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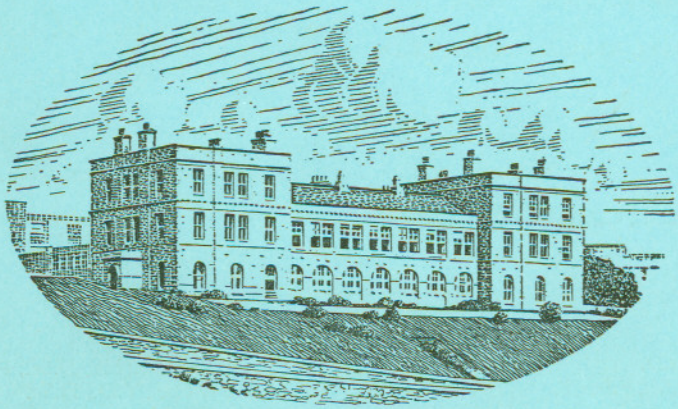

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THE FURTHER SPREAD OF *ELMINIUS MODESTUS* IN THE BRITISH ISLES TO 1959

By D. J. CRISP and A. J. SOUTHWARD

Marine Biology Station, Menai Bridge, Anglesey and The Plymouth Laboratory

(Text-fig. 1)

Since the surveys described in our last report (Crisp, 1958) on the distribution in north-west Europe of the immigrant Australasian barnacle, *Elminius modestus* Darwin, the species has become much commoner along the western coasts and has finally spread to Ireland (Beard, 1957). In this report we present the results of surveys made in 1958–9 along the eastern side of the Irish Channel and in the Isle of Man, as well as full records of a survey of the Irish coast in 1958. The methods employed were similar to those described previously (Crisp, 1958; Bishop & Crisp, 1958).

We are indebted to the Browne Research Fund of the Royal Society and to the Marine Biological Association for financial assistance. Dr J. R. Lewis and Mr W. J. Ballantine have kindly supplied additional information for Ireland.

EASTERN SIDE OF THE IRISH CHANNEL

The changes in distribution of *Elminius* along the west coast of England and Wales have been described up to 1956 (Crisp, 1958). At that time an extension was just beginning around the Lleyn peninsula into the north end of Cardigan Bay. Between 1956 and 1958 the species spread southward over most of the north end of the bay; but, except for the area between Pwlheli and Barmouth, where heavy settlements occurred, it was otherwise confined to harbours and estuarine areas (Fig. 1).

At the south end of Cardigan Bay a relatively local settlement was discovered around the port of Fishguard. This colony, and a smaller one in the harbour at Solva, are believed to have originated from Milford Haven, where *Elminius* has been common for 10 years. The slow spread from this direction, in comparison with the rapid extension from the north, must be due to the lack of suitable habitats for the species along the wave-beaten Pembroke coast and the probable presence of adverse currents inshore. Similar factors may have prevented the spread of the species around the Lizard and Lands End in west Cornwall.

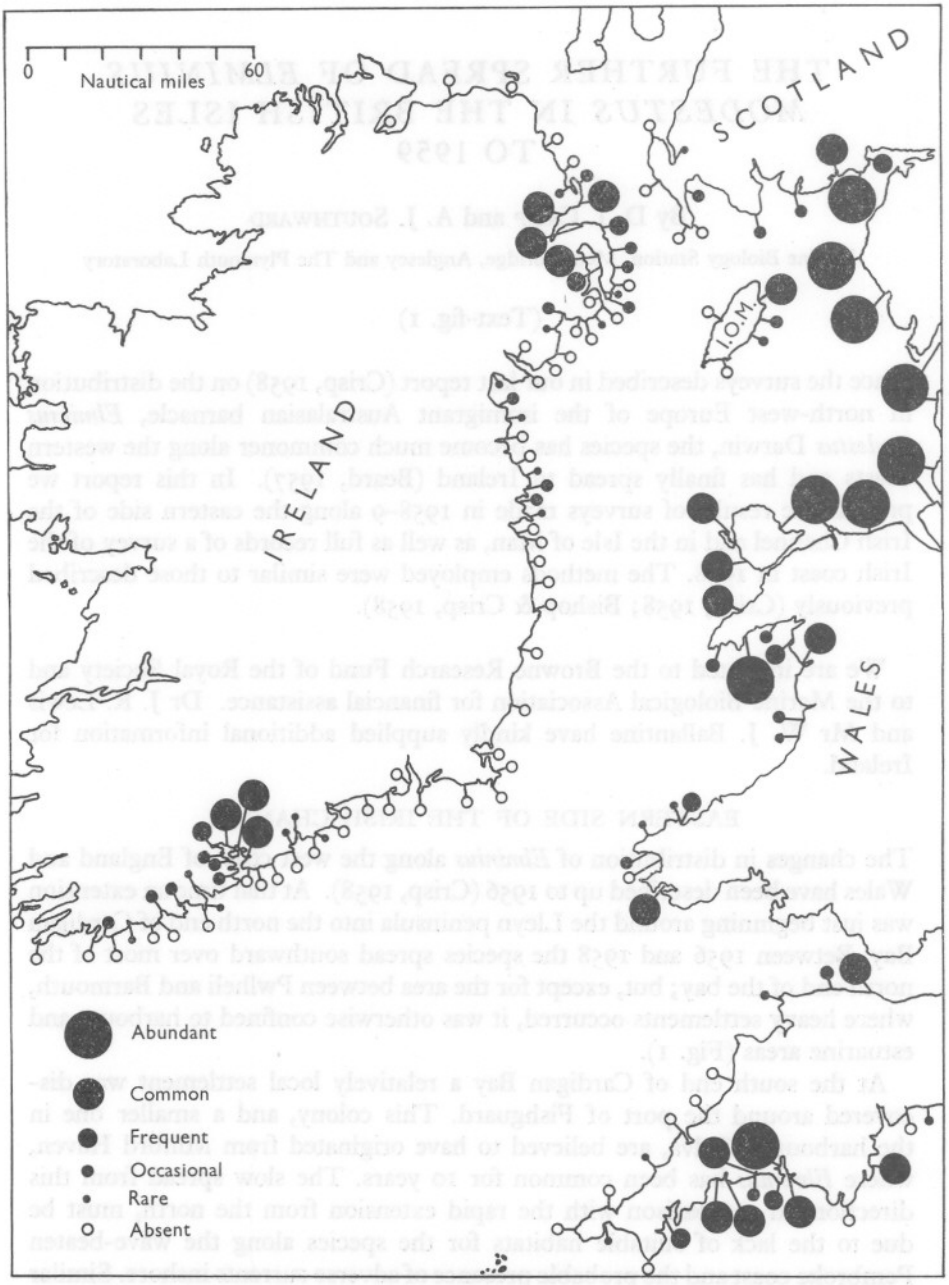

Fig. 1. The distribution of *Elminius modestus* in 1958–9. Stations visited in Ireland in 1958 are shown in full only for the east and south coasts. Isle of Man records made in 1959, those on west coast of Britain in 1958 or 1959. In localities where the species is common or abundant not all stations can be shown. For quantitative meaning of symbols refer to Table 1.

Outside the Bristol Channel, *Elminius* has, so far, failed to establish itself on the exposed coasts of north Devon and Cornwall, or even in Mounts Bay. Along the south Devon and Cornish coasts, from the Exe to the Helford estuaries, *Elminius* is present, and often abundant, in most of the harbours, and since our last report has been slowly increasing on the wave-beaten open coast. West of the Lizard, however, the previously reported settlement at Penzance appears to have failed: only a few old specimens could be found there in 1959, and the filling in of the inner harbour appears to have removed the only suitable habitat for *Elminius* at that end of Mounts Bay. The earlier record (rare) for St Ives in 1955 (Crisp, 1958) has not been confirmed, in spite of extensive searches, and since the specimen was not kept, we cannot now be sure whether this was a true sporadic settlement or a mis-identification of a distorted example of *Balanus balanoides*. Genuine sporadic settlements seem to have occurred in the Newquay area in 1958, when one individual was seen on the open coast, and in 1959, when another was found in the harbour. The species was not seen elsewhere along this coast, and it would be an idle speculation to discuss the probable origin of such few specimens.

Along the northern part of the Irish Channel there has been little further extension of range. *Elminius* is, as in 1956, abundant in the Solway Firth and common in Wigtown Bay. It is now present on wave-beaten promontories such as Burrow Head and Meikle Ross, but is still relatively rare in Luce Bay and has not yet passed the Mull of Galloway. Similarly, there has been little change in the isolated and sparse colony in Lough Ryan at the entrance to the Clyde Sea.

ISLE OF MAN

Except at Ramsey, where the species has become more common, there has been little change in the distribution and abundance of *Elminius* in the Isle of Man (Fig. 1). It is present in small numbers in the harbour at Laxey, south of Ramsey, and one specimen was found at Douglas after more than 1 h searching of suitable habitats, but is absent from the west and south coasts of the Island. The apparent failure of *Elminius* to spread southward from Ramsey must be due to the local hydrography. The residual flow through the Irish Sea is northward, and the set of the tidal currents is such that there is little chance of larvae being carried direct from the north end of the island to the south.

IRELAND

An earlier survey of the entire coast of Ireland was made in 1952, and further observation in 1953 between Lough Foyle and Dublin showed that *Elminius* had not then been able to colonize Ireland (Crisp & Southward, 1953; Southward & Crisp, 1954). Its failure to reach Ireland was attributed to the isolation of the coast from the mainland of Britain by the wide channel of deep water, which prevented normal dissemination of the planktonic larvae

by water-currents. In 1957, however, *Elminius* was reported to be present in small numbers outside Lough Ine, Co. Cork (Beard, 1957), while we were planning another survey. The new survey was made over the whole coast, as well as in the neighbourhood of this record, particular attention being paid to the eastern coast which might be vulnerable to direct infection from the stronger populations now present on the opposite side of the Irish Channel (see p. 429).

Full details of localities where *Elminius* was found in Ireland in 1958 are given in Table 1; the distribution on both sides of the Irish Channel is shown in Fig. 1. The whole of the north and west coast, from Glenarm to Bantry Bay, was apparently free of *Elminius*, even in the vicinity of the ports of Londonderry and Galway. On the south and east coasts there were several distinct regions where *Elminius* was found. The most northerly colonization extended from Larne Harbour to Newcastle, Co. Down; dense populations were found in Belfast Lough and Strangford Lough, and in places reached one individual per cm^2 of surface.

South of this strong entrenchment there were two thinly settled patches, one from Dundalk to Port Oriel, another from Skerries to Howth. These settlements nowhere exceeded 100 per m^2 and the density was often much less. Farther south along the east coast we did not find any more specimens of *Elminius*, not even in the busy harbours at Dun Laoghaire, Wicklow, Arklow and Wexford, nor in the estuary at Waterford.

The remaining area in which *Elminius* was found corresponded to that first reported by Beard (1957). The settlement apparently extended from Ardmore to Lough Ine, but the species was common only in the sheltered bays and channels of Cork harbour. Here, in favourable places such as Rathcoursey, populations up to one individual per cm^2 were sometimes found, with spat settling at the rate of 3 per cm^2, but over most of this large enclosed area the density did not exceed 0·1 per cm^2. No specimens were found at any of the wave-beaten open coast stations in this region.

The origin of the Elminius *colonization of Ireland*

Before discussing the way *Elminius* spread to Ireland it is worth while considering how suitable the Irish coast is for the establishment of the species. By analogy with the known distribution of *Elminius* in other parts of Europe (see Den Hartog, 1953; Crisp, 1958; Bishop & Crisp, 1958), most of the north and west coasts would be expected to be as unfavourable for the species as are the equally wave-beaten coasts of Cornwall and Brittany. The larger estuaries and bays, such as Lough Foyle, the River Shannon and Bantry Bay should eventually support isolated populations similar to those of the drowned valley systems of Cornwall and Brittany. The south-east and east coasts of Ireland, which are sheltered from swell and the prevailing south-westerly winds might be expected to be more favourable to *Elminius*, but these shores

have nowhere the drowned alluvial coast, termed 'côtes à estuaires' by de Martonne (1935), which in south-east England and the Netherlands is so particularly favourable to the species. The stretch of coast from Wicklow to Wexford comes nearest to these conditions, but the sand and gravel shores are subject to severe scouring, the tidal range is small and the shore fauna as

TABLE 1. DISTRIBUTION OF *ELMINIUS MODESTUS* IN IRELAND, 1958

Observations were made between May and September: many negative records are omitted here, as are the whole of the stations on the north and west coasts where the species was not found.

A Density over 1 per cm^2, covering 30% or more of available area.
C Density from 1 to 0·1 per cm^2, most specimens close enough to breed.
F Density, from 0·1 to 0·01 per cm^2, some of them close enough to breed.
O Density 100 to 1 per m^2; often local and needing to be searched for; rarely close enough to breed.
R Less than 1 per m^2; only a few isolated specimens in a 1 h search.
N None found in 1 h search in suitable place.

Place	Shore*	Abundance	Place	Shore*	Abundance
Larne, coast road	R, B, I	N	Dun Laoghaire	R, P	N
Larne Harbour	P, B, M	R	Dalkey	R, B, G	N
Port Muck	RIE (chalk), B	O	Ardmore	RE	R
Whitehead	P, B, G,	C	Ardmore Head	RS	N
Carrickfergus	R, P, B, S, M	C (spat A)	Youghal, bridge	P	R
Bangor Harbour	P, S, M	C	Pilmore, bridge	P	R
Bangor	RI	F	Knockadoon Head	RS	N
Donaghadee, north end	RI, B	F	Ballycotton	RIE, P	N
			Power Head	RS	N
Donaghadee Harbour	P, S, M	F	Inch Strand	R	N
			Gyleen	RE, B	N
Donaghadee, south	RE, B	N	Farsid, nr. Aghada	RI, P, G, S, M	C–F (little spat)
Ballywalter	P, S, M	O–R	Cobh	P	F
Burr Pt., Ballyhalbert	RE, B, S	O–R	Rathcoursey	P, B, G, M	C (spat A)
			Ballynacorra	B, M (fresh water)	N
Kearney Pt.	RSIE	N			
Ballyquintin Pt.	B	O–R	Belvelly	P, B, G, M	C (spat C)
Portaferry	B, S, M	R	Cobh Junction	B, M	O–R
Kirkubbin	P, B, S, M	F (spat A)	Cork	P	F
Greyabbey	B, S	C	Passage West	RS, M	O(W.J.B.)
Kilclief	R, S	N	Monkstown	B, G, M	O(W.J.B.)
Ballyhornan	RS, G	N	Myrtleville	RSI	O(W.J.B.)
Ardglass Harbour	P, M	R	Kinsale Harbour	P	R
St John's Pt.	RSE, B	N	Oldhead Harbour	P, B, G	R
Rossglass	R, B, S	N	Kinsale		
Dundrum	B, G, S, M	O–R	Courtmacsherry	B, G, M	O
Newcastle	R, B, S	R	Clonakilty, North Ring	P	O–R
Annalong	R, P, B	N			
Clashbridge	RS, B	N	Inchydoney	P	N
Rostrevor	P, B, M	N	Dunowen	RIE	N
Greenore Pt	P, B, S	N	Ballinglanna	RIE, B, G, S	N
Dundalk Harbour	P, B, S, M	R (1 only)	Galley Head	RSI	N
Annagassan	B, S	O–R	Glandore Harbour	P, M	O
Port Oriel	R, P, B	R	Toe Head	RSI	N
Baltray	P, M	N	Toe Head Bay	RIE, B	N
Balbriggan	R, B, S	N	Tragumna	RSI, B	N
Skerries	RI, B	R	Barloge Creek, harbour	P, B, G	O–R
Rush	P, S	O–R			
Malahide	R, S, M	O–R	Baltimore	R, P, B, G, M	N
Howth	R, P, G	R	Berehaven	R, B, G	N

W.J.B.: records by Mr W. J. Ballantine.

* R, rocks (S, steep; I, irregular; E, extensive); B, boulders; G, gravel; S, sand; M, mud; P, artificial substrata.

a whole is impoverished (Southward & Crisp, 1954). Under such conditions it is unlikely that *Elminius* would be common on the open coast, although it would be expected to be present in areas of sheltered water such as at Rosslare and Wexford. Apart from the loughs and estuaries, the region of the east coast around Dundrum and Dundalk bays would appear to be the most favourable in Ireland to *Elminius*. Wave action is less and the extensive bays and areas of shallow water should result in local warming-up during the summer and hence increase breeding and settlement of the species (Southward & Crisp, 1954; Crisp & Davies, 1955; Crisp, 1958).

In addition to suitability of the coast we must consider its accessibility to invasion by the two possible means of dispersal: marginal dispersal by larvae liberated into the sea and carried by currents, and remote dispersal, by adults carried on ships, shellfish and floating objects later liberating larvae when in harbour or washed up on the shore (see Crisp. 1958). The nearest accessible point on the Irish coast is Co. Down, which lies some 40 miles from the populations of *Elminius* in Wigtown Bay and the Isle of Man. The Co. Dublin coast is about 60 miles from the population in Anglesey. These distances exceed, though not greatly, the apparent minimum critical distance for colonization by *Elminius* across a sea barrier in British Waters (Crisp & Southward, 1953). However, the northward flow through the Irish Sea passes to the east of the Isle of Man, and thus close to the dense populations of *Elminius* on that side of the channel, before travelling through the North Channel. Although, in the earlier parts of the year, at least (Williamson, 1956), the northward flow is separated from the Antrim and Down coasts by a tongue of southgoing cold water, the possibility of colonization of this coast by marginal dispersal cannot, therefore, be ruled out.

Remote dispersal by ships carrying the adults depends on the likelihood of the infected ships remaining long enough in port to liberate larvae, and of the port being in an area suitable for the retention of larvae during development and for their later settlement. Belfast, lying at the head of a suitable lough, and frequently visited by ships of all kinds from across the Irish Sea, is a likely port of entry, as to a lesser degree are the ports of Londonderry, Larne, Wexford, Waterford and Cork. The ports of Dublin, Dun Laoghaire, Wicklow and Arklow, though likely to receive infected shipping, are less suitable for the establishment of *Elminius*.

The probable history of the colonization

All stretches of coast where *Elminius* was found in 1958 were situated on the more favourable east and south coasts, and two were clearly centred on the ports of Belfast and Cork. Remote dispersal is therefore indicated.

The population on the north-east coast may in fact be presumed to have been introduced by ships in, or close to, the port of Belfast. Within the lough, the size-groups of adult barnacles indicated an initial settlement prior to 1957.

The other dense population, in this region, in Strangford Lough, may be presumed to have been colonized by marginal dispersal from Belfast, and subsequently increased within the lough. If we assume that the open coast and the other loughs were also settled by marginal dispersal from Belfast Lough, the maximum spread northwards has been little more than 10 miles, whereas to the south it has extended to Newcastle, some 40 miles down the coast. This relative failure of the species to extend northwards may be ascribed to the presence of the south-going current along the Antrim and Down coasts. In addition, the region north of Belfast Lough experiences lower sea temperatures which would discourage an animal such as *Elminius* which depends on high summer temperatures for rapid breeding (Crisp & Davies, 1955). In contrast, conditions south of Strangford Lough would appear much more suitable for *Elminius*, as for the several southern forms that occur there in some abundance (Southward & Crisp, 1954). In general, the whole of the Antrim coast north of Belfast Lough seems unsuitable for barnacles; the native species are often sparse, and may rarely be present at all on the predominantly chalk rocks. It is worth noting that similar factors to these, viz. south-going currents, lower temperatures and less favourable substrata, are believed to have prevented the extension of *Elminius* north of the Humber on the east coast of England since 1950 (Crisp, 1958).

The areas of colonization lying between the Belfast settlement and Dublin Bay are smaller and more thinly populated than the other Irish populations. The main reason for regarding them as distinct from Belfast centre is that no trace of settlement was found in Carlingford Lough. This lough appears to offer as suitable an environment as Belfast Lough or Strangford Lough, and it is difficult to believe that marginal dispersal along the coast could have reached Dundalk without some larvae entering Carlingford Lough and establishing themselves there. Similar, but less cogent, arguments can be applied to the gap separating the two minor settlements, for the mouth of the River Boyne and the port of Balbriggan would seem to be no less suitable than the settled harbours of Port Oriel and Skerries on either side. In both areas of settlement the population density was so low that few, if any, individuals could have been capable of breeding. It is therefore difficult to account for these settlements unless there exists one or more centres of dispersal not discovered during the survey, containing individuals close enough to breed. There is a distinct possibility that these sparse settlements might have been derived from numbers of small craft which had become infected in other ports, and one such vessel, carrying many adult specimens was indeed observed at the quayside in Howth, where *Elminius* was scarce. The difficulty in accepting this possibility of multiple dissemination is that *Elminius* was entirely absent south of Howth. The harbours at Dun Laoghaire, Bray, Wicklow and Arklow would be expected to receive as many infected craft as

the smaller and more exposed harbours of Port Oriel and Skerries. Further investigations after the passage of time may allow the correct interpretation to be chosen from these alternatives.

The most widespread settlement of *Elminius* in Ireland, that in the south-west, shows every sign of having arisen from an initial introduction somewhere in the sheltered channels and inlets of Cork harbour. From this area it has extended over 40 miles to the west but little more than 20 miles eastward: here again we have the possibility of a coastal current, this time from the east, influencing the dispersal of larvae. On account of the exposed nature of the coast, the spread has been confined to enclosed bays and harbours, as on the Cornish coast.

In the case of the Cork settlement, we are fortunate in having two means of estimating, albeit very approximately, the probable date of introduction of *Elminius*. The rate of spread along a coast of similar type, that of south Devon and Cornwall, is of the order of 10–15 miles a year (Crisp, 1958). Measuring from the Lough Ine and Glandore settlements, this would place the original entry some time in 1955. Further evidence was obtained from settlements present on piles which had been removed from the estuary just downstream of Cork, and were lying on the bank. According to local workmen the piles had been removed at various times, some that year, some in 1957, and some as early as 1956, but unfortunately they had not been kept separate. The oldest looking piles had lost all growth, but others had a mixed population of *Balanus improvisus* and *Elminius modestus* still attached, the latter at densities of up to 0·03 per cm^2. Many of the *Elminius* measured 9–10 mm diameter, and must therefore have settled in 1957, probably quite early in the season, and certainly in considerable numbers. This evidence suggests that the original settlement must have been present as long ago as 1956, and possibly earlier. *Elminius* was absent (or present in numbers too small to be revealed by survey) in 1952 when we inspected several stations in Cork harbour, and in 1953 when Dr J. R. Lewis examined the same area. From the two independent estimates, and the negative records, it seems reasonably probable that *Elminius* was established between 1954 and 1956. This original population must have been introduced by remote dispersal, since Cork harbour is far removed from any other locality where *Elminius* is plentiful.

SUMMARY

Since 1956, *Elminius modestus*, the immigrant Australasian barnacle, has increased its range and abundance along the west coast of Britain. At the same time it has established itself in Ireland, and is now common in two areas, centred on Belfast and Cork, respectively. Both areas have been independently colonized since 1953, the former probably and the latter certainly by remote dispersal on ships. Two further sparse settlements were discovered on

the east coast of Ireland from Dundalk to Howth, but further evidence is needed to determine whether these finds represent a southerly extension of the Belfast population, or independent invasions.

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Note added in proof

One of us (D.J.C.) was able to re-examine part of the north and east coasts of Ireland during a brief visit in July 1959, and *Elminius* was found at the following stations (to be added to Table 1):

Londonderry, main channel in L. Foyle	R, P, M	R
Dublin, North Wall	P, M	O–F

The species seemed well-established at Dublin, with a fresh spatfall of 2–3 per cm^2; this confirms the existence of a separate invasion of the Co. Dublin area. The record for L. Foyle cannot be regarded as establishing the species in that area. Only three old specimens of *Elminius* were found, all on piles marking the channel, while none were present on the rocks along the shore. This clearly represents a sporadic settlement, probably derived from infected ships.

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TWO NEW SPECIES OF POGONOPHORA FROM THE NORTH-EAST ATLANTIC

By EVE C. SOUTHWARD
From the Plymouth Laboratory

(Text-figs. 1–2)

In May 1958 it was thought that all species of Pogonophora so far discovered in the north-east Atlantic belonged to the genus *Siboglinum* Caullery (Southward & Southward, 1958). Since then I have had the opportunity of examining further collections from deep water off the British Isles and Spain. At least two species of multitentaculate pogonophores have been discovered, and re-examination of some material collected in May 1958 shows that one of the species was also present in these collections. Although considerable material belonging to other species remains to be worked up it has been thought worth while to describe these two species first. One is widespread and can be locally abundant, while the other is the largest pogonophore so far found in the Atlantic. Both are the first Atlantic representatives of their respective genera.

All my material was collected during cruises of R.V. 'Sarsia'. I am grateful to Captain C. A. Hoodless and the crew of 'Sarsia' for their continued interest in deep-sea dredging. I am also indebted to my husband and to Dr J. B. Gilpin–Brown who picked out the specimens, and to Dr J. S. Alexandrowicz who very kindly assisted me with the translation of Russian and German.

Oligobrachia ivanovi sp.nov.

Only one specimen of this species has been found. Its tube is black and completely opaque, and was 25 cm long before being broken to remove the animal. The diameter of the tube varies along its length from 0·5 to 0·9 mm. The wall is stiff and is made of several layers of brown material with a transparent lining layer; its outer surface has narrow raised rings at fairly regular intervals (Fig. 1, A). The animal is 95 mm long, and since only 10 mm of the post-annular region of the trunk is present the complete animal must have been considerably longer. Its diameter is about 0·5 mm and its colour is greenish brown with brown tentacles and red blood. There are seven tentacles, coiled together, which are 12 mm long. Each has a double row of pinnules along the inner side (the length of the pinnules being about equal to the diameter of the tentacle) and a band of brown spots on the outer side (Fig. 1, D). The tentacles are joined to the protosoma in a circle just in front of a slight

transverse groove (Fig. 1, B). The protosoma is about 0·8 mm long and is separated from the mesosoma by an oblique groove which reaches the anterior point of the bridle on the dorsal side. Patches of white glands are present on the proto- and mesosoma and their positions are shown in Fig. 1, B and C, by heavy stipple. The anterior part of the metasoma or trunk, called the metameric region in other genera, has white epidermal glands arranged in two

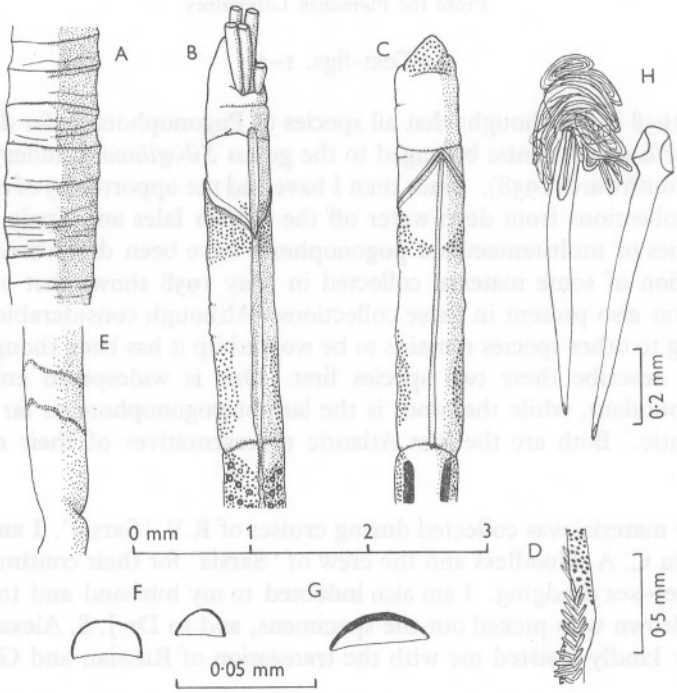

Fig. 1. *Oligobrachia ivanovi*: A, part of tube (surface view); B, anterior end, ventral view; C, anterior end, dorsal view; D, part of tentacle; E, girdle region; F, G, adhesive plates from smaller and larger papillae of trunk; H, spermatophores, filament removed from one.

lateral bands separated by a deep groove ventrally and by two dark brown stripes dorsally. Between the brown stripes lies the dorsal ciliated band which is pinkish white. The white bands are spotted with 3 to 4 rows of clear patches which mark the openings of internal glands. These patches lie in regular oblique lines and show up clearly because they are surrounded by brown rings. This glandular region of the body is 10 mm long. The second region of the metasoma lacks epidermal glands but bears numerous small papillae arranged in two irregular rows along the ventral side for about 50 mm. The rows of small papillae are followed by large papillae, arranged in 3 to 4 rows, extending for 10 mm, while the last 5 mm of the pre-annular region is devoid of all but a few small papillae. Both large and small papillae are armed with

half-moon-shaped adhesive plates (Fig. 1, G), but the plates are difficult to see because they are almost colourless. There are two girdles (annuli), which both encircle the body completely, and each is made up of 2 to 3 rows of platelets. The last 10 mm of the body has no papillae, adhesive plates or epidermal glands and is broken off abruptly. The pre-annular part of the trunk contains spermatophores which are 0·85 mm long and spindle-shaped. When the filament is removed the filamentar end of the spermatophore is seen to be flattened and drawn out into two flaps, which are usually folded inwards (Fig. 1, H).

The specimen on which this description is based was collected on 28 November 1958 at 48° 26′ N., 10° 8′ W.; depth 730–780 fm.

This new species has been named after Prof. A. V. Ivanov of Leningrad, who has already described 17 species of Pogonophora from the Pacific and Arctic Oceans, including the only other species of *Oligobrachia*.

O. ivanovi differs from *O. dogieli* Ivanov (1957) in having adhesive plates on the trunk papillae, and in this respect it resembles *Birsteinia vitjasi* Ivanov (1952) and confirms Ivanov's thesis that the two genera are closely related. The spermatophores of *Oligobrachia dogieli* and *O. ivanovi* both have wing-like flaps at the filamentar end while there are no such flaps in *Birsteinia* or any other pogonophore yet described. Other characters in common between the two species of *Oligobrachia* are the curious patches or spots on the tentacles, and the comparatively long pinnules. The tentacles in *Birsteinia* have very short pinnules and, apparently, no spots or glands of any sort (Ivanov, 1952).

Polybrachia capillaris sp.nov.

The tubes are dark chestnut brown and look very much like hairs; the specific name *capillaris* refers to this likeness. The tube wall is stiff and, in the anterior part at least, is made up of short overlapping segments (Fig. 2, A). Occasionally the colourless lining layer extends a few mm beyond the first segment. The first few segments are pale brown and unringed; the later segments and the unsegmented part of the tube are marked with regular light and dark brown rings (Fig. 2, A), or half rings. The posterior end of the tube is yellow; the wall is thinner, but still ringed, and is occasionally surrounded by short segments of a darker outer layer. The diameter is constant along any individual tube and may be from 0·12 to 0·17 mm in different individuals, but is usually between 0·13 and 0·14 mm.

It is difficult to assess the length of the complete tube or animal, since all the specimens seem to be broken, but the longest fragment of tube is 12 cm long. The animal itself may possibly reach 55 mm. A specimen (from the Spanish material) that seems to be nearly complete has been chosen as the type and its measurements are given below, followed by the range, in parentheses, found in other specimens: diameter 0·12 mm (0·10–0·12); total length 30·6 mm;

length of pre-annular part 10 mm (5–15); length of post-annular part 20·6 mm (up to 39); length of proto- and mesosoma together 0·75 mm (0·5–1·0); length of coiled tentacles 1·5 mm (0·5–2·2).

The pre-annular part of the body grows longer as the gonads and gametes begin to develop. It is less than 7 mm long in immature specimens; 7–10 mm long in females and 10–15 mm long in males.

There are from two to four tentacles but two is the most common number (11 specimens have 2, 3 specimens have 3, 2 specimens have 4) and the type

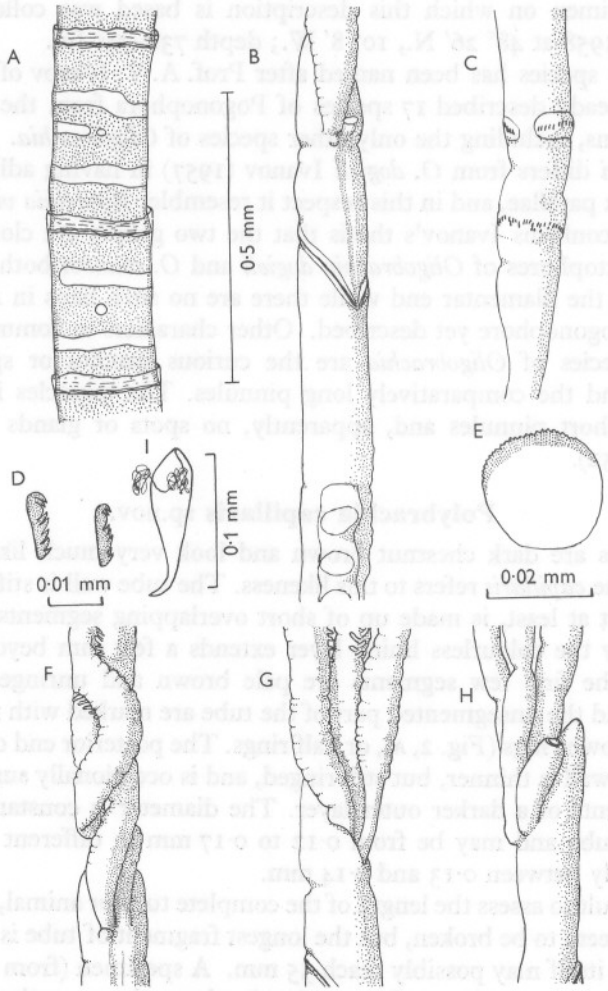

Fig. 2. *Polybrachia capillaris*: A, part of tube; B, anterior end of type specimen, ventral view; C, girdle region; D, girdle platelets (uncini), side view; E, adhesive plate; F, G, H, anterior ends of specimens with 2, 3 and 4 tentacles; I, spermatophore.

specimen has two; they are coiled together into a tight spiral inside the tube and each one has a double row of pinnules (Fig. 2, F, G). Specimens with two tentacles often have a small swelling behind the base of one of them, which may be the first sign of the development of a third. The protosoma is very short (about one-quarter to one-fifth the length of the mesosoma) and is separated from the mesosoma by a shallow groove on the ventral side only (Fig. 2, B). The bridle lies on a broad ridge and is not complete on the dorsal side, but on the ventral side the two halves are sometimes separate (Fig. 2, B) and sometimes joined. There is a wide groove along the ventral side between the base of the tentacles and the bridle.

The septum between the mesosoma and metasoma can be seen through the body wall, but there is no external groove. The metameric region is short and has only 6–13 pairs of glandular papillae (7 in the type specimen). The first 4 or 5 of these papillae are devoid of adhesive plates, but all the other papillae on the metasoma bear small round plates (Fig. 2, E). Behind the paired papillae is a long region bearing occasional isolated papillae and ending in a close row of up to 15 large papillae just before the girdles. The two girdles are made up of single or semi-double rows of small toothed platelets (Fig. 2, C, D). The post-annular region bears transverse rows of two or three papillae at intervals of about 1 mm.

Mature males contain flat, leaf-like spermatophores, with very fine filaments (Fig. 2, I).

Three species of *Polybrachia* are already known. They are: *P. gorbunovi* (Ivanov, 1949), *P. annulata* Ivanov (1952) and *P. barbata* Ivanov (1952). *P. capillaris* has the following characters in common with other species of *Polybrachia*: part of the tube is built up of short overlapping segments; the tentacles bear pinnules; both paired and unpaired trunk papillae bear adhesive plates; the post-annular papillae are arranged in transverse rows, and the spermatophores are of similar shape to those so far described for *Polybrachia*. There are some differences which suggest that the new species might belong to a separate, new, genus: the number of tentacles is small compared with 18–73 in other species; there are no circular grooves on the proto- and mesosoma, apart from the incomplete one separating the two segments; the new species is only about half the size of the smallest species previously known. For the present it seems best not to propose a new generic name for a species obviously closely related to *Polybrachia*.

P. capillaris has been collected at six stations, which are listed below:

Date	Position	Depth (fm.)	No. of specimens
16. v. 58	47° 56′ N., 7° 57′ W.	340–350	1 empty tube
16. v. 58	47° 50′ N., 7° 57′ W.	300–450	3 (and 1 empty)
6. viii. 58	43° 43′ N., 4° 02′ W.	680–970	12 (and many empty)
28. xi. 58	48° 26′ N., 10° 08′ W.	730–780	2 (and 2 empty)
30. xi. 58	48° 39′ N., 9° 50′ W.	750–850	2
30. xi. 58	48° 40′ N., 9° 48′ W.	600–680	1

Some of these specimens were previously recorded as empty tubes of *Siboglinum inerme*[1] (Southward & Southward, 1958), but the description of *S. inerme* was based entirely on genuine specimens.

Type specimens of *Oligobrachia ivanovi* and *Polybrachia capillaris* have been sent to the British Museum (Natural History).

SUMMARY

Two new species of Pogonophora are described. *Oligobrachia ivanovi* is recorded from a depth of 730–780 fm. off the mouth of the English Channel; *Polybrachia capillaris* is recorded from depths of 340–970 fm. at various stations off the mouth of the English Channel and one station off the north coast of Spain.

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[1] The name *S. inermis* was used in the original description, it should of course be *S. inerme*.

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OBSERVATIONS ON HERRING SPAWNING AND LARVAL DISTRIBUTION IN THE FIRTH OF CLYDE IN 1958

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(With Plate I and Text-fig. 1)

Apart from the extensive egg surveys carried out by Norwegian workers (Runnstrom, 1941) most of the investigations on the spawning of the Atlantic Herring have depended on studies of the distribution of the spawning fish, on captures of newly hatched larvae, and on records of the occurrence of herring eggs in the stomachs of predatory fish species (principally haddock). With the exception of recent observations by Bolster and Bridger (1957), attempts to sample egg concentrations quantitatively in the North Sea and neighbouring areas have usually proved abortive. In consequence little is known of the distribution and density of eggs on the spawning grounds, their percentage fertilization, mortality during the egg stage, hatching rate, and the relationship between the distribution of eggs and the nature of the sea-bed.

To study these items, and also the subsequent production, dispersal, and mortality of larvae, and the relationship of these to subsequent year-class strength, it was decided to concentrate effort on the spring spawning in the Firth of Clyde (see Fig. 1). This area had been studied much earlier by Cossor Ewart (1884), and it was considered very suitable for intensive study for the following reasons: (i) spawning occurs in a relatively small, well defined, area of shallow depth (13–24 m); (ii) the spawning season is short, extending over a period of about one month; (iii) the main spawning ground is the scene of a small commercial fishery from which detailed information on the distribution and composition of the spawning shoals can be readily obtained; (iv) the dispersal of the larvae can be followed more closely than in the much more extensive regions of the northern North Sea; (v) the region is one in which accurate fixing of position by Decca and land bearings is possible.

The general features of the biology of the herring in the Clyde have been described by Marshall, Nicholls and Orr (1937, 1939) and Wood (1951, 1958, 1959). Spawning takes place in late February and March in two localities in the outer reaches of the firth, a major one on Ballantrae Bank off the Ayrshire coast of the Scottish mainland, and a smaller one off the south-west corner of the island of Arran. These are shown in Fig. 1. Hatching occurs throughout

March and early April, followed by the dispersal of the larvae from the vicinity of the spawning grounds. The main nursery areas for young herring are located in the upper reaches of the firth where they remain until the onset of first maturity in their third or fourth years of life. After spawning the adults move out of the firth. Only a small proportion of these adults return to the Clyde spawning grounds in subsequent years.

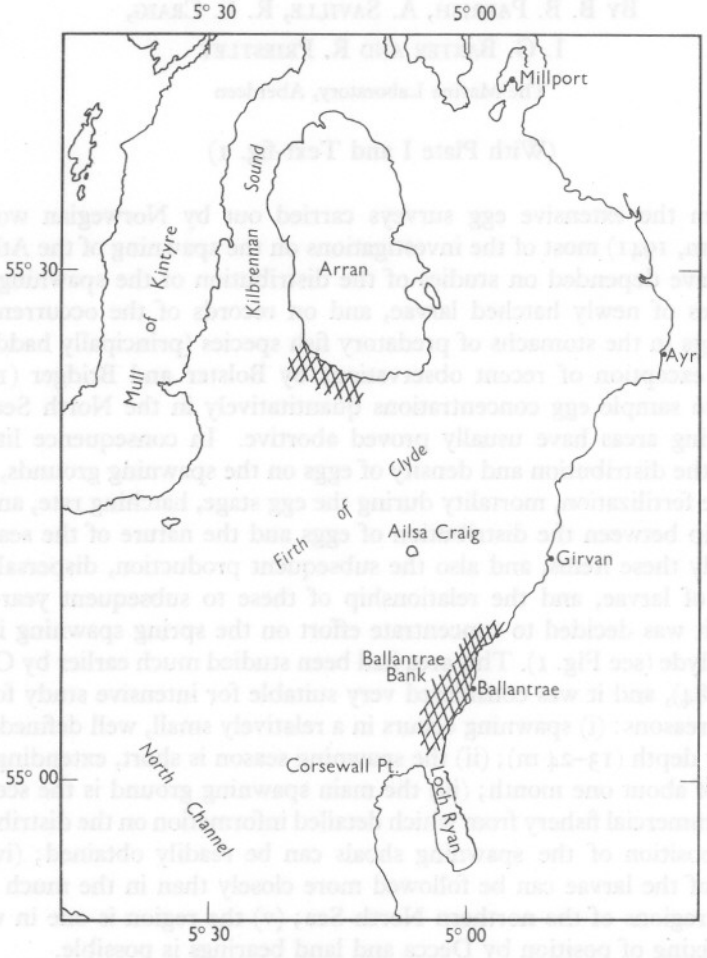

Fig. 1. Chart of outer reaches of Firth of Clyde showing main spawning areas (hatched).

SAMPLING OF EGG CONCENTRATIONS

In 1957 and 1958, surveys of egg concentrations have been restricted to the larger of the two spawning grounds, Ballantrae Bank. Preliminary work in February and March 1957, and again in greater detail during the same period

in 1958, consisted of an intensive survey by a continuous series of dredge hauls over the full extent of the bank. This provided a detailed picture of the nature of the sea-bed and the presence or absence of eggs. An egg patch was located in both 1957 and 1958, but only in 1958 was it possible to investigate the extent of the patch and the distribution and density of the eggs.

The procedure in 1958, after the egg patch had been located by dredge on 4 March, was to mark the position accurately by buoys and with Decca fixes, and then to sample the full extent of the patch with a small grab (mouth aperture 20 × 20 cm), designed for sampling in conjunction with the laboratory's underwater T.V. unit (shown in Pl. IA). By this means, and using the Decca track plotter, it was possible to delimit accurately the boundaries of the patch, to determine the distribution of the eggs and to estimate their total abundance and density. A series of underwater colour transparencies was also taken over the patch using the laboratory's Mk V camera equipped with electronic flash. This proved very satisfactory as a survey instrument for this type of work, and black and white prints, taken from the colour transparencies, are shown in Pl. IB.

The most important results obtained from the dredge, grab and photographic surveys are as follows.

(1) The eggs sampled in both years were located in an area of small stones and gravel. No eggs were found in areas of large stones, boulders or rock.

(2) The egg patch surveyed in detail in 1958 was sharply delimited, the boundaries coinciding with a change in the substratum from gravel and small stones to large stones and rock. The patch was approximately square, measuring 320 × 320 m.

(3) Eggs were distributed in an almost continuous carpet, ranging from 1–2 eggs thick at the boundaries to 4–8 eggs thick over the greater part of the patch.

(4) Detailed examination of eggs taken from different parts of the patch indicated that no hatching had taken place up to the time of locating it. The stage of development varied markedly throughout the egg layer. The eggs in the surface layer were in an advanced stage of development near to hatching, while those in the lower layers were in earlier stages of development. These observations suggest that the eggs were all deposited at approximately the same time, and that the development of the eggs in the lower layers was retarded, due possibly to reduced oxygen supply.

(5) Very few unfertilized or dead eggs were observed in the samples taken from the patch, and there was no significant increase in the thickest layers of eggs. In the samples of eggs examined, the proportion of unfertilized or dead eggs did not exceed 1%.

(6) No invertebrate fauna was observed over the whole extent of the egg patch, although dense concentrations of ophiuroids were sampled on a similar substratum in the vicinity of the patch and on other parts of the bank.

Abundance of eggs and numbers of spawners

From a knowledge of the size of the egg patch, the distribution of eggs within it, the thickness of the egg layers, and the average egg diameter, it is possible to estimate the total number of eggs in the spawning patch. The number of spawning females and the total number of spawners producing the patch can then be calculated from data on the average fecundity and sex ratio.

Estimates of these quantities are given in Table 1. These estimates are based on there being a continuous carpet of eggs over the patch, and they are given for upper (5) and lower (2) levels of egg layer thickness, within which the actual average egg layer thickness certainly lay. (The average thickness of 50 samples of eggs taken over the patch was 4.) The fecundity value used was for fish of 26–27 cm, which corresponded to the mode of the length composition of the catches taken by the commercial fishery on the bank, and the 50:50 sex ratio used does not differ significantly from that observed in the commercial samples.

TABLE 1. ESTIMATES OF EGGS IN PATCH AND NUMBER OF SPAWNING FISH

	Size of egg patch (m^2)	Diameter of egg (mm)	Estimated total no. of eggs	Average fecundity	Sex ratio	Estimated no. of spawners		
						♀	♂	Total
Egg layer thickness = 2	$1{\cdot}01 \times 10^5$	1·4	$1{\cdot}03 \times 10^{11}$	$3{\cdot}0 \times 10^4$	50:50	$3{\cdot}4 \times 10^6$	$3{\cdot}4 \times 10^6$	$6{\cdot}8 \times 10^6$
Egg layer thickness = 5	$1{\cdot}01 \times 10^5$	1·4	$2{\cdot}58 \times 10^{11}$	$3{\cdot}0 \times 10^4$	50:50	$8{\cdot}6 \times 10^6$	$8{\cdot}6 \times 10^6$	$17{\cdot}2 \times 10^6$

These calculations indicate that the total number of herring of both sexes contributing to the spawning patch lay between 7 million and 17 million fish. The estimates of average egg layer thickness obtained during the survey suggest that the actual number was nearer the upper of these two limits, and that 10 million fish represents a reasonable conservative estimate.

That this spawning patch did not constitute the total spawning on Ballantrae Bank during the whole of the spawning season in 1958 is evident from other information. Ripe herring were caught by the commercial fishery on the bank after the time that the egg patch had been located; larval records obtained subsequent to the location of the egg patch indicated that substantial spawning took place after this time. Also running fish and a few spents were caught in the fishery on the bank at a time before the formation of the sampled egg patch. The general course of the fishery and the subsequent larval data indicate that the sampled patch was formed near the beginning of the spawning season, and that substantial spawning took place on the bank subsequently. From these data it is estimated that the total number of spawners on the bank during the spawning season was 4–5 times greater than the number contributing to the sampled egg patch. This gives rough limits for the total spawning stock size of between 30 million and 85 million fish.

Rate of exploitation

A comparison of these estimates of the size of the spawning stock with the catches taken by the commercial fishery on the bank, during the spawning season, provides information on the rate of exploitation. This fishery is pursued with anchored gill nets during February and March. The anchored nets are shot in fleets of 10 across the bank; they are set to fish throughout 24 h, and are usually hauled in the morning. The positions of the nets are changed from time to time according to the regions on the bank at which the heaviest catches are being taken.

The statistics of this fishery in 1958, from its commencement at the beginning of February to its termination in late March, are given in Table 2.

TABLE 2

Week	Total no. net-days	Total catch (crans)
3–8 February	120	39·5
10–15 February	750	185·0
17–22 February	750	207·75
24 February–1 March	750	65·5
3–8 March	750	66·5
10–15 March	570	77·5
17–22 March	450	16·25
Total	4140	658·0

Estimates of the proportion of the total spawning stock taken by the fishery can be made from these catch data and the estimates of spawning stock size, both for the total spawning season and for the spawners contributing to the sampled egg patch. The number of herring per cran in this fishery averaged approximately 1000, which gives a total catch during the season of about 658000 herring. This constitutes a fraction of between

$$\frac{0{\cdot}658 \times 10^6}{85{\cdot}0 \times 10^6} = 0{\cdot}8\,\% \quad \text{and} \quad \frac{0{\cdot}685 \times 10^6}{30{\cdot}0 \times 10^6} = 2{\cdot}2\,\%$$

of the total number of spawners on the bank.

Similarly, estimates can be made of the herring contributing to the sampled spawning patch which were taken by the fishery. From the stage of development of the eggs in the patch it is estimated that they were fertilized 12–13 days previously. This fixes the spawning date as on or about 22 February. The catch by the fishery up to this date amounted to 432 crans, and on the assumption that the herring available to the fishery up to this time all contributed to the sampled spawning patch, the estimate of the proportion taken by the fishery up to this time lies between

$$\frac{0{\cdot}432 \times 10^6}{17{\cdot}2 \times 10^6} = 2{\cdot}5\,\% \quad \text{and} \quad \frac{0{\cdot}432 \times 10^6}{6{\cdot}8 \times 10^6} = 6{\cdot}4\,\%$$

These estimates are in fact over-estimates, since it is known that in the early part of the season some spawning took place over parts of the bank other than the sampled patch. It is evident therefore that the seasonal fishing mortality rate of this spawning stock in 1958 was probably between 1 and 3%.

LARVAL ABUNDANCE AND DISTRIBUTION

The larval surveys carried out in 1958 subsequent to the egg surveys were planned with the two primary objectives of assessing the numbers of eggs spawned over the season, and of tracing the subsequent dispersal of the larvae produced from them. The first of these aims demanded a close 'spawning' grid of stations covering the limited area on Ballantrae Bank within which spawning was known to take place, to be worked and repeated at frequent intervals; the second required a grid of more widely spaced stations over the Clyde estuary. The attempt to fulfil both of these requirements inevitably resulted in sampling falling rather short of the ideal in both cases.

The 'spawning' grid of sixteen stations was completed fifteen times between 21 February and 21 April. Sampling was by means of oblique hauls from bottom to surface with a one metre net of bolting silk with 60 threads to the inch.

Recently hatched larvae were found from 3 March to 9 April. Hatching was greatest, however, between 10 and 14 March, with a distinct but very secondary mode around 7 April. Only negligible production of larvae occurred in the intervening period.

An indispensable preliminary to assessing the numbers of larvae produced was to estimate the rate at which larvae were removed from the bank. It was possible to estimate this from the rate at which the numbers on the bank fell off between 18 and 31 March, and again after 7 April, during which periods no, or only negligible, new production of larvae was taking place. In both of these periods the rate of loss from the bank was estimated to be about 75% per day. This loss is of course compounded of two factors, dispersion and mortality, but for this purpose it is not necessary to distinguish between them. This rate has been taken as applicable throughout the entire hatching period. Other assumptions made in estimating the number of larvae hatched were that the distribution was linear in space between stations, and in time between surveys. The former is not likely to lead to serious error in this case, where the stations were less than 1 mile apart. The latter is less reliable where, as happened on one occasion, surveys were separated by a period of 6 days, with a marked fall in larval numbers in the intervening period.

On this basis the number of larvae hatched on Ballantrae Bank during the 1958 spawning season was estimated at $5{\cdot}4 \times 10^{11}$. This would be the progeny of about $21\frac{1}{2}$ million spawning females if one assumes that all the eggs spawned

subsequently hatched. (It has been shown in an earlier section that the proportion of dead or unfertilized eggs in the sampled patch was low, and no large concentrations of predators were encountered. It is likely, therefore, that mortality in the egg stage was low and that this assumption is reasonable.) This estimate is in broad agreement with that deduced from the estimation of the egg patch. It would suggest, as shown earlier, that there were perhaps four or five egg patches present on the bank during the course of the spawning season.

The larger grids, designed to follow the fate of the larvae subsequent to hatching, were completed eight times between 10 March and 22 April. The first two surveys were carried out on 10 and 12 March. On both of these larvae were sharply restricted to a narrow belt in the vicinity of Ballantrae Bank, and were evidently drifting southwards towards Loch Ryan and round Corsewall Point. Unfortunately there was then a gap of six days before the wide grid was resampled. During this period two marked changes had occurred. First, the numbers of larvae present in the survey area had fallen to less than 1% of their previous abundance; secondly, the sharp western boundary to the distribution of larvae had broken down and the few that remained were widely dispersed westwards to the limit of the survey area. That the marked reduction in abundance of these larvae was the result of drift westwards beyond the limits of the area surveyed, and probably out of the Clyde altogether, is supported by returns from drift bottles released over Ballantrae Bank during this period. The percentage return from these releases was very low, the few recovered being returned from the shores of Kintyre. The most plausible explanation of the low returns is that the remaining bottles were lost to the Atlantic.

The remnants of this group of larvae, which represented the preponderance of the season's production, only suffered a moderate mortality thereafter. By 3 April they were split into a smaller group in Kilbrennan Sound and a larger body off Corsewall Point and the mouth of Loch Ryan. These two groups retained their identity thereafter, the Kilbrennan Sound one being later found to the east of Arran, while that to the south showed a pulse-like movement, first moving south into the Irish Sea and then north again into the Clyde. This was also true of the larvae hatched during the second peak in early April, which showed distinct evidence of the same oscillation of the main body between the Clyde and the Irish Sea, with smaller bodies of larvae subsequently drifting northwards. These movements also are in substantial agreement with returns from later releases of drift bottles, which gave evidence of a southwards drift into the Irish Sea and subsequent return to the Clyde, with a predominantly northerly drift to the Ayrshire coast in the latter half of April.

Surveys of the sea lochs in the upper reaches of the firth were undertaken in late April and early May from M.V. 'Calanus', by kind permission of the

Director of the Scottish Marine Biological Association Laboratory at Millport. They showed no evidence of larvae in these regions, in striking contrast to results in 1957, when large numbers of larvae were captured in these lochs at that time. The difference would seem to be due primarily to the different water movements in the two years. In 1958 there was a large loss of larvae from the Clyde due to predominantly westerly and southerly transport, while in 1957 the northerly drift tended to retain the larvae within the Clyde estuary. These water movements may be largely wind determined. In 1957 winds were predominantly southerly during the last three weeks of March, whilst in 1958 during this period the prevailing winds were from the east.

SUMMARY

In the period February–April 1958 an intensive survey was carried out on the herring spawning grounds of Ballantrae Bank, in the outer reaches of the Firth of Clyde, and over the regions of subsequent larval dispersal.

During a detailed dredge and grab survey of the bank an egg patch, measuring approximately 320×320 m was located. This was confined to a gravel and small stone substratum. The eggs were distributed as an almost continuous carpet, with the layers of eggs ranging from two at the boundaries of the patch to eight in its densest parts. Very few dead or unfertilized eggs were observed, but the stage of development varied through the egg layer.

Estimates of the number of eggs in the patch lay between $1{\cdot}03$ and $2{\cdot}58 \times 10^{11}$ and of the number of spawners giving rise to them between 7 million and 17 million. These gave estimates for the total number of herring spawning on the bank during the season of between 30 million and 85 million.

Using these estimates of total spawning stock size, the seasonal fishing mortality rate was estimated to lie between 1 and 3%.

Estimates of total spawning stock size obtained from data collected on subsequent quantitative larval surveys over the bank were in broad agreement with those derived from the egg survey. The dispersal of larvae from the bank was rapid and widespread, and a large proportion of the larvae were probably carried out of the Clyde estuary. The dispersion differed from that in 1957, when the bulk of the larvae were retained within the Firth of Clyde, and it corresponded with the dispersion of drift bottles, liberated on Ballantrae Bank during the hatching season.

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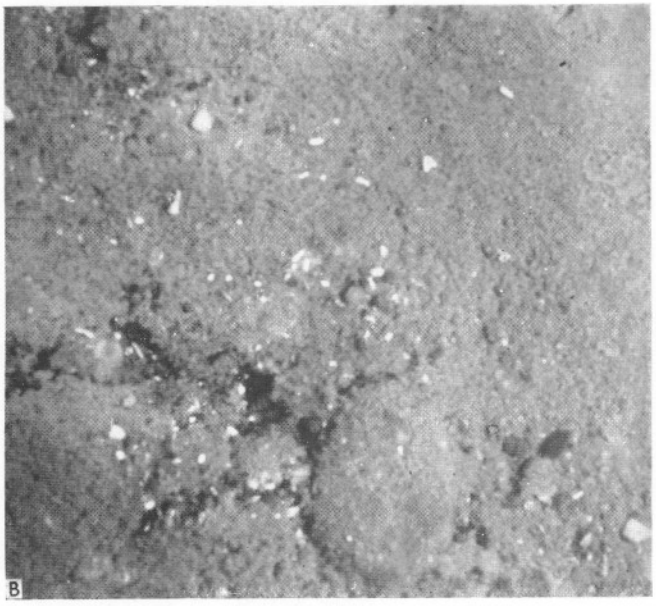

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EXPLANATION OF PLATE I

A. Grab used in egg survey, with egg sample taken in a single haul.
B. Print of eggs *in situ* taken with underwater camera.

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NAPHTHAQUINONE PIGMENTS IN *PSAMMECHINUS MILIARIS* (GMELIN)

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(Text-figs. 1–3)

Since MacMunn (1885), the nature and distribution of naphthaquinone pigments in sea urchins have been widely surveyed, and the main findings have been summarized in reviews by Lederer (1952), Fox (1953) and Thomson (1957). Among spinochromes and echinochromes, the most unusual one is the Spinochrome E discovered by Lederer (1952), which is insoluble in ether.

Recently, the author has examined the napthaquinone pigment in the echinoid, *Psammechinus miliaris* (Gmel.), and has found a similar pigment to Spinochrome E together with other common spinochromes.

SPINOCHROME E

Methods

Tests and spines were digested with concentrated HCl and extracted with re-distilled diethyl ether. The spectral characteristics were measured on Unicam SP 500. The crude ether phase showed absorption maxima at 269, from 315 to 318 and at 480 mμ with a hump at 360 mμ (Fig. 2*a*). When this was left for a short time at room temperature, needle-like crystals occasionally appeared, which, when dissolved in methanol, showed maxima at 267, between 359 and 360 and at 476 mμ (Fig. 2*b*).

A considerable amount of orange red pigment remained in the acid phase ($\lambda_{max.}$; 261, 330 and 465 mμ in HCl), which on adding a few drops of pyridine turned purple. Dilution with methanol produced an amorphous purple precipitate, which re-dissolved in methanol on acidification with a drop of concentrated HCl. From this solution, fine needle crystals separated out at room temperature. These, when dissolved in methanol, gave $\lambda_{max.}$ at 267, 358 and 476 mμ and the absorption curve (Fig. 1*b*) was exactly the same as that from the ether phase.

A more effective method of crystallization was as follows. Tests and spines were digested with a small amount of concentrated HCl and the solution was diluted at once with methanol, which gave a solution with the absorption spectrum shown in Fig. 1*d*. On adding a small amount of pyridine, the purple pigment precipitated in quantity. This was washed several times with methanol, dried and re-dissolved in acidified methanol or acetone. Crystals separated out at room temperature, which after washing with distilled water and drying, were re-crystallized from acidified methanol.

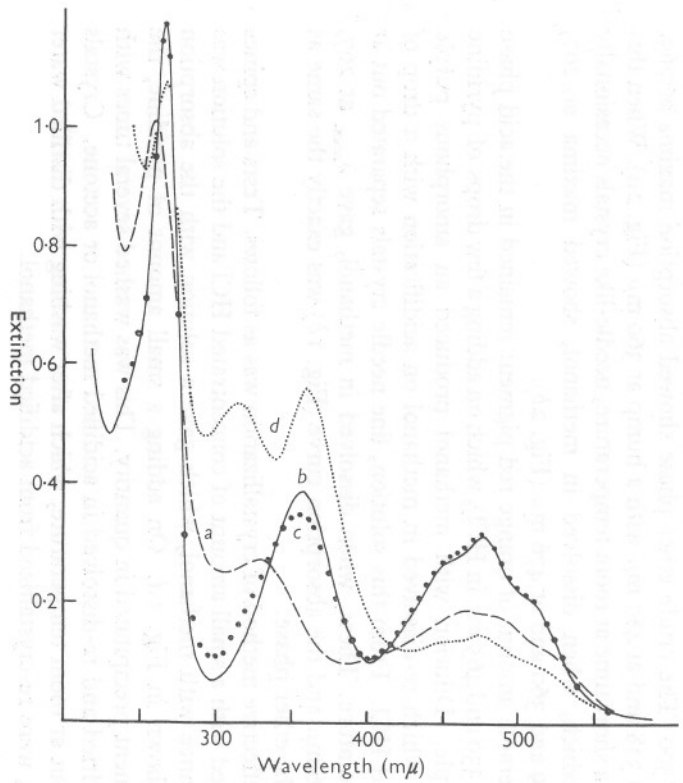

Fig. 1. Absorption spectra of pigment extracted from *Psammechinus miliaris*. HCl phase. *a* (broken line), crude extract in HCl; *b* (continuous line), crystallized pigment in methanol; *c* (dots), samples of Spinochrome E, furnished by Dr Lederer; *d* (dotted line), crude extract made with HCl methanol.

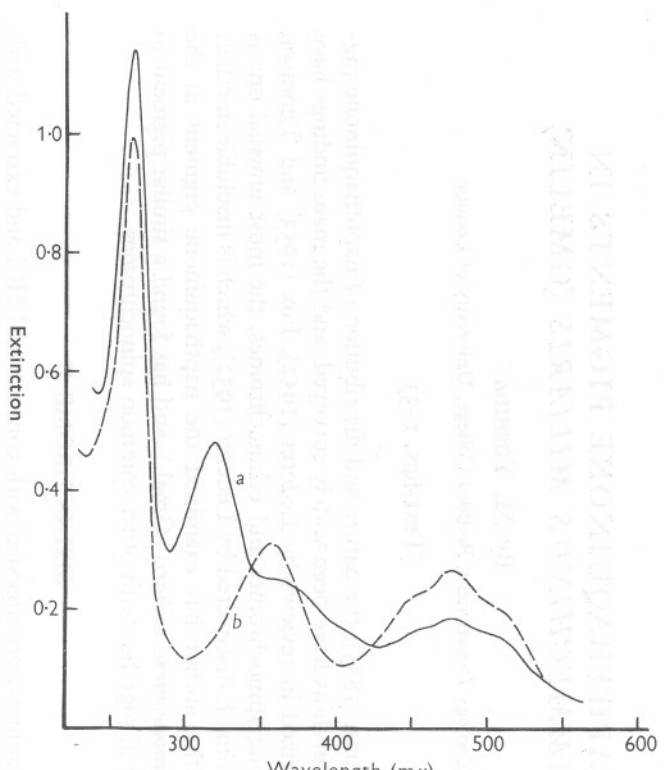

Fig. 2. Absorption spectra of pigment extracted from *Psammechinus miliaris*. Ether phase. *a* (continuous line), crude extract in ether, *b* (broken line), Spinochrome E in methanol.

