of the programme could be completed, however, it would have held up publication of existing facts inconveniently long, at

the risk also of producing a communication too large for publication in one piece.

On the experimental side we have not so far succeeded in observing the actual process of production of the motile phase from the dormant non-motile phase, nor have we demonstrated any process of sexual reproduction in these forms though negative evidence does not exclude it. There is indeed suggestive evidence, from the apparently invariable production of the small, walled daughter-cells in fours that here, if anywhere, is the place to look for some more direct indication of either a sexual fusion or a meiotic process without which these stages cannot be further interpreted.

With regard to that most characteristic organ, the *haptonema*, we can by no means yet specify its full biological significance in spite of the care with which its behaviour has been examined. That the detection of phagotrophic feeding was an immediate consequence of this examination can perhaps too easily suggest that there is a causal connexion. Such a suggestion admittedly is strengthened by finding that phagotrophic feeding is in fact practised by all our new organisms in which a haptonema has been demonstrated, and it was also reported in the analogous case of *Prymnesium saltans* by Conrad (1941). We cannot, however, state that a haptonema or analogous organ is essential to such feeding since this is also practised by the non-motile amoeboid stages and by other flagellates lacking the organ, notably some freshwater species of *Ochromonas* (Lund, 1942; Pringsheim, 1952) and *Chromulina* (Hutner & Provasoli, 1951). The reasons for the apparent association of a haptonema with phagotrophic feeding in the marine plankton are therefore not as self-evident as might have been expected and such description as we have so far been able to give of its behaviour has not yet provided the complete answer.

The formidable task of a biochemical analysis of the nutritional needs with respect to which phagotrophy is practised has been outside our scope of inquiry, though it would obviously be a matter of the very greatest interest if it could be undertaken as successfully as in recent work on *Ochromonas* (Pringsheim, 1952; Hutner, Provasoli & Filfus, 1953; Hutner & Provasoli, 1955). Another field in which we still lack information concerns the relative frequency of these organisms in the sea. We hope at a later stage, when a greater number of the commonest flagellates have been isolated and described, to attempt to obtain some quantitative figures for their frequency. At present, however, the undescribed species are so numerous that a quantitative estimate of individual species is scarcely obtainable. The most that we can state is that this type of organism is abundant both in species and in individuals, previous failure to detect them being solely due to their fragility and not at all to their rarity. In the total metabolic turnover in the plankton as a whole, therefore, they cannot fail to be playing an integral and perhaps an important part.

SUMMARY

Diagnoses and descriptions are given of three new species of marine plankton flagellates in the class Chrysophyceae. All possess two equal, homodynamic flagella and a third filiform appendage to which the name *haptonema* has been given. Phagotrophic feeding has been demonstrated in all. The descriptions include structural details visible only with the electron microscope as well as observations on behaviour and life-history visible only in the living material. The reasons for the use of the generic name *Chrysochromulina* Lackey are discussed in a preliminary way.

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STUDIES ON PIGMENT-ACTIVATING SUBSTANCES IN ANIMALS

I. THE SEPARATION BY PAPER ELECTROPHORESIS OF CHROMACTIVATING SUBSTANCES IN ARTHROPODS

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(Plates I and II in colour and Text-figs. 1–6)

Introduction

It has been established that many substances can bring about a change in position of pigments in crustacean chromatophores (Florey, 1951). The extracts of certain animal tissues (pituitary, crustacean sinus-gland and post-commissure organs, insect brain and corpora cardiaca) are especially active (Brown, 1940; Knowles, 1953; Dupont-Raabe, 1952; Thomsen, 1943); there is evidence that these tissues intervene in the normal colour change of the animals which possess them and that their products may properly be considered as hormones. On the other hand, many species which do not themselves possess chromatophores (oligochaetes, molluscs, and many insects) have nevertheless been shown to contain substances in their tissues which will, after injection into crustaceans, initiate pigment movements (Scharrer, 1954). It has not yet been ascertained whether these pigment-activating substances chemically resemble normal colour-change hormones or whether the pigment movements they produce are pharmacodynamic effects irrelevant in the study of colour physiology.

Many workers have made reciprocal-injection experiments in their studies of colour-change, and have found that for the most part the pigment-activating hormones are not species-specific, a fact which might seem to indicate some degree of chemical resemblance between chromactivating substances, though a number of observations have indicated that a chemical identity is unlikely (Brown, 1944). The interpretation, however, of the early reciprocal-injection experiments is complicated by the fact that many workers have referred to pigment changes after the injection of extracts without specifying with sufficient precision which pigments responded. The colour patterns of crustaceans

are very complex, and it is now recognized that chromatophores with apparently identical pigments may differ physiologically in relation to their position on the body (Brown & Wulff, 1941; Knowles, 1955) and that, for instance, an injection may be followed by the concentration of some red pigments but by the dispersal of others. Injection and extirpation experiments have given rise to the suspicion that in a single crustacean species there may be a number of distinct chromactivating substances, each playing a specific role in the changing colour pattern of the individual. The probability of many colour-change hormones in a single individual makes comparison by reciprocal injection experiments difficult to assess, and it is clear that the ultimate resolution of the problem of the number and the identity of the various pigment-activating substances which have been extracted from animal tissues must await chemical separation and purification.

Chemical studies on the pigment-activating substances of crustaceans have hitherto been restrained by the difficulty of obtaining adequate material for analysis. For this reason the attempts by Abramowitz (1940) to purify the crustacean eye-stalk extract did not yield conclusive results (Brown, 1944). The techniques of paper chromatography and of paper electrophoresis overcome to some extent the problem of the separation of minute quantities of substances for study, and we have therefore used these methods in the present studies on pigment-activating substances. In this preliminary paper we give an account of experiments designed to provide at least a partial solution to two fundamental problems in the endocrinology of colour-change in arthropods, namely (1) whether there are chemical similarities between the pigment-activating substances which have been extracted from the sinus-glands and the post-commissure organs of crustaceans and from the brain and the corpora cardiaca of insects, and (2) how many pigment-activating substances are present in a single crustacean or insect species.

Materials and Methods

Animals

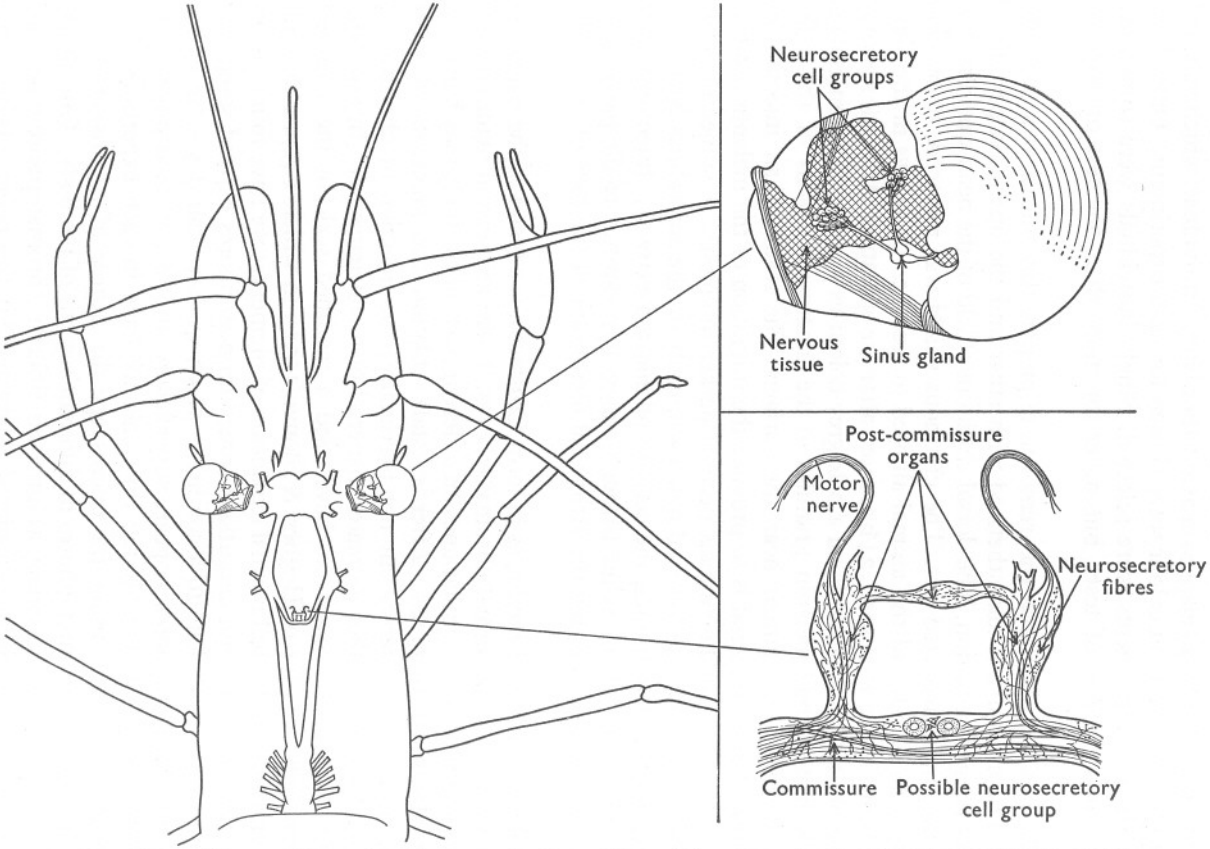
Three species of animals were used for testing the chromactivating potency of extracts, the crustaceans *Leander serratus* and *Crangon vulgaris* and the stick insect *Carausius morosus*, although others were used in some of the experiments as sources of extracts. *Leander* and *Crangon* were taken from various habitats near Plymouth and Roscoff, and stored until required for use in the aquarium tanks in running sea-water. *Carausius* were taken from the colony maintained by one of us (M.D.-R.) at the Sorbonne, and from that in the insect house of the Zoological Society of London.

Leander and *Crangon* were prepared as test subjects for this work by eye-stalk ablation. One eye of a male or non-ovigerous female was removed at the base by cutting through the arthrodial membrane with sharp-pointed scissors;

no precautions of sterility or of cauterization were taken. Twenty-four hours later the other eyestalk was removed in the same way. Animals were used after a further 24 h had elapsed or at any time later. Individuals which matched each other perfectly in colour were chosen for each experiment. Only those with dispersed pigments were selected. Single individuals were sometimes used for a number of tests, but never for more than one test on any one day.

The brain of *Carausius* was removed to prepare this animal as a test subject. The animal was anaesthetized with ether and the operation performed under Ringer's solution. The dorsal chitinous shield of the head was removed and the brain dissected out. The chitinous shield was replaced, to be held by clotting blood, and the animal allowed to recover. *Carausius* in the sixth or seventh instar were used, 24 h or more after the operation. After the removal of the brain *Carausius* takes on a pale grey colour as a result of the migration of the hypodermal melanin granules to the innermost depths of the cells, thus leaving less pigment near the surface. The result of an injection of chromactivating material is to provoke the migration of the melanin towards the surface so that the animals become darker in colour. A subjective scale of degree of darkening assessed macroscopically on the animal has been used throughout in determining the activity of injected extracts. Injections were made of about 0·05 ml. saline through a glass hypodermic needle passed in a forward direction through the arthrodial membrane at the base of one of the legs.

Colour change in *Leander*, as in most Crustacea, is a result of the migration of pigment in hypodermal chromatophores. There are several main types of these, and apparently similar ones on different parts of the body may behave in quite dissimilar manners. After eyestalk ablation the pigments of most chromatophores disperse, but not maximally; the injection of chromactive extracts produced either a greater dispersal or a concentration. In assessing colour change in *Leander* we have used a modified scale of the accepted chromatophore index of Hogben & Slome (1931), in which 1 represents full concentration and 5 full normal expansion of pigments; we have found, however, that under certain conditions some chromatophores expand some way beyond the normal state to a condition which we have called 5+, in which adjacent chromatophores appear, superficially at least, to anastomose (see Knowles, 1955, fig. 12). Using this scale we have in each experiment endeavoured to assess under the microscope the degree of expansion of the various types of chromatophores in different regions of the body. Our observations were made on as many as nineteen different chromatophore types, but only a few of these will be considered in any detail in this paper. Assessments were made 15, 30, 60 and sometimes 120 min or more after injection. Injections were made through a no. 28 steel hypodermic needle passed forward through the arthrodial membrane between the terga of the third and



Text-fig. 1. Diagram illustrating the approximate positions of the sinus glands and post-commissure organs of *Leander serratus*.

fourth abdominal segments, slightly to one side of the mid-line; the needle thus entered through muscle and was passed forward, directed towards the mid-line and slightly dorsally, till the point came to lie in the pericardium. 0·05 ml., or a little more, was injected each time into the main blood stream in this way, and the lateral muscle blocks served, by their contraction, to seal the hole, and so to reduce bleeding.

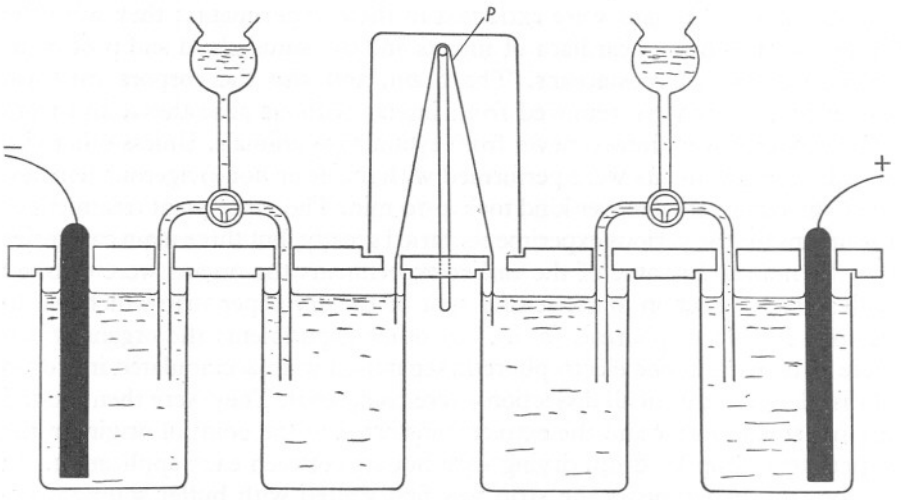
Preparation of Extracts

Four different organs were extracted in these experiments; they were the brain and the corpora cardiaca of insects and the sinus gland and post-commissure organs of crustaceans. The brain, and also the corpora cardiaca, which lie near it were removed from insects without anaesthesia and from animals which were intact, never from eyestalkless animals. Unless otherwise stated, all experiments were performed with males or non-ovigerous females. Each dissection of whatever kind took 4–10 min. The subsequent treatment of the organs for the various experiments varied greatly, but three main categories may be noted. In some of the earlier experiments the organs were crushed with forceps direct on to the central spot of the dry paper strip which was to be used for electrophoresis (*v. i.*). In other experiments the organs which were to be used in one electrophoretic separation were accumulated in a drop of distilled water until all dissections were completed. They were then ground up in a small mortar and the extract transferred to the point of origin on the paper strip, drop by drop, drying with hot air between each application. In all the later experiments the strip was first wetted with buffer solution, extractions were made in the same solution and applied to the wet paper immediately before electrophoresis; the extracts were not allowed to become dry at any time. All three methods were used in experiments on each of the four organs.

Electrophoresis

Electrophoresis was carried out upon filter-paper in two kinds of apparatus. In the earlier experiments at Plymouth the paper strip, 3 cm wide, was draped over a horizontal glass support and hung down on either side into the buffer vessels (Text-fig. 2). In the apparatus used at Roscoff the paper strips, several of which could be used simultaneously, were stretched horizontally. A more fundamental difference was that in the power pack used at Plymouth the current was stabilized, but the voltage allowed to fluctuate somewhat, while in that at Roscoff the voltage was stabilized but the current rose sometimes as much as 100% during the course of the experiment. Since it is not possible to stabilize both at the same time, it appears of more theoretical importance to stabilize the voltage, but in practice stabilization of either leads to adequate uniformity of results. In all runs phosphate buffer was used,

N/15 or N/30. Unless otherwise stated, the pH was 7·5, each run at Plymouth lasted 16 h (less at Roscoff, but variable) and the length of paper was 28 cm. The extract which was to be separated by electrophoresis was applied to the central point of the paper, midway between the electrodes. In some of the later experiments a spot of LD-leucine was placed alongside to act as a marker; this was stained with ninhydrin at the end of the run and gave some measure of the constancy of conditions from one run to the next. Whatman's paper no. 1 was used.



Text-fig. 2. A diagrammatic section through the electrophoresis apparatus used at Plymouth; *p*, the filter-paper strip.

After electrophoresis the paper was cut into sections, usually after preliminary drying with hot air. In the earlier experiments the band extending 0·5 cm on either side of the origin was cut out and numbered 0. The remaining portions of the paper were cut transversely into five equal parts, 2·7 cm wide, on each side of the origin, numbered −1 to −5 at the cathode and +1 to +5 at the anode. The arrangement of the pieces was thus:

anode +5 +4 +3 +2 +1 0 −1 −2 −3 −4 −5 cathode.

In some experiments the region numbered −1, as it proved of exceptional interest, was further subdivided into three pieces, each 0·9 cm wide, numbered $-\frac{1}{3}$, $-\frac{2}{3}$, and −1. In the experiments performed at Roscoff, bands 1 cm wide on each side of the origin were cut out separately and numbered +0 and −0; the other bands, each 2 cm wide, started correspondingly farther out from the centre than in the Plymouth experiments. These various bands were eluted with 0·2 ml. of an appropriate saline or sometimes distilled water

for a period of 15–30 min. The eluates were then injected, as described above, and the results recorded.

Further details of special methods used are mentioned in the experimental sections.

The Post-Commissure Organs

The effect of an injection of a post-commissure extract into an eyeless prawn differs significantly from the effects produced by the injection of a sinus-gland extract. A sinus-gland extract concentrates the small red and the large red chromatophores of the body and of the tail and is generally without effect on the white pigments (sometimes a slight concentration is observed after injection). During the first half hour after injection the effects on the large red chromatophores and on the small red chromatophores are indistinguishable, maximum contraction being produced in both type of chromatophore, but later the large red chromatophores begin to re-expand before any change in the small red chromatophores can be observed. On the other hand, injection of a post-commissure extract is followed by a contraction of all chromatophore pigments (including the white pigments), which lasts for 5–10 min., but thereafter the small red chromatophores of the body and certain red chromatophores of the tail begin to expand and in due course become more expanded than they had been before the injection. The large red and the white chromatophores, however, remain contracted for an hour or longer. Brown & Klotz (1947) reported that they could extract two substances from the commissure of *Crago* (= *Crangon*) by making use of differential solubility in alcohol. One of these (which they called CBLH) concentrated dark pigments in the body and tail of *Crago*, and the other (CDH) in the absence of the first, darkened the animal by promoting expansion of the dark chromatophores of the body and of the tail. We have by electrophoresis separated these two substances and have shown that there are also other chromatophorotropically active substances in the post-commissure organs.

The electrophoresis of post-commissure organ extracts was carried out at Plymouth and at Roscoff. In the Plymouth experiments care was taken to ensure that the current did not fluctuate during an experiment, but stabilization of the voltage was not complete. A relatively low current, ranging from 0·2 mA to 1 mA/cm width of paper was applied in this first series of experiments. In a later series of experiments, carried out at Roscoff, higher voltages, higher current strengths and shorter times were employed than in the first series and the apparatus used delivered a stabilized voltage, but the current increased during the course of an experiment. Each extract of the post-commissure organs was generally prepared from that portion of the post-commissure complex posterior to the commissure, namely the two post-commissure nerves and their lamellae (Knowles, 1953); in none of the later experiments

did the extract contain any commissure material. The post-commissure organs used were dissected from large male individuals or non-ovigerous females, measuring 50–55 mm in length.

Plymouth Experiments

A series of experiments was carried out at Plymouth during January and July–August, 1954. In the preliminary experiments some of the commissure material was included with the post-commissure organs when the extracts were made. Each extract contained ten post-commissure organs and the extract was allowed to stand at room temperature while the organs were being dissected, an operation which generally took up to an hour to complete. The extract was then applied to the centre of a strip of Whatman no. 1 paper and dried under an electric hair-drier; after this operation the paper strip was subjected to electrophoresis, sometimes immediately, but usually a period of about $\frac{1}{2}$ h was allowed for equilibration before the current was switched on. In later experiments at Plymouth the organs from five individuals were used each time and the extracts were applied immediately to the paper: they were not allowed to stand at room temperature.

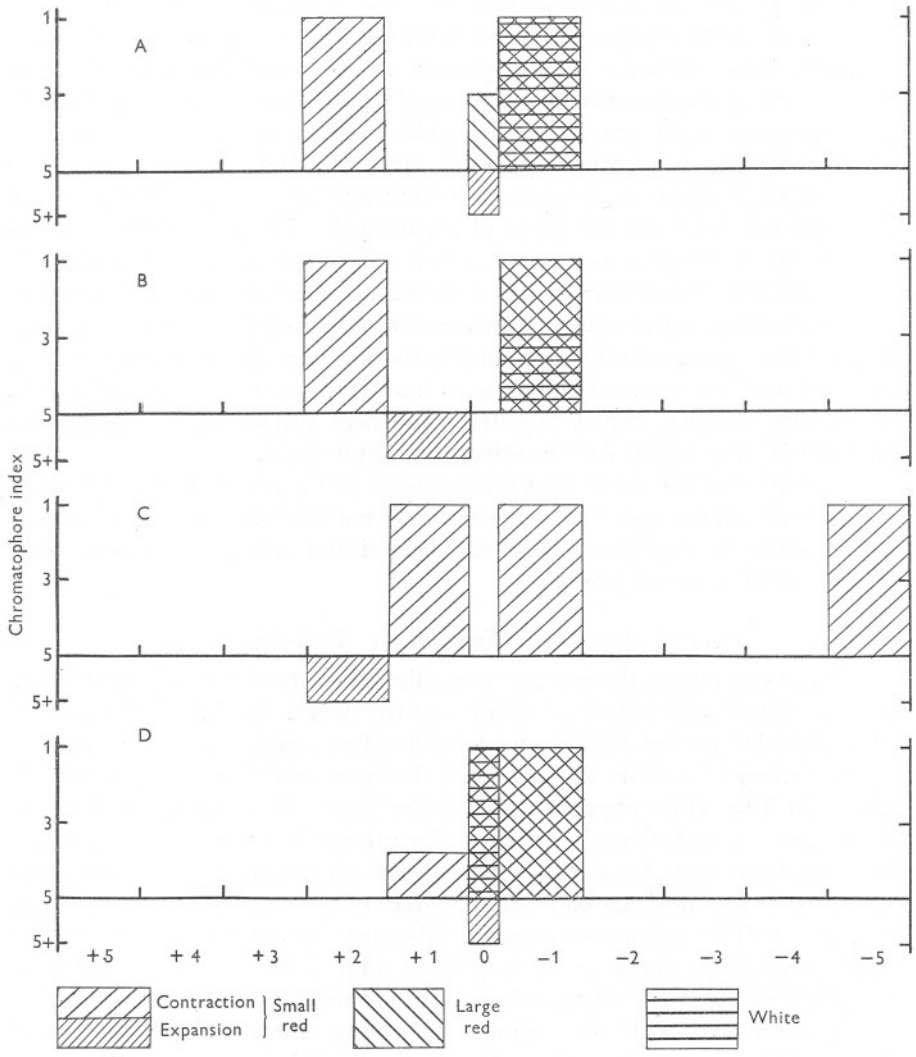
After electrophoresis the strip was dried once more under an electric hair-drier and then cut into portions each 2·7 cm long, with the exception of the central portion, containing the point of application of the extract, which was only 1 cm long. Each portion was then eluted separately in distilled water and the resultant solutions were injected into prawns from which the eyes and their stalks had been removed a week previously. The resultant effects are shown graphically at Text-fig. 3, in which the generally accepted chromatophore index has been modified slightly to give a means of expressing an expansion of pigments greater than that normally found in eyeless individuals. This extraordinary expansion is referred to as 5+.

Immediate Application (Text-fig. 3A)

In three experiments the organs were macerated first in water and then in methanol. Aqueous and alcoholic portions were applied separately to point of origin immediately after removal from the body and the resultant extract was applied within a few minutes to the paper which was then dried. It was hoped in this way to reduce to a minimum the possibility of chemical change of the extract before electrophoresis began. Electrophoresis was carried out at pH 7·5, 320–350 V, current 0·3 mA/cm, applied for 16 h.

In each of these experiments three portions of the paper yielded active extracts after electrophoresis (Text-fig. 3A), namely +2, zero and −1, and the effects of these extracts were different.

An injection of the −1 extract was followed by a contraction of all the chromatophores of the body and of the tail, namely the large red, the small red and the white chromatophores. The effects of this extract resembled those



Text-fig. 3. Diagrams of the results of typical experiments with injection of post-commissure extract into *Leander*, after electrophoresis. In each graph the horizontal axis represents the length of the paper strip, cut into numbered portions (see section on methods), and the vertical axis the chromatophore index after 60 min, in the prawns which were injected with the eluates of the corresponding fragment of paper. Only striking departures from the initial chromatophore index at the beginning of the experiment are recorded; minor variations are omitted. A, after immediate extraction, pH 7·5, 18° C, 16 h, 320–350 V, 0·3 mA/cm; B, organs left 1 h in water at room temperature before extracting, pH 7·5, 18° C, 16 h, 230–250 V, 0·2 mA/cm; C, organs left 4 h in water at room temperature before extraction, pH 7·5, 18° C, 16 h, 430–460 V, 0·5 mA/cm; D, extract boiled, pH 7·5, 18° C, 12 h, 230–250 V, 0·2 mA/cm.

produced by the −1 extract yielded by sinus gland and corpus cardiacum extracts but differed from them by reason of the presence of the white-pigment activator. The substance at −1, present in the three organs enumerated, we have called the A-substance (Carlisle, Dupont-Raabe & Knowles, 1955).

An injection of the zero extract was followed by a contraction of the large red chromatophores of the body and of the tail and of the white chromatophores of the body but by an expansion of the small red chromatophores of the body and the tail, especially those of the uropods. The general effect of this injection was to produce an individual with a dark body and with a scarlet tail; during the first 10 min after injection the bands of colour on the body turned blue as the large red chromatophores contracted sharply and a blue pigment diffused from them into the surrounding tissues; later this blue pigment disappeared and the bands of colour were hardly apparent. A maximally dark prawn with minimal display of pattern had been produced. We have called the material responsible for this effect the B-substance.

An injection of the +2 extract was followed by the contraction of the small red chromatophores only; the large red chromatophores remained dispersed and the white chromatophores were not affected. We call such a substance with a single effect an α-substance.

Delayed Application Experiments (Text-fig. 3B, C)

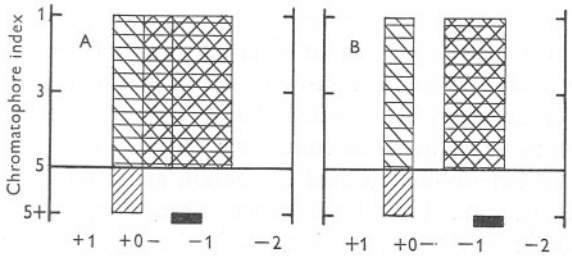
In four experiments the extracts were allowed to stand for an hour or more at room temperature before application to the paper. In one experiment the extract was left on the damp paper for 6 h before electrophoresis began.

The pattern of activity differed from that previously described in several ways. (1) The white pigment concentrator gradually disappeared from its position at −1, and after 3 h was no longer present in a detectable concentration. Sandeen (1950) has already reported that a white pigment-concentrating substance slowly disappeared from an extract of the tritocerebral connectives of *Uca*. (2) The pigment-dispersing substance becomes more mobile and moves towards the anode as far as 4 cm or more from the point of application of the extract. The effect of large red chromatophore concentration previously associated with the pigment-dispersing substance was no longer present, and one may speculate whether the large red chromatophore concentrating substance was originally linked to the pigment dispersing substance, and that the rupture of this link rendered the dispersing substance more mobile. If, this is so, the B-substance contains two components active on red pigments; one ('B_X') disperses red pigments; the other ('B_C') concentrates the red pigments of the large red chromatophores. (3) The −5 portion of the paper yielded an extract which concentrated the small red chromatophores. This additional α-substance had not been detected in the immediate extraction experiments and it seems likely to be a disintegration product of some larger molecule.

Roscoff Experiments

The first series of experiments having shown that when fresh extracts were used the greater part of the substances with effect on the chromatophores were relatively immobile under the conditions used in the experiments, a further series of experiments were undertaken with the express intention of separating those relatively immobile substances. A higher voltage and current were used but for a shorter time; and the zero portion of the paper was cut into two portions, one on each side of the centre, in order that any slight migration might be detected.

In the second series of experiments the extracts, which were made in buffer solution, were not allowed to become dry at any time; they were applied to *damp* paper, which was then immediately subjected to electrophoresis. A spot of DL-leucine was added at the edge of the paper in these experiments to serve as a marker.



Text-fig. 4. As Text-fig. 3, but showing two experiments performed at Roscoff. The black rectangle represents the position taken up by a spot of DL-leucine. pH 7·8, 18·5° C, 5 h, 320 V, 0·8 mA/cm.

After electrophoresis the still damp paper was cut into portions and extracted at once with sea water, while a thin strip along the edge of the paper was dried and stained with ninhydrin to detect the leucine. The immediate application method was used in each experiment.

The most significant feature of these later experiments was the complete separation of the A- and the B-substances (Text-fig. 4), and the demonstration that the B substance bears a slight negative charge, even when both B_X and B_C effects are produced. Another interesting feature of these later experiments was the distribution of the white-pigment-concentrating substance. At 320 V and 0·8 mA/cm it did not apparently move far from the point of application and sometimes not so far as did the A-substance; its distribution in this case strengthens the suspicion that it is not normally bound to the A-substance. If it is bound to any other substance, it would seem more probable that it may be bound to the B-substance, for in a boiled extract it is found associated with the B- rather than with the A-substance (Text-fig. 3D). This suggestion would also help to explain the otherwise somewhat baffling

distribution of the white-pigment-concentrating substances in the experiment illustrated in Text-fig. 4A. The distribution becomes more reasonable if we suppose that some of the white-pigment-concentrating substance has become detached from the B molecule but that some still remains, but we have not yet sufficient evidence to establish that the white-pigment-concentrating substance forms part of a larger molecule.

Environmental and Sex Differences

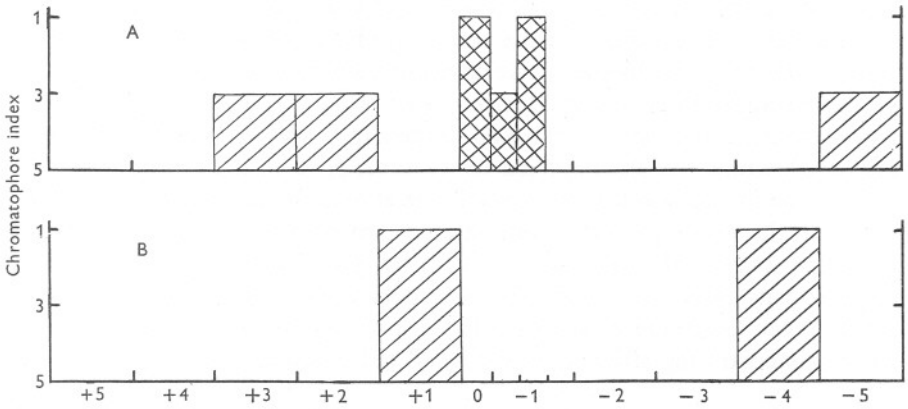
Most of the experiments have been carried out using extracts prepared from male individuals or from a mixture of males and non-ovigerous females, but we have sometimes perforce used females only. The prawns have generally been gathered close inshore in water 1–6 m in depth, but once prawns were used which had come from greater depths—40 or more metres. We have not yet sufficient data to dogmatize, and it is hoped that an investigation of environmental and sex differences, if indeed they do exist, may form the subject of a later publication, but it is worth putting on record here that the electrophoretic patterns which we have so far described may only be typical of shallow-water, male animals. As we have shown, these seem to contain a mixture of A- and B-substances, but when only females were used in the preparation of the extracts little or none of the B-substance was found. On the other hand, male prawns from greater depths seemed to contain an abundance of the B-substance, but little or none of the A-substance. These deeper-water prawns were noticed to be different from those gathered in shallower waters, being almost scarlet in colour and with maximal expansion of the small red chromatophores.

The Sinus Gland

The general pattern of these experiments resembled that of the experiments already described. Fresh and stored extracts were used from prawns of both sexes gathered from various localities in the Plymouth area. Normally ten glands were used for each extraction, but in later extractions the number was sometimes smaller.

Immediate Application Experiments (Text-fig. 5A)

In these experiments the glands were dissected out under isotonic saline, immediately crushed on to the spot of origin on the filter-paper strip and dried with a blast of hot air. Each dissection took 4–7 min. As soon as all the glands



Text-fig. 5. As Text-fig. 3, but extracts of sinus glands. Band −1 was subdivided into three. A, after immediate extraction; extract derived from large, blue, non-ovigerous females, pH 7·5, 18° C, 16 h, 320–350 V, 0·3 mA/cm; B, organs left for 12 h in water before extraction, pH 7·5, 18° C, 12 h, 320–350 V, 0·3 mA/cm.

had been applied in this manner the paper strip was placed in the electrophoresis apparatus and, after equilibration, the current was switched on. In such a run the distribution of chromactive substances was similar to that found with extracts of the post-commissure organs, except that no consistent effect was observed on the white chromatophores, nor was any sign apparent of any substance tending to provoke expansion of the small red chromatophores (Text-fig. 5A). About the middle of the −1 band (which was frequently subdivided for elution into smaller sections) was a substance provoking strong concentration of all red pigments, but with a rather stronger action on the small red than on the large red chromatophores. At zero a substance effective in concentrating red pigments was sometimes found, but we have not sufficient evidence that this is a distinct substance. At about +2 and −5 were found small quantities of more labile substances—the α-substances—acting only on

the small red chromatophores. These α-substances appeared similar in all respects to those of the post-commissure organ extracts.

In a small number of experiments a slight concentration of the pigment of the white chromatophores was found after injection of material from about −3. This may be an artifact, for the white chromatophores are directly light-sensitive and may be affected by slight alterations in the intensity of illumination (Knowles, 1940). We have, however, sometimes observed a similar effect after injection of whole fresh extracts of sinus gland (see also Knowles, 1939).

Delayed Application Experiments (Text-fig. 5B)

For these experiments the first gland dissected out was placed in 0·1 ml. distilled water, and each succeeding gland added to this. When all the dissections were completed the material was ground in a small mortar, with addition of a further 0·1 ml. water, and the extract applied, after a variable interval of time, to the dry paper by successive applications with a capillary pipette. Each successive application was dried with a blast of hot air. The residue, after the aqueous extract had been transferred to the paper, was extracted further with methanol, which was then applied to the same spot on the paper.

When an extract was kept for some hours at room temperature before application to the paper the electrophoretic pattern obtained was quite different from that found with fresh extracts (Text-fig. 5B). Little or no chromactivating substance was found at zero or −1 under these conditions, but an abundance of the α-substances was found at −4 or −5 and at +1 or +2, with a strong concentrating effect upon the small red chromatophores.

Corpora cardiaca and Brain of Insects

For the comparative study of the chromactivating substances of the corpora cardiaca and brain of insects we have chosen a species, *Carausius morosus*, which itself shows colour changes. The active substances in the extracts of these organs, after separation by electrophoresis, were tested on the living insect and on the crustaceans *Leander serratus* and *Crangon vulgaris*.

Whole extracts of the brain and of the corpora cardiaca of insects are each active upon the pigments but their effects differ, both upon insects and upon Crustacea. Whole extracts of corpora cardiaca provoke a strong concentration of the red chromatophores of *Leander* (Thomsen, 1943; Brown & Meglitsch, 1940) and the black of *Crangon* and *Ligia* (Dupont-Raabe, 1952), but their activity is only moderate upon the pigments of *Carausius*: after injection of a total extract of corpora cardiaca the animals do not take on the black colour characteristic of complete darkening, but pass only from light grey to dark grey. Total extracts of the brain, on the other hand, are completely inactive on the pigments of *Leander*, but act very strongly on the pigments of insects and

possess some degree of activity on the black pigments of crustaceans such as *Crangon* and *Ligia* (Dupont-Raabe, 1949, 1951, 1952).

The conditions of electrophoresis were as described above. Ten organs were usually used for each separation. Dissections were made rapidly under Ringer's solution ('Rabbit Ringer'); the organs immediately after injection were placed in a small quantity of distilled water, ground up and transferred directly, or sometimes after boiling, to the paper strip.

Corpora cardiaca

Injection of eluates, of different portions of the paper strip after electrophoresis, into *Leander* revealed the existence of a band at -1 which was very strongly active upon the small and large red chromatophores. It provoked a strong contraction of all the red chromatophores of the body and tail, but had no effect on the white pigments. In its effects and in its electrophoresis migration it seems comparable to substance A present in extracts of the post-commissure organs and sinus glands of *Leander*. At pH 7·5 eluates of the part of the paper near the origin and 1 cm towards the cathode (0 and $-\frac{1}{3}$) were often rather strongly active, especially upon the small red chromatophores. At pH 5·0 the migration of this substance towards the cathode was much stronger: it was not present in portions 0 and $-\frac{1}{3}$, but revealed its presence at -1 and -2.

Besides substance A, another substance is evidently present in extracts of corpora cardiaca—a more mobile substance which provokes concentration of only the small red chromatophores. After electrophoresis at pH 7·5 for 16 h at 0·3 mA/cm, 320–350 V the band corresponding to this substance was to be found at $+2$ and $+3$. With a higher current and voltage (1 mA/cm, 540–580 V) it migrated to $+5$. This substance may correspond to the α-substance found at $+2$ in sinus gland and post-commissure organ extracts, representing a disintegration product of the A-substance. Such an hypothesis is supported by the fact that this α-substance cannot be detected if electrophoresis is performed with a boiled extract (in which presumably the enzymes are destroyed), which, however, retains the A-substance, at -1 in full strength.

Substance A is responsible for the action of corpora cardiaca extracts upon the melanophores of *Crangon*. In a series of experiments in which the eluates of the central portions of the paper strip were injected simultaneously into *Leander* and *Crangon* the responses obtained, though different in kind—concentration of the red pigments in *Leander* and of the black in *Crangon*—were always coincident.

The identification of that substance of the corpora cardiaca which is active upon the pigments of insects offers some difficulties, and the results are not yet clear. It will be recalled that total extracts of corpora cardiaca provoke only a moderate darkening of insects, and after electrophoresis, with its attendant losses of activity, the concentration in the eluates is lower and the

reactions obtained still weaker, so that the delimitation of the zones of activity is difficult. The positions of the active zones, so far as they have been determined, sometimes coincide with substance A and substance α, but sometimes we have obtained no response at -1, while positive responses have been observed with the eluates of portions of the paper inactive upon crustacean pigments, notably $+1$ and -4. Thus it seems improbable that it is the A- and α-substances in total extracts of corpora cardiaca which influence the insect pigments, the more so as in the only two exploratory experiments we have performed with sinus gland extracts we have not found an exact correspondence in position of substances active upon insects and Crustacea. In later experiments, performed at Roscoff, with a higher voltage and an apparatus permitting several simultaneous runs, a detailed comparison was made, in the central region of the paper from $+2$ to -2, between the positions of substances active upon insects from extracts of the corpora cardiaca and brain. These experiments showed clearly that the tritocerebral chromactivating substance of the brain (substance C) which was so strongly active upon the pigments of the insect, was not present in extracts of corpora cardiaca.

The Brain

Electrophoresis of extracts of the brain at pH 7·5 revealed the existence of a substance of very low mobility, which was to be found on both sides of the origin ($+0$ and -0). This substance brought about a maximal darkening of the insect, analogous to that obtained with total extracts, and also a concentration of the melanophores of *Crangon*, an effect which was, however, not like that of substance A of the corpora cardiaca. It is totally inactive on pigments of *Leander*. The position of this substance on the paper did not coincide with either substance A or substance B and we have called it the C-substance.

It appears probable that, as with substance A, the C-substance may decompose into more mobile substances of lower molecular weight; parts of the paper situated rather far from the point of application of the extract often showed quite strong activity upon *Carausius*, particularly $+3$, $+4$ and $+5$. We may call this tentatively the γ-substance(s). It will be interesting in the future to compare in detail these substances with the mobile α-substances of the corpora cardiaca which are active upon insects and to establish if they are similar or perhaps identical.

No portion of the paper provoked concentration of the red pigments of *Leander*: substance A does not exist in the brain. Injection of total extracts had revealed no activity, but this might have been due to the existence of masking by an antagonistic substance; this possibility appears to be eliminated by the results of the electrophoresis experiments. Nor has any substance been found in the brain of insects which provokes expansion of any pigments of *Leander* or *Crangon*.

The insects thus possess at least two different chromactivating substances: substance A in the corpora cardiaca and substance C in the brain, localized in the tritocerebral region (Dupont-Raabe, 1954). It is difficult to pronounce any opinion on the origin of the A-substance, present in the corpora cardiaca, and upon its significance in the physiology of the insect, since its presence does not seem to be necessary for the normal colour changes. It does not seem to have its origin in the pars intercerebralis of the brain, where are situated the cells from which the internal cardiac nerves run; nor in the lateral protocerebral neurosecretory cells. Extracts of these parts dissected out from the brain, and of the internal cardiac nerves, are quite inactive, even after boiling, upon the pigments of *Leander* and those of the insect. Perhaps substance A is a transformation product of substance C, arriving at the corpora cardiaca by migration along a third pair of tritocerebral-cardiac nerves whose existence has only just been discovered (Dupont-Raabe, 1955). Yet a single experiment performed with a *Carausius* deprived of its brain 5 days previously has shown no diminution in the content of A-substance in the corpora cardiaca; this would suggest (though this is against the current of present day speculation) that the cells of the corpora cardiaca themselves may elaborate substance A.

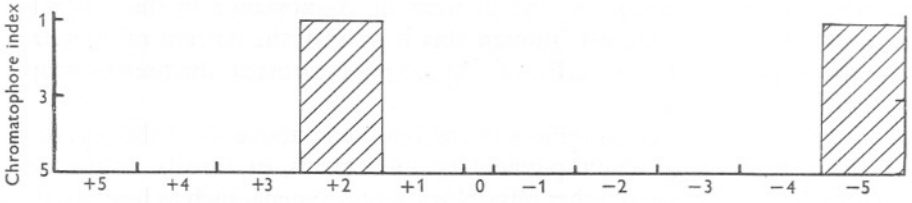
It is possible that the substances of the brain and above all of the corpora cardiaca which bring about pigmentary movements in insects, intervene equally in the regulation of other physiological phenomena, such as heart-beat, water metabolism or the genesis of pigments—all phenomena whose regulation certainly involves the brain and corpora cardiaca (Wigglesworth, 1954; Cameron, 1953; Stutinsky, 1953; L'Hélias, 1955)—and that a chromactivating potentiality is possessed in various degrees at times (perhaps incidentally) by a precursor and by different products of disintegration.