Properties

The properties of this pigment resemble very closely those of Spinochrome E. On adding sodium hydrosulphite, the solution turns colourless but the colour soon comes back on contact with air. The pigment forms a slightly reddish yellow sodium salt when sodium bicarbonate is added to a methanolic solution. On acidifying with HCl, the spectral character of the original solution is restored.

The spectral absorption of a sample of Spinochrome E, kindly sent to me by Dr E. Lederer, was found to be almost identical with that of the above pigment, showing only a slight difference in the relative extinction at the vicinity of the middle peak (Fig. 1*c*).

The two pigments were compared by paper-chromatography, by running them in parallel with 2N-HCl/methanol (1:5) as developer. The R_F was found to be 0·39–0·41 for Lederer's and 0·38–0·41 for my samples of pigment. None of the pigments moved from the origin when the chromatogram was developed by butanol–water–1% formic acid. This solvent system, devised by Dr Barbier, was recommended to me privately by Dr E. Lederer.

The crystals from *Psammechinus* do not melt below 320° C, the highest temperature tested, and sublime at 280° C (according to Lederer (1952) his pigment did not melt below 350° C).

Both pigments were found to be insoluble in ether, carbon disulphide, chloroform and benzene, but very soluble in acetone, methanol and ethanol.

Thus the two pigments are closely similar in solubility, absorption spectra, melting-points and in their behaviour on paper chromatograms. The pigment found in *Psammechinus*, therefore, appears to be Spinochrome E.

An elementary analysis of the pigment after two successive re-crystallizations showed C = 41·66%, H = 3·82% and O (by difference) = 54·52%. The empirical formula which corresponds most closely to this is therefore $C_{10}H_{11}O_{10}$.

On addition of weak sodium hydroxide, the optical density in the visible relative to that in the ultraviolet range decreases, with an enhancement of the hump at 455 mμ, as well as a shift of the first peak to 270 mμ. If 0·5 c.c. of 20% NaOH is added to 5 c.c. of methanolic solution, the second peak (at 358 mμ) and the third (476 mμ) disappear completely, leaving a peak at 278 mμ and a hump at 300 mμ.

It is noteworthy that the pigment from *Psammechinus* shows a change during extraction. Thus after it has crystallized out of a crude extract made with either ether or HCl, it will not re-dissolve in either solvent.

Distribution

The pigment was found not only in tests and spines but also in the coelomic fluid. It was not detectable, however, in extracts of guts and gonads.

Discussion

In general, though the properties described above are well known and characteristic of hydroxynaphthaquinones, certain reservations must be made. First, the pigment is remarkably unstable. Although the crystallized pigment is so stable that it gives exactly the same absorption curve after several months storage, crude extracts go black overnight even when acid. Again, when a drop of 2% hydrogen peroxide is added to 10 c.c. of pigment solution, the colour disappears almost instantaneously. This may explain the fact that, if a crude HCl solution is shaken with ordinary ether, which usually contains small quantities of peroxide, the red colour fades within 30 min. Therefore, only pure re-distilled ether should be used during the course of extraction. Again, the absorption spectrum of the pigment eluted from alumina columns by acid methanol is altered greatly.

Secondly, there is the question of the elementary composition, which does not correspond closely to that expected for a hydroxynaphthaquinone, the value for O being too high.

Nevertheless, the three absorption peaks are characteristic of a hydroxynaphthaquinone (Spruit, 1949), as is the behaviour of the pigment towards sodium hydrosulphite, sodium bicarbonate and sodium hydroxide. Thus the shift of the peak towards the longer wavelength in alkali (see p. 457) is more or less in harmony with Spruit's ideas. The infra-red spectrum reveals nothing unusual for a hydroxynaphthaquinone.

The change in solubility occurring during the course of extraction is noteworthy and may indicate that the naturally occurring pigment differs somewhat from that which is crystallized from extracts. A similar change occurs when pyridine is used as described on p. 455, for the crystals which are deposited are insoluble in HCl, although the original pigment was extracted with it. Spinochrome E cannot therefore be accepted unreservedly as a naturally occurring pigment.

However, the spectral absorption of the pigment is consistent. Crude extracts with both HCl-methanol (Fig. 1*d*) and ether (Fig. 2*a*) show either a small peak or a hump at the vicinity of 360 mμ in addition to a peak at 320 mμ; the latter is clearly due to other spinochromes (see p. 459). Thus the peaks in the crystallized pigment at 267, 358 and 476 mμ correspond to those at 268, 360 and 478 mμ in the crude extract.

OTHER PIGMENTS

Other pigments in the ether phase were also studied by the following means. Tests and spines were digested by 2N-HCl and extracted with re-distilled ether. The ethereal solution was washed with distilled water and dried over anhydrous Na_2CO_3. The concentrated pigment solution was chromatographed on $CaCO_3$, which was activated for 3 h at 180° C before use.

By developing with ether, the pigment can be separated into three fractions on the column: (1) a red pigment which is only weakly adsorbed, (2) a blue (sometimes green) zone, which descends slowly, and (3) a violet top zone. The

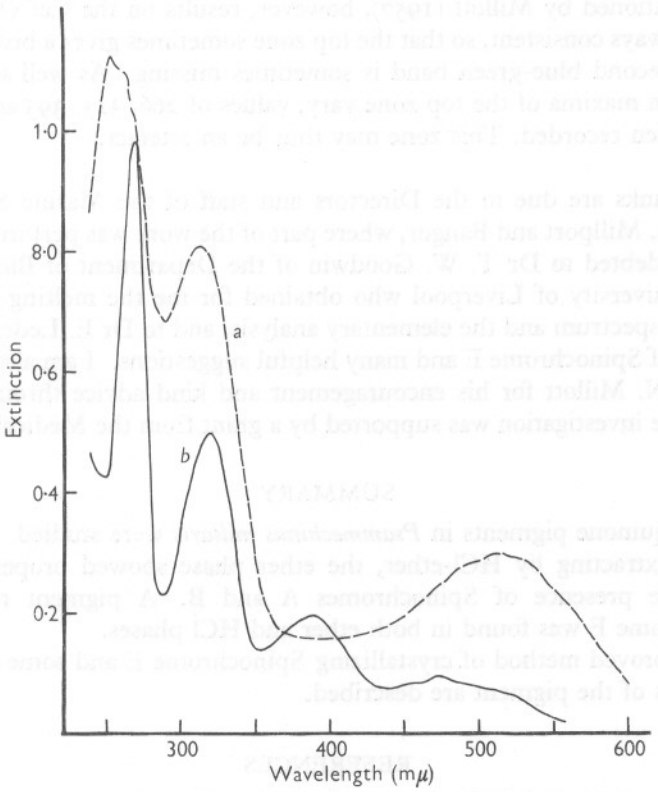

Fig. 3. Absorption spectra of pigment extracted from *Psammechinus miliaris*. Ether phase. *a* (broken line), Spinochrome A in ether; *b* (continuous line), Spinochrome B in ether.

second and the third zones were separated by dissecting the column, dissolving each part in 2N-HCl and taking up the pigment liberated into diethyl ether.

The absorption maxima of the three eluates were as follows and the curves are shown in Fig. 3:

Zone	Solvent	$\lambda_{max.}$ (mμ)			
Red	Ether	269	320	390	470
Blue or green	Ether (Fig. 3*b*)	272	320/2	388	470
	Methanol	270	318	—	480
Violet	Ether (Fig. 3*a*)	255	315	—	510
	Chloroform	255	318	—	515 535

Although no further tests were performed, the behaviour of these pigments on the chromatogram, their spectral absorption and their coloration on calcium columns, suggest that the violet and blue-green zones are Spinochromes A and B respectively (Goodwin & Srisukh, 1950; Lederer, 1952).

As mentioned by Millott (1957), however, results on the $CaCO_3$ columns are not always consistent, so that the top zone sometimes gives a brown colour and the second blue-green band is sometimes missing. As well as this, the absorption maxima of the top zone vary, values of 266, 315, 395 and 480 mμ having been recorded. This zone may thus be an artefact.

My thanks are due to the Directors and staff of the Marine Stations at Plymouth, Millport and Bangor, where part of the work was performed. I am deeply indebted to Dr T. W. Goodwin of the Department of Biochemistry at the University of Liverpool who obtained for me the melting-point, the infra-red spectrum and the elementary analysis, and to Dr E. Lederer for the samples of Spinochrome E and many helpful suggestions. I am also indebted to Prof. N. Millott for his encouragement and kind advice throughout the work. The investigation was supported by a grant from the Medical Research Council.

SUMMARY

Naphthaquinone pigments in *Psammechinus miliaris* were studied.

After extracting by HCl-ether, the ether phase showed properties indicating the presence of Spinochromes A and B. A pigment resembling Spinochrome E was found in both ether and HCl phases.

An improved method of crystallizing Spinochrome E and some additional properties of the pigment are described.

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THE LARVAL DEVELOPMENT OF TWO SPECIES OF GASTROCOTYLID TREMATODE PARASITES FROM THE GILLS OF *TRACHURUS TRACHURUS*

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(Text-figs. 1–9)

Plymouth scad (horse-mackerel) *Trachurus trachurus* (L.) are known to harbour three species of monogenean trematode gill parasites, of which two belong to the Gastrocotylidae and one to the Microcotylidae. The oncomiracidia (= newly hatched larvae) of these parasites have already been described (Llewellyn, 1957*a*), but nothing is known of the developmental stages intervening between the oncomiracidia and the adults. Bychowsky (1957) has stated that some information is available about the larval development of 13 monogenean families, leaving 15 families, including the Gastrocotylidae, about whose larval development nothing is known. The ontogenetic development of the Gastrocotylidae is of especial interest since the oncomiracidia are bilaterally symmetrical, but the adults invariably show an extreme degree of asymmetry.

In spite of a rigorous search, no larval monogeneans were found on mature *Trachurus* (20–30 cm long) at Plymouth during July and August 1954–8. Even in smaller scad (10–15 cm) examined in the same months in 1957 and 1958, all the parasites were found to be mature and egg-laying.

On 6 May 1959 about 50 specimens of *Trachurus trachurus*, of 9·3 to 12·6 cm length, were landed at Plymouth and sent in ice to Birmingham. Here 19 out of a sample of 20 of these young scad were found to bear an abundance of larval monogeneans, and immediately some new problems presented themselves: (*a*) is there a seasonal rhythm in the reproductive cycle of the monogenean parasites? and (*b*) do these particular monogenean larvae become parasitic only on young scad? It was of course realized that these questions would be best answered by continuous observations over a long period, but since this was impracticable, it was thought that some indications might emerge from comparing the parasite populations from hosts of different ages. Accordingly, through the most helpful co-operation of the staff of the Plymouth Laboratory, some large *Trachurus trachurus* caught off Plymouth on 20 May were measured individually to permit estimates to be made of their ages, then deep-frozen, and later the heads and gills were sent in vacuum flasks to Birmingham.

The gills of all the fishes were searched carefully with a stereomicroscope, and the dead parasites were rinsed in sea water and preserved in 4% formaldehyde. Some specimens were stained in haematoxylin or carmine and mounted in Canada Balsam, and some were sectioned at 6 μ, but most were mounted freely without pressure of a coverglass, in 4% formaldehyde, to permit measurements to be made.

The parasites were identified as *Gastrocotyle trachuri* van Beneden & Hesse (present relatively abundantly) and *Pseudaxine trachuri* Parona & Perugia (present relatively rarely); no microcotylids were found. A combined total of 182 larval and adult *Gastrocotyle* was found to be distributed among 24 host fishes as indicated in Table 1.

TABLE 1

Length of host (cm)	Probable age of host* (yr)	No. of host specimens examined	No. infested	Total no. of parasites	Mean no. of parasites/ host	Mean and range of no. of clamps/ parasite
Under 12·9	Up to 1	10	9	116	11·6	12·5 (0–21)
13·0–18·9	1–2	3	3	37	12·3	20·4 (9–35)
19·0–23·9	2–3	6	5	25	4·2	21·0 (0–36)
Over 24·0	Over 3	5	1	4	0·8	15·8 (12–17)

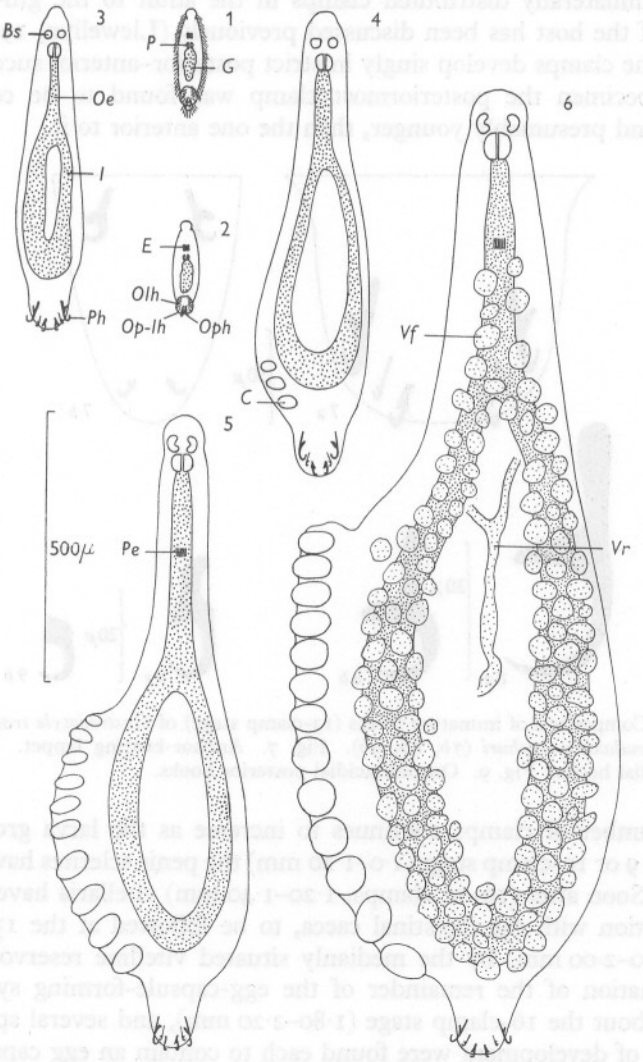
* Based on information kindly supplied by the late Dr G. A. Steven (Marine Biological Association of the United Kingdom, Report of the Council for 1957–58, p. 15).

THE LARVAL DEVELOPMENT OF *GASTROCOTYLE TRACHURI*

The oncomiracidium, about 0·16–0·20 mm in length (Figs. 1, 2) has been described previously (Llewellyn, 1957*a*), but subsequent observations during the course of abortive attempts to infect adult *Trachurus* with oncomiracidia have shown that the postero-lateral hooks may be as long as 26 μ, and a more accurate expression of their size range is 23 μ (19–26 μ). The posterior hooks are 26 μ (23–28 μ) in length.

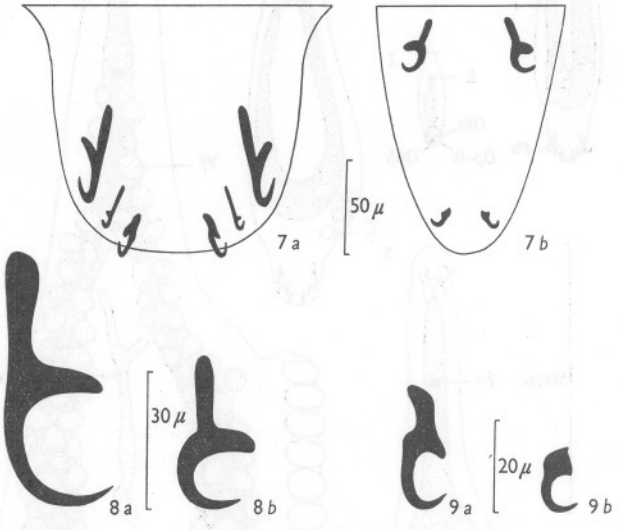
The earliest post-oncomiracidial larvae found were bilaterally symmetrical and about 0·50–0·85 mm long (Fig. 3). These larvae have lost the eyes and the 4 pairs of lateral hooks of the oncomiracidium, the place of the lateral hooks being taken, functionally if not topographically, by a pair of relatively large hooks each 54 μ (52–56 μ) long (Figs. 3, 8*a*). These hooks in *Gastrocotyle* obviously correspond to those hooks in *Microcotyle labracis* which I described as 'primordial adult hooks' (Llewellyn, 1957*a*). It is noteworthy that all three pairs of hooks (i.e. the oncomiracidial postero-lateral and posterior hooks, and the newly acquired 'large hooks') present in *Gastrocotyle* at this stage persist without change of size or shape throughout all succeeding larval stages and survive in the definitive adult on the 'anchor-bearing lappet' (Fig. 7*a*) that has been described in many monogeneans. The alimentary canal, which in the oncomiracidium consisted merely of a mouth, pharynx, and simple sacculate intestine, is now provided with a pair of buccal suckers; the gut is differentiated into an oesophagus that bifurcates into two intestinal

limbs and these become confluent posteriorly. The walls of the oesophagus and the intestine are lined by scattered pigment cells indicating, by analogy with what is known about similar cells in adult polyopisthocotylineans, that the larvae have already been feeding on blood (Llewellyn, 1954).



Figs. 1–6. The larval development of *Gastrocotyle trachuri*; all diagrams drawn to the same scale. Fig. 1. Newly hatched oncomiracidium. Fig. 2. Oncomiracidium after shedding of ciliated epidermis. Fig. 3. Bilaterally symmetrical post-oncomiracidial larva. Figs. 4–6. Immature stages with 3, 10, and 13 clamps respectively. *Bs*, buccal sucker; *C*, clamp; *E*, eye; *G*, gut; *I*, intestine; *Oe*, oesophagus; *Olh*, *Op-lh*, *Oph*, lateral, postero-lateral, and posterior hooks respectively of oncomiracidium; *P*, pharynx; *Pe*, penis; *Ph*, post-oncomiracidial hook; *Vf*, vitelline follicle; *Vr*, vitelline reservoir.

During the next phase (0·60–1·00 mm) asymmetrical development begins: clamps develop on one side only of the posterior region of the body, which becomes slightly wider to accommodate them (Fig. 4). There is a complete absence of clamp development on the other side of the body. The relationship of these unilaterally distributed clamps in the adult to the gill-ventilating current of the host has been discussed previously (Llewellyn, 1956, 1957*b*). Usually the clamps develop singly in strict posterior–anterior succession, but in one specimen the posteriormost clamp was found to be considerably smaller, and presumably younger, than the one anterior to it.



Figs. 7–9. Comparison of immature forms (13-clamp stage) of *Gastrocotyle trachuri* (7*a*, 8*a*, 9*a*) and *Pseudaxine trachuri* (7*b*, 8*b*, 9*b*). Fig. 7. Anchor-bearing lappet. Fig. 8. Post-oncomiracidial hooks. Fig. 9. Oncomiracidial posterior hooks.

The number of clamps continues to increase as the larva grows bigger, and at the 9 or 10-clamp stage (1·0–1·20 mm) the penis sclerites have appeared (Fig. 5). Soon after (10–12 clamps, 1·20–1·40 mm) vitellaria have developed in association with the intestinal caeca, to be followed at the 13–16-clamp stage (1·50–2·00 mm) by the medianly situated vitelline reservoir (Fig. 6). The formation of the remainder of the egg-capsule-forming system takes place at about the 16-clamp stage (1·80–2·20 mm), and several specimens at this stage of development were found each to contain an egg capsule which, however, appeared to be without embryonic (ovum/oocyte/zygote) contents. In fact, no germarium could be identified in whole mount preparations, and so paraffin sections of 13-, 14-, 15- and 16-clamp larvae were prepared. The specimens, having been preserved in ice before histological treatment, yielded comparatively poor sections, but it was possible to determine that the

testes and germarium were still immature. The inference is, then, that the egg-capsule-forming apparatus (the vitellarium and the ootype and its associated glands) becomes functional before the truly germinal portions of the genitalia (the testes and germarium). Egg capsules yielding oncomiracidia have been collected from a specimen of *Gastrocotyle* with 19 clamps. A continued steady increase in the total length of the body is accompanied by a correspondingly steady increase in the number of clamps until eventually a maximum of 35 to 40 clamps is present in parasites of about 2·80–3·20 mm long.

THE LARVAL DEVELOPMENT OF *PSEUDAXINE TRACHURI*

The larval development of *Pseudaxine trachuri* is generally similar to that of *Gastrocotyle trachuri*, but the following differences have been found to occur.

Morphologically the oncomiracidium of *Pseudaxine* may be reliably distinguished from that of *Gastrocotyle* only by the smaller posterior hooks, which are 19 μ (18–20 μ) in *Pseudaxine* and 26 μ (23–28 μ) in *Gastrocotyle*. The difference in size of the postero-lateral hooks, which have been found to be 19 μ (18–20 μ) in *Pseudaxine* and 21 μ (20–26 μ) in *Gastrocotyle*, is likely to be a less reliable distinction, especially since the postero-lateral hooks of *Pseudaxine* are destined to reach a length of 24–26 μ before they eventually disappear.

With the acquisition of the post-oncomiracidial hooks the two species of larvae may be readily distinguished from each other: in *Pseudaxine* these hooks are 32 μ (31–33 μ) long whereas those of *Gastrocotyle* are 54 μ (52–56 μ) long. The shapes of these hooks are also different in the two parasites (Fig. 8).

While in *Gastrocotyle* the posterior hooks retain their oncomiracidial size and form without any alteration throughout development, in *Pseudaxine*, between the 5- and 12-clamp stages, the proximal regions of the posterior hooks disappear so that these hooks decrease in length from 19 μ to about 12 μ, and assume a different shape (Fig. 9*b*). The posterior hooks of *Pseudaxine* persist in this reduced form throughout adult life. While the reduction in size of the posterior hooks of *Pseudaxine* is taking place, i.e. between the 5- and 12-clamp stages, the postero-lateral hooks are lost completely.

Whereas the positions relative to each other of the oncomiracidial and post-oncomiracidial hooks remain constant throughout life in *Gastrocotyle*, in *Pseudaxine* the 'anchor-bearing lappet' elongates considerably so that in the adult the surviving oncomiracidial posterior hooks and the post-oncomiracidial hooks are relatively further apart (Fig. 7*b*).

Other features of the larval development of *Pseudaxine* are generally similar to those of *Gastrocotyle*.

DISCUSSION

Both larvae remain bilaterally symmetrical for a considerable period of post-oncomiracidial development, sufficient for the acquisition of buccal suckers and a new pair of large hooks. It is possible that this period could be used to investigate experimentally the factors, environmental (unilateral incidence of the gill-ventilating current, see Llewellyn, 1957*b*) or genetical, which determine the side of the subsequent asymmetrical development of the clamps.

In both species a pair of relatively large hooks develops in the first post-oncomiracidial stage. There is no trace of these in the newly hatched larvae. Bychowsky (1957) expressed the opinion that the corresponding hooks in the Microcotylidae and Mazocraeidae were not homologous with any of the six pairs of hooks present in the larvae of the majority of diclidophorideans, and, quite independently, I referred to such hooks in *Microcotyle labracis* not as larval hooks, but as 'primordia of adult hooks' (Llewellyn, 1957*a*). The present study supports the view that these 'large hooks' are post-oncomiracidial, but since they are relatively most prominent and have reached their definitive size during early larval development, namely, after the loss of the oncomiracidial lateral hooks, and before the development of the clamps of the adult, these hooks are probably best regarded as essentially *larval* features. Their appearance during the embryonic development of *M. labracis* is probably no more than an example of the kind of precocious development met elsewhere in polyopisthocotylineans, e.g. the appearance in the *embryo* of *Diplozoon paradoxum* of the first pair of clamps of the adult (Zeller, 1872).

The finding of larval and immature stages of *Gastrocotyle* and *Pseudaxine* in May, but of only adults in July and August, suggests that a seasonal rhythm is present in the reproductive cycle of these parasites.

The recovery from hosts of over two years of age of four immature specimens of *Pseudaxine* and 15 immature specimens of *Gastrocotyle*, one of which was a very early larva at the stage before any clamps had developed, indicates that it is not only 'young' (under two years old) *Trachurus* which are susceptible to infestation by these monogeneans, but older fishes also.

The main difference between the larval development of *Gastrocotyle* and *Pseudaxine* and those of *Microcotyle spinicirrus* as described by Remley (1942) and *Diclidophora denticulata* as described by Frankland (1955) is the survival of the 'hook-bearing lappet' with at least some of its hooks in the first two species, and its complete disappearance in the other two species.

I am happy to acknowledge the help I have received from the Director and Staff of the Plymouth Laboratory, and especially that given by Mr J. E. Green and Mr A. D. Mattacola.

SUMMARY

Larval forms of *Gastrocotyle trachuri* and *Pseudaxine trachuri* are common on *Trachurus trachurus*, especially on young fishes, at Plymouth in May, but only adult parasites are found in July and August.

The larval development of these monogeneans includes a bilaterally symmetrical post-oncomiracidial stage in which the most prominent adhesive organs are a pair of relatively large post-oncomiracidial hooks. Asymmetrical development begins with the formation of the principal adult adhesive organs, the clamps.

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DIGESTION IN SEA ANEMONES

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(Plate I and Text-fig. 1)

A sea anemone, normally a passive-looking animal, reacts to suitable foodstuffs by a series of fairly complicated activities. When its tentacles encounter solid food there is, first of all, a discharge of cnidae, which poison living prey and adhere to the food mass. These cnidae are independent effectors, responding directly to external excitation (Pantin, 1942). Next, the tentacles clasp the food and bend towards the mouth; they push the food into the mouth, and the pharynx draws it down into the coelenteric cavity where it is digested (Pantin & Pantin, 1943).

The food bolus lying in the coelenteric cavity gradually disintegrates, and the particulate and soluble products of digestion are absorbed. The indigestible residue is finally expelled from the mouth. Batham & Pantin (1950) have recorded the phasic activities of *Metridium* following feeding: these include elongation of the column, peristaltic movements and, finally, defaecation and shrivelling after about 48 h. The faecal pellets which are extruded are covered with thick mucus.

According to Yonge (1931, 1937, 1954), some preliminary digestion of protein takes place extracellularly in coelenterates, whereas the digestion of carbohydrates and fats, and the final degradation of proteins are all carried out intracellularly. Previous workers have had difficulty in demonstrating an extracellular protease in actinians, since fluid drawn from the coelenteron usually possesses little or no proteolytic activity (Mesnil, 1901; Bodansky, 1924). Many lines of evidence show, however, that extracellular digestion does take place (Boschma, 1925; Yonge & Nicholls, 1930; Krijgsman & Talbot, 1953). There is, seemingly, a paradox between the low proteolytic activity of coelenteric fluid, and the demonstrated efficacy of coelenteric digestion, and some experiments were made to investigate certain aspects of digestive secretion in sea anemones.

METHODS AND EXPERIMENTS

Most of the observations and experiments were made on the anemone *Calliactis parasitica* (Couch). For *in vitro* studies mesenteric filaments, together with neighbouring regions of the mesenteries, were removed from

anaesthetized sea anemones. Roughly weighed samples of these tissues were used for investigations of protease activity.

For estimating protease activity, the photoelectric method of Riggs & Stadie (1943) was used. In this method, changes in the turbidity of a suspension of homogenized boiled egg white during the course of digestion are measured in an absorptiometer. From suitable dilutions of the substrate, a curve is obtained relating concentration to density. The initial concentration is taken as 100%. Density readings made of the digest at suitable times are converted to concentrations by means of this curve. Concomitantly with the digests, blanks are run that contain fluid to be tested and preservative, but no substrate. Values for the blanks are subtracted from the digests. Digests were carried out at pH 7·4, using boric acid–borate buffer. This hydrogen-ion concentration was chosen somewhat arbitrarily after reviewing the literature. Previous workers have shown that the optimal pH for intracellular proteases and the hydrogen-ion concentrations of coelenteric fluids of diverse Anthozoa range from pH 6·7 to 8·75 (Yonge & Nicholls, 1930; Krijgsman & Talbot, 1953).

Digestive activity of extracts

Tests were made of the proteolytic activity of crude extracts of mesenteric filaments of *Calliactis*. Filaments from an animal were ground with sand, 10 ml. of water were added, and the suspension was centrifuged and filtered. Results are given in Table 1. The rate of digestion, computed as a monomolecular reaction, is shown in Text-fig. 1. The velocity constant, k, falls off with time. The extract contains a strong protease acting at pH 7·4.

Proteolytic activity of coelenteric fluid

Samples were drawn of coelenteric fluid of some anemones to test for proteolytic activity. At most a few ml. were obtained from each animal. Out of 14 specimens (2 *Tealia felina* and 12 *Calliactis parasitica*), only 1 (*Tealia*) showed slight digestive activity (substrate concentration reduced to 73% in 18 hours).

Rate of digestion of a meal in a normal animal

The rate at which *Calliactis* can digest a meal was investigated as follows. Animals were fed weighed pellets of denatured gelatine. At selected intervals animals were sacrificed, the residual pellets (if any) recovered and weighed. Kjeldahl analyses were made of the latter.

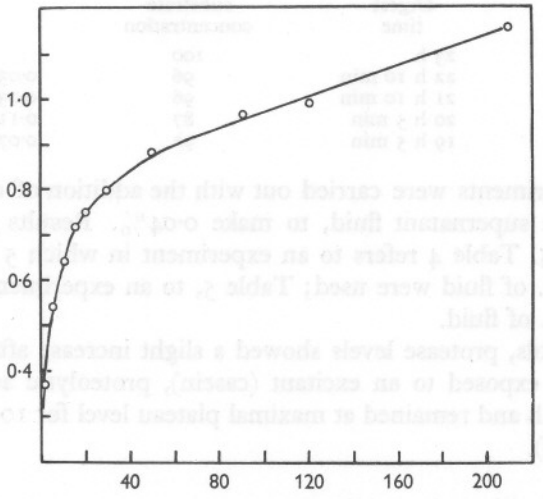
The pellets were prepared from a 10% solution of gelatine, denatured with formalin, and washed in sea water.

Fed sea anemones were sacrificed at 2, 4, 8, 16 and 24 hr. At least 3 animals were tested for each of these periods. Sea-water temperatures were about

TABLE 1. PROTEOLYTIC ACTIVITY OF EXTRACT OF MESENTERIC FILAMENTS OF *CALLIACTIS*

Period (time after mixing) (min)	Concentration of egg white (%)	k*
1	70·2	0·364
6	57·8	0·091
11	52·3	0·059
16	48·3	0·045
21	46·9	0·036
31	44·8	0·026
51	41·4	0·017
91	38·0	0·011
121	37·2	0·008
211	31·6	0·005

* $k = 2{\cdot}3/t \log_{10} C_0/C_t$ (proportion of protein hydrolysed per min.)



Text-fig. 1. Rate of digestion of egg white substrate by an extract of the mesenteric filaments of *Calliactis*. Abscissae, $2{\cdot}3 \times \log_{10} C_0/C_t$; ordinates, time in min after mixing extract and substrate.

TABLE 2. DIGESTION OF DENATURED GELATINE PELLETS BY *CALLIACTIS*

Digestion time (h)	No. of trials	Original wet weight (g)	Percentage of pellet digested	
			Basis wet weight	Basis N
2	5	0·766–1·035	44·5	45·4
4	5	0·592–0·847	65·9	79·1
8	8	0·906–1·022	53·9	48·9
16	5	0·663–1·019	76·5	80·7
24	3	0·687–1·013	98·7	98·6

12°–13° C. The results are summarized in Table 2. The gelatine blocks were about half digested in the first 8 h and completely digested in 24 h.

Progress of secretion with time

The temporal course of secretion *in vitro* was determined by the following procedure. In control experiments, 5 g of *Calliactis* tissue (isolated mesenteries and filaments) were placed in a vessel and covered with 25 c.c. of sea water. Samples of 1 c.c. were drawn from the supernatant fluid at the start and at 1 h intervals and were tested for protease activity. Room temperature was 18° C. The results appear in Table 3.

TABLE 3. PROTEASE ACTIVITY BY *CALLIACTIS* FILAMENTS *IN VITRO*

Time of drawing sample	Digest time	Final substrate concentration	k
0	23 h	100	—
1 h	22 h 10 min	96	$0{\cdot}030 \times 10^{-3}$
2 h	21 h 10 min	96	$0{\cdot}032 \times 10^{-3}$
3 h	20 h 5 min	87	$0{\cdot}117 \times 10^{-3}$
4 h	19 h 5 min	92	$0{\cdot}072 \times 10^{-3}$

Similar experiments were carried out with the addition of casein (B.D.H. soluble) to the supernatant fluid, to make 0·04%. Results are shown in Tables 4 and 5. Table 4 refers to an experiment in which 5 g of *Calliactis* tissue in 25 c.c. of fluid were used; Table 5, to an experiment with 10 g of tissue in 50 c.c. of fluid.

In the controls, protease levels showed a slight increase after 3 h. In the experimentals, exposed to an excitant (casein), proteolytic activity became marked after 4 h and remained at maximal plateau level for 10 h (duration of the experiment).

In vitro *studies with various excitants*

The results obtained in the experiments described in the previous section suggested the possibility of testing the relative efficacy of various excitants. Accordingly, a long series of experiments was carried out, using 1 g samples of mesenteric filaments in 5 c.c. of sea water. Excitatory substances tried were proteins, proteose, peptones, glutathione and many amino acids (0·02%). Test samples were drawn after 4 h (temp. 17°–19° C). Digest time was 19 h. Since the results were very variable, it is not proposed to describe them in detail. Controls (mean of 12 tests) showed a final substrate concentration of 86%; proteins (casein and egg albumen, 12 tests), 66–73%; peptones (bacteriological, 14 tests), 55–83%; proteose, glutathione, and amino acids (52 tests), 83–100%. Proteins and peptones were secretagogues, producing stronger secretory activity than that occurring in the controls.

TABLE 4*

Time of drawing sample	Digest time	Final substrate concentration	k
0 h	23 h 30 min	100	—
1 h	22 h 35 min	100	—
2 h	21 h 40 min	100	—
3 h	21 h	83	$0{\cdot}170 \times 10^{-3}$
4 h 20 min	19 h 40 min	66·5	$0{\cdot}343 \times 10^{-3}$

TABLE 5*

Time of drawing sample	Digest time	Final substrate concentration	k
0 h	23 h	95	—
2 h	23 h	66	$0{\cdot}298 \times 10^{-3}$
4 h	23 h	61	$0{\cdot}358 \times 10^{-3}$
6 h	23 h	59	$0{\cdot}383 \times 10^{-3}$
8 h	23 h	63	$0{\cdot}335 \times 10^{-3}$
10 h	21 h 5 min	62	$0{\cdot}370 \times 10^{-3}$

* Details in text.

DISCUSSION AND CONCLUSIONS

There is now clear evidence, both from previous observations and the present work, that a protease is secreted into the coelenteron of sea anemones. Earlier studies are reviewed by Boschma (1925) and Yonge (1931). Especially pertinent are the experiments of Jordan (1913), who enclosed fibrin in sacs of filter-paper and fed them to anemones (*Anemonia sulcata*). Digestion of the fibrin occurred and, since the mesenteric filaments could not come into contact with the food, he concluded that extracellular protease diffused into the sacs and caused hydrolysis of the fibrin. Krijgsman & Talbot (1953) fed *Pseudactinia flagellifera* with pieces of sponge soaked in meat extract. The sponges were removed, the juice expressed and tested for proteolytic activity (casein precipitation method and alcohol titration). With casein substrate, there was pronounced digestion after 3 h. With gelatine substrate, marked digestive activity was found after 3 h (increase of acidity measured by alcohol titration method of Waldschmidt–Leitz).

Studies dealing with excitation of digestive secretion in lower animals are rather infrequent. In the experiments of Krijgsman & Talbot (1953), cited above, the coelenteric fluid collected from anemones which were fed pieces of sponge containing meat extract showed pronounced proteolytic activity, but, as no similar experiments were recorded of feeding sponge alone, it is difficult to draw any conclusions concerning the stimulatory effect of meat extract on the secretory mechanism. Ishida (1936) collected coelenteric fluid from *Actinia mesembryanthemum* by cutting off the pedal disc; starved and fed animals were compared for proteinase activity. Three hours after

feeding cooked egg white there was a marked increase in the proteinase ('tryptase') activity of the collected fluid.

Only a small amount of coelenteric fluid can be collected from sea anemones by suction or by cutting open the animals, and this fluid shows little proteolytic activity, even in fed animals (Bodansky, 1924). Yet there is secretion of protease, extracellular digestion proceeds rapidly and large food masses are quickly cleared. How are these apparent contradictions to be explained? Extracellular protease has been detected in fluids collected from the intact animal (*Hydra*, anemones), and secretion of protease has been demonstrated by using *in vitro* preparations (anemones). Krijgsman & Talbot (1953) believed that a food mass becomes coated with stiff mucus in the coelenteron of an anemone. The mucus coating is impregnated with proteinase, and forms a protective barrier against dilution by sea water; digestion takes place within the mucus coating. The particulate products of extracellular digestion are then phagocytized and digestion is completed intracellularly.

We have found no stiff masses of mucus about partially digested food boluses in *Calliactis*. In an expanded fed animal very little fluid can be collected from the base of the coelenteron. When fed sea anemones are quickly frozen (solid carbon dioxide in acetone) and cut open in the frozen state, the food bolus is found completely invested by mesenteric filaments at the base of the coelenteron, below the stomodaeum, and there is no fluid filled cavity in this region (Pl. I). This would explain why it is difficult to draw a sample of uncontaminated coelenteric fluid through the stomodaeum. Fluid which is obtained comes mostly from the upper regions of the coelenteron, or contains sea water drawn down the stomodaeum.

Mesenteric filaments of anemones are very active structures which move over, and closely adhere to the food bolus. Boschma (1925) and Yonge (1930) have made similar observations on corals (*Astrangia* and *Euphyllia*): they found that mesenterial filaments close quickly over plankton organisms, completely obscuring them. These filaments are in continual motion, one replacing another as soon as the first becomes gorged with food. The gastrozooids of *Physalia* behave in an analogous manner, closely enveloping the prey. Digestive enzymes are secreted, and partially digested food material passes up the lumina of the gastrozooids (Wilson, 1947).

The structure of the coelenteron favours enzymic activity. The food bolus is enveloped in a sac-like mass of mesenteric filaments which adhere closely to the surface of the food (Pl. I). Proteolytic secretion is stimulated by protein components in the food mass. The enzymes act on the food practically at the surface of the filaments, and suffer little dilution; as the food is dissolved and absorbed the filaments continue to press against the shrinking mass. This situation is in contrast to that found in many higher Metazoa, notably vertebrates, which discharge extracellular enzymes into large sacs or tubes, in the open lumina of which digestion takes place. The apparently feeble

proteolytic activity of gastric fluids or *in vitro* samples, therefore, should be compared with the *in vivo* condition, in which the enzyme is concentrated in small volume, immediately over the food mass.

Corals and anemones digest food masses quickly. In the present experiments it was found that *Calliactis* would dissolve sticks of insoluble gelatine, weighing 0·6–1 g and containing 5–9 mg N, within 24 h (12° C). Yonge & Nicholls (1930) found that the corals *Fungia*, *Symphyllia* and *Favia* digested large plankton organisms (copepods, mysids, etc.) in 4–13 h (around 25° C). Masses of coagulated blood were digested by *Fungia* in 4 h.

It is generally believed that the extracellular protease of coelenterates hydrolyses some part of the protein fraction of the meal to polypeptides; the disintegrated food bolus is then phagocytized, and digestion is continued intracellularly (Yonge, 1937).

The mesenteric filaments of corals and anemones contain a powerful proteinase. This is regarded as being of the trypsin type (i.e. an endopeptidase acting in an alkaline medium on peptide linkages adjacent to arginine or lysine); it has the same pH optimum as extracellular protease, and may be identical with the latter (Krijgsman & Talbot, 1953). It would be reasonable to find the precursor of the extracellular protease in the secretory tissue. If digestion of proteins proceeds beyond the polypeptide stage, then other intracellular proteases must be involved.

SUMMARY

Proteolytic activity of *Calliactis parasitica* was investigated by a photoelectric method.

The filaments contain a strong protease; the coelenteric fluid shows little or no proteolytic action.

Gelatine pellets (up to 1 g wet weight and 9 mg N) were digested within 24 h (12° C).

Secretion by mesenteric filaments *in vitro* was followed. Proteins and peptones acted as secretagogues. Secretion reached a maximum in 4 h.

The mesenteric filaments closely invest the food bolus. Digestion, initiated by extracellular protease, takes place at the surface of the filaments, and partially decomposed food material is absorbed.

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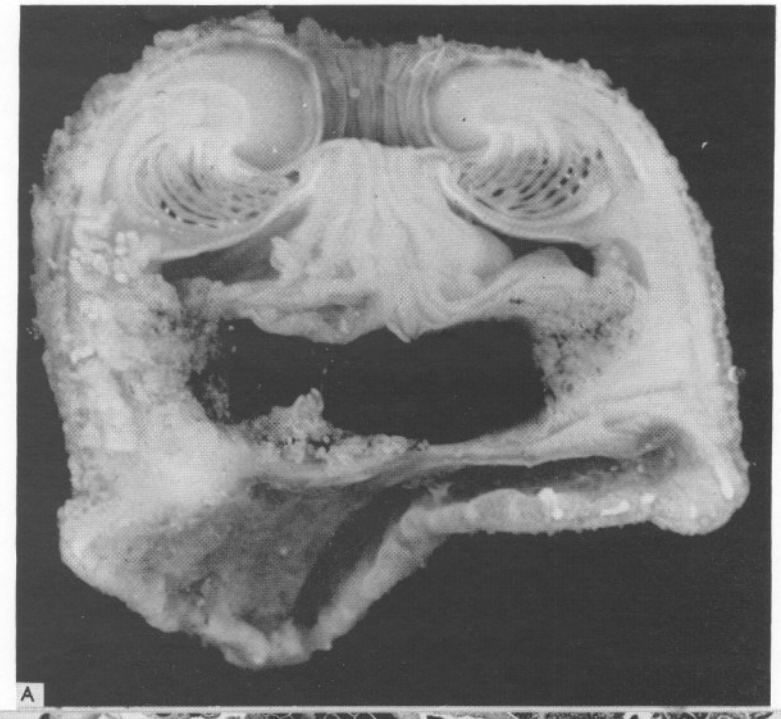
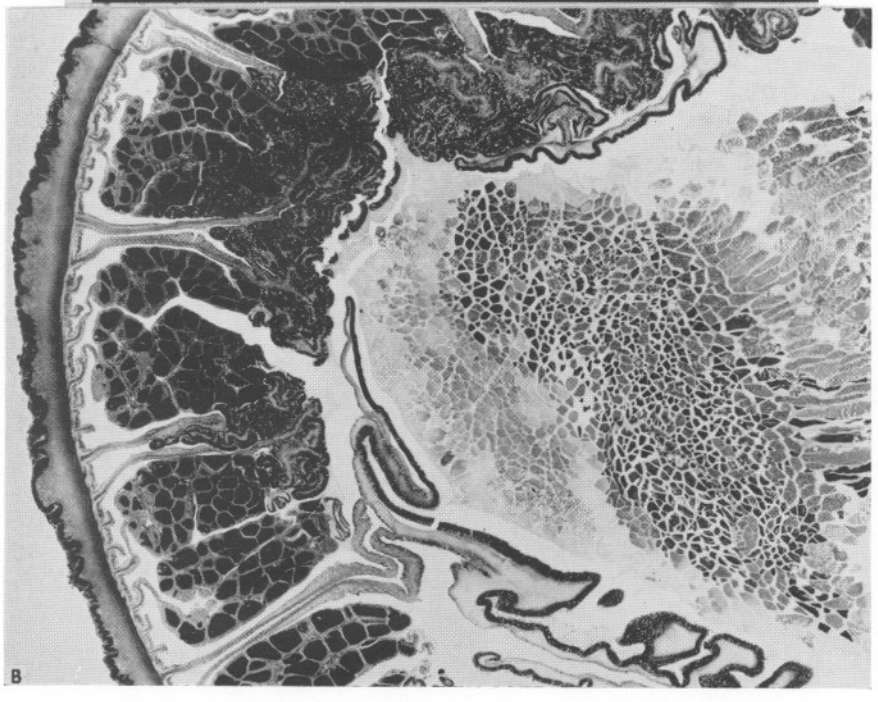
EXPLANATION OF PLATE I

A. A frozen *Calliactis*, cut vertically through the centre. The black rectangle, below the stomodaeum, is a gelatine pellet coloured red with carmine. It is closely invested by mesenteric filaments (× 5).

B. Horizontal celloidin section across the body of a fixed *Calliactis* that had been digesting a meal of plaice muscle. A mass of muscle lies in the coelenteron; note the change in density at the margin, due to partial digestion. Mesenteric filaments press closely against the muscle mass (a little separation has occurred during fixation) (× 12·5).





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STUDIES ON LUMINESCENCE. ATTRACTION OF ANIMALS TO A WEAK LIGHT

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(Text-fig. 1)

In order to evaluate the biological significance of luminescence, it is desirable to know how weak light affects the behaviour of marine animals, and what intensities they can see. Much information is available concerning the attractive power of bright lights, and light directed movements (see Verheijen, 1958, for example), but little for weak point sources. Weak coloured lights have been used by Baylor & Smith (1953) to trap freshwater arthropods.

In the present study, the reaction was tested of animals to a small light-lure, having the colour and intensity of animal luminescence. The light-lure was a conical light-guide, consisting of frosted glass 20 mm long, 4·4 mm in diameter at the base, and tapering to a point (Fig. 1). The light-lure was mounted in a light-proof case, containing a small light-bulb. A blue filter was placed between the bulb and the base of the light guide. The light which was emitted by the light-lure had a spectral range of 420–540 mμ, and maximal emission at 475 mμ. Intensities used were judged by eye to be equivalent to those observed in various marine animals, such as deep-sea fish with luminous barbels and deep-sea shrimp with photophores.

Animals were tested in a black tray, 51 × 41 × 10 cm (Fig. 1). This was divided into three compartments, left (*L*), centre (*C*) and right (*R*), separated from each other by clear plastic ('Perspex') partitions. The latter were > shaped, the apex pointing away from *C* to *L* or *R*. At the apex was a vertical slit, variable in width (1–3 cm), through which the animals could pass at will from the centre to the lateral compartments. The light-lure was hung vertically in one of the lateral compartments, 10 cm from a vertical slit. The tray was filled with sea water.

An experiment or test was carried out as follows. A batch of animals was dark-adapted for 1 h, following which they were placed in *C* and left in the dark for 1–5 h. Then the numbers of animals in the three compartments were counted. If the animals remained in *C* or distributed themselves equally among the *L*, *C* and *R*, it was concluded that they were not attracted by the light. If they tended to congregate in the lateral compartment containing the light, they were considered to be attracted by the latter.

Animals tried were small free-swimming crustacea and fish, easily obtained in inshore waters. The light intensity was varied with a rheostat.

Animals attracted by the light (protocols in Appendix 1) were: a decapod crustacean *Palaemonetes varians*, a mysid *Praunus neglectus*, and a copepod *Tigriopus fulvus*. Two other decapods *Crangon vulgaris* and *Pandalina brevirostris* were probably also attracted. Under the conditions of the experiment a decapod *Palaemon serratus* and a fish *Gobius flavescens* were not.

Praunus neglectus gave a clear-cut response, and was employed to determine the minimal intensity of the source to which the animals would respond

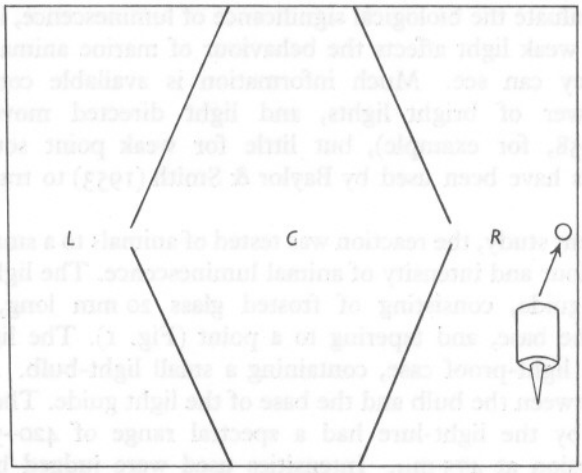

Fig. 1. Test-tray and light-guide in its holder (details in text).

(see Appendix 2 for protocols). This was $28{\cdot}7 \times 10^{-6}\,\mu W/cm^2$ receptor surface at 10 cm distance. Therefore, the animal responds to a flux of $29 \times 10^{-6}\,\mu W$ falling upon a flat surface of 1 cm square in the plane of its eye and at right angles to a line extending from the light-lure to the eye. In the dark-adapted state the eyes lighten, owing to withdrawal of iris-pigments ('superposition' condition). Let us assume that the eye of *P. neglectus*, diameter 0·68 mm, has an effective area of 0·36 mm^2 for incident light from a point-source. This is the area of a flat plane in the maximal diameter of the eye at right angles to the axis of the light-beam. Then, *P. neglectus* responds to $10{\cdot}5 \times 10^{-8}\,\mu W$ falling into each eye.

It is not clear what should be regarded as the effective area of the dark-adapted ('superposition') compound eye of a malacostracan. The visual area involved is certainly less than the value just presented, since only a fraction of the ommatidia are affected by a directional light. In the superposition eye of *Lampyris*, some thirty neighbouring ommatidia may concentrate light from a point source upon a single rhabdome (Exner, in Wigglesworth, 1939).

Pirenne & Marriott (1955) have determined that a freshwater planarian, *Dendrocoelum lacteum*, responds to a radiant flux of 15×10^{-9} erg/sec falling into one eye of diameter 0·08 mm (i.e. $1{\cdot}5 \times 10^{-9}$ μW/eye). Values for radiant fluxes of animal-luminescences range from about 1×10^{-5} μW/cm^2 at 1 cm for a single radiolarian cell to 4×10^{-1} μW/cm^2 at 1 cm for *Pyrosoma* (Nicol, 1958).

SUMMARY

The attraction for animals of a small light-lure, emitting a feeble blue light, was tested in a multiple choice apparatus. *Palaemonetes varians*, *Praunus neglectus* and *Tigriopus fulvus* were attracted. The minimal intensity to which *P. neglectus* responded was 29×10^{-6} μW/cm^2 receptor surface.

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APPENDIX

(1) *Test of animals with the light-lure*

Animal	Duration of experiment (h)	Numbers in compartments		
		L	*C*	*R*
Palaemonetes varians	1	1	3	11*
P. varians	1	2	0	13*
Palaemon serratus	1	5	3	7*
Pandalina brevirostris	5	6*	9	1
Praunus neglectus	1	1	7	7*
P. neglectus	2½	3	2	11*
Tigriopus fulvus	1	3	16	30*
Gobius ruthensparri	1	1	13	0*

(2) *Response of* Praunus neglectus *to various light intensities (test lasted 5 h)*

Intensity of source μW/cm^2 at 10 cm	Numbers in compartments		
	L	*C*	*R*
23×10^{-6}	7	2	7*
$77{\cdot}5 \times 10^{-6}$	1	3	15*
$48{\cdot}8 \times 10^{-6}$	3	5	12*
$36{\cdot}2 \times 10^{-6}$	9	2	12*
$31{\cdot}6 \times 10^{-6}$	7	3	17*
$28{\cdot}7 \times 10^{-6}$	6	1	11*
23×10^{-6}	8	1	9*

* Light-lure in this compartment.

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ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

II. THE TERMINATION OF THE MALE PHASE

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The Plymouth Laboratory

Pandalus borealis Krøyer is a protandric hermaphrodite. In some populations, e.g. that found off the Northumberland coast, the hermaphroditism is partial (Allen, 1959). That is to say only certain individuals undergo sex reversal, while others are primary females, never going through a male phase; all the males undergo sex reversal. In the terminology proposed by Carlisle (1959*a*) such populations exhibit partial obligatory protandric hermaphroditism. By contrast, the population of the Gullmarfjord, Sweden, which I have been investigating, appears to exhibit full obligatory protandric hermaphroditism: the population contains no primary females; all the females have passed through a male phase. The data presented in this paper have no bearing, therefore, on the factors regulating the production of primary females, since I have never encountered one.

In most of the southern populations of *P. borealis* (a useful summary is provided by Rasmussen, 1953), the eggs are carried for about 6 months. Since there is only one breeding season per year this means that copulation takes place between animals which are about $1\frac{1}{2}$, $2\frac{1}{2}$, or $3\frac{1}{2}$ years old. The breeding season is somewhat prolonged, however, lasting up to 4 months. The evidence presented by Jägersten (1936), Rasmussen (1953) and Allen (1959) for different southern populations suggests that the male is not mature at 6 months old and cannot therefore breed until about $1\frac{1}{2}$ years old when it is approximately 90 mm long. A few individuals may already by the end of this breeding season be functioning as females. By the next breeding season, when the animals are $2\frac{1}{2}$ years old and measure about 110 mm, the great majority have reversed sex and copulate as females. A year later, at about 140 mm, they copulate again as females. Egg laying follows almost immediately upon copulation, in contrast to the condition found in Reptantia, where the interval may be as long as 6 months. According to Allen (1959) there are in the Northumberland population normally five moults between the fully functional male phase and the assumption of the breeding dress of the female; the last of the five moults is a moult of copulation. In the population of the Gullmarfjord the number of moults appears to be four, giving three intermoults between the male and the functional female states. In some individuals however, especially at certain seasons of the year, this may be cut to two

intermoults or even to one; in experimental conditions a single individual has been observed to complete the change from male to female at one moult and breed as both male and female in one season. An individual taking only two moults (one intermoult) for the completion of sex reversal will also breed in both sexes in the one season under natural conditions; this is rare. The sex reversal takes place in the Gullmarfjord population at between $1\frac{1}{2}$ and $2\frac{1}{2}$ years old. All individuals seem to partake in the breeding season as males in their first season of maturity, all take part as females in their second season; some few may complete sex reversal in time to partake as females in the first season, after having earlier in the same season functioned as males.

In the first paper of this series (Carlisle, 1959*b*) I have described incretory organs which I have reason to believe may exert a controlling influence on the reversal of sex. In this paper I shall be concerned only with influences which bear upon the first part of the sex reversal—the loss of the male characters. For the purposes of these observations all individuals as far as possible were observed through one moult after experimental interference. Results are expressed in terms of loss or otherwise of a functional male condition after this one moult.

EXPERIMENTAL DATA

Vasectomy

Effective vasectomy may be achieved in a prawn by blocking the genital openings with small pellets of plasticine, soft wax, dental cement or cold-cure araldite. It is a commonplace that the operation performed on mammals (by ligating the vas deferens), besides leading to sterility, often leads also to impotence and to loss of libido (e.g. in the tom cat). In my experience the same is also true of prawns, including both *Pandalus borealis* and *Palaemon* (= *Leander*) *serratus*. This is most easily seen in the behaviour. As described by Höglund (1943), Burkenroad (1947) and Forster (1951), a ripe female which has just moulted, i.e. one which has just undergone the moult of copulation, proves attractive to males. Forster states of *P. serratus*: 'If an "attractive" female is placed in a tank with males nothing happens until the antenna of the male touches some part of the female. At once the male's behaviour alters, he swims very rapidly in no particular direction, often making small circles, until he again makes contact....' Essentially the same behaviour characterizes *Pandalus borealis*: the male, once he has touched an attractive female with his antenna, swims rapidly in circles, then with quick sharp motions, once he has found the female again, palpates her all over with his chelae. Copulation normally follows, but if several males are in a tank there may be some fighting.

When an attractive female is offered to 'vasectomized' males the response depends on the interval which has elapsed between the operation and the offer. No 'vasectomized' males were used until 48 h had elapsed, to allow

time for postoperative recovery. Controls were animals in which the pellet was first inserted into each genital opening and then removed.

Up to 5 days after the operation the response of the 'vasectomized' males to the proffered females was normal, even including the fighting between rival suitors. Copulation was attempted but never completed. Six offerings were made to a tank containing nineteen males between 2 and 5 days after the operation. Each female was then removed after the failure at copulation and offered to a similar tank containing twenty controls. In each of these offerings copulation was completed satisfactorily.

Between 5 and 10 days post-operatively the interest exhibited by the operated males dropped progressively. In this interval I was able to offer them seven attractive females. The first of these on the sixth post-operative day elicited all the earlier part of the response without, however, any attempt at copulation. On the tenth day the offer of an attractive female elicited the swimming-in-circles response in about half the animals, but no further response. By the twelfth day an attractive female elicited no response. Every one of these females completed copulation successfully with one of the control animals. One operated animal moulted on the third day after the operation, shedding with the old shell the blocking pellets. The new shell was that of a fully functional male. Five days after the moult an attractive female was offered to him and they completed copulation satisfactorily. Histological examination after this event revealed a normal testis with spermatogenesis continuing, a normal vas deferens gland and a vas deferens still half full of sperm.

Other operated animals moulted from the seventh day onwards, and before my departure from the Kristineberg Laboratory nine had moulted (besides the one mentioned above), together with seven of the controls. All nine of the operated animals after moulting showed male appendages characteristic of the first non-sexual inter-moult, with reduction of the appendix masculina and loss of its setae. All of them at moulting shed the plug from the genital ducts. Histological examination revealed that spermatogenesis had ceased in these animals, the vas deferens was normal, but the vas deferens gland, though not characteristic of a normal first stage non-sexual animal, was greatly reduced and showed many pycnotic nuclei. Of the controls which moulted, only one passed into the non-sexual phase, and histologically it showed the conditions characteristic of this phase, with loss of the massive portion of the vas deferens gland, retaining only the strand. The other controls remained as functional males and retained the histological structures appropriate to this stage.

The conclusion is inevitable that occlusion of the spermduct in *P. borealis* causes sterility, loss of libido, and degeneration of the male genital system, and promotes the assumption of the non-sexual condition at the succeeding moult.

Eyestalk removal

This operation has been shown in other species of Natantia to have a profound effect on the development of the ovary and in particular on vitellogenesis (Panouse, 1943–6; Carlisle, 1953). The operation is followed by the initiation or acceleration of ovarian development and by vitellogenesis. The same is true of crabs (Brown & Jones, 1949), and in male crabs eyestalk removal is followed by a rapid increase in weight of the testes and vasa deferentia (Démeusy, 1953). It might be expected, therefore, that eyestalk removal would have some influence on the change from male to female in a protandric hermaphrodite. On the other hand, in the protandric hermaphrodite *Lysmata seticaudata* I have found neither eyestalk removal nor injection of eyestalk extracts to have any significant effect upon the weight of the testis or of the vas deferens (Carlisle, 1954). Tables 1 and 2 show that the same is true of *P. borealis*. The animals used in these experiments were all mature, sexually active males. Under the conditions of the experiments neither eyestalk removal nor injection of whole eyestalk extracts had any influence upon the weight of the testicular portion of the gonad, nor had starving, while the weight of the vas deferens was affected significantly only by starvation, not by either of the endocrine disturbances. It seems unlikely, therefore, that any tissue within the eyestalk has any effect upon testicular development.

The same result is apparent when the effect on the numbers of individuals losing the external male characters is considered. During April about half the large males which moult (in the samples I have examined) lose their secondary and accessory male characters (93 out of 197 moults observed) and change to the non-sexual phase. Interference with the eyestalks did not in any way alter this proportion. The results are presented in Table 3. Neither eyestalk removal nor injection of eyestalk extracts had any effect on the numbers moulting to the non-sexual state.

The vas deferens gland

The degeneration of this gland shortly before the termination of the male phase made it seem plausible that this event might be the immediate cause of the loss of the male character, since it is known that removal of the gland in *Orchestia* leads to loss of maleness (Charniaux-Cotton, 1954, 1955, 1956). Preliminary attempts at removing the gland by surgical methods in *Palaemon* proved abortive, because of total mortality of all operated animals within 24 h. The use of radio cautery, however, met with some success in *Palaemon* with less than 90% mortality from the operation. The results of these experiments will be reported elsewhere. When the method was applied to *Pandalus borealis*, however, I met with no success at first, most of the animals dying even before completion of the operation, and the remainder within a few

hours. By modifying my techniques I eventually achieved success with no more than 75% mortality, better even than the earlier results with *Palaemon*. The technique of operation which I adopted may therefore perhaps be usefully described in some detail.

On the stage of the microscope was mounted a heavy, silver-plated brass plate with a central rectangular hole 20 × 7 mm. This was connected to the earth of the radio cautery outfit and served as the neutral electrode. On the brass plate were mounted two containers, whose bottoms were formed by the plate itself, in which could be placed crushed ice. These were mounted at the front and back of the stage to leave room for the hands at the sides. The source of illumination was filtered through copper sulphate solution to remove the infra-red rays. Before operation each animal was refrigerated at -1° C for 30 min. The fingers of my left hand were repeatedly cooled in ice water throughout the operation and were used only for holding and orientating the animal. By transmitted light it is just possible to distinguish the position of the vas deferens and of the gland in the intact animal. With the active electrode of the radio-cautery apparatus (a nickel-chrome (80/20) wire, 40 μ diameter, mounted in a glass holder) and with the apparatus turned to a rather high power I burned a hole near the base of the fifth walking leg immediately over the visible gland. The power was then reduced, the electrode inserted into the gland and the massive portion destroyed on one side. The animal was then immediately returned to the dish of ice-cold sea water and allowed to recover in the constant temperature room maintained at 4° C where the stock of animals was kept. Provided my left hand was kept cold and the operation was completed within about 2–3 min this stage resulted in negligible mortality. The other side of each animal was operated in like manner 24–48 h later. The longer period resulted in less deaths, which in my best series amounted to no more than 50%, while with a period between the two operations of only 24 h, even under the best conditions of cooling, deaths were usually more than 80%. Those animals which survived the first 48 h after the completion of the double operation showed a death rate not significantly different from unoperated controls.

It must be stated that in these experiments only the massive portion of the gland was destroyed, not the strand which runs the entire length of the vas deferens. Destruction has been checked histologically in all specimens. The control animals had the hole burned in the shell over the vas deferens gland, without the destruction of the gland itself; it was apparent that this part of the operation was the prime cause of the heavy mortality, since the deaths in this control group of animals almost equalled those in the operated group.

The operations to be reported here were all performed during the month of September, a few weeks before the start of the mating and egg-laying season or as it was just beginning. In over 300 moults observed amongst large mature males during this period not a single individual was observed

to lose the male characters, while a sample of 2000 animals contained only two specimens in the inter-sexual condition. It is abundantly clear that sex reversal does not normally take place at this time of the year—all individuals seem to enter upon the mating season either as fully functional males or fully functional females. In the females vitellogenesis was well advanced and ovaries dark green. Any augmentation of the sex reversal is therefore most evident at this season.

The results of the operations in terms of loss of external male characters are presented in Table 4, and in terms of testis weight in Table 5. It is obvious that destruction of the massive portion of the vas deferens gland has led to degeneration of the testis and to loss of secondary and accessory male characters.

Examination of sections of the testes of these animals and of the sperm ducts provided additional confirmation of the degeneration of the former. The seminiferous tissue was shrunken and no sign of spermatogenesis could be seen. The sperm ducts by contrast were little altered and remained full of sperm.

A few days after the start of the above experiment a further series of eighty males was operated. Of these thirty-eight survived the operation and were used for injection experiments. Half received an intravenous injection of 0·15 ml. of distilled water 48 h after the completion of the operation and further similar injections 3 and 6 days later; these served as the controls. The other half had a similar series of injections of extract of vas deferens gland. This extract was prepared by homogenizing the fresh glands in distilled water in a Potter all-glass homogenizer. At each injection each animal received the extract of the glands from one individual. The animals were killed nine days after the first injection, 11 days after the end of the operation. The results are presented in Tables 6 and 7. The effects of the destruction of the vas deferens gland are partially but significantly countered by the injection of the extracts. Histological examination of the testes of these animals confirms that although they are somewhat degenerate with shrunken seminiferous tissue and absence of spermatogenesis, yet the shrinkage is not nearly so marked as in the uninjected controls.

DISCUSSION

The experiments reported in this paper make it plain that the hormones of the eyestalks have little or nothing to do with the loss of the male characters at the termination of the male phase in *Pandalus borealis*. On the other hand, it seems probable that this event is in large measure directly controlled by the secretions of the vas deferens gland. The gland degenerates in animals which are approaching the end of the male phase, its extirpation has resulted experimentally in the premature termination of the male phase at a time of

year when this does not normally happen, and injection of extracts of the gland have partially prevented this result of operation. It seems probable also that the similar results which followed occlusion of the male aperture may in fact be ascribed to interference with this gland. Certainly it is most likely that some hormonal influence is involved since such occlusion results also in behavioural changes.

Such a conclusion about the importance of the role of the vas deferens gland does not of course preclude the possibility, or indeed the probability, that other endocrine organs are playing a part. Allen (1959) has implicated the follicle cells of the testis, for example, while it seems *a priori* probable that the endocrine complex of the protocerebrum might be exerting an over-riding endocrinokinetic control (Carlisle & Jenkin, 1959).

Despite such considerations, however, it seems to me that the immediate cause of the termination of the male phase is the degeneration of the vas deferens gland which was described in the first paper of this series (Carlisle, 1959). Other endocrine factors may exert a modifying influence or may operate through the vas deferens gland.

I should like to acknowledge the friendly assistance afforded me in this work by the former director, Dr G. Gustafson, and the staff of K. Svenska Vetenskapakadamien Kristinebergs Zoologiska Station.

SUMMARY

In the Gullmarfjord population of *Pandalus borealis* all individuals seem to be protandric hermaphrodites; no primary females have been seen by me in the years 1956, 1957 and 1958. Bilateral destruction of the vas deferens gland in mature males is followed by degeneration of the testes and by loss of the external male characters at the next moult, so that the animal moults into the non-sexual condition characteristic of the first stage of the normal sex reversal. These two effects of destruction of the gland have been partially prevented by injection of extracts from fresh glands. The same results follow occlusion of the male openings by means of pellets of cement. It is surmised that this is a result of damage to the vas deferens gland. Such occlusion of the vasa deferentia is followed also by loss of libido. Eyestalk removal and injection of eyestalk extracts have no significant effect on the testis, vasa deferentia, or rate of loss of male characters. Prolonged starvation results in loss of weight of the sperm-filled vasa deferentia, but has no noticeable effect on testis weight or histology. It is concluded that the immediate cause of the termination of the male phase in *P. borealis* is the degeneration (and consequent cessation of endocrine activity) of the vas deferens gland which always precedes this event.

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TABLE 1

Group	Treatment	No. in group	A	B
1	Intact. Fed *ad lib.*	27	3·157 ± 0·432	7·710 ± 0·554
2	Intact. Starved	26	3·263 ± 0·391	7·419 ± 0·516
3	Both eyestalks removed. Fed *ad lib.*	23	3·315 ± 0·302	7·895 ± 0·600
4	Both eyestalks removed. Starved	17	3·240 ± 0·611	7·314 ± 0·708

All animals were killed 15 days after the start of the experiment; animals dying before the close are not included. The animals which are recorded as 'starved' obtained a little food by scavenging in the tank.

A, mean weights and standard deviations (mg) of pairs of testes; B, mean weights and standard deviations (mg) of pairs of vasa deferentia.

ANALYSES OF VARIANCE

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Testis weights				
Operation	1	0·122	0·122	N.S.
Feeding	1	0·013	0·013	N.S.
Interaction	1	0·228	0·228	N.S.
Error	89	16·292	0·183	—
Total	92	16·654	—	—
	$s = 0·428$.			
Vas deferens weights				
Operation	1	0·144	0·144	N.S.
Feeding	1	4·064	4·064	< 0·001
Interaction	1	0·172	0·172	N.S.
Error	89	26·197	0·294	—
Total	92	30·576	—	—
	$s = 0·543$.			

N.S. = not significant.

TABLE 2

Group	Treatment	No. in group	A	B
5	Intact. Injected distilled water. Fed *ad lib.*	23	3·258 ± 0·408	7·777 ± 0·532
6	Intact. Injected distilled water. Starved.	25	3·250 ± 0·439	7·431 ± 0·517
7	Intact. Injected eyestalk extract. Fed *ad lib.*	20	3·201 ± 0·397	7·938 ± 0·505
8	Intact. Injected eyestalk extract. Starved.	22	3·111 ± 0·515	7·505 ± 0·539
9	Both eyestalks removed. Injected distilled water. Fed *ad lib.*	18	3·330 ± 0·430	7·818 ± 0·590
10	Both eyestalks removed. Injected distilled water. Starved.	16	3·090 ± 0·319	7·475 ± 0·611
11	Both eyestalks removed. Injected eyestalk extract. Fed *ad lib.*	20	3·210 ± 0·592	7·793 ± 0·58
12	Both eyestalks removed. Injected eyestalk extract.	18	3·227 ± 0·577	7·390 ± 0·680
		162		

All animals were killed 13 days after the start of the experiment; animals dying before the close are not included. The animals which are recorded as starved obtained a little food by scavenging in the tank. All animals were injected at the start and again on the sixth day with 0·05 ml. either of distilled water or of a whole eyestalk extract made by grinding fresh male eyestalks with sand and distilled water and centrifuging; the extract was equivalent to 40 eyestalks/ml.

A, mean weights and standard deviations (mg) of pairs of testes; B, mean weights and standard deviations (mg) of pairs of vasa deferentia.

ANALYSES OF VARIANCE

Nature of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Testis weights				
Operation	1	0·001	0·001	N.S.
Feeding	1	0·269	0·269	N.S.
Injection	1	0·155	0·155	N.S.
Interaction-OF	1	0·007	0·007	N.S.
Interaction-FI	1	0·063	0·063	N.S.
Interaction-OI	1	0·170	0·170	N.S.
Triple interaction	1	0·255	0·255	N.S.
Error	154	32·920	0·214	—
Total	161	33·840	—	—
		$s = 0·462$.		
Vas Deferens Weights				
Operation	1	0·022	0·022	N.S.
Feeding	1	5·856	5·856	< 0·001
Injection	1	0·059	0·059	N.S.
Interaction-OF	1	0·095	0·095	N.S.
Interaction-FI	1	0·036	0·036	N.S.
Interaction-OI	1	0·304	0·304	N.S.
Triple interaction	1	0·034	0·034	N.S.
Error	154	43·119	0·280	—
Total	161	49·491	—	—
		$s = 0·529$.		

N.S. = not significant.

TABLE 3. THE LOSS OF THE EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING EYESTALK REMOVAL OR INJECTION OF EYESTALK EXTRACT

Group	Eyestalks removed	Injected	No. of moults	No. losing male characters	Proportion losing male characters
14	−	−	197	93	0·472
15	+	−	94	43	0·457
16	−	+	87	40	0·460
17	+	+	103	49	0·476
Total			481	225	0·468

$P \simeq 0{\cdot}7$.

TABLE 4. THE LOSS OF EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING DESTRUCTION OF THE VAS DEFERENS GLAND

	Operated	Controls
Retaining male characters	0	9 (11)
Losing male characters	7 (11)	0

$P < 0{\cdot}001$.

Moults 4 or more days after the completion of the operation; two earlier moults in the control group are omitted. The figures in parentheses include animals which were sufficiently advanced in proecdysis at the end of the experiment for the new appendages to be dissected free of the old integument.

TABLE 5. TESTIS WEIGHT 12 DAYS AFTER VAS DEFERENS GLAND DESTRUCTION

	No. surviving	Testis weight (mg) and standard deviation
Gland destroyed	16	$2{\cdot}110 \pm 0{\cdot}225$
Controls	20	$3{\cdot}725 \pm 0{\cdot}371$

$P < 0{\cdot}001$.

TABLE 6. THE LOSS OF EXTERNAL MALE CHARACTERS AT THE MOULT FOLLOWING DESTRUCTION OF THE VAS DEFERENS AND INJECTIONS OF EXTRACTS OF THE GLAND

	Injected	Controls
Retaining male characters	0	4 (5)
Losing male characters	6 (7)	3

$P = 0{\cdot}049$, $P' = 0{\cdot}022$.

Moults 4 or more days after the completion of the operation. The figures in parentheses include animals which were sufficiently advanced in proecdysis at the end of the experiment for the new appendages to be dissected free of the old integument. P' is the probability including these animals, P that excluding them, utilizing only the actual moult figures.

TABLE 7. TESTIS WEIGHT 11 DAYS AFTER VAS DEFERENS GLAND DESTRUCTION FOLLOWED BY INJECTION OF EXTRACTS OF THE GLAND

	No. surviving	Testis weight (mg) and standard deviation
Injected	14	$2{\cdot}795 \pm 0{\cdot}230$
Controls	15	$2{\cdot}011 \pm 0{\cdot}222$

$P < 0{\cdot}001$.

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ON THE SEXUAL BIOLOGY OF *PANDALUS BOREALIS* (CRUSTACEA DECAPODA)

III. THE INITIATION OF THE FEMALE PHASE

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(Text-figs. 1–2)

In the second paper of this series (Carlisle, 1959*b*) I have described the results of various experimental manipulations upon the termination of the male phase in the protandric hermaphrodite *Pandalus borealis* Krøyer. In this paper I shall be concerned with the effects of such interference upon the initiation of the female phase of the life history.

In a number of papers Panouse (1943–8) provided evidence, based upon extirpation experiments, that the sinus gland of the eyestalks of the common European prawn *Palaemon* (= *Leander*) *serratus* secretes a hormone which inhibits the development of the ovary and in particular vitellogenesis. His results were confirmed by Takewaki & Yamamoto (1950*a*, *b*), who worked upon the Japanese prawn *Paratya*. Since the above work was completed much evidence has accumulated that the sinus gland is not in itself an endocrine organ, but a neurohaemal organ (Carlisle & Knowles, 1953); that is to say it is the terminal organ of a neurosecretory system, whose main function is to release into the blood hormones produced elsewhere, within neurosecretory cells. It is generally (but not universally) accepted that the hormones stored in the sinus gland, and released into the blood there, are produced mainly within the neurosecretory cells of the ganglionic X organs (see discussion in Carlisle & Knowles, 1959). In agreement with this view I have found that, both in *Palaemon* (Carlisle, unpublished) and in the Mediterranean prawn *Lysmata seticaudata* (Carlisle, 1953*a*, *b*), it is possible to extract an ovary-inhibiting substance from the sinus gland and from the ganglionic X organ which lies in the medulla terminalis of the brain (see Carlisle, 1953*c*). This substance, after intravenous injection, produces involution of the developing ovary in the intact animal, or effectively counteracts the result of eyestalk ablation, so far as ovarian growth is concerned. Extirpation of the sinus glands alone leads to an increase in ovarian weight significantly less than does total eyestalk ablation—as indeed Panouse also found. Ablation of the ganglionic X organ of the medulla terminalis leads to a delayed onset of increase in ovarian size, but once this increase has begun it proceeds as rapidly as after total eyestalk ablation (Carlisle, unpublished). We may

suppose that the delay is a result of the hormone stored within the sinus gland: once this has been utilized, then ovarian increase can begin. The sinus gland seems nevertheless to be necessary for the efficient release of the hormone, for its removal does lead to some increase in ovarian size, until a new sinus gland is regenerated from the stump of the X organ-sinus gland neurosecretory tract.

The evidence seems satisfactory that ovarian growth in these two species of prawns is inhibited by a hormone secreted by the X organ-sinus gland complex. This complex in *Pandalus* has been described in the first part of this series (Carlisle, 1959*a*). Unpublished data suggest that in *Palaemon*, during the juvenile phase, the ovary is inhibited by the action of this hormone; before the onset of maturity the secretion of the hormone ceases and the ovary begins to develop; ovulation succeeds and once more the ovary is inhibited by the hormone until the breeding season of the following year. There is thus an annual cycle in the secretion of the hormone once the animal is mature. Arvy, Echalier & Gabe (1954) have provided evidence that the immature gonad may be influenced by a secretion of the Y organ in the crab *Carcinus*, but this has not yet been confirmed in *Palaemon*, nor indeed in any natantian, nor do these authors suggest that this organ has any influence on the gonad after sexual maturity.

Whereas most of the Decapoda are normal bisexual creatures, three isolated genera have been found to show a functional sex reversal, at least in some of their species. The blind burrowing decapod *Calocaris macandreae* is known to show this phenomenon (Runnström, 1925; Balss, 1930); *Lysmata seticaudata* and *L. nilita* apparently show full obligatory protandric hermaphroditism, so that every male which survives long enough reverses sex to become a female, while there are no primary females in the population (Spitschakoff, 1912; Caroli, 1917; Dohrn, 1950; Dohrn & Holthuis, 1950; Carlisle & Dohrn, 1952, 1953; the terminology is that proposed by Carlisle, 1959*c*); certain species of the genus *Pandalus*, notably the deep-sea arctic prawn *P. borealis*, the subject of the present communication, *P. montagui* and *P. kessleri* are also protandric hermaphrodites (Berkeley, 1930; Jägersten, 1936; Rasmussen, 1953; Aoto, 1952) though according to Pike (1952) *P. bonnieri* is dioecious. In *P. montagui* and in *P. borealis* most populations show a variable percentage of primary females so that the hermaphroditism is partial but obligatory (Mistakidis, 1957; Allen, 1959). In some populations, however, including that upon which I have worked in the Gullmarfjord, Sweden, the hermaphroditism is full and there are no primary females in the population (Carlisle, 1959*b*). The analysis of the sex reversal is of course easier in such a population.

I have shown (Carlisle, 1953*a*, 1954) that the assumption of functional female form in *Lysmata seticaudata* is inhibited by a hormone of the X organ-sinus gland complex. I then suggested that it seemed probable that the

hormone responsible for this inhibition might well be the ovary-inhibiting hormone. The evidence that I am about to present suggests that in *Pandalus borealis* also, the ovary is inhibited by a hormone of the X organ-sinus gland complex, that the assumption of the female form is also inhibited by a hormone of this complex, and that there is a high degree of correlation between the two actions.

EXPERIMENTAL DATA

Change of the apparent sex of the external accessory and secondary sexual characters can only take place in a crustacean when the animal moults, shedding the old shell and growing a new one of different dimensions and with differing proportions. In a prawn possessing both functional ovarian and functional testicular tissue the functional sex is that corresponding to the external characters, for even if the testis is producing sperm, the prawn cannot mate as a male if it lacks the copulatory appendages and if the vasa deferentia terminate blindly with no opening to the outside; nor can it mate as a female, though possessing a functional ovary, if it lacks the female apertures and the ovigerous hairs. A prawn which has not moulted during the period of observation has had no chance to modify its sexual condition. In all experiments reported here, therefore, only individuals which had moulted during the experimental period are included in the data. All animals which did not moult before the end of the experiment were disregarded.

OVARIAN INHIBITION

Effects of the X organ-sinus gland complex

Eight groups of animals were selected, four of females and four of large males. Two groups of each sex were left intact and two groups had the eyestalks extirpated. One group from each division received a single injection of 4 sinus gland equivalents of an extract prepared from the sinus glands of females. Table 1 summarizes the treatments accorded to the different groups together with the mean ovarian weight in each group after a period of ten days had elapsed. Table 2 supplies a summary of the analysis of variance of these data. It will be seen that the operation of eyestalk removal is followed by a very significant increase in ovarian weight, while the injection of the extract is followed by the reverse. In the females, when both the operation and the injection are performed upon the same group of animals (group 4), it is the operation which has the greater effect—the injection is evidently inadequate to counteract the effect of the operation. In the smaller males, however, which received the same dose of the extract as the larger females and hence a larger dose per unit body weight, the effects of the injection and of the operation have effectively cancelled each other out, so that the mean ovarian weight in group 8 is the same as in group 5.

It is to be noted in the table of analysis of variance (Table 2) that there is a highly significant entry for 'interactions'. This is not unexpected for of course the males and females are in different physiological states, and it is not to be expected that either the injection or the operation will have exactly the same degree of action on the ovarian weight in the two sexes. Moreover, since all individuals, whatever their size, received the same dose of extract,

TABLE 1. OVARIAN INHIBITION

Group	No. in group	Sex	Operated	Injected	Mean ovarian weight (mg)	Standard deviation	Standard error of mean
1	15	♀	−	−	34·79	9·58	2·56
2	11	♀	−	+	23·63	8·22	2·60
3	14	♀	+	−	123·71	30·33	8·41
4	10	♀	+	+	42·51	4·41	1·47
5	24	♂	−	−	19·58	6·95	1·45
6	25	♂	−	+	16·45	7·94	1·62
7	19	♂	+	−	27·86	8·49	2·00
8	27	♂	+	+	19·44	6·99	1·37

TABLE 2. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 1 (OVARIAN INHIBITION)

Source of variation	Degrees of freedom	Sum of squares	Probability
Sex	1	48,313	< 0·001
Operation	1	20,346	< 0·001
Injection	1	19,136	< 0·001
Interactions	4	45,918	< 0·001
Error	137	20,626	—
Total	144	154,339	—

$s^2 = 150{\cdot}59$. $s = 12{\cdot}25$.

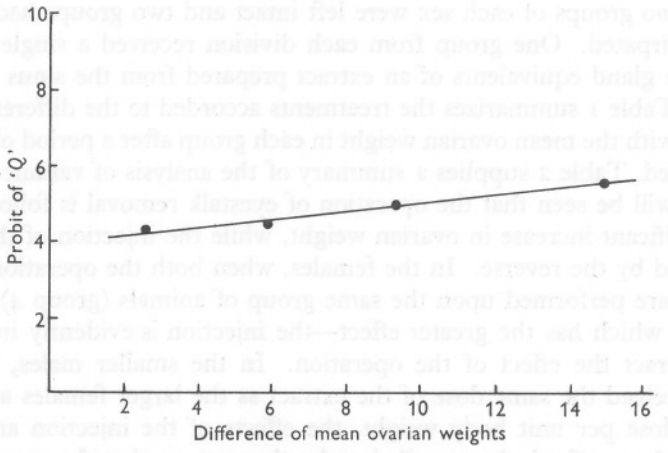

Fig. 1. The correlation in response to various treatments of the ovarian weight, with proportion becoming female.

whereas the operation would have, presumably, a uniform effect per individual, it might be expected that these two factors would interact. In fact, if the analysis of variance is carried further to separate the various possible interactions orthogonally, each of them is found to be significant. This, however, is irrelevant to the main argument. There is no doubt that eyestalk ablation leads to a rapid increase in ovarian weight and that injection of sinus gland extract prevents this wholly or in part, while in the intact animal such an injection is followed by involution of the ovaries. The case is clear for the hormonal inhibition of ovarian development by the X organ-sinus gland complex.

Effects of other organs and tissues

Complete selective extirpation of other tissues has not been attempted in this series of experiments. Removal of the eyestalk necessarily involves the ablation of other organs besides the sinus glands and the ganglionic X organs. Attempts at replacement therapy with tissues of the eyestalk other than these had no effect. In particular injections of extracts of the sensory pore X organ (*SPX*), of retroretinal tissue (*RT*) or of the medulla externa (*ME*) had no effect on ovarian weight in either males or females, intact or lacking eyestalks. Injections of extracts of the medulla terminalis ganglionic X organ (*MTGX*), however, acted like sinus gland extract injections. The data are summarized in Tables 3 and 4. The experiment was carried out in like manner with like dosages and times as in the first experiment reported above. In each group only the first twelve animals caught at the end of experiment were dissected and find inclusion in these tables. The remainder were discarded. It is abundantly clear that injection of extracts of the *SPX*, *ME* and *RT* are without any effect on ovarian weight in any of the conditions of the experiment.

The vas deferens gland

This gland, which has been shown to be necessary for maintaining the male phase (Carlisle, 1959*b*), is absent from the later non-sexual stages and from the female. Injection of extracts of the gland in dosages which had a profound effect upon the male (Carlisle, 1959*b*) had no effect upon oocytes that were undergoing vitellogenesis, or upon ovulation. Ovaries which were sectioned ten days after a single injection of vas deferens gland extract, however, showed the presence of cells which appeared to have the character of spermatogonia, while these were more clearly defined in animals which had had two such injections. Much more work is required upon this point, especially in view of the results of Charniaux-Cotton (1954) with *Orchestia*, and I will refrain from further comment until I have had the opportunity to experiment once again upon the vas deferens gland of *Pandalus*.

TABLE 3. OVARIAN INHIBITION

Group	No. in group	Sex	Operated	Injected *MTGX*	Injected *SPX*	Injected *ME*	Injected *RT*	Mean ovarian weight (mg)	Standard deviation
9	12	♀	−	−	−	−	−	35·01	8·4
10	12	♀	−	−	−	−	+	35·27	8·93
11	12	♀	−	−	−	+	−	34·20	9·01
12	12	♀	−	−	−	+	+	34·35	8·70
13	12	♀	−	−	+	−	−	36·07	8·25
14	12	♀	−	−	+	−	+	35·05	8·26
15	12	♀	−	−	+	+	−	34·76	8·78
16	12	♀	−	−	+	+	+	34·60	8·99
17	12	♀	−	+	−	−	−	23·14	8·21
18	12	♀	−	+	−	−	+	23·77	8·20
19	12	♀	−	+	−	+	−	23·09	7·95
20	12	♀	−	+	−	+	+	22·91	8·12
21	12	♀	−	+	+	−	−	25·05	8·88
22	12	♀	−	+	+	−	+	24·73	8·70
23	12	♀	−	+	+	+	−	23·68	8·76
24	12	♀	−	+	+	+	+	23·50	8·90
25	12	♀	+	−	−	−	−	120·01	30·24
26	12	♀	+	−	−	−	+	110·37	33·76
27	12	♀	+	−	−	+	−	130·25	31·09
28	12	♀	+	−	−	+	+	104·38	33·02
29	12	♀	+	−	+	−	−	114·46	27·89
30	12	♀	+	−	+	−	+	137·50	26·66
31	12	♀	+	−	+	+	−	90·92	27·84
32	12	♀	+	−	+	+	+	127·76	35·21
33	12	♀	+	+	−	−	−	44·26	7·22
34	12	♀	+	+	−	−	+	42·57	7·20
35	12	♀	+	+	−	+	−	39·20	7·91
36	12	♀	+	+	−	+	+	40·11	6·93
37	12	♀	+	+	+	−	−	39·34	7·29
38	12	♀	+	+	+	−	+	40·31	7·28
39	12	♀	+	+	+	+	−	42·41	6·91
40	12	♀	+	+	+	+	+	42·00	7·35
41	12	♂	−	−	−	−	−	18·75	7·04
42	12	♂	−	−	−	−	+	18·85	6·82
43	12	♂	−	−	−	+	−	19·34	6·64
44	12	♂	−	−	−	+	+	19·39	7·32
45	12	♂	−	−	+	−	−	19·42	7·49
46	12	♂	−	−	+	−	+	19·04	7·34
47	12	♂	−	−	+	+	−	19·58	7·14
48	12	♂	−	−	+	+	+	18·63	7·06
49	12	♂	−	+	−	−	−	15·82	7·03
50	12	♂	−	+	−	−	+	16·13	7·09
51	12	♂	−	+	−	+	−	16·60	6·88
52	12	♂	−	+	−	+	+	16·55	7·41
53	12	♂	−	+	+	−	−	15·90	6·97
54	12	♂	−	+	+	−	+	16·03	7·32
55	12	♂	−	+	+	+	−	16·07	7·58
56	12	♂	−	+	+	+	+	16·51	6·99
57	12	♂	+	−	−	−	−	27·83	7·95
58	12	♂	+	−	−	−	+	25·97	8·47
59	12	♂	+	−	−	+	−	25·96	8·99
60	12	♂	+	−	−	+	+	26·34	7·63
61	12	♂	+	−	+	−	−	26·51	8·60
62	12	♂	+	−	+	−	+	26·70	7·64
63	12	♂	+	−	+	+	−	26·03	7·70
64	12	♂	+	−	+	+	+	27·58	8·94
65	12	♂	+	+	−	−	−	19·91	7·00
66	12	♂	+	+	−	−	+	18·87	7·32
67	12	♂	+	+	−	+	−	18·92	7·56
68	12	♂	+	+	−	+	+	19·73	8·39
69	12	♂	+	+	+	−	−	19·21	7·34
70	12	♂	+	+	+	−	+	19·87	7·43
71	12	♂	+	+	+	+	−	20·03	8·09
72	12	♂	+	+	+	+	+	20·11	8·81

TABLE 4. ANALYSIS OF VARIANCE OF THE DATA OF TABLE 3

Source of variation	Degrees of freedom	Sum of squares	Mean square	Probability
Sex	1	219,882·8	219,883	$\ll$ 0·001
Operation	1	146,440·1	146,440	$\ll$ 0·001
MTGX	1	112,447·7	112,448	$\ll$ 0·001
Interactions of Sex, op., *MTGX*	4	265,671·1	66,418	$\ll$ 0·001
Other injections	3	372·8	124·3	N.S.
Other interactions	53	17,798·2	335·8	*c.* 0·01
Error	704	121,591·9	172·7	
Total	767	885,701·8		

s = 13·1. N.S., not significant.

ATTAINMENT OF THE FUNCTIONAL FEMALE STATE

Effects of the X organ-sinus gland complex

To determine the effects of the X organ-sinus gland complex upon the attainment of the functional female state animals in the intersexual condition were chosen. The individuals so chosen were allocated to groups at random. The animals were treated like those of groups 5–8 in the first experiment reported above, but three types of extract were used:

(1) Extract of the sinus gland of females, which at this season of the year (April) might be presumed to be moderately active in secreting ovary-inhibiting hormones;

(2) Extract of the sinus glands of young males, which might be presumed to be fully active in the secretion of the ovary-inhibiting hormone;

(3) Extract of the sinus gland of old males and non-sexual individuals, which might be presumed to be relatively inactive in the ovary-inhibiting hormone.

The animals in the first experiment received an injection of type 1 and the data from groups 5–8 of this experiment will be considered here also. The treatments accorded to the various groups are summarized in Table 5, together with the numbers which did and which did not attain a functional female condition at the succeeding moult. Table 6 summarizes the statistical analysis of these data. It will be seen that eyestalk removal has led to a very significantly higher proportion of attainment than in the unoperated animals, while injection of sinus gland extracts has had the opposite effect. The most effective type of extract was type 2 and the least effective type 3 which had an insignificant effect. Perusal of Table 5 will show that an injection of type 1 or type 2 effectively counteracted the effects of the operation, affording adequate replacement therapy in this respect; statistical analysis confirms this impression. It is evident that the attainment of a functional female state from the non-sexual condition is inhibited by some hormonal emanation from the sinus gland.

TABLE 5. ATTAINMENT OF FEMALE STATE: TREATMENTS AND RESULTS IN THE VARIOUS GROUPS

Group	No. in group	Operated	Injected (type 1)	Injected (type 2)	Injected (type 3)	Number becoming female	Number remaining non-sexual
73	24	−	−	−	−	12	12
74	25	−	+	−	−	9	16
75	19	+	−	−	−	16	3
76	27	+	+	−	−	14	13
77	28	−	−	−	−	15	13
78	26	−	+	−	−	9	17
79	23	+	−	−	−	18	5
80	28	+	+	−	−	13	15
81	30	−	−	−	−	14	16
82	23	−	−	+	−	5	18
83	22	+	−	−	−	17	5
84	31	+	−	+	−	12	19
85	29	−	−	−	−	15	14
86	31	−	−	−	+	15	16
87	31	+	−	−	−	24	7
88	30	+	−	−	+	23	7

Effects of other organs and tissues

Once more no attempt has been made at complete selective extirpation of other organs. An experiment similar to that summarized in Tables 3 and 4, but utilizing non-sexual animals, made it plain that injection of extracts of the *MTGX* acted in precisely the same way as extracts of sinus glands upon the attainment of the functional female condition, while injection of extracts of the *SPX*, *ME* and *RT* had no significant effect. The results of this experiment will not be reported in full.

I could find no evidence that injection of vas deferens gland extract affected the attainment of the functional female state, but this requires further investigation.

THE CORRELATION BETWEEN OVARIAN GROWTH AND ATTAINMENT OF THE FUNCTIONAL FEMALE STATE

As a measure of the effectiveness of the various treatments in interfering with the normal rate of attainment of the female state the coefficient of association (Q) was calculated (Yule, 1900; Yule & Kendall, 1945). The values of Q are listed in Table 6 and repeated in Table 7. The differences in mean ovarian weight brought about in the same individuals by the same treatments were calculated, as a measure of the effectiveness of these treatments in interfering with ovarian weight; these values are also listed in Table 7. If the phenomena of hormonal inhibition of attainment of the female status and of hormonal inhibition of ovarian growth are not interconnected these values should show no significant regression one upon another, whereas if they are connected there should be a significant regression.

Now Q is a coefficient which has the limits $+1$ and -1, and the graph of a function which is constrained to lie between limits is of necessity sigmoid, hence some linearizing transformation is needed if we are to calculate a linear regression of Q upon the difference in mean ovarian weights. According to Finney (1952) there is little to choose between the various linearizing transformations of sigmoid curves especially over the middle of the range, when we do not know on *a priori* grounds which sigmoid to expect. It is possible that the logit transformation might give a closer fit, but for ease of computation the probit transformation was chosen. The expected probits corresponding

TABLE 6. ATTAINMENT OF FEMALE STATE: χ^2 AND COEFFICIENTS OF ASSOCIATION (Q) BETWEEN ATTAINMENT AND DIFFERENT TREATMENTS

Treatment	$\chi^2_{[1]}$	Probability	'Q'	Standard error of 'Q'
Operation	19·112	< 0·001	+0·412	0·083
Injection (type 1)	10·718	= 0·001	−0·430	0·120
Injection (type 2)	8·462	< 0·01	−0·525	0·155
Injection (type 3)	0·96	—	−0·058	0·377

Second order comparisons:

Injection (type 1): Injection (type 2) 1·808 = 0·15.
Injection (type 1): Injection (type 3) 6·098 = 0·02.
Injection (type 2): Injection (type 3) 9·672 < 0·01.

TABLE 7. CORRELATION BETWEEN EFFECT ON OVARIAN WEIGHT AND EFFECT ON ATTAINMENT OF FEMALE STATE

Source of variation	No. of animals	Difference of mean ovarian weights (x)	'Q' (from Table 6)	$p = \frac{Q+1}{2}$	Expected probit (y)
Injection (type 1)	200	4·9367	−0·430	0·2853	4·432
Injection (type 2)	106	2·5979	−0·525	0·2375	4·287
Injection (type 3)	121	9·3511	−0·058	0·4708	4·927
Operation	427	14·9577	+0·412	0·7060	5·542

After appropriate weighting of the data the regression equation is $y = 3{\cdot}95 + 0{\cdot}106x$ with variance of gradient 0·000 084 67, so that gradient = $0{\cdot}106 \pm 0{\cdot}0092$; hence $t_{[400]} = 11{\cdot}538$ and $P < 0{\cdot}001$. Columns 3 and 6 and the regression equation are illustrated graphically in Fig. 1.

to each value of Q are listed in Table 7. A regression equation of these expected probits (y), suitably weighted, upon the difference in mean ovarian weights (x), was then computed by the usual probit method (Finney, 1952). After appropriate weighting the equation obtained was

$$y = 3{\cdot}95 + 0{\cdot}106x.$$

The regression coefficient was $0{\cdot}106 \pm 0{\cdot}0092$, whence $t_{[400]} = 11{\cdot}583$ and $P < 0{\cdot}001$. The equation and the four points upon which it is based are illustrated graphically in Fig. 1. It will be seen that the regression of the probit of Q upon difference in mean ovarian weight is highly significant; that

is to say there is an extremely close correlation between the effects of the various treatments upon sex reversal and the effects of these same treatments upon ovarian growth.

OVARIAN SIZE AT THE MOMENT OF ATTAINMENT OF A FUNCTIONAL FEMALE CONDITION

In all groups it was found that those animals, regardless of body weight, which had the largest ovaries had become females, while those with the smallest ovaries had remained in the non-sexual condition; there was a very narrow zone of overlap, i.e. the animal with the smallest ovary of those which had become females, had, in some groups, an ovary just a little smaller than the largest of those which remained non-sexual. Fig. 2 illustrates the range

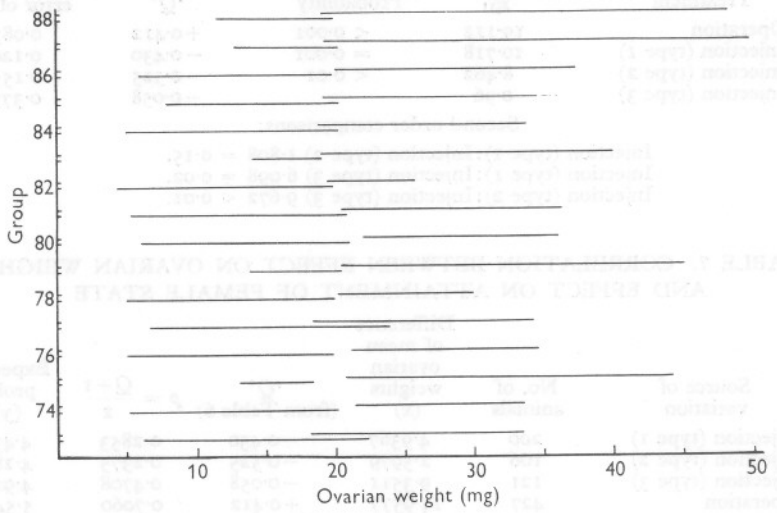

Fig. 2. The range of ovarian weights in animals of groups 73–88 (see Table 5). The lower line of each pair represents the range of weights found in those animals which remained non-sexual after the moult following experimental interference; the upper line the range in those which became functional females.

of ovarian weights in the various groups of animals referred to in the previous sections. Statistical analysis confirms visual impressions that there is a very strong correlation between a large ovary and attainment of the female condition. The correlation coefficient is 0·99. Inspection of the graph also suggests that there is little variation in the ovarian weight at the boundary between those which had and those which had not become females. In all groups the largest ovary of a prawn which had remained non-sexual and the smallest ovary of a prawn which had become female were approximately 20 mg. Once more statistical analysis confirms this impression. Curve-fitting techniques applied to this whole assembly of ovarian weights and to the individual

groups reveal insignificant departures from expectation with a $\chi^2_{[15]}$ of 11·85, whence $P = 0{\cdot}7$. In other words the ovarian weight at the moment of attainment of a functional female state was not modified by any of the treatments which I had applied.

DISCUSSION

These experiments make it evident that ovarian growth and maturation in *Pandalus borealis*, as in *Palaemon*, *Lysmata*, *Carcinus* and other species of decapods, is regulated, at least in part, by an ovary-inhibiting hormone produced by the X organ-sinus gland complex. This hormone is found in the medulla terminalis ganglionic X organ and in the sinus gland, but has not been found in the sensory pore X organ. It seems probable, therefore, that it is produced in the *MTGX*, stored in the sinus gland and released into the blood from there. No other tissue in the eyestalk which has been investigated had any effect upon ovarian growth or maturation. This is to be contrasted with the conclusions of Aoto & Nishida (1956) who, working with *Pandalus kessleri*, likewise found that eyestalk removal led to premature ovarian growth (at least during early summer). On histological grounds, however, they concluded that an ovary-inhibiting hormone was produced by the X organ (by which term they appear to mean the sensory pore X organ), while an antagonistic hormone was produced by the 'circum-orbital gland'. In *P. borealis* I have been able to find no trace of any hormone affecting gonadal or sexual development in the sensory pore X organ. Certainly there is a seasonal cycle in the histological appearance of this organ (Carlisle, 1959*a*), and since breeding is also seasonal, these two cycles must perforce be correlated. This is not, however, to be taken as a causal relationship without any other supporting evidence, and a diligent search has failed to produce any such evidence. We must conclude then that the seasonal changes in the *SPX* do not correspond to seasonal secretion of an ovary-inhibiting hormone, but rather are an expression of some other seasonal secretion, or annual cycle of metabolism. The same arguments apply also to the retroretinal tissue, which likewise undergoes a seasonal cycle in histological appearance. Here too I have failed to find any evidence of the secretion of hormones regulating any aspect of the sexual cycle. I believe that this tissue is probably homologous with that described as the circum-orbital gland by Aoto & Nishida. I have been able to find no evidence for any eyestalk hormone controlling any aspect of the sexual cycle of *P. borealis*, except the ovary-inhibiting hormone secreted by the neurosecretory cells of the *MTGX* and released into the blood by the neurohaemal endings of the sinus gland.

Similarly I could find no evidence that any hormone except one emanating from the *MTGX*-sinus gland complex, played any part in governing the change from the non-sexual to the female phase. Extracts of the *SPX*, of the

retroretinal tissue or of the medulla externa were without effect, while eyestalk removal increased the proportion which became females at the next moult, an effect which was countered by the injection of extracts of sinus gland or of *MTGX*. We must conclude that some hormone produced in the *MTGX* and released into the blood at the sinus gland is responsible for restraining the attainment of the functional female condition. It is possible, but unlikely, that the vas deferens gland plays some part in this; the point requires further investigation. I have no evidence as to any part played by the Y organ.

The high degree of correlation found between the effects of the various treatments upon the attainment of the female form and upon ovarian inhibition makes it seem probable that but one hormone is concerned in inhibiting premature ovarian growth and in restraining the change from the non-sexual to the female condition. At the moment of this change the ovary seems always to have reached a certain minimum size. This seems unlikely to be a causal relationship but rather an expression of the same agent acting upon both the change of status and upon ovarian development.

I should like to acknowledge the friendly assistance afforded to me by the former director, Dr G. Gustafson, of the K. Svenska Vetenskapsakadamien Kristinebergs Zoologiska Station, and to thank him for all he has done to make three visits to the laboratory pleasurable as well as profitable.

SUMMARY

Ovarian growth and vitellogenesis are inhibited by the ovary-inhibiting hormone secreted by the medulla terminalis ganglionic X organ and the sinus gland. No other tissue within the eyestalk has been found to have any action upon ovarian growth. Similarly the change from the non-sexual condition to the functional female state is inhibited by a hormone emanating from the same centres, while no other tissue within the eyestalk has been found to have any effect. The two inhibitions show a high degree of correlation which makes it probable that they represent two responses to the same hormone.

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ON THE BIOLOGY OF THE OPISTHOBRANCH *PLEUROBRANCHUS MEMBRANACEUS*

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(Plate I and Text-figs. 1–8)

On 5 November 1958 our attention was drawn, by Mr A. K. Nagabhushanam, to the presence in Port Erin bay of large numbers of swimming gastropods. These gastropods proved on examination to be *Pleurobranchus membranaceus* (Montagu), a species generally believed to be exclusively benthic in habit. This paper is a report on this occurrence and on some aspects of the biology of *P. membranaceus*.

For 4 days these animals were abundant in the bay and hundreds could have been captured with a hand-net from the steps of the Raglan Pier. Only approximately forty specimens were taken, this number being ample for our purpose. Between 5 and 9 November, numbers were stranded on the beach by the receding tide. Although many local fishermen consented to look out for these easily recognizable animals, and the Port Erin research vessels were out each day, no record of their swimming outside the bay was obtained, although specimens were occasionally dredged. On 9 November they disappeared from the bay without trace, and otter trawl and D-net hauls in the bay yielded only two further specimens; none were found on frequent shore-collecting expeditions. However, on 2 December five more specimens were captured swimming near the Raglan Pier and occasional individuals were seen in the same place on 9 December. During the periods of their abundance it was ascertained that they swam during the hours of darkness as well as in daytime.

Hydrographic conditions were not unusual during this invasion; temperatures taken in Port Erin bay during November and December 1958 were only about 1° C above the grand monthly means (see Bowden, 1955), while the salinities of samples collected at a position 54° 05′ N., 4° 50′ W. (about 3 miles N. 65° W. of Port Erin and 3 miles N. 12° E. of Chicken Rock) were about 0·3‰ below the mean for the area.

A search of the literature brought to light only three references to swimming in *P. membranaceus*. Pruvot-Fol (1954) states that this species (referred to as *Oscanius tuberculatus*, pp. 220–2) 'nage avec des contorsions du pied', but gives no details. Garstang (1890, pp. 418–20) quotes observations made by A. R. Hunt on an invasion of Torbay by numbers of *P. membranaceus* in

December 1873 and January 1874; Hunt observed that the animal swims on its back, 'alternately flapping, with wave-like contractions from before backwards, the two halves of its broad foot. The mantle flaps assist also in the action.' Garstang (1890) also mentions that these animals can secrete a substance which will redden blue litmus and he states (without giving reasons) that this substance is sulphuric acid. Garstang (1892) mentions an invasion of Plymouth Sound in September 1892 by young *P. membranaceus*; numbers were seen on the night of 21 September swimming at the surface of the sea. Hartley (1940) states that Saltash was invaded by large numbers of pleurobranchids in October 1936, but he does not say whether these animals were swimming. Except that Yonge (1949, p. 252) mentions that *P. membranaceus* feeds on simple ascidians (also inferred by Hunt (1925)), this is all that is known of the natural history of the species.

Nomenclature of British animals referred to herein is according to the Plymouth Marine Fauna list (Marine Biological Association, 1957); in the case of foreign species, the name used by the author cited is employed.

Material for sectioning was fixed in Zenker's fluid (with or without acetic acid), cleared in amyl acetate (Barron, 1934) and embedded in Hance's rubber wax (Gurr). The stains employed were Heidenhain's alum haematoxylin, Mayer's haemalum and, as counterstains, eosin and alcian blue 8GS (Steedman, 1950). Methods used in the investigation of the nature of the acid secretion will be described in context.

DESCRIPTION OF THE SPECIMENS

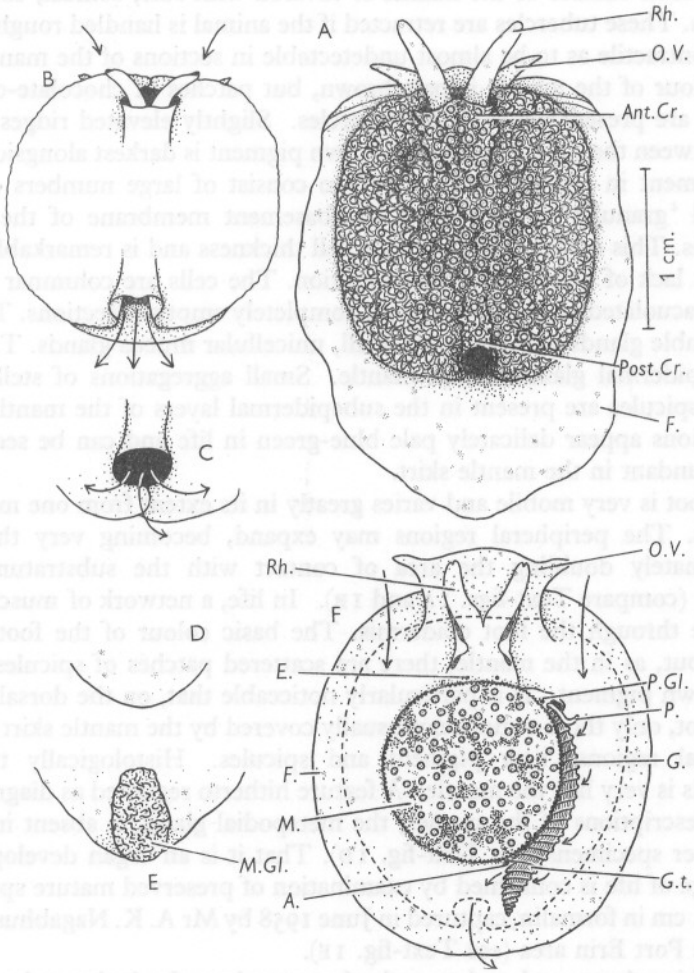
The external features and parts of the internal anatomy are shown in Text-figs. 1–6. Only those features which are not immediately apparent from these illustrations will be described. The description in the first instance will apply to animals which are creeping; the changes in form when swimming will be dealt with later.

External features

The lengths of the November specimens varied between $1\frac{1}{2}$ and $3\frac{1}{2}$ cm, measured while they were extended in creeping. Individuals of up to $4\frac{1}{2}$ cm in length were found in December. According to Pruvot-Fol (1954) adults may attain a length of 6 cm. In aquaria in the laboratory they spent by far the greater part of the time creeping and usually would swim only if disturbed.

Text-fig. 1A shows a specimen in the act of creeping on the bottom of a glass vessel in the typical gastropodan fashion. Noteworthy features are the anterior and posterior crenations in the mantle, the former giving rise to a wide temporary sheath for the rhinophoreal tentacles (Text-fig. 1A, *Ant.Cr.*), while the latter forms an exhalent aperture for the respiratory current (Text-fig. 1A, *Post.Cr.*). In some individuals the posterior edges of the mantle

skirt may temporarily meet below this exhalent aperture, rendering it functionally a circular orifice (Text-fig. 1 C). When the animal is at rest (Text-fig. 1 B), with the foot contracted so that it does not project laterally



Text-fig. 1. External features of *P. membranaceus*, all drawings to the same scale: A, dorsal aspect of November specimen while creeping; B, dorsal aspect of November specimen while at rest; C, as for B, but showing alternative condition of posterior crenation; D, ventral aspect of tip of metapodium of November specimen; E, ventral aspect of tip of metapodium of mature specimen of June, 1958; F, dorsal aspect of November specimen with mantle skirt dissected away; the interrupted line represents the normal extent of the mantle skirt; the cut edge of the mantle is represented by a jagged line. The arrows in all cases show the direction in which ciliary currents impelled fine carmine particles. *A.*, anus; *Ant.Cr.*, anterior mantle crenation; *E.*, eye; *F.*, foot; *G.*, gill; *G.t.*, free tip of gill; *M.*, mantle; *M.Gl.*, metapodial gland; *O.V.*, oral veil; *P.*, penis; *Post.Cr.*, posterior mantle crenation; *P.Gl.*, prebranchial gland opening; *Rh.*, rhinophore.

beyond the mantle skirt, this posterior crenation is relatively much wider than in the active animal. It is usually undetectable after death, which in part explains why it has not been described hitherto.

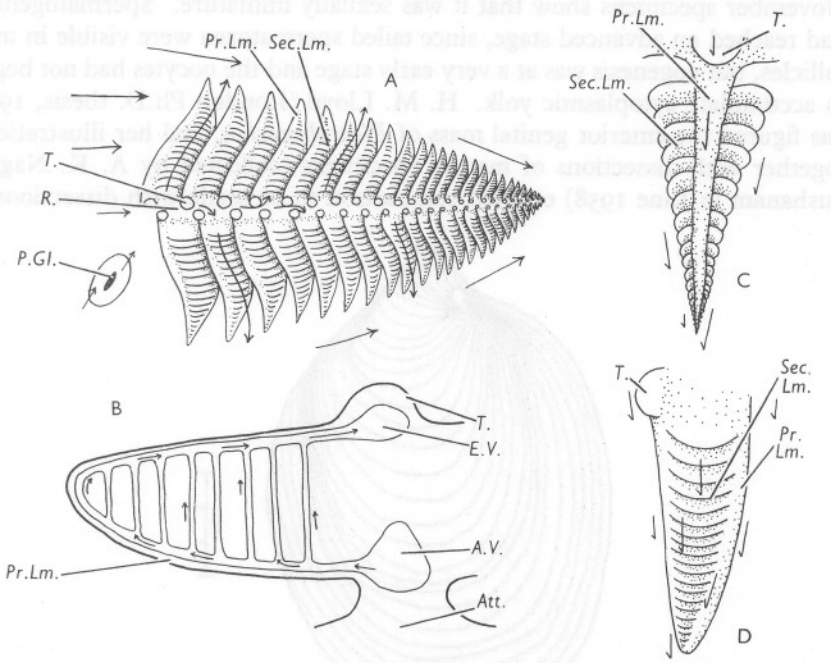
The dorsal surface of the mantle is covered with soft, conical, contractile tubercles. These tubercles are retracted if the animal is handled roughly; they are so contractile as to be almost undetectable in sections of the mantle. The basic colour of the mantle is pale brown, but patches of chocolate-coloured pigment are present between the tubercles. Slightly elevated ridges anastomose between the tubercles and the brown pigment is darkest alongside these. The pigment in sections can be seen to consist of large numbers of small spherical 'granules' lying against the basement membrane of the mantle epidermis. This epidermis is of single cell thickness and is remarkable for its apparent lack of histological differentiation. The cells are columnar and are greatly vacuolated so that they appear completely empty in sections. The only recognizable glands are scattered, small, unicellular mucus glands. There are no subepidermal glands in the mantle. Small aggregations of stellate calcareous spicules are present in the subepidermal layers of the mantle; these aggregations appear delicately pale blue-green in life and can be seen to be most abundant in the mantle skirt.

The foot is very mobile and varies greatly in its extent from one minute to the next. The peripheral regions may expand, becoming very thin, and approximately doubling the area of contact with the substratum when required (compare Text-figs. 1A and 1B). In life, a network of muscle fibres is visible through the foot epidermis. The basic colour of the foot is pale brown, but, as in the mantle, there are scattered patches of spicules and of dark brown pigment. It is particularly noticeable that, on the dorsal surface of the foot, only those regions not usually covered by the mantle skirt (i.e. the peripheral regions) bear pigment and spicules. Histologically the foot epidermis is very like the mantle. A feature hitherto regarded as diagnostic in earlier descriptions of the species, the metapodial gland, is absent in all the November specimens (see Text-fig. 1D). That it is an organ developed at a later stage of life is confirmed by examination of preserved mature specimens (length 4 cm in formalin, captured in June 1958 by Mr A. K. Nagabhushanam) from the Port Erin area (see Text-fig. 1E).

The rhinophores and oral tentacles bear patches of spicules and of brown pigment. The black-pigmented eyes (Text-fig. 1F, *E.*), at the bases of the rhinophores, are hidden by the mantle when the animal is creeping.

The single gill is of the 'pectinate' type (Pelseneer, 1906) and is attached by its rachis to the lateral body wall on the right side along approximately three-quarters of its length. The free tip is muscular and mobile. The mantle skirt hides the gill from view when the animal is creeping. The respiratory currents set up by the cilia of the foot, mantle and gill lamellae are shown in Text-figs. 1 and 2. The respiratory chamber is elongated and tubular, bounded above by

the mantle and below by the foot. It is open anteriorly, lateral to the right rhinophore, and posteriorly, at the temporary exhalent aperture formed by the mantle crenation (Text-fig. 1A, *Post.Cr.*). Sections show the gill lamellae to bear large numbers of unicellular mucus glands, and the tubercles on the rachis (Text-fig. 2, *T.*) are rich in such glands, as well as being loaded with stellate spicules. Anterior to the gill is the opening of the pre-branchial gland



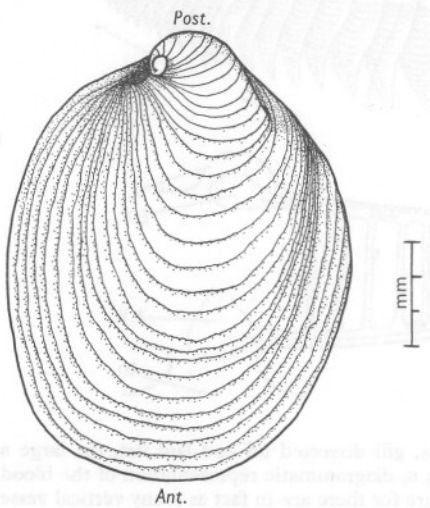
Text-fig. 2. The gill: A, gill dissected off and laid flat, the large arrow points towards the posterior of the animal; B, diagrammatic representation of the blood vessels in a gill pinnule, this is a simplified picture for there are in fact as many vertical vessels in the pinnule as there are secondary lamellae; C, single pinnule viewed from above; D, single pinnule viewed from the side. The arrows in A, C and D show the direction in which ciliary currents impelled fine carmine particles. In B they show the direction of the flow of blood in the branchial vessels. *Att.*, attachment of the gill rachis to the lateral body wall; *A.V.*, afferent vessel; *E.V.*, efferent vessel; *P.Gl.*, opening of prebranchial gland; *Pr.Lm.*, primary lamella; *R.*, rachis; *Sec.Lm.*, secondary lamella; *T.*, tubercle.

(the poison gland of Bourne (1885) or excretory organ of Bojanus (Vayssière, 1898; Abbott, 1949)), whose functions are not understood. Near the posterior extremity of the gill is the anal opening (Text-fig. 1F, *A.*). After feeding on *Ascidia* the faeces are very dark in colour and are expelled as elongated pellets, which, being on the 'downstream' side of the gill, present no problem in sanitation. Text-fig. 2B shows the blood supply of a gill pinnule. The course of the flow of blood in the gill can be seen readily if a dilute suspension of indian ink is injected into the haemocoel.

The ciliary currents over the rest of the body impel particles towards the posterior of the animal. No evidence of ciliary collection of food was obtained.

State of maturity

Microtome sections of the ovotestis of one of the larger (length $3\frac{1}{2}$ cm) November specimens show that it was sexually immature. Spermatogenesis had reached an advanced stage, since tailed spermatozoa were visible in male follicles, but oogenesis was at a very early stage and the oocytes had not begun to accumulate cytoplasmic yolk. H. M. Lloyd (London Ph.D. thesis, 1952) has figured the anterior genital mass of *Pleurobranchus*, and her illustrations, together with dissections of mature specimens (collected by A. K. Nagabhushanam in June 1958) enabled comparison to be made with dissections of



Text-fig. 3. The shell; dorsal aspect.

the sexual organs of our November specimens. This showed clearly that the genital mass of our specimens was in all respects in a rudimentary condition, although all the organs were present. The *external* genitalia, however, have all the appearance of maturity and the penis is long, and, in life, very mobile. This emphasizes the danger of judging a gastropod mature merely because the externally visible genital organs have the appearance of full development.

None of our specimens was observed to copulate or lay eggs either in the sea or in the laboratory.

FEEDING BEHAVIOUR

Specimens killed immediately after capture while swimming had no recognizable matter in the stomach. A little white flocculent matter of unknown nature was all that could be found. This white matter was certainly not composed of any of the usual planktonic organisms.

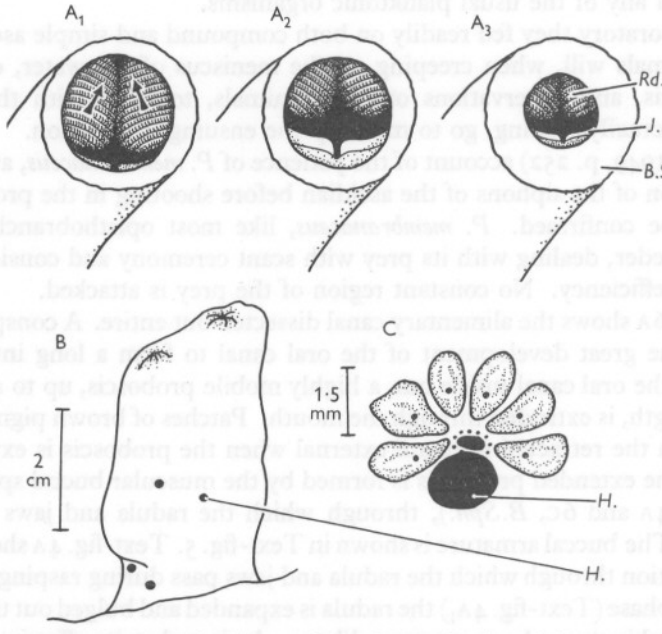
In the laboratory they fed readily on both compound and simple ascidians. Starved animals will, when creeping on the meniscus of the water, extrude the proboscis, and observations on such animals, together with those on specimens actually feeding, go to make up the ensuing description.

Yonge's (1949, p. 252) account of the patience of *P. membranaceus*, awaiting the relaxation of the siphons of the ascidian before shooting in the proboscis, could not be confirmed. *P. membranaceus*, like most opisthobranchs, is a voracious feeder, dealing with its prey with scant ceremony and considerable mechanical efficiency. No constant region of the prey is attacked.

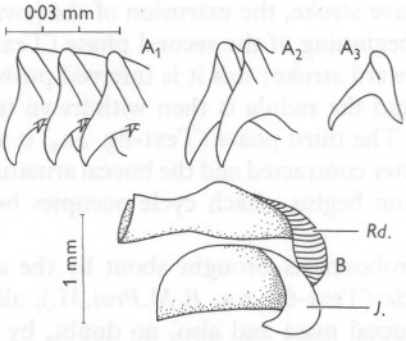
Text-fig. 6A shows the alimentary canal dissected out entire. A conspicuous feature is the great development of the oral canal to form a long introvert. By turning the oral canal inside out, a highly mobile proboscis, up to a centimetre in length, is extruded through the mouth. Patches of brown pigment on the inside in the retracted state are external when the proboscis is extruded. The tip of the extended proboscis is formed by the muscular buccal sphincter (Text-figs. 4A and 6C, *B.Sph.*), through which the radula and jaws can be protruded. The buccal armature is shown in Text-fig. 5. Text-fig. 4A shows the phases of action through which the radula and jaws pass during rasping. During the first phase (Text-fig. $4A_1$) the radula is expanded and bulged out through the buccal sphincter, whose aperture dilates. As it makes its effective stroke (in the direction indicated by arrows in Text-fig. $4A_1$) and is withdrawn, the two halves of the radula are brought together. The effect is to carry particles caught on the radular teeth upwards and inwards. While the radula is halfway through its effective stroke, the extrusion of the jaws through the buccal sphincter marks the beginning of the second phase (Text-fig. $4A_2$). The jaws then make a short upward stroke; this it is inferred pushes further matter on to the radular teeth and the radula is then withdrawn into the buccal mass, followed by the jaws. The third phase (Text-fig. $4A_3$) is a brief resting period with the buccal sphincter contracted and the buccal armature withdrawn before the next cycle of action begins. Each cycle occupies between one and two seconds.

Extrusion of the proboscis is brought about by the action of the buccal mass protractor muscle (Text-fig. 6B, *B.M.Prot.M.*), aided by the extrinsic musculature of the buccal mass and also, no doubt, by pressure changes in the haemocoel. Its withdrawal is accomplished by a pair of stout buccal mass retractor muscles (Text-fig. 6A–C, *B.M.Ret.M.*), running from the buccal mass to the posterior body wall. Anatomical features associated with the

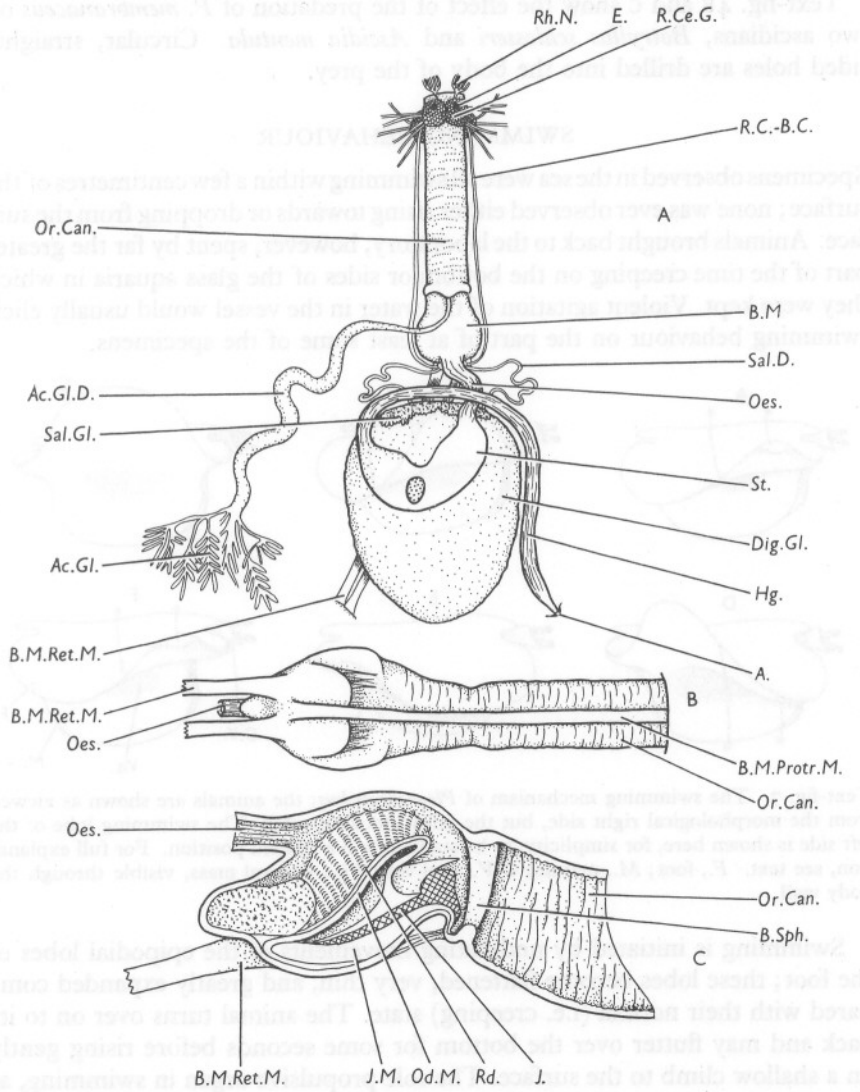
great development of the oral canal are the relatively great length of the cerebro-buccal connectives (Text-fig. 6A, *R.C.-B.C.*), of the salivary gland ducts (Text-fig. 6A, *Sal.D.*) (the salivary glands themselves lying attached to the anterior face of the digestive gland) and the extreme extensibility of the oesophagus.



Text-fig. 4. The feeding mechanism: A, three phases of action of the radula and jaws at the tip of the extruded proboscis (see text for explanation); B, *Ascidia mentula* attacked by *Pleurobranchus*; C, *Botryllus schlosseri* attacked by *Pleurobranchus*. *B.Sph.*, buccal sphincter; *H.*, hole drilled by *Pleurobranchus*; *J.*, jaw; *Rd.*, radula.