Dialysis Experiments

In these experiments extracts were dialysed against distilled water or saline through a variety of semipermeable membranes. No significant variations were observed when the following membranes were used: British Cellophane Company's cellophanes PT 600, PT 400, PT 300; British Cellophane Company's wet cellulose film ref. no. 2/468/EHD/WR; two different samples of synthetic sausage skin. All these membranes allowed the passage of eosin, methylene blue (or at least some component of it), and a pink constituent of trypan blue, but failed to allow the passage of congo red. For experiments the extract selected was placed in a cavity slide and covered with a membrane; 0·25 ml. of distilled water or of saline was then placed on top of the membrane and the whole covered with an inverted Petri dish. Under these conditions an hour's dialysis sufficed for the passage of detectable quantities of chromactivating materials from the fresh whole extracts of sinus gland of *Leander*

serratus, *Homarus vulgaris*, *Carcinus maenas*, *Maia squinado* and *Cancer pagurus* from fresh whole extracts of post-commissure organs of *Leander serratus* and of corpora cardiaca of *Carausius morosus*. When, however, extracts of the brain of *Carausius* were dialysed the dialysate was much less active than the residue; it produced its action more quickly after injection, but the action was ephemeral, disappearing after 2–3 h, while that of the residue persisted for at least 4 h.

It was evident in these preliminary experiments that not all the material in the extracts was able to pass through the membranes which we used, for the activity of the dialysate was frequently different in kind from that of the residue. This was especially noticeable with extracts of the post-commissure organs, where apparently the B-substance did not pass, for the dialysate had a concentrating effect on the red pigments while the residue had a strong dispersing effect (Pl. II, fig. 10).



Text-fig. 6. As Text-fig. 3, but extract derived by leaving intact post-commissure organs in saline for ½ h and then removing them from the saline without maceration; saline used without further treatment. Note absence of the A-substance. pH 7·5, 18° C, 16 h, 380–430 V, 0·4 mA/cm.

In order to determine which fractions were and were not able to pass the membranes dialysis was performed on the eluates of various portions of filter-paper strips after electrophoresis of the extracts. It was found that the A- and B-substances and the C-substance were incapable of passing the membranes (Pl. II, fig. 12). The more transient α-substances which are to be found at about $+2$ and -5 on the papers and the γ-substances readily passed the membranes. These results hold for extracts of sinus gland, post-commissure organ and corpora cardiaca and insect brain.

With the intention of using the cell membranes as the semi-permeable membrane for dialysis experiments, post-commissure organs were dissected out carefully with a portion of the commissures and connectives attached. These were allowed to stand in isotonic saline for half an hour and then the saline was subjected to electrophoresis. The resulting pattern (Text-fig. 6) lacked substances A and B, possessing only the more labile α-substances. In further experiments the dissection was performed as before and the preparation left to stand for five minutes in saline. When this saline was injected

it was found to contain no detectable chromactivating substances. Fresh saline was then added to the preparation and the connectives stimulated with a square-wave stimulator (100 c.p.s., 1·5–2 V) for 2 min only. Some of the saline was then subjected to electrophoresis and some of it injected direct into *Leander*. This showed a strong effect in concentrating the red pigments. The electrophoretic pattern once more lacked the A- and B-substances; the α-substances, however, were present in abundance. It appears likely, therefore, that the chromactivity of the dialysates we have tested is due to the α-substances (and γ-substances) and that the A-, B-, and C-substances, by reason of the size of their molecules or the charges they bear, do not readily pass the membranes.

Discussion

It is evident that paper electrophoresis provides a precise means of dealing with the very small amounts of pigment-activating substances that are present in insect and crustacean organs. Our preliminary studies have shown that the method provides a simpler and cleaner separation than the method of differential solubility in alcohol adopted by Brown and his collaborators, while at the same time supporting their contention that a number of different chromactivating substances are present in crustaceans. Brown & Klotz (1947) showed that the alcohol-soluble fraction of the commissure, which they called CBLH, concentrated the body chromatophores of *Crago* but was without effect on the tail chromatophores; it seems probable that this may correspond to the 'A-substance' which we have found in the extracts of the post-commissure organs as well as in the sinus-gland and the corpora cardiaca. The alcohol-insoluble fraction of the commissure (CDH) darkened both the body and the tail of *Crago*; it seems likely that this is the B-substance that we have isolated from commissure organ extracts. Clearly therefore our paper-electrophoresis studies thus far corroborate the work of Brown & Klotz, but in addition they provide evidence that other pigment-activating substances besides the A- and the B-substance may be present in extracts of the sinus-gland and the post-commissure organs.

The early work on the 'eyestalk hormone' suggested that it was chemically a very stable substance. Abramowitz (1940) found that boiling, even in HCl, appeared to increase the activity of the extracts used. Perkins & Snook (1931) reported that desiccated eyestalks would retain their activity for long periods of time. Our electrophoresis studies suggest that this appearance of stability can be to some extent illusory. While it is true that sinus-gland and post-commissure organ extracts of *Leander* still retain pigment activating potency after some hours in solution at room temperature it is clear that the active substances present after some hours are not those which are present in the neurohaemal organs immediately after they are removed from the body. It has already been reported that the white-pigment activator disappears fairly

rapidly from *Uca* commissure extracts which are allowed to stand at room temperature and that the black-dispersing factor disappears, though more slowly (Sandeen, 1950). Our experiments suggest, moreover, that a red-pigment concentrator (the A-substance) becomes chemically transformed if the extracts are allowed to stand at room temperature, although what appear to be the transformation products (the α-substances) still retain some red-pigment-activating potency. We did not find α-substances after electrophoresis of boiled extracts; this agrees with Sandeen's observation that brief boiling retarded the disappearance of activity from *Uca* extracts.

We have not yet determined whether the α-substances are normally produced and released into the blood of *Leander*; if in fact they are the true hormones and the A-substance is a precursor. The greater mobility of the α-substances and the fact that they pass more readily through a cellophane membrane than do the A- and B-substances supports the idea that they would, if present in the animal, pass freely into the blood-stream, but we cannot yet with certainty state that they are normal blood-borne substances; the evidence suggests that they are fragmentation products of the A- or B-substances, but it is possible that they may be artificially produced during the preparation of the extracts. The results of the experiments in which the connectives were stimulated electrically support the idea that these α-substances are indeed among the actual blood-borne hormones, while the A and B substances are not, but they offer no proof of this. It is certain, however, that immediate extraction of a fresh organ gives different results from a slightly more delayed extraction, and any consideration of earlier work on the chemistry of the eye-stalk extracts and deductions from injection experiments should be reviewed in the light of this knowledge. It is clear that any future studies on the chemical analysis of chromactivators must distinguish between true hormones and precursor substances. The mode of preparation of extracts would seem to be all-important in this respect.

It is interesting to find that the sinus-gland and the post-commissure organs of crustaceans possess a chromactivating substance that resembles physico-chemically a substance present in the corpora cardiaca of insects. Hanström (1940), Brown & Meglitsch (1940) and Thomsen (1943) showed that extracts of the corpora cardiaca concentrated the red pigments in crustacean chromatophores but they did not bring forward any chemical evidence to suggest that the corpora cardiaca substance resembled the sinus-gland substance. Indeed, Brown (1944) has suggested that the chromactivating content of the sinus-gland and the corpora cardiaca may differ. In his experiments in collaboration with C. M. Stuter (unpublished) he claimed that the results indicated that the sinus-glands contained a precursor of the pigment-activating hormone but that the corpora cardiaca did not. The position of the pigment-activating substances after electrophoresis of sinus-gland and corpora cardiaca extracts indicates that the A-substance in the insects and the crus-

taceans may be chemically similar though as yet we cannot prove identity. The significance of the A-substance in insects is not yet clear, unless, as our electrophoresis studies have suggested, it may itself be a precursor of other substances.

Our studies have shown that the behaviour of the brain pigment-activating substance of *Carausius* after electrophoresis differs from any pigment-activating substance that we have separated from the neurohaemal organs of crustaceans. It is also clear that this substance, which we have termed the C-substance to distinguish it from the crustacean chromactivating substances, is ineffective when injected into *Leander*. It does, however, bring about pigment-concentration in the chromatophores of *Ligia* and *Crangon* (Dupont-Raabe, 1952). We may therefore speculate whether the C-substance is absent from crustaceans, or whether it is a physiologically active colour-change hormone of some species. It is perhaps significant in this respect that *Crangon* and *Ligia* differ from *Leander* but resemble *Carausius* in the presence of melanin in their pigmentary effector systems.

It is interesting to find that the method of paper electrophoresis separates from crustacean organs more than one substance which concentrates red pigments in the chromatophores. This finding suggests that both precursor and active substances may be present in extracts of crustacean neurohaemal organs, and that the precursor substances too have some pigment-activating potency. Such a concept would provide a possible explanation for the presence of pigment-activating substances in organs of species which do not themselves possess chromatophores.

Acknowledgements

We wish to acknowledge the assistance afforded to us in many ways by individuals and institutions, and in particular we desire to thank the Nuffield Foundation and Le Centre National de la Recherche Scientifique for financial assistance, the proprietors and publishers of *Endeavour* for permission to use the colour blocks which grace this paper, and the block for Text-fig. 1, and the Zoological Society of London for the gift of a colony of *Carausius morosus*.

Summary

Pigment activating substances in the prawn *Leander serratus* and the stick insect *Carausius morosus* have been compared. Paper electrophoresis has been used successfully to separate substances in extracts. A substance which we have called substance A appears to be present in extracts of sinus glands and post-commissure organs of *Leander* and in extracts of corpora cardiaca of *Carausius*: it seems very similar or possibly even identical in all three types of extracts. It stimulates contraction of all the red pigments of the body of *Leander*. It is incapable of passing the semipermeable membranes which we

have used, and possesses a low mobility at pH 7·5. There is evidence suggesting that it is not the definitive hormone which is released into the blood, but a precursor which is split before release. What appear to be disintegration products of substance A are present in extracts and increase in amount at its expense if an extract is left standing. These substances—the α-substances—pass freely through a dialysis membrane and have a high mobility at pH 7·5. Only the α-substances are released under the effect of electrical stimulus of the commissure when the post-commissure organs are lying in a saline bath. The α-substances affect only the small red chromatophores of *Leander*.

Another substance of low mobility was found only in extracts of post-commissure organs. This substance B concentrated the pigment of the large red chromatophores only and expanded the pigment of the small red chromatophores of the body and tail. It is antagonistic to substance A. It is incapable of passing a dialysis membrane.

Substance C is present only in extracts of the brain of *Carausius*. This substance provoked a darkening of *Carausius* and concentration of the black pigments of *Crangon*, but was without effect on *Leander*. Substance C has low mobility and cannot pass a dialysis membrane.

At least two chromactivating substances are present in *Leander*, and two in *Carausius*. The mechanism of colour change appears to be totally different in the two species, but *Carausius* possesses one of the substances which is concerned in the colour change of *Leander*, and another of its chromactivating substances is active upon *Crangon*, which, unlike *Leander*, possesses melanophores. It may be that melanin is under the same control in both insects and crustaceans.

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EXPLANATION OF PLATES

Colour photographs of *Leander serratus*, taken on Kodachrome film (Figs. 1 and 2) or on Ferraniacolor film (Figs. 3–12) with electronic flash.

PLATE I

Fig. 1. A dorsal view of the cephalothorax of *Leander* illustrating some of the chromatophore types—the white reflecting chromatophores, the large red-yellow chromatophores forming the bands, and the small red-yellow chromatophores between.

Fig. 2. A group of expanded chromatophores (chromatophore index 5) from the cephalothorax; white, large and small red, and faintly under each red chromatophore its yellow component; note the red component of the white chromatophore.

Fig. 3. The edge of one of the uropods, showing all pigments moderately dispersed (chromatophore index 4–5).

Fig. 4. Two large red chromatophores of one of the uropods, moderately contracted (chromatophore index 2).

Fig. 5. The same two chromatophores moderately expanded (chromatophore index 4–5).

Fig. 6. An eyestalkless *Leander serratus* which had been injected $\frac{1}{2}$ h previously with 0·05 ml. sea water (control).

Fig. 7. A similar animal which had been injected with an extract of one sinus gland in 0·05 ml. sea water—concentration especially of small red chromatophores, thus enhancing the pattern.

Fig. 8. A similar animal which had been injected with an extract of one post-commissure organ in 0·05 ml. sea water—concentration especially of the large red chromatophores of the bands, thus obscuring the pattern.

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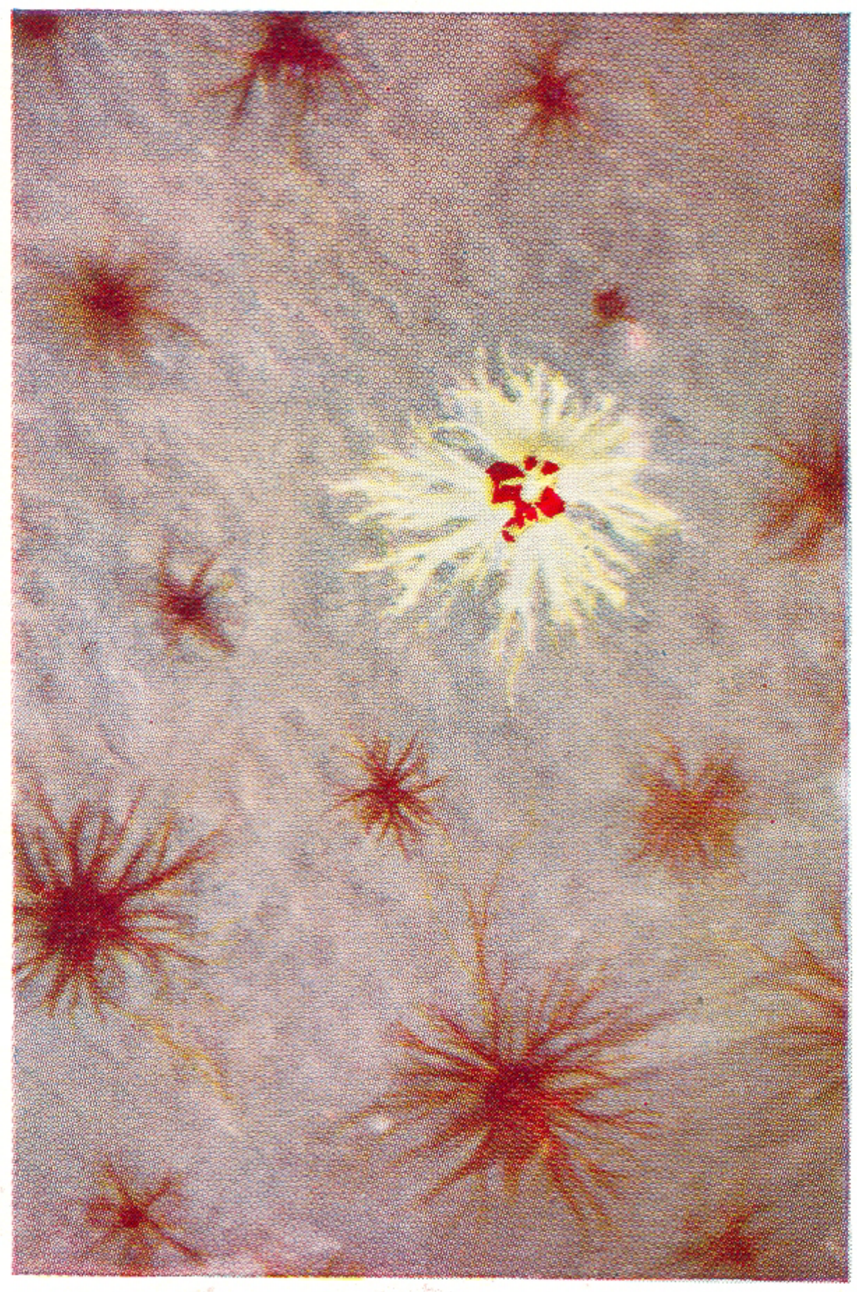

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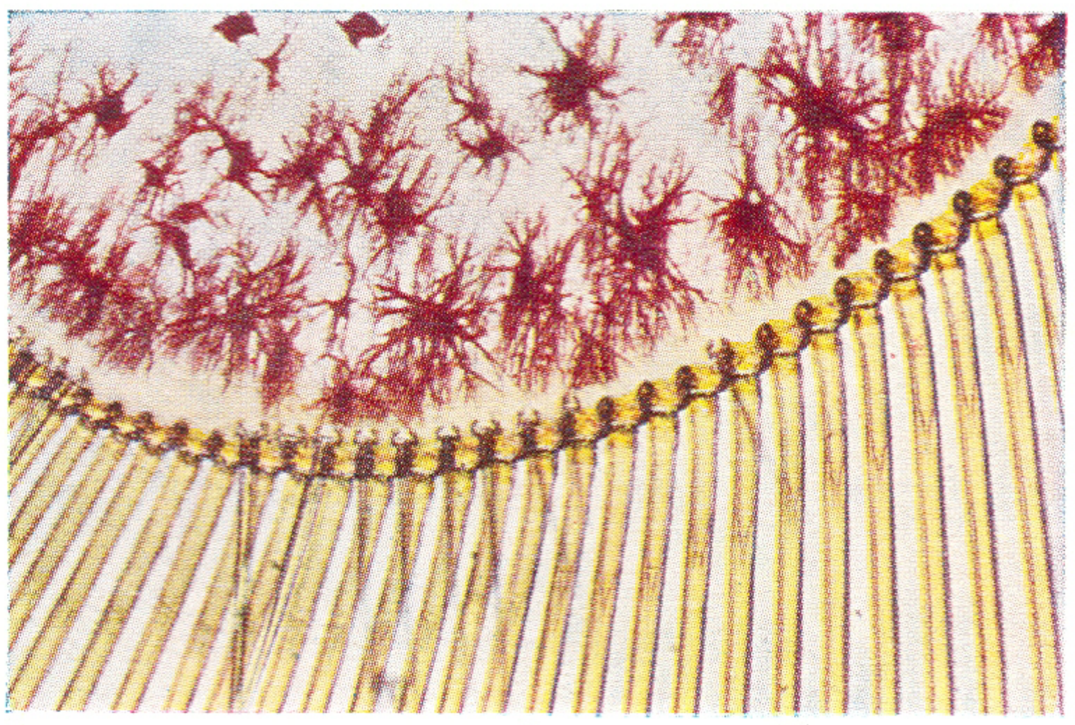

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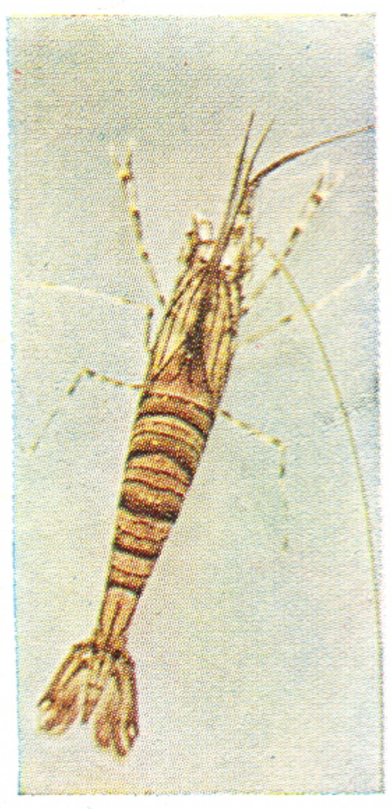

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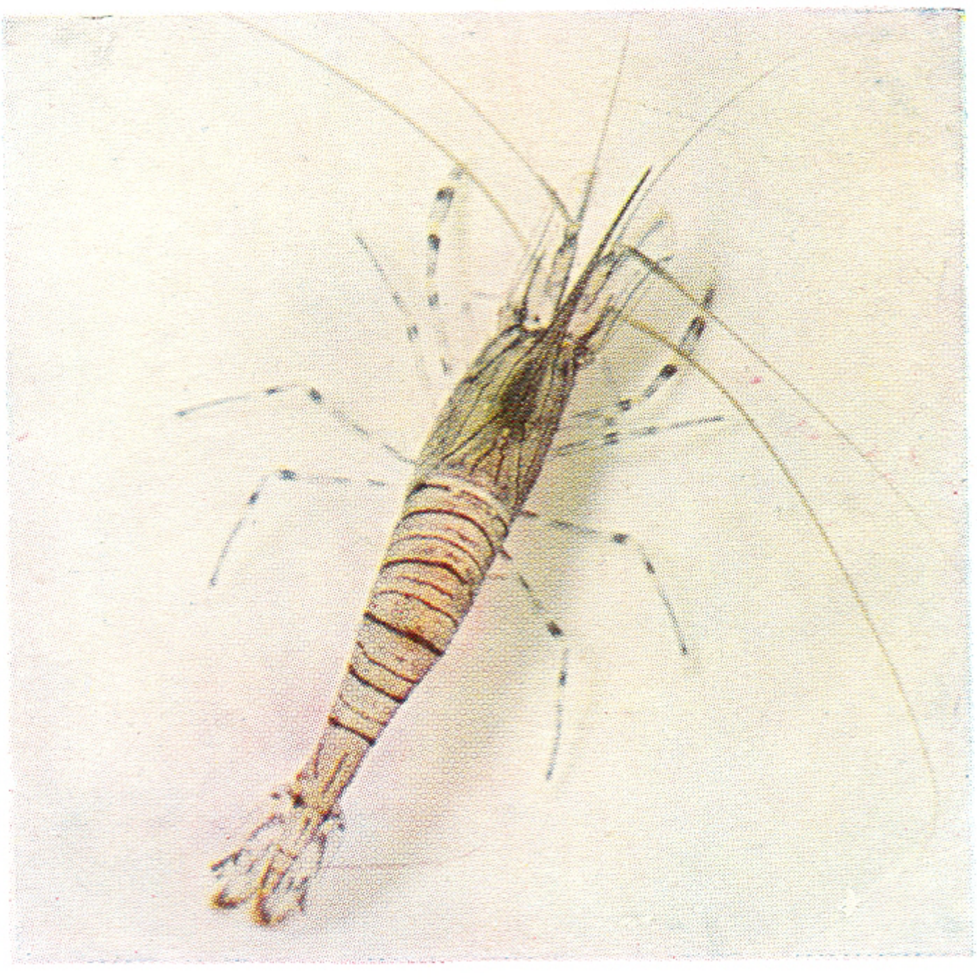