Text-fig. 5. The buccal armature: A, radular teeth, showing teeth from centre of radula (A_1), more lateral teeth (A_2) and a tooth from the extreme edge (A_3); B, radula and jaws dissected out entire seen from lateral aspect, with anterior to the right of the page, dorsal to the top. *J.*, jaws; *Rd.*, radula.

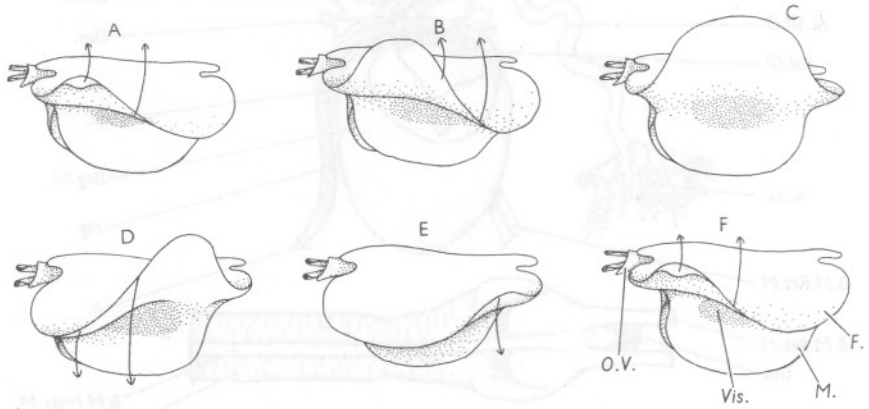


Text-fig. 6. The alimentary canal: A, alimentary canal dissected out entire from dorsal aspect with buccal mass retracted; B, ventral view of retracted buccal mass; C, left sagittal half of retracted buccal mass, cut surfaces left blank. *A.*, anus; *Ac.Gl.*, acid gland; *Ac.Gl.D.*, acid gland duct; *B.M.*, buccal mass; *B.M.Protr.M.*, buccal mass protractor muscle; *B.M. Ret.M.*, buccal mass retractor muscle; *Dig.Gl.*, digestive gland; *Hg.*, hindgut; *J.*, jaw; *J.M.*, jaw muscle; *Oes.*, oesophagus; *Od.M.*, odontophore muscle; *Or.Can.*, oral canal; *R.C.-B.C.*, right cerebro-buccal connective; *R.Ce.G.*, right cerebro-pleural ganglion; *Rd.*, radular membrane; *Rh.N.*, nerves to rhinophores; *Sal.D.*, duct of salivary gland; *Sal.Gl.*, salivary gland; *St.*, stomach.

Text-fig. 4B and C show the effect of the predation of *P. membranaceus* on two ascidians, *Botryllus schlosseri* and *Ascidia mentula*. Circular, straight-sided holes are drilled into the body of the prey.

SWIMMING BEHAVIOUR

Specimens observed in the sea were all swimming within a few centimetres of the surface; none was ever observed either rising towards or dropping from the surface. Animals brought back to the laboratory, however, spent by far the greater part of the time creeping on the bottom or sides of the glass aquaria in which they were kept. Violent agitation of the water in the vessel would usually elicit swimming behaviour on the part of at least some of the specimens.

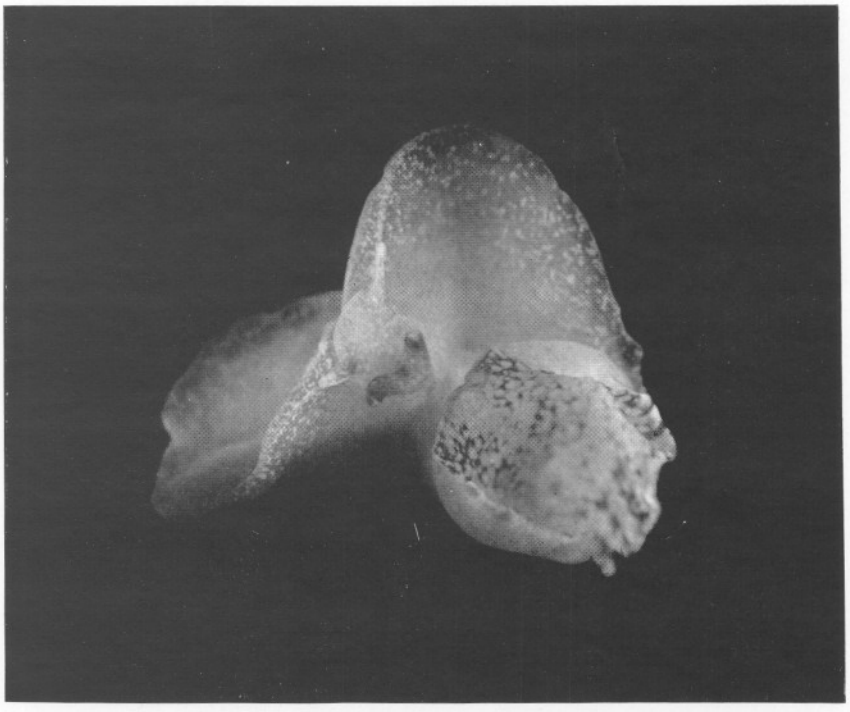


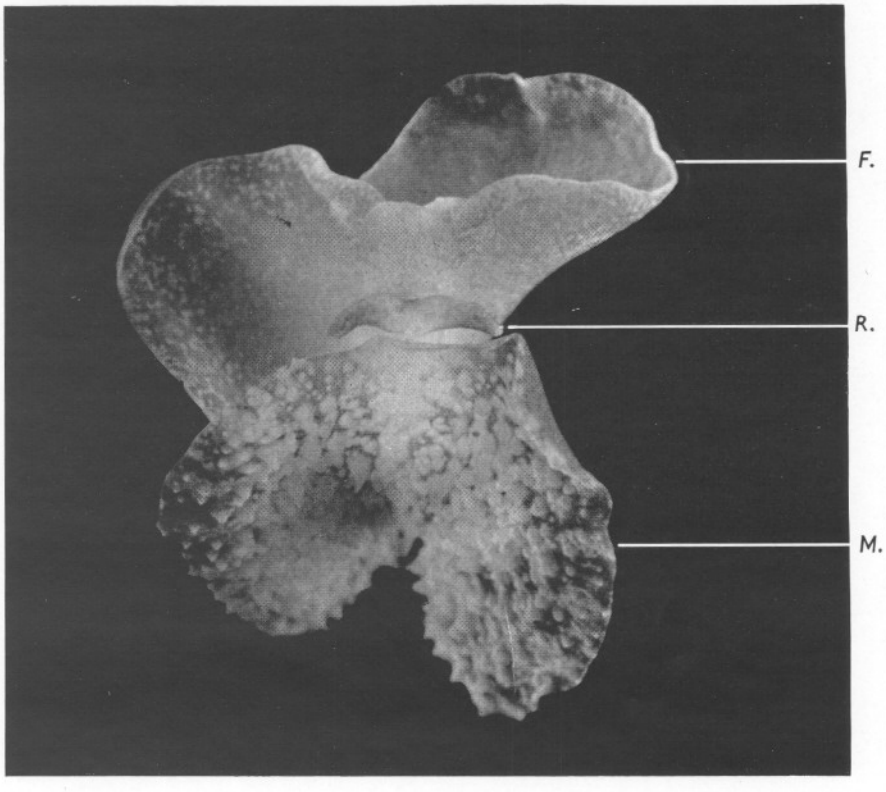
Text-fig. 7. The swimming mechanism of *Pleurobranchus*: the animals are shown as viewed from the morphological right side, but the gill is not illustrated. The swimming lobe of the left side is shown here, for simplicity, to be stationary in a relaxed position. For full explanation, see text. *F.*, foot; *M.*, mantle; *O.V.*, oral veil; *Vis.*, visceral mass, visible through the body wall.

Swimming is initiated by undulating movements of the epipodial lobes of the foot; these lobes become flattened, very thin, and greatly expanded compared with their normal (i.e. creeping) state. The animal turns over on to its back and may flutter over the bottom for some seconds before rising gently on a shallow climb to the surface. The sole propulsive organ in swimming, as in creeping, is the foot. The mantle is reflected away from the broad epipodial lobes; posteriorly the edges of the mantle skirt touch, forming a keel at the rear. The most striking feature of the swimming movements of the foot is the fact that the two lobes are not synchronous in action; that of one side under-

EXPLANATION OF PLATE I

Photographs taken through the side of a glass vessel of swimming *Pleurobranchus membranaceus*, viewed from the anterior; by electronic flash. ×2. *F.*, foot; *M*, mantle; *R.*, rhinophore.





(*Facing p.* 516)

goes its recovery stroke at the precise time when the other is in the phase of effective beat. This causes a high degree of instability in progression: the roll to either side is approximately 45° and so a movement through about 90° occurs between each pair of strokes. The anterior and posterior extremities of the foot are deeply notched (see Text-fig. 7), marking the line of discontinuity of action of the two lateral halves of the foot.

The cycle of activity through which each epipodial lobe passes may be best explained by reference to the figures. Text-fig. 7A shows the lobe at the commencement of the recovery stroke; this is initiated anteriorly, a wave of action passing back (Text-fig. 7B) until the whole lobe is poised above the animal (Text-fig. 7C). The effective stroke again begins anteriorly (Text-fig. 7D), passing backwards; the result is quite a powerful beat, providing a lifting component, with the thinner trailing margin of the lobe helping to provide a posterior component resulting in a small amount of forward progression (Text-fig. 7E). These swimming movements are not unlike those of a skate, except that, as stated above, the two swimming lobes act asynchronously in *Pleurobranchus*, and the wave of action in this gastropod is of relatively greater magnitude.

The mantle, reflected away from the body, provides no protection for the gill during swimming; this organ is widely exposed at each recovery stroke of the right epipodial lobe. This contrasts with the behaviour of the animal when creeping, with the gill completely enclosed from view in a furrow between the mantle skirt and the foot. While the animal is swimming the mantle acts as a double keel, counteracting to some extent the rolling and yawing consequent on the nature of the mode of progression.

Counts of from 55 to 60 strokes per minute (of each epipodial lobe) were made with animals in sea water at 15° C, in the laboratory.

While in the sea the animals did not appear to be swimming in any particular direction, and were completely at the mercy of the currents. *P. membranaceus* at this stage of its life cycle is more planktonic than pelagic.

ACID SECRETION

The opportunity was taken to re-investigate the statement of Garstang (1890) that *P. membranaceus* rendered itself distasteful to predators by the secretion of sulphuric acid. It is clear that Garstang was referring to previous work by some other naturalist, but we have been unable to trace the original source.

Preliminary tests were made by applying pH papers to the mantle and foot of a roughly stimulated animal. Blue litmus immediately turned red at the point of contact, and Johnson's pH papers indicated a pH in the region of 1. Repeated stimulation (with a glass rod) of a small area of the mantle soon brought about local exhaustion of the capacity to produce this acid. The secretion does not seem to be produced continuously, but only when the

animal is abruptly disturbed; pH papers resting lightly on the mantle of a creeping specimen exhibited no colour change.

Some simple qualitative tests were then made in order to identify the anions present. To obtain samples of the acid secretion, the animal was passed rapidly through three changes of distilled water and excess water removed with filter paper. It was then placed in a Petri dish and the mantle stimulated with a glass rod. A few drops of distilled water were placed on it and then immediately drawn off with a fine pipette. Drops of this fluid were then tested for anions (see, for example, Belcher & Wilson, 1946). Chloride and sulphate were found to be present, but nitrite, nitrate and such organic radicals as oxalate, tartrate and citrate were not detected.

Gravimetric methods were used in an attempt to determine the sulphate: chloride ratio of the acid secretion, although the very small quantities of material available for testing rendered the results variable. Several animals were passed quickly through three changes of distilled water and then roughly stimulated with a glass rod. After stimulation the animals were washed in a minimum amount of distilled water, and determinations carried out on the combined washings (about 1–2 ml.).

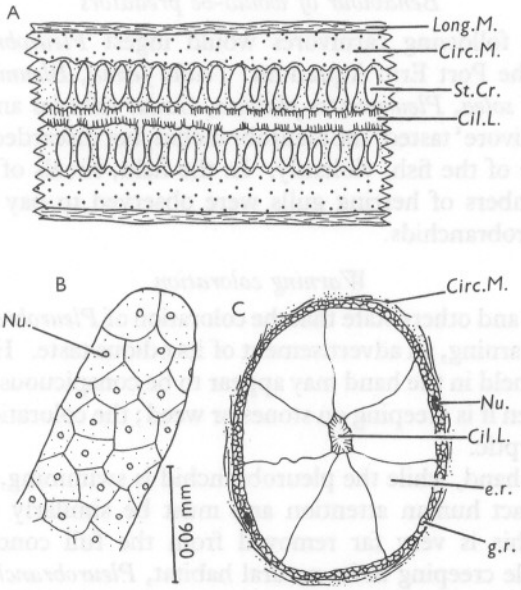
Tests for proteins, with such coagulating reagents as mercuric chloride, ethyl alcohol and concentrated nitric acid were negative. Therefore it was assumed that proteins were not present in sufficient quantities to interfere with the gravimetric determinations.

Sulphate and chloride were precipitated as barium sulphate and silver chloride respectively, according to the procedure recommended by Vogel (1951). To reduce the effects of co-precipitation of the sulphates of sodium and potassium, this precipitation was carried out in the presence of picric acid. Precipitates were separated from the fluid by means of a centrifuge. The results of four separate determinations varied rather widely, giving a mean SO_4'':Cl′ ratio of $0{\cdot}75 \pm 0{\cdot}19$:1. Two determinations carried out on local sea water using the same technique gave ratios of 0·13:1 and 0·14:1, compared with a mean value of 0·13991:1 found by Bather & Riley (1954) for the sulphate-chlorinity ratio of Irish Sea water. This fairly close agreement suggests that the technique used to obtain the fluid is the cause of the variation in our results, rather than the method of analysis.

Thus, when disturbed, *Pleurobranchus* produces an acid fluid of approximately pH 1, in which sulphate and chloride are the only anions present in appreciable amounts. The proportion of sulphate to chloride is much greater than in sea water. No analyses were made of the internal body fluids of *Pleurobranchus* due to the uncertainty of knowing whether a sample was contaminated with acid fluid from the epidermis or the acid gland. Robertson (1949) has given figures for the ionic composition of the body fluid of *P. membranaceus*, and he found the sulphate:chloride ratio to be about 0·143:1, only slightly above that of sea water.

Sites of production of the acid secretion

The general body surface. As mentioned above, the mantle and foot are both able to produce the acid fluid. The histological structure of the epidermis in *Pleurobranchus* is apparently simple and is quite unlike that of a dorid nudibranch (Thompson, 1958), in which large numbers of unicellular and multicellular epidermal and subepidermal glands open to the surface all over the mantle and its tubercles. In *Pleurobranchus* there are no subepidermal glands associated with the mantle or foot; there are only scattered small unicellular mucus glands of the usual type associated with ciliated epithelia.



Text-fig. 8. The acid gland: A, diagrammatic longitudinal section through the acid gland duct; B, branch of the acid gland, stained intra-vitally with methylene blue; C, transverse section through a branch of the acid gland, fixed Zenker-without-acetic, stained Heidenhain's alum haematoxylin. *Cil.L.*, ciliated central lumen; *Circ.M.*, circular muscle fibres; *e.r.*, empty region; *g.r.*, granular region; *Long.M.*, longitudinal muscle fibres; *Nu.*, nucleus; *St.Cr.*, storage crypts.

The acid gland (Text-fig. 6A, *Ac.Gl.*, and Text-fig. 8). This is a large, ramifying gland, communicating with the roof of the buccal mass by a stout duct. Tests performed on extracts of this gland showed that it produced a strongly acid fluid. Text-fig. 8B shows an ultimate branch of the gland and Text-fig. 8C a section through such a branch. The gland cells (see Text-fig. 8C) have the same appearance in sections as the cells of the mantle and foot epidermis. The main duct (Text-fig. 6A, *Ac.Gl.D.*, and Text-fig. 8A) has two muscle layers and huge numbers of crypts opening off the central ciliated lumen. It is inferred that the secretion of the gland cells is stored in this

spongy duct, to be expelled rapidly when required, by contraction of the muscle layers.

To summarize: acid secretions are produced by the mantle and foot epidermis and by the ramifying median buccal gland (therefore called the 'acid gland'). The primary characteristic of the cells responsible for the secretions is a striking histological anonymity. They appear completely empty in sections; this, it is interesting to note, contrasts with the appearance of mammalian oxyntic cells, whose function is believed to be the secretion of hydrochloric acid (see Carleton & Leach, 1949).

Behaviour of would-be predators

None of the following carnivores would ingest *Pleurobranchus* in tests carried out at the Port Erin Aquarium: *Tealia felina*, *Blennius pholis*, *Pholis gunnellus*, *Solea solea*, *Pleuronectes platessa*, *Gadus calarias* and *G. virens*. In all cases the carnivore 'tasted' the pleurobranchid, but discarded it immediately, and, in the case of the fish, violently. In the field, shoals of coalfish (*Gadus virens*) and numbers of herring gulls were observed to pay no attention to swimming pleurobranchids.

Warning coloration

Cooke (1895) and others state that the coloration of *Pleurobranchus membranaceus* acts as a warning, an advertisement of its odious taste. However, while a *Pleurobranchus* held in the hand may appear to be conspicuous, this is certainly not the case when it is creeping on stones or weed; the coloration of the mantle is efficiently cryptic.

On the other hand, while the pleurobranchid is swimming, the movements of the foot attract human attention and must be similarly obvious to fish. Nevertheless, this is very far removed from the full concept of warning coloration. While creeping in its natural habitat, *Pleurobranchus* is effectively camouflaged; while swimming it does not advertise its presence by striking coloration, but neither does it make the least effort at concealment.

DISCUSSION

Swimming is a well-documented phenomenon in gastropods; it is widespread in the group and the possession of a swimming mechanism can be only warily accepted as evidence of close relationship. The means by which swimming is effected are as follows. (1) By lateral waves passing backwards along the whole body, e.g. the nudibranchs *Dendronotus giganteus* and *Melibe leonina* (Agersborg, 1922), *Scyllaea pelagica* (Collingwood, 1881), *Phyllirhoe* and *Cephalopyge* (Morton, 1958*b*). The waves pass down each side alternately, and the mechanism may be assisted either by the presence of an oar-like tail, as in *Lomanotus* (Trinchese, quoted by Colgan, 1908), or vertical undulations of the head, as in *Plocamopherus* (Lowe, quoted by Alder & Hancock, 1845–55). (2) By

vertical waves passing backwards along the mantle skirt, e.g. some tropical dorid nudibranchs (personal communication from J. S. Colman). (3) By movements of expanded lobes of the foot, as in most swimming gastropods. The action may resemble (as Morton, 1958*a*, points out) in essence either sculling, e.g. *Clione* (Morton, 1958*a*), Heteropoda (Morton, 1958*b*), or rowing (or even 'flying'), in which the lateral halves of the foot may either beat synchronously, e.g. *Akera* (Morton & Holme, 1955), *Gastropteron* (Morton, 1958*b*), *Limacina* (Morton, 1954), or beat asynchronously, e.g. *Pleurobranchus membranaceus*.

The majority of these gastropods spend most of their lives swimming; this is plainly not the case in *Pleurobranchus*, which has only occasionally been seen swimming. Pelagic seasonal swarming is a familiar phenomenon in marine animals and it is usually associated with feeding, reproduction or dispersal. None of these fits the facts concerning *P. membranaceus*, for this species apparently does not feed while swimming, is sexually immature during the requisite phase and already has (in the shape of a pelagic veliger phase) a dispersal mechanism of the usual molluscan type. The only other suggestion which we can advance at present is that this is a phenomenon similar to the much-debated inshore migrations of nudibranchs, namely, a means of bringing about aggregation for reproduction at a much later date. However, since it is unlikely that drifting in the surface layers of the sea will bring about any such aggregation, and since the existence of migrations in nudibranchs is denied by several authorities (a summary has been given by M. C. Miller, Liverpool Ph. D. thesis, 1958), this hypothesis is of questionable value. The true explanation will only be obtained when more facts concerning the natural history of *P. membranaceus* are discovered.

This report would be incomplete without some comments regarding the systematic position of the pleurobranchids. It is known that in the nervous system, the reproductive system,[1] the alimentary canal (with its 'holohepatic' arrangement—see Text-fig. 6), and features of the development (compare the mode of formation of the adult mantle in the metamorphosing larvae of *Berthellina* (Gohar & Abul-Ela, 1957) and *Adalaria* (Thompson, 1958)), the pleurobranchids are closely related to the dorid nudibranchs. This was recognized by Thiele (1931) who placed the pleurobranchids in the suborder Notaspidea, which, together with the suborder Nudibranchia, made up his order Acoela. In support of this is the arrangement of the exhalent respiratory aperture, formed by a temporary crenation in the mantle skirt (see Text-fig. 1A, *Post.Cr.*), rendering the anus in *Pleurobranchus* in the same position *functionally* as that of the dorid nudibranchs. A simple change in the position of the gill would turn the pleurobranchid into a dorid. A homology of the dorid branchial circlet with the 'tectibranch' gill was first suggested by Evans (1914). In addition the way in which the mantle skirt encloses the basal parts

[1] H. M. Lloyd, London Ph.D. thesis, 1952.

of the rhinophoreal tentacles in *Pleurobranchus* (see Text-fig. 1A, *Ant.Cr.*) is strikingly similar to a developmental stage of the dorid *Adalaria* (Thompson, 1958); in *Adalaria* the anteriorly extending mantle fold during metamorphosis encircles and isolates the developing rhinophores. *Pleurobranchus* clearly shows an evolutionary stage in the production of the dorid rhinophore, which in the adult dorid appears to arise from the mantle.

The authors are indebted to Mr J. S. Colman for critical reading of the manuscript and for the provision of laboratory facilities. The work was done while one of us (T. E. T.) was the holder of a Leverhulme Fellowship in the University of Liverpool. We are very grateful for field observations on *Pleurobranchus* to Mr L. Collister, Mr J. Faragher, Mr K. Woodworth, Mr P. J. Miller and Mr R. G. Hartnoll. Mr A. K. Nagabhushanam kindly provided trawled specimens for dissection.

Note added in proof

Specimens dredged and trawled during the spring and summer of 1959 were, through the kindness of Mr P. J. Miller and Mr R. G. Hartnoll, made available to us. Specimens of up to 7 cm long were collected during April and May and these deposited spawn masses in captivity. The ability to swim if abruptly disturbed is possessed even by the largest individuals. No specimens were found on the shore during 1959. None was dredged or trawled after the end of May; the inference is that *Pleurobranchus membranaceus* has an annual life cycle (like that of many dorid nudibranchs), the adults dying at the close of the breeding season.

SUMMARY

An account is given of an invasion of Port Erin bay by large numbers of swimming *Pleurobranchus membranaceus* (Montagu).

Descriptions are given of the external features of the specimens, the state of maturity, feeding behaviour and swimming behaviour.

The sites of production and the nature of the acid secretions are described.

Thiele's placing of the pleurobranchids close to the dorid nudibranchs is discussed and additional evidence to support this view is furnished.

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A HOPPER FOR USE WHEN SIEVING BOTTOM SAMPLES AT SEA

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(Text-figs. 1 and 2)

When large quantities of bottom soil are brought up by the dredge or grab, the problem of sieving the material within a reasonable time presents itself. The usual practice is to wash the soil through a sieve or series of sieves, the finest being of the order of 1–2 mm aperture, according to the nature of the soil or the size of the animals to be collected. It is usually necessary to agitate the soil either by spraying with a hose or by shaking the sieve in a bath of water, in order that finer particles may pass through the sieve. Many of the more delicate animals are damaged by these methods, and Spooner & Moore (1940), working on the Tamar estuary, found it necessary to mix the mud carefully by hand with water to make a 'soup' before it could be sieved without damage to the fauna. For work at sea Hartman (1955) describes a machine by which sieving was accomplished with the aid of water sprays and mechanical agitation of the sieves with power provided by an electric motor.

The apparatus to be described provides a semi-automatic means of washing soil into suspension in sea water, the resulting suspension passing fairly readily through a sieve. The animals are little damaged by this method, as once the soil has been placed in the hopper it remains undisturbed until washed over into the sieve. The method is probably no faster than others, but the physical labour of sieving is almost eliminated and the apparatus can be left to run unattended while further samples are being taken. The apparatus may be used on any ship having an adequate water supply to the deck.

It consists essentially of a square wooden hopper, some 18 in. (46 cm) across, into which the whole sample of soil is tipped, standing on a sloping V-shaped wooden base. The base is supported on four legs, to such a height that a sieve can be placed beneath the spout at its lower end. The two halves of the base are each inclined at about 23° to the horizontal, while along its axis the base slopes down towards the spout at an angle of 10°. The base has low walls at its sides which stop the water spilling out as the ship rolls. There is a gap of 4 cm between the base and three sides of the hopper, around which water from the overhead jets flows, slowly, eroding the outside of the pile. At the lower side of the hopper is a rising 'gate' which is lowered as necessary to retain the sample when the hopper is being filled.

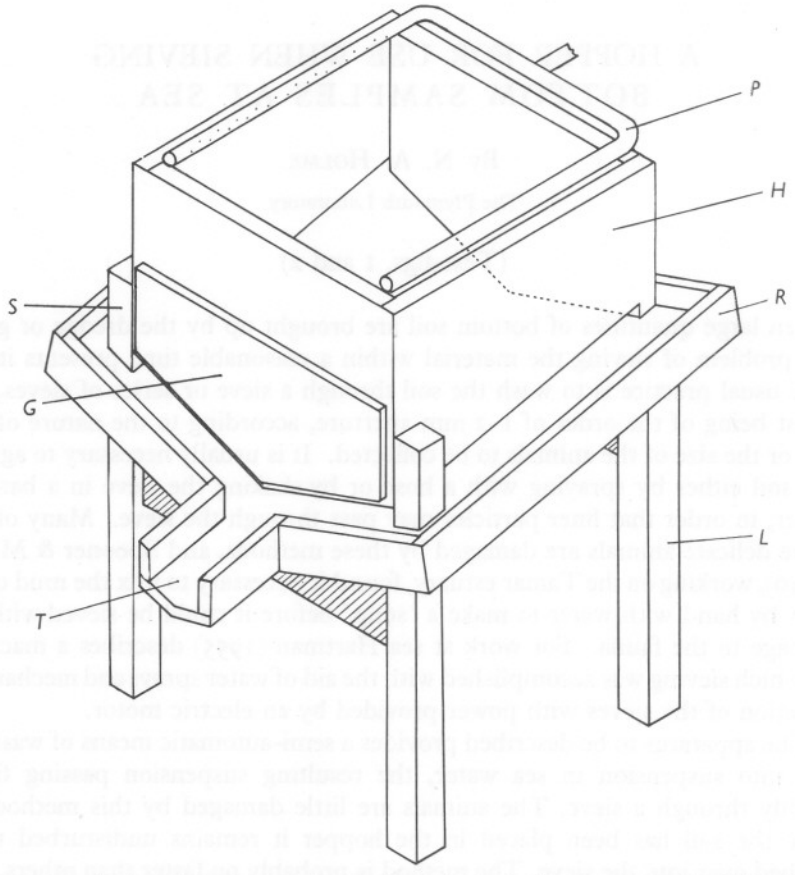

Fig. 1. General view of the hopper (isometric projection). Some constructional details are omitted. *P*, pipe supplying the jets along the top of the hopper; *H*, side wall of the hopper; *R*, retaining wall at side of base; *T*, spout; *G*, rising gate; *S*, short legs supporting lower side of hopper clear of base; *L*, legs supporting base.

The soil is broken down by jets of water issuing from rows of small holes in two lengths of pipe running along the top of two sides of the hopper. One inch bore polythene pipe, sealed at the outer end, has been used, and by using Yorkshire 'plastronga' elbow joints it is possible to vary the angle of the jets as required. The jets are arranged to fall at different angles to cover the surface of the soil in the hopper as completely as possible. Water accumulating on the surface excavates for itself cavities at the side of the heap, so draining down to the bottom of the hopper. Thence it travels around the base of the pile, out through the spout and into the sieve. It has not been found necessary to make an overflow near the top of the hopper, as the water always drains

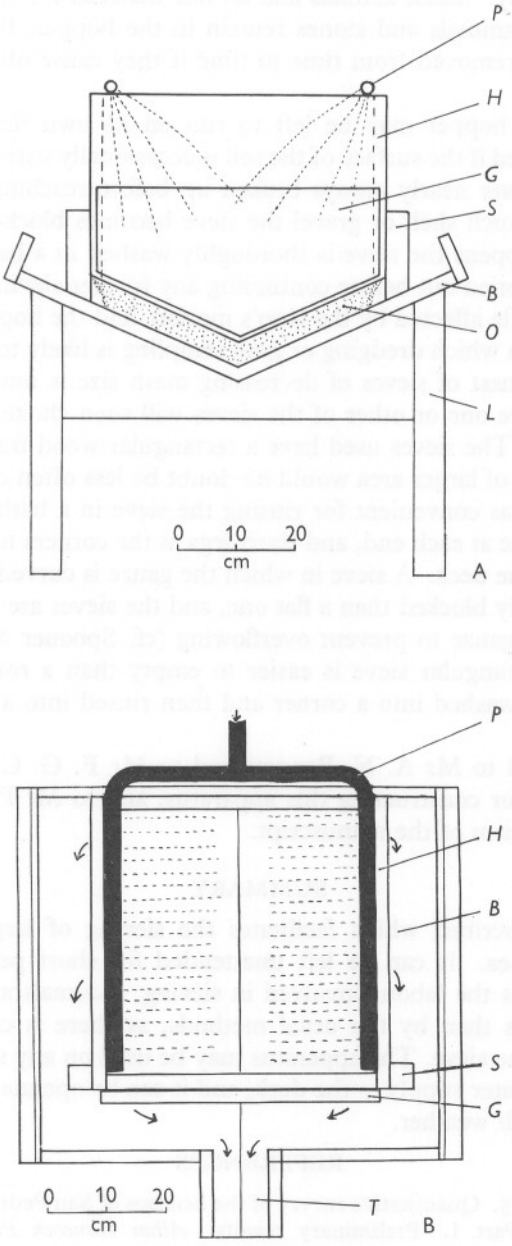

Fig. 2. A, Transverse section of the hopper just above the spout. *B*, base; *O*, soil in hopper, showing eroding outer edge of the pile. Other lettering as in Fig. 1. B, Top view of the hopper.

away satisfactorily. Small animals and all fine material are washed over into the sieve; large animals and stones remain in the hopper, from which they may have to be removed from time to time if they cause obstruction to the flow of water.

Although the hopper may be left to run on its own for short periods, washing is speeded if the surface of the soil is occasionally stirred with a trowel. Lumps of mud are nearly always broken up before reaching the sieve, but where there is much shell or gravel the sieve becomes blocked from time to time. If this happens the sieve is thoroughly washed in a bath of water and the contents emptied out before continuing any further sieving.

Washing is little affected by the ship's motion, and the hopper can be used in any weather in which dredging or grab sampling is likely to be undertaken.

The use of a nest of sieves of decreasing mesh size is unsatisfactory with this hopper, since one or other of the sieves will soon choke and overflow if left unattended. The sieves used have a rectangular wood frame $45 \times 35 \times 20$ cm deep (a sieve of larger area would no doubt be less often clogged, but this size was chosen as convenient for rinsing the sieve in a bath of water). The sieve has a handle at each end, and short legs at the corners to raise the gauze screen clear of the deck. A sieve in which the gauze is curved into an arc of a circle is less easily blocked than a flat one, and the sieves are provided with a side-window of gauze to prevent overflowing (cf. Spooner & Moore, 1940). A square or rectangular sieve is easier to empty than a round one, as the sievings can be washed into a corner and then rinsed into a jar with a little water.

I am indebted to Mr A. N. Bennett and to Mr F. G. C. Ryder and his workshop staff for constructing this apparatus, and to Mr P. G. Corbin for his helpful criticism of the manuscript.

SUMMARY

A hopper is described which facilitates the sieving of large quantities of bottom soil at sea. It can be left unattended for short periods, and considerably reduces the labour involved in sieving. Animals are probably less damaged by this than by the usual methods, as there is considerably less disturbance in the sieve. The apparatus may be used on any ship where there is an adequate water supply to the deck, and it can be operated successfully in moderately rough weather.

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WIND CURRENTS, TIDAL STREAMS AND PLANKTON OFF THE NORTHUMBERLAND COAST

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(Text-figs. 1–7)

Investigations of the water movements off the southern Northumberland coast have until now been confined to the surface region. While important for navigational and similar purposes such investigations can give us no more than an indication of the total water flow along the coast nor can they elucidate the effects of currents on planktonic organisms which spend part or all of their existence away from the surface. It is for the latter reason that the series of investigations of sea surface currents made aboard the R.V. 'Alexander Meek' in 1956–7 (Evans, 1957) has now been supplemented by further series at two deeper levels.

The work of measurement was again undertaken on the stretch of coast between the River Tyne entrance (55° 01′ N., 1° 24′ W.) and Newbiggin Point (55° 11′ N., 1° 30′ W.) at a distance of about a mile from the shore. There now exist for this area records of (aperiodic) wind currents and tidal streams for 2 fathoms below the surface, for 6 fathoms below the surface and for approximately 3 fathoms off the bottom. In the work to be described the method used in measuring water flow followed that of the 1956–7 series; the track of a large, freely drifting drogue and buoy was plotted over half a lunar day. This was done to isolate the wind current from the general flow, observations beginning at various states of tidal stream and continuing for between 12 and 13 h, the exact period to be spent in tracking the buoy being determined from an inspection of the tide tables.

A description of the form of drogue and buoy used has been given in my earlier account; briefly, each drogue was an open-ended box 4 ft 6 in. high, of wood and canvas, cross-stayed with steel bar and attached by 1 in. circumference wire to a fisherman's dan buoy. Using this equipment runs at 2 and 6 fathoms were made by interposing the appropriate length of wire between the buoy and the suspended drogue. Runs with the drogue riding approximately 3 fathoms off the bottom were accomplished in the following way: a wire 15 fathoms long was used, this being 3 fathoms less than the extreme depth of water in which the gear was worked. The wire was shackled

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to both buoy and drogue, and close beneath the buoy a bight was taken out of the wire and stopped off with a bulldog grip (Figs. 1 & 2). The effective length of wire between the buoy and the drogue could be increased or decreased by slacking back the bulldog grip and adjusting the length of the bight. During the work soundings were taken on the ship's echo sounder and the length of wire in use varied accordingly.

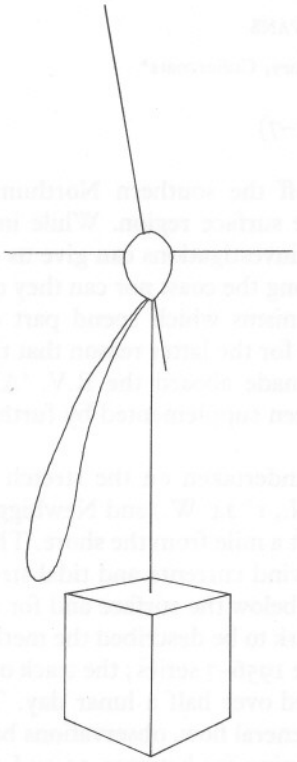

Fig. 1

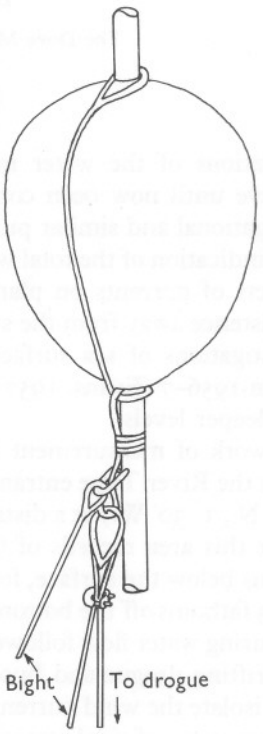

Fig. 2

Fig. 1. Attachment of the drogue to the buoy for runs near the bottom.

Fig. 2. Details of the attachment of the near-bottom drogue to the buoy.

Between January and July 1958, 5 runs were completed with a drogue riding at 2 fathoms' depth, 20 runs with a drogue riding at 6 fathoms' depth and 13 runs with a drogue riding about 3 fathoms off the bottom (Table 1). The results of the shallow water runs of 23 May and 4 July in which only a 6-fathom drogue was used have been utilized in both the 6-fathom records and those of 3 fathoms off the bottom. During the observations the mean depth of water was 11·2 fathoms (Table 2); consequently the 2-fathom drogue sampled near-surface currents, the 6-fathom drogue sampled mid-water currents and the drogue riding at 3 fathoms off the sea floor sampled near-bottom

currents. For the sake of brevity, currents at these depths will therefore be referred to as near-surface, mid-water and near-bottom.

Drogue positions were fixed every 45 min by horizontal sextant angles of conspicuous marks ashore and, since the installation of a Decca Navigator in our ship, by an occasional Decca fix in thick weather. Wind speed and direction,

TABLE 1. WIND CURRENT AND WIND OBSERVATIONS DURING THE YEAR

(Directions are true and measured from north throughout this account)

Date 1958	Set (°) Near surface	Set (°) Mid water	Set (°) Near bottom	Drift (miles) Near surface	Drift (miles) Mid water	Drift (miles) Near bottom	Downwind direction (°)	Downwind speed (knots)
29 Jan.	080	090	—	0·4	0·3	—	070	3·7
12 Feb.	135	358	—	1·4	0·5	—	097	13·5
20 Feb.	131	136	—	4·5	3·7	—	117	18·8
4 Mar.	112	128	—	3·8	2·1	—	090	17·4
11 Mar.	139	247	—	0·6	0·4	—	124	7·8
2 Apr.	—	169	164	—	0·5	0·9	253	6·0
21 Apr.	—	128	129	—	2·2	1·2	090	12·3
23 Apr.	—	357	340	—	0·7	0·6	018	12·0
29 Apr.	—	128	082	—	1·0	1·1	083	13·3
1 May	—	297	310	—	0·5	0·6	320	2·8
8 May	—	024	020	—	3·0	2·7	035	23·3
9 May	—	250	250	—	0·3	0·2	145	3·7
14 May	—	337	329	—	1·9	1·7	277	9·6
20 May	—	057	047	—	1·1	0·9	078	21·0
23 May	—	309	(309)	—	1·2	(1·2)	256	14·3
28 May	—	230	260	—	0·3	0·1	297	2·0
30 June	—	080	105	—	0·3	0·4	200	4·3
4 July	—	167	(167)	—	1·1	(1·1)	198	13·0
7 July	—	337	329	—	1·1	1·0	315	4·0
14 July	—	152	129	—	2·1	2·0	096	16·8

TABLE 2. MEAN DEPTH OF WATER DURING THE RUNS AND MEAN DEPTH OF THE NEAR-BOTTOM DROGUE

(Depths in fathoms)

Date 1958	Water depth	Drogue depth	Diff.	Date 1958	Water depth	Drogue depth	Diff.
29 Jan.	8·3	—	—	8 May	12·6	8·0	4·6
12 Feb.	12·8	—	—	9 May	9·8	7·5	2·3
20 Feb.	14·1	—	—	14 May	9·4	8·0	1·4
4 Mar.	14·7	—	—	20 May	12·9	9·5	3·4
11 Mar.	9·0	—	—	23 May	8·3	(6·0)	2·3
2 Apr.	8·7	7·0	1·7	28 May	9·6	8·0	1·6
21 Apr.	15·3	10·4	4·9	30 June	10·5	7·2	3·3
23 Apr.	11·7	9·6	2·1	4 July	7·6	(6·0)	1·6
29 Apr.	12·5	8·8	3·7	7 July	10·3	8·0	2·3
1 May	11·0	8·0	3·0	14 July	15·5	11·2	4·3
						Mean difference	2·8

measured by anemometer and compass, were noted at each fix, and a sounding was taken. Drogues were used in pairs, sampling two depths simultaneously. While this doubled the sampling rate over that of the 1956–7 series it possessed the disadvantage that after some hours the two drogues sometimes separated

so much that it was impossible to supervise them both from the ship and one had to be picked up and launched again close to the other, allowance for such relaunching being made in the plot of the buoy's track. At one time I had hoped to install a radio transmitter on one of the buoys whose signal could be received on the ship's direction finder but technical difficulties prevented this.

From the results obtained wind current and tidal stream data have been derived. The wind current was taken to be the residual current of each run, since over half a lunar day the tidal streams were held to have cancelled out. The set and drift of the wind currents together with accompanying wind speed are shown in Table 1. The five near-surface results confirm

TABLE 3. DOWNWIND-CURRENT ANGLES DERIVED FROM TABLE 1

(Angles in degrees)

Date 1958	Near surface	Mid water	Near bottom	Date 1958	Mid water	Near bottom
29 Jan.	+10	+20	—	8 May	−11	−15
12 Feb.	+38	−99	—	9 May	+105	+105
20 Feb.	+14	+19	—	14 May	+60	+52
4 Mar.	+22	+38	—	20 May	−21	−31
11 Mar.	+15	+123	—	23 May	+53	(+53)
2 Apr.	—	−84	−89	28 May	−67	−37
21 Apr.	—	+38	+39	30 June	−120	−95
23 Apr.	—	−21	−38	4 July	−31	(−31)
29 Apr.	—	+45	−1	7 July	+22	+14
1 May	—	−23	−10	14 July	+56	+33

Mid-water average +5°

Near-bottom average −3°.

that surface water off the Northumberland coast moves in general into the quadrant to the right of downwind. This is not so at mid water nor near the bottom. Here currents flow as freely to the left of downwind as to the right and about one current in five has an upwind component. During the observations the mean vector downwind blew 092° at 4·9 knots or 61 miles per half lunar day, compared with 093° and 5·4 knots, or 67 miles per half lunar day during the 1956–7 observations. Direct comparison of current speed and direction between the series of 1956–7 and those of 1958 may therefore be made without undue error.

From Table 1 the mean vector current at mid water is 101° and 0·37 miles per half lunar day. Near the bottom it is 040° and 0·25 miles per half lunar day. This compares with 114° and 1·30 miles per half lunar day found near the surface in 1956–7. The mean vector current is thus reduced to 28% of the near-surface value at mid water and to 19% near the bottom. The average current, disregarding direction, is at mid water 1·22 miles per half lunar day and near the bottom 1·05 miles per half lunar day. These are 63% and 54%

respectively of the near-surface value of 1·95 miles per half lunar day recorded in 1956–7.

The proportional discrepancy between the mean vector currents and non-directional averages at the three levels is largely due to the greater directional randomness of the deeper currents. That it is not due to more random speeds of the deeper currents is shown by an examination of the simple expression *wind speed/current speed*. For the near-surface observations of 1956–7 we have an average value of 65·2, for mid-water 140·4 and for near the bottom 145·4. The coefficients of variability of these values are 56%, 51% and 49% respectively; i.e. randomness of wind current speed does not increase from surface to bottom.

From Table 3 the average of downwind-current angles for all observations works out to be, at mid-water +5° (current to the right of downwind) with standard deviation 63°, and near the bottom −3° with standard deviation 45°. During 1956–7 near-surface current deflexion was +40° with standard deviation 26°. Hence, away from the surface, deflection is less but deviation from the mean is greater. At neither mid water nor near the bottom is there a predominant current as defined in my earlier account (Evans, 1957, p. 496).

To a subscriber to Ekman's theory relating wind and ocean current direction these results are puzzling, for Ekman, it will be recalled, postulated that close to the surface a wind current would be deflected 45° to the right of downwind in the northern hemisphere and that the deflexion would increase with depth. The near-surface currents, but not the deeper currents, agree with this finding. None of the later modifications to Ekman's theory, including that of a wind blowing offshore across a land barrier (Proudman, 1953, p. 187) appear to remove the difficulty.

Current directions were tested against winds recorded during the 24 h preceding each observation, wind data being compiled from 3-hourly readings of an anemometer at Tynemouth. Agreement was poorer than with winds blowing at the time of observation. Current deflexion at mid-water was +19° with standard deviation 82° and near the bottom +6° with standard deviation 95°. It is clear that, as with near-surface water, the onset of a given wind and the appearance of the induced current are separated by only a short interval of time.

For comparison, the 9 a.m. winds observed at sea and at Tynemouth are shown in Tables 4 & 5. The winds encountered at sea form a good sample of the daily winds observed ashore and it is assumed that the wind currents found are likewise representative of conditions during the first 7 months of 1958.

The measurement of tidal streams was accomplished by subtracting from the plot of each day's run that portion which represented wind current. Of necessity it was assumed that the wind current flowed steadily and in a

constant direction throughout each run. Then, by construction (Fig. 3), it was possible to find the points of slack water had there been no wind current. The tidal stream curve off Northumberland is approximately elliptical; therefore a line joining the two points of slack water is the major axis of the ellipse and represents the mean flow of both ebb and flood streams in magnitude and direction. Such tidal stream axes had a mean orientation of 164°–344° at all depths, maximum deviations being ±17°. Since the bearing of Newbiggin Point from the North Tyne Light is 343° the mean flow of tidal streams, unlike that of wind currents, is seen to be parallel to the coast.

TABLE 4. 9 A.M. WIND FORCES AT TYNEMOUTH AND AT SEA, EXPRESSED AS PERCENTAGE OF TOTAL OBSERVATIONS

	At Tynemouth	At sea
Observations	212	20
Calm	5·7	5
Force 1	10·8	5
Force 2	14·6	20
Force 3	25·0	25
Force 4	27·6	25
Force 5	9·9	5
Force 6	4·2	15
Force 7	0·5	0
Force 8	1·9	0

TABLE 5. 9 A.M. DOWNWIND DIRECTIONS AT TYNEMOUTH AND AT SEA, EXPRESSED AS PERCENTAGE OF TOTAL OBSERVATIONS

	At Tynemouth	At sea
Observations	212	20
Calm	5·4	5
000°–089°	24·0	15
090°–179°	37·3	45
180°–269°	14·6	25
270°–359°	18·4	10

The magnitude of the major axis of the tidal stream ellipse may appropriately be called the stream range, corresponding to the vertically measured tidal range. The stream range is found to vary rather less in proportion over the three levels than the wind currents. Near the surface 25 observations including those of 1956-7 yield an average range of 2·8 miles, at mid-water the range averages 2·7 miles and near the bottom, 2·5 miles.

Stream range varies under soli-lunar influence in the same manner as tidal range. Relationships have been established between stream range and predicted tidal range at the Tyne entrance. In Figs. 4–6 the stream range is compared with the mean tidal range for each day of observation. Regression lines are plotted for the following values: near the surface $t = 5{\cdot}3 + 1{\cdot}88s$; at mid-water $t = 4{\cdot}9 + 2{\cdot}29s$; and near the bottom $t = 6{\cdot}8 + 1{\cdot}79s$; where t is the predicted tidal range in feet and s the stream range in miles.

If the mean tidal range at the Tyne entrance is taken as 11 ft, then by Figs. 4–6 the mean stream range is 3·03 miles near the surface, 2·66 miles at mid-water and 2·35 miles near the bottom. Thus under average and parallel tidal conditions the stream range near the surface is about 0·4 miles greater than at mid-water and about 0·7 miles greater than near the bottom.

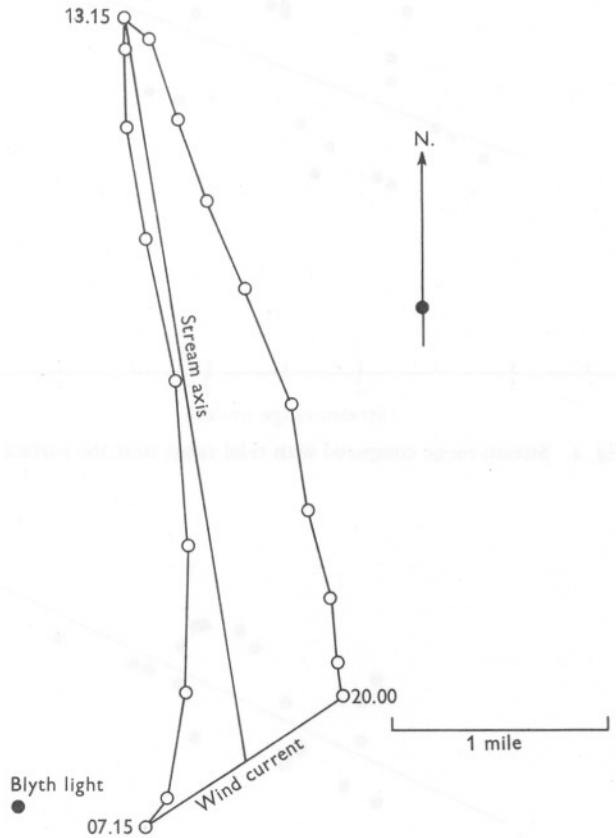

Fig. 3. Plot of the mid-water run of 20 May 1958 showing the extraction of wind current and stream range.

As a footnote to the stream range data the following may throw some light on the manner and time at which drifting material and the larvae of littoral animals are brought ashore on the Northumberland coast. (Until currents in and near the surf zone have been studied the final track before grounding must remain conjectural.) Near the surface the shape of the tidal stream ellipse is largely obscured by the wind current. The prevailing westerly wind combined with Coriolis force produces a predominant current moving southwards along the coast and somewhat offshore. Away from the surface,

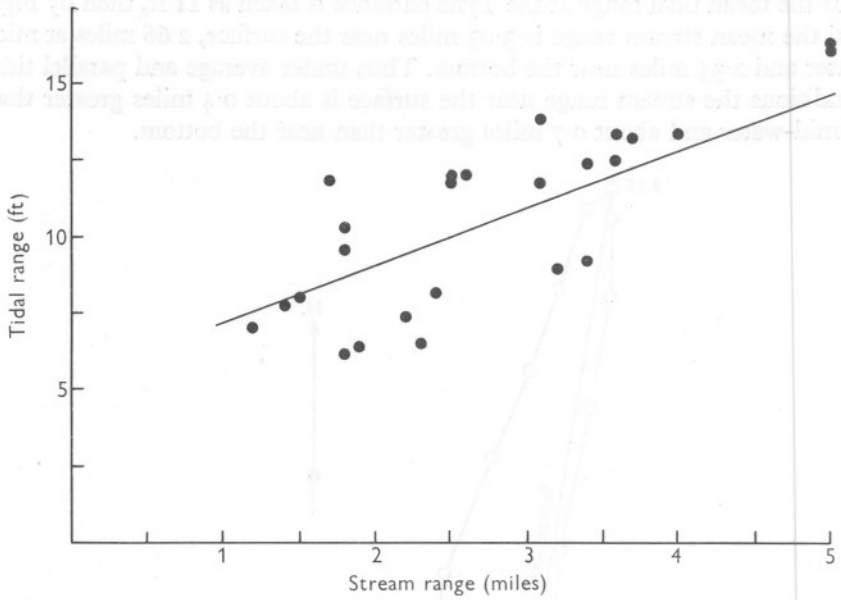

Fig. 4. Stream range compared with tidal range near the surface.

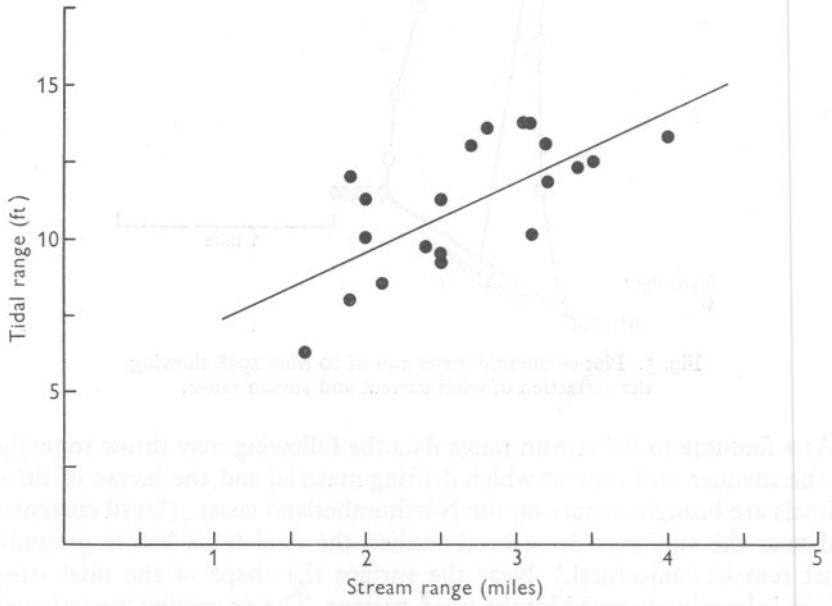

Fig. 5. Stream range compared with tidal range at mid-water.

however, where the wind current is less strong, true stream movement becomes more apparent. Off Northumberland still water is rarely encountered and at the period referred to as slack water the stream, having attained its maximum reach northwards or southwards along the coast, is setting either onshore or offshore. From Table 6 it is seen that while the near-surface water usually turns offshore at each slack water this tendency is modified at mid-water and

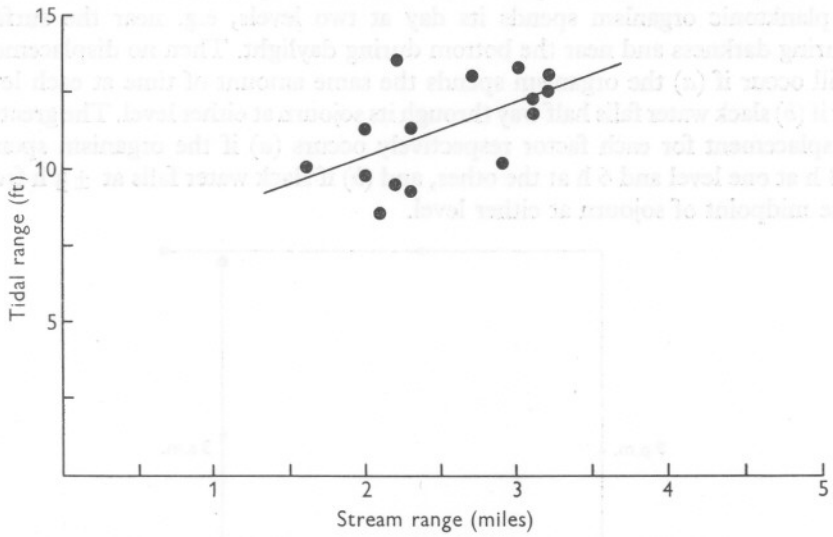

Fig. 6. Stream range compared with tidal range near the bottom.

TABLE 6. 120 OBSERVATIONS OF THE TURN OF THE TIDE OFF NORTHUMBERLAND

	Flood end		Ebb end	
	Offshore	Onshore	Offshore	Onshore
Near surface	20	5	18	7
Mid water	12	8	7	13
Near bottom	10	5	3	12

near the bottom, and as the depth increases a pattern of onshore sets at the end of the ebb and offshore sets at the end of the flood becomes manifest, i.e. the stream ellipse has an anticlockwise sense. This is in agreement with Doodson & Warburg's (1941, p. 179) analysis of the behaviour of a progressive wave having a shelving coast on the right of its direction of travel. Such a pattern suggests that drifting and suspended material would tend to ground at the end of the ebb, which in the region under consideration is about $2\frac{1}{2}$ h after low water at the Tyne entrance, low water and slack water being there separated by this interval.

Both wind currents and tidal streams may effect a permanent displacement of position of planktonic organisms. This is obviously so for wind currents but less so for tidal streams, whose mean vector is zero. In the latter case it is brought about by the combination of differing stream ranges at different levels and the vertical movement of plankton (which, off Northumberland, takes place readily in 10 fathoms of water and less). Even so, certain conditions are necessary to effect such displacement. As an example, let it be assumed that a planktonic organism spends its day at two levels, e.g. near the surface during darkness and near the bottom during daylight. Then no displacement will occur if (*a*) the organism spends the same amount of time at each level or if (*b*) slack water falls half way through its sojourn at either level. The greatest displacement for each factor respectively occurs (*a*) if the organism spends 18 h at one level and 6 h at the other, and (*b*) if slack water falls at ± 3 h from the midpoint of sojourn at either level.

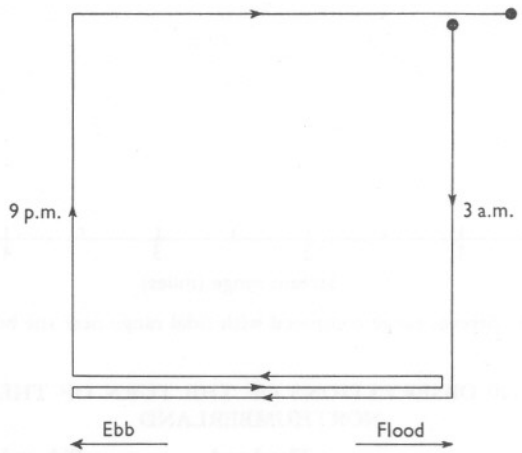

Fig. 7. The tidal stream forces acting on a planktonic organism which migrates vertically at 3 a.m. and 9 p.m. slack waters.

To illustrate this (Fig. 7), with the end of flood occurring at 3 a.m., if a planktonic organism retires to near the bottom at this time and returns to near the surface at 9 p.m. then over a day it will spend two ebb streams and one flood stream near the bottom and one flood stream near the surface. Since stream range is, on average, 0·7 miles greater near the surface than near the bottom it will, in a day and by stream influence alone, be moved this average distance southwards along the Northumberland coast.

In addition it may be noted that tidal streams, whose mean vectors at any one level are zero, may yet affect the spatial separation of pairs of planktonic organisms, provided such organisms undertake vertical migration at different times of day; tidal streams, like wind currents, can thus contribute to patterns of plankton distribution.

With the aid of the foregoing information and armed with records of wind, tidal range and hours of daylight it is possible to plot for a small number of days the movement of plankton along the Northumberland coast, together with some estimate of the error in our final position. Using local weather forecasts we may also attempt short-term prognosis. Over the short intervals envisaged the wind speed/current speed and stream range/tidal range relationships should be employed, not the mean vectors.

TABLE 7. FREQUENCY DISTRIBUTION OF REPRESENTATIVE ANIMALS TAKEN BY VERTICAL HENSEN NET

	Times of hauls and numbers of animals						
	08.00	09.30	11.00	12.30	14.00	15.30	17.00
29 Jan.							
Nyctiphanes couchii	0	0	8	4	0	8	12
Sagitta elegans	0	2	2	6	2	2	2
Calanus finmarchicus	27	10	58	96	99	45	40
12 Feb.							
Nyctiphanes couchii	14	123	259	117	69	197	
Sagitta elegans	8	16	29	30	19	17	
Calanus finmarchicus	198	155	287	277	251	522	
20 Feb.							
Nyctiphanes couchii	3	4	30	76	21	271	103
Sagitta elegans	1	24	35	74	33	38	20
Calanus finmarchicus	23	33	145	269	66	414	254
4 Mar.							
Nyctiphanes couchii	2	0	0	27	5	54	77
Sagitta elegans	1	8	3	9	13	9	8
Calanus finmarchicus	5	2	1	13	33	72	13
11 Mar.							
Nyctiphanes couchii	1	0	0	2	1	5	
Sagitta elegans	0	2	11	13	8	1	
Calanus finmarchicus	2	1	1	3	8	12	

Plankton in the Northumberland seas is unevenly distributed as it is elsewhere. Anraku (1956) has shown that uneven distribution occurs within very small limits of sampling. During the present work small-scale patchiness was again shown during attempts to secure replicate plankton samples from close beside the mid-water drogue. Samples were taken at $1\frac{1}{2}$ h intervals on 5 days, with a Hensen net which was hauled vertically from bottom to surface. By hauling the net close to the mid-water drogue much the same water was fished on each occasion, especially in the deeper regions where the bulk of the plankton was known to be lying. The frequency distribution of a few representative planktonic animals is shown in Table 7. Clearly, even allowing for small changes in depth, the uneven distribution of plankton extends to smaller limits than these.

What follows is presented as a promising but so far poorly pursued line of enquiry into plankton patchiness. It is concerned with the turbulent

nature of all sea current flow (Sverdrup, Johnson & Fleming, 1956, p. 90). Turbulence has the effect of altering the spatial relationships of water particles in a random manner; it is that diffusing component of flow (Ludlam & Scorer, 1954) which leads to the dispersal or aggregation of particles in a turbulent medium.

In August 1958 small-scale turbulence in water movement off Northumberland was encountered during an investigation of the accuracy of individual current measurements using drogues. Pairs of drogues used in the studies of gross currents were employed, both drogues being set to ride 6 fathoms below the surface. They were launched together one mile east of Blyth Light in 10 fathoms of water and their tracks were plotted over a period of 2 h. The bearing and distance of one drogue from the other was ascertained by bringing the ship in transit with the buoys and taking a compass bearing and vertical

TABLE 8. SEPARATION OF A PAIR OF DROGUES OVER TWO HOURS

(Separation in feet)

	Time interval (minutes)							Current speed	Wind speed
Date	0	20	40	60	80	100	120	(knots)	(knots)
5 Aug.	25	95	80	135	125	140	190	0·5	9
6 Aug.	15	40	60	75	50	65	75	0·2	13
7 Aug.	15	25	40	50	95	130	145	0·2	12
8 Aug.	15	60	45	100	90	130	140	0·2	5
11 Aug.	25	50	60	45	75	85	105	0·3	2
12 Aug.	40	45	70	75	95	85	140	0·3	1
13 Aug.	20	15	0	0	25	60	55	0·2	5
14 Aug.	35	10	40	70	95	145	170	0·4	11
15 Aug.	25	165	155	460	690	660	840	0·7	6
18 Aug.	15	30	5	25	65	110	130	1·0	6

sextant angle of the buoy poles which were 7·5 ft long. Distances were then extracted from table 15 of the Admiralty Manual of Hydrographic Surveying (1952). Runs with pairs of drogues were made on 10 occasions (Table 8); the maximum separation of the two drogues occurred on 15 August and was 840 ft. In view of the identical form of the buoys and drogues their separation can best be explained by invoking the principle of turbulent flow known to be associated with all sea currents.

The number of observations is too small for detailed statistical treatment and the results give little more than a qualitative impression of the turbulent forces present. Stommell (1949), in reviewing studies of turbulence, notes that by the so-called '4/3 law' dispersing forces increase as the separation between particles grows. Off Northumberland the separation during the second hour averaged 1·17 times that of the first hour, and the mean separation after 2 h was 200 ft. This suggests that separation after half a lunar day averages at least $\frac{1}{4}$ mile.

From the results of these studies the proposition is advanced that, regardless of other factors, the combination of varying wind currents and tidal streams,

turbulent flow, and the vertical migration of much of the plankton ensures that plankton distribution off Northumberland is of a dynamic and irregular nature; and that even when the intrinsic behaviour of the planktonic organisms is left out of account there remains a tendency directed towards patchiness.

My acknowledgements are due to the Coast Guards of Tynemouth for providing wind records. I remain greatly indebted to Skipper R. Harrison of the R.V. 'Alexander Meek' for his extensive co-operation in this work.

SUMMARY

Using free drogues observations have been made of the wind currents and tidal streams at three depths off the Northumberland coast. Records of current speed and direction for the near-surface, mid-water and near-bottom levels are given, together with tidal stream ranges. A relationship is established between wind current and wind, and between stream range and tidal range. Using pairs of drogues the characteristics of oceanic turbulence over a short period were investigated. The relationship of all these factors to plankton patchiness is considered.

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A NEW SPECIES OF COPEPOD FROM THE EDDYSTONE SHELL GRAVEL

By S. Krishnaswamy

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(Text-figs. 1–2)

Recent investigations of the bottom fauna off Plymouth by Spooner (1959*a*, *b*) have revealed the existence of a number of unusual and interesting crustaceans. The copepods collected during this investigation were very kindly placed at the disposal of the writer for study. A careful examination of the collection revealed the presence of a second and new species of *Paramisophria* Scott. This genus was established in 1897 by Scott on the basis of females collected in Scotland. Sars (1903) described the male for the first time. *Paramisophria cluthae*, the only species known previously, has been recorded from Plymouth.

Paramisophria spooneri nov.sp.

The Female (Fig. 1 A–L)

Body cyclopoid in shape and slightly compressed laterally. The anterior end of the cephalosome is rounded while the posterior margin is slightly produced and bears a small tooth about its middle (Fig. 1B). The urosome is short and is about a third of the total length. Caudal rami (Fig. 1D) are wider than long and each ramus bears six setae. Its inner margin is hirsute.

The antennule is short and only 20 segments could be made out clearly (Fig. 1C). It carries a number of aesthetes. The basal segment is nearly $4\frac{1}{2}$ times longer than the succeeding joint. The antenna is biramous, both the rami being two-jointed (Fig. 1F). The endopod is short and the terminal joint which is short carries one apical and two lateral setae. The exopod is long and slender, its terminal joint being slightly shorter than the basal joint, carrying two lateral and four apical setae. Thus the antenna differs from that of *Paramisophria cluthae* Scott, where the endopod is 6-jointed and also carries a larger number of setae. Other oral appendages are as in *P. cluthae*. The maxillule however differs in that it shows a reduction in the number of setae it carries (Fig. 1G).

The rami of the first four pairs of the swimming feet are 3-segmented. The first pair (Fig. 1H) is slightly shorter than the other pairs. $Basal_2$ has an outer seta. The first and the second joints of the exopod carry an outer spine and an inner seta each and the terminal joint one apical and two outer spines and four inner setae. The endopod is nearly as long as the exopod and the outer distal corner of the second and the third joints is produced into a spinous projection. The first joint carries an inner seta, the second joint two inner setae and the terminal joint a short plumose outer spine and four inner setae. The seta formulae of the second, third and fourth legs are shown in Table 1.

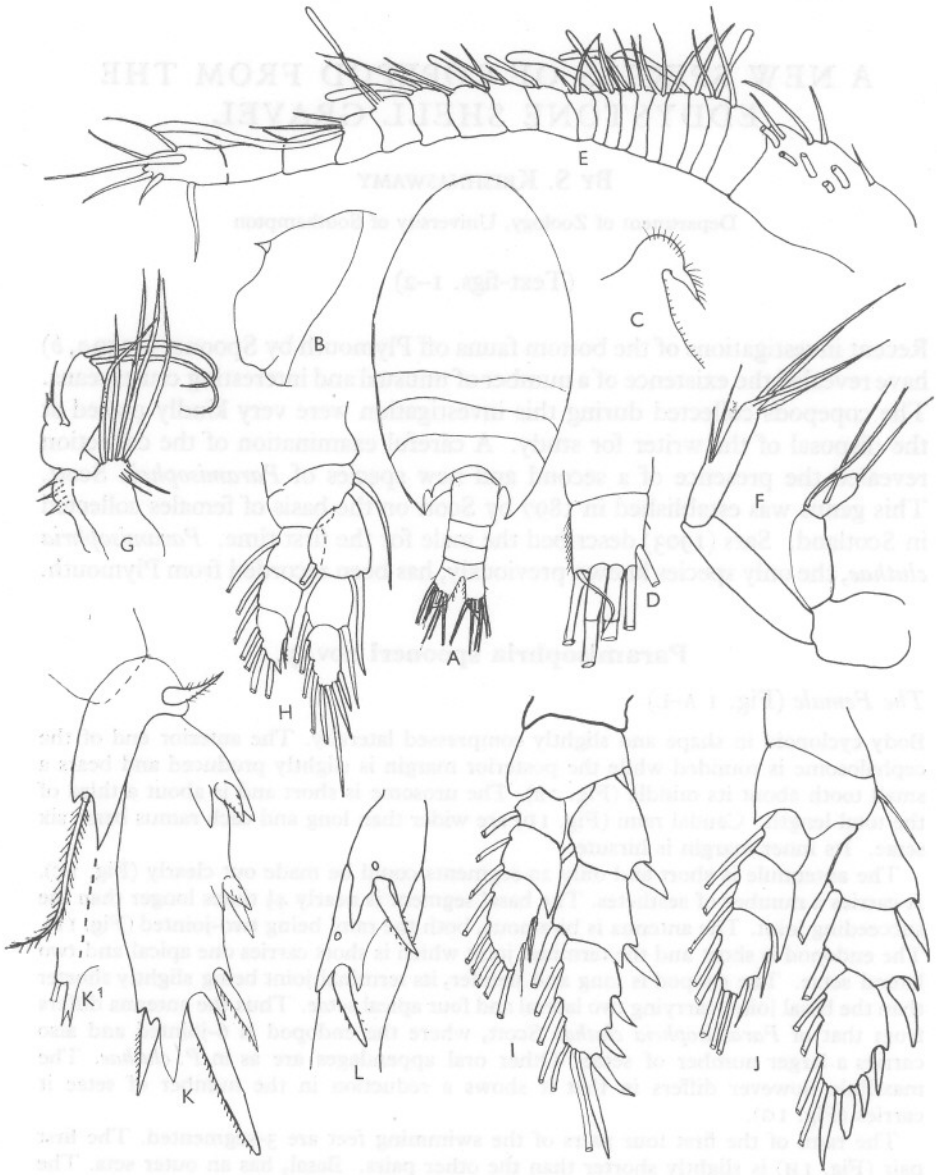

Fig. 1. *Paramisophria spooneri* n. sp. Female. A, dorsal view; B, posterior margin of metasome, lateral view; C, labrum, lateral view; D, caudal ramus; E, antennule; F, antenna; G, maxillule; H, I, J, swimming feet 1, 2 and 3; K, fifth leg; K^1, end of the inner projection with the bifid end; L, lateral view of the fifth leg showing the insertion of spines.

Fifth leg (Fig. 1K) consists of a small basal joint and a long and slender distal joint. The basal joint has an inner as well as an outer seta and is produced on the inner side into a process with a bifid end. The distal joint, which is three times longer than wide, is produced into a spinous process terminally. It carries an outer apical and three outer lateral spines. The outer lateral spines are borne on processes which are produced on either side, as may be clearly seen in a lateral view (Fig. 1L). In *P. cluthae* on the other hand, the inner margin of the basal joint is not produced into a bifid process. Length: 0·62 mm.

TABLE 1

	Exopod		Endopod	
	Outer spine	Inner setae	Outer spine	Inner setae
Second leg	1, 1, 4	1, 1, 5	0, 0, 2	1, 2, 6
Third leg	1, 1, 4	1, 1, 5	0, 0, 2	1, 2, 5
Fourth leg	1, 1, 4	1, 1, 4	0, 0, 2	1, 2, 5

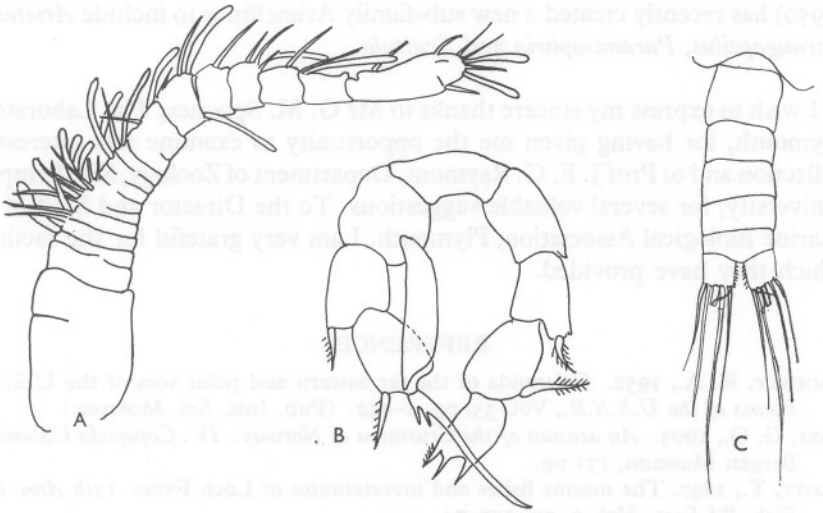

Fig. 2. *Paramisophria spooneri* n.sp. Male. A, antennule; B, fifth leg; C, urosome.

The Male

Resembles the female in general shape of the body. The urosome is however 5-jointed (Fig. 2C). The antennule is 19-jointed and is geniculate on the left side, having the last segment feebly and imperfectly hinged to the penultimate segment (Fig. 2A). The antenna and oral appendages as well as the first four pairs of swimming feet are as in the female. The fifth pair is modified and is 5-jointed (Fig. 2B). On the left the third and the fourth joints carry an outer plumose spine each while the terminal joint which is spatulate is produced into three processes which are spinous, thus giving the appearance of a three-pronged process to the joint. On the right side, the second joint carries a blunt process which is long and reaches up to the end of the fourth joint. The third and the fourth joints carry an outer plumose spine each. The terminal joint is a long slender process with two teeth at its base on the outer side. Length: 0·62 mm.

Remarks

The present species resembles *Paramisophria cluthae* Scott very closely. It differs from it, however, in the structure of the antenna and fifth legs. The presence of a long unarmed blunt process on the second joint on the right side of the male fifth leg is a feature unique for this species. In view of these differences the present species is treated as a new one. I have great pleasure in naming it after Mr G. M. Spooner of the Plymouth Laboratory.

The presence of an inner lobe on the right side of the male fifth leg necessitates the alteration of the generic diagnosis given by Sars (1903, p. 127) to read as follows: 'those in male 5-articulate, *with* or without any lobe inside the 2nd joint'.

Sars (1903) included this genus under the family Arietellidae, and Brodsky (1950) has recently created a new sub-family Arietellinae to include *Arietellus*, *Paraugaptilus*, *Paramisophria* and *Scottula*.

I wish to express my sincere thanks to Mr G. M. Spooner, The Laboratory, Plymouth, for having given me the opportunity to examine this interesting collection and to Prof J. E. G. Raymont, Department of Zoology, Southampton University, for several valuable suggestions. To the Director and Staff of the Marine Biological Association, Plymouth, I am very grateful for the facilities which they have provided.

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THE POLYCHAETE *MAGELONA FILIFORMIS* SP. NOV. AND NOTES ON OTHER SPECIES OF *MAGELONA*

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(Text-figs. 1 and 2)

As already mentioned (Wilson, 1958), a species of *Magelona* from clean sand near low water at Mill Bay, Salcombe, has not yet been described. This worm was first noticed in 1939; it is recorded in the 1957 edition of the *Plymouth Marine Fauna* as '*Magelona* sp.' and it is there mentioned that artificial fertilizations were made and larvae reared in April–September 1939, and that these larvae differed from those of the other two *Magelona* species from the Plymouth district (*papillicornis* F. Müller and *alleni* Wilson). The worm is still common in the same locality at Salcombe, in the same ground and often in the same spade-full as *papillicornis*, but it is not as abundant as the latter and probably not as abundant as it was when first seen in 1939. A very good low tide receding below datum is needed to collect it; it is easily overlooked on account of its fragility and fine thread-like appearance, mature females coloured pink by their contained eggs being more readily seen while digging than translucent immature worms or white males. Worms are difficult to collect whole, the tail end usually being left behind in the sand.

Until recently this worm had only been seen at Salcombe. Now, through the kindness of Mr A. D. McIntyre, specimens of a dwarf variety from inshore waters off the east coast of Scotland have also been studied and are discussed below.

The following description of the species is based on specimens collected at Salcombe in 1939 and recently. Some specimens were simply preserved in formalin, others narcotized in 7% magnesium chloride in tap water and fixed in Bouin's fluid used hot and then preserved in alcohol. Most of the drawings in Fig. 1 are from such fixed and preserved specimens mounted unstained in euparal.

Special thanks are due to Dr Olga Hartman, Allan Hancock Foundation, Los Angeles, for her continued interest in my worm since she first saw specimens in 1939, and for her helpful correspondence then and recently.

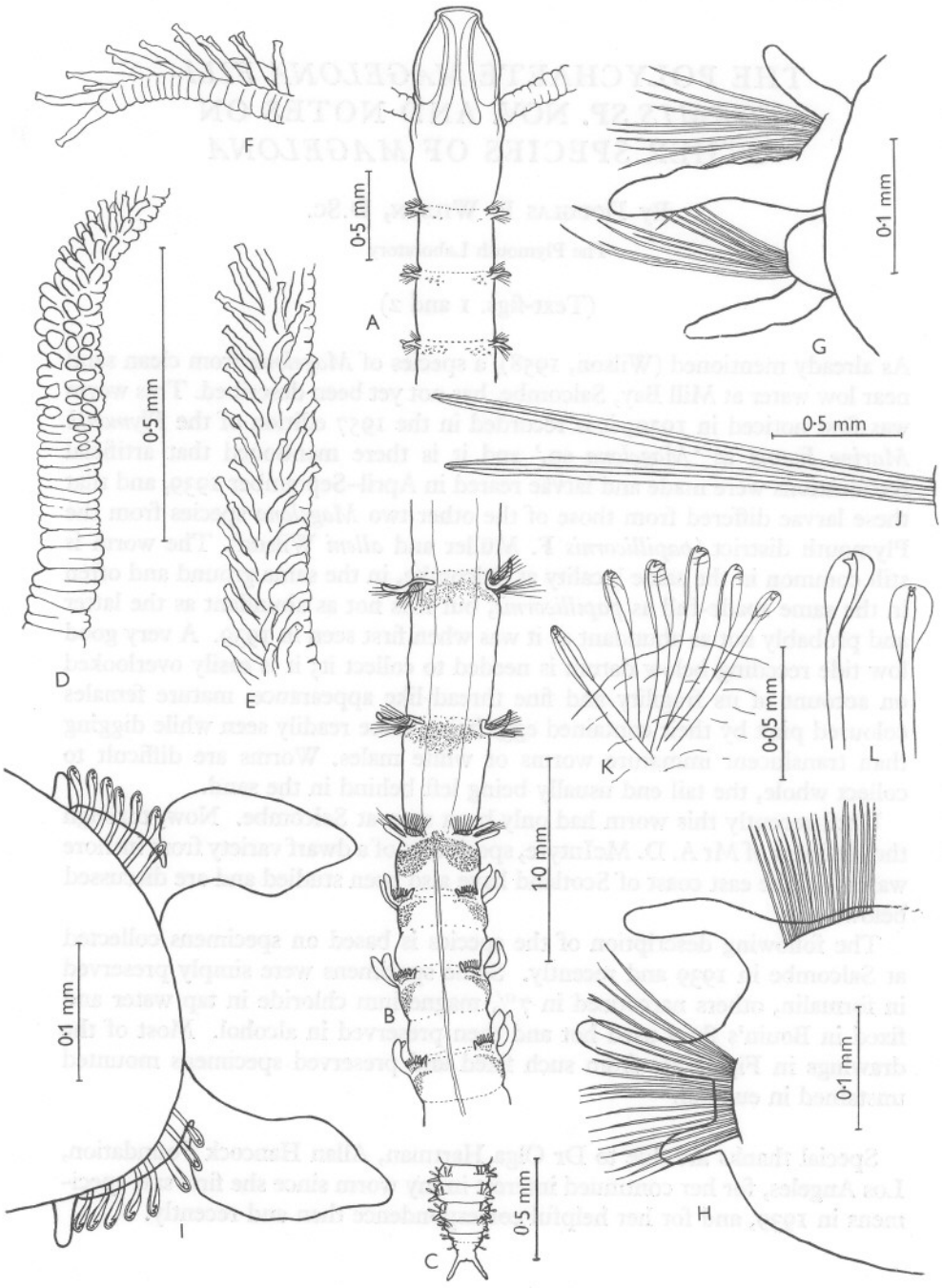

Fig 1. For legend see bottom of facing page.

Magelona filiformis sp.nov.

Adult specimens in the relaxed state reach lengths of more than 80 mm, possibly 100 mm, but are only about 0·3–0·5 mm wide. The width is fairly uniform for most of the body length except that the extreme posterior end tapers gradually to the anus, and there is a slight constriction at the ninth setiger. Total number of setigers in a complete specimen was 142; some individuals probably have more. Prostomium (Fig. 1A) eyeless, spatulate, longer than wide with two low dorsal longitudinal ridges; anterior border relatively wide, slightly convex and with corners or slight horns on each side. The everted proboscis is globular, longitudinally ridged. On either side of the mouth, below the postero-lateral corners of the prostomium, there arises a long slender tentacle roughly twice as long as the anterior region of the body (in narcotized and fixed specimens). A short proximal portion is transversely wrinkled and without papillae; for most of its length the tentacle carries on one face long capitate papillae arranged in two main rows (Fig. 1D–F), the other face being as wrinkled as the base.

The first nine pairs of parapodia carry only double-winged bristles (Fig. 1J); these wings are most readily seen in formalin preserved material examined in water. The first eight pairs of parapodia are all of similar structure (Fig. 1G), the notopodium with a short dorsal cirrus and a long ventral finger-like process above and below the fifteen (approximately) bristles, the neuropodium with a ventral finger-like cirrus of moderate length and approx. ten bristles. Segmental limits are difficult to observe, the anterior region being largely free from definite annulations. Slight grooving sometimes occurs immediately anterior to the parapodia but sections show that the septa are in advance of these grooves, usually no annulations marking their positions. The parapodia are situated towards the front end of each segment. Parapodia are spaced at progressively increasing distances apart from the first pair to the eighth, the hinder segments being markedly longer than broad. The limits of the ninth setiger are ill-defined externally and need to be worked out in sections. The ninth parapodia (Fig. 1H) are of special construction; the notopodium has an almost comb-like row of 25–30 fairly straight double-winged bristles and a ventral finger-like process of moderate length; the neuropodium has two finger-like processes, the dorsal moderately long the ventral short, and 25–30 curved double-winged bristles arising from a setal-sac anterior to the processes.

The parapodia of the long posterior region from the tenth setiger backwards are all similar with rather widely separated noto- and neuropodia (Fig. 1, I), each with a foliacious lamella on a short stalk and a row of hooks on a torus dorsal or ventral to it, and a short dorsal or ventral cirrus. The lamellae are approximately equal in size and are slightly larger on anterior than on posterior segments. The hooded hooks each have one large tooth surmounted by two smaller teeth (Fig. 1K, L). The hoods appear larger and more widely spread in formalin preserved material examined in water (Fig. 1L) than in fixed specimens mounted in euparal (Fig. 1K). At the anterior end

Legend to fig. 1

Fig. 1. *Magelona filiformis* sp.nov. A, dorsal view of prostomium and first three setigers; B, dorsal view of body in the region of setigers 7 to 12; C, dorsal view of anal extremity; D, E and F, proximal, middle and extreme distal portions of a tentacle showing arrangement of capitate papillae; G, 6th parapodium; H, anterior view of 9th parapodium; I, 13th parapodium; J, emergent portions of two adjacent bristles, from a formalin preserved specimen examined in water; K, some hooded hooks from a specimen mounted in euparal, the full lengths of the shafts not being drawn; L, lateral and front views of hooded hooks from a formalin preserved specimen examined in water.

of the posterior region the intersegmental grooves are ill-defined; they appear to lie mid-way between the parapodia (Fig. 1B). Farther back segmental limits are more clearly visible and in passing back along the body of the worm there is a gradual transformation in the position of the parapodia which come to lie at the posterior end of each segment, which except when contracted or swollen with genital products are markedly longer than broad. The last few segments have incompletely developed parapodia. There are two anal cirri (Fig. 1C).

Living specimens are in general colourless with a transparent body wall through which the gut, pale pink in the anterior region, transparent in the mid region and brownish posteriorly, is seen. In mature specimens pink ova or creamy-white sperm showing through the body wall colour the region of the body in which they lie, that is most of the posterior region except for some of the anterior segments. In a mature specimen swollen genital segments give a moniliform appearance to the worm. Careful examination reveals a pattern composed of patches of epithelia cells with brownish yellow intracellular granules. The dorsal aspect of this pattern is indicated in Fig. 1A and B. The granules occur dorsal to and a little behind the parapodia, reaching their greatest density on a few segments just in front of and behind the transition region between the eighth and tenth setigers, there being a particularly large and dense patch of granular cells just behind each ninth parapodium. On the ventral surface there are more of these cells with granules, at the bases of the parapodia from about the fourth pair and in groups mid-ventrally anterior to the parapodia, and there is again concentration in the region of the ninth parapodia. Farther back they form a thin interrupted band on each side of the mid-ventral line and there are prominent longitudinally elongate patches on the sides of the segments, forming on each side of the worm an almost continuous band of brownish pigment interrupted only at the parapodia.

The following characters in combination are sufficient to distinguish *filiformis* from all other known species: (1) the presence of small prostomial horns; (2) the structurally modified ninth parapodium in which the dorsal cirrus is absent and the bristles are similar to those in front; (3) the finger-like processes of all anterior region parapodia, the first eight pairs with dorsal and longer ventral cirri and the notopodium with an even longer process below the bristles; (4) the widely separated and approximately equal sized foliacious lamellae of the tenth and succeeding setigers.

The typical form is known only from sand (fairly clean to rather muddy) near E.L.W.S.T., Mill Bay, Salcombe, south Devon, in the same ground with *M. papillicornis* F. Müller. It lives in fragile tubes, which may be no more than the walls of burrows lined with a secretion to which sand grains adhere. A minute form (see below) is found in muddy ground off the north-east coast of Scotland.

The above description and drawings are based on several specimens from Salcombe. A specimen has been chosen as the holotype and deposited in the British Museum (Natural History) and given the number 1959.4.2.1. It is a complete worm of 142 setigers in two portions, the last few segments having broken off during preservation. Both portions are mounted in euparal. Other specimens have also been deposited and given the paratype numbers 1959.4.2.2/10.

Magelona filiformis sp.nov. **minuta** var.nov.

McIntyre (1958) has recorded as *M. rosea* Moore, a small magelonid from muddy sand off the north-east coast of Scotland, it being particularly abundant in the Aberdeen coastal area (at one station 26 were found in $\frac{1}{5}m^2$). It was also present in his bottom samples from St Andrews Bay and to a lesser extent from Smith Bank. At my request Mr A. D. McIntyre kindly supplied me with some of his specimens (preserved in formalin). The immediate impression was of their small size and transparent tissues, in these respects recalling to mind large magelonid larvae from the plankton, but they had, of course, been obtained from grab hauls in muddy sand. A typical specimen of 60 segments incomplete posteriorly was 15 mm long and varied in width from 0·20 to 0·30 mm. For comparison 60 segments of a Salcombe worm measured 38 mm long and varied in width from 0·30 to 0·50 mm. Close examination has shown these North Sea worms to agree in structural detail with the much larger *filiformis* specimens from Salcombe, in fact except for size they cannot be separated from them. Mr McIntyre has himself compared them with Salcombe specimens and with tracings from my drawings and he is in agreement with this. He informs me that he has never had any larger specimens than these very small ones, although his collections have been made throughout the year, and he has never had them from the shore or from water of a depth of less than 10 m. These small worms begin to appear with *papillicornis* (which is found in shallower water) at depths a little greater than 10 m; they were common at one station at 18 m and were found down to 59 m. It would appear therefore that these worms are genuinely a small variety and not merely young ones. Unfortunately I have not been able to satisfy myself that genital products are present in any of the specimens I have seen (collected January, April and July), but as they were incomplete posteriorly it is just possible that genital segments had been lost or the products were shed during preservation.

On these small transparent worms the pigmented patches of intracellular granules show up strikingly; by reflected light against a dark background the pigment is yellow rather than brownish yellow, but it follows exactly the pattern already described for the Salcombe worms.

Specimens of this dwarf variety have also been deposited in the British Museum (Natural History).

COMPARISON WITH OTHER KNOWN SPECIES

It has become the practice to divide the genus *Magelona* into two convenient groups, those with prostomial horns and those without. The latter includes *papillicornis* F. Müller, 1858; *obockensis* Gravier, 1906; *rosea* Moore, 1907; *pitelkai* Hartman, 1944*a*; *californica* Hartman, 1944*b*; an unnamed species

near *californica* mentioned by Hartman (1951); *alleni* Wilson, 1958. The horned species include *cincta* Ehlers, 1908 (see Wilson, 1958); *pacifica* Monro, 1933; *japonica* Okuda, 1937, and *japonica* var. *koreana* Okuda, 1937; *cornuta* Wesenberg-Lund, 1949; *cerae* Hartman & Reish, 1950. By virtue of its small horns *filiformis* would thus be included in this latter group; parapodial characters clearly separate it from *cincta*, *pacifica*, *japonica*, *japonica* var. *koreana*, and *cerae*. Parapodial characters separate it also from *cornuta*, some notes on which follow.

There remains the doubtful species *longicornis* Johnson, 1901, described by Johnson from two imperfect specimens collected at West Seattle in 1899. It is difficult on the basis of Johnson's incomplete description and poor figures (when he wrote only one other species was known, namely *papillicornis*, and he was easily able to separate his worms from that) to point to clear-cut distinctions between his species and *filiformis*. He mentions that the proboscis lacks corrugations (grooved in *filiformis*) and that the hooks are bidentate (tridentate in *filiformis*). Hartman (1944*b*, pp. 318–19) has discussed *longicornis*; she points out that 'it is not certain whether the prostomium has frontal horns' and discusses an ambiguity concerning the setae. Her conclusion is that *longicornis* be 'regarded as a species *incertae sedis*'. In a private letter (from which she very kindly allows me to quote) she describes the species as unrecognizable, mentioning that there are at least five recognizable species in the north eastern Pacific (excluding Okuda's two species from Japan) and that 'which one of these (if any) might be *M. longicornis* of Johnson from Washington, would be sheer guess, since Johnson left no types and the published account is useless. There may be specimens, so labelled, in the U.S. Nat. Mus. but they can hardly be regarded as type specimens.'

In the British Museum (Natural History) there is a tube of specimens labelled *Magelona longicornis* 1924.5.5.58/62 and I am indebted to the Museum for the loan of this. The inside label indicates that the worms were collected on Pleasant Beach, Seattle, and were determined by F. A. Potts (who probably collected them). This tube contained one nereid worm and five *Magelona*; three of the latter are *papillicornis* or a closely allied species, while the remaining two agree with Johnson's imperfect description and may possibly be the species he saw. An examination of these two worms shows the following features: a prostomium horned a little more prominently than in *filiformis*: proboscis lightly grooved longitudinally; foliacious, not finger-like, lobes below the notopodial bristles; ninth parapodia with foliacious lobes, dorsal cirri but no ventral cirri; lamellae of tenth and succeeding setigers foliacious, dorsal and ventral approximately equal in size and apparently not stalked but springing from broad bases; hooks definitely bidentate, the main tooth being surmounted by a single smaller tooth. In almost all these points these specimens differ from *filiformis*. The two specimens are both incomplete posteriorly; one is of about 30 setigers and measures approximately 23 mm

long and has widths of about 1·0 and 1·5 mm in the anterior and posterior regions respectively.

Fauvel (1936, p. 63) has identified as *Magelona rosea* Moore a worm from the Atlantic coast of Morocco, but his figures and description of it do not agree with Moore (1907, pp. 201–4, pl. XVI). Fauvel, for instance, draws a markedly horned prostomium, whereas Moore shows a rounded frontal margin. Fauvel's worm cannot from his figures and description be identified with any known species. *M. rosea* has been recorded by Southern (1914, p. 105) from Killary Harbour, Ireland, largely it would seem because the setae of the ninth setiger tapered to a point. Moore himself confirmed the identification from specimens sent by Southern. Eliason (1920, p. 52) records three fragments of the same species from the Öresund. It is desirable that these records be checked in the light of modern knowledge of the genus.

COMPARISON WITH *MAGELONA CORNUTA* WESENBERG-LUND

M. filiformis bears some resemblance to *M. cornuta* Wesenberg-Lund (1949, p. 328 and fig. 36) from the Gulf of Iran. As in that species each posterior parapodium bears two large foliacious lamellae of equal size and the anterior parapodia as drawn in Wesenberg-Lund's figure appear to be similar to those of *filiformis*. Both species have frontal horns, but those of *cornuta* are much more pronounced. Moreover, *cornuta* is decidedly larger than *filiformis* and is differently coloured, and the hook as shown in Wesenberg-Lund's figure and description ('a blunt bidentate tip') would appear to be of an aberrant type for the genus. However, normal *Magelona* hooks have a similar appearance to that shown in her drawing when they are seen in partial front view. Normally they do not arise from papillate processes as indicated by Wesenberg-Lund's artist, but are arranged in transverse rows dorsally and ventrally. To check these details and to enable a better comparison to be made between what appeared to be two closely similar species I have, through the kindness of Dr Wesenberg-Lund, examined her type specimen of *cornuta*. This has enabled me to add to and amend the original description.

Fig. 2A is a drawing of the eighth parapodia of *cornuta* seen *in situ* in dorso-lateral view, the view presented by the specimen as it lies in a dish. These parapodia seem to be similar to all those anterior to them, more doubtfully so to the ninth parapodia. One of the latter had been removed for the purpose of the original description and the other is difficult to examine in detail without spoiling the specimen. In order to avoid further damage to the specimen I have done no more than examine the parapodia *in situ*, or in microscopical preparations of them loaned to me by Dr Wesenberg-Lund. Examination *in situ* is difficult, but by critically positioning a spot-light most details can be made out. In the eighth and anterior parapodia the notopodial bristles arise between a minute dorsal cirrus and a foliacious lamella of very transparent

tissue. On the eighth setiger this lamella is clearly visible on the right side of the worm but on the left it is seen nearly edge-on (see Fig. 2A). The neuropodial bristles spring between two finger-like processes the lower one being the ventral cirrus; in Fig. 2A these are clearly indicated on the left side.

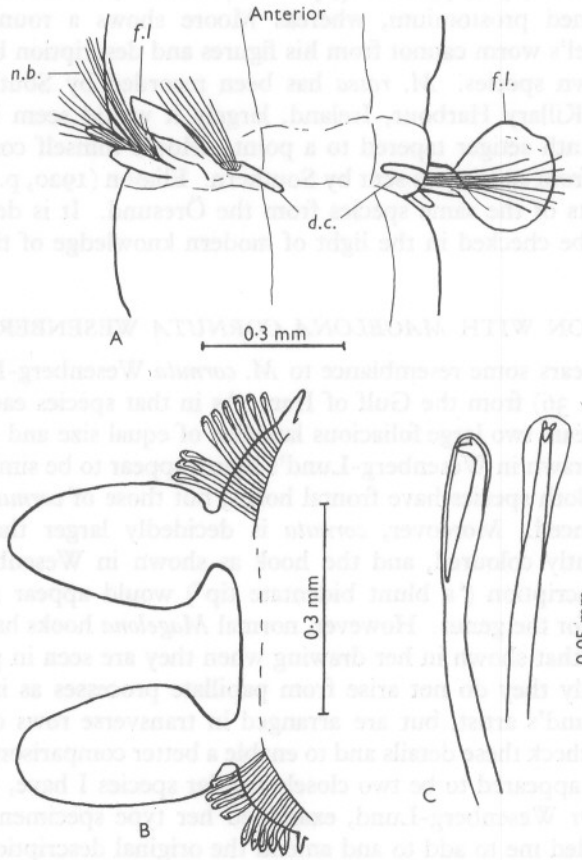

Fig. 2. *Magelona cornuta* Wesenberg-Lund. Some details from the type specimen. A, semi-dorsal view of the eighth pair of parapodia, with the worm heeled over to the right and showing more of the left parapodium than of the right. *d.c.*, dorsal cirri; *f.l.*, foliacious lobe below notopodial bristles; *n.b.*, neuropodial bristles with finger-like process above and ventral cirrus below. B, a parapodium of about the fifteenth setiger. C, hooded hooks in lateral and front views.

In two features therefore these anterior parapodia differ from those of *filiformis* which has a finger-like process below the notopodial bristles and not a foliacious lamella, and which lacks the upper of the two neuropodial processes of *cornuta*. These parapodial differences clearly separate the two species.

The posterior parapodia of the two species are very much alike (compare

Figs. 1, 1 and 2B), as already mentioned. The hooded hooks of *cornuta* are arranged in short transverse rows (about ten hooks to a row) dorsally and ventrally and do not arise from papillate processes (see above). The hooks (Fig. 2C) are tri-dentate; in front view two minute teeth above the main tooth are clearly visible. In partial front view they do look similar to Wesenberg-Lund's figure. A minute dorsal and ventral cirrus at the extremity of each row of hooks can be seen with careful illumination. The large lamellae are extremely transparent.

SUMMARY

A *Magelona* first found at Salcombe in 1939 is described as a new species and given the specific name *filiformis*. It is distinguished from all other species of the genus by its parapodial characters and from some species by its small prostomial horns. So far it has been obtained in its typical form only at Salcombe.

A markedly dwarf variety from inshore waters off the east coast of Scotland is given the variety name *minuta*. Formerly recorded by Mr A. D. McIntyre, who found it, as *M. rosea* Moore it is structurally identical with *filiformis* from Salcombe but differs from it by being very much smaller when adult.

M. filiformis is compared with other species of *Magelona*, in particular *longicornis* Johnson which is shown to have been imperfectly established, and with *cornuta* Wesenberg-Lund, the original account of which is amended and expanded following a re-examination of the type specimen.

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THE BRITISH SPECIES OF *LUTRARIA* (LAMELLIBRANCHIA), WITH A DESCRIPTION OF *L. ANGUSTIOR* PHILIPPI

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(Plates I and II, and Text-figs. 1–4)

The Lutrariidae are large lamellibranchs which burrow deeply in bottom-deposits on the shore and in shallow water. The foot is relatively weak, and they live permanently buried 30 cm or so beneath the surface, maintaining connexion with the overlying water through their long siphons, which are united in a common sheath for practically their whole length. The commonest British species, *Lutraria lutraria* (L.) is found at low-water mark on shores of muddy sand, and offshore its siphons are sometimes taken by the dredge or grab. Another species, *L. magna* (da Costa) is more local, and appears to inhabit muddy deposits near the mouths of estuaries. It is a southern form, being occasionally found on the south and west coasts of the British Isles, and, like *L. lutraria*, its range extends southward to the Mediterranean.

Examination of shells from offshore shell-gravel deposits near Plymouth has revealed the presence of a third form, which appears to correspond with the *L. elliptica* var. *angustior* of Philippi (1844), a variety subsequently noted by several other authors under different names. Although a distinct form, it has never been assigned specific rank, perhaps because many of the specimens found would have been dead shells washed up on the beach, in which the hinge-teeth, an important character for identification, are usually worn or broken. This form was taken by Ford (1923, 1925) at Plymouth, but was identified as *L. magna*, and I have also collected specimens from off other parts of the south coast of England (see Appendix for list of localities).

LUTRARIA ANGUSTIOR PHILIPPI

?*Mactra lutraria* variety. Turton, 1819, p. 85.
Lutraria elliptica (form). Brown, 1827, plate xii, fig. 3.
Lutraria elliptica (form). Brown, 1844, plate xliii, fig. 3.
Lutraria elliptica var. *angustior* Philippi, 1844.
Lutraria elliptica var. *intermedia* Sowerby, 1859, plate iv, fig. 1, *nec.* fig. 2.
Lutraria elliptica var. *alterutra* Jeffreys, 1863.
Lutraria elliptica var. *angustior* Philippi. Hidalgo, 1870, plate 6, fig. 2.
Lutraria elliptica var. *attenuata* Monterosato, 1878.
Lutraria lutraria var. *angustior* Philippi. Bucquoy *et al.* 1896.

A form of *L. lutraria* with a shell narrower than the type has been noted by a number of authors, but where the description is brief or unaccompanied by an illustration, some doubt must remain as to the identity of the specimen. Descriptions in the literature do not indicate that the hinge-teeth or pallial scar are different from those of typical *L. lutraria*, so that identity with the form here described is based solely on the shape and proportions of the valves. The first illustrations of *L. angustior* are in Brown (1827, 1844), while those of Sowerby (1859), Hidalgo (1870), and especially Bucquoy *et al.* (1896) are also of the same species. The description, unaccompanied by a figure, by Jeffreys (1863) of var. *alterutra* corresponds with that of *L. angustior*, but although he mentions Philippi's record of *L. lutraria* from Sicily he evidently does not regard the variety as synonymous with Philippi's var. *angustior*. Philippi's description is brief, giving dimensions of the shell, and the opinion that it is intermediate between *L. lutraria* and *L. magna*.

Philippi's original specimens are apparently no longer in existence, but his opinion that it is intermediate between the two other species strongly suggests that it is the species described here. Bucquoy *et al.* (1896) give the most detailed description, accompanied by photographs, of *L. lutraria* var. *angustior*, based on specimens from the French Mediterranean coast, but unfortunately do not illustrate or describe characters on the inside of the shell. These authors considered that the variety they describe corresponds not only with Philippi's description but with that of specimens from British waters described by Brown, Sowerby and Jeffreys. This lends additional support to the use of Philippi's name *angustior* for the British specimens described here.

Shell

The shell is equivalve, and of an oval-elongate shape, the specimen shown in Pl. I, figs 2 and 3, measuring 11 cm long × 5·9 cm high. The ventral and posterior dorsal margins are moderately straight and parallel with one another. The posterior end is fairly evenly rounded, but the anterior end is usually more sharply curved towards the dorsal side (Fig. 1B). The valves gape slightly in front, and more so posteriorly, but the arrangement of the hinge in all members of the genus is such that the valves can rock longitudinally about the hinge cartilage, so that the relative gape at either end can be varied. The shell is moderately thick, and is marked on the outside with concentric striae corresponding to the lines of growth. The periostracum on the smallest specimens is colourless and transparent, on larger specimens it is brown and slightly glossy, but on all the adults examined it was totally abraded, exposing the white prismatic layer. The beaks are not very acute, and are scarcely directed forward. They are situated about two-fifths of the shell's length from its anterior end. The ligament, as in the other species, is small and mainly internal. There is no lunule and the escutcheon is virtually absent, as in the other species.

The inside of the shell is white and slightly glossy, with well-marked muscle scars and pallial line. There is a deep U-shaped pallial sinus which extends slightly forward of the mid-point of the length of the shell. The scar marking the lower edge of the sinus runs almost parallel and close to the ventral pallial scar for the greater part of its length, the two usually merging for a part of the length of the sinus. The range of variation of this character is between that shown in Fig. 1B and that in Pl. I. The distal end is usually marked by an oval enlargement of the scar. The hinge plate is fairly flat, and bears a triangular pit to accommodate the large cartilage. The posterior dorsal edge of this pit is almost straight, and makes an angle of about 15° with the adjacent dorsal margin of the shell. Each valve bears two erect cardinal teeth. In the right valve these are both laminar and diverge from one another at rather less than a right angle. The first tooth curves downwards so that distally it makes an angle of 60–80° to the second tooth. The first tooth consists of two imbricating teeth, one lying beyond the other, and both point in the same direction. In a worn shell they may appear as a single tooth. Behind the cartilage is a low lateral tooth set in an oval-elongate depression. There is no anterior lateral tooth. The inner dorsal margins of the shell are not grooved, as in *L. lutraria*, and the dorsal surfaces tend to curve inwards so that the outer, striated, surface is visible in a lateral view of the interior (Fig. 2B).

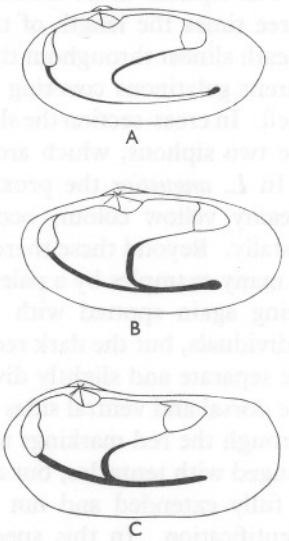

Fig. 1. Interior of the right valve of *Lutraria* shells, showing muscle and pallial scars. A, *L. lutraria*; B, *L. angustior*; C, *L. magna*.

The two cardinal teeth in the left valve engage between the cardinals of the right valve; the first is slightly convex on its outer side, the second straight. On their inner sides the teeth are thickened, and they are fused together for about a third of their lengths. There is a thin tongue-shaped tooth at the side of the cartilage pit, behind the second cardinal tooth. It is present in all three species (see Pl. II), but is broken off in the majority of dead shells collected. Behind the cartilage pit is a low lateral tooth, as in the right valve, and there is a curved anterior lateral tooth running almost parallel with and outside the first cardinal tooth. Both lateral teeth are set in shallow depressions, but the remainder of the inner dorsal margin is convex.

Siphons

This description is based on a single whole adult specimen, and on a number of siphons cut off by the dredge. Since the siphons are all that may be captured on offshore grounds, the differences between those of the three species are of some importance for identification.

The siphons of all Lutrarias are very long, and can be extended to two or three times the length of the shell. The two tubes are united in a common sheath almost throughout their lengths, the sheath being invested with a transparent gelatinous covering which is an extension of the periostracum of the shell. In cross-section the sheath is oval with a slight groove or 'waist' between the two siphons, which are of about the same cross-sectional dimensions.

In *L. angustior* the proximal four-fifths or so of the sheath is a uniform creamy yellow colour, becoming marked with small strawberry-red spots distally. Beyond these there is a circular band of a uniform dark red, followed in many examples by a pale whitish band, the extreme tips of the two siphons being again spotted with strawberry red. The colour pattern varies with individuals, but the dark red band is present in all examples. The two siphons are separate and slightly diverging at their tips (Fig. 3B and Pl. I), and along the dorsal and ventral sides of the common sheath there is a pale line running through the red markings to the tip of each siphon. The siphon openings are fringed with tentacles, but as these are only to be seen when the living animal is fully extended and not disturbed in any way, they are of little use for identification. In this species the dorsal siphon is fringed with numerous small tentacles, while the ventral siphon has eight (or perhaps nine) longer tentacles. There are probably small tentacles between the longer tentacles, as in *L. lutraria*, but these were not distinguished. In *L. lutraria* there are two circles of smaller tentacles outside the opening of the ventral siphon. These were not observed in the living specimen of *L. angustior*, but it cannot be said with certainty that they are absent in this species.

LUTRARIA LUTRARIA (L.)

Shell

The shell grows a little larger than in the previous species, a fairly large specimen measuring 13 cm. long × 7·2 cm. in height. The dorsal margins are more convex and the anterior end more evenly rounded, so that the outline is more nearly elliptical (Fig. 1A). Some specimens, however, have shell proportions similar to those of *L. angustior*. The valves gape to about the same extent as in that species. The shell substance is a little thinner, and the outside rather smoother. The periostracum in all but the smallest shells is an olive-brown colour, glossy, and in addition to the concentric lines of growth, is marked towards the umbo with faint radial lines, which are of use in identifying small specimens (p. 564). The beaks are more acute than

in *L. angustior*, and are situated at about the same position relative to the shell's length.

The pallial scar differs from that of *L. angustior*. The scar marking the lower edge of the sinus meets the ventral pallial scar in a narrow V, the two remaining well separated until they meet at the posterior end of the sinus (Fig. 1A). At this point the scar tends to be expanded as in *L. angustior*. Even in narrow shells having similar proportions to *L. angustior* the two scars remain well separated.

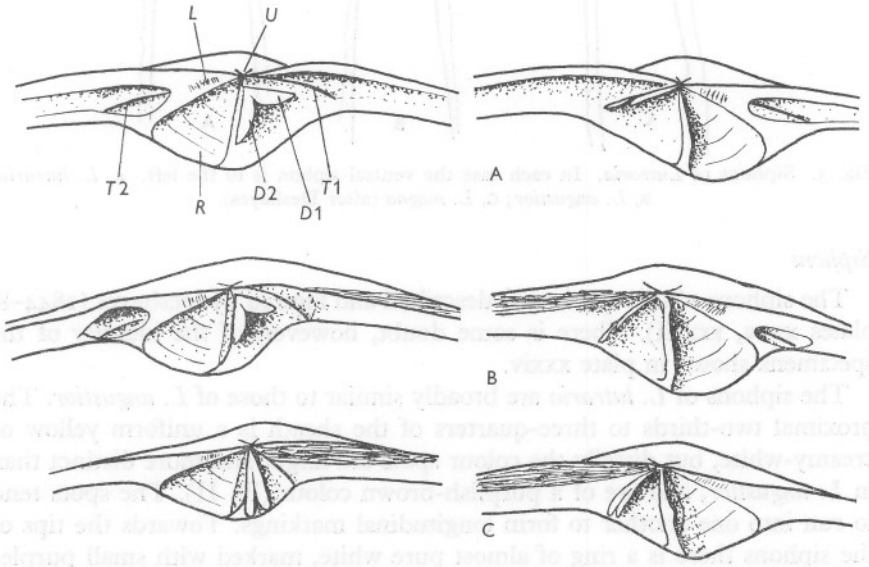

Fig. 2. Hinges of the left and right valves of *Lutraria* shells. The linguiform tooth between the second cardinal tooth and the cartilage in the left valve has been omitted in each case as it is so frequently broken off. *D*1, *D*2, 1st and 2nd cardinal tooth; *T*1, *T*2, 1st and 2nd lateral teeth; *L*, ligament attachment area; *R*, cartilage pit; *U*, umbo. Note that in no case is a pair of valves from a single specimen drawn, so that the left and right valves drawn do not exactly correspond. *A*, *L. lutraria*; B, *L. angustior*; C, *L. magna*.

The hinge (Fig. 2A) differs only in detail from the preceding species. The posterior dorsal outline of the cartilage pit is straight or slightly convex, and makes a greater angle (about 30°) with the adjacent dorsal margin of the shell. In the right valve the two cardinal teeth are straight and diverge at 80°–90°. The first tooth is made up of two imbricating teeth in line, as in *L. angustior*. There is a low posterior lateral tooth, but the depression in which it is set is continued posteriorly as a shallow rounded groove inside the dorsal margin of the shell. A similar but rather deeper groove runs forwards from the umbo, passing dorsally to the first cardinal tooth. The dorsal edges of the shell are produced upwards and inwards in this species, so accentuating the depth of these grooves.

In the left valve the two cardinal teeth diverge at a slightly greater angle than in *L. angustior*, and the first tooth is straight on its dorsal side. There is a low anterior and posterior lateral tooth, and the inner dorsal margin of the shell is grooved, as in the right valve.

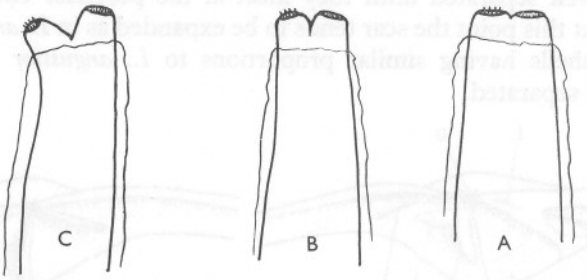

Fig. 3. Siphons of *Lutraria*. In each case the ventral siphon is to the left. A, *L. lutraria*; B, *L. angustior*; C, *L. magna* (after Deshayes).

Siphons

The siphons of this species are described and figured by Deshayes (1844–8, plates xxxv, xxxvii). There is some doubt, however, of the identity of the specimens shown in plate xxxiv.

The siphons of *L. lutraria* are broadly similar to those of *L. angustior*. The proximal two-thirds to three-quarters of the sheath is a uniform yellow or creamy-white, but distally the colour spots are larger and more distinct than in *L. angustior*, and are of a purplish-brown colour (Pl. II). The spots tend to run into one another to form longitudinal markings. Towards the tips of the siphons there is a ring of almost pure white, marked with small purple-brown spots, and the pale lines along the dorsal and ventral sides of the sheath are very distinct. The siphons are scarcely separated, and do not diverge, at their tips (Fig. 3A). The form of the tentacles at the tips of the siphons is described by Deshayes.

LUTRARIA MAGNA (DA COSTA)

Shell

The shell is more elongate than in the other species, a fairly large specimen measuring 12 cm. long × 5·2 cm. high. The umbo lies rather far forward, being about one-quarter of the shell's length from the anterior end (Fig. 1C). The ventral edge is fairly evenly curved, and the dorsal edge behind the umbo is curved upwards to about the same extent, so that the posterior dorsal outline is concave. The anterior end is evenly rounded, but the posterior is obliquely truncate. The posterior gape is much wider than in the other species. The valves are moderately thick, and are covered by a dark brown periostracum. The outer surface is rather irregular, and is marked with concentric growth lines. An indefinite line from the umbo to the ventral side of the truncated

posterior divides the surface into a smoother anterior and a more wrinkled and irregular posterior part. The beaks are rather obtuse, and are directed if anything slightly forward.

There is deep U-shaped pallial sinus, the scar marking the lower edge of the sinus merging completely with the ventral pallial scar for practically the whole length of the sinus (Fig. 1 c). The distal end of the scar is not enlarged as in the other species.

The posterior dorsal outline of the cartilage pit is straight or slightly concave, and lies at a small angle (*ca.* 10°) to the adjacent dorsal edge of the shell. In the right valve the first cardinal tooth is cloven longitudinally into two subequal halves (Fig. 2 c). It is therefore totally different from the corresponding tooth in the other two species. The first cardinal tooth curves downward so that the axis of its more distal part lies at only 30–40° to the second tooth, which is laminar, as in the other species. In both valves the anterior part of the cartilage pit is more deeply excavated than the remainder. Posterior lateral teeth are absent in both valves, and the inner dorsal surfaces are flat or convex. In front of the umbo in both valves the striated outer surface is curved inward to an even greater degree than in *L. angustior*, so that it practically merges with the inner dorsal surface of the shell. In the left valve the two cardinal teeth are almost straight on their outer surfaces, and diverge at 30–40°. Their inner surfaces are fused together for about two-thirds of their length. There is a low anterior lateral tooth, as in the other species.

Siphons

These are described by Deshayes (1844–8, plate xxxvii), and I have not myself seen any specimens. The sheath is white proximally, becoming a shade of purple posteriorly, which gradually increases in intensity towards the tips of the siphons, often terminating in a very deep reddish-purple colour. Coloration is uniform, and there are no colour spots as in the other species, and the pale lines along the dorsal and ventral edges of the sheath are absent. The two siphons are separated at some little distance from their tips, and diverge from one another at almost a right angle (Fig. 3 c). The tentacles at the tips of the siphons are a little different from those of *L. lutraria*, and the two outer circles of small tentacles on the ventral siphon are lacking.

IDENTIFICATION OF ADULTS

Identification of *Lutraria magna* presents no problem, as it may at once be recognized by the longitudinally cleft first cardinal tooth in the right valve, the more acute angle between the two cardinal teeth, the absence of posterior lateral teeth in both valves, and by the completely linear scar below the pallial sinus.

It is difficult to find a single character which will invariably separate *L. angustior* from *L. lutraria*. Where the periostracum is intact, the presence

of fine radial striae in the older parts of the shell (not to be confused with wrinkling in the later *brown* periostracum) is a sure recognition character for *L. lutraria*. The longitudinal grooving of the inner dorsal surface in *L. lutraria* and the shape of the pallial scars, although showing some degree of variation, are also valuable characters. The degree of curvature of the first cardinal tooth in the right valve, the angle between the teeth in this valve, and the outline of the shell are all rather variable, and should only be used in combination with the more diagnostic characters noted above. Additional characters are the more pointed umbo of *L. lutraria*, and the greater thickness of the shell in *L. angustior*.

IDENTIFICATION OF YOUNG STAGES

I have not examined small specimens of *L. magna*, but this species should be readily identifiable at all sizes from hinge characters, particularly the form of the first cardinal tooth in the right valve.

Mr Ford's collection contains small shells (length range 3–22 mm) of *L. lutraria* and of another form (labelled *L. magna*) from shell-gravel deposits in Plymouth Sound. The latter appears to be *L. angustior*.

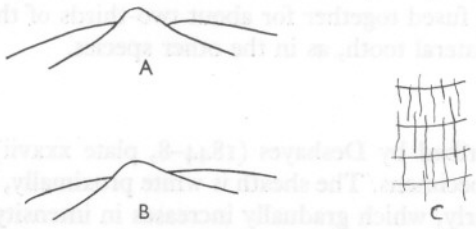

Fig. 4. Identification of young stages. A, lateral view of umbo and dorsal side of left valve of *L. lutraria* (4–5 mm long); B, similar view of *L. angustior*; C, radial markings on periostracum of *L. lutraria*.

At these sizes the shells are white, glossy, and translucent. Comparison of the two species shows that some of the specific differences between the adults are of no use in identifying the young stages. For example, the pallial line is difficult or impossible to distinguish at this stage. While the hinge of small *L. lutraria* is similar to that of the adult, that of *L. angustior* differs in that the two cardinal teeth diverge at almost a right angle, and the inner dorsal surfaces of the shell are grooved as in *L. lutraria*. The form of the cardinal teeth is explained by the fact that the first tooth in the right valve is curved in the adult, and actually diverges from the second at nearly a right angle, so that the angle between the teeth in a young shell would be greater than in the adult. The grooving of the inner dorsal surfaces is discussed in the next section.

The two species may, however, be distinguished by:

(i) The shape of the beak, which is more acute in *L. lutraria* (Fig. 4A, B).

(ii) The presence of irregular *radial* striae in the periostracum of *L. lutraria* (Fig. 4C), these being absent in *L. angustior*.

STATUS OF *L. ANGUSTIOR*

It is evident that *L. magna* is a species quite distinct from the other two, the question at issue being whether *L. angustior* is a variety of *L. lutraria* or a separate species.

Since *L. angustior* inhabits shell gravel while *L. lutraria* inhabits muddy sand, some of the differences between them might be attributed to environmental influences. The looser and possibly more disturbed substratum inhabited by *L. angustior* may require a greater degree of burrowing activity, which might be shown, for example, in a shell which was relatively narrower than in *L. lutraria*. Greater activity might also affect growth of the shell along the inner dorsal surface, so producing a convex rather than a concave surface in larger specimens. However other differences remain, notably the presence of fine radial markings in *L. lutraria*, the angle between the cardinal teeth, the form of the pallial scar, and the colour and degree of separation of the siphons, which cannot readily be attributed to environmental effects.

In spite of a certain variability of some characters there appears to be no intergrading between the two forms, and it therefore seems reasonable to infer that *L. angustior* is a separate species, the name being taken from Philippi's (1844) description of a variety of *Lutraria elliptica* from Sicily.

I am indebted to Mr A. W. Battin for assistance with some of the drawings, and to Mrs E. A. Peace for taking the photographs for Plates I and II. Much of the material was obtained by the Plymouth Laboratory's research vessels, and I am grateful to their captains and crews for their assistance in making these collections.

Note added in proof

Through the kindness of Dr H. A. Rehder of the U.S. National Museum, Washington, I have received photographs of a pair of valves of *L. elliptica* var. *alterutra* from the Jeffreys collection. These leave no doubt as to the identity of Jeffrey's variety with the species *L. augustior* described here. Details of locality are lacking, and this lectotype has been recatalogued under the reference USNM Cat. No. 622510.

Dr R. Kilias of the Zoologisches Museum der Humboldt-Universität, Berlin, has kindly loaned me a pair of valves of an adult specimen labelled '*Lutraria elliptica* var. *augustior* Phil. Sicilien. Benoit. 6223'. These are of particular value as they come from the same country as Philippi's specimens. The shell material is a little thinner and is less abraded than in the British

specimens, suggesting that the habitat was more sheltered from wave or current disturbance. The shells agree with the description of *L. angustior* given in this paper in most particulars, and since the periostracum is intact it is possible to see that the fine radial markings on the outer surface are absent, so confirming that the specimens are not *L. lutraria*. The only significant difference is in the dorsal edges, which are slightly produced upwards and inwards so that the inner surfaces are slightly concave. The longitudinal grooves so formed are not as deep as in *L. lutraria* however, and this upward extension of the shell is no doubt the normal condition when living in more tranquil conditions.

SUMMARY

Two species of the genus *Lutraria*, *L. lutraria* (L.) and *L. magna* (da Costa) are well known in British waters. To these is added a third, originally described as *L. elliptica* var. *angustior* by Philippi, but which is considered sufficiently distinct to merit specific rank. A description of the third species, to be called *L. angustior* Philippi, is given, and differences between it and the other two species described. Small specimens taken by Mr Ford in Plymouth Sound and identified as *L. magna* are considered to be *L. angustior*, so that there are at present no records of *L. magna* from the Plymouth area.

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APPENDIX

RECORDS OF *L. ANGUSTIOR*

Philippi's type locality is in Sicily, and Bucquoy *et al.* (1896) record it from the beaches of La Franqui and Leucate. Records from British waters attributed to this species are to be found in Turton (1819), Brown (1827, 1844) and Jeffreys (1863).

Localities from which I have recorded *L. angustior* are as follows:

St Austell Bay, Cornwall

50° 18·7′ N, 04° 44·8′ W. Muddy gravel, 11 m. One small living specimen. 16/11/50.

50° 19·9′ N, 04° 43·8′ W. *Lithothamnion* gravel, 9 m. One small living specimen. 16/11/50.

Plymouth area

Eddystone *Amphioxus* shell gravel. Occasional living specimens; siphons and dead shells often taken. 42–47·5 m.

Mewstone shell gravel. Dead shells and siphons. *ca.* 27 m. March, 1959.

Plymouth Sound. Living specimens at several stations in shell gravel. *See* Ford (1923, as *L. magna*).

Great West Bay

Station 6 (*see* Holme, 1950). Muddy sand and gravel, 19 m. One small living specimen (listed as *L. lutraria*). 29/7/48.

Dawlish Warren

A pair of valves (? living) washed up on beach. 3/2/50.

Weymouth Bay

50° 36·95′ N, 02° 22·9′ W. 18 m. Dead shells. 11/11/58. 50° 37·0′ N, 02° 20·6′ W. 18 m. Dead shells. 11/11/58. 50° 36·4′ N, 02° 19·75′ W. 19 m. Dead shell. 11/11/58. 50° 34·6′ N, 02° 14·45′ W. 27 m. Dead shell. 11/11/58. 50° 34·0′ N, 02° 08·7′ W. 26 m. Dead shell. 11/11/58.

Swanage Bay

50° 37·65′ N, 01° 53·7′ W. *ca.* 18 m. Dead shells. 28/1/59.

Additional records, added in proof

Torbay

Several, living, washed up at Three Beaches, Goodrington after E. gale. 3/4/58.

Falmouth Bay

One small living specimen at each of two stations:

50° 06·65′ N, 05° 03·4′ W. *ca.* 18 m. *Lithothammion* gravel. 17/7/59.
50° 07·2′ N, 05° 04·3′ W. *ca.* 37 m. *Lithothammion* gravel. 17/7/59.

Mount's Bay

One small living specimen at:

50° 06·3′ N, 05° 30·3′ W. *ca.* 22 m. Gravel and stones. 16/7/59.

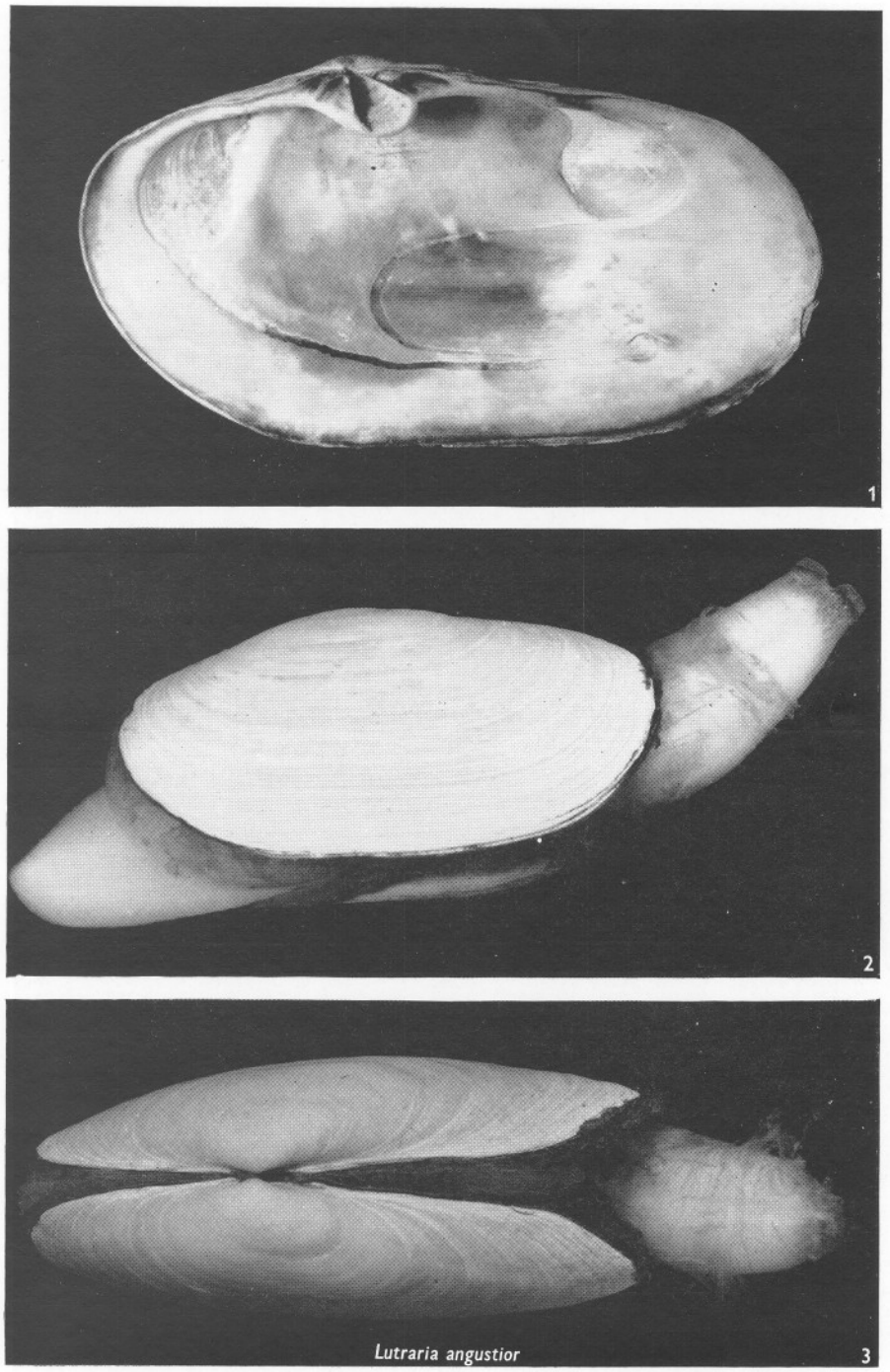
EXPLANATION OF PLATES

PLATE I. *Lutraria angustior*

Fig. 1. Interior of right valve. Dead shell, Swanage Bay. ×1·2.
Fig. 2. View with foot and siphons moderately extended. Eddystone shell-gravel. ×0·65.
Fig. 3. Dorsal view. Eddystone shell-gravel. ×0·75.