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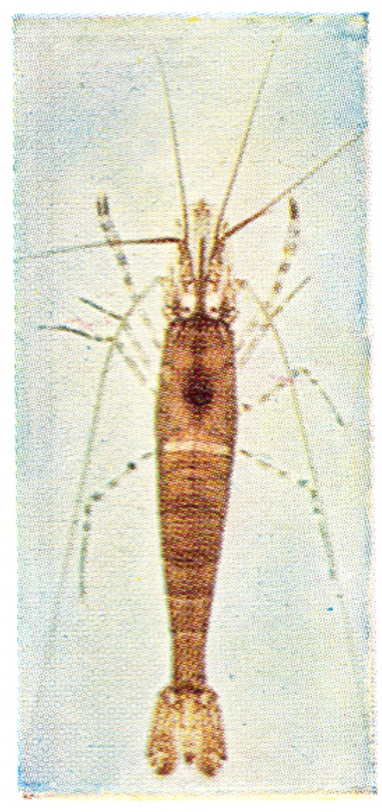

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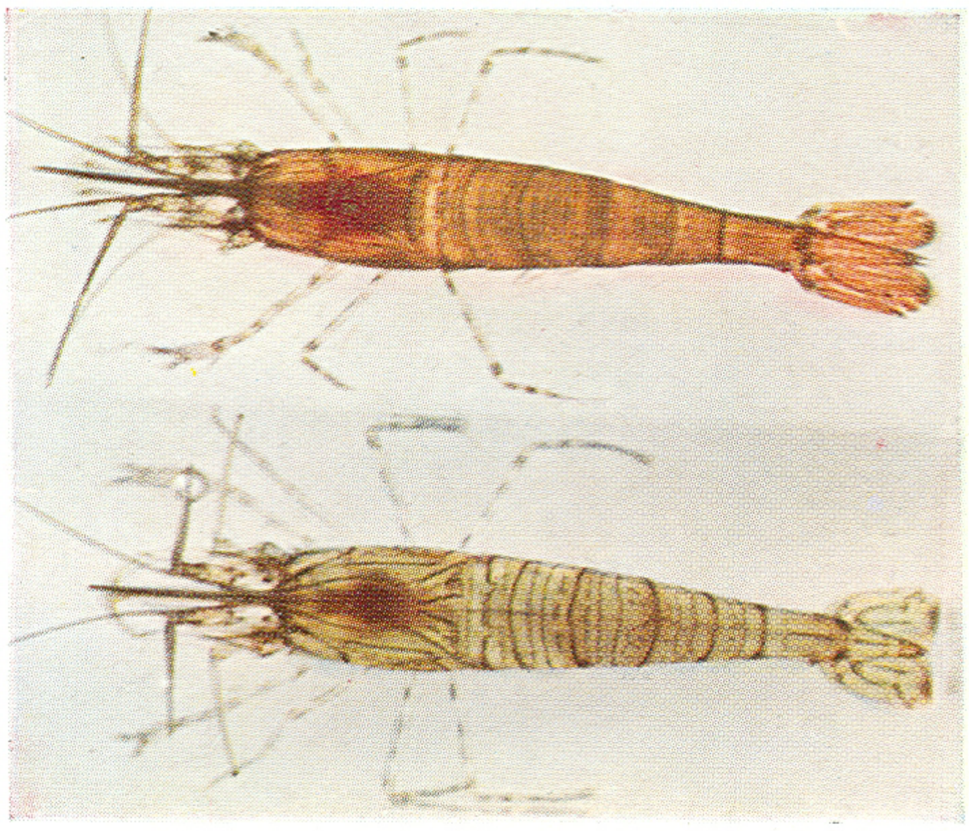

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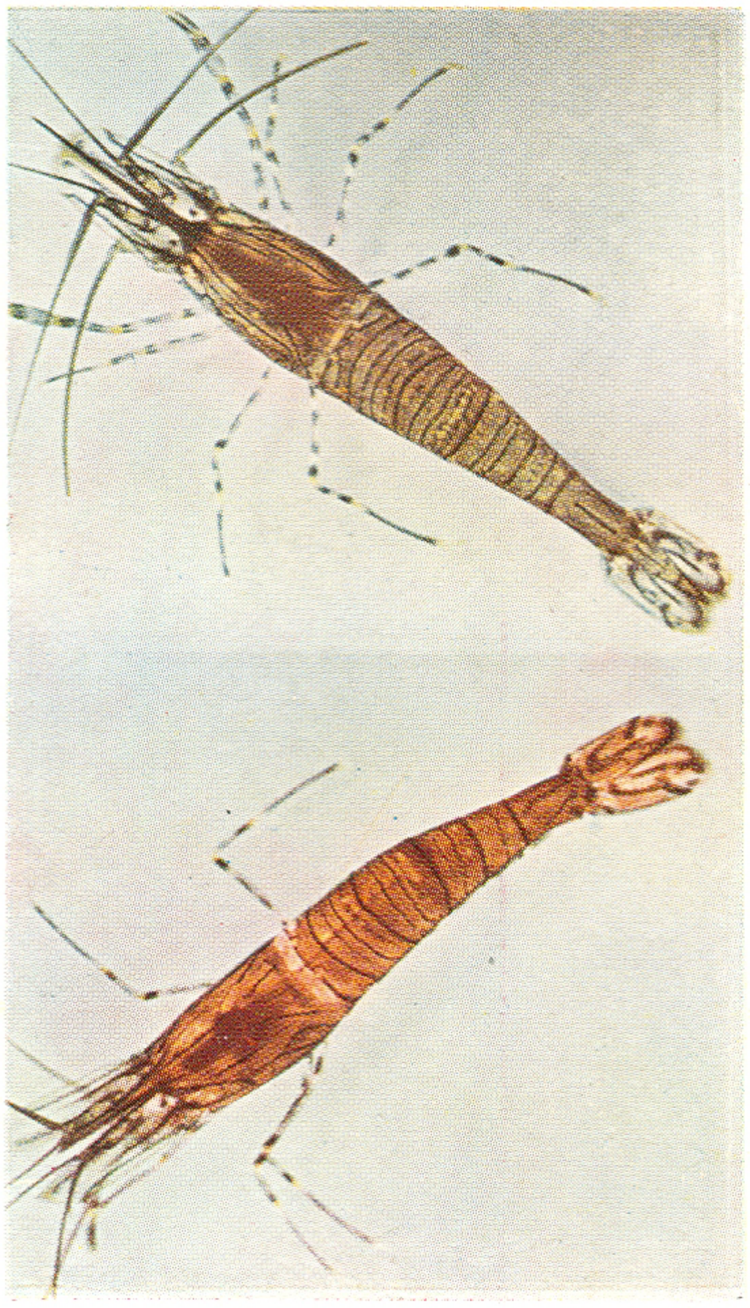

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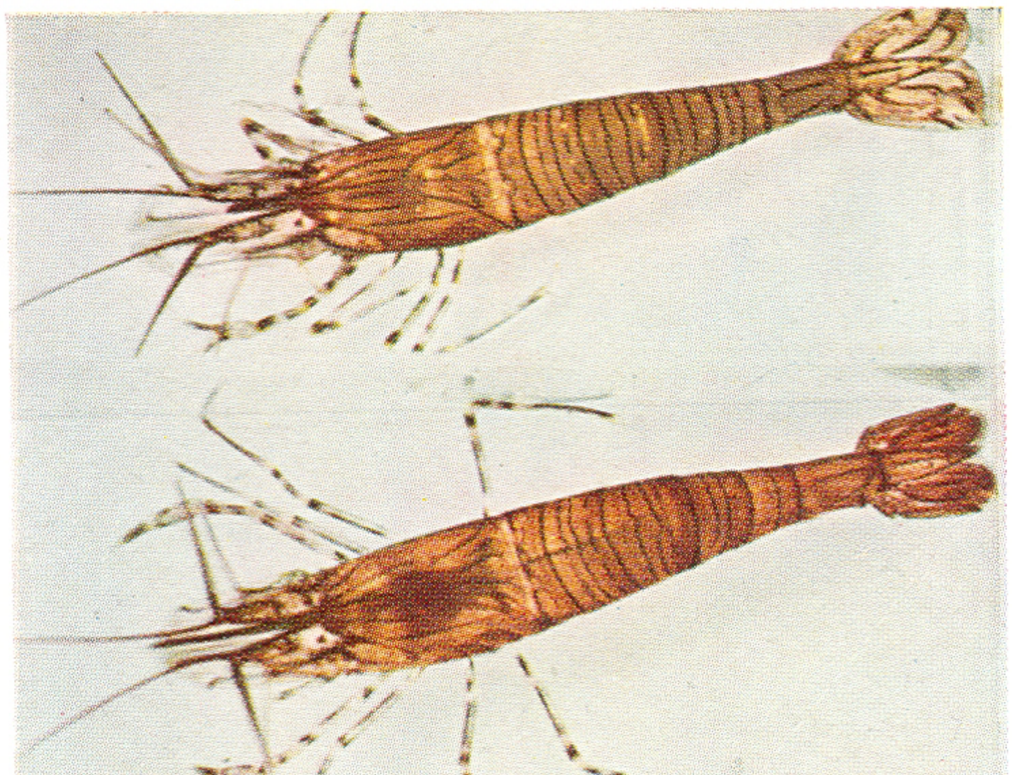

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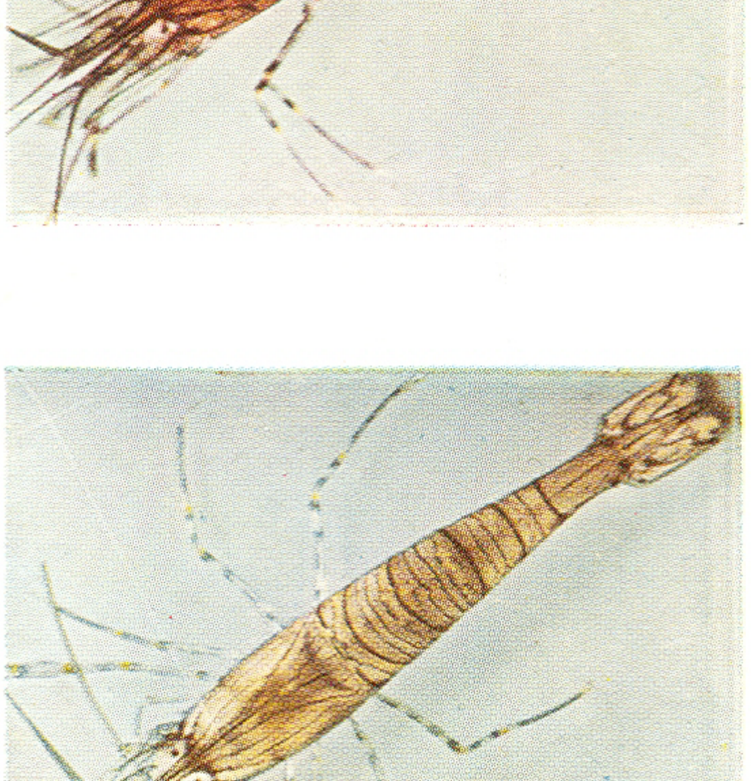

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PLATE II

Fig. 9. Electrophoretic separation of chromactivating substances from an extract of post-commissure organs. Under the conditions of this experiment substance B remains at 0, producing a hyperexpansion of the small red chromatophores to 5+ but a concentration of the large red chromatophores; substance A migrates to −1, concentrating all the red chromatophores. Photograph taken 30 min after injection.

Fig. 10. Separation of chromactivating substance by dialysis. Half an hour before this photograph was taken the left animal had received the fraction of a post-commissure organ extract that had passed through a cellophane membrane: this fraction contained α-substances; the animal on the right received the fraction that did not pass through the membrane: the effect is predominantly that of substance C.

Fig. 11. Separation of chromactivating substances by dialysis. The animal on the left received the fraction of a sinus gland extract which had passed through a cellophane membrane; the right-hand animal that which had not passed through.

Fig. 12. As Fig. 11, but using the eluate of the −1 fraction of the paper strip after electrophoresis of the sinus gland extract: the A-substance is incapable of passing the cellophane membrane.

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BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

IV. MAGNESIUM EXCRETION

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(Text-figs. 1–3)

From our previous publications (Brull & Nizet, 1953; Brull, Nizet & Verney, 1953; Brull & Cuypers, 1954), it appears that the power of concentration of the aglomerular kidney of *Lophius* is rather low, the density of the urine being usually less than that of the plasma concentration. Most individual urinary constituents do not reach concentrations that are more than once or twice those in the plasma, magnesium being an exception.

The kidney of *Lophius* possesses a high power for concentrating magnesium, to a degree approaching a hundred-fold. The plasma Mg usually increases after the fish has been caught, and therefore it seems likely that trauma is a factor in increased Mg absorption.[2] We referred in our previous work (1953) to other authors who had already noticed that urine secreted by *Lophius* in the aquarium is not the same as that secreted by fishes living under normal conditions. Yet, as *Lophius* kidneys are endowed with a particular power for excreting Mg, we thought it would be interesting to investigate this function further. What is the normal amount of Mg in *Lophius* plasma? Is it higher or similar to the figures found in mammals? Is there a threshold below which the secretion stops? Is exogenous Mg excreted in the same manner as the Mg that enters the blood stream through normal channels? These are the first questions which arise and seem worthy of investigation.

Methods

The problem was studied with isolated kidneys perfused with heparinized blood, according to the method described by Brull & Cuypers (1954). As we demonstrated that there is a maximum or optimum perfusion pressure above which no further increase of urine secretion is obtained (250–300 mm water), our perfusions were made at pressures of about 250–300 mm of water, at

[1] With the collaboration of A. Dujardin, L. Dubois and L. Wilsens.

[2] In dialysis experiments on *Lophius* skin E. Nizel (unpublished) found that rubbing the skin increases its permeability to minerals.

room temperature and with oxygenated blood (in July–August). Leaving aside a first series of experiments, in which the initial plasma level of Mg was high, we shall describe briefly three experiments only in which, during prolonged perfusion of the kidney, total exhaustion of the plasma Mg was reached.

Determinations of Mg were made by the method of Fister (1950), with a modification described by M. Orange & H. C. Rhein (1951).

Some of the determinations were checked with the ammonium magnesium phosphate method, and the figures agreed. Trichloracetic deproteinization was used. When the plasma figures were low, 2 ml. of plasma were used instead of 0·5 ml. Urine samples were diluted.

Experimental Results

Figs. 1–3 illustrate three perfusion experiments in which, with the use of two kidneys, the plasma Mg could be exhausted in 3–5 h, Mg being added afterwards.

In Expt. 54B (Fig. 1) a perfusion was started with 300 ml. of blood with a cell volume of 16%, and an initial plasma Mg of 16·2 mg. Two kidneys, weighing 12·5 and 12 g respectively, were perfused. As usual, there was an initial rise in urinary Mg, probably due to the fact that the kidneys came from a *Lophius*, the plasma Mg of which was lower than that of the pool of blood contained in the reservoir, the first two samples of urine being a wash-out.

Owing to the progressive exhaustion of the Mg reserve in the plasma, there is a regular drop of Mg, down to 0·47 mg % at the fifth hour of perfusion. With a delay of about 80 min, there is a parallel drop in the Mg concentration in urine and in plasma. In 80 min each kidney gives ±2·5 ml. urine. When a total amount of 15 ml. of urine have been excreted by the two kidneys, the plasma Mg has dropped to 0·47 mg %, showing that excretion continues even when practically all the available Mg has been removed. Some blood was lost during the perfusion, so that it is impossible to compare the available Mg with the excreted amount.

In Expt. 54C (Fig. 2) the initial level of Mg in the plasma was 13 mg %, with a pool of blood of 300 ml., and a red-cell volume of 15·8%. (Loss of blood during perfusion: 10 ml., sampling: 30 ml., total: 40 ml.)

After perfusing two kidneys weighing 12 and 11 g respectively, for 4 h 37 min, the Mg level in the plasma is down to 0·9 mg; there is a parallel drop of Mg in the urine (one kidney only is represented in the diagram) with a delay of 140 min. Of the 33·8 mg of Mg contained in the pool of blood (minus the losses and samples), 29·1 mg had been excreted after that time; thus ±4·7 mg are left in about 218 ml. of plasma, which would represent 2·1 mg % against 0·9 actually found. This means an error of 2·6 mg in all measurements and determinations involved. 73 min later, when two more ml.

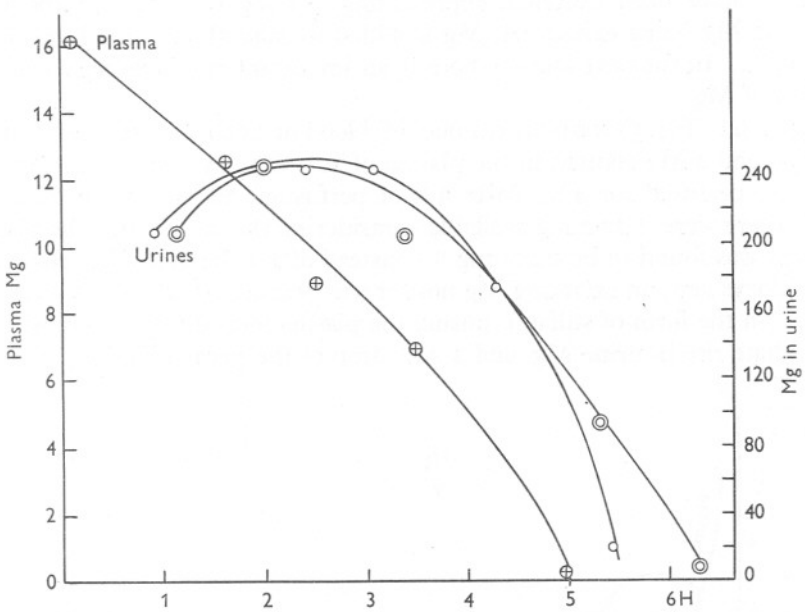

Fig. 1. Two kidneys are perfused until the plasma Mg is exhausted. All figures are given in mg per 100 ml.

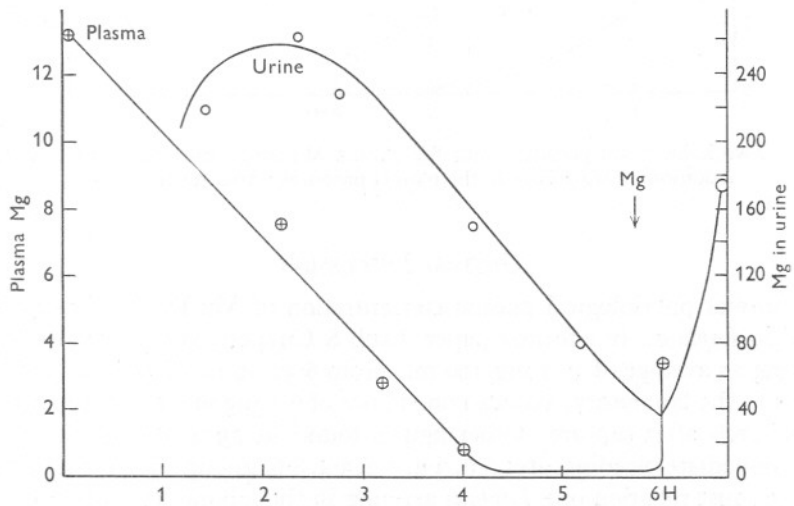

Fig. 2. Two kidneys are perfused until the plasma Mg drops down to 0·9 mg %. After addition of Mg sulfate to the blood, there is a rise of Mg in the urine.

of urine have been excreted, representing ±2 mg of Mg, and the total available Mg being exhausted, Mg is added to raise the plasma level up to 3·3 mg %. In the next sample there is an important rise in urinary concentration of Mg.

Expt. 54L (Fig. 3) starts with a pool of blood of 260 ml., a red-cell volume of 8%, and a Mg content in the plasma of 10·1 mg. Two kidneys of 13 and 11 g are perfused for 4 h. After 3 h of perfusion, 20·6 mg were excreted, while there were ±22·2 mg available (considering the sampling). The plasma content was found to be 0·225 mg %, instead of 1·1 theoretically. However, the kidneys kept on excreting Mg until it was practically exhausted. Mg was added (in the form of sulfate), raising the plasma level up to 2·5 mg with an immediate rise in urine Mg, and a new drop of the plasma level.