PLATE II

Fig. 1. *L. lutraria.* Interior of right valve. From a living specimen. Millport. About natural size.
Figs. 2, 3. *L. lutraria.* Hinges of left and right valves. Note the accessory tooth, often broken off, in the left valve behind the 2nd cardinal tooth. From living specimens. Millport. ×*ca.* 1⅓.
Figs. 4, 5. *L. angustior.* Hinges of left and right valves. Dead shells. Swanage Bay. ×*ca.* 1⅓.
Fig. 6. *L. lutraria.* Posterior part of shell, and partially extended siphons. The dorsal side is uppermost. Cornwall. ×0·87.



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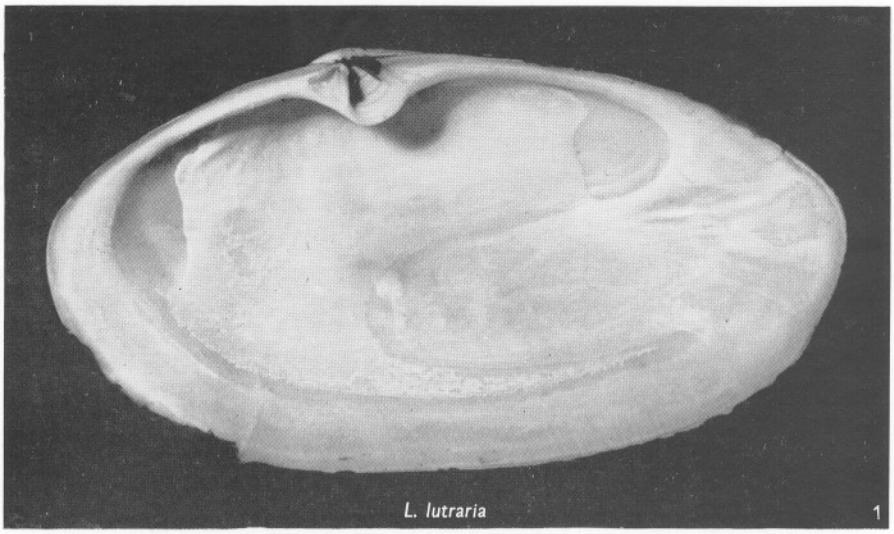
L. lutraria
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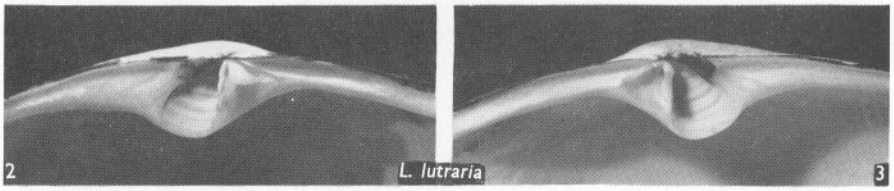
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L. lutraria
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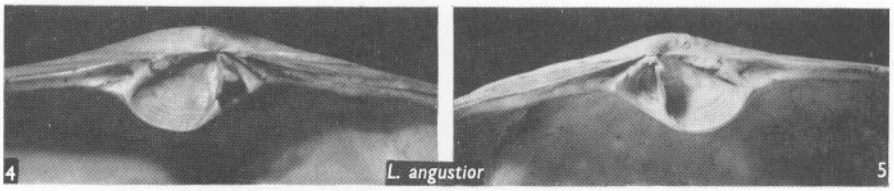
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L. angustior
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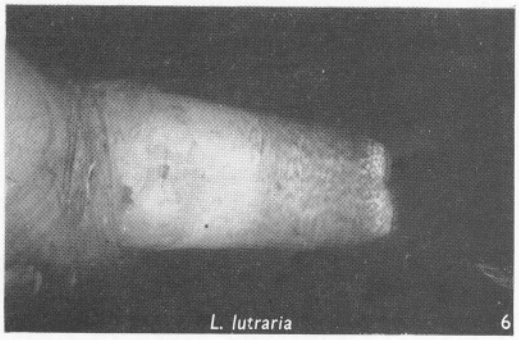
L. lutraria
6

J. mar. biol. Ass. U.K. (1959) 38, 569–580
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THE REACTIONS OF THE LIMPET, *PATELLA VULGATA* L., TO CERTAIN OF THE IONIC CONSTITUENTS OF SEA WATER

By D. C. Arnold

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(Text-figs. 1–8)

It has been shown (Arnold, 1957) that when exposed to air the common British limpet, *Patella vulgata* L., reacts to splashing with normal or moderately diluted sea water by raising the front edge of the shell, and that the amount of this movement can be correlated with the degree of dilution and with the tide level from which the animals are obtained. A basically similar result was obtained by the use of solutions of sodium chloride. The precise factors influencing the limpets were not identified. Additional data can now be presented on these and other reactions of *P. vulgata* as a contribution towards further definition of these factors.

MATERIALS AND METHODS

The limpets were obtained from a rocky ledge lying just below H.W.O.N.T. at the Pier Rocks, St Andrews, and were collected by chipping away fragments of the rock to which they were attached. In the laboratory they were placed in running sea water until they moved from the shattered rock. Each was then placed in a small polythene cup. Though they could easily climb out, the majority adhered firmly and remained within the cups for several days. Difficulties due to horizontal movement of the animals, encountered in the previous experiments, were thus largely avoided. Specimens which at any stage were found to be damaged were discarded. The shell length of those used ranged from 36 to 47 mm, mean length 41 mm.

As in the earlier work, vertical movements were normally recorded by means of a heart lever attached to a hook inserted beneath the front edge of the shell. An upward movement of the shell was thus recorded as an upward movement on the trace. The presence of a hook caused a local retraction of the mantle fringe, but seemed not to affect qualitatively the behaviour of the animal. A slower drum speed (0·048 mm/sec) and longer lever (magnification 35:1) were used than in the preceding experiments. Stimulation was provided by dropping about 2 ml. of fluid onto the apex of the shell, a small amount

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passing beneath its edge and coming into contact with the mantle fringe. The amount of liquid used in this manner was found not to be critical; presumably once the space beneath the shell had been filled any excess drained off without effect. Stimuli were customarily given at 5 min intervals, though in a few experiments 10 min intervals were used. After each stimulus the limpets were washed with about 5 ml. distilled water in order to remove any of the stimulating liquid still adhering to the shell. Holes drilled in the bases of the polythene cups allowed these to drain between each test.

The solutions used (sodium chloride, calcium chloride, magnesium chloride, potassium chloride, sodium sulphate, sodium bicarbonate and an artificial sea water composed of these salts) were made up from Analar reagents to the concentrations given by Pantin (1946) as approximately isotonic with sea water of salinity 34·6‰.

All experiments were performed in daylight during the first three days after collection of the limpets. Further details of the methods employed are given in the accounts of the experiments concerned.

PATTERN OF MOVEMENT OF UNSTIMULATED ANIMALS

Despite all care it was impossible fully to prevent vibrations from reaching the experimental animals. A number of records of otherwise unstimulated limpets were therefore made in order to assess how far they might be affected by these unavoidable mechanical stimuli. It was found that under the conditions generally prevailing in the laboratory the limpets, as upon the shore, slowly raised their shells so as to form a slight gap between the edge and the substratum, then remained quiescent except for a series of, usually small, downward movements. The periodicity and amplitude of these contractions varied between different animals (Fig. 1). Occasionally, those animals which had not been long out of water showed considerable activity, and for this reason a period of 8–10 h dryness prior to each experiment was adopted as standard procedure throughout the rest of the investigation. The occurrence of the movements made by the unstimulated limpets could not be related to the general vibrations of the laboratory. Indeed, almost identical traces were given by two animals, one recorded on a day when the laboratory was entirely empty, the other during a visit by a large party of school-children. Only jarring of the apparatus or of the bench upon which it stood produced an unequivocal response, invariably a sharp downward movement. However, care was needed to avoid sudden decreases in illumination, to which the limpets responded in a similar manner. The presence or absence of this shadow reaction formed a useful test for their general well-being during experiments.

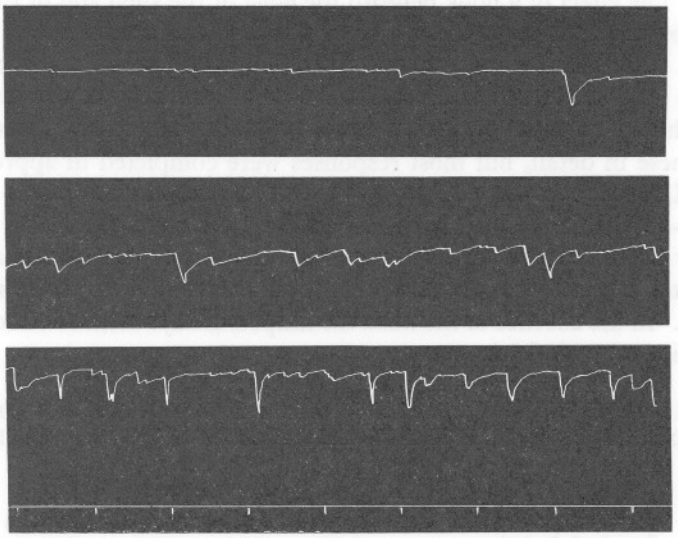

Fig. 1. Part of kymograph records of vertical movements of unstimulated limpets. Time marks at 15 min intervals. Shell lengths 43 mm (upper), 36 mm (middle) and 37 mm (lower).

MODIFICATION OF THE PATTERN THROUGH STIMULATION

Since the limpet is not fully contracted against the substratum when at rest, it may respond to splashing either by an upward movement of the front edge of the shell or by a downward movement. An upward movement was given to sea water, to isotonic solutions of sodium chloride, calcium chloride and magnesium chloride, and to artificial sea water; a downward movement was given to distilled and tap water and to solutions of sodium sulphate, potassium chloride and sodium bicarbonate isotonic with sea water.

Effect of experimental conditions

Since the conditions under which the experiments were performed might be expected to affect the behaviour of the limpets, a series of comparisons were made between animals left undisturbed within the collecting area and those brought into the laboratory, using the responses to sea and fresh water as a guide. It was found that animals upon the shore reacted in a manner qualitatively similar to those studied in the laboratory. Accurate measurements of their movements could not be made, however, and for this purpose a number of specimens attached to small boulders were brought into the laboratory and studied under a binocular dissecting microscope fitted with a micrometer eyepiece. It was thus possible, though tedious, to measure fairly accurately the heights to which the limpets rose upon stimulation, without removing them from their scars or disturbing them in any further

manner. The effect of a hook beneath the edge of the shell and of the tension exerted by the heart lever could also be studied in this manner.

It was found that the magnitude of the responses to sea water, measured from the position of maximal contraction of the animal, showed great regularity, while their time relations were also regular. No attempt was made to record these in detail, but most responses were completed in approximately 2 min. Limpets left on their scars gave rather smaller responses than did those re-settled in polythene cups (Table 1). The presence of the hook naturally prevented complete contraction of the animal upon stimulation with fresh water and in these cases the downward movements might be somewhat irregular. In the absence of the hook the limpets always contracted completely when irrigated with fresh water.

TABLE 1. THE EFFECT OF EXPERIMENTAL CONDITIONS UPON THE RESPONSES OF *PATELLA* TO STIMULATION BY SEA WATER

	A	B	C	D
Number of stimuli	20	20	19	10
Mean height attained	1·0	1·5	2·6	1·8 mm
Standard deviation	0·24	0·27	0·22	0·11 mm

A. 38 mm limpet undisturbed on small boulder.
B. Same specimen attached to lever, but still on scar.
C. 40 mm limpet attached to lever and resettled in polythene cup.
D. 36 mm limpet, conditions as in C.
Measurements of A and B made under binocular microscope, those of C and D calculated from kymograph traces.

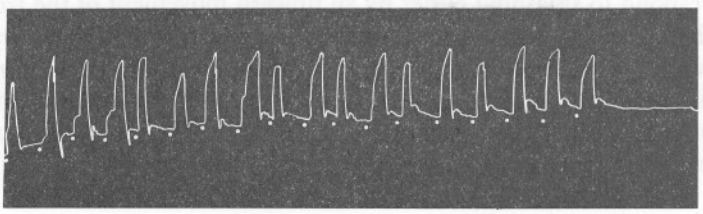

Fig. 2. Kymograph record of response to sea water. Stimuli every 5 min. Shell length 40 mm.

Response to sea water

The response to sea water had certain well-defined characteristics, the majority of which are shown in Fig. 2. The movement commenced abruptly and soon after stimulation and consisted of a smooth, rapid rise to a clear maximum, followed by an equally rapid fall. Occasionally this 'peak' response was preceded by a very small initial contraction of the order of 0·1 mm actual movement. A noticeable feature of the response was that the movements, except for the first one or two in a series, were extremely regular and showed but little variation in their form, general time relations or height attained (by no means the maximum height to which the animals were capable of moving)

and the amount of the subsequent downward movement. Under continued stimulation, about half the limpets studied showed a tendency to slight progressive increase in the height of the responses, accompanied by a gradual diminution in the amount of the subsequent downward movements, while the others gave responses of even size. None showed decreased height of movement on repeated stimulation.

Effect of osmotic pressure

As described in the earlier account, the height of response given to sea water diminished with dilution. In order to determine whether this was due to alteration of the ionic concentration or whether it could be ascribed simply to alteration of the osmotic pressure, limpets were tested with normal sea

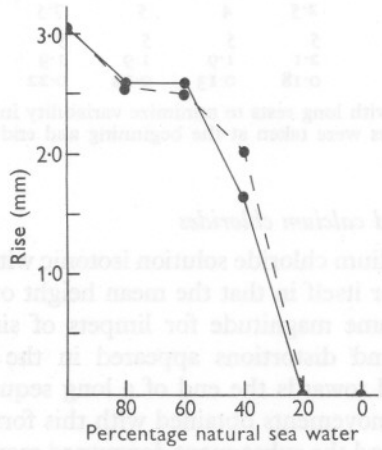

Fig. 3. Response to sea water diluted with distilled water (continuous line) or 0·9 M dextrose solution (broken line). Both distilled water and 0·9 M dextrose solution caused contraction when applied to the extended limpet. Shell length 39 mm.

water and with sea water the relative salinity of which had been reduced to 80, 60, 40 and 20% of normal by dilution with either distilled water or 0·9 M dextrose solution. Both series of dilutions, though of different osmotic pressures, elicited responses which showed considerable similarity in size and form at each level, while differences in height between the two series (Fig. 3) were not consistent.

Influence of pH

Since the reaction of sea water alters slightly upon dilution, while the other solutions used varied considerably in their pH, the influence of this factor was determined by comparison of the reactions to sea water and to isotonic sodium chloride solutions, the pH of which was varied by addition of small amounts of hydrochloric acid or sodium hydroxide. The results of a typical experiment

are given in Table 2. In experiments where more alkaline media were used the responses remained regular up to pH 9·5. At pH 10 upward movements were given to the first 2–3 stimuli only, later stimuli eliciting downward movements. Above pH 10·5 the limpets always contracted strongly upon stimulation. If the pH was lowered by use of sulphuric in place of hydrochloric acid the limpets normally moved downward when stimulated and any upward movements given were always smaller than were the responses to sea water.

TABLE 2. EFFECT OF DIFFERENT pH VALUES UPON RESPONSES OF A SINGLE *PATELLA*

	Sodium chloride solutions. Approximate pH					Sea water	
	2·5	4	5	7·5	8		
No. of stimuli	5	5	5	5	5	5	5
Mean height attained	2·1	1·9	1·9	1·9	2·2	2·2	2·1
Standard deviation	0·18	0·13	0·22	0·22	0·22	0·18	0·13

Short series were used with long rests to minimize variability inherent in stimulation with NaCl. The sea-water series were taken at the beginning and end of the experiment. Shell length 40 mm.

Response to sodium and calcium chlorides

The response to sodium chloride solution isotonic with sea water resembled that given to sea water itself in that the mean height of a series of responses was usually of the same magnitude for limpets of similar size. However, many irregularities and distortions appeared in the records and became especially pronounced towards the end of a long sequence of tests (Fig. 4). Most of the upward movements obtained with this form of stimulation were peaks, but both they and the subsequent downward movements showed much greater variation in height than did those given to sea water, while after a time the rapidity of the response was also affected and the movements began to lose their well-defined form, thus giving a transition to a form of response more aptly to be described as a 'hump'. Sodium chloride alone was not an entirely favourable stimulus, since when the isotonic solution was placed upon a portion of the mantle fringe there was a sharp local contraction. After about the tenth stimulation by sodium chloride the shadow reaction was lost, while after about the twentieth stimulation the limpet ceased to react further to either sodium chloride or fresh water and reacted in a quite irregular and unpredictable manner when stimulated with sea water.

The use of isotonic calcium chloride solution as a stimulus resulted in an upward movement, but one of very different form to that elicited by sodium chloride or by sea water. The first few responses were all of the peak type, but thereafter the movements slowed and the maxima became less well-defined, while the subsequent downward movements became slower and smaller until after about the tenth stimulation the limpets failed to react further to either

calcium chloride or fresh water (Fig. 5), remaining in an expanded condition with a wide gap between shell and substratum. Responses to calcium chloride were very nearly as regular in the height attained, measured from the level of maximum contraction, as were those given to sea water. The mantle fringe gave no local reaction to isotonic calcium chloride solution.

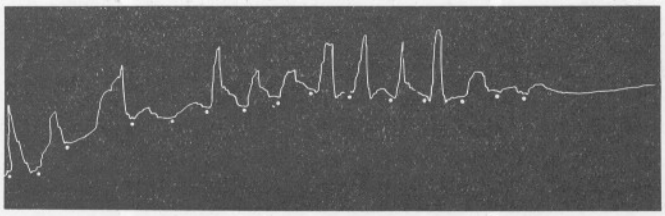

Fig. 4. Kymograph record of response to isotonic NaCl solution. Stimuli every 5 min. Shell length 40 mm.

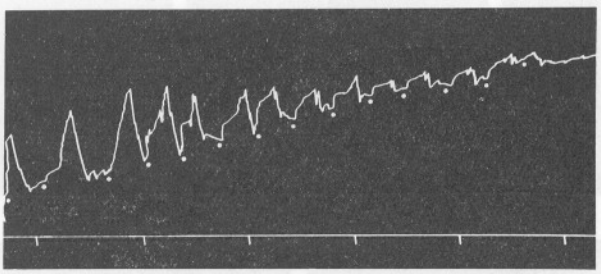

Fig. 5. Kymograph record of response to isotonic $CaCl_2$ solution. Stimuli at 5–10 min intervals. Time mark every 15 min. In this and following records the level of the time mark has been adjusted to the position of maximum contraction of the limpet. Shell length 42 mm.

Since the response to sodium chloride solution resembled that to sea water in its general form, while that to calcium chloride solution did so in its regularity of height attained, it was of interest to examine the effect of mixtures of these two solutions. This proved a matter of some difficulty. The continued use of either one alone was already known to result in characteristic anomalies of behaviour and ultimate cessation of response, while during a long series of tests upon the same animal fatigue and the possible residual influence at each stage of the experiment of the mixture used in the preceding stage had to be considered. The method finally adopted was to group the stimuli in sets of 6 for each test and to alternate each set with a similar group of sea-water stimuli as control. Thus each result could be immediately compared with the behaviour to natural sea water and the stability of this behaviour during the course of the experiment could be assessed. Since such an experiment would involve some scores of stimuli, the limpet was left untouched for 30 min between each set of responses. The combinations of chlorides used were

made up from the original solutions isotonic with sea water and contained 1·5, 2·5, 3, 5 and 20% calcium chloride.

An example of a series of traces obtained from a single limpet is given in Fig. 6. The response to sea water showed the same pattern throughout the experiment. The height above base, indicated by the time-trace, remained

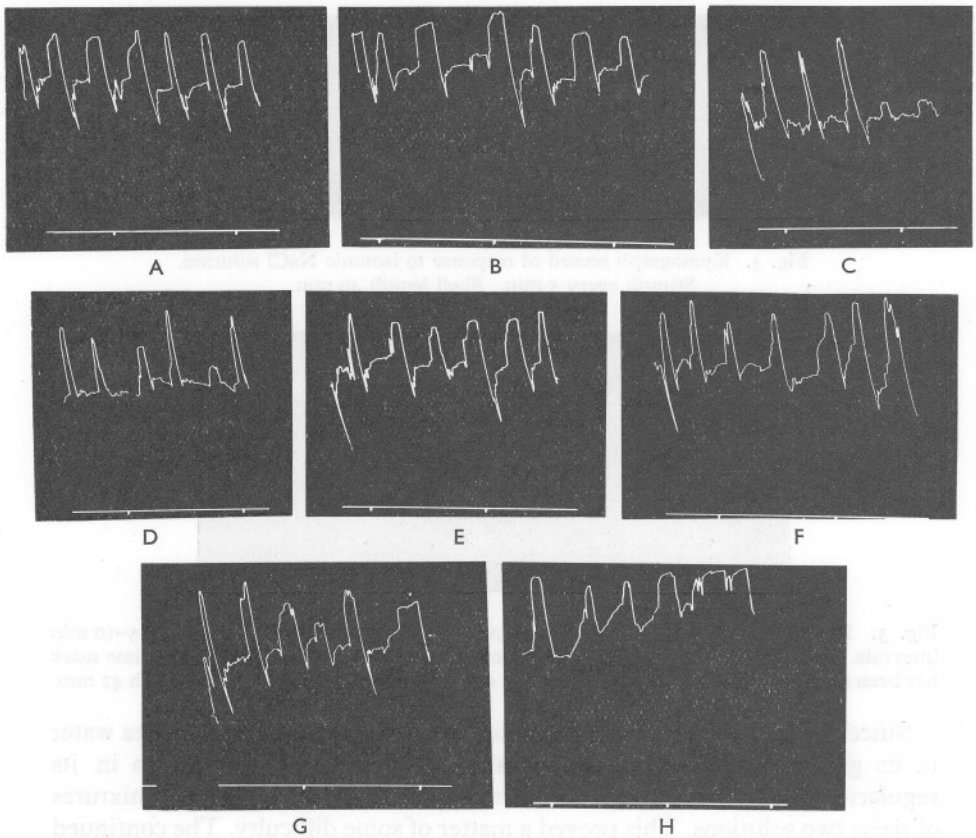

Fig. 6. Kymograph record of short sequences of responses to solutions of varying calcium content. For explanation, see text. Stimuli every 5 min. Time mark every 15 min. Shell length 40 mm.

constant for each upward movement, as did the amount of the subsequent downward movements. Individual peaks were narrow, indicating rapidity of movement, and had clear-cut points (Fig. 6A). Response to an artificial sea water showed a similar pattern, but with the peaks two or three times as wide, indicating a corresponding slowness of movement (Fig. 6B). The pattern of response to isotonic sodium chloride solution resembled the two foregoing patterns in certain respects, but was markedly irregular in the heights attained at each upward movement (Fig. 6C).

When isotonic sodium and calcium chloride mixtures were used, that containing 1·5% calcium chloride resulted in a pattern similar to that induced by sodium chloride alone (Fig. 6D). Responses to mixed solutions containing 2·5 and 3% calcium chloride more closely resembled the responses to sea water (Fig. 6E and F). The solutions which contained 5 and 20% calcium chloride induced patterns of movement closer to that elicited by stimulation with isotonic calcium chloride solution alone, namely slowing of all movements and reduction of the downward component with successive stimuli, though the heights attained with the upward movement remained regular (Fig. 6G and H). The trace shown in Fig. 6G is more irregular in the heights attained than was usual for limpets stimulated in this manner; as it was actually the last test conducted in this experiment the pattern may have been modified by fatigue.

By calculation, the mixture containing 2·5% calcium chloride solution, considered to give the best approximation to the pattern of movement characteristic of sea water, contained 0·36 g/l. Ca″, while the artificial sea water should contain 0·40 g/l. Ca″. The limpets might therefore be adapted to a slightly lower concentration of calcium ion than is normally present in sea water. To check this hypothesis the calcium content of the artificial sea water sample used and of inshore water at the time the experiments were conducted (mid-June, 1958) were determined by the method of Kirk & Moberg (1933). The artificial sea water was found to contain 0·41 g/l. Ca″, while two samples of sea water contained 0·37 and 0·38 g/l. Ca″ (inclusive of strontium). These values differ from those for the open sea (Harvey, 1955), but explanation of the discrepancy is beyond the scope of this paper.

Response to magnesium and potassium chloride

Magnesium chloride solution, isotonic with sea water, induced an upward movement similar to that given to sodium and calcium chlorides, but marked by its own specific characters (Fig. 7). The heights attained in response to successive stimuli were quite regular and the movements themselves reasonably smooth and fairly rapid. For a while at least the amount of each downward movement was also stable. However, each upward movement was succeeded by a period in which the limpet remained extended and almost motionless and the onset of contraction was often much delayed, though rapid enough when once it started. As the experiment proceeded these characteristics became ever more pronounced until after about the tenth stimulus a superficial narcosis ensued. In this condition the limpet would respond, albeit slowly, to direct mechanical stimulation of the foot, but it no longer reacted to fresh water, reduction of light intensity or jarring of the bench or apparatus.

In contrast, isotonic potassium chloride solution dropped upon a slightly expanded limpet induced an immediate and complete contraction, followed by a rapid sequence of abrupt, jerky, up-and-down movements, after which

the majority of limpets tested in this manner climbed out of the polythene cup and so terminated the experiment.

The effect of absence of magnesium and potassium ions was tested by use of artificial sea waters in which their chlorides were replaced by an osmotically equivalent amount of sodium chloride. These replacements did not have any effect upon the size of the responses as compared with those given to natural and the complete, artificial sea water.

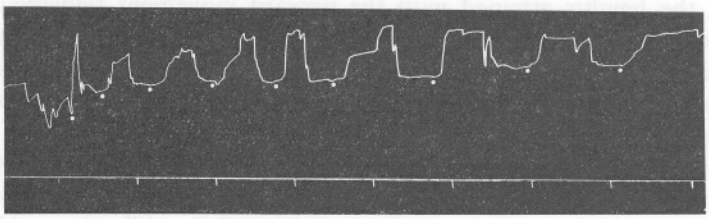

Fig. 7. Kymograph record of response to isotonic $MgCl_2$ solution. Stimuli at approximately 10 min intervals. Time mark every 15 min. Shell length 41 mm.

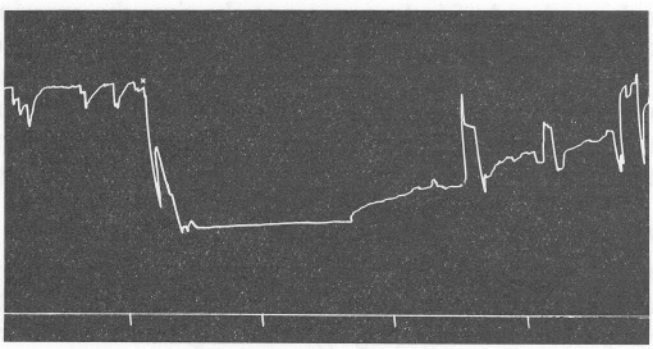

Fig. 8. First part of kymograph record of response to isotonic Na_2SO_4 solution. Stimulus given at *x*. Time mark every 5 min. Shell length 37 mm.

Response to sodium carbonate and sulphate

Isotonic solutions of these two salts induced downward movements. With sodium carbonate the limpet contracted as far as possible upon stimulation, then began a series of fairly rapid up-and-down movements which gradually died away into the normal steady state with its periodic small contractions. With sodium sulphate solution as stimulus the initial contraction was followed by a prolonged period of inactivity (Fig. 8), succeeded by a series of rapid up-and-down movements which might continue for two or more hours before the normal pattern of the quiescent animal was restored.

DISCUSSION

The experiments here described have confirmed the earlier results and show that the responses of *Patella vulgata* to natural and diluted sea water cannot be attributed to recognition of variation in either pH or osmotic pressure of the media. Further, the occurrence of similar reactions when a simple artificial sea water is used as stimulus shows that the responses are not due to the influence of minor ions since, apart from contaminating traces of arsenic and iron in the reagents used, these would be absent. Upward movements have been obtained with simple chloride solutions (except with potassium chloride), but not with sodium sulphate or carbonate, and it follows that the reactions may most easily be attributed to sensitivity to the concentration of chloride ions. Calcium ions apparently exert a stabilizing influence when in optimum concentration and the behaviour of the animal soon indicates whether the amount of calcium present is above or below that of the inshore water to which it is accustomed.

The response of *P. vulgata* to dilution of the external medium differs in its nature from that found in other animals so far investigated. The estuarine lamellibranch *Scrobicularia plana* (da Costa) exhibits behavioural changes when placed in sea water diluted with distilled water, but is unaffected when dilution is secured by means of 0·9 M sucrose solution (Freeman & Rigler, 1957). Loss of activity is shown by the copepod *Tigriopus fulvus* (Fischer) when subjected to salinities of 90‰ and upwards, recovering when the medium is once again diluted (Ranade, 1957), but it is not clear whether the reaction denotes sensitivity to osmotic pressure or to ionic concentration.

The effects of various ions upon *P. vulgata* show interesting similarities to those found for animals belonging to other groups. Thus the branchiopod *Artemia salina* (L.), placed in sodium chloride solution isotonic with sea water survives for several days, but in other solutions the survival time is greatly reduced, the animal becoming moribund in similar solutions of magnesium and calcium chlorides (6–9 h), potassium chloride (30 min) and sodium bicarbonate (5 min) (Croghan, 1958). The importance of calcium ions in resistance to reduced salinity has been demonstrated (Pantin, 1931) for the turbellarian *Procerodes ulvae* (Oersted).

The ecological importance of these reactions in *Patella* can be in little doubt, for they provide a means whereby the feeding period of the limpet may be prolonged beyond the time during which the animal is covered by the tide. In the first place, they enable *P. vulgata* to take advantage of spray cast by breaking waves or carried by an onshore wind; but more than this, the sensitivity to chloride ions and tolerance to a lowered concentration provide a mechanism which would allow the limpet to utilize those periods when the rocks are damp through high humidity or light rain, while ensuring that the animals do not leave their scars when heavy rain temporarily washes the rocks

and vegetation free of salt. It is known that *Patella* spp. do browse upon damp rocks during periods of exposure (Orton, 1929; Stevenson, cited by Thorpe, 1956), especially at night, and this has also been observed at St Andrews. Browsing during the period of exposure is least important to those animals which inhabit the lower tide levels, most important to those near the upper limits of the shore. The increased responsiveness to splash and tolerance to diminished salt content of the water shown by limpets near high water mark (Arnold, 1957) can be readily interpreted as an adaptation to adverse environmental conditions which, together with increased radula length (Brian & Owen, 1952), enables these animals to maintain the minimum feeding period necessary for their growth and reproduction.

SUMMARY

Further experiments on the response of *Patella vulgata* to sea water and to the major ionic constituents of sea water are described. These responses cannot be attributed to recognition of variation in pH, osmotic pressure or minor ions. Instead they can be related to chloride ion concentration, modulated by the presence of calcium ions. The calcium ion concentration appears quite critical and the nature of the response given by the limpet alters when this is varied. It is suggested that these responses provide a means by which animals living on the higher portions of the shore can take advantage of spray or of periods of light rain or high humidity to prolong the feeding time into the periods of exposure by the tide.

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SOME PARAMETERS OF GROWTH IN THE COMMON INTERTIDAL BARNACLE, *BALANUS BALANOIDES* (L.)

By H. Barnes and Margaret Barnes

The Marine Station, Millport, Scotland

(Text-figs. 1–6)

It is difficult to determine the weight or nitrogen content of the living tissues of an operculate barnacle without destroying it. It has been customary, therefore, in ecological work to express growth in terms of various parameters determined from repeated measurements of the shell. Moore (1934) employed shell volume calculated from the height and basal diameters; others (Costlow & Bookhout, 1953, 1956; Mawatari, Hirosaki & Kobayashi, 1954*a*, *b*) have used the area of the basis. Most commonly, however, in growth-rate studies the length of the basis measured through the rostro-carinal axis has been used (Hatton, 1938; Barnes & Powell, 1953). Recently, working with animals cultured in the laboratory when the cast of an individual could be obtained subsequent to ecdysis, Costlow & Bookhout (1957) have used the size of the mouthparts as a measure of growth after first establishing their relation to body size. The space, both areal and volumetric, occupied by a sedentary animal is of primary importance in studies of its ecology and measurements of shell-size are, therefore, adequate for many purposes. For some aspects of growth and ecology it is, nevertheless, very desirable that the relation between such parameters and others, more directly connected with the living material, should be established.

THE MATERIALS AND METHODS

The specimens of *Balanus balanoides* (L.) were collected from near mid-tide level, individuals being selected that had had unrestricted growth and were, therefore, of the typical conical shape. Two collections were made; the first was in the autumn, just prior to the time at which fertilization takes place, and when the reproductive products were fully developed, and the second in the early winter after fertilization when the reproductive products were minimal. The shell dimensions were obtained using a travelling microscope. The body (prosoma and thorax) was then carefully separated, by cutting the attachment to the opercular valves, and weighed. Ovarian tissue when present was removed and weighed separately. After weighing, the body or ovary was

transferred to a Kjeldahl flask, wet ashed in the usual way and the nitrogen determined as ammonia by a standard procedure for milligram quantities; the ammonia was absorbed in a saturated solution of boric acid and titrated with N/40 or N/80 hydrochloric acid. For the smallest animals pooled samples were used. Total shell weight and shell volume were determined on a separate series of individuals in which the animal's body was carefully removed without destroying the operculum. The shells were weighed dry and then carefully filled with plasticine and re-weighed; the shell volume (mantle cavity) was calculated from the increase in weight and the previously determined density of plasticine.

Wet weight of the body (W), wet weight of ovary (O), their total nitrogen contents (N_W and N_O), and the weight of calcareous shell (W_S) are expressed in mg, length of the rostro-carinal diameter (L) along the basis in mm, and volume of the mantle cavity (V) is given in μl.

RESULTS

Body weight and total nitrogen

The relation between body weight and its total nitrogen content is shown in Fig. 1. It is clear that the relation is linear and that the results fall on two separate lines which represent animals collected in autumn and winter. In the former the tissues include the fully developed male reproductive organs and their products which in the latter are only poorly developed. The regression of total nitrogen on body weight is

$$N_W = 0{\cdot}0086 + 0{\cdot}0215W$$

for the autumn animals, and

$$N_W = 0{\cdot}0530 + 0{\cdot}0143W$$

for the early winter animals. For a given body weight, above about 5 mg, which represents the approximate size at which the animals reach sexual maturity, the autumn animals have a higher nitrogen content than those collected in early winter, and this is consistent with the fact that a high nitrogen content (3·1 % wet weight) was found for the semen expressed from ripe animals.

Body weight and ovarian tissue

The female reproductive organs and their products are conspicuous in the base of the mantle cavity of animals collected in the autumn and may be easily separated from the body. Fig. 2 suggests that at smaller body weights the relation is not linear, the relative weight of female reproductive tissue falling off with decreasing body weight. When tested by the appropriate statistical technique the deviation from linearity is not, however, significant

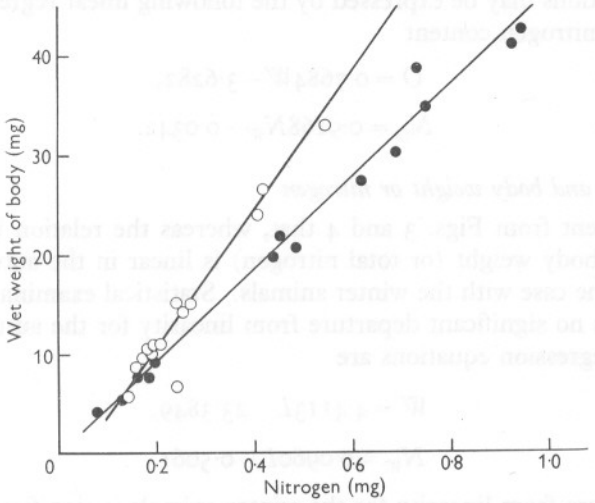

Fig. 1. *B. balanoides*: relation between body weight and total nitrogen content; —●—, animals taken in early autumn with well-developed male organs and reproductive products; —○—, animals taken in early winter with poorly developed male organs: regression lines drawn.

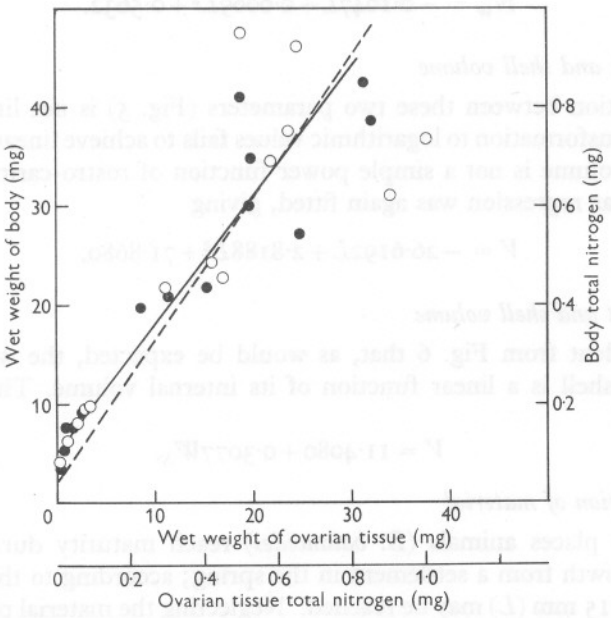

Fig. 2. *B. balanoides*: relation between body weight and ovarian tissue weight (—●—) and body total nitrogen and ovarian tissue total nitrogen (--○--): regression lines drawn.

and the relations may be expressed by the following linear regressions for the weight and nitrogen content

$$O = 0{\cdot}7684W - 3{\cdot}6282,$$

and

$$N_O = 0{\cdot}9168N_W - 0{\cdot}0342.$$

Shell length and body weight or nitrogen

It is evident from Figs. 3 and 4 that, whereas the relation between shell length and body weight (or total nitrogen) is linear in the autumn animals, this is not the case with the winter animals. Statistical examination indicates that there is no significant departure from linearity for the autumn animals; the linear regression equations are

$$W = 4{\cdot}4113L - 23{\cdot}3849,$$

and

$$N_W = 0{\cdot}0960L - 0{\cdot}5061.$$

The departure from linearity for the winter animals is significant and curvilinear regression equations were, therefore, determined. They are as follows:

$$W = -4{\cdot}6938L + 0{\cdot}3593L^2 + 22{\cdot}4883,$$

and

$$N_W = -0{\cdot}1047L + 0{\cdot}0069L^2 + 0{\cdot}5632.$$

Shell length and shell volume

The relation between these two parameters (Fig. 5) is not linear and the fact that transformation to logarithmic values fails to achieve linearity indicates that shell volume is not a simple power function of rostro-carinal diameter. A curvilinear regression was again fitted, giving

$$V = -26{\cdot}6192L + 2{\cdot}8188L^2 + 71{\cdot}8680.$$

Shell weight and shell volume

It is evident from Fig. 6 that, as would be expected, the weight of the calcareous shell is a linear function of its internal volume. The regression equation is

$$V = 11{\cdot}4080 + 0{\cdot}3077W_S.$$

The acquisition of material

In many places animals (*B. balanoides*) reach maturity during the first season's growth from a settlement in the spring; according to the conditions a size up to 15 mm (L) may be reached. Neglecting the material present in the original cyprid, the organic and inorganic matter acquired by an animal of any given size between settlement and the early autumn when the reproductive organs are fully ripe may be readily estimated from the preceding regression

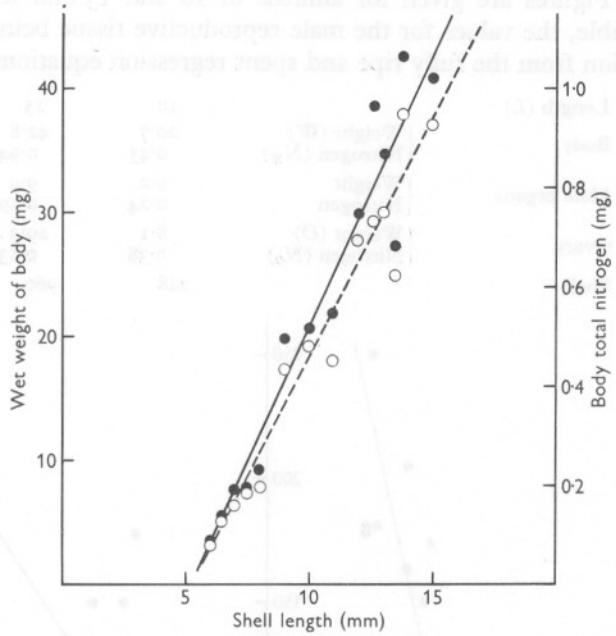

Fig. 3. *B. balanoides*: relation between body weight and shell length (—●—) and body total nitrogen and shell length (--○--) for animals taken in early autumn: regression lines drawn.

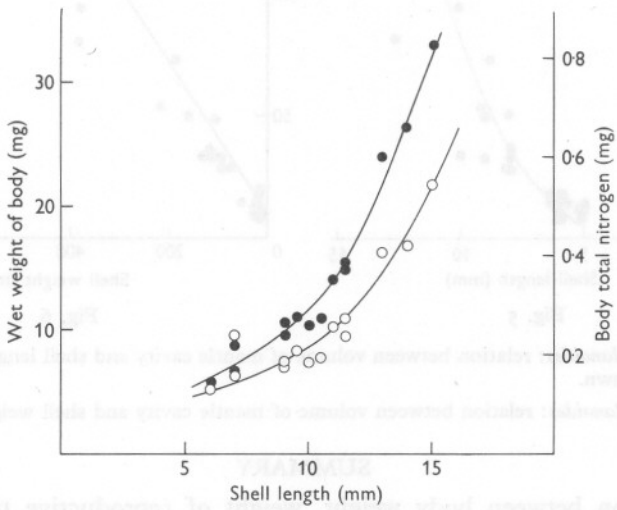

Fig. 4. *B. balanoides*: relation between body weight and shell length (—●—) and body total nitrogen and shell length (—○—) for animals taken in early winter: curvilinear regressions drawn.

equations. Figures are given for animals of 10 and 15 mm length in the following table, the values for the male reproductive tissue being calculated by subtraction from the fully ripe and spent regression equations:

Length (L)		10	15
Body	Weight (W)	20·7	42·8
	Nitrogen (N_W)	0·45	0·94
Male organs	Weight	9·2	9·9
	Nitrogen	0·24	0·39
Ovary	Weight (O)	8·1	29·3
	Nitrogen (N_O)	0·38	0·83
Shell		248	960

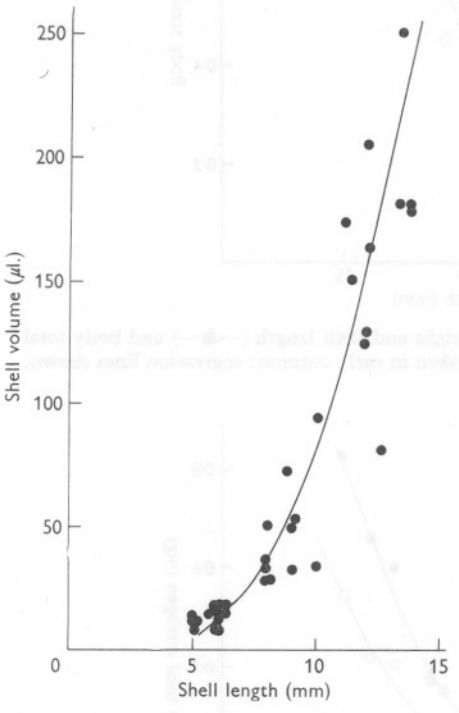

Fig. 5

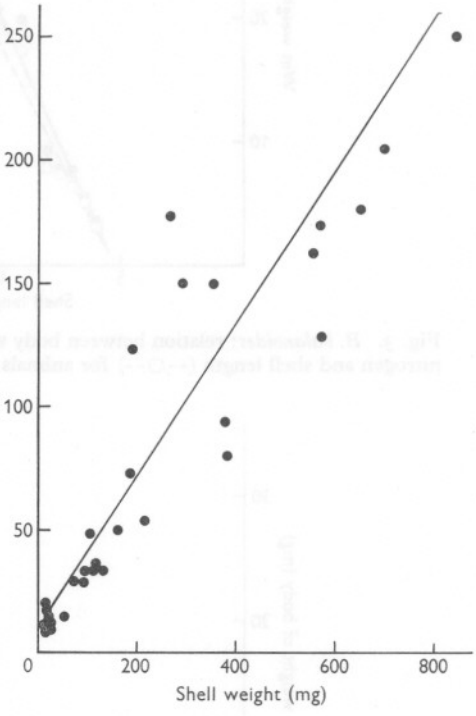

Fig. 6

Fig. 5. *B. balanoides*: relation between volume of mantle cavity and shell length: curvilinear regression drawn.

Fig. 6. *B. balanoides*: relation between volume of mantle cavity and shell weight: regression line drawn.

SUMMARY

The relation between body weight, weight of reproductive tissues, their nitrogen content, and shell length and volume, have been investigated for *Balanus balanoides*. The results are presented in graphs and as regression equations.

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THE INFLUENCE OF TEMPERATURE ON THE REPRODUCTION AND MOULTING OF *LEPAS ANATIFERA* L. UNDER LABORATORY CONDITIONS

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(Text-figs. 1–2)

Lepas anatifera L. is generally found on floating objects in tropical and sub-tropical oceanic waters, where sea temperatures exceed 18–20° C.

A drifted buoy covered with recently settled and adult *Lepas anatifera* L., washed ashore near Cable Bay on the south-west of Anglesey, however, provided an opportunity to study in detail the processes of moulting and reproduction in this species under laboratory conditions.

Animals were carefully detached from the buoy and were kept in glass crystallizing dishes. Sea water was circulated through a long thin-walled coiled glass tube immersed in a tank controlled by a thermostat. A constant flow of warm water was thus maintained over the animals throughout the experiment. This was found necessary to keep them healthy and in normal condition; they rapidly became lethargic and moribund if the water was allowed to become stagnant.

The barnacles were fed on *Artemia* larvae and on a paste prepared from powdered mammalian liver. Small pieces of *Mytilus* tissue (1–2 mm) were also offered; these were rapidly grasped by the cirri and engulfed. Very large pieces (about 5 mm) were accepted, but the animals had difficulty in swallowing them (cf. Howard & Scott, 1959). However, uneaten *Mytilus* tissue and liver caused fouling of the water, so *Artemia* larvae were generally used and the animals seemed more healthy when fed on live food.

We found that in isolation the barnacles lived healthily with the stalks unattached. The stalks which had been carefully freed from their original attachment showed no evidence of refixing themselves, for example to the glass dish. However, when several such individuals were kept in the same dish, they were often found grasping and attempting to feed on each other's stalk, thus occasionally causing injury. The injured stalks decayed, followed often by the death of the animal. In one instance the stalk started to decay and a few days later cirral activity ceased and the animal appeared dead. When the stalk of the animal was lifted the thick outer integument separated, leaving the muscular part of the stalk attached to the animal. On the following

day the fleshy stalk disappeared, the other barnacles in the dish having devoured it. The stalkless animal was then isolated; it resumed its normal activity and lived for a further 2 weeks. It is unlikely that this type of damage would arise if the animals were growing together attached to a common object, because then only the leathery outer surface of the stalk would be exposed to attack and the individuals would also be able to move away from each other by bending the stalk.

Colour of ovary

The normal colour of the ovary in *Lepas* is deep blue (Darwin, 1851; von Willemöes Suhm, 1876; Groom, 1894) as are the recently fertilized egg masses, though later the embryos turn to purple, red and pink as development proceeds. The original colour of the ovaries of the specimens washed ashore was blue, as also were the egg masses. On the Munsell Colour Chart they matched purple blue 5·0, value 4/chroma 6. However, after the animals had been kept in the laboratory and fed on *Artemia* larvae, the newly developed ovaries were seen to be distinctly pink (Munsell red 5·0, value 6/chroma 10) and the colour changed only slightly to a salmon peach tint (Munsell red 5·0, value 8/chroma 4) by the time the embryos were ready to hatch. A similar though less dramatic difference in colour of the ovary has been noticed also in operculate barnacles, such as *Balanus crenatus* Bruguière, *B. amphitrite* var. *denticulata* (Broch), *B. perforatus* Bruguière and *Chthamalus stellatus* Poli, when fed on *Artemia* in the laboratory, the ovaries being slightly more peach coloured in comparison with the yellow or orange ovaries of naturally fed specimens. The type of food thus plays an important role in determining the ovary colour.

In *Lepas anatifera* at least, this difference appears not to be due simply to an additional pigment derived from the *Artemia*, for there is no sign of the presence of any deep blue colour. The blue colour in *Lepas* eggs is considered to be an astaxanthin-protein complex (Ball, 1944), as also is the blue pigment of the oceanic Siphonophore, *Velella lata*, which Fox and Haxo (1958) believe to be derived from crustacean food. It seems probable therefore that an element in the oceanic plankton on which these animals feed contains an essential precursor of the blue astaxanthin-protein complex.

Reproduction

Like operculate Cirripedes and unlike a few pedunculate forms, *Lepas* is an hermaphrodite animal; it bears embryos four to five weeks after settlement (Skerman, 1958; Evans, 1958). Groom (1894) observed in detail the process of fertilization in *Lepas anatifera* L. and our observations confirm his. Occasionally some undeveloped and cytolysed eggs were observed in the egg mass; it was thought that these eggs may not have been fertilized. Failure to effect complete fertilization of the egg mass was considered not unlikely,

because the animals, not being attached to their natural substratum, were unable to copulate and insert the penis normally in the mantle space of the adjacent specimens. Laboratory conditions might also have reduced the potency of spermatozoa. At every successful copulation the individual acting as female contracted its stalk as though to squeeze the ova through the oviducts and up into the mantle cavity where they became attached to the fraenae, the semi-circular folds of skin at the base of the mantle space (Darwin, 1851).

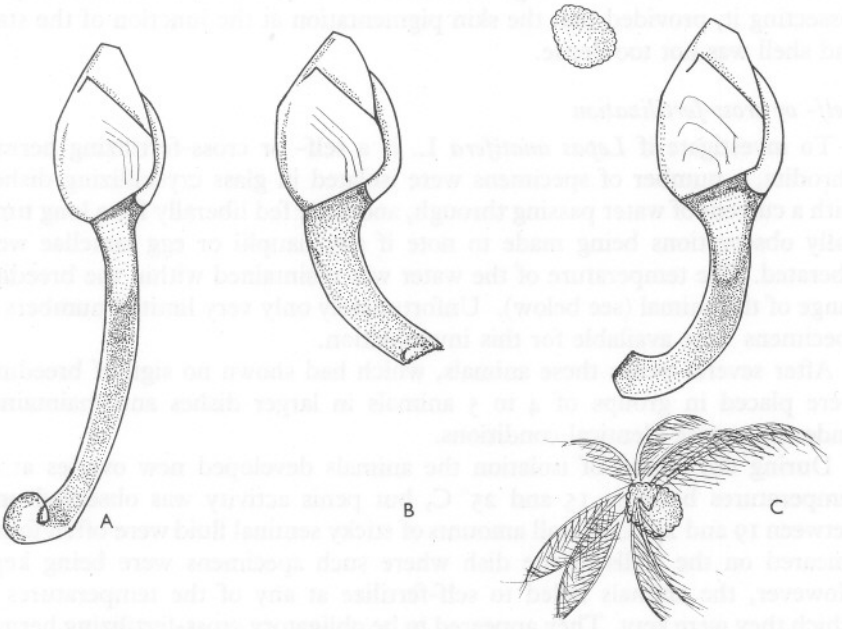

Fig. 1. A single individual of *Lepas anatifera* L. at different phases of breeding cycle. A, Before copulation. The large ovary is visible through the stalk. B, Just after copulation. The ovary has disappeared and the stalk is contracted. C, After liberation and moulting. The two egg lamillae are visible, one still attached to the cast skin. The ovary has redeveloped.

The ovary, originally visible through the stalk, was observed to have disappeared after copulation was completed. This fact could be used to ascertain when an animal had been fertilized. The clear stalk generally remained contracted for two to three days till a new ovary was formed and began to show through the stalk.

The sequence of changes visible outside the barnacle is presented in Fig. 1. One *Lepas*, which had a clearly defined pink ovary showing through the stalk, was marked and photographed (Fig. 1A). It was then put into contact with other animals. Some time later, the marked individual was found in copula; after copulation the ovary disappeared and the stalk remained contracted (Fig. 1B). The *Lepas* was then kept isolated and fed liberally. Three to four

days later a new ovary appeared. On completion of the embryonic development (after seven days at 25° C) it liberated egg masses which were seen to be hatching as stage I nauplii and were accompanied by the cast skin. In Fig. 1 C one of the egg masses is still attached to the cast skin. This sequence of events could be observed in most specimens. The changes in the appearance of the ovary as seen through the upper part of the stalk might usefully be employed in the field to determine whether an animal had recently been fertilized or whether it still carried a large unfertilized ovary, without the necessity of dissecting it, provided that the skin pigmentation at the junction of the stalk and shell was not too dense.

Self- or cross-fertilization

To investigate if *Lepas anatifera* L. is a self- or cross-fertilizing hermaphrodite, a number of specimens were isolated in glass crystallizing dishes, with a current of water passing through, and were fed liberally for a long time, daily observations being made to note if any nauplii or egg lamellae were liberated. The temperature of the water was maintained within the breeding range of the animal (see below). Unfortunately only very limited numbers of specimens were available for this investigation.

After several weeks these animals, which had shown no sign of breeding, were placed in groups of 4 to 5 animals in larger dishes and maintained under otherwise identical conditions.

During the period of isolation the animals developed new ovaries at all temperatures between 15 and 25° C, but penis activity was observed only between 19 and 25° C. Small amounts of sticky seminal fluid were often found smeared on the walls of the dish where such specimens were being kept. However, the animals failed to self-fertilize at any of the temperatures at which they were kept. They appeared to be obligatory cross-fertilizing hermaphrodites like *B. balanoides* L., *B. balanus* (= *porcatus*, da Costa) (Crisp, 1954; Barnes & Crisp, 1956) and *Elminius modestus* (Crisp, 1958). No sooner were they grouped than copulations were observed at temperatures between 19 and 25° C. Clearly under the conditions of these experiments self-fertilization does not occur, but cross-fertilization takes place readily (Table 1).

Breeding temperature

The results of a number of experiments in which groups of animals were maintained for several weeks at approximately constant temperatures are shown in Table 2. It will be noted that breeding was possible between 19 and 25° C but not at or below 15° C, nor at temperatures higher than 30° C. These results were confirmed by the disappearance of ovaries only from the barnacles in dishes kept at 19 and 25° C, and later by the presence of liberated nauplii only in the same dishes. Probably 16° C is close to the lower limit of the breeding range because Skerman (1958) found *L. anatifera* var. *testudinata*

in New Zealand waters bearing embryos at temperatures varying from 17 to 20° C, while Darwin's records (1851), with one exception, are from areas where the temperatures generally exceed this range. Darwin includes a record from the Bass Straits, where the range lies between 13 and 17° C. Evans' (1958) observations during a voyage from Dakar to Barbados indicate that this species breeds readily between 24 and 26° C, and also agree with the behaviour of specimens in the laboratory.

TABLE 1. ABSENCE OF SELF-FERTILIZATION IN *LEPAS ANATIFERA* L. IN THE LABORATORY

(The temperature range of the experiment was 19–25° C)

Number of *Lepas* available during experiment	Condition of specimens	Total no. of barnacle days*	No. of liberations of nauplii seen
25	Isolated	667	0
31	Grouped	619	26

TABLE 2. INFLUENCE OF TEMPERATURE ON BREEDING ACTIVITY IN *L. ANATIFERA* L.

Temperature (°C)	No. of barnacles used	Total no. of barnacle days	No. of liberations observed	Embryonic period (days)	Size of ripe embryos
8–10	9	53	0	—	—
15–16	4	96	0	—	—
19–20	19	431	12	11–12	290 × 136 μ
24–25	12	188	14	6–7	266 × 122 μ
30–31	7	160	0	—	—
34–36	12	Died	—	—	—

* 'Barnacle days' = no. of specimens × no. of days under observation.

It is interesting to note that the breeding optimum, 19–25° C, is close to the optimum range of cirral activity as shown by Southward (1957), (Fig. 2). It is surprising that Boëtius (1952–3) found recently settled *Lepas* in Danish waters where, except in very shallow and locally warm pools, such temperatures would not be expected, though it is also possible that, if its normal planktonic food were available, breeding might occur over a wider temperature range than when fed on *Artemia*. However, the specimens kept in the laboratory at 15–16° C appeared healthy, developed large ovaries, and became fertilized within a few days of their temperature being raised to 24–25° C.

Rate of embryonic development

Groom (1894), following the work of von Willemöes Suhm (1876), studied all the stages of embryonic development, but did not mention the time required for complete development. The exact time required by a fertilized egg to reach its final stage capable of hatching as a stage I nauplius is important in an animal which breeds continuously, as this determines its fecundity

(Crisp & Davies, 1955). The time of the embryonic development was investigated as follows:

The fertilized animals were removed and kept isolated as soon as they were observed in copula or when their ovaries had disappeared from the stalk. They were kept under observation and examined every 6–12 h until liberation of egg masses or nauplii occurred. This was always coincident with moulting.

The time of the embryonic development varied with temperature as shown in Table 2.

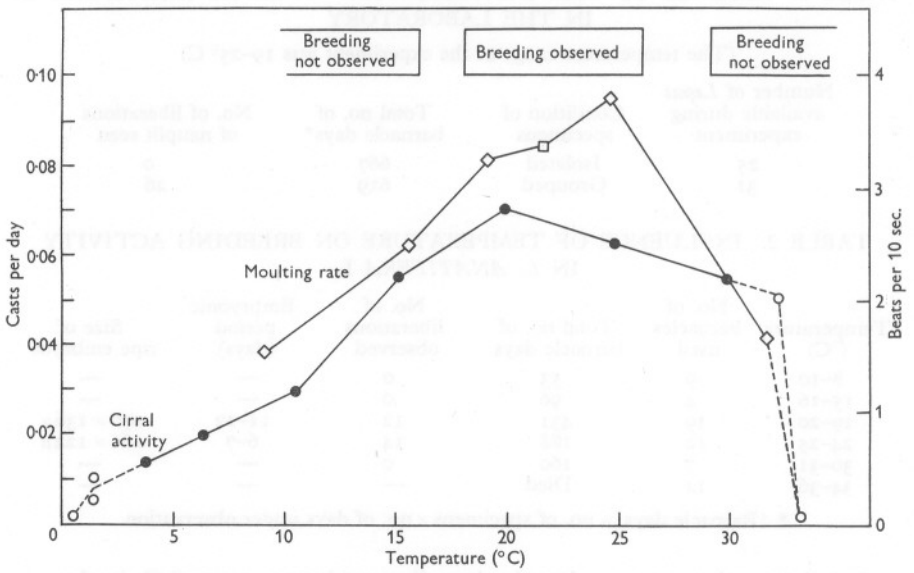

Fig. 2. The effect of temperature on cirral activity (after Southward, 1957), on moulting rhythm and on breeding activity in *Lepas anatifera* L.

The ripe embryos were measured and were found larger both in length and in breadth when the parents had been maintained at the lower temperature and smaller in size when the parent was kept at the higher temperature (Table 2). This phenomenon has been found also in other operculate barnacles. Groom (1894) measured the sizes of the eggs containing ripe embryos from *Lepas anatifera* L. grown under natural conditions, and found their length to be 250 μ. His measurement of the breadth of ripe embryos as stated seems absurdly small and is probably so due to a misprint; it should surely read 145 μ not 45 μ. His figures are then of the same order as ours. Evidently the eggs produced in the laboratory with abnormal food were of the normal size found in nature, as also were the stage I nauplii.

Ecdysis

Within the sub-class Cirripedia the mode of ecdysis differs between sessile and pedunculate forms, as Darwin (1851) noticed. In the sessile forms the chitinous layers of the animal body and also the inner lining of the operculum and the shell are moulted regularly. We confirmed that in *Lepas* the chitinous layers of the prosoma, cirri and penis, together with the lining of the oesophagus and rectum, were regularly cast, but on no occasion did the cast skin include the membrane lining the walls, nor was there any ecdysis of the integument of the stalk. On average an animal took about 15–30 min in completely relieving itself of the cast.

TABLE 3. EFFECT OF TEMPERATURE ON THE FREQUENCY OF MOULTING IN *LEPAS ANATIFERA* L.

	Isolated specimens				Grouped specimens				All specimens		
Temperature (°C)	No. of *Lepas*	Barnacle days	No. of casts	Inter-moult period (days)	No. of *Lepas*	Barnacle days	No. of casts	Inter-moult period (days)	Barnacle days	No. of casts	Moulting rate (cast/day/barnacle)
8–10	—	—	—	—	9	53	2	26·5	53	2	0·0380
15–16	29	1214	76	16·0	4	96	6	16·0	1310	82	0·0625
18–20	3	84	6	14·0	19	431	33	13·0	515	39	0·0760
21–22	11	363	30	12·0	—	—	—	—	363	30	0·0840
24–25	11	220	26	8·5	12	188	22	8·5	508	48	0·0950
30–31	3	9	—	—*	7	160	7	23·0	169	7	0·0415
	Starved specimens										
15–16	11	495	16	31·0	—	—	—	—	495	16	0·0320

* Only three *Lepas* were kept for about 3 days. None moulted during this period, hence no definite figure for the inter-moult period.

All except the last row were fed liberally on *Artemia* and dried liver.

During the experiments described in the section on reproduction observations were made on moulting. Table 3 shows the effect of temperature on the rate of moulting (Fig. 2). It will be seen that with the increase of temperature from 10 to 25° C, the rate of moulting increased in an approximately linear fashion, but at 30–31° C the rate of moulting decreased and the animals subsequently died at 34–35° C. It can be seen from Fig. 1 that the three activities, namely cirral activity (Southward, 1957), moulting rate and breeding increased regularly to an optimum at about 20–25° C, and thereafter decreased sharply.