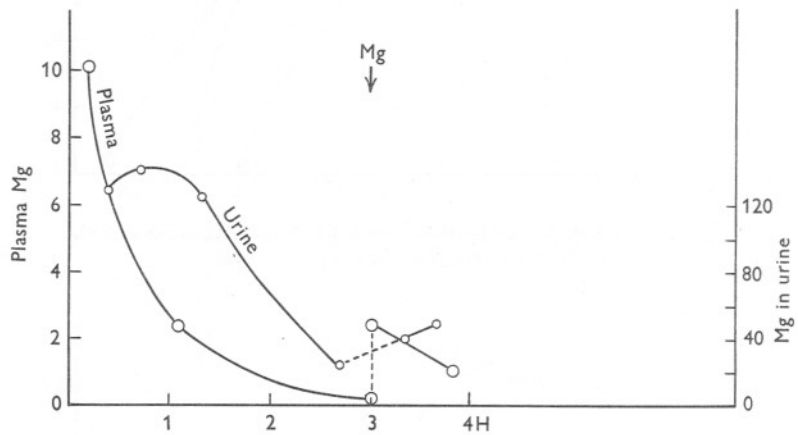

Fig. 3. Two kidneys are perfused until the plasma Mg drops from 10·1 to 0·225 mg %. Addition of Mg sulfate to the plasma produces a rise in urinary Mg.

General Discussion

The normal physiological plasma concentration of Mg has not been established in *Lophius*. In a former paper (Brull & Cuypers, 1954) we gave figures showing an average of 12·1 mg/100 ml. (from 6·32 up to 16·7), in fish bled on arrival in the laboratory, and also one figure of 6·5 mg in a fish bled on board immediately after capture. Other figures found in 1954 aboard the *Sarsia*, after immediate bleeding, are: 2·6, 3·2, 7·2, 3·4, 8·6, giving an average of 5 mg, and we must mention one *Lophius* arriving in the laboratory with 1·98 mg.

It is well known that mammalian plasma contains an average Mg content of about 2 mg/100 ml. It is likely that while the fish are being trawled, unusual quantities of sea water are swallowed and absorbed, thereby increasing the Mg concentration in the plasma on the way to the laboratory. We may however

suppose, from the above figures, that the normal concentration is about 2 mg or less.

As long as the plasma figures are higher than, let us say, 5 mg, the percentages in urine are high, up to 200 or even nearly 300 mg/100 ml. This concentration seems to be the maximum attainable for Mg concentration by *Lophius* kidneys. At first sight, when we performed perfusions with high initial plasma figures, and lasting only for 3–4 h, it looked as if urinary percentages of Mg were independent of the plasma concentration; this is true down to a certain level. But it is clear, from the three experiments reported, that when the plasma Mg drops down to 4, 3 or 2 mg, there is a parallel drop in urinary percentage.

But the most striking fact of all is that, while we believe we may consider Mg as a normal constituent of the plasma, the perfused *Lophius* kidneys keep on excreting Mg until its total, or practically total exhaustion, as if there were no threshold.

In our opinion, this finding is capable of two explanations. (1) The isolated perfused kidney of *Lophius piscatorius* lacks some (hormonal?) control to maintain its Mg threshold. (2) *Lophius* is a very greedy fish in the stomach of which enormous amounts of sea animal food can be found; sea water may be unavoidably ingested with the food, thus producing a continuous inflow of Mg, the latter to be neutralized by a very active power of excretion the kidney possesses. No mechanism for the preservation of Mg is required. On the contrary, the problem of its constant removal is by far more urgent. The plasma level may be more variable than in terrestrial or freshwater animals, in which Mg may eventually be lacking.

Under normal conditions of life, a minimum amount of sea water is swallowed, and the excess Mg is easily excreted by the kidneys. After trawling, there is a surfeit of Mg in the blood, representing up to 16 or 17 mg %.

As far as we know, in mammals, there is a very accurate homeostatic mechanism for minerals. One of us demonstrated in 1927 a threshold for phosphates (Brull, 1927), which is under control of the hypophysis (Brull, 1928), and of the parathyroids (Brull, 1936). This author also produced evidence that usually protein-bound and ionized Ca is not excreted by the kidney, but that a small quantity of Ca combined in a complex form with organic acids, is the origin of the urinary leakage of Ca (Brull, 1930).

It is the first time we come across conditions under which a normal inorganic constituent of the plasma is excreted by the kidney until exhaustion. Further research will have to show whether this phenomenon, observed in the perfused kidney, may be confirmed with the whole animal.

Moreover, exogenous Mg, added to the plasma, seems to be excreted in the same way as absorbed Mg.

SUMMARY

Kidneys of *Lophius piscatorius*, perfused with heparinized *Lophius* blood, concentrate magnesium up to one hundred times. This degree of concentration is independent of the plasma level as long as the blood contains high amounts of magnesium, as occurs in the hours following capture of the fish. Below 5 mg % or less, there is a parallel drop in urinary concentration of magnesium and in plasma level. When kidneys are perfused with a limited pool of blood, they excrete magnesium until total exhaustion of this substance. Under such conditions there is no threshold for magnesium in *Lophius*. Exogenous magnesium added to the plasma is excreted in the same manner as endogenous (absorbed) magnesium.

We are much indebted to the Marine Biological Laboratory, Plymouth, for all facilities provided year after year for our research.

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J. Mar. biol. Ass. U.K. (1955) 34, 643–647
Printed in Great Britain

THE GROWTH RATE OF THE LONG ROUGH DAB *HIPPOGLOSSOIDES PLATESSOIDES* (FABR.)—A CORRECTION

By T. B. Bagenal
The Marine Station, Millport

(Text-fig. 1)

In a recent paper (Bagenal, 1955) on the growth rate of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.) in the Clyde Sea area, a serious mistake was made.

The ages of 1561 Long Rough Dabs, which were collected from October 1953 to September 1954, were determined from an examination of the otoliths, and from the age analysis each fish was allocated to a population *f* to *a*, depending on when it was supposed to have spawned. This procedure was designed to obviate the difficulty during the winter and spring when fish of the same year-class would be grouped differently according to the condition of the otolith edge. The mean lengths of the fish of populations *f–a* were taken as the mean lengths of Long Rough Dabs after 1–6 years. This, however, is wrong, and they should be taken as the mean lengths after 2–7 years. I wish to apologize for this inexcusable mistake, and to give the correct figures at the earliest opportunity. They will be found in Table I.

Turning next to the mathematical treatment of the data, and applying the correct figures to the same formulae as were used before, it is apparent that the regression equation

$$l_{n+1} = ml_n + C, \qquad \text{(i)}$$

where l_n is the mean length at age n, will be exactly the same, as will the asymptotic value, l_∞, to which the growth approaches. This is because the regression is based on the l_{n+1} to l_n ratio, and the actual values of n are immaterial. The figures obtained for m and l_∞ $(= C/1 - m)$ may again be incorporated in the exponential equation modified from Bertalanffy (1938, 1949):

$$l_t = l_\infty (1 - e^{-kt}), \qquad \text{(ii)}$$

where $e^{-k} = m$ of equation (i). However, with the correct figures there is a large discrepancy between the values of C and what would be reasonable figures for l_1; for equation (i) and (ii) to be comparable these should be the same:

for the females $C = 11{\cdot}262$ cm, the calculated $l_1 = 2{\cdot}130$ cm,

for the males $C = 7{\cdot}368$ cm, the calculated $l_1 = 5{\cdot}335$ cm.

It is clear, therefore that the growth is only described by the equations given in the previous paper over the upper range of the curve, and in order to describe the growth over the whole life span a sigmoid curve which above the point of inflexion approximates to the exponential type given in equation (ii) is required.

TABLE I. THE MEAN LENGTHS OF LONG ROUGH DABS, FROM POPULATIONS *f–a*

Population	Age	Mean lengths (cm.) Males	Females
—	1	—	—
f	2	10·5	12·6
e	3	13·5	18·3
d	4	15·3	22·1
c	5	16·3	23·4
b	6	—	25·3
a	7	—	25·3

Of the commoner growth equations the most appropriate would appear to be the Gompertz equation whose properties have been given by Winsor (1932). This equation has the form

$$l_t = l_\infty \ \exp[-be^{-kt}]. \qquad \text{(iii)}$$

Its relation to equation (ii) is seen by considering the graph of l_{n+1} plotted against l_n. With the Gompertz equation one obtains a curve which cuts the 45° line at the origin and at l_∞, and a straight line regression can be got by plotting the logarithms of the lengths. In this case the regression is

$$\log l_{n+1} = m' \log l_n + C'. \qquad \text{(iv)}$$

The value of l_∞ is obtained from the equation

$$\log l_\infty = \frac{C'}{(1-m')}. \qquad \text{(v)}$$

The slope of the curve on the simple $l_{n+1} - l_n$ plane at any fraction $(1/x)$ of l_∞ is equal to $\frac{m'}{x^{m'-1}}$. The curve rises rapidly from the origin and at its intersect with the 45° line has the same slope as that for equation (iv). It is therefore apparent that the two curves are indeed similar, particularly at their upper ranges when they approach their asymptotic levels.

Applying equation (iv) to the Long Rough Dab data of Table I gives

$$\text{for the females: } \log l_{n+1} = 0{\cdot}475 \log l_n + 0{\cdot}742,$$

$$\text{for the males: } \log l_{n+1} = 0{\cdot}501 \log l_n + 0{\cdot}619,$$

and equation (v) gives:

$$\text{for the females: } \log l_\infty = \frac{0{\cdot}742}{(1-0{\cdot}475)} = 1{\cdot}4133 . l_\infty = 25{\cdot}90,$$

$$\text{for the males: } \log l_\infty = \frac{0{\cdot}619}{(1-0{\cdot}501)} = 1{\cdot}2405 . l_\infty = 17{\cdot}40.$$

These may be compared with 26·437 and 17·754 for the females and males respectively given in the previous paper for equation (ii). The value for b for the curve to pass through the origin is given by

$$b = \frac{\log l_\infty}{\log e}, \tag{vi}$$

$$\text{for the females: } b = \frac{1{\cdot}4133}{0{\cdot}4343} = 3{\cdot}2542,$$

$$\text{for the males: } b = \frac{1{\cdot}2405}{0{\cdot}4343} = 2{\cdot}8563,$$

and e^{-k} equals m' of equation (iv). The Gompertz equations (iii), for the Long Rough Dabs, are therefore

$$\left.\begin{aligned} &\text{for the females: } l_t = 25{\cdot}90 . 2{\cdot}7183^{-3{\cdot}2542 . 0{\cdot}475^t} \\ &\text{for the males: } l_t = 17{\cdot}40 . 2{\cdot}7183^{-2{\cdot}8563 . 0{\cdot}501^t} \end{aligned}\right\} \tag{vii}$$

The calculated values from these equations are given in Table II, and in Fig. 1.

TABLE II. THE OBSERVED MEAN LENGTHS OF THE LONG ROUGH DABS, AND THE VALUES CALCULATED FROM THE GOMPERTZ EQUATION (vii)

	Females		Males	
t	Observed	Calculated	Observed	Calculated
1	—	5·52	—	4·16
2	12·6	12·43	10·5	8·50
3	18·3	18·27	13·5	12·14
4	22·1	21·95	15·3	14·87
5	23·4	23·94	16·3	15·90
6	25·3	24·96	—	—
7	25·3	25·44	—	—

In the previous paper it was argued that the calculated mean lengths are less liable to bias (as might, for example, be produced by net selection) than are the observed values. This is because the regression equation is based on the ratios of the lengths and not on their absolute values. The same argument applies to the present treatment with the $\log l_{n+1} - \log l_n$ plane and the Gompertz curve. It will be seen that the agreement between the observed and calculated values

is remarkably good with the females, but there is an appreciable discrepancy with the males. The reason for this is not readily apparent, and it can only be suggested that different reactions of the fish to the trawl might be responsible, in leading to greater escape by the males. In this connexion one should remember that there is a marked difference between the numbers of males and females caught; even in comparable size-groups the females are much more numerous.

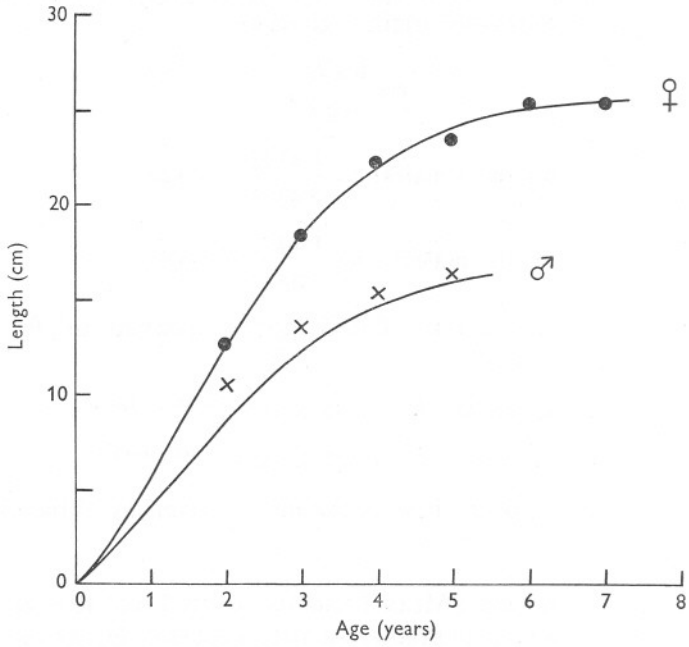

Fig. 1. The growth curves of male and female Long Rough Dabs given by the Gompertz equations (viii), together with the observed mean lengths for the males (×) and females (●). Data from Table II.

Summary

A serious mistake in a previous paper on the growth rate of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.) is corrected. The growth equation originally used is shown to be inapplicable to the correct figures over the whole life span, though for suitable data the method of fitting the curve would still appear to be the most accurate. More suitable for the correct Long Rough Dab data is the Gompertz equation, and this approximates to the previous equation over the upper ranges.

The method previously developed of finding estimates of the coefficients to be used for the growth curve is shown to be equally applicable to the Gompertz

equation. The agreement between the calculated and observed values is very close with the females, but less so with the males. It is suggested that this may in part be connected with the scarcity of the males in the samples, since a greater ability to escape would produce both effects.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

PYTHIUM THALASSIUM N.SP. INFECTING THE EGG-MASS OF THE PEA-CRAB, *PINNOTHERES PISUM*

By **D. Atkins**

Trans. Brit. mycol. Soc., Vol. 38, 1955, pp. 31–46

A marine *Pythium*, *P. thalassium* n.sp., has been found parasitic and saprophytic in the eggs of the pea-crab, *Pinnotheres pisum*, and of other Crustacea. The sporangia are filamentous, the method of asexual reproduction is that characteristic of the genus *Pythium*: proliferation occurs. Some variation in the behaviour of the contents of the sporangium was noted. Asexual bodies, similar to those described in certain other species of *Pythium*, are borne extramatrically at the apex of hyphae. Various stages in the germination of spores were seen. Sexual organs were not present in the material examined. The affinities of *P. thalassium* are discussed. D.A.

THE POST-EMBRYONIC DEVELOPMENT OF BRITISH *PINNOTHERES* (CRUSTACEA)

By **D. Atkins**

Proc. zool. Soc. Lond., Vol. 124, 1955, pp. 687–715

The two species of *Pinnotheres* occurring at Plymouth have been reared in the laboratory from egg to megalopa for the first time.

P. pisum has four zoeal stages and a megalopa. The megalopa stage was reached in 11 weeks from spawning and 6 from hatching. These times are probably considerably longer than under natural conditions.

P. pinnotheres has two zoeal stages and a megalopa. The megalopa stage was reached in about 9 weeks from early egg and three weeks from hatching.

The stages are described and some observations are given on habits and moulting of the zoeae. D.A.

FIRST-STAGE LARVAE HATCHED FROM NEW ZEALAND DECAPOD CRUSTACEA

By **M. V. Lebour**

Ann. Mag. nat. Hist., Ser. 12, Vol. 8, 1955, pp. 43–48

Larvae hatched by Dr E. Batham at the Portobello Marine Laboratory, New Zealand, were sent to the author and are here described. These are *Palaemon affinis*, *Alope spinifrons* and *Callianassa* (*Trypaea*) *filholi*. No *Alope* larva has

as yet been described. These are very like *Hippolyte* with a few minor differences. The larva of *Callianassa filholi* is here described for the first time. It is peculiar in the armature of the abdomen and in this respect unlike any species known. M.V.L.

Observations on the Food and the Gut Pigment of the Polyopisthocotylea (Trematoda: Monogenea)

By J. Llewellyn

Parasitology, Vol. 44, 1954, pp. 428–37

The Polyopisthocotylea is a suborder (upwards of 250 species) of monogenetic trematodes that are parasitic mainly on the gills of fishes, but the nature of the food of these parasites has not previously been determined.

An investigation by spectroscopic and histochemical methods of six representative trematodes from the gills of marine fishes at Plymouth and of trout from elsewhere, and of the monogenean parasitic in the bladder of the frog, has shown that these parasites feed mainly on the blood of their hosts. The blood is probably haemolysed fairly rapidly and subsequently absorbed by amoeboid ingestion, the globin moiety of the haemoglobin forming the chief nutriment of the parasite and the unaltered haematin being eliminated either by its discharge from epithelial cells into the gut lumen or by the sloughing off of intact epithelial cells.

In a more limited sample of skin- and cloaca-inhabiting Monopisthocotylea there was no evidence of a blood-feeding habit. J.L.

The Structure and Function of the British Pholadidae (Rockboring Lamellibranchia)

By R. D. Purchon

Proc. zool. Soc. Lond., Vol. 124, 1955, pp. 859–911

Exhaustive study of the Pholadidae, the least specialized family in the Adesmacea, revealed two features of potential systematic importance. The appendix of the stomach is homologous with the caecum of the Teredinidae and with the postero-dorsal caecum of the Tellinacea, thus indicating relationship between the Adesmacea and the Tellinacea. An accessory visceral ganglion is present in the Pholadidae, and such a structure has been reported elsewhere only in *Dreissena*.

When all other ciliary activity appeared normal, cilia in the proximal oral groove were usually inactive, suggesting that feeding is intermittent. It was concluded that the ciliary mechanisms in the stomach served chiefly for trapping and rejection of particles, and that food material could not be passed

from the stomach into the digestive diverticula solely by ciliary action. Probably during a period of ciliary inactivity in the proximal oral groove, the stomach contents are prepared for presentation to the digestive diverticula, during which time the stomach contents are not contaminated by entry of fresh food material. Finally the stomach contents are passed to the digestive diverticula by relative volumetric changes in the stomach and digestive diverticula. These volumetric changes are brought about by the adductor and pedal muscles, and by the muscle fibres in the walls of the stomach and digestive diverticula.

R.D.P.

Growth Changes in the myelin Sheath of peripheral nerve Fibres in Fishes

By P. K. Thomas

Proc. roy. Soc. B, Vol. 143, 1955, pp. 380–391

A quantitative study of the growth of the myelin sheath of fibres of the lateral line nerve in *Salmo trutta* and *Raia clavata* has been made. This nerve is especially favourable for growth studies because of the magnitude of the changes taking place. In a large specimen of *Salmo*, the largest fibres attain a diameter in excess of 30μ and possess internodes 4 mm in length. In *Raia*, internodes of up to 8 mm in length are found.

Contrary to earlier reports, but in agreement with a recent investigation on mammalian nerves, the relative thickness of the myelin sheath is found to decrease progressively during growth as fibre size increases. Comparison between fishes of differing length demonstrates that the thickness of the myelin sheath for a given axon diameter is constant, irrespective of the size of the fish.

Measurement of the internodal length of the largest fibres in the nerve in specimens of differing length confirms the close dependence of the length of the myelin segments on growth. The form of the relationship between internodal length and fibre diameter depends upon their relative growth rates. Growth of internodal distance follows that of the part in which the nerve lies, but this is not true of diameter. The changes in the relationship between internodal length and diameter during growth have been analysed and the applicability of the equation employed to other vertebrate groups considered.

The contribution that quantitative investigations of the changes taking place during the growth of nerve fibres can make towards the understanding of the way in which their structural organization is achieved and maintained is discussed.

P.K.T.

The Distribution of certain intertidal Animals around the Irish Coast

By **A. J. Southward** and **D. J. Crisp**

Proc. R. Irish Acad., B, Vol. 57, No. 1, 29 pp.

During the period 1950–53, the distribution of some animals found mainly on the upper half of the shore was investigated at ninety-six stations round the Irish Coast.

The barnacle *Chthamalus stellatus* was found on exposed shores of all coasts, except for occasional areas of chalk in Co. Antrim, and was the dominant barnacle of the south and west coasts. It was generally less common on the east coast, but was quite abundant in a limited area of the coasts of S. Co. Down and Co. Louth. *Balanus balanoides*, on the other hand, was practically absent from the extreme South-west, its distribution being almost the inverse of that of *Chthamalus*.

The top-shells *Gibbula umbilicalis* and *Osilinus lineatus* followed the main features in the distribution of *Chthamalus*, but *Osilinus* was absent from most of the north and east coasts except for an area in S. Co. Down and Co. Louth. The sea-urchin *Paracentrotus lividus* was found only on the west coast.

Of several other animals investigated, the parasite *Hemioniscus balani* was more common in the South-west, and *Littorina neritoides*, though common on all exposed shores, was generally less abundant on the east coast.

The distributions of the southern species, notably *Chthamalus*, *Osilinus*, *Gibbula umbilicalis* and *Paracentrotus* have a common basis, apparently the effect of temperature rather than any other known or unknown factor in the environment. In some of these animals the effect of low summer temperatures on the breeding activities must restrict the distribution in certain areas. The anomalous abundance of *Chthamalus*, *Osilinus* and *Gibbula umbilicalis* on a limited part of the east coast may be due to a combination of local warming during the summer, with suitable rock topography and tidal conditions. Similar examples of the possible beneficial examples of local warming during the summer are given in the case of Lough Ine (for *Paracentrotus*), and the Scottish Lochs Sween and W. Tarbert (for *Chthamalus*). A.J.S.

BOOK REVIEWS

Aspects of Deep-sea Biology

By N. B. Marshall

Published by Hutchinson's Scientific and Technical Publications, London. Pp. 380. Illustrated by Olga Marshall; with 5 coloured plates, and many black and white illustrations. Price 35*s*. 1954.

In seeking to understand the activities of deep-sea organisms, one is impressed by the scantiness of our present knowledge and the host of unsolved problems which life in the depths presents. In this book Mr Marshall has given a clear and factual account of environmental conditions in the deep sea, and the ways in which plants and animals live in deep waters. It is so written that it can be understood by the non-scientific reader and yet prove of interest and value to professional biologists. After describing background conditions in the sea the author devotes special attention to certain subjects, among which may be mentioned: food chains, counter-acting gravity, sensory perception, sound production, luminescence and reproduction. A great many scattered observations are assembled and are employed to interpret how deep-sea organisms adjust themselves to life at different levels in the open oceans. Most of this information is necessarily indirect for obvious reasons. Not the least interesting feature of the book is the way in which information is assembled from many different branches of biology, when capable of throwing light on the problems under discussion. Many of the problems raised cannot yet be answered, but it is useful to have them clearly stated.

There is a short list of the references at the end of each chapter and the book is amply illustrated by line blocks, half-tones, and coloured plates.

J. A. C. N.

The Indian Journal of Fisheries

Vol. 1, Nos. 1 and 2, May 1954. Price 7 rupees 8 annas net.

It is unusual, in these days, to find oneself welcoming the appearance of yet another scientific journal or other scientific periodical. But to the new *Indian Journal of Fisheries* a welcome must be accorded. It is published by the Indian Ministry of Food and Agriculture to provide a medium for the publication of original contributions and critical reviews in the field of Indian fisheries science. Each volume is planned to consist of about 400 pages to be issued in two numbers. The proposed intervals between publication are not stated.

Volume 1, nos. 1 and 2, has already appeared and contains many papers covering a wide range of subjects. The editorial board appears to have set a high standard which it will no doubt maintain. Fisheries research and development in India are now being pursued with such vigour and success that a special publication solely concerned with these subjects has become essential. We therefore extend a hearty welcome to the new *Journal* and wish it every success.

G. A. S.

Biology of a Marine Copepod

By S. M. Marshall, D.Sc., F.R.S.E. and A. P. Orr, M.A., D.Sc., F.R.S.E.

Published by Oliver & Boyd, Ltd., Edinburgh. Pp. 1–188, with 63 figs. Price 21*s*. 1955.

This is a very valuable book. *Calanus* is one of the most ubiquitous and abundant animals in the plankton. It is a main source of food for whales and fish, and plays a large part itself in the utilization of the phyto-plankton. It is also a representative of the largest group of Crustacea in the plankton. It is natural, therefore, that more research has been done on the biology of this copepod than on that of any other. Dr S. M. Marshall and Dr A. P. Orr have brought together all existing knowledge on every aspect of the biology of this copepod. They cover its systematics and anatomy, its reproduction and life cycle, and the general physiology of its growth, feeding and respiration, together with information on its behaviour and its parasites. In all of these aspects of the biology of *Calanus* both Dr Marshall and Dr Orr have themselves contributed much to our knowledge by their own researches, which have covered a period of more than thirty years. This book should be of interest not only to those engaged in plankton research, but to all biologists, since it covers problems of fundamental importance concerning life in the sea. The authors are to be congratulated on making available to their fellow workers in so interesting a manner and over so wide a field our knowledge to date on the biology of *Calanus*.

The book is well illustrated both with line and half-tone figures, and is in every way a most pleasing production.

F. S. RUSSELL

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1954–55

The Council have to record with great regret the deaths of Dr E. S. Russell, O.B.E., a former member of Council and a Vice-President from 1948, of Prof. F. E. Fritsch, F.R.S., also a former member of Council, and of Miss M. J. Delap, an Associate Member since 1937.

During the year Major E. G. Christie-Miller, our Honorary Treasurer, has been elected a Vice-President.

The Council and Officers

Four meetings of the Council have been held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was eighteen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

The Plymouth Laboratory

The extension to the library is approaching completion. This new building, which has three floors, will give nearly as much floor space as the existing library. Besides providing extra shelf accommodation the extension will give much needed space for reading. The new specimen store has now been finished and is in use.

The Council are pleased to report that H.M. Treasury have sanctioned a Capital Grant from the Development Fund to meet the cost of constructing new sea-water reservoirs and rebuilding and extending the existing outside storage tanks.

The Aquarium

The Aquarium has continued to attract large numbers of visitors, many of whom have remarked favourably on the improvements made last year.

Some additional picture labels have been painted by Miss Jenefer Peter so that most, though not yet all, of the animals shown can be identified by the visitor.

During October it was possible to show a special exhibit of living Portuguese Men-of-War (*Physalia physalis*), at a time when they were being stranded on the shores of south-west England.

Research Ships

During the year R.V. *Sarsia* has been in continuous service and is giving great satisfaction. The Laboratory is deriving great benefit from her greater range of activities which enable her, among other things, to bring back living organisms from much farther afield and from greater depths.

Sula has undergone Lloyds ordinary special hull survey and the hull has been found to be in excellent condition. Unfortunately, routine inspection of the machinery revealed unexpectedly heavy wear of the main bearings of the engine and in the gear box. Lloyds therefore ordered a complete overhaul of the machinery. This has been carried out at considerable expense.

Gammarus has worked normally throughout the year.

The Staff

Dr W. R. G. Atkins, C.B.E., F.R.S., retired from the Staff of the Plymouth laboratory on 31 March 1955. The Council wish to record their deep appreciation of Dr Atkins's services to the Association as Head of the Department of General Physiology for 34 years, and of his own distinguished research which has gained for the Plymouth laboratory an international reputation. They wish him many years of happy retirement.

Miss D. Ballantine joined the staff of the Plymouth Laboratory in the grade of Scientific Officer, on 1 October 1954.

Dr G. A. Steven attended the meeting of the International Council for the Exploration of the Sea in Paris in October 1954.

Dr D. P. Wilson spent the month of October 1954 at Naples by invitation of Dr R. Dohrn to discuss improvements in the sea-water circulation at the Stazione Zoologica.

Dr L. H. N. Cooper attended the opening of the new Laboratory of Oceanography of the U.S. Office of Naval Research at Woods Hole in June 1954, and read a paper at a subsequent convocation. While in America Dr Cooper visited the Fish and Wild Life Service Laboratories at Galveston in Texas, and at Stanford in California, the marine laboratories at Miami, the Scripps Institution of Oceanography, and Yale University.

In September 1954 Dr L. H. N. Cooper attended the meeting of the International Union of Geodesy and Geophysics in Rome, after which he spent a few days at Monaco and Villefranche-sur-Mer.

Dr Mary Parke attended the VIIIth International Botanical Congress in Paris in July 1954, where for the first time meetings of a Phycological section were held. After the Paris meeting she spent a few days at Roscoff.

Mr P. G. Corbin accompanied the cruise of the R.R.S. *Discovery II* in September 1954, by kind invitation of the Director of the National Institute of Oceanography, to test his large pelagic net.

Dr J. A. C. Nicol returned to Plymouth in September 1954 after spending twelve months in the United States, while holding a Guggenheim Fellowship. The greater part of this time was spent at the Scripps Institution of Oceanography, but Dr Nicol also visited Santa Barbara and Friday Harbour.

Mr F. S. Russell, F.R.S., was appointed a Commander of the Order of the British Empire in the New Year's Honours for 1955.

International Paints Research Fellowship

The Directors of International Paints Ltd. have, by arrangement with the Council of the Association, endowed a research fellowship for investigation of the effects of toxic substances on marine organisms. This is to be known as the International Paints Research Fellowship and is tenable for three years at the Plymouth laboratory.

Dr E. D. S. Corner, of the Department of Biochemistry, St Thomas's Hospital Medical School, has been appointed to the Fellowship, and he started work at Plymouth on 1 September 1954.

Occupation of Tables

The following one hundred and fifty-seven workers have occupied tables at the Plymouth Laboratory during the year:

E. ADAMS, Plymouth (Library).
Miss J. M. ALLEN, London (General).
Señora A. A. ALVARIÑO, Madrid, British Council Scholar (Plankton).
D. C. ARNOLD, Oxford (Biology of Carapidae).
Miss D. E. ASHHURST, Oxford (Blood groups in fishes).
Dr DAPHNE ATKINS, London (Ciliary feeding mechanisms of brachiopods and *Phoronis*).

Miss D. BALLANTINE, Development Commission (Taxonomy and culture of marine flagellates).
Miss S. BAUR, Basel (Scientific drawings).
D. S. BENDALL, Cambridge (Chromatography of nucleotides).
Dr ANNA M. BIDDER, Cambridge (Library).
Dr H. M. BISHAI, Cullercoats (Biology of fish larvae).
Dr H. BLASCHKO, Oxford (Amine metabolism in invertebrates).
B. BOCZAROW, Plymouth (Library).
A. D. BONEY, Plymouth (Shore ecology).
Prof. A. A. BOYDEN, New Jersey (Comparative serology).
L. R. BRIGHTWELL, Peacehaven, Sussex (Biology of pagurids).
Dr L. G. BROCK, Cambridge (Mode of excitation of electric organ in *Raia*).
Dr ELEANOR M. BROWN, London (Digestive system of mysids and *Nebalia*; *Syndinium*).
Prof. & Mme L. H. BRULL, Liége (Physiology of kidney in *Lophius*).
Dr P. BRUNET, Oxford (Byssus of *Mytilus*).
Dr M. BULJAN, Split (Chemical hydrography).

Dr P. C. CALDWELL, London (Giant nerve fibres of *Loligo*).
Dr J. N. CARRUTHERS, National Institute of Oceanography (Testing oceanographic equipment).
Dr J. D. CARTHY, London (Biology of larval *Geranomya*).
P. CHILDS, Plymouth (Comparative anatomy of biliary tract).
B. I. CLARKE, Leeds (Marine flagellates).
R. D. COHEN, Plymouth (Library).
Dr H. A. COLE, Burnham-on-Crouch (Oyster fisheries).
D. J. COLLINS, Plymouth (Library).
R. J. CONOVER, Southampton (General).
Dr E. D. S. CORNER, International Paints Research Fellow (Effects of toxic substances on marine organisms).
C. A. COSWAY, Torquay (Library).
Miss M. COURTNEY, London (Cirratulids).
Dr Y. CUYPERS, Liége (Physiology of kidney in *Lophius*).

Dr R. PHILLIPS DALES, London (Feeding and morphology of the gut of terebellid polychaetes).
R. D. DESHPANDE, Southampton (Biology of *Acartia*; ecology of Trochidae).
Mr and Mrs P. S. B. DIGBY, London (Feeding of copepods).
Miss E. J. DIMELOW, Reading (Functional morphology of *Antedon*).
D. A. DORSETT, London (Digestive cycles in *Gibbula*).
Mme M. DUPONT-RAABE, Paris (Colour change in insects and crustaceans).

C. EDWARDS, Belfast (Ecology of *Marinogammarus*).
J. H. ELGOOD, Rustington (Library).
S. K. ELTRINGHAM, Southampton (Biology of *Limnoria*).

R. W. FEARNHEAD, Cambridge (Development of fish teeth).
Dr MARIA FELINSKA, Buckden, Huntingdon (Ciliates).
Dr L. R. FISHER, Reading (Vitamin A and carotenoids).
Dr E. W. FLÜCKIGER, Basel (Giant nerve fibres of *Loligo*).
A. R. FONTAINE, Oxford (Feeding of ophiuroids).
B. A. FOSTER, Aberdeen (Culture of marine flagellates).
Cdr R. H. C. FRAMPTON, R.N. (Rtd.), Plymouth (Library).
Dr. C. B. FRANKENHAEUSER, Stockholm (Giant nerve fibres in *Loligo*).
Dr VERA FRETTER, London (Uptake of ^{24}Na by *Nereis*).

J. B. GILPIN-BROWN, Bristol (Nervous system of *Nereis*).
Dr SYLVIA J. GILPIN-BROWN, Bristol (Behaviour of littoral gastropods).
Dr D. R. GLASSON, Plymouth (Library).
R. U. GOODING, Cambridge (Underwater ecology).
Mr & Mrs T. F. GOREAU, Jamaica (Phosphates in corals).
Prof. A. GRAHAM, Reading (Library).
A. V. GRIMSTONE, Cambridge (Gastropods).

Miss J. E. HANNAFORD, Plymouth (Library).
R. E. HARRIS, Manchester (*Callithamnion*).
B. T. HEPPER, Burnham-on-Crouch (Oyster fisheries).
Miss J. M. HIMMS, Oxford (Amine metabolism in invertebrates).
Miss A. HINDS, Cambridge (Chromatography of nucleotides).
Prof. A. L. HODGKIN, F.R.S., Cambridge (Giant nerve fibres in *Loligo*).

D. B. HOPE, Oxford (Amine metabolism in invertebrates).
Dr G. A. HORRIDGE, Cambridge (Ephyra larvae).
G. HOYLE, Glasgow (Neuromuscular physiology of ascidians).
Dr A. F. W. HUGHES, Cambridge (Cytology of fish nervous systems).
Surg.-Lt. C. O. HUGHES, R.N., Alverstoke (Collection of fish juices).
Mrs S. HUGHES, Cambridge (Development of fish teeth).

L. A. J. JACKMAN, Paignton (Library).
F. J. JEFFERY, Plymouth (Library).
Dr C. H. JELLARD, Plymouth (Bacteriological tests).
Dr PENELOPE M. JENKIN, Bristol (Feeding of flamingoes; Library).

Miss J. M. KAIN, London (Culture of dinoflagellates).
Dr G. Y. KENNEDY, Sheffield (Porphyrins in marine animals).
Dr R. D. KEYNES, Cambridge (Giant nerve fibres in *Loligo*).
M. C. KINGWELL, South Brent (Library).
P. KIRTISINGHE, Ceylon (Parasitic copepods).
Sir FRANCIS KNOWLES, Bart., Marlborough (Neurosecretion in crustaceans).
Dr S. K. KON, Reading (Vitamin A and carotenoids).
M. E. KORN, London (Holothurians).
Dr P. L. KRAMP, Copenhagen (Hydroids and medusae).
Prof. E. KROGH, Aarhus (Nucleic acid in *Aplysia* nerve cells).

Dr MARIE V. LEBOUR, Plymouth (Decapod crustaceans).
H. LEIVESTAD, Bergen (Endocrinology).
D. LESTON, London (*Aëpophilus*).
Dr J. LLEWELLYN, Birmingham (Trematode ectoparasites of fishes).
A. P. M. LOCKWOOD, Cambridge (Green glands of crustaceans).
P. A. LONGTON, Plymouth (Library).
Prof. O. E. LOWENSTEIN, F.R.S., Birmingham (Sense physiology of *Raia* and *Motella*).
Dr A. G. LOWNDES, Plymouth (Mineral contents of filtered sea water).

Mrs F. J. H. MACKERETH, Windermere (Amphipods).
Prof. IRENE MANTON, Leeds (Marine flagellates).
P. T. MARSHALL, Cambridge (Library).
Dr SHEINA M. MARSHALL, Millport (Feeding experiments on *Calanus*).
Mlle M. M. MARTIN, Paris (Marine animal painting).
R. B. MAYNE, Plymouth (Library).
J. S. MCDONALD, Edinburgh (General).
Prof. A. E. W. MILES, London (Development of fish teeth).
P. L. MILLER, Cambridge (Gastropods).
Prof. N. MILLOTT, Jamaica (Echinoderm pigments).
Dr J. E. MORTON, London (Daily rhythms in *Lasaea*).
Dr R. W. MURRAY, Birmingham (Sense physiology in elasmobranchs).

Dr G. R. E. NAYLER, Cambridge (*Maia* blood).
Dr MARGARET NAYLOR, London (Life histories of Phaeophyceae).
Dr J. NEEDHAM, F.R.S., Cambridge (Chromatography of nucleotides).
Miss M. E. NEEDLER, London (Neuromuscular physiology of sea anemones).
B. S. NEWELL, Zanzibar (Library).
Dr WHEELER J. NORTH, Cambridge and La Jolla (Light sensitivity of *Metridium*).

Dr A. P. ORR, Millport (Feeding experiments on *Calanus*).

Dr C. F. A. PANTIN, F.R.S. Cambridge (Light sensitivity of sea anemones).
A. H. PAPWORTH, Northampton (Plankton).
F. G. E. PAUTARD, Leeds (Locomotive organization in Protozoa).
Prof. B. PEYER, Zürich (General).
D. G. POLLARD, Plymouth (Library).
Mme S. PONTUS, Liége (Physiology of kidney in *Lophius*).
Dr H. H. POOLE, Dublin (Photoelectric measurements of submarine illumination).
Miss E. C. POPE, Sydney (Cirripedes).
L. B. PRADHAN, Madras (Mackerel fisheries).

Dr W. J. REES, London (E. T. Browne Collection of Medusae).
Dr K. REICH, Jerusalem (General).
Miss B. RICKARD, Plymouth (Library).
Miss J. E. RIGBY, London (Library).
Dr F. H. RIGLER, Atomic Energy of Canada, Ltd. (Ion exchange in plant cells).
Miss E. A. ROBSON, Cambridge (Motor elements in *Metridium*).
Dr D. M. ROSS, London (Behaviour in sea anemones).
M. J. S. RUDWICK, Cambridge (Brachiopods).

A. SHARPLES, Plymouth (Library).
Dr E. M. SHELSWELL, Mitcham, Surrey (Comparative serology).
Dr R. I. SMITH, Berkeley, California (Chloride regulation in *Nereis diversicolor*).
R. J. SNELL, Ruislip (Pelagic fisheries).
R. E. SOERIAATMADJA, Indonesia (Chemical hydrography).
Dr A. J. SOUTHWARD, D.S.I.R. (Shore ecology).
Mrs A. J. SOUTHWARD, Plymouth (Polychaetes).
Miss F. A. STANBURY, Plymouth (*Cladophora*).
Miss J. E. STEINBERG, California (Opisthobranchs and caprellids).
Dr F. C. STOTT, Ewell (*Holothuria*).
G. SUNDNES, Bergen (Endocrinology).
J. C. SWALLOW, National Institute of Oceanography (Geophysical methods).

Dr Olive S. TATTERSALL, Hayling Island, Hants. (Biology of mysids).
I. M. THOMAS, Adelaide (Iodine uptake in lower chordates).
A. K. TOTTON, London (Morphology of *Physalia*).

E. URSIN, Denmark (Bottom sampling methods).

H. L. VAN DE VYVER, Louvain (Muscle physiology).
J. I. VISFELDT, Aarhus (Nucleic acid in *Aplysia* nerve cells).

Prof. G. P. WELLS, F.R.S., London (*Arenicola*).
Miss J. A. WESTFALL, Naples (Opisthobranchs).
Dr B. WHITTINGTON, London (*Aëpophilus*).
G. WIKKENHAUSER, London (Testing oceanographic equipment).
Mlle L. WILSENS, Liége (Physiology of kidney in *Lophius*).
A. J. WOODS, London (Testing oceanographic equipment).
H. V. WYATT, Plymouth (*Calyptraea chinensis*).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Lt.-Cdr. B. M. Bary, R.N.Z.N., W. Macnae, S. Africa; Dr Ellinor H. Behre, Louisiana; Dr E. S. Deevey, Canada; Miss Mary Samuel, S. India; Prof. W. S. Hoar, Canada; Dr G. J. Hunter, Canada; Dr J. R. Nursall, Alberta; Dr H. Pektas, Turkey; G. A. van Erkel, Utrecht; Prof. B. T. Scheer, California; Prof. A. D. Hasler, Wisconsin; Prof. Isao Motomura, Japan; Dr R. S. Dietz, U.S.A.; C. R. Elsey, Vancouver; B. V. Hamon, Sydney; Dr W. Templeman, Newfoundland; Prof. J. W. Durham, California; Dr M. Buljan, Split; Miss M. I. Garcia, Spain; Mr A. Ben-Turia, Haifa.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-two students from the following Universities and University Colleges: Oxford, Cambridge, Aberdeen, London, Liverpool, Sheffield, Reading, Southampton, Aberystwyth, Cardiff, Bangor, Exeter and the Chelsea Polytechnic.

Also during the Easter Vacation Mr R. J. Jones brought a party of eighteen boys from Whitgift School, and Mr K. W. Wilkes brought three boys from Harrow School. Mr J. F. Eggleston brought eleven pupils from Hinckley Grammar School.

A Joint Meeting of the Challenger Society and Representatives from the Marine Laboratories (Development Commissioners Scheme) was held at the Plymouth Laboratory on 1 and 2 July 1954. Members of the staff provided the papers and demonstrations for the meeting, which was attended by about ninety persons.