A few animals were kept without food at 16° C and compared with animals fed on *Artemia* the moulting rate fell considerably (Table 3), cirral activity ceased in the starved individuals, and eventually many died. The fact that even at the lower part of its temperature range *Lepas* cannot survive long without food suggests that it is less able to withstand starvation than most sessile barnacles, since forms such as *Balanus balanoides* L. and *Chthamalus stellatus* Poli can be kept for many months in the laboratory without food.

Relation between moulting and reproduction

Crisp and Patel (1958) showed for the first time that there was generally a relationship between moulting and reproduction in sessile Cirripedes.

In *Lepas*, fertilized specimens in which the ovaries had disappeared from the stalk were placed each in a separate dish and examined daily. None of these individuals moulted whilst carrying embryos. After embryonic development was completed, they usually liberated nauplii and simultaneously underwent ecdysis. In a few instances the animals liberated first and moulted on the following day, but in most cases the salmon-peach-coloured egg masses containing unfertilized eggs with free stage I nauplii (and sometimes a few stage II nauplii) were given off at the same time as, or within an hour or so of, the act of moulting. In one instance only, a *Lepas* kept at 24–25° C moulted although bearing egg masses. This occurred eight days after the ovary had disappeared, by which time the eggs should have been hatching. At the end of another three days it gave off salmon-peach-coloured egg masses. On microscopical examination it was found that none of the eggs, though normally oviposited and cemented together, had developed, and they appeared to be unfertilized.

From Tables 2 and 3 it may be seen that the duration of embryonic development is on average slightly less at all temperatures than the inter-moult period of unfertilized specimens. Thus if the recently moulted individual is able to be fertilized immediately, there would be no interference between the moulting cycle and breeding cycle, the moult normally taking place simultaneously with, or soon after, the completion of embryonic development. On one occasion two animals were put together at 19–20° C, one of them moulted (it was marked) and after a few hours it was found receiving sperms. After the ovary had disappeared from the stalk this specimen was separated and it liberated nauplii as expected after 12 days and moulted on the 13th day.

I am greatly indebted to Dr D. J. Crisp for his generous help and guidance in the experimental work and in the preparation of the manuscript.

SUMMARY

The moulting and breeding activities of *Lepas anatifera* L. were studied under laboratory conditions.

When removed from the substratum and fed on *Artemia* and powdered mammalian liver the animals remained healthy and resumed their normal activities if kept in constantly changing sea water. If the animals were not fed or water ceased to circulate they became lethargic and slowly died.

The ova, normally of a blue colour, develop instead to a pink when the animals are fed on *Artemia* larvae.

Isolated specimens showed penis activity but did not self-fertilize; when

grouped together they became fertilized and produced viable nauplii at temperatures between 19 and 25° C. After successful copulation the acting female shed the ova by contraction of the stalk into the mantle space, and a new ovary developed after 4–5 days.

The rate of moulting increased with rise in temperature from 10 to 25° C but fell after further rise in temperature, 34–36° C being rapidly lethal.

Gravid *Lepas* did not moult while they were carrying embryos, and the liberation of nauplii was always accompanied or followed shortly by ecdysis.

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A NOTE ON SOME PHYSICAL CONDITIONS FOR CULTIVATING *OXYRRHIS MARINA*

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(Text-figs. 1–4)

This note concerns a series of experiments to determine the best conditions of salinity, temperature and pH for cultivating the euryhaline phagotrophic dinoflagellate *Oxyrrhis marina* Dujardin.

The strain of *Oxyrrhis* employed was isolated from a brackish pool at Tvärminne, Finland (Droop, 1953*a*, *b*). The culture medium for the experiments contained soil extract and an artificial sea water, SW 1 (NaCl, $MgCl_26H_2O$, KCl, and $CaSO_42H_2O$ in the proportions by weight 15:2·5:0·4:0·5), and for food a small quantity of the yeast *Saccharomyces exiguus* was administered daily from an agar culture with a wire loop.

The rate of cell division during the logarithmic phase of growth is a measure of the suitability of conditions prevailing. Since growth is by binary fission the number of divisions per day is conveniently given by the relative growth rate when expressed as the binary logarithm of the relative increase in cell numbers per day. This parameter, denoted by k, is simply the slope of the growth curve when cell numbers are expressed as binary logarithms.

Cell counts were made in a deep chamber which allowed the whole of a 0·1 ml. sample to be counted if required. Five samples were usually counted but when numbers exceeded 100 per sample it was more convenient to count several areas within the sample with the aid of a squared eyepiece graticule and compute accordingly.

The statistical treatment in the salinity experiments followed conventional procedures of regression analysis (Snedecor, 1946) and was carried out on the transformed counts. I am indebted to my colleague T. B. Bagenal for advice and for undertaking the analysis.

SALINITY

Different salinities were obtained by varying the amount of SW 1 in the medium. Cultures of *Oxyrrhis* can thrive if the salinity lies between 4‰ and 130‰.[1] Greater salinities were not tested, but below 4‰ cultures failed.

The salinity experiments were required to determine the optimum salinity and also the effect of transfer from one salinity to another. They consisted of

[1] ‰, titrated chloride expressed as g NaCl per l.

two sets of cultures grown at room temperatures and initial pH 7·4 in the following salinities: 4, 8, 16, 32 and 64‰, from inocula adapted to 8‰ in the one set and 64‰ in the other. The parameters to be determined were, relative growth rate, 'apparent initial viable count', and 'initial total count'.

TABLE 1. GROWTH OF *OXYRRHIS* IN CULTURES OF DIFFERENT SALINITY AND INOCULA FROM TWO DIFFERENT SOURCES

(Counts expressed as $\log_2$ cells per ml. Those shown in italics lie off the logarithmic phase of growth and were not used in calculating the regression coefficients.)

	Inoculum from 8‰ culture					Inoculum from 64‰ culture				
Culture salinity	4‰	8‰	16‰	32‰	64‰	4‰	8‰	16‰	32‰	64‰
3rd day	4·9	6·75	8·41	5·32	—	6·64	8·99	10·00	8·82	8·02
	4·32	6·58	8·45	5·64	—	7·90	9·09	10·57	8·32	7·96
	5·32	6·58	8·49	6·32	—	7·84	8·87	10·26	8·53	7·13
	3·32	5·58	8·64	6·13	—	7·02	9·60	9·64	8·82	7·71
	4·9	6·75	8·12	4·32	—	7·49	6·27	9·22	8·90	7·64
5th day	6·13	9·46	12·84	9·86	—	8·36	10·92	13·32	12·32	9·10
	5·90	9·70	12·23	9·88	4·32	8·45	12·71	13·58	12·00	10·07
	6·49	9·66	12·02	9·80	4·32	8·32	11·23	13·45	10·57	9·34
	6·13	9·45	11·32	9·94	3·32	8·22	12·50	12·81	11·82	9·45
	6·32	9·71	11·49	9·88	3·32	8·17	11·91	13·32	11·32	9·34
7th day	5·90	12·57	16·52	11·74	5·32	*8·41*	14·91	16·02	13·58	10·14
	5·64	12·82	16·48	12·57	5·32	*8·12*	15·00	16·21	13·90	10·54
	6·49	13·11	16·63	12·23	5·64	*7·96*	15·58	16·39	14·17	9·64
	6·64	13·12	16·22	13·18	5·32	*8·07*	15·52	16·47	13·58	10·26
	6·9	12·78	16·71	11·49	5·64	*7·64*	15·58	16·08	13·90	10·38
10th day	*6·79*	*15·32*	*16·28*	*15·16*	—	—	*14·96*	*15·94*	*15·12*	12·52
	7·13	*15·46*	*16·28*	*15·58*	8·18	—	*14·70*	*16·92*	*15·64*	12·21
	5·32	*15·80*	*16·68*	*15·75*	8·23	—	*15·00*	*15·83*	*15·12*	12·32
	6·91	*15·52*	*16·86*	*15·58*	6·49	—	*15·09*	*15·96*	*15·64*	13·21
	7·23	*15·52*	*16·52*	*15·64*	—	—	*15·25*	*15·49*	*15·78*	12·75
12th day	—	*16·52*	—	*16·52*	—	—	—	—	—	—
	—	*16·64*	—	*16·04*	10·85	—	—	—	—	—
	—	*16·55*	—	*16·20*	10·81	—	—	—	—	—
	—	*16·46*	—	*16·58*	10·93	—	—	—	—	—
	—	*16·35*	—	*16·24*	—	—	—	—	—	—
14th day	—	—	—	—	12·32	—	—	—	—	—
	—	—	—	—	12·13	—	—	—	—	—
	—	—	—	—	12·77	—	—	—	—	—
	—	—	—	—	11·09	—	—	—	—	—
	—	—	—	—	11·09	—	—	—	—	—

The ten growth curves are shown in Table 1. The earlier counts of each cover the logarithmic phase of growth, and are, therefore, fitted statistically by the general equation

$$n = kt + c,$$

when n represents $\log_2$ cells per ml. at time t, k and c being constants, the former the relative growth rate, the latter the logarithm of the 'apparent initial viable count', i.e. n at $t = 0$. The two constants and their 95% fiducial limits for the ten regressions are plotted against salinity in Figs. 1 and 2.

Since a clear maximum for k occurred at 16‰ in both experiments (Fig. 1) it can be concluded that the optimum salinity at 16‰ was not influenced by the salinity of the inoculum.

In contrast to k, the second constant c did depend to some extent on the source of the inoculum. The value of c must be determined primarily by the 'initial total count', that is the number of cells introduced, but it would be influenced either by mortality of cells on transfer or by an initial lag preceding the start of regular cell division. Either effect could result from a change in the composition of the medium. They are not distinguished from each other in the regressions and are conveniently treated as wholly mortality.

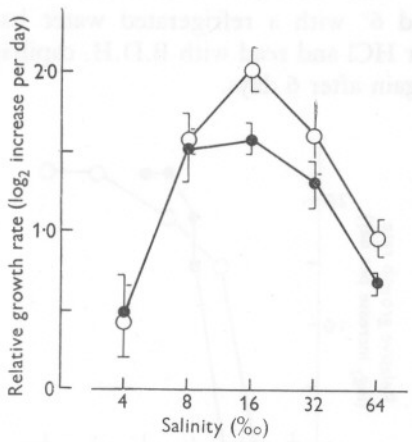

Fig. 1

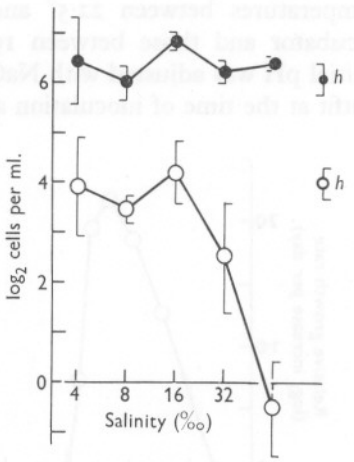

Fig. 2

Fig. 1. Relative growth rate (k) as a function of salinity. Open circles, 8‰ adapted inoculum; filled circles, 64‰ adapted inoculum. 95% fiducial limits indicated.

Fig. 2. Log 'apparent initial viable count' (c) as a function of salinity. Open circles, 8‰ adapted inoculum; filled circles, 64‰ adapted inoculum. Log 'initial total count' (h) in each case is shown on the right. 95% fiducial limits indicated.

The 'initial total count' was obtained by counting sample 0·06 ml. aliquots from the inoculum cultures and dividing by 6 (since an inoculum of 0·06 ml. was being given to 6·0 ml. of culture medium). h, the logarithm of this, in the 8‰ experiment was 3·95 (fiducial limits $\pm 0{\cdot}30$), and in the 64‰ experiment 6·08 (limits $\pm 0{\cdot}31$). Fig. 2 shows that h is significantly higher than c only in the case of the two cultures of highest salinity in the 8‰ experiment, from which the conclusion is drawn that, while the transfer from a high to low salinity was tolerated without shock, the more extreme cases of reverse transfer were not experienced without harm to the population.

Microscopic observation of the behaviour of cells on transfer confirmed this conclusion and, moreover, showed that the correct interpretation of the

phenomenon was mortality on transfer and not initial lag. Thus, cells suddenly transferred from 8 to 64‰ quickly became lean and angular and apparently dehydrated, shedding their flagella in most cases and all but 2 or 3% failing to recover; whereas they became swollen and almost completely spherical and sluggish on transfer in the downward direction and they did not shed their flagella but regained shape and activity within the hour.

TEMPERATURE AND pH

A medium of 16‰ salinity was used in the temperature and pH experiments. Initial pH in the former was 7·4 and temperature in the latter 22·5°. The temperatures between 22·5° and 34° were obtained with a conventional incubator and those between 19° and 6° with a refrigerated water bath. Initial pH was adjusted with NaOH or HCl and read with B.D.H. capillator outfit at the time of inoculation and again after 6 days.

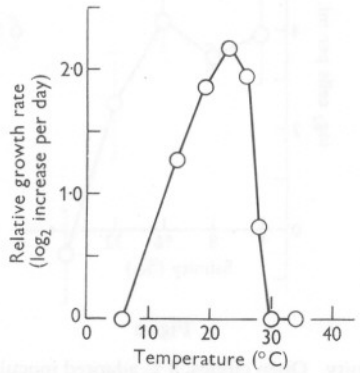

Fig. 3

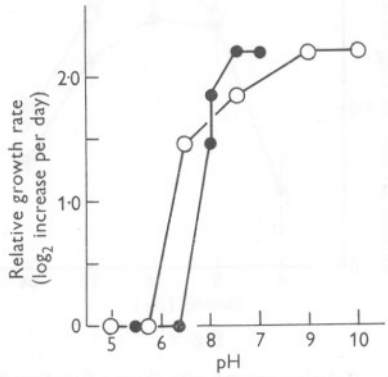

Fig. 4

Fig. 3. Relative growth rate (k) as a function of temperature.

Fig. 4. Relative growth rate (k) as a function of pH. pH measured: open circles, at start; filled circles, after 6 days.

The results of the two experiments were not subjected to statistical analysis, since the sole parameter required was relative growth rate. They are shown in Figs. 3 and 4, where relative growth rate is graphed against temperature or pH. There was a temperature optimum of 22°–23°, an upper tolerance limit, of 28°, and a Q_{10} of about 2·7 between 10° and 20°. A pH of 7 or over appears to be suitable.

DISCUSSION

A maximum division rate of 2·2 per day is higher than is normally met among dinoflagellates and may be correlated with *Oxyrrhis*'s phagotrophic habits. Braarud (1951) obtained maxima of 0·32 for *Exuviaella baltica*, 0·55 for

Amphidinium sp., 0·95 for *Peridinium trochoideum*; while Braarud & Rossavik (1951) obtained 0·55 for *Prorocentrum micans*, Braarud & Pappas (1951) 0·89 for *Peridinium triquetrum*, and Sweeney (1954) 0·67 for *Gymnodinium splendens*.

Oxyrrhis is most often encountered in brackish rock pools above the high-water mark and sometimes occurs in water of very high salinity. At the other extreme, it is seldom found in pools whose salinity is lower than 4‰, in which respect it differs from many other supra-littoral species which tolerate or even prefer the lower salinities (Droop, 1953*a*, table 10; 1955, table 1). The optimum salinity for the neritic species studied by Braarud (1951) ranged from 16 to 20‰ with upper and lower toleration limits only slightly narrower than those reported here for *Oxyrrhis*. It seems that salinity tolerance *per se* cannot satisfactorily account for the absence of neritic species from supra-littoral pools or of *Oxyrrhis* from the sea.

The response to sudden changes in salinity is interesting and shows *Oxyrrhis* well fitted to its habitat, for the salinity of sea-water marks the limit to which a pool can suddenly be raised by sea splash, any further rise being necessarily by evaporation and therefore slow. In south Finland, where this strain originated, this figure is 6‰. On the other hand, the reverse change, which is brought about by flooding with rain water, can be quite rapid, especially if mixing is not delayed. It has been observed previously (Hopkins, 1938) that the physiological response to changes in salinity of marine Protozoa not possessing a rigid periplast or contractile vacuoles is to contract or to swell. This is due to a temporary unbalance between the osmotic pressure of the internal and external media. Recovery is stated to be due to adjustment by the passage of salts through the membrane, though it might equally be brought about by mobilization or immobilization of carbohydrate reserves of high osmotic pressure. *Oxyrrhis* certainly has great powers of adjustment, particularly when the change is made gradually. The fact that dehydration is a greater hazard than bursting speaks for the tensile strength of the periplast.

The response to pH was as expected, for a pH 8–9 is normal for a pool containing *Oxyrrhis* and one below 7 is seldom encountered in the aerobic layers of supra-littoral pools. The temperature curve is conventional: its peak suggests that *Oxyrrhis* is a summer organism, which indeed it is, though the high rainfall in this country or ice cover in Finland would in any case keep the pools empty of flagellates during the winter months. The upper laboratory temperature limit of 28° is often exceeded for short periods in pools containing large populations of *Oxyrrhis* during the summer; for instance, I obtained midday records of 30° in Finland (Droop, 1953*a*). Possibly other more southern races of this species tolerate higher continuous temperatures.

SUMMARY

Maximum division rate of the dinoflagellate *Oxyrrhis marina* Dujardin feeding on *Saccharomyces exiguus* occurred in cultures of salinity 16‰, temperature 22·5° and pH 8–10, and was 2·2 per day.

Salinities below 4‰, pH below 6·5 and continuous temperatures over 28° were not tolerated.

Sudden extreme changes in salinity were tolerated in the downward but not in the upward direction.

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WATER-SOLUBLE FACTORS IN THE NUTRITION OF *OXYRRHIS MARINA*

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(Text-figs. 1–8)

Oxyrrhis marina Dujardin is a non-photosynthetic dinoflagellate whose obvious mode of nutrition is phagotrophy. The method of feeding was described by Barker (1935). It is an extremely euryhaline and hardy organism and is apparently very successful in brackish habitats such as supralittoral rock pools and ditches (Droop, 1953*a*). Owing to the ease with which it can be cultivated *Oxyrrhis* is a choice for the initial study of phagotrophy among the predominantly plant-like Dinoflagellata.

Axenic cultures (i.e. cultures free from other organisms of any kind) are required for nutritional studies, but they are not often easily established when the subject is phagotrophic. *Oxyrrhis* was no exception, despite the ease with which it can be kept in the laboratory. Bacteria-free cultures of a Finnish race were established in 1951 (Droop, 1953*b*), but there then appeared to be a requirement for a living food organism such as a yeast or alga. Dr J. J. A. McLaughlin (Haskins Laboratories, New York, personal communication) succeeded, however, with axenic cultures of another strain in 1954, but unfortunately was not in a position to pursue the matter.

Dr McLaughlin's media contained, in addition to sources of water-soluble nutrients, such substances as soya meal, corpus luteum extract, cream, and beef serum in an attempt to meet the suspected fat requirement. These ingredients proved difficult to handle and unreliable in Dr McLaughlin's experience and, moreover, in my hands they entirely failed to support axenic growth. The first reliably successful axenic cultures at Millport were obtained in 1955 with a medium supplemented with 4 ml./l. of neutralized, strained but unfiltered, lemon juice. At a later date the juice was replaced by a carbon tetrachloride extract of the rind, which has the advantage of being composed entirely of fat-solubles. Isolation and identification of this fat-soluble 'lemon factor' is not completed, and will not be discussed further in this paper, which is concerned with water-soluble components of the medium.

MATERIALS AND METHODS

The strain of *Oxyrrhis* referred to as the Finnish race (Millport No. 18; Culture Collection of Algae and Protozoa, the Botany School, Cambridge, No. LB 1133/1) and used in these experiments was isolated by myself from

a supra-littoral pool at Tvärminne, Finland, in 1951. To obtain axenic cultures, monoxenic ones, in which the food organism was an obligate phototroph (*Nannochloris oculata*), were simply incubated in the dark with lemon juice. Tests showed these cultures to be free of algae after two transfers.

Stocks were maintained in medium E 6 (Provasoli, McLaughlin & Droop, 1957) supplemented with the lemon factor.[1] The extract referred to as 'LF' henceforth is a Sohxlet carbon tetrachloride extract of autoclaved, then oven-dried (70° C) lemon rind, concentrated to 10 ml. per lemon, and kept well-stoppered in complete darkness. LF was shaken up with the medium beforehand and the solvent driven off on autoclaving, leaving the extractives in fine suspension. Alternatively, in later experiments not concerned with carbon nutrition, LF was administered from ethanolic solution, which obviated shaking.

Glassware was cleaned with 'Lux' then chrome-sulphuric acid followed by repeated rinsing in tap, then distilled, water. Synthetic media were prepared from AR quality chemicals with glass-distilled water. Approximately 0·6 ml. of inoculum was used to start experimental cultures of 6 ml. capacity in 15 × 150 mm Pyrex test-tubes plugged with cotton-wool (aluminium caps in some experiments as a check against chemical contamination from cotton-wool). Cultures were incubated in darkness at 22° C. Elimination of carry-over from the complex to simpler media in the experiments was effected by serial transfers, ten being regarded as sufficient for this purpose. A bacteriological peptone or medium E 6 was used for frequent routine sterility tests, a necessary precaution since phagotrophy can obscure bacterial contamination in cultures. Cell counts were made in a chamber 0·67 mm deep with the aid of a squared eyepiece graticule, and are expressed as cells per mm^3 to the nearest whole number.

DEVELOPMENT OF A DEFINED BASAL MEDIUM

The first step in simplifying the stock medium E 6 was to replace the liver extract and Bacto Tryptone by 3 mg/l. tryptophane, 400 mg/l. Bacto Vitamin-free Casamino Acids (VFC) and 3 ml./l. of the B vitamin mixtures, D 7, 8 and 9 (Table 1). Yield of cultures then increased to over 100 cells per mm^3 and was maintained thus over many transfers.

The next stage was to replace VFC by synthetic mixtures of amino-acids (AA I–AA V, Table 1) with, however, only limited success, as the amino-acids gave a reduced yield which was restored by 200 mg/l. VFC. It later transpired that the new medium lacked metabolizable carbon.

[1] 1 l. of supplemented E6 contains: Oxo Liver Infusion (L25), 250 mg; Bacto Tryptone, 250 mg; glucose, 250 mg; soil extract, 12·5 mg; KNO_3, 50 mg; K_2HPO_4, 5 mg; $MgSO_47H_2O$, 5 mg; LF, 3 ml.; natural sea water, 500 ml.

Soil extract could be omitted and artificial solutions SW 1 and SW 2 (Table 6; Provasoli *et al.*, 1957) and a dispersing agent for LF, sodium taurocholate, could substitute for the natural sea water in E 6, provided the liver infusion was kept. But with liver infusion and Tryptone replaced as above neither natural sea water nor soil extract were replaceable by the buffered trace metal solutions usually used for this purpose (e.g. TM2 and the buffer tris(hydroxymethyl)aminomethane (TRIS) as used in S36 and S46, etc. (Provasoli *et al.*, 1957).

An alternative to the obvious hypothesis that unknown growth factors were being supplied by both soil extract and natural sea water was that the mixtures replacing them were toxic. Both TRIS and EDTA (ethylenediaminetetraacetic acid, the chelating agent in TM2) were known to be toxic to some organisms, and the possibility that TM2 was unbalanced could not be ruled out. So indeed it proved, for eventually soil extract and natural sea water were successfully replaced by a synthetic medium containing neither TRIS nor EDTA.

TABLE 1. S49, PARTIALLY DEFINED 'UNTAILORED' MEDIUM
(LF being the only undefined component)

SW 1*	250 ml.
SW 2*	5 ml.
TM 11B*	10 ml.
Glycylglycine	500 mg
L-Histidine	200 mg
K_2HPO_4	10 mg
KNO_3	100 mg
Na taurocholate	3·0 mg
LF	3·0 ml.
Fourteen supplementary solutions†	each 4·0 ml.
H_2O to	1·0 l.
pH adjusted to 8·0 before autoclaving	

* Cation mixtures, see Table 6.

† Supplementary mixtures (amounts unless otherwise stated in mg per l.):

D7: putrecine, 100; spermine, 100; choline, 500.

D8: niacin, 100; Ca pantothenate, 100; pyridoxine, 20; riboflavin, 5·0; *p*-aminobenzoic acid, 10; inositol, 1000.

D9: thiamine, 200; biotin, 0·5; vitamin B_{12}, 0·05; folic acid, 1·0; folinic acid, 0·2; thioctic acid, 0·5.

PP I: guanine, 1000; adenine, 500; uracil, 300; thymine, 300; cytidylic acid, 300.

AA I: DL-alanine, 10,000; DL-aspartic acid, 10,000; glutamic acid, 10,000; glycine, 10,000.

AA II: L-arginine, 10,000; L-histidine, 5000; DL-lysine, 10,000.

AA III: DL-isoleucine, 1000; DL-phenylalanine, 1000; DL-leucine, 1000; L-tyrosine, 1000.

AA IV: DL-methionine, 1000; DL-threonine, 2000; DL-tryptophane, 1000.

AA V: DL-serine, 2000; L-proline, 1000; L-valine, 1000.

FA I: Na acetate (anhyd.), 10,000; butyric acid, 1·0 ml.; propionic acid, 1·0 ml.; valeric acid, 1·0 ml.

FA II: succinic acid, 10,000; α-ketoglutaric acid, 10,000; fumaric acid, 10,000; malic acid, 10,000.

FA III: pyruvic acid, 10 ml.; lactic acid, 10 ml.; citric acid, 10,000.

CHHO I: arabinose, 10,000; rhamnose, 10,000; xylose, 10,000; glucose, 10,000; fructose, 10,000; galactose, 10,000; mannose, 10,000.

CHHO II: lactose, 20,000; sucrose, 20,000; maltose, 20,000.

In this, glycylglycine acted as pH buffer, and glycine or histidine as chelating agent in a new trace metal mixture (10 ml. of which contained such amounts of the metals as might occur in 1 g of algal protoplasm). The new medium retained the three B vitamin mixtures, the five amino-acid mixtures, SW 1, SW 2, potassium nitrate and phosphate, LF, and sodium taurocholate, but glucose was replaced by five solutions embracing a total of 22 carbon compounds.

The quantity of chelator was varied (glycine: 2, 10 and 100 mg/l.; histidine: 4, 20 and 200 mg/l.), but after two transfers it was clear that the two lower values of either amino-acid were unsuitable.

A medium with high histidine, S 49, was therefore adopted; it is given in full in Table 1. This was the first essentially synthetic basal medium to allow more than a couple of transfers. In its 'untailored' state it gave smaller yields than the original E 6, but in subsequent transfers, as the essential constituents were identified and brought up to adequate concentration, the yield came to surpass that of the original medium and no component of the latter effected any improvement, although mature cultures lasted rather longer in E 6.

Simplification of this appallingly complex medium was in principle a relatively straightforward undertaking, although it took many months, the work involved being comparatively great.

Carbon source

Identification of essential carbon sources involved a basal medium lacking the five carbon solutions, FA I–III and CHHO I and II. This medium supported a negligible amount of growth and, moreover, was scarcely improved by the single addition of any one of the carbon solutions (Table 2). But when the solutions were added in pairs (Table 3) FA III was shown to be essential and to give best results in combination with FA I, though it was not clear at this stage whether the other solutions also contained available compounds. A breakdown of FA III (Table 4) showed citrate to be the active component of this solution. The active component of FA I was similarly identified as acetate. Citrate, together with a high concentration of acetate, allowed good repeatable growth.

Subsequently no other compound included in solutions FA I–III and CHHO I–II proved to be available as a source of carbon, and the following were also ineffective: glycerol, erithritol, mannitol, sorbitol, dulcitol, raffinose, melizitose, glycogen, starch, inulin, dextrin, salicin and aesculin. Unavailable also was the carbon skeleton of any amino acid included in solutions AA I–V, or of asparagine. Ethanol, however, was utilized and was not toxic at a concentration of 4 ml./l. In contrast, propionic, butyric, valeric and lactic acids were toxic in concentrations over 0·04 ml./l.

By way of confirmation, yield in response to graded doses of sodium acetate, glucose, citric acid, ethanol and valine is shown in Fig. 1. The inoculum for

these cultures had been carried for four transfers in medium S49. Citrate thus proved not to be available as a bulk carbon source, whereas acetate served just this purpose since yield appeared as directly proportional to sodium acetate concentrations up to 0·2%. The requirement for citrate is discussed later.

TABLE 2. EFFECT OF SINGLE CARBON SOLUTIONS

(Basal medium: S49 with the five carbon solutions omitted. Cells per mm³ after 3 weeks' growth.)

No addition	FA I	FA II	FA III	CHHO I	CHHO II	All five solutions
			First transfer			
11	9	9	18	9	8	120
			Second transfer			
4	2	2	6	5	5	37

TABLE 3. EFFECT OF CARBON SOLUTIONS IN PAIRS

(Basal medium: S49 with the five carbon solutions omitted. First transfer. Cells per mm³ after 3 weeks' growth.)

No addition	FA I and FA II	FA I and FA III	FA I and CHHO I	FA I and CHHO II	FA II and FA III
9	10	122	9	9	19

FA II and CHHO I	FA II and CHHO II	FA III and CHHO I	FA III and CHHO II	CHHO I and CHHO II
9	8	22	28	8

TABLE 4. BREAKDOWN OF FA III

(Basal medium: S49 with FA III omitted. Cells per mm³ after 3 weeks' growth.)

No addition	Pyruvate	Lactate	Citrate	Pyruvate and lactate	Pyruvate and citrate	Lactate and citrate	Pyruvate and lactate and citrate
			First transfer				
11	9	8	89	9	82	69	64
			Second transfer				
6	5	0	32	3	42	20	26

Nitrogen source

When amino-acid solutions AA I–V were omitted, medium S49 did not support more than a few cells per mm³ in the first and later transfers. Furthermore, potassium nitrate had no effect on the medium either in the presence of the amino-acids or in their absence and it was subsequently omitted from all media. Oxidized nitrogen is evidently unavailable.

The single addition of AA I or especially AA V restored the depleted medium, whereas AA II–IV were without effect. A breakdown of AA I and V showed valine, proline and alanine as active, and aspartic and glutamic acids, glycine and serine as inactive. In later experiments valine proved the most readily available of the three amino-acids and yielded the best dose/response curves, although activity of the other two was of the same order (Fig. 2). No response was obtained to the ammonium ion, which was apparently too toxic at pH 8 to be of any use, nor to urea, uric acid or asparagine.

Valine was therefore adopted as N source, but a little proline was also retained, as it appeared to be stimulatory in early phases of cultures.

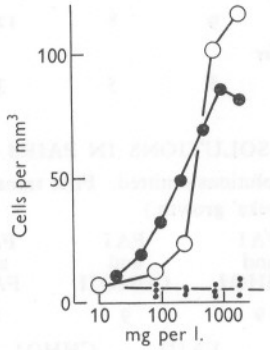

Fig. 1

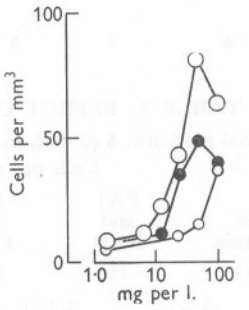

Fig. 2

Fig. 1. Carbon sources. Yield in response to increasing amounts of: sodium acetate (open circles); ethanol (filled circles); glucose, citric acid and valine (dots).

Fig. 2. Nitrogen sources. Yield in response to increasing amounts of: valine (large open circles; proline (filled circles); alanine (small open circles).

Growth factors

In the untailored medium S 49 possible growth factors were being supplied in solutions PP 1, D 7, D 8 and D 9. A conventional process of elimination proved D 9 to be the only essential mixture. Similarly, in D 9, thiamine, vitamin B_{12} and biotin proved essential and folic, folinic and thioctic acids unessential. In these analyses it was, of course, necessary to resort to serial transfers, owing to the minuteness of the requirements. The requirements for thiamine and vitamin B_{12} were absolute, that for biotin possibly not so (Table 5 and Fig. 7).

Dose/response curves for thiamine and vitamin B_{12} are given in Fig. 3. The thiamine requirement was previously reported in this *Journal* (Droop, 1958); thiazole replaces thiamine completely. Specificity towards the vitamin B_{12} analogues proved to be of the '*Ochromonas*' pattern (Kon, 1955) with the exception that factor A, a natural analogue containing 2-methyl-adenine in place of 4, 6-dimethyl-benzimidazole, showed slight activity in my experiments.

On the other hand, pseudo-vitamin B_{12}, the analogue containing adenine in the nucleotide, was quite inactive. A similar specificity has been reported by McLaughlin & Provasoli (1957) for two photosynthetic species of *Amphidinium*.

TABLE 5. REQUIREMENT FOR VITAMIN B_{12} DEMONSTRATED BY SERIAL TRANSFERS

(Dose level: 100 mμg/l. Yield in cells per mm^3)

No addition	With vitamin B_{12}	With factor A	With pseudo-vitamin B_{12}	With factor B
		First tranfer		
14	269	—	—	—
		Second transfer		
1	41	—	—	—
		Second transfer (repeat)		
1	133	11	1	1

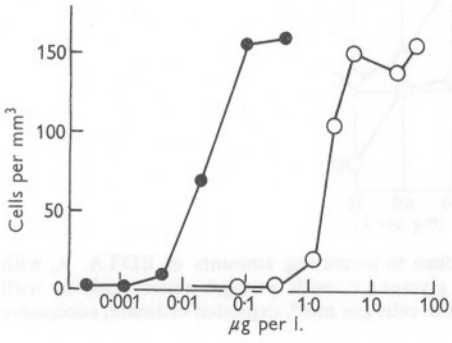

Fig. 3

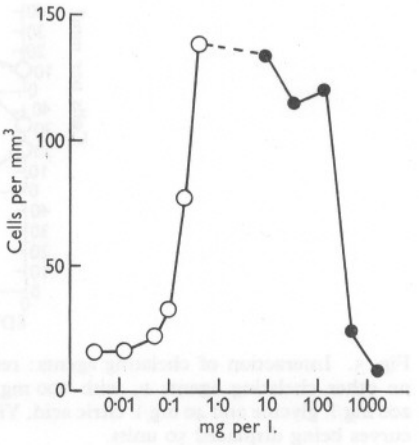

Fig. 4

Fig. 3. Response to increasing amounts of: thiamine (open circles); vitamin B_{12} (filled circles).
Fig. 4. Response to increasing amounts of citric acid (two experiments).

Citric acid

It was established that citrate, though apparently required, was not available as a bulk carbon source. It might, therefore, be functioning either as a growth factor or as a chelating agent.

Response to graded doses of citrate is shown in Fig. 4. The basal medium contained histidine and the metals of TM 11 B, but citrate was omitted. The inoculum had passed two passages in citrate-free medium. The response is seen to be sensitive to citric acid concentrations between 10 and 100 μg/l., while concentrations over 100 mg/l. were toxic. One would suppose that such

a high order of activity would rule out chelation. However, in another experiment with a medium containing 50 mg/l. EDTA there was no response to citrate at the low end of the scale but growth was uniformly depressed, which suggested that chelation was, after all, involved.

A further set of experiments, undertaken with a basal medium lacking both citrate and histidine but containing the trace metals of TM 11 B, not only showed that it was possible to dispense with citrate if the correct amount of EDTA were present but also that glycine, citrate and EDTA all interacted in such a way as to suggest that their chelating properties were additive (Fig. 5). Thus, while best growth was obtained with 6·0 mg/l. EDTA in the absence of

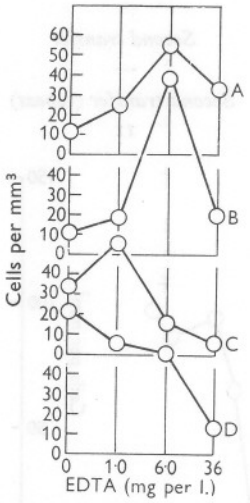

Fig. 5. Interaction of chelating agents: response to increasing amounts of EDTA. A, with no other chelating agent; B, with 200 mg/l. glycine; C, with 40 mg/l. citric acid; D, with 200 mg/l. glycine and 40 mg/l. citric acid. Yield in cells per mm³, exploded ordinate, successive curves being displaced 50 units.

citrate, in its presence (40 mg/l. citric acid) only 1·0 mg/l. EDTA was required. Furthermore, with citrate and glycine both in the medium no EDTA was required.

The apparent requirement for citrate is thus seen to be due to inadequate chelation by glycine or histidine. The fact that the ferric ion forms no complex with α-amino acids (Albert, 1950) should have made this conclusion obvious; indeed, in the absence of both citrate and EDTA a precipitate, which could be iron hydroxide, forms in the medium upon autoclaving.

On the other hand, the reason for poor growth in media with high (20–60 mg/l.) EDTA is not at all clear. Over-chelation is ruled out because calculation[1] shows the concentrations of the free ions of each of the metals in the

[1] By a method suggested by Spencer (1958), with the aid of published stability constants.

mixture actually to be lower with the citrate-glycine or histidine mixtures than with even the highest concentrations of EDTA used. EDTA may indeed be toxic to *Oxyrrhis* for some entirely unconnected reason.

It is possible, however, to conclude empirically, that in a half-strength artificial sea-water medium with cationic concentrations as shown in Table 6, and in which the strongest chelators otherwise are valine and glycylglycine, the main function of chelation can be discharged by a variety of combinations of EDTA, citrate and glycine or histidine, bearing in mind that glycine and histidine do not chelate trivalent iron and that EDTA behaves as if it were inherently toxic.

pH *control*

The use of synthetic media introduces an urgent need for pH control, for a great many variables are influenced by pH. In particular, the degree of ionization and penetration of weak acids, bases and amphoteric substances is affected and consequently their utilization or toxicity. pH also enters as a term in the mass-action equations controlling the behaviour of heavy metals with chelating agents and on that account may not be ignored. It can be said with truth that a synthetic culture medium must be developed in relation to, and may only be useful over, a limited pH range.

pH control during growth does not present great problems except in certain circumstances. But the act of autoclaving a culture medium does create difficulties. Permanent damage may be done to a medium in which pH buffering is left to the carbonate-bicarbonate system, because the great rise in pH occasioned by the loss of carbon dioxide causes uncontrolled, usually irreversible, precipitation of various components. However, the practice of incorporating artificial buffers overcomes this difficulty to a large extent (Provasoli *et al.* 1957).

Since one seeks to prevent pH *rise* during autoclaving, the region where high buffer capacity is needed is immediately and for some distance above the setting. Thus, for a setting of pH 8, media buffered with glycylglycine ($pK_2 = 8{\cdot}1$)[1] should be improved by the presence of compounds having higher pK values. Valine ($pK_2 = 9{\cdot}6$), histidine ($pK_2 = 9{\cdot}7$) or glycine ($pK_2 = 9{\cdot}7$) serve this purpose in addition to their other functions (Fig. 6).

There are, however, valid objections to the use of organic acids in culture media on the grounds that they complicate the situation needlessly. Some pH buffers are also chelating agents; many of them (e.g. glycylglycine) are weak and can be ignored in adequately balanced media, but others have considerable influence and must be taken into account, as has been done in the case of histidine and glycine in the previous section. It is also undesirable that substances introduced for their physical effects should be metabolized, and due account should be taken of this possibility in any instance. But the

[1] The relatively more toxic TRIS buffers in the same region.

converse is equally true: the physical properties of compounds introduced as nutrients cannot always be ignored.

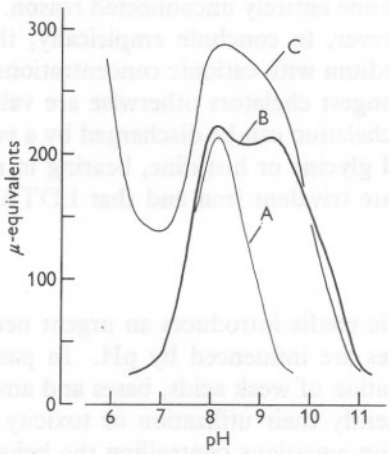

Fig. 6. Buffer capacity of some culture media in μ-equivalents of acid or alkali required to displace pH by 0·1. A, media with 3·8 mM glycylglycine or TRIS alone (e.g. S36, Provasoli *et al.* 1957); B, media with 3·8 mM glycylglycine and 3·3 mM glycine (e.g. S50, Droop, 1958); C, media with 3·8 mM glycylglycine, 1·3 mM histidine, 2·1 mM valine and 25 mM acetic acid (S69, Table 6). (Based on data from the '*Bufferule*' by the California Corporation for Biochemical Research.)

FINAL MEDIUM

The conclusions expressed in the previous pages are embodied in the two 'final' media S68 and S69 set out and annotated in Table 6. If handled carefully *Oxyrrhis* maintains heavy yields on continued subculturing in S69 (Fig. 7); consequently it is likely that all *absolute* water-soluble requirements are now defined.[1]

In Fig. 8 a typical growth curve in S69 and one in OX7 (a maintenance medium[2]) are contrasted with growth on living *Saccharomyces exiguus* (Droop, 1959). The contrast is sufficiently striking and is in a sense a measure of failure to substitute a non-living for a living diet. In this connexion it should be mentioned that although whole milk, egg yolk and lipids such as olive, linseed and palm oils can be ingested none have been found which improve the rate of growth or replace acetic acid. It would appear to be a living diet and not phagotrophy *per se* which makes for the high growth rate (Droop, 1953*b*).

Two further characteristics of axenic cultures which have been responsible for many delays and frustrations in this investigation are the inability of

[1] Proline and histidine have now also been rigorously eliminated from the list of possible absolute requirements.

[2] One litre of OX7, which is now recommended for maintenance of axenic stocks, contains: Bacto Casitone, 125 mg; soil extract, 30 mg; Na acetate, 2 g; valine, 250 mg; D9 vitamins, 20 ml.; K_2HPO_4, 10 mg; LF, 3 ml.; natural sea water, 500 ml.

isolated cells to multiply, even in media which support normal subculturing indefinitely, and the inability of a population whose growth has been arrested by nutritional depletion to pick up again on being transferred to a complete

TABLE 6. MEDIA S68 AND S69

(Amounts per l.; pH adjusted to 8·0 before autoclaving.
Note: glycine can replace L-histidine in S69.)

NaCl	15 g	Major ions of sea water (i.e. 250 ml. of 'SW1')
KCl	400 mg	
$MgCl_26H_2O$	2·5 g	
$CaSO_42H_2O$	500 mg	
$SrCl_26H_2O$	6·5 mg	Minor constituents (i.e. 5·0 ml. of 'SW2'. This can very likely be omitted)
KBr	33 mg	
$AlCl_36H_2O$	250 μg	
RbCl	100 μg	
KI	50 μg	
$FeSO_47H_2O$	2·5 mg	Trace metals (i.e. 10 ml. of TM11B)
$MnSO_4H_2O$	100 μg	
$ZnSO_47H_2O$	100 μg	
$CuSO_45H_2O$	20 μg	
$CoSO_47H_2O$	2·5 μg	
$NaMoO_42H_2O$	1·2 μg	
EDTA*	6·0 mg	Chelating agent in medium S68
Citric acid†	40 mg	Chelating agent in medium S69
L-Histidine†	200 mg	Chelating agent and pH buffer at 9·2 in medium S69
Glycylglycine	500 mg	pH buffer at 8·1
L-Valine	250 mg	N source and pH buffer at 9·6
K_2HPO_4	10 mg	P source
Na acetate (anhydr.)	2·0 g	C source
L-Proline	40 mg	Water-soluble growth factors
Thiamine	100 μg	
Biotin	50 μg	
Vitamin B_{12}	200 mμg	
Na taurocholate	3·0 mg	Dispersing agent for LF
LF	3·0 ml.	CCl_4 extract of lemon rind, containing an unknown lipid factor

* Omitted from S69. † Omitted from S68.

medium. The rate of growth once depressed may stay so over many transfers, or even indefinitely. As can be imagined, these characteristics render the serial subculturing technique extremely difficult to handle. I am of the opinion that the explanation of this behaviour will prove to lie with the lipid factor and not the water-soluble nutrients, but one cannot be sure. However, no aqueous extract or hydrolysate of natural materials yet tried has been of any avail; and filter-sterilizing crude media to avoid destroying heat-labile substances has likewise been useless.

NUTRITIONAL STATUS

Dinophyceae have since Pascher (1914) and Fritsch (1935) been generally recognized as one of the great algal series; albeit one represented almost entirely by motile unicells.[1] They are not alone among algae in having phagotrophic

[1] The possible affinity of *O. marina* with the Cryptophyceae (Dragesco, 1952) emphasizes Pascher's views regarding the near relationship between the two series.

representatives; this character they share with Euglenophyceae and Chrysophyceae. Starch staining blue with iodine is among the reserve products, though in my experience in many species, including *Oxyrrhis marina*, starch is only found in the cysts. Lwoff (1944, p. 215) and Hutner & Provasoli (1955, p. 19), however, stress the animal or protozoan tendencies shown by dinoflagellates.

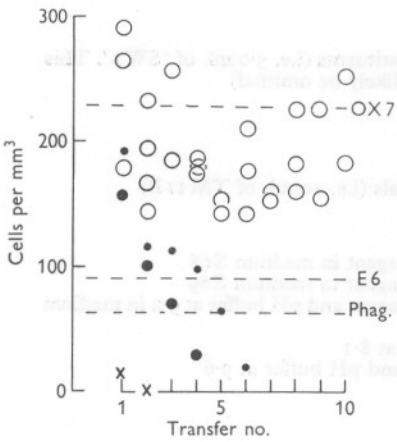

Fig. 7

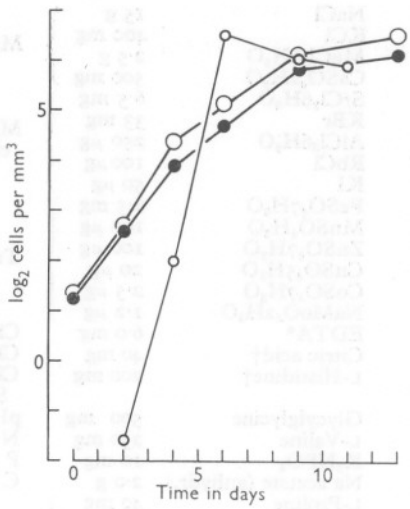

Fig. 8

Fig. 7. Yield in successive transfers in S69 (open circles); S69 without biotin (filled circles) (two experiments); S69 without either thiamine or vitamin B_{12} (crosses). Yields normally obtained in undefined media indicated by broken lines: axenic cultures in OX7 and E6; monoxenic cultures on *Saccharomyces exiguus* in Erdschreiber ('phag').

Fig. 8. Typical growth curves: axenic cultures in OX7 (large open circles) and S69, 10th transfer (filled circles); monoxenic cultures on *Saccharomyces exiguus* in Erdschreiber (small open circles).

Superficially *Oxyrrhis* has an entirely animal-like nutrition, for it can rely, and probably does so in nature, on phagotrophy for all its major and accessory nutrients. Only when phagotrophy is denied it and an analysis then made of its nutritional requirements and abilities does a more plant-like nature emerge.

Taking carbon nutrition first: *Oxyrrhis* proves to be an 'acetate flagellate' (Pringsheim, 1935; Pringsheim & Hovasse, 1948; Hutner & Provasoli, 1951) since it uses acetic acid and ethanol, not glucose nor the carbon of amino-acids, for growth. Lwoff (1944) termed this kind of nutrition 'oxytrophy' in contrast to the 'haplotrophy' of organisms that use a wider range of substrates.[1] Lwoff's designations 'chlorophyte', 'leucophyte', 'protozoon',

[1] These are subdivisions of what Lwoff called 'allotrophy', but now more generally known as chemotrophy.

broadly correspond to the respective nutritional categories phototroph, oxytroph, haplotroph. However, a great many chlorophytes are oxytrophic in the dark while many others, including isolated tissues of phanerogams, utilize carbohydrate as well and to that extent may be regarded as being haplotrophic (Provasoli, 1938; Algeus, 1946; White, 1951; Pringsheim, 1952; Lewin, 1953). Although the number of possible substrates is even greater in animals such as ciliates, insects and vertebrates, so it is also in some fungi and bacteria (Kidder & Dewey, 1951; Trager, 1953; Albritton, 1954; Foster, 1949; Stephenson, 1949).

Acetate organisms are thus characterized by limitations which they share with neither animals nor higher plants. They would not, therefore, appear to be in direct evolutionary line with plants or animals, which are probably derived from more versatile ancestors. The limitation according to current views (Lwoff, 1951) is probably one of phosphorylating enzymes rather than membrane permeability. Limited permeability is implied, however, in the resistance shown by many acetate flagellates to high concentrations of such penetrating and toxic substrates as acetic acid and ethanol, or, for that matter, to their typically highly polluted habitat (Hutner & Provasoli, 1951).

As regards nitrogen nutrition: although ability to employ nitrate as sole nitrogen source is confined to the plant kingdom, loss of the ability is found in some members of most phyla of plants. *Oxyrrhis* shares this state of slight but obligate heterotrophy (mesotrophy) with such photosynthetic algae as *Hemiselmis virescens* (Cryptophyceae) and *Chlamydomonas pulsatilla* (Chlorophyceae). Here nitrogen requirements are met by the ammonium ion or, failing that, any one of a number of simple organic compounds provided they can be made to yield an amino group. In fungi and bacteria greater and varying degrees of heterotrophy are to be found; they are exemplified by *specific* requirements for one or more amino-acids (Snell, 1951). But it is in animals (ciliates, insects, vertebrates) that nitrogen heterotrophy has proceeded farthest and become so stereotyped that it is possible to speak of the 'ten amino acids essential for protein synthesis' with little ambiguity (Kidder & Dewey, 1951; Trager, 1953; Rose, 1938). Against this background the mere inability to reduce nitrate would seem not to remove *Oxyrrhis* very far from the higher green plant.

Oxyrrhis requires but thiamine, vitamin B_{12} and biotin among B vitamins, thus showing a self-sufficiency toward the remainder that is typical of auxotrophic lower algae (Droop, 1957; Provasoli, 1958). Provasoli (1957) regards auxotrophy as indicating 'animality' ('vegetality' being typified by the need for plant hormones). Certainly, the lower members of the plant kingdom are likely to be more animal-like than the higher ones. On the other hand, many isolated phanerogamic tissues are also auxotrophic; fungi are commonly so. Even a vitamin B_{12} requirement is now recorded in a fungus (Adair & Vishniac, 1958) and in tumour tissues of spruce (Reinert & White, 1956). Furthermore,

the pattern of specificity towards the vitamin B_{12} analogues typical of vertebrates and, by inference therefore, the most animal-like of the three patterns, is also shown by all the Chlorophyceae requiring vitamin B_{12} which I have examined.[1]

One is struck by the simplicity of the growth requirements in *Oxyrrhis*. But for the 'lemon factor' which in truth has been responsible for past failures with this organism, it is a typical acetate flagellate with an acetate flagellate's requirements and abilities, no more and no less plant-like. Phagotrophy thus emerges as an anomalous element in a conventional nutritional pattern. A progression *phototroph–oxytroph–phagotroph* could now be put beside the more usual (though equally theoretical) sequence *phototroph–haplotroph–phagotroph*. Such a progression might be characteristic of dinoflagellates and euglenids, but our knowledge is scanty. Although oxytrophy is well documented in the latter, the principal substrate for phagotrophic members is unknown (Storm & Hutner, 1953); and the only other non-photosynthetic dinoflagellate being studied is *Gyrodinium cohnii* for which there are as yet no published data.[2]

Phagotrophy appears to be commoner than saprotrophy in colourless dinoflagellates; photosynthetic phagotrophs are also reported. This might mean that in this alliance phagotrophy usually arises before the loss of photosynthetic pigments. It is also likely to have preceded the advent of any lipid requirements, which would otherwise be greatly disadvantageous to a free-living aquatic organism. Conversely, such requirements would survive without hindrance in established phagotrophs, with the result that their lipid metabolism would tend in the long run to become atrophied. On the other hand, organisms as large as *Oxyrrhis* have relatively limited cell surface, and are likely to find phagotrophy the most efficient method of feeding—particularly in nutritionally depleted environments. Isolation has many advantages; its disadvantages are thus avoided. An acetate organism which is also a phagotroph gets indeed the best of both worlds.

SUMMARY

The phagotrophic dinoflagellate *Oxyrrhis marina* can be cultivated indefinitely in the absence of other organisms of all kinds provided it is supplied with certain vegetable lipids.

Development of culture media in which all the water-soluble components are defined enabled the water-soluble nutrient and absolute growth factor requirements to be ascertained. The media were equivalent to a half-strength sea water buffered with glycylglycine and histidine with a trace metal mixture chelated with histidine and citrate.

[1] *Balticola droebakensis*, *B. buetschlii*, *Brachiomonas submarina*, *Chlamydomonas pulsatilla*, *Platymonas tetrathele* and *Stephanosphaera pluvialis*.

[2] Dr L. Provasoli, however, tells me that glucose is utilized by *G. cohnii*.

Acetate or ethanol (not any carbohydrate nor the carbon of amino-acids) serve as carbon source, and alanine, proline or especially valine (not NO_3^-, NH_4^+ or urea or other amino-acids) as nitrogen source. Auxotrophic requirements are met by the thiazole moiety of thiamine, vitamin B_{12} (near '*Ochromonas*' specificity), and biotin.

The nutritional status of *Oxyrrhis marina* is discussed.

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THE BIOLOGY OF THE PRAWN, *PALAEMON (=LEANDER) SERRATUS* (PENNANT)

By G. R. Forster
The Plymouth Laboratory

In a recent paper Cole (1958) has offered a new interpretation of the results obtained in 1949 and 1950 during my investigations of the biology of prawns. The present paper is the outcome of a re-examination of these results and of further prawn measurements which were continued on a small scale until 1955. The later investigations consisted of an annual survey of the prawns, particularly the O-group, caught from the rocks on the south side of the R.A.F. Station, Mount Batten, usually during the first low spring tides every October. The total number of prawns measured in the whole period was over 8,600.

THE 1949 AND 1950 SAMPLES

The length measurements of the prawns caught during a 12-month period, starting in October 1949, have been re-analysed into millimetre instead of $\frac{1}{2}$-centimetre groups and the results plotted on probability paper. As Harding (1949) has pointed out, this paper can help considerably in the analysis of polymodal samples; though with the prawn's long breeding season one cannot necessarily expect year-groups to be sharply defined. During the winter, catches have been combined and plotted at monthly or bimonthly intervals, but during the summer when rapid growth may occur it was found desirable to limit the inclusive period for any one group to a maximum of about a fortnight.

The results are shown in Fig. 1, with the different symbols indicating my interpretation of the year-groups. The appearance of the O-group prawns in August and September can be clearly distinguished both with males and females. In July and August there were also many tiny prawns taken by a stramin net which could not be sexed. These have not been included.

The September samples of the O-group appear in Fig. 1 as reasonably straight lines, i.e. showing a normal distribution. But by October, after further growth and recruitment, the population has been widely spread and when plotted can be considered as two or three separate but overlapping populations. These populations probably represent fluctuations in the numbers of larvae settling in the early part of the summer. A separate late 'brood' in the smallest stages is clearly shown with the males in October 1949, and with the females in October 1950. Cole (1958, p. 20) preferred to consider these 3-cm prawns as the true O-group; but they only represent about

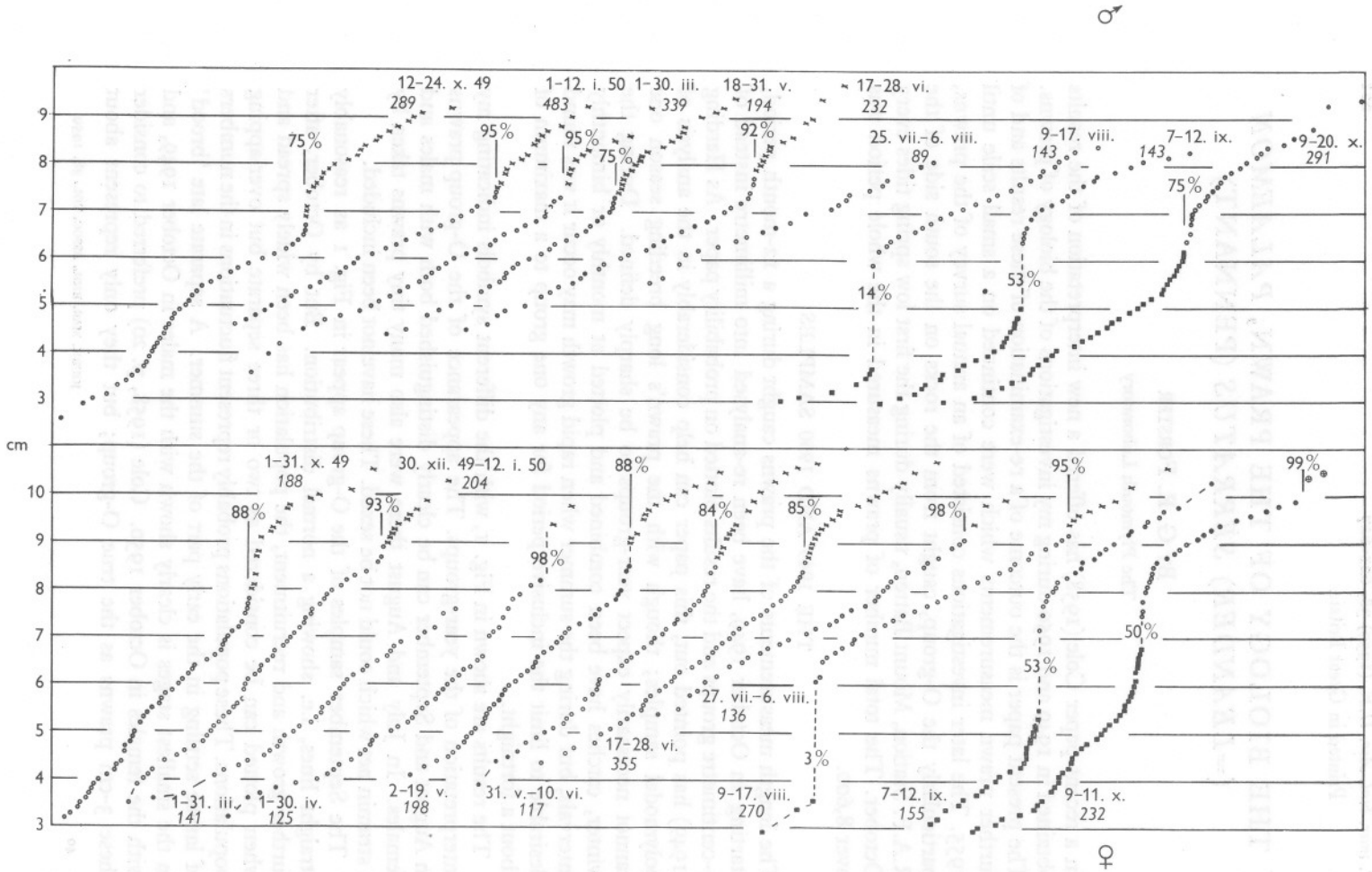

Fig. 1. Catches of male and female prawns in 1949 and 1950 plotted as cumulative percentages on log scale (probability paper). The year-groups from 1948–50 are represented by crosses, open circles and solid dots respectively.

5% of the total catch, and are even smaller than the clearly defined O-group of later, colder years. The 1949 males soon grew sufficiently to merge with the main bulk of the O-group. The exceptionally warm conditions during the spring and summer of 1949 probably led to a very early settlement of larvae, beginning in May or June. Lebour (1947), during her 1940–5 investigations, found larvae all the year round except in November and December, but with the greatest numbers in June, July and August.

The sequence of replacement of the 1948 and 1949 year-groups can be seen in Fig. 1 and calls for no detailed re-description. It is possible that some of the prawns included in the 1948 year-group might in fact be somewhat older; in particular the January 1950 catch of males shows two populations at 8 cm and 9 cm but these numbers are very small; the distinction is not evident for several months and the whole group disappear from the catches after the end of June. The 1948 group females continued to appear in the August samples, i.e. after their third birthday. But they too had apparently died off by mid-September, though two at 10·4 cm and 10·5 cm caught in October might represent the last survivors; however, they form only 1% of the total sample.

CATCHES FROM 1951–1955

In 1952 and 1955 only the O-group were sampled, but for the other three years large as well as small size-groups were measured. The results are shown in histogram form in Fig. 2. Both males and females exhibit very clear bimodal distributions, the O-group appearing at 3–4 cm and the 1-year-old at 7–8 cm. The 1-year-old females have outgrown the males by about 1 cm. In 1951 there is a small 'tail' of very large prawns. When their distribution is plotted on probability paper, these prawns show up clearly as a separate group and are presumably the few surviving 2-year-olds. Their numbers have dwindled to less than 2% of the total. The various O-group distributed in Fig. 2 are all much smaller than their counterparts in 1949–50 but correspond closely to the results of Cole from N. Wales in the same period. The mean lengths of the O-group for each year 1949 to 1955 have been extracted and plotted together with the sea temperature in Table 1.

The figure for 1952 is approximate as only the percentages of each $\frac{1}{2}$-cm group were available, the original measurements having been lost. The temperatures have been taken from Cooper (1958). From the Table it can be seen that there is a general agreement between the temperatures and lengths particularly in the two warmer years 1949 and 1950. Mr. G M. Spooner had kindly undertaken a statistical analysis of the figures and has found a significant correlation between them.