Scientific work of the Plymouth Laboratory Staff

Sea Water and Plankton

In collaboration with Dr H. H. Poole, who again visited the laboratory, Dr W. R. G. Atkins has worked on a method for measuring the extinction coefficient of light in the sea using two submerged photometers and thus avoiding certain errors inherent in a comparison with a cell in air. The apparatus has now been used in the sea. A method has also been developed for measuring the light at a point on the sea, as opposed to the vertical component of daylight. In this, readings are made with the six cells of the cube photometer and compared with those got with a globe photometer in the ship. The cube photometer has also afforded further information on the angular distribution of submarine light. The use of the globe does away with the use of gimbals, as the incidence of sunlight is always normal. Daylight factors in the laboratory have also been determined, using a pair of globe photometers.

Though selenium rectifier cells have an advantage for use under water in their high sensitivity and colour sensitivity none of them is really stable on

account of the inherent properties of selenium; as a result the form of the relation between current and light intensity alters and re-standardizations are necessary. With a view to reverting to vacuum emission cells Mr F. J. Warren and Dr Atkins used an amplifier to extend the depth range of this type of cell and were able to measure blue and green light at 70 m some years ago. After a discussion with Dr Poole it now appears possible to use the amplifier for work near the surface also, as a comparatively 'fool proof' method, and this will be tried.

Further measurements have been made, both in the river and at sea, using the 'balance by depth method' to determine the extinction coefficient. The magnitude of the drift error has also been determined under various conditions with the pressure-operated Admiralty depth-recorder. As a control it has also been tried in windless weather and slack water. On account of the rapidity of working the balance by depth method is but little affected by the drift error, nor does alteration in daylight affect it.

Dr Atkins and Mr Warren have tested the stability of a vacuum emission cell of the CMV6 type exposed on the roof for over three years. No change can be detected, nor has the sodium cell of the Burt type changed during this period. It has been on the roof since 1929 save for some of the war years, during which the fire altered its sensitivity. Selenium rectifier cells were also exposed in specially designed water-cooled cases, the current being limited to about that given by a vacuum cell. All infra-red and some red was removed from the daylight by a filter placed outside the water-cooled vessel. The three different types of cell all deteriorated, though one remained constant for over a year. None could be recommended for use in the tropics, as could the CMV6 it seems—for in its manufacture it is baked at a high temperature, well above the melting-point of sodium.

In response to a request from Prof. Hugh C. Trumble, who had visited Africa on behalf of the International Bank for Reconstruction and Development, Dr Atkins has examined samples of West African river and lake waters. Dr Atkins has also modified the diphenylbenzidine method for the determination of nitrate in waters to reduce the effect of any organic matter present. The reagent is easily prepared, stable, sensitive and the colour produced is stable to light.

Using the 'Code des Couleurs' of Klincksieck and Valette and also Ridgway's plates, Miss P. G. Jenkins and Dr Atkins have been able to provide another method for the identification of water masses by comparison of the colour of mineral matter retained by collodion filters after extraction of the phytoplankton pigments. Examination of the residue on filter disks given by water taken at intervals from the surface to bottom, indicates that the mineral matter originates from the surface, not from the bottom, save probably in the autumn equalization of temperature. It appears to come out in fresh water and to be carried long distances in suspension; thus the colour shown at E1

on certain dates was probably due to water from the River Teign with a mineral content derived from the Permian sandstone near Dawlish. They are indebted to Dr A. G. Lowndes for identifying as microcline, a potash felspar, the material found most abundantly from the 5 m water at E 1 on 29 March 1954. Dr Lowndes is now engaged on a systematic examination of such disk deposits and of material from the bottom. The application of petrological methods to marine suspensions and deposits may be expected to give valuable information.

Dr H. H. Poole and Dr Atkins have continued the study of the scattering of light due to suspended matter, and of the effect of the colour of the light. The flask has now been blackened in an attempt to minimize reflexion errors, which are important in their effect upon the residual back scatter. Alterations in design have also been planned for the same purpose. A number of depth series from station E 1 have been examined to follow the seasonal changes. The importance of the small back-scatter arises from its value as an indication of the opacity of the suspension, for opaque obviously reflect more than do translucent particles. As an offshoot from this work on scattering Mr Warren has been able to supply Dr J. A. C. Nicol with helpful information about the vagaries of the photo-multiplier cell and the conditions required for obtaining reliable results.

Miss Jenkins continued her work on the phytoplankton at station E 1. The chlorophyll was determined spectro-photometrically as before. The winter and June minima were quantitatively in close agreement, though the times of occurrence varied. Rough weather prevented observations at the time when the spring outburst was at its maximum in 1954. The years differed remarkably in some respects. Thus *Thalassiosira decipiens*, absent in 1952, was abundant in late months of 1953, whereas *T. gravida*, common in spring and autumn 1952, with some in the spring of 1953, was altogether absent in the autumn of 1953, but very abundant in the spring of 1954. Also *Chaetoceros debilis* was common later in 1952 and 1953, absent in the spring of 1953, and very common in the spring of 1954.

Dr H. W. Harvey has investigated the growth in culture of several species of marine diatoms and flagellates. The addition of vitamin B_{12} (cobalamin) was found necessary to produce considerable population densities of the inshore diatom *Skeletonema costatum* and of two species which are at times abundant at the weather-ship stations in the Atlantic—*Nitzschia seriata* and *Chaetoceras decipiens*. The cells used for insemination had been maintained in cultures containing soil extract, which itself contains this vitamin, but it appears that the cells had not lost their capability of synthesizing the vitamin during the period of culture; a water collected in April, which had a *Skeletonema*-dominant flora, after enrichment and illumination produced few cells without the addition of B_{12}, the addition of which led to dense populations.

Unlike these species of widely distributed planktonic diatoms, *Phaeodactylum* (*Nitzschia closterium* forma *minutissima*), which is very readily cultured

and has been used for most of the classical experiments on the physiology of marine phytoplankton, produces very dense population densities without the addition of B_{12}. Also unlike planktonic diatoms, it appears to require very little, perhaps no, silicate, although it rapidly absorbs silicate if this is provided. Another difference is its ability to utilize carbon dioxide in the medium until almost all the bicarbonate is converted to carbonate and the pH has risen to 10·0.

The supply of silicate to *Skeletonema costatum* has been examined. This lightly silicified species, collected off-shore in the spring, contained some 50 times more silica than phosphorus. When grown in enriched sea water with added soil extract, and containing some 2 mg SiO_2 per litre, this diatom rapidly uses the silicate and then becomes silica deficient, further proliferation being then dependent upon the rate of solution of silicate from the glass vessel.

During various attempts to load sea water with sufficient silicate for heavy growths of diatoms without their becoming silica deficient, it was observed that in many sea waters, but not all, *Skeletonema* grew in bent or tangled chains. This could be prevented by the addition of divalent sulphur, as sulphide or thiourea.

This was in line with observations made both here and in Japan which suggested that the deposition of amorphous silica to form the frustules of diatoms is in some way linked with their sulphur metabolism. This conclusion has now been independently arrived at and verified by Mrs Lewin of Halifax, Nova Scotia, using a freshwater pennate diatom.

Experiments on the growth of natural mixed phytoplankton communities in samples of raw water which has been enriched have shown several instances where diatom growth has soon ceased, unless divalent sulphur was also added.

Although these various experiments have shown that natural sea waters may often contain insufficient B_{12} or insufficient 'sulphur factor' for the growth of dense populations of certain diatom species, they provide no evidence of insufficiency affecting the growth rate of the low population densities which occur naturally in the sea.

The co-operation of Mr F. A. J. Armstrong and Mr C. B. Cowey in these experiments is gratefully acknowledged.

Dr Harvey has also made experiments which bear out the conclusion that zooplankton, when feeding heavily on diatoms during the spring outburst and voiding many more broken diatoms than they can digest, may set free material quantities of inorganic phosphate into the euphotic zone, due to dephosphorylation of organic phosphorus compounds by the enzymes set free in the plant tissue when the cells are broken during their passage through the animals.

During his visits to the United States, Italy, France and Monaco, Dr L. H. N. Cooper was able to discuss the similarities between his problems in the Eastern North Atlantic related to biological productivity in Northern

European waters, and those of the Western North Atlantic, and Eastern North Pacific Oceans, the Gulf of Mexico and the Mediterranean. He read a paper at Woods Hole on his investigations on deep-water movements in the North Atlantic as a link between climatic changes around Iceland and the biological productivity of the English Channel and Celtic Sea. This paper will be published in the *Journal of Marine Research*. He is also collaborating with Dr E. G. Goldberg of the Scripps Institution in the preparation of a detailed account of the occurrence of chemical elements in sea water.

Dr Cooper has also been studying the implications on marine research of the draft articles for international legislation on the regime of the high seas prepared by the International Law Commission. A leading article by him appeared in *Nature*, and the subject has since been considered by a number of interested national and international scientific bodies.

Mr F. A. J. Armstrong has continued the regular cruises to station E 1, and analyses for phosphate, total phosphorus and silicon. During spring and summer 1954 extra depths have been sampled. Surface temperatures in this period have been lower than in the past four years, and the silicate figures below 40 m have shown an interestingly steady rise after the thermocline was established. Some aluminium determinations during the period gave figures from 0·2 to 2·0 μg atom Al/l. with no obvious relationship to the silicon concentrations. Some earlier (unpublished) analyses of Western Atlantic water showed values in excess of 10 μg atom Al/l. in water below 1000 m.

The series of experiments on biological differences between sea waters was continued by Dr D. P. Wilson and Mr F. A. J. Armstrong during the spring of 1954. Unfortunately weather conditions much interfered with the collection of the water samples, and on several occasions experiments could not be carried out as planned. None the less, some preliminary results indicated that there is a marked difference between bottom and surface waters from the same hydrographical position, the eggs and larvae of *Echinus esculentus* developing more normally in one than in the other. However, in view of the fact that these experiments cannot be completed and confirmed until another year, a description of what has been done might ultimately prove to be misleading. In addition, some further attempts were made to free water samples from bacteria at the time of collection. Few of these attempts were completely successful, but those that were gave little support to the hypothesis that the major differences which have been observed can be accounted for by changes brought about by the multiplication of bacteria in the water samples in the period between collection and testing.

No noticeable change in the poor macroplankton production level association with the prevailing *Sagitta setosa* conditions has been observed by Mr P. G. Corbin during 1954.

During her tenure of a British Council Scholarship, Señora A. Alvariño, biologist on the staff of the Spanish Institute of Oceanography, assisted

Mr Corbin in the analysis of the routine weekly macroplankton samples taken during 1950–53 in half-hour hauls of the 2 m stramin ring trawl off the Eddystone. It is intended to publish these observations in one paper.

In September 1954 Mr Corbin had an opportunity aboard R.R.S. *Discovery II* of carrying out some preliminary trials of the 15 ft, self-depressing mid-water trawl referred to in the last Report of Council. It was evident from the lie of the net and its stability when towing that the principle of design is sound, but to attain a good fishing depth when towed at some speed without paying out a disproportionately long length of warp will entail certain modifications.

Mr Corbin gave some assistance to Señora A. Alvariño in her plankton work and accompanied her on the May 1954 cruise in R.V. *Sarsia*. This cruise extended southwards from Ushant along the French Biscayan continental shelf to the latitude of Belle Ile. Señora Alvariño's analysis of the collections taken revealed some interesting new records of *Sagitta frederici*, a species not previously taken in the Atlantic farther north than the French Moroccan shelf.

The collection of species-pure cultures of marine phytoplankton organisms has been maintained by Dr Mary Parke throughout the year, and a number of new forms have been added to the collection. During the year cultures for research purposes have been sent abroad and have also been produced for the use of research workers in the laboratory.

Work is continuing on new members of the Chrysophyceae. These new organisms and also forms already described are being studied by means of the electron microscope, and Prof. Irene Manton is collaborating with Dr Parke in this part of the investigation.

Miss D. Ballantine has continued work on the two species of *Gymnodinium* in the culture collection, and it is hoped that descriptions of them will shortly be published.

One of these species (*G. veneficum* nom. prov.) has been shown to be highly toxic to fish and to some other marine animals, notably molluscs, and the physiological effect of the toxin on whole animals has been studied. Dr Abbott, Dr Carlisle and Miss Ballantine have been checking the effect of the toxin on isolated preparations of nerves and muscles and on perfused hearts, in an attempt to learn something of its pharmacology and they are also trying to isolate the toxin both from the cells and from the supernatant culture water.

Miss Ballantine and Dr J. E. Morton have been studying the feeding rate and digestive capabilities of the small lamellibranch, *Lasaea rubra*, when fed on a variety of cultures of marine algae. It was found that *Lasaea* was capable of filtering organisms and particles from about 1–40 μ in size, but that digestion of armoured forms (dinoflagellates and diatoms) was very slow, if any.

Macro-Fauna and Flora

Dr D. P. Wilson's experiments on the settlement of the larvae of *Ophelia bicornis*, continued during the summer of 1954, indicated very clearly that the presence of living micro-organisms on the sand grains is a primary factor in inducing larvae to settle and metamorphose. It appears that these organisms, perhaps mainly bacteria, must be neither too few nor too abundant to make an otherwise clean sand fully attractive to the larvae. It is this coating of living organisms which constitutes the food of the adult worms, which swallow the sand grains. On the other hand, dead organic matter is unattractive to the larvae and may be repellent. It is possible that not only the relative abundance of the micro-organisms present influences the larvae testing the sand grains, but that different kinds are more attractive than others. So far very few experiments to determine this aspect of the problem have been possible. A paper describing the latest results will be published in a future number of the *Journal*.

Dr G. A. Steven's routine studies of the spring mackerel fishery at Newlyn, Cornwall, have been continued. The moderate success achieved by that fishery in the spring of this year was in good agreement with his forecast. The outlook for next year is that only moderately successful fishing is again to be expected.

The trawling surveys mentioned in last year's report have been continued. No new trends of particular significance have become evident.

The experiments with artificial bait for crab and lobster pots have now reached a stage when small-scale field experiments are called for. Several members of the trade have offered to collaborate in such experiments, and preparations for them are now well in hand.

In carrying out an echo-sounder survey in an area known as La Chapelle Bank, about 100 miles west of the north-west corner of France, Dr Steven obtained echo traces showing that the sea floor in the vicinity of the 100 fm contour is thrown into a remarkably uniform series of undulations running parallel with the 100 fm contour which marks the beginning of the continental slope in that area. Bottom sampling has confirmed that these undulations are sand waves which extend eastwards (=inwards) from the continental edge for some 10 miles. The 'waves' are of very uniform height—about 35 ft from crest to trough—and roughly half a mile from crest to crest. It seems probable that these sand waves reflect the energy pattern of waves in the overlying water. This deserves further investigation.

Mr G. M. Spooner has continued the faunistic work on isopods and amphipods referred to previously, the results of which will be largely incorporated in the revised fauna list of these groups. Amongst material derived from Mr G. R. Forster's hand-collected rocks and weeds, that from the southern reef of the Eddystone is noteworthy. The fauna of the tufted weeds

growing here on exposed rocks intertidally is dominated by *Parajassa pelagica* and *Idothea pelagica*, accompanied by *Hyale pontica*, *H. perrieri*, and, a species new to the British fauna, *Stenothoë spinimana*. This last was previously known from Brittany (rarely) to W. Africa.

Attention has been given by Mr Spooner to insects inhabiting the intertidal zone, in particular the Diptera. Ecologically these fall into two distinct groups. (*a*) Flies that breed in seaweed litter accumulated in the H.W. zones, or at a somewhat lower level in gullies. These include one small, but numerous, sphaerocerid (*Thoracochaeta zosterae*), four coelopids or 'Kelp flies', and five small aberrant empidids predatory on the others. (*b*) A more varied class whose larvae live amongst algae or in rock crevices, etc., in the intertidal zone, the adults of the more active species ranging over the whole tidal area when uncovered. The predatory cordylurids are represented by *Ceratinostoma ostiorum*; and the muscids by the abundant *Fucellia maritima*, also predatory to some degree as an adult. The Aphrosylines are a distinct marine subfamily of the Dolichopodidae, represented in this area by the larger *Aphrosylus celtiber* and *A. raptor*, and the smaller *A. ferox* and *A. mitis*, the first and third being generally common on the rocky shore. Two separable dolichopodid larvae have been recognized from algal tufts in the mid-tide zone, and these are expected to belong to *celtiber* and *ferox*. *Aphrosylus*, too, are predatory in the adult stage, and like *Ceratinostoma* and *Fucellia*, have a predominantly grey colour, though this is quite atypical for the family. Nematocerous families represented are Tipulidae and Chironomidae. Larvae of the tipulid *Geranomyia unicolor* are now well-known inhabitants of the upper half of the tidal zone, sheltering amongst the lichen *Pygmaea pumila* or alga *Catenella repens*; while the adults fly round shaded rocks or damp patches on cliffs in the H.W. region. Five chironomid species regularly occur on the shore in the Plymouth Area; the orthocladines *Hydrobaenus thalassophila*, *Cricotopus fucicola*, and *C. vitripennis*, and the clunionines *Thalassomyia frauenfeldi* and *Clunio marinus*. The larvae are frequent in various intertidal weeds, and an attempt is being made to assign these to their correct adult.

Mr Corbin has continued his observations on the Lucernariidae and on the sucker fish, *Lepadogaster couchii*.

Dr H. G. Vevers has resumed observations, started some years ago, on the animals which live sheltered in clumps of the bryozoans *Cellaria* and *Lepralia*. These clumps occur in the trawling grounds and a measure of their frequency has been obtained from a study of underwater photographs. In particular, the delicate calcareous meshwork of the *Cellaria* colonies form safe nurseries for the young of many benthonic invertebrates. Among these may be mentioned the regularly occurring populations of young queen scallops (*Chlamys opercularis*)—a cubic foot of *Cellaria* colony may contain 100–200 of these molluscs, with a mean shell length of 60 mm.

Dr Vevers is also working with Miss D. Ballantine on the pigments of

certain marine flagellates, and in collaboration with Dr G. Y. Kennedy, of the University of Sheffield, has published a paper in Vol. 33, No. 3, of the *Journal*, on the occurrence of porphyrins in certain marine invertebrates. This work has shown the presence of uroporphyrin I in the integument of two molluscs (one tectibranch and one nudibranch), of coproporphyrin III in the viscera of three polychaetes, and of protoporphyrin in three echinoderms. Of particular interest is the occurrence of free chlorocruoroporphyrin in two of these three echinoderms, the phanerozonian starfishes *Luidia ciliaris* and *Astropecten irregularis*.

Mr N. A. Holme has started taking samples of the bottom fauna at a position near the Eddystone. These will be repeated at regular intervals in an attempt to correlate changes with the hydrography of the area. The first samples, taken in August 1954, are comparable with those previously taken in 1950, but there have been appreciable changes in numbers of a few species, notably an increase in numbers of the lamellibranch *Abra alba*. Sampling will have to be continued over a period of years, however, before the significance of any such changes can be appreciated.

Work has continued on the ecology of lamellibranchs on the sea bed, with particular reference to the Veneridae, some of which are important constituents of the bottom fauna. A number of collections have been made on beaches in Devon and Cornwall, but little work has been possible at sea during 1954.

For taking a small sample of the sea bed an improved form of the Hunt 'suction-sampler' has been made. The instrument is easier to handle, as the glass plate sealing the air-chamber has been eliminated, and a number of other modifications have been made. The sampler is effective to depths of at least 200 m, and seldom fails to take a sample.

Observations on the opisthobranch mollusc *Akera bullata* have been made, and notes on the ecology and swimming movements of this species are to be published in a joint paper with Dr J. E. Morton in Vol. 34, No. 1, of the *Journal*. Mr Holme has collaborated with Mr Armstrong in the production of a short film to show the swimming movements of this mollusc.

Dr Carlisle has continued his studies of Plymouth tunicates and has contributed papers on this subject to the *Journal*.

Mr G. R. Forster has completed a note on the sublittoral fauna of Stoke Point Reef. As it was found from the 1953 underwater observations that sponges were frequently abundant, a general study has been made of this group to facilitate their identification. One of the commoner sublittoral sponges has been found to be *Hemimycale* (*Stylotella*) *columella*, only once previously recorded from Plymouth.

The diving technique and collecting methods have been improved, and much more material has been obtained for rock fauna studies. In August and September a total of twenty-nine dives was made outside the Sound, with the

assistance of Mr R. U. Gooding. This total includes twelve dives made during a fortnight's cruise to Dartmouth and Torbay in Mr Forster's own yacht *Sunset*.

Additions have been made during the year to the Laboratory's museum collection of algal material and many gaps have been filled both in the Herbarium collection and in the preserved collection. The spread of some of the more southern forms which have appeared on the south coast is being followed.

Physiology of Marine Organisms

During his tenure of a Guggenheim Fellowship Dr J. A. C. Nicol spent most of his time at the Scripps Institution of Oceanography, La Jolla, California, where he continued his researches on the nervous control of luminescence. Most of his attention was devoted to a study of luminescence in the pennatulid *Renilla*. Bright luminous waves, very characteristic of this animal, are controlled by a nerve net which shows non-polarized through-conduction and neuro-effector facilitation of a mode very similar to that discovered in neuromuscular responses of other coelenterates. Information was also obtained for recovery of luminescence after light exposure. Two papers dealing with this work have been accepted for publication in the *Journal of Experimental Biology*. Opportunities were also taken to investigate luminescence in sea pens (*Leioptilus*), California singing fish (*Porichthys*), and in hydromedusae, both at the Scripps Institution, and at the Friday Harbor Laboratories. Visits were paid to other campuses of the University of California, and to the Kerckhoff and Hopkins Marine Laboratories. Dr Nicol also attended a conference on luminescence at Pacific Grove, California, the proceedings of which will shortly be published.

Dr J. S. Alexandrowicz has continued his studies on the muscle receptor organs of crustaceans. The structure of these elements appears to follow the same pattern in various groups of crustaceans, but certain peculiar arrangements were observed in isopods and mysids. In *Ligia oceanica* and *Idotea emarginata* there are also receptors with muscles which extend over two segments, and in *Praunus flexuosus* the muscle components of the receptors of three segments can be fused end to end.

Dr Alexandrowicz has also continued his studies on the innervation of the crustacean heart. An account of his investigations of this system in *Marinogammarus marinus* has been published in Vol. 33, No. 3, of the *Journal*. The observations on *Praunus flexuosus* have shown that the Mysidacea also have three systems of heart nerves (as found previously in other crustaceans), i.e. a local system made up of no less than six neurons, the nerves connecting the local system with the central nervous system, and the nerves of the arterial valves. These results have been published in the *Journal*. A peculiar arrangement of the heart nerves has been observed in *Leander serratus*, in that the nerves coming from the central nervous system enter the ganglionic trunk

at its posterior end. The nerve fibres supplying the pericardial muscles have been found to travel by two different ways, some being carried by the segmental nerves, while others are branches of an unpaired nerve emerging on the dorsal surface of the infraoesophageal ganglion.

Dr D. B. Carlisle has continued his work on the endocrinology of moulting in Crustacea. The topography of the endocrine organs of the protocerebrum of Isopoda is similar to that of Natantia, and in the hormonal control of moulting this group appears to follow the same pattern. Eyestalk ablation performed on our native stock of *Leander serratus* led to slower moulting, whereas in *L. serratus* collected at Roscoff the opposite was true, as previously reported by P. Drach. These results are taken as supporting the hypothesis that the eyestalk secretes both a moult-inhibiting and a moult-accelerating hormone.

In collaboration with Mme M. Dupont-Raabe and Sir Francis Knowles, Dr Carlisle has begun an investigation into the separation and characterization of chromactivating substances in prawns and insects. Some measure of success has attended this attempt.

Dr B. C. Abbott has continued measurements on the uptake by marine organisms of uranium fission products—using in particular yttrium, ruthenium and niobium. He and Dr Carlisle have started an investigation into the uptake by ascidians of radioactive niobium, an element which Dr Carlisle has recently found to be present in relatively large amounts in some ascidians. Slow accumulation and retention of niobium has been demonstrated in *Molgula* using the isotope ^{95}Nb, but none is retained in *Ciona*. Autoradiographs have confirmed the uptake in *Molgula* and its absence in *Ciona*.

Dr Abbott has used the radioisotope ^{42}K in a study of potassium exchange in green algae, and has estimated the ionic flux across cell membranes at various levels of illumination.

The rate of leaching of iodide from frog and other muscles after previous equilibration with Ringer solution in which iodide replaces a portion of the chloride ion has been measured in conjunction with experiments of Prof. A. V. Hill on the effects of iodide ion on isolated muscle twitches.

Preliminary experiments on the heat production in isolated byssal retractor muscles of *Mytilus* show a very small heat rate in a maintained tetanus. The effect of width of stimulus pulse and repetition frequency on the threshold voltage for isolated *Mytilus* muscle was investigated in collaboration with M. Van de Vyver from Louvain.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

Dr Anna M. Bidder and Mrs Barclay Russell have generously presented to the Library a collection of reprints which belonged to the late Dr G. P. Bidder.

During the year the library of books and reprints presented by Dr Marie V. Lebour has been catalogued and incorporated in the general index.

The Library has again been much used by visiting members of the Association.

Published Memoirs

Volume 33, No. 1, of the *Journal* was published in February 1954, No. 2 in June 1954 and No. 3 in October 1954; and Vol. 34, No. 1 in February 1955.

The following papers, the outcome of work done at the Plymouth Laboratory, have been published elsewhere than in the *Journal* of the Association:

ABBOTT, B. C. & LOWY, J., 1953. Mechanical properties of *Mytilus* muscle. *Proc. Physiol. Soc.* 20–21 March 1953, *J. Physiol.*, Vol. 120, 50*P*.

ALEXANDROWICZ, J. S., 1954. Notes on the nervous system in the Stomatopoda. IV. Muscle receptor organs. *Pubbl. Staz. zool. Nap.*, Vol. 25, pp. 94–111.

ATKINS, D., 1955. The post-embryonic development of British *Pinnotheres* (Crustacea). *Proc. zool. Soc. Lond.*, Vol. 124, pp. 687–715.

ATKINS, W. R. G., 1953. Chemical evidence on the abundance and botanical composition of the marine phytoplankton. *Proc. Seventh Int. bot. Congr.*, Stockholm, 1950, p. 262, publ. 1953.

ATKINS, W. R. G., 1954. Note on the use of diphenylbenzidine for the estimation of nitrate in sea water. *J. Cons. int. Explor. Mer*, Vol. 20, pp. 153–5.

ATKINS, W. R. G. & POOLE, H. H., 1954. The angular scattering of blue, green, and red light by sea water. *Sci. Proc. R. Dublin Soc.*, Vol. 26, pp. 313–23.

BRULL, L. & CUYPERS, Y. (avec la collaboration de L. Dubois et L. Wilsens), 1954. Quelques caractéristiques biologiques de '*Lophius piscatorius* L.' *Arch. int. Physiol.*, T. 62, pp. 70–5.

CARLISLE, D. B., 1954. On the relationship between mammary, sweat, and sebaceous glands. *Quart. J. micr. Sci.*, Vol. 95, pp. 79–83.

CARLISLE, D. B., 1954. Studies on *Lysmata seticaudata* Risso (Crustaces Decapods). VIII. The lack of influence of eyestalk ablation and of injection of eyestalk extracts on testicular weight and degree of development of the male genital ducts. *Pubbl. Staz. zool. Nap.*, Vol. 25, pp. 241–5.

CARLISLE, D., DUPONT-RAABE, MARIE & KNOWLES, Sir F., 1955. Recherches préliminaires relatives à la séparation et à la comparaison des substances chromactives des Crustacés et des Insectes. *C.R. Acad. Sci., Paris*, Vol. 240, pp. 665–7.

GUELIN, A., 1954. La contamination des poissons et le problème des eaux polluées. *Ann. Inst. Pasteur*, T. 86, pp. 303–8.

JACKMAN, L. A. J., 1954. The early development stages of the Bass, *Morone labrax* (L.). *Proc. zool. Soc. Lond.*, B, Vol. 124, pp. 531–4.

JONES, WALTER CLIFFORD, 1954. Spicule form in *Leucosolenia complicata*. *Quart. J. micr. Sci.*, Vol. 95, pp. 191–203.

KERKUT, G. A., 1954. The mechanisms of coordination of the starfish tube feet. *Behaviour*, Vol. 6, pp. 206–32.

LEBOUR, MARIE V., 1954. The planktonic decapod Crustacea and Stomatopoda of the Benguela Current. Part I. First survey, R.R.S. 'William Scoresby', March 1950. *'Discovery' Rep.*, Vol. 27, pp. 219–34.

LEBOUR, MARIE V., 1955. First-stage larvae hatched from New Zealand decapod Crustacea. *Ann. Mag. nat. Hist.*, 12th ser., Vol. 8, pp. 43–8.

LLEWELLYN, J., 1954. Observations on the food and the gut pigment of the Polyopisthocotylea (Trematoda: Monogenea). *Parasitology*, Vol. 44, pp. 428–37.

LOWENSTEIN, O., 1955. The effect of galvanic polarization on the impulse discharge from sense endings in the isolated labyrinth of the thornback ray (*Raja clavata*). *J. Physiol.*, Vol. 127, pp. 104–17.

LOWNDES, ASHLEY G., 1954. A first tracer mineral. *Discovery*, Vol. 15, pp. 153–54.

LOWY, J., 1954. Contraction and relaxation in the adductor muscles of *Pecten maximus*. *J. Physiol.*, Vol. 124, pp. 100–5.

MORTON, J. E., 1954. The pelagic mollusca of the Benguela current. Pt. I. First survey, R.R.S. 'William Scoresby', March 1950 with an account of the reproductive system and sexual succession of *Limacina bulimoides*. 'Discovery' Rep., Vol. 27, pp. 163–200.

PASSANO, L. M. & PANTIN, C. F. A., 1955. Mechanical stimulation in the sea-anemone *Calliactis parasitica*. *Proc. roy. Soc.*, Ser. B, Vol. 143, pp. 226–38.

PURCHON, R. D., 1955. The structure and function of the British Pholadidae (Rock-Boring Lamellibranchia). *Proc. zool. Soc. Lond.*, Vol. 124, pp. 859–911.

RUSSELL, F. S., 1954. James Herbert Orton, 1884–1953. *Obit. Not. roy. Soc.*, Vol. 9, pp. 201–15.

SOUTHWARD, A. J. & CRISP, D. J., 1954. The distribution of certain intertidal animals around the Irish coast. *Proc. R. Irish Acad.*, Vol. 57 (B), 29 pp.

SOUTHWARD, EVE C., 1955. Polychaeta new to the British Isles. *Nature, Lond.*, Vol. 175, p. 264.

THOMAS, P. K., 1955. Growth changes in the myelin sheath of peripheral nerve fibres in fishes. *Proc. roy. Soc.*, Ser. B, Vol. 143, pp. 380–91.

VEVERS, H. G., 1954. Photography of the sea floor. *Advanc. Sci., Lond.*, Vol. 11, pp. 49–51.

VEVERS, H. G., 1954. *The British Seashore*. London, 160 pp.

VEVERS, H. G., 1954. Photography of the sea floor. *New Biol.*, no. 17, pp. 91–5.

VEVERS, HENRY GWYNNE, 1954. The experimental analysis of feather pattern in the Amherst Pheasant, *Chrysolophus amherstiae* (Leadbeater). *Trans. zool. Soc. Lond.*, Vol. 28, pp. 305–49.

WELLS, G. P., 1954. The mechanism of proboscis movement in *Arenicola*. *Quart. J. micr. Sci.*, Vol. 95, pp. 251–70.

WELSH, JOHN H., 1954. Marine invertebrate preparations useful in the bioassay of acetylcholine and 5-hydroxytryptamine. *Nature, Lond.*, Vol. 173, pp. 955–6.

Membership of the Association

The total number of members on 31 March 1955 was 827, being 57 more than on 31 March 1954; of these the number of life members was 93 and of annual members 734. The number of associate members is five.

Momber Bequest

Under the terms of the will of the late E. R. T. Momber, who died in 1911, the Marine Biological Association has received a bequest of £1072.

The Council have decided to use this money for the construction of new outside seawater tanks for research purposes.

Grant from Rockefeller Foundation

The thanks of the Council are due to the Trustees of the Rockefeller Foundation for a generous grant of 30,000 dollars for the promotion of research at the Plymouth laboratory. The grant is to be spread over a period of five years as from 1 October 1954, and not more than 7500 dollars are to be spent in any one year.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Capital Grant. The Council wish to record their thanks to the Development Commissioners for a substantial Capital Grant to meet the cost of extending the library and constructing a new store for dogfish and other large marine specimens.

Private Income. The Council gratefully acknowledges the following generous grants received during the year:

From the Fishmongers' Company (£500), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s.*), Leeds (£20), Durham (£10. 10*s.*), Manchester (£10. 10*s.*), Sheffield (£10. 10*s.*), Reading (£10. 10*s.*), Nottingham (£10. 10*s.*), Hull (£10. 10*s.*), Exeter (£10. 10*s.*), Leicester (£10. 10*s.*), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1955–56:

President

Prof. A. V. HILL, C.H., O.B.E., Sc.D., F.R.S.

Vice-Presidents

The Earl of IVEAGH, K.G., C.B., C.M.G.
Sir NICHOLAS E. WATERHOUSE, K.B.E.
Col. Sir EDWARD T. PEEL, K.B.E., D.S.O., M.C.
Vice-Admiral Sir JOHN A. EDGELL, K.B.E., C.B., F.R.S.
Sir EDWARD J. SALISBURY, Kt., C.B.E., D.Sc., Sec.R.S.
Admiral Sir AUBREY C. H. SMITH, K.B.E., C.B., M.V.O.
A. T. A. DOBSON, C.B., C.V.O., C.B.E.
Major E. G. CHRISTIE-MILLER
MORLEY H. NEALE, C.B.E.
The Earl of VERULAM
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

COUNCIL

To retire in 1956

J. N. CARRUTHERS, D.Sc.
Prof. H. MUNRO FOX, F.R.S.
Prof. A. L. HODGKIN, F.R.S.
Prof. J. E. SMITH, Sc.D.
Prof. V. C. WYNNE-EDWARDS

To retire in 1957

Miss ANNA M. BIDDER, Ph.D.
D. J. CRISP, Ph.D.
Prof. J. E. HARRIS, Ph.D.
C. E. LUCAS, D.Sc.
Prof. C. M. YONGE, C.B.E., D.Sc., F.R.S.

To retire in 1958

Miss VERA FRETTER, D.Sc.
MICHAEL GRAHAM, C.M.G., O.B.E.
N. A. MACKINTOSH, C.B.E., D.Sc.
G. E. NEWELL, Ph.D.
Prof. LILY NEWTON, D.Sc.

Hon. Treasurer

Major E. G. CHRISTIE-MILLER, 38 Hyde Park Street, London, W. 2.

Secretary

F. S. RUSSELL, C.B.E., D.S.C., D.F.C., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. WALL (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
The Prime Warden
Major E. G. CHRISTIE-MILLER
HARRISON S. EDWARDS
Prof. A. C. HARDY, D.Sc., F.R.S. (Oxford University)
S. SMITH, Ph.D. (Cambridge University)
EDWARD HINDLE, Sc.D., F.R.S. (British Association)
H. W. PARKER, D.Sc. (Zoological Society)
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1954–55

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 MARCH 1955

	£	£
CAPITAL RESERVE ACCOUNT:		
As at 31st March 1954	170,447	
Add: Expenditure on fixed assets recovered	525	
		170,972
SURPLUS ACCOUNT:		
As at 31st March 1954	11,452	
Less: Excess of expenditure over income for the year	3,306	
		8,146
		179,118
BALANCES ON SPECIAL FUNDS		10,214
CURRENT LIABILITIES:		
Sundry Creditors and Accrued Expenses	2,137	
Subscriptions and Grants received in advance	146	
Equipment and Apparatus—R.V. 'Sarsia'	838	
		3,121
Note: Capital commitments outstanding amount to £8,522 (1954 £18,072) in respect of the Library Extension and Dogfish House of which £6,815 will be recoverable under a Development Fund Grant.		
		£192,453

	£	£
FIXED ASSETS:		
At valuations as estimated by the Director at 31st March 1955:		
Boats and Equipment:		
M.F.V. 'Sula'	11,000	
R.L. 'Gammarus'	200	
	11,200	
Laboratory Apparatus, Equipment and Machinery at cost	11,060	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost	20,900	
R.V. 'Sarsia' at cost	137,761	
		180,921
INVESTMENTS:		
General Fund, at cost (Market value £217; last year £228)	232	
Composition Fees Fund, at cost (Market value £1,110; last year £1,013)	1,219	
E. T. Browne Bequest Funds at cost (Market value £3,440; last year £3,441)	4,013	
		5,464
CURRENT ASSETS:		
Stocks on Hand as valued by the Director	1,550	
Sundry Debtors	791	
Prepayments	135	
Balance at Bank and Cash in Hand	3,592	
		6,068
JOHN E. HARRIS, O. LOWENSTEIN } *Members of the Council*		
		£192,453

REPORT OF THE AUDITORS TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1955, and of the excess of expenditure over income for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
20 May 1955

PRICE WATERHOUSE & CO
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1955

	£	£
SALARIES, NATIONAL INSURANCE AND SUPERANNUATION SCHEME CONTRIBUTIONS		27,537
LABORATORY AND BOATS' CREWS' WAGES, NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND EMPLOYER'S LIABILITY INSURANCE		21,643
UPKEEP OF LIBRARY		651
SCIENTIFIC PUBLICATIONS, *less* Sales		1,531
UPKEEP OF LABORATORIES:		
Buildings and machinery	434	
Electricity, Gas, Coal and Water	1,351	
Chemicals and Apparatus	1,127	
Rents and Insurances	161	
Travelling Expenses	711	
Audit Fees (including £63 in respect of previous year)	189	
Stationery, Postage, Telephone and Sundries	888	
Specimens	202	
		5,063
MAINTENANCE AND HIRE OF BOATS:		
Petrol, Oil, Paraffin, etc.	1,298	
Maintenance and Repairs	4,680	
Collecting Expenses and Upkeep of Truck	293	
Insurances	2,472	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
Hire of R.V. 'Sabella'	—	
		9,138
ENTERTAINMENT EXPENSES		42
BALANCE being excess of income over expenditure for the year		—
		£65,605

Note: No depreciation is provided on fixed assets.

	£	£
GRANTS AND TABLE RENTS:		
Ministry of Agriculture, Fisheries and Food Grant from Development Fund	55,866	
Fishmongers' Company	400	
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Reading £10. 10*s*. and Sheffield £10. 10*s*., Imperial College £10, Ministry of Works £104, Imperial Chemical Industries Ltd £52. 10*s*. and International Paints Ltd. £39. 7*s*. 6*d*.)	1,233	
		57,499
SUBSCRIPTIONS (excluding those received in advance)		699
SALES:		
Specimens	2,220	
Fish	358	
	£	
Nets, Gear and Hydrographical Apparatus	439	
Less: Cost of Materials	400	
	39	
		2,617
INTEREST ON INVESTMENTS		44
INTEREST ON BANK DEPOSITS, *less* Charges		39
SALE OF DR M. V. LEBOUR'S BOOK		5
AQUARIUM:		
Admission Fees	1,655	
Sale of Guides	116	
	1,771	
Less: Maintenance, Printing and Advertising	375	
		1,396
BALANCE being excess of expenditure over income for the year		3,306
		£65,605

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31ST MARCH 1955

	Aquarium Sinking Fund	Composition Fees	E. T. Browne Bequest			A. R. T. Momber Bequest	Rockefeller Foundation	War Damage Compensation Part II	Buildings Reconstruction	Library Extension and Dogfish House	Research Funds*	TOTAL
			Library	Special Apparatus	Scientific Publications							
	£	£	£	£	£	£	£	£	£	£	£	£
BALANCE AT 31ST MARCH 1954	307	1,093	1,296	2,795	(21)	942	—	957	1,136	—	760	9,265
Add: Receipts during year:												
Grants	—	—	—	—	—	—	1,343	—	—	9,550	1,956	12,849
Income from investments	—	—	39	81	—	—	—	—	—	—	—	120
Bank deposit interest	4	—	—	—	—	14	—	—	—	—	—	18
Other receipts	30	158	—	—	—	134	—	—	—	—	—	322
Inter-Fund transfers	—	—	—	—	—	—	—	(564)	(1,136)	1,700	—	—
	341	1,251	1,335	2,876	(21)	1,090	1,343	393	—	11,250	2,716	22,574
Deduct: Expenditure	—	—	—	—	—	—	825	348	—	9,683	1,504	12,360
BALANCE AT 31ST MARCH 1955	£341	£1,251	£1,335	£2,876	£(21)	£1,090	£518	£45	—	£1,567	£1,212	£10,214

* Including International Paints Research Fellowship.

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

THE ASSOCIATION was founded in 1884 to promote accurate researches leading to the advancement of zoological and botanical science and to an increase in our knowledge of the food, life, conditions and habits of British fishes. The work of the Association is controlled by a Council elected annually by its subscribing members.

Professor T. H. Huxley took the chair at the initial meeting held in the rooms of the Royal Society and was elected the first President. Among those present were Sir John Lubbock (afterwards Lord Avebury), Sir Joseph Hooker, Professor H. N. Moseley, Mr G. J. Romanes, and Sir E. Ray Lankester who, after Professor Huxley, was for many years president of the Association. It was decided that a laboratory should be established at Plymouth, where a rich and varied fauna is to be found.

The Plymouth Laboratory was opened in June 1888, and, since that date, a new library and further laboratory accommodation have been added.

The Association is maintained by subscriptions and donations from private members, universities, scientific societies and other public bodies; a generous annual grant has been made by the Fishmongers' Company since the Association began. Practical investigations upon matters connected with sea-fishing are carried on under the direction of the Council, and from the beginning a Government Grant in aid of the maintenance of the laboratory has been made; in recent years this grant has been greatly increased in view of the assistance which the Association has been able to render in fishery problems and in fundamental work on the environment of marine organisms. Accounts of the laboratory and aquarium and the scope of the researches will be found in Vol. XXVII (p. 761) and Vol. XXXI (p. 193) of this *Journal*.

The laboratory is open throughout the year and its work is carried out by a fully qualified research staff under the supervision of the Director. The names of the members of the staff will be found at the beginning of this number. Accommodation is available for British and foreign scientific workers who wish to carry out independent research in marine biology, physiology and other branches of science. Arrangements are made for courses for advanced students to be held at Easter, and marine animals and plants are supplied to educational institutions.

Work at sea is undertaken by two research vessels and by a motor boat, and these also collect the specimens required in the laboratory.

TERMS OF MEMBERSHIP

		£	s.	d.
Annual Members	per annum	1	1	0
Life Members	Composition fee	15	15	0
Founders		100	0	0
Governors		500	0	0

Members of the Association have the following rights and privileges: they elect annually the Officers and Council; they receive the Journal of the Association free by post; they are admitted to view the laboratory at Plymouth, and may introduce friends with them; they have the first claim to rent a place in the laboratory for research, with use of tanks, boats, etc.; they have the privilege of occupying a table for one week in each year free of charge; and they have access to the books in the library at Plymouth.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

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