Fig. 2 also shows a sample of prawns taken in July 1954. In both sexes there are two distinct year-groups which are assumed to be those of 1953 and 1952, with the 1953-group females having grown considerably since the

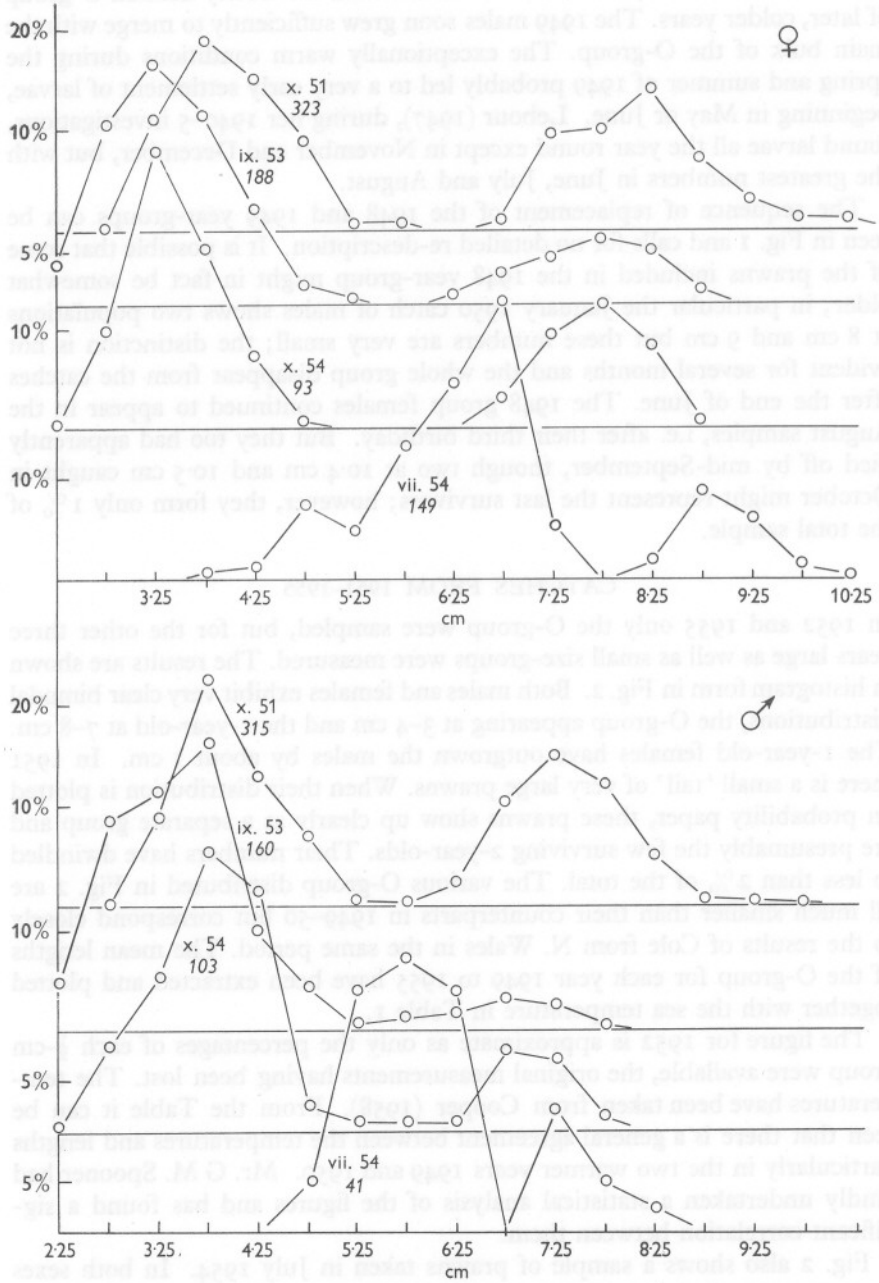

Fig. 2. Samples of male amd female prawns from Rum Bay, 1951–4, in ½-cm groups.

previous autumn. Table 2 gives the breeding condition of these female prawns.

It is very probable that some of the non-breeding prawns about 6 cm in length had already hatched one brood, and also the two-year-olds (7–8 cm) would be carrying their second brood.

TABLE 1

Year	Mean length of O-group. Oct. sample ♂+♀	No. in sample	Difference ±39 mm (mm)	Agg. temp.* (°C)
1949	56·1	366	+17·1	+9·6
1950	47·5	332	+8·5	+3·6
1951	38·6	354	−0·4	−2·8
1952	39·9		+0·9	+1·4
1953	35·9	104	−3·1	−0·6
1954	35·5	148	−3·5	−3·2
1955	37·9	197	−1·1	−1·8

* Temperature heading: aggregate differences of each monthly mean sea temp. for March–October from 10-year average 1947–56.

TABLE 2. PRAWNS CAUGHT ON 2 JULY 1954

Length (cm)	Non-breeding	Total berried	Berried with eggs near hatching	Cemented
3·5–3·9	1	0	0	0
4·0–4·5	2	0	0	0
4·5–4·9	11	0	0	0
5·0–5·4	7	0	0	0
5·5–5·9	8	11	3	1
6·0–6·4	12	16	9	1
6·5–6·9	23	17	11	2
7·0–7·4	4	3	3	1
7·5–7·9	0	0	0	0
8·0–8·4	0	3	0	0
8·5–8·9	4	9	1	0
9·0–9·4	0	8	1	1
9·5–9·9	0	2	1	0
10·0–10·4	0	1	1	1

The 1953 year-group will have spawned in April or May and must therefore have continued to grow rapidly in the late autumn. Its mean length in July was 61 mm. The small non-breeding prawns would have been growing in May and June as would the larger ones after hatching their eggs, so the mean length of the whole group may be estimated at about 55 mm before spawning. The group therefore grew from a mean length of 32 mm at the end of September to 55 mm in the spring. At first sight this might appear an impossibly high rate of growth for the colder part of the year. However, in the fortnight from September 1953, when the sample was taken, until 9 October, the mean length of the group increased by 4 mm; as the sea temperature did not decline rapidly until January, a length of 50–55 mm might well have been attained by the end of December.

DISCUSSION

Apart from the particularly warm years 1949 and 1950 the growth rate of prawns at Plymouth is comparable to that found by Cole (1958) in N. Wales. The O-group in October averages about 38 mm total length and 7 mm carapace length. There is a marked difference in the age at which the female prawns mature. Whereas in Wales only a few females breed in their first year, at Plymouth the bulk of the female population spawn before their first birthday.

The breeding period seems, in general, to be a little earlier than that found by Cole (1958) at Holyhead in 1953, but much later than at Plymouth in the warmer year, 1950. By their second October there is little difference in size between the Plymouth prawns and their Welsh counterparts.

Cole (1958) questions my earlier interpretation of the autumn 1949 and 1950 catches, maintaining that it involved an impossibly high growth rate because of a large size difference between samples at the end of August and the early part of September. But the August samples were taken with a stramin net which would not catch nearly as many of the 3–4 cm prawns as the normal hand net with $\frac{1}{2}$ in.-mesh netting. The O-group samples taken in August, September and October shown in Fig. 1 present a consistent pattern of moderately rapid growth, together with an increasing spread as later settling juveniles come into the samples. Nor is there any trace of the group suggested by Cole to lie between my O-group–1-year-old group, either in 1949–50 (Fig. 1) or in the later years, Fig. 2.

With regard to the older prawns which Cole considers to live several years longer than at Plymouth, it is difficult to follow the interpretation of his results. Concerning the males (p. 12) he states 'a group of prawns completing their second year with a peak at 13·0–14·2 mm in June can also be discerned,' but in the next paragraph Cole describes 'the group at 14·8 mm in June as attaining 3 years in July.' In my view these groups are so similar in size that they should both be considered as nearly two years old. Similarly with the female prawns it seems that 'the strong group about 16·6 mm in June' described on p. 15 could more reasonably be regarded as nearly two years old rather than three. Cole describes his fig. 13 as providing clear evidence of the existence of at least 5 year-groups, even though the older groups are merged, but by comparison with his fig. 12 the main bulk of catch in fig. 13 (October–November) would seem to be composed of 1 and 2-year-olds and the few larger prawns both in this and other samples of female prawns would, to my mind, be more reasonably taken as the remnants of the 3-year-old group rather than 4 or 5-year-olds. The largest prawns of all might well be somewhat older but they form a very small proportion of the catches.

SUMMARY

Data on the age and growth of the prawn *Palaemon serratus* (Pennant) collected in 1949 and 1950 have been re-examined in the light of further investigation at Plymouth from 1951–5 and of Cole's results from North Wales. Contrary to Cole's view the original interpretation of the growth rate is considered to be valid.

The length of the O-group in October is significantly correlated with the sea temperatures from March till October. Growth rates of the Plymouth and North Wales population are similar but the females mature much sooner at Plymouth. The age reached by the N. Wales prawns is thought to be rather less than Cole has suggested.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

DENTON, E. J., 1959. The contributions of the orientated photosensitive and other molecules to the absorption of whole retina. *Proc. roy. Soc.*, B, Vol. 150, pp. 78–94.

Various animals were chosen to give golden, red and purple retinae. It is confirmed, by a method giving the absorption curve of unbleached retina, that deep-sea fish have golden photosensitive pigments in densities so high that they absorb over 90% of blue-green light striking the retina. Although light has passed through all retinal layers the spectral total density curves correspond closely to those obtained on highly purified retinal extracts. After making a small correction for losses of light in the layers of retina other than the receptor layer the $D_{\text{min.}}/D_{\text{max.}}$ ratios were about 0·25 for chrysopsin retinae and 0·45 for porphyropsin retinae. The losses of light in retinae from which the rod and cone layer has been brushed off, although varying little with wavelength, rise slowly in the near ultra-violet and red from a minimum in the yellow.

The edge-fold preparation, useful for studying the dichroism of the retinal rods is described. It is shown that the spectral curves of dichroic difference in density are very close to those of total retinal density and therefore there can be little material absorbing light between 400 and 720 mμ orientated along the axes of unbleached rods. There is such material absorbing near ultra-violet light present in unbleached retinae whose principal photosensitive pigment is derived from vitamin A_2. For both vitamin A_1 and vitamin A_2 retinae the sense of the dichroism in the near ultra-violet is reversed in the first hour following the bleaching of the photosensitive pigments. This shows that the molecules of vitamin A in the bleached isolated retina are orientated with their axes of resonance parallel to the axes of the rods, a conclusion confirmed by studies of the polarization of the fluorescence of visual white in the bleached retina.

E.J.D.

MURRAY, R. W., 1959. The response of the ampullae of Lorenzini to combined stimulation by temperature change and weak direct currents. *J. Physiol.*, Vol. 145, pp. 1–13.

When single units in the ampullae of Lorenzini are stimulated by weak direct currents their impulse frequency varies linearly with current in most preparations. When thermal stimuli are applied during the adapted response to maintained D.C. the change in frequency due to the combined stimuli equals the sum of the changes due to each separately. When the unadapted response to D.C. is combined with the thermal response, interaction occurs. The direction of the spatial gradient of temperature is not significant for the thermal response. Some anomalous thermal responses are described.

R.W.M.

NICHOLS, D., 1959. Changes in the Chalk heart-urchin *Micraster* interpreted in relation to living forms. *Phil. Trans.*, B, Vol. 242, pp. 347–437.

Seven species of irregular echinoids, whose morphology, behaviour and ecology show important comparative features, were studied, and many aspects of their mode of life, in particular their burrowing, feeding, sanitation and locomotion, are shown to be

closely correlated with the particle-sizes of the substrata in which they live, and this adaptation is expressed in many features of their tests, in particular the position and degree of development of the fascioles and the division of labour in the tube-feet.

The fossil heart-urchin *Micraster* is one of the best examples of continuous and directional evolution of individual characters, and the study of living forms has been used to suggest the changes in mode of life which accompanied the structural changes. The main change appears to have been a gradual increase in the depth to which the Micrasters burrowed below the sea-floor. When the shallow-burrowing forms ceased to occur, another closely-related form, *Isomicraster*, arose in the English area, probably by immigration. By analogy with living forms, in particular the number and arrangement of tube-feet in the petaloid parts of the paired ambulacra, this form most likely lived only partially submerged in the substratum. The taxonomy of the English *Micraster* complex is discussed in the light of this approach. D.N.

SPOONER, G. M., 1959. New members of the British marine bottom fauna. *Nature, Lond.*, Vol. 183, pp. 1695–6.

Some unfamiliar small invertebrates are recorded from bottom gravels off Plymouth, more particularly the Eddystone shell gravel (at depth of about 25 fm.). Acochlidiacean gastropods are reported from Britain for the first time and represented by *Microhedyle lactea* Hertling, *Hedylopsis suecica* Odhner, and *Philinoglossa helgolandica* Hertling. The holothurian *Leptosynapta minuta* (Becher) was also found.

The Eddystone shell gravel harbours one oligochaete and five species of mites, including *Scaptognathas tridens* Trouess. and *Halacarus bisulcus* Viets which are new to Britain; and, furthermore, it is particularly rich in small malacostracans. Out of 56 species listed, 20 are new to the Plymouth Fauna and five or six new to science.

Tanaidacea are dominated by *Typhlotanais microcheles* Sars and *Strongyurella indivisa* Hansen. Isopoda include *Paramunna bilobata* Sars, *Eurycope pygmaea* Sars, *Microjaera anisopoda* Bocquet and Lévi, and *Microcharon harrisi* Spooner. The last belongs to a genus of small blind colourless forms of which six of the nine species recognized live in terrestrial ground-water in southern and eastern Europe, and are truly 'interstitial' or 'phreatic'.

Among many Amphipoda, there is a new metopid and a new syrrhoid, but the most interesting and unexpected finds were representative of two cosmopolitan 'phreatic' families—Bogidiellidae and Ingolfiellidae. The bogidiellid will require a new generic name. Previously species have been found in fresh water or coastal sand of southern Europe or Brazil. The genus *Ingolfiella* includes six species from the most diverse range of habitats (from the abyssal Atlantic to a cave in the Belgian Congo): the two specimens from the Eddystone are very close to *I. acherontis* Karaman from the Macedonian uplands. G.M.S.

BOOK REVIEW

Endocrine Control in Crustaceans

By D. B. Carlisle and Sir Francis Knowles

Cambridge University Press, 1959.

This is Vol. 10 of the *Cambridge Monographs in Experimental Biology* and it is a worthy member of a series of publications of great merit.

Thirty years ago Perkins and Koller discovered the endocrine mechanism of colour change in the Crustacea, and when a few years later Hanström demonstrated that the sinus gland was an endocrine organ, the scene was set for a new epoch in comparative endocrinology.

The authors sub-divide progress in this field into three phases. During the first phase the nature of the newly discovered chromactivating principles associated with the sinus gland was further substantiated. The second phase brought to light the fact that functions other than chromatic mechanisms are under hormonal control, and the third phase, seemingly approaching its end now, resulted in our knowledge that modified neurons act as producers of hormones, and that hormone transport takes place along nerve axons.

Of the achievements during these three periods of research the authors give a lucid and excellently illustrated account, the authoritative nature of which stems from the fact that both have made substantial original contributions to our knowledge in this field.

There are chapters on the neurosecretory system of the head and thorax, on the mechanism of colour change, on the pericardial organs and their influence on the heart beat, on the hormonal control of growth, moulting, development, metabolism, and breeding. In conclusion the authors point to the future fourth phase of progress and look forward to the extension of research beyond the decapod crustaceans and to a synthesis of the results with those gained in other fields of endocrinology.

There is an extensive list of references and a very useful index. O.E.L.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1958–59

The Council have to report with deep regret the death of Dr G. A. Steven, F.R.S.E., who had been a member of the Scientific Staff of the Plymouth Laboratory since 1928. During this time he did much to develop the sea-going work of the laboratory, and made notable contributions to the biology of fishes.

The Council also regret to report the deaths of Mrs E. W. Sexton, who had been a member of the staff of the Plymouth laboratory for many years, and was especially well known for her research on amphipod crustacea; of Dr Å. Vedel Tåning, President of the International Council for the Exploration of the Sea, who was an Honorary Member of the Association; and of Prof. James Ritchie, C.B.E., LL.D., who had been a member of Council.

The Council and Officers

Four ordinary meetings of the Council were held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was nineteen.

The Plymouth Laboratory

During the year a small building was erected in the laboratory grounds for the storage of inflammable chemicals. The passages of the north building and the bridge connecting the two buildings have been redecorated; this has also necessitated considerable electrical rewiring.

Aquarium

The aquarium attracted unusual numbers of the public during the summer, probably because of the wet weather, the attendance on August Bank Holiday being a record.

The tank room was closed to the public on 27 September 1958, and work on the modernization and reconstruction of the tanks is well in hand.

Research Ships

The research ships 'Sarsia' and 'Sula' have operated regularly throughout the year apart from normal overhauls. In November and December 1958 M.L. 'Gammarus' was completely overhauled. Her woodwork was generally

in very good condition. A fibre-glass dinghy and an outboard motor have been purchased for use with the 'Gammarus'.

R.V. 'Sarsia', Lt. Cdr. C. A. Hoodless, D.S.C., R.N.R. in command, has now been in commission for five years, and has made many cruises over an area stretching from the eastern end of the English Channel to the continental slope south-west of Ireland and off the coast of Spain. The interesting results of the extension of the Plymouth laboratory's working area, and of the facilities for doing physiological research at sea are now becoming apparent.

R.V. 'Sula', Mr W. J. Creese in command, has been in commission for over ten years. With the increasing number of cruises undertaken annually by R.V. 'Sarsia' the major part of the daily collecting of specimens is now done by R.V. 'Sula'.

The collection of specimens from inshore waters and intertidal areas continues to be undertaken by M.L. 'Gammarus' under the charge of Mr A. C. Briggs.

STAFF

On 1 October 1958 Mr W. H. Searle, B.E.M., retired from the staff of the Plymouth laboratory, after 63 years of devoted service to the Association. He will have the best wishes of all members of the Association and of the many visiting research workers for whom he has collected specimens. The building up of our knowledge of the fauna from its early beginnings owes much to his skill and powers of observation. He will be remembered with affection by all.

Dr L. H. N. Cooper and Mr G. M. Spooner have been promoted to the grade of Senior Principal Scientific Officer as from 1 April 1958.

Dr D. B. Carlisle has been promoted to the grade of Principal Scientific Officer as from 1 April 1958.

Dr T. I. Shaw joined the staff of the Plymouth laboratory as Senior Scientific Officer on 1 September 1958.

Mr G. W. Bryan joined the staff of the Plymouth laboratory on 1 October 1958 in a special temporary appointment.

Mr Q. Bone joined the staff of the Plymouth laboratory as Scientific Officer on 1 January 1959.

Dr B. C. Abbott left the staff of the Plymouth laboratory on 1 September 1958 to continue his appointment in the University of California at Los Angeles.

Dr Mary Parke attended the Third International Seaweed Symposium held in Galway, Eire, in August 1958.

Dr E. J. Denton attended the meeting of the International Council for the Exploration of the Sea held in Copenhagen in September and October 1958.

Dr D. B. Carlisle spent a month in August and September 1958 working at the Kristineberg Marine Laboratory, Sweden.

Dr A. J. Southward spent seven weeks in August and September 1958 making a survey of the distribution of barnacles round the Irish coasts.

Through the kindness of the Director of the National Institute of Oceanography Mr P. G. Corbin and Dr L. H. N. Cooper took part in cruises of R.R.S. 'Discovery II' in April and November to December 1958 respectively. In March 1959, through the kindness of the Director of Fisheries Research, Ministry of Agriculture, Fisheries and Food, Dr A. J. Southward accompanied a cruise of the fisheries research vessel 'Sir Lancelot'.

International Paints Research Fellowship

The Directors of International Paints Ltd. have, by arrangement with the Council of the Association, continued the endowment of their Research Fellowship at the Plymouth laboratory for a further three years.

Dr G. T. Boalch has been appointed to the Fellowship, and he started work at Plymouth on 1 October 1958.

Occupation of Tables

The following one hundred and sixty workers have occupied tables at the Plymouth laboratory during the year:

E. Adams, Plymouth (Library).
R. McN. Alexander, Cambridge (Swim bladders of fish).
Dr J. S. Alexandrowicz, Plymouth (Invertebrate nervous systems).
F. P. Anderson, Cape Town (Physical oceanography).
S. R. Armstrong, Bryanston School, Dorset (Mollusca).
Dr Daphne Atkins, Plymouth (Ciliary mechanisms of brachiopods).
Miss D. Ballantine (Mrs B. T. Hepper), Conway (Dinoflagellates).
W. J. Ballantine, London (*Patella*).
Dr B. McK. Bary, Edinburgh (High-speed plankton sampling).
Dr Elizabeth J. Batham, Cambridge (Nervous system of *Metridium*).
Dr J. H. Belcher, London (Bangioideae and Nemalionales).
E. Bernabe, Philippines (Plankton).
Miss M. Beynon, Aberystwyth (General).
Dr Anna M. Bidder, Cambridge (Biology of cephalopods).
Dr G. T. Boalch, International Paints Research Fellow (Effects of toxic substances on *Ectocarpus*).
Dr B. P. Boden, La Jolla (Penetration of light and vertical migration of plankton).
Q. Bone, Oxford (Feeding behaviour of *Amphioxus*).
A. D. Boney, Plymouth (Ecology of red algae).
Dr J. S. Bradshaw, La Jolla (Ecology of estuarine Foraminifera).
L. R. Brightwell, Truro (Holothurians)
R. W. Brimblecombe, Porton (Shark repellants).
J. D. Bromhall, Hong Kong (Underwater photography).
Dr Eleanor M. Brown, London (Plankton).
Dr P. C. J. Brunet, Oxford (*Chaetopterus* and Pogonophora tubes).
Dr A. J. Bruce, Glasgow (Penaeid prawns).
B. M. H. Bush, Cambridge (Crustacean neuro-muscular physiology).
Mrs P. A. Caldwell, Plymouth (Library).

Dr P. C. CALDWELL, Alan Johnston, Lawrence and Mosely Research Fellow of the Royal Society (Muscle and nerve physiology).
C. CARRÉ, Villefranche-sur-Mer (Culture of phytoplankton).
Prof. M. R. CARRIKER, North Carolina (Malacology).
Prof. L. S. CIERESZKO, Oklahoma (Vanadium in ascidian eggs and larvae).
Miss E. CLAY, Brixham (Library).
Dr H. A. COLE, Lowestoft (Saltash oyster fishery).
J. W. COLES, London (Seaweed nematodes).
J. S. COLMAN, Port Erin (Vertical distribution of oceanic plankton).
Dr J. D. COSTLOW, North Carolina (Barnacles).
C. A. COSWAY, Torquay (Library).
Dr WILHELMINA A. M. COURTNEY, Nottingham (*Amphioxus*).
C. J. CROFT, Plymouth (Library).
Dr R. I. T. CROMARTIE, Cambridge (Pigments of *Antedon*).
J. G. C. CUBB, Winfrith Heath (Analysis of sea-water).
K. W. DAISLEY, Shinfield (Vitamin B_{12} in sea-water).
Dr R. PHILLIPS DALES, London (Coelomic cells of *Sabella*).
J. B. DAVIES, Oxford (General).
E. W. DAWSON, Cambridge (Hydrostatic skeleton in invertebrates).
P. S. B. DIGBY, London (Zooplankton).
Miss E. J. DIMELOW, New Brunswick (Biology of *Antedon*).
J. D. DODGE, London (Cytology of Protista).
Dr D. T. DONOVAN, Bristol (Submarine geology).
Dr PATRICIA L. DUDLEY, Seattle (Notodelphyid copepods).
Dr R. ENDEAN, Bristol (Role of vanadocytes in tunicin formation).
J. ERNST, Paris (Underwater study of algae).
Dr L. EUZET, Sète (Platyhelminths of fish).
Dr MARIA M. FELINSKA, Nottingham (Ciliates).
Dr UNA FIELDING, London (Central nervous system of fish).
Prof. P. H. FISCHER, Paris (*Otina*).
Dr L. R. FISHER, Shinfield (Pigments of invertebrate eyes).
D. N. FLETCHER, Harwell (Trace analysis).
Prof. E. FLOREY, Seattle (Crustacean inhibitory nerve fibres).
I. H. FORD, Bristol (Submarine geology).
P. FOXTON, National Institute of Oceanography (High-speed plankton sampling).
R. F. H. FREEMAN, London (Metabolism of *Scrobicularia*).
Dr VERA M. FRETTER, Reading (Prosobranchs).
Dr M. M. FRODYMA, Hawaii (Chemistry of sea-water).
M. H. W. GALL, London (Marine photometry).
Dr J. B. GILPIN-BROWN, Plymouth (Biology of nereids; pelagic squid).
Miss E. H. GOLDIE, Shinfield (Pigments of invertebrate eyes).
Prof. A. GRAHAM, Reading (Prosobranchs).
Dr E. C. HADERLIE, Monterey and Bristol (Ecology of polychaetes).
D. N. F. HALL, Colonial Office (Indo-West-Pacific Penaeidae).
Prof. Sir ALISTER C. HARDY, F.R.S., Oxford (Drawing marine animals).
M. G. HARDY, Reading (Nerve histology of lamellibranch siphons).
Prof. J. E. HARRIS, F.R.S., Ray Lankester Investigator, Bristol (Vertical migration of plankton).
Dr H. W. HARVEY, F.R.S., Plymouth (Productivity of sea-water).
D. HEDDLE, Oxford (Asteroid skeletons).
Miss M. HENDERSON, London (Shore ecology).
B. T. HEPPER, Conway (Lobster marking).

Prof. A. L. HODGKIN, F.R.S., Cambridge (Nerve physiology).
M. HORNSEY, Cambridge (Light reactions in *Procerodes*).
Dr G. M. HUGHES, Cambridge (Respiratory movements of teleosts).
O. D. HUNT, Newton Ferrers (Library).
Dr G. M. JARMAN, Bristol (Vertical migration of plankton).
Dr C. H. JELLARD, Plymouth (Library).
Dr PENELOPE M. JENKIN, Bristol (Library).
Dr J. B. JENNINGS, Leeds (Feeding and digestion in Nemertea).
Dr R. J. JONES, Croydon (Drawing fish).
Dr JOANNA M. KAIN, Port Erin (Sublittoral algae).
Dr ELIZABETH M. KAMPA (Mrs B. P. BODEN), La Jolla (Penetration of light and vertical migration of plankton).
Dr P. KARLSON, Munich (Chromactivating hormones).
Dr G. Y. KENNEDY, Sheffield (Sponge regeneration; chlorophyll pigments).
Prof. H. KINOSITA, Tokyo (Ciliary response in invertebrates).
Dr P. L. KRAMP, Copenhagen (Medusae).
Dr S. KRISHNASWAMY, Southampton (Respiratory metabolism of parasitic copepods).
Dr K. LAGERSPETZ, Turku, Finland (*Mytilus edulis*).
Miss E. C. LEACH, Cambridge (Discharge of nematocysts of *Actinia*).
Dr MARIE V. LEBOUR, Plymouth (Decapod larvae).
Prof. B. LEVRING, Göteborg (Algae).
Dr J. LLEWELLYN, Birmingham (Trematode parasites of fishes).
Prof. O. E. LOWENSTEIN, F.R.S., Birmingham (Elasmobranch labyrinths).
Miss M. R. LUNT, Oxford (Biochemistry in arthropods).
Prof. IRENE MANTON, Leeds (Flagellates and motile cells of algae).
A. L. MARTIN, London (Digestive gland in *Gammarus*).
Dr A. B. MEGGY, Plymouth (Library).
Dr N. A. MEINKOTH, Swarthmore, Pennsylvania (Cestodes).
Prof. J. L. MOHR, Los Angeles (Protozoan parasites of Crustacea; Mesozoa).
Dr J. E. MORTON, London (Pleurobranchoids).
Dr R. W. MURRAY, Birmingham (Sensory physiology of rays).
Dr E. NAYLOR, Swansea (Isopods).
Dr MARGARET NAYLOR, Hull (Cytology of Phaeophyceae).
Dr F. ÖZTIG, Banyuls (Red algae).
Dr C. F. A. PANTIN, F.R.S., Cambridge (Fine structure of *Metridium*).
Dr A. M. PATIL, Reading (Taxonomy of Rissoidae).
Mrs J. L. PEARSON, Cambridge (Embryology of *Sycon*).
C. J. PENNYCUICK, Cambridge (Physiology of dogfish muscle).
W. J. PHILLIPPS, Wellington, N.Z. (Teeth of spiny dogfish).
Dr W. T. W. POTTS, Birmingham (Free amino acids in *Gammarus*).
Cdr. C. F. B. POWELL, R.N. (Rtd.), Plymouth (Library).
J. D. PYE, London (Colour change in fishes).
A. M. QURESHY, London (Haemogregarines of fishes).
Miss P. M. RALPH, Wellington, N.Z. (Hydroids and medusae).
Miss M. RAZARIHELISOA, Madagascar (Trematode parasites of fish).
Dr W. J. REES, London (Cnidaria of sand and mud).
Dr J. D. ROBERTSON, Glasgow (Chemistry of cephalopod muscle)
Prof. M. ROCKSTEIN, New York (Echinoderm orientation and photosensitive pigments).
G. RODRIGUEZ, Caracas, Venezuela (Ecology of sandy shores).
Dr D. M. ROSS, London (Behaviour of *Calliactis*).
Prof. FINDLAY E. RUSSELL, Los Angeles (Toxins of weevers).

Prof. P. SAWAYA, São Paulo (Body fluid of Holothuria).
D. J. SCARRATT, D.S.I.R. (Fauna of *Laminaria* holdfasts).
Prof. K. SEMBRAT, Breslau (Dogfish thyroid).
Prof. Z. SEMBRATOWA, Breslau (Neurosecretion in Nemertini and Polychaeta).
A. B. SIDLE, London (Algae).
A. C. SIMPSON, Lowestoft (Saltash oyster fishery).
R. J. SKAER, Cambridge (Rhabdite discharge in *Procerodes*).
Dr DOROTHY M. SKINNER, Harvard (Crustacean moult cycle).
Dr EVE C. SOUTHWARD, Plymouth (Pogonophora; polychaetes).
B. W. SPARROW, Newton Ferrers (Library).
Miss F. A. STANBURY, Plymouth (*Cladophora*).
Dr D. C. STEWART, Chicago and Harwell (Trace analysis).
Dr J. H. STOCK, Amsterdam (Crustacean parasites of invertebrates).
Miss E. M. F. SWALE (Mrs J. H. BELCHER), London (Bangioideae and Nemalionales).
R. V. TAIT, London (Shore ecology and plankton).
Prof. E. TORTONESE, Genoa (Channel fauna).
M. TUFAIL, Exeter (Development of *Porcellana*).
Dr J. VERWEY, den Helder (Library).
Dr H. G. VEVERS, London (Library).
G. E. WALSTER, Plymouth (Glycolysis in *Maia*).
Surg.-Cdr. G. WEDD, R.N., Porton (Shark repellants).
Prof. G. P. WELLS, F.R.S., London (*Arenicola*).
Dr J. O. WERSÄLL, Stockholm (Elasmobranch labyrinth).
Dr H. P. WHITING, Bristol (Nervous system of *Amphioxus* and *Scyliorhinus*).
Prof. W. F. WHITTARD, F.R.S., Bristol (Submarine geology).
J. H. WICKSTEAD, Colonial Office (Tropical plankton).
Prof. C. A. G. WIERSMA, Pasadena (Proprioreceptors in crustacean legs).
Dr H. HARFORD WILLIAMS, Aberystwyth (Cestodes).
Dr D. I. WILLIAMSON, Port Erin (Decapod larvae).
Dr SHEILA M. WILLMOTT, London (Digenea of fish).
Dr M. ALISON WILSON, Plymouth (Library).
Miss M. J. WOOD, London (Algae).
M. YOSHIDA, London (*Psammechinus* pigments).

Among the many other 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: Dr Talbot H. Waterman (U.S.A.), Dr A. E. J. Went (Dublin), W. G. Morison (Sarawak), J. L. Reid, Jr. (U.S.A.), Prof. J. Tokida (Japan), Dr R. W. Hiatt (Hawaii), Dr M. S. Gordon (U.S.A.), S. C. Otuka (Nigeria), J. Davids (Holland), B. V. Hamon (Australia), Dr W. B. Hartman (U.S.A.), Dr T. F. Goreau (U.S.A.), P. Gabel (U.S.A.), Dr Dorothy C. Saunders (U.S.A.), Dr A. E. Parr (U.S.A.), Dr L. Provasoli (U.S.A.), G. Michanek (Sweden), Prof. Ki-Chul Choi (S. Korea), Prof. B. B. Benson (U.S.A.), Prof. N. W. Rakestraw (U.S.A.), F. B. Pithavadian (Madras), W. B. Bailey (Canada), Dr K. B. Markman (Sweden), Miss T. Vucetic (Yugoslavia), Prof. S. Motoda (Japan), Dr B. A. Ketchum (U.S.A.), Prof. Melom J. Cohen (U.S.A.).

About 120 delegates of the XVth International Congress of Zoology took the opportunity of visiting the Plymouth laboratory during their stay in this

country. These included a party of thirty-three Russian scientists. Some of the delegates spent a day or so in the laboratory, and among these were: Dr H. L. Sanders (Woods Hole), Dr W. Trager (New York), Dr and Mrs K. Lagerspetz (Finland), Prof. E. Witschi (Iowa), Dr A. Bourdillon (Marseilles), Prof. Jean A. Lynsdale (Burma), Prof. and Mrs R. Defretin (Lille), Prof. and Mrs F. Bernard (Algiers), Dr and Mrs V. L. Loosanoff (Connecticut), Prof. and Mrs I. McT. Cowan (Vancouver), Prof. Erwin Kamptner (Vienna), Dr Lucia Rossi (Turin), Dr H. Stieve (Hamburg), Prof. and Mrs Carl Hubbs (La Jolla), Prof. Marie Gontcharoff (Sète), Dr H. G. Godlewski (Poland), Prof. J. H. Day (Cape Town), Prof. and Mrs R. Buchsbaum (Pittsburg), Dr O. Wetzel (Germany), Prof. Denzaburo Miyadi (Seto, Japan), Prof. P. Sawaya (São Paulo, Brazil).

The West German research vessel 'Anton Dohrn' visited Plymouth on 21 and 22 January 1959. The Captain, scientists and technical assistants were entertained at the Laboratory, including Dr G. Hempel, Dr E. Rogalla, Dr W. Gunkel and Dr H. J. Münzing.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-one students from the following Universities: Oxford, Cambridge, St Andrews, Glasgow, Durham, London, Sheffield, Reading, Exeter, Hull, Swansea, Aberystwyth and Regent Street Polytechnic.

Also during the Easter Vacation Dr R. J. Jones and Dr C. T. Prime brought a party of twenty-one boys from Whitgift School, and Mr J. Peirson brought four boys from Rugby School.

Two University Courses were held at the Plymouth laboratory in September; a course in marine biology for ten students supervised by Miss J. E. Rigby and Miss S. Rogers of Queen Elizabeth College, and a course in marine botany for eighteen students supervised by Dr Margaret Naylor of the University of Hull.

Scientific work of the Plymouth Laboratory Staff

Sea Water and Plankton

For a third of a century many and varied studies on sea water and plankton have been concentrated upon a single accessible shallow-water station in the English Channel (E 1), supplemented by occasional more extensive excursions. This concentration of effort has been richly rewarded. A similar approach is appropriate in deep-water oceanography; a standard station has, therefore, been selected in a position, 46° 31′ N., 8° 00′ W., already worked twice by the Danish Research vessel 'Dana' in 1922 and 1930. This is the nearest deep-water station to Plymouth, and it has best possible coverage by the Decca navigator system. Since 1952 it has been repeatedly worked from R.R.S. 'Discovery II' and R.V. 'Sarsia'. During 1958 the programme has been co-ordinated with the requirements of the International Geophysical Year. Three

cruises on R.V. 'Sarsia' in March, April and September have been organized by Dr L. H. N. Cooper, together with work from R.R.S. 'Discovery II' in the late autumn. This station, with continued study, should eventually provide part of a framework not obtainable by other IGY ships widely dispersed over the ocean.

A picture of the very considerable fluctuations which take place in the deep ocean has already emerged. Water which is physically homogeneous is not always chemically so. The mean oxygen content of the deep water decreased from a maximum in 1922 through 1930 to 1952, but is now once more steadily approaching the high figure of 1922. The results bear on the vexed issue of the 'age' of deep oceanic water.

The main oceanic position is complemented by two further standard stations over the continental slope off La Chapelle Bank and one at the nearby break of slope. Only after seven years are the data from these stations enabling the very important processes of vertical and horizontal mixing, and the leat currents which occur only in the blanket of water over continental slopes, to be differentiated. It seems likely that mixing processes are trifling in the deep open ocean, and that the appearance of such at any depth is a consequence of mixing processes against slopes and horizontal displacements of the mixed waters created there.

For practical reasons the distribution of the rarer constituents of sea water has everywhere been studied largely in the shallow seas. The reservoir stocks in the deep ocean remain unknown because of the difficulty of getting uncontaminated samples. Thus, much attention is being given to the technique of deep sampling for trace constituents for analyses to be made at other laboratories. During cruises in 1958 a range of samples to the bottom (4700 m) at the standard station were obtained for vitamin B_{12} (Dr K. Daisley, Shinfield), cadmium (Dr J. P. Riley, Liverpool), copper (Mr F. A. J. Armstrong), uranium (Mr G. C. Milner, Harwell), and state of oxidation of uranium (Dr D. C. Stewart, Argonne National Laboratory, Chicago and Harwell). From the same hoists deep-water samples were collected for studies on deep-dwelling flagellates and allied organisms (Dr Mary Parke). Mr E. I. Butler has helped much on these cruises.

It is known in broad terms that the continental slope south-west of the British Isles has a very rugged topography, but the present experimental approach requires concentration of effort on a few selected small areas whose topography is accurately charted. With the assistance of Mr G. W. Battin the necessary close surveys have been started at the edge of La Chapelle Bank. Measurements of the current at the break of slope of the bottom water in this area have been initiated by Mr E. I. Butler.

Dr Cooper and Dr T. I. Shaw have published their theoretical studies on the state of iodine in sea water in *Nature, Lond.*, Vol. 180, p. 250, and Vol. 182, p. 251.

Mr F. A. J. Armstrong has continued his analyses for ammonia and total inorganic nitrogen in monthly samples from the International Hydrographic Station E 1. In July 1958 inorganic nitrogen fell to less than 5% of the winter maximum values, whereas phosphate fell only to about 15%, so that nitrogen seems to be the limiting micronutrient for plant growth. Nitrogen-phosphorus ratios were about 20:1 by atoms or 9·2:1 by weight; these are a little higher than those found (16–17:1 by atoms) in 1931 by Dr Cooper. Recently nitrogen has been a little higher and phosphate lower than in 1931; silicate values have been similar. Phosphate and silicate analyses for Station E 1 during 1956 have been published in Vol. 37, No. 2, of the *Journal*, and those for 1957, together with some nitrogen figures, have been described jointly with Mr E. I. Butler, in Vol. 38, No. 1, of the *Journal*.

In an attempt to define the accuracy and precision of phosphate determinations Mr Armstrong has investigated three of the more reliable molybdenum blue methods, two using stannous chloride and the other ascorbic acid as reductant. Eight or ten replicate analyses are run at a time, and if filtered sea water is used, and all absorbancies measured on the same spectrophotometer, all three methods are surprisingly precise. The three methods give, however, three different results with the same sample of sea water, with differences of more than 0·1 mg atom P/l. between two methods. Part of these differences may be put down to the effect of arsenic in the water, since the methods are subject to differing degrees of interference from this element. This may make it very difficult to compare phosphate analyses from different laboratories.

Reduction of dissolved oxygen by corrosion of the metal when sea water is held in brass hydrographic bottles has been measured. In an extreme case the loss of oxygen was 0·3 ml. O_2/l. in $1\frac{3}{4}$ h at 16° C, and a rough equivalence has been found between the amount of oxygen reduced and the quantities of copper and zinc dissolved. Coating the insides of bottles with a cold-curing epoxy resin (Araldite No. 820 RH) was found to reduce oxygen loss to negligible proportions, and all the hydrographic bottles have been so treated. After repeated use at sea this year the coating shows no sign of deterioration.

In order to obtain large samples of deep ocean water, for some investigations in which the Laboratory assisted Dr G. C. Milner and Dr D. G. Stewart of Harwell, Mr Armstrong has designed and tested a 5 l. sampling bottle in which the sample does not come into contact with metal. A set of these bottles, of welded polythene, with rustless steel frames and release mechanisms was made by the Engineering Services Division of A.E.R.E. Harwell. After some initial difficulties the bottles have worked well at sea, and Mr Armstrong has been able to use them for collection of water for analysis for copper, trying out a very simple method using bis-cyclohexanone oxalyl dihydrazone, for which no solvent extraction is necessary.

Mr Armstrong has visited the laboratory of the Ministry of Agriculture, Fisheries and Food at Lowestoft where, by courtesy of the Director and with

instruction from the Staff, he recalibrated about eighty of the deep-sea reversing thermometers belonging to the Plymouth laboratory. Some of them appear to have altered markedly since they were first calibrated, and the new corrections have generally given more consistent agreement between pairs of thermometers when applied to the observations made in the last 2 or 3 years.

With the co-operation of Mr E. I. Butler monthly samples of sea water have been taken throughout the year, usually from R.V. 'Sula', at Stations L 2–L 6 and E 1 (surface to 70 m) for the examination of the nanoplankton by Dr Mary Parke and Miss I. M. Adams. From these routine samples very numerous temporary cultures have been set up and grown on a special apparatus designed and built by Mr F. G. C. Ryder. With Dr L. H. N. Cooper's co-operation additional sea-water samples, taken at a series of stations out over the continental shelf into oceanic water (from surface down to 4000 m) during the March, April and September cruises of R.V. 'Sarsia', have also been cultured and have given useful data for comparison with those obtained from the more inshore stations. In addition to the culturing of samples, quantitative and qualitative examinations have also been made of a series of samples for Mr R. I. Currie of the National Institute of Oceanography to give him information to use with his results obtained by pigment analysis and by the ^{14}C method.

From this study of the nanoplankton interesting information is being accumulated. It had been found that the very small 1–2 μ pigmented flagellates are very widely distributed and are at times extremely common. A number of these forms have now been isolated and studied, but because of their very small size it is extremely difficult to be certain to which class of algae they belong, and therefore co-operation is being given by Prof. Irene Manton of Leeds University in their examination with the electron microscope, and by Dr G. Y. Kennedy of Sheffield University in analysing their pigments. All the forms so far examined have been found to belong to the Chlorophyceae, including the very commonly occurring organism originally described as a member of the Chrysophyceae, *Chromulina pusilla* Butcher.

This study has also confirmed that members of the genus *Chrysochromulina* (Chrysophyceae) are extremely well represented in the nanoplankton and it has also shown that species of the genus *Pseudopedinella* (Chrysophyceae), both pigmented and unpigmented, are of very frequent occurrence. A number of different species of this genus, therefore, including the type species, have already been isolated for future study by Dr Parke and Miss Adams. Isolations and the culturing of the 'Phaeocystis' type colonies, occurring at all times of the year, sometimes in great abundance, are also being continued for the study of the different types of motile stages produced by them. Some time has also been spent on the isolation and culture of numerous clones of the very commonly occurring *Coccolithus huxleyi* (Lohm.) Kamptner for a study of its form range and life history. Isolations have been made at different

depths from inshore samples and from samples from oceanic stations as two strains differing in size may be involved, the larger strain occurring in oceanic water. Prof. E. Kamptner of Vienna, at his own request, is examining all the clone cultures that have been isolated.

From the temporary cultures set up a number of other interesting new forms have been isolated for future study, including new species of the genus *Chrysochromulina* and other forms belonging to the Chrysophyceae which will extend our knowledge of the form range to be found among marine members of this class. For example, a find of great botanical interest was made when Miss Adams, who has been responsible for the isolation of all new nanoplankton forms, found an organism she recognized as a new type which, with the help of electron micrographs taken by Prof. Manton, Dr Parke has now identified as *Crystallolithus hyalinus* Gaarder & Markali recently described (1956) from preserved material as a new genus and species of the Coccolithophoraceae. Two strains of this organism, isolated from different localities, are now being grown successfully in culture. The study of this coccolith-bearing organism in the living state has shown that it is an extremely valuable find as its structure gives further evidence for classifying the Coccolithophoraceae in the class Chrysophyceae. Also, like species of the genus *Chrysochromulina*, it is phagotrophic and in addition to possessing two flagella (not observed in the preserved material when the organism was described), it has also an unmistakable haptonema similar to that possessed by the members of the genus *Chrysochromulina*, on the form range of which Dr Parke is continuing to work. A further paper on this genus, in collaboration with Prof. Irene Manton, has been published in Vol. 38, No. 1, of the *Journal*. In this paper a species very common in the English Channel is described, and for the first time records for distribution and seasonal occurrence at six stations are included. Unlike any of the previously known species this new form is characterized by a very long haptonema capable of attaching at any point along its length and a two-layered covering of small scales, the scales of the two layers being of different size and pattern. The anatomy of the haptonema of this species is similar to that described for *C. chiton*, except that the number of fibres in the ring is six and not seven.

The collection of unialgal cultures of marine phytoplankton organisms continues to be in great demand by research workers in this country and abroad to whom over 200 cultures have been distributed.

Dr F. S. Russell has made a study of the biology of the deep-sea scyphomedusa *Atolla*. It was hoped that regular observations over the year on size and maturity would give an indication of length of life. It was found, however, that although mature specimens occurred in two size ranges, small and large, both groups were present throughout the year. A detailed examination of the small mature specimens has now shown that they are specifically distinct from the large mature *Atolla wyvillei*. The new species has been named *A. parva*

and was briefly described in *Nature, Lond.*, Vol. 181, p. 1811. A full account of these observations has been published in Vol. 38, No. 1, of the *Journal*.

Work by Mr G. M. Spooner on the planktonic Amphipoda Hyperiidea has been mainly devoted to *Cystosoma*, a peculiar deep-water genus of large transparent forms, much of whose biology has hitherto remained obscure. Thanks to a collection made by Dr E. J. Denton on a cruise to the Bay of Biscay in R.V. 'Sarsia', by which the number of recorded adults of one of the species was doubled, the breeding cycle could be followed. The numerous small eggs are carried in an invaginated brood pouch in which they develop, as it proves, into a peculiar larval form unlike anything previously known in Amphipoda, resembling in appearance the micronisicid larvae of Isopoda. A considerable change in structure must occur between this stage and the 'Physosoma' stage which precedes the final adult form.

Dr A. J. Southward has been examining rapid methods for sampling the plankton 'indicator' species in the Channel, and has made several surveys with a Lowestoft type Gulf III high-speed plankton sampler. This instrument makes good catches of euphausids and chaetognaths, and most of the Western Channel can be sampled in a few days. Results are still being analysed, but already they are producing a picture of the changing conditions of offshore Channel waters in relation to those in the vicinity of Plymouth. In the first half of 1958 *Sagitta elegans* was dominant throughout the Western Channel, and off the North Cornish coast, but appeared in comparatively large numbers at the Plymouth stations (Eddystone and E1) only in March.

In agreement with this, Mr P. G. Corbin has observed no major change in the low level of macro-plankton associated with the prevailing *Sagitta setosa* in the 2 m. stramin ring trawl collections during 1958.

Macro-Fauna and Flora

During the year many additions have been made to the herbarium and collection of preserved marine algae.

In 1939 Dr D. P. Wilson began a study of the developments of three species of the polychaete family Magelonidae, but the war made it impossible to continue. Of the three species one was the common *Magelona papillicornis* F. Müller, one a new and still undescribed species from Salcombe, whilst the third, abundant in the Rame Mud, was provisionally identified by him as *M. cincta* Ehlers. Recent searches of the Rame Mud and other likely muddy-sandy areas have shown that this latter species is no longer abundant, and only a few fragments of the worm have this year been obtained. A close study of these and of specimens collected in 1939 has established that it is a previously unrecognized species differing from *M. cincta* in several major features. Dr Wilson has described the worm and named it *M. alleni* after the late Dr E. J. Allen. He has also partially redescribed and refigured *M. cincta* Ehlers from the type specimen (kindly loaned by the Zoologisches Museum,

Berlin) and from specimens recently collected on South African coasts by Prof. J. H. Day. From records by several workers and from specimens loaned by the British Museum (Natural History) it has appeared that *M. alleni* is widely distributed in the north-eastern Atlantic, while *M. cincta* is known only from southern and south-eastern coasts of Africa influenced by the warm Mozambique and Agulhas Current. A paper on this work has been published in Vol. 37, No. 3, of the *Journal*.

Dr Wilson has also published in Vol. 37, No. 2, of the *Journal* a third series of notes on observations made in the aquarium. In this series an account is given of the breeding behaviour of the Black Sea-bream, *Spondyliosoma cantharus* (Gmelin), of which no adequate description existed. Of even more interest are the observations on the previously unknown sexual display of the male Cuckoo Wrasse, *Labrus ossifagus* L., noteworthy for the remarkable white patch which appears on its head and shoulders during periods of intense sexual excitement, and which is not visible at other times.

Populations of the isopod *Jaera albifrons* (sens. lat.) have been examined by Mr G. M. Spooner in the Plymouth area, and elsewhere, to investigate the distribution of the so-called microspecies, which depend on differences in the secondary sexual development of certain limbs of the male. The animals are normally collected from the under side of stones, but *J. a. praehirsuta* typically confines itself to fucoid weeds. Investigation is handicapped because the females are apparently indistinguishable, and there is a low sex-ratio of males, because of the trivalent sex chromosome. Out of 2999 animals examined only 739 were male—a ratio not significantly different from that of 1 in 4 expected on theoretical grounds. In the Plymouth area *J. a. albifrons*, *J. a. forsmani*, *J. a. ischiosetosa* and *J. a. praehirsuta* are all common. There is wide over-lapping between these four, but only a single hybrid individual has been recognized. The 'microspecies' have rather different average optima, in the field—apart from the special substratum preference of *praehirsuta*—but they do not occupy clear zones on the shore as has been emphatically claimed on the Continent. The distribution of the very different *J. nordmanni* (now confirmed from Plymouth for the first time since the days of Spence Bate) has also been studied. It is confined to sites of much reduced salinity.

Mr Spooner has now found that the Eddystone shell gravel (depth 25–28 fathoms) is rich in the smaller species of malacostracan crustaceans, when appropriate means are taken to isolate the microfauna from samples of raw gravel. Out of thirty species so far seen, thirteen are new to the Plymouth fauna, some being hitherto undescribed. Among these are members of the isopod genus *Microcharon* and the amphipod family Bogidiellidae, both typical underground 'interstitial' groups, of which the majority of the known species (all small, blind, colourless) live in freshwater habitats. Others are known from intertidal sands usually affected by land drainage. These are the first true

offshore records from anywhere. *Microcharon harrisi* has been described in Vol. 38, No. 1, of the *Journal*. The bogidiellid, represented at present by eleven damaged specimens, is being assigned to a new genus. These finds distinctly strengthen the idea, already suggested by the distribution of the aberrant amphipod *Ingolfiella*, that there exists an 'underworld' faunal element, spread universally through the earth's surface strata, irrespective of whether this is covered by sea, fresh water, or land, and independent of these elements for effective distribution.

The new species of Goby discovered by Mr G. R. Forster, while diving with aqualung equipment off the open coast-line, where rough reefs and cliffs form the shore, was described by Mr P. G. Corbin in *Nature, Lond.*, Vol. 181, p. 1659, and named *Gobius forsteri*.

Since Leach's (1815) original description of the swimming crab, *Portunus marmoreus*, it has remained a matter of difficulty to distinguish it from the closely related *P. holsatus* Fabricius and there has been much discussion about these two species in the literature, some authors considering them to be only variants of the same species. Mr Corbin has recently found a difference in the setation on the carpus and propus of the cheliped which readily separates the two species: it is a clear-cut difference and not one of superfine comparison.

Mr N. A. Holme has continued working through grab samples taken in previous years, and has completed a series taken in and around Quiberon Bay in 1955. Deposits in the bay are mainly of mud or muddy sand with a rich polychaete fauna, and are characterized by such molluscs as *Turritella communis*, *Thyasira flexuosa*, *Lucina spinifera* and *Dentalium* spp. The channel inside Belle Ile is scoured by currents, and the bottom is mainly of stones or gravel. *Amphioxus lanceolatus* was found at one or two stations, and the ophiuroid *Ophiopsila annulosa* was also recorded. Sorting of the sievings has been speeded up with no apparent loss of accuracy by using the flotation technique recently described by Birkett. Trichlorethylene was used, however, as it is less toxic than carbon tetrachloride.

A record of specimens of *Saxicavella jeffreysi* Winckworth taken off Plymouth has been published in the *Journal of Conchology*. Although reported by Jeffreys from among trawl refuse from Plymouth, this species had not since been recorded from the area.

Further measurements of populations of *Venerupis rhomboides* have been made in order to determine if the form *sarniensis* described by Turton is in fact a separate species. It now seems clear that the proportions of the shell are directly related to depth of water, the broader and stouter form (*sarniensis*) occurring on the shore, while the more slender typical form occurs in 20–30 fathoms. It is possible to trace a gradation in shell proportions with increasing depth, and this has been confirmed for other populations outside the Plymouth area.

Mr G. R. Forster has continued his underwater studies on the rock fauna in the vicinity of Plymouth, for which the selection of suitable positions for diving has been made much easier by the provision of a small visual-type echo-sounder. During the summer many dives have been made to try to assess the effect which the population of large spiny sea-urchins, *Echinus esculentus* L., have on the sessile animals and algae.

The numbers of *Echinus* have been estimated quantitatively by counting all the specimens seen while swimming with a $2\frac{1}{2}$ m. rod along a 50 m rope which has been laid out in a straight line along the sea bottom. Up to the present 598 *Echinus* have been counted; the total area covered being just over 3300 m^2. This gives a ratio of one *Echinus* to slightly more than 5·5 m^2 of rock surface.

The rate of browsing has been studied by placing six *Echinus* under a wire frame 1 m^2 in area covered with large mesh nylon netting. Two of these frames were arranged on the sea bottom in the natural habitat of the *Echinus*. After a few weeks the rock surface on which the *Echinus* had been able to browse was carefully observed and compared with that under the second frame which contained no *Echinus*. Three such tests have been made during the summer, two were unsuccessful owing to the frames being moved or disturbed by gales. In the successful test in September, however, in rather less than four weeks the rock surface appeared to have been almost completely swept clean of its thin covering of Bryozoa, small barnacles, encrusting ascidians, besides some brown and red algae.

Dr A. J. Southward has continued observations on the distribution and abundance of barnacles and other shore animals. No marked changes have occurred in the Channel for the past year. Near Plymouth, the immigrant barnacle *Elminius*, which has been very common in the estuaries for several years, is now present on the wave-beaten open coast and is increasing slowly in abundance. In collaboration with Dr D. J. Crisp, of the Marine Biology Station, Anglesey, Dr Southward spent seven weeks in Ireland re-investigating in detail the distribution of the shore fauna and flora. Among other things it was found that the abundance of the northern barnacle *Balanus balanoides* had increased considerably in the south and west compared with 1952, while *Elminius* is established at several centres on the east and south coasts. Dr Southward is continuing work on the use of electronic flash cinematography for the study of barnacle behaviour. Some preliminary results were incorporated in a film on barnacle behaviour shown at the International Zoological Congress in London in July, as part of a joint contribution with Dr Crisp (*Proc. XVth Int. Congr. Zool.*, Sect. III, paper 5).

Dr A. J. Southward and Dr Eve Southward are studying the distribution and systematics of the Pogonophora of the Atlantic. The discovery that these interesting relatives of the chordates are common on the continental slope is a rewarding result of continued prosecution of deep water dredging, and

owes a great deal to the perseverance of Captain C. A. Hoodless and the crew of R.V. 'Sarsia'. Preliminary reports on the Pogonophora have been published in *Nature, Lond.*, Vol. 181, p. 1607, and Vol. 182, p. 272; and two new species are described in Vol. 37, No. 3, of the *Journal*. A note on the systematics and behaviour of two deep-sea barnacles, *Hexalasma* and *Verruca*, was also published in the same issue of the *Journal*.

Physiology of Marine Organisms

Dr T. I. Shaw has prepared an account of his experiments upon ^{131}I uptake by the sea weed *Laminaria digitata*, and this has been accepted for publication in the *Proceedings of the Royal Society* B. Currently he has investigated the state of iodine in the tissues of the weed. Chemical analyses, based on precipitations with silver nitrate, have indicated that some three-quarters of the iodine taken into the tissues is retained there as inorganic iodide. Preliminary experiments using chromatography upon the unhydrolysed weed tissues have both confirmed this finding and have also shown that the remaining iodine, being immobile upon the chromatographs, can be neither mono-, nor di-iodotyrosine.

Further studies upon the intermediate compounds of carbohydrate metabolism have shown that citric acid is increased in concentration in the tissues of the weed during the uptake of iodide.

In collaboration with Prof. A. L. Hodgkin, F.R.S., of Cambridge, and Dr P. C. Caldwell, Alan Johnston, Lawrence and Mosely Research Fellow of the Royal Society, Dr Shaw is also investigating the influence of phosphate esters upon the active transport of sodium and potassium across the membrane of the giant nerve fibres of *Loligo*. Factors influencing the active transport of sodium and potassium are of the widest general interest in biology, and it is important to try and obtain an understanding of the mechanisms involved. A great deal of work in the field has already been carried out at Plymouth by Dr R. D. Keynes of Cambridge and Dr Caldwell, and the present investigations arise directly from their earlier observations. This season's work has principally centred upon the effects of injecting arginine phosphate into the fibres poisoned with either dinitrophenol or cyanide. It has been shown that the effects upon the sodium efflux are markedly dependent both upon the state of poisoning and upon the presence or absence of potassium in the external sea water. For example, arginine phosphate reduces the sodium efflux from fibres poisoned with dinitrophenol (applied in artificial sea water containing bicarbonate) provided that potassium is absent from the external medium, but markedly increases the sodium efflux from fibres poisoned with cyanide. Experiments are also being carried out to determine the time course of changes in the sodium efflux induced by altering the external potassium concentration.

Most work on luminescent animals has hitherto been carried out on large multicellular species (Metazoa), but there are certain advantages in studying

protozoans. *Noctiluca* is especially suitable, since the cells are large enough to be seen and handled easily. An experimental study of *Noctiluca* has been carried out by Dr J. A. C. Nicol, both in the laboratory and at sea. This species sometimes occurs in dense patches, in concentration sufficient to produce 'red tides', and producing bright phosphorescence at night. Although the light from the aggregation of cells appears like a bright glow or wave, the light from each cell is a quick flash, some 0·1 sec in duration. By controlled electrical and mechanical stimulation of single cells, it has been possible to show that luminescence is subject to fatigue. The cells recover in about a minute; consecutive flashes may become brighter (facilitation). The luminescent responses are much like those observed in higher animals possessing nervous systems. Presumably, a similar system of excitation is involved: some form of surface action takes place when the cell is excited, leading to a controlled release of a brief photogenic reaction. In higher animals the excitatory stimulus is normally nervous.

A further study has also been made by Dr Nicol of sea pens collected by R.V. 'Sarsia' in the Bay of Biscay. Earlier studies have shown how the nervous system (a nerve net) controls the transmission of light waves across these animals. In the present investigation isolated zooids or polyps have been studied. These emit brief flashes, lasting about $\frac{1}{5}$th of a sec, and having an intensity of $0{\cdot}1 \times 10^{-9}$ to 63×10^{-9} μJ/cm^2 receptor surface at 1 cm distance. The additive effect of the flashes of separate zooids produces waves lasting about 1 sec at each locus, and progressing over the animal at a velocity of 6 cm/sec. The intensity of light emitted by the whole animal ranges from $0{\cdot}8 \times 10^{-6}$ to 7×10^{-6} μJ/cm^2 receptor surface at 1 cm distance. Luminescence in sea pens is accompanied by contraction and withdrawal. In its normal habitat, the animal, when disturbed, would give a flash visible for several metres, and then contract and pull itself down into the mud, in which it is anchored. It would be desirable to have some information about how quick weak flashes of this kind affect other active animals. The results of this research have appeared in Vol. 37, No. 3, of the *Journal*.

Dr Nicol has also published in Vol. 37, No. 3, of the *Journal* the results of his work on the luminescence of animals in a number of different groups, some of which was outlined in last year's report. In this paper observations are also given on the reflecting properties of red prawns and black fish. It was shown that the absorption characteristics are such as to provide minimum reflection of blue light prevailing in the deep sea and of luminescent light, thus having adaptive significance. Estimates have also been made of the distances at which the luminescence of animals can be seen in the sea ranging from below 10 to about 100 m according to the brightness of the organism.

Dr E. J. Denton has continued his experiments on the vision of fishes. The layers of retinae other than the receptor layer have been shown to have a very small absorption of light and, since this absorption varies little with wave-

length, it could only account for very small changes in spectral sensitivity. The observations on the polarization of the fluorescence of the vitamin A in the bleached retinal rods which was described last year have been confirmed on other species. An account of this work consisting of observations on the retinae of various animals chosen so as to give examples of golden, red and purple retinae has been published in the *Proceedings of the Royal Society* B.

Experiments have been made to show that the retinal rods, because of their high refractive index, channel light coming to them. This allows the layer of receptors, although often relatively very thick, to act effectively as a 'thin emulsion' and thus to allow accurate vision. It has been shown that to give the best retinal image the lens must form its image on the internal end of the receptors. This has been demonstrated for several coastal fish, the frog and for several species of deep-sea fish. The retinae of deep-sea fish are extremely favourable for this experiment because the rods are very long and the other layers of retina very thin.

The experiments undertaken by Dr Denton in collaboration with Dr D. B. Carlisle on the change in visual pigments during the metamorphosis from the yellow to the silver eel have been completed by histological measurements. These showed that although the area of retina increases about fourfold on metamorphosis the number of receptors remains constant. The retinal rods about double, however, in diameter, and this accounts for the fact that the optical density of pigment in the retina remains high. A deep-sea eel, *Synaphobranchus* sp., has been shown to have a golden retinal pigment almost identical with those of the silver fresh-water eel and the conger eel. The results of this work have been published in Vol. 38, No. 1, of the *Journal*.

Further observations have been made, with the assistance of Mr F. J. Warren, on the spectral absorption of light by the crystalline lenses and corneas of fish and squid. In the freshwater perch a curious system exists in which the selective absorption of light on its way to the retinae takes place in two stages. The crystalline lens absorbs all the ultra-violet light below 400 mμ whilst the cornea, which is transparent in the ultra-violet, absorbs the blue region of the spectrum. In other species all this absorption is done by the lens alone.

Dr Denton's work with Mr N. B. Marshall, of the British Museum (Natural History), on the buoyancy of deep-sea fish without a swimbladder has been completed and has been published in Vol. 37, No. 3, of the *Journal*.

Dr Denton and Dr Shaw have studied the physiology of a very notable adaptation for buoyancy found in the Cranchid squid. Dr J. Gilpin Brown, a visiting research worker, is collaborating with them and is studying the histology and anatomy of these squid with particular reference to this problem. These squid are in buoyancy equilibrium with sea water. They achieve this equilibrium by balancing their heavier parts by a very large volume of coelomic fluid which is less dense than sea water, its density being 1·010. This coelomic fluid accounts for about two-thirds of the mass of squid. The fluid is almost

isotonic with sea water showing that buoyancy is not achieved by simple exclusion of solutes. The low density results from replacing most of the cation of sea water by ammonium ions which exist in the fluid in a concentration of almost 500 mM. The anion content is almost exclusively chloride. The fluid is very acid, pH 5·2, and this is doubtless connected with the retention of ammonia in the coelom. In design these squids are strikingly similar to the bathyscaphe, relatively recently designed by Professor Piccard, in that they balance their useful but dense parts by a large flotation chamber containing a fluid of lower specific gravity than sea water. A brief account of this work has been published in *Nature, Lond.*

An investigation begun last year by Dr D. B. Carlisle, in collaboration with Dr Peter Karlson of Munich, into the chemistry of crustacean hormones is bearing some fruit. A sample of chromactivating substances of which 10^{-16} g is enough to produce a response in an individual prawn has been prepared. This substance is, however, not yet pure, and work is in progress to obtain a much purer sample of this material for chemical analysis. It has been shown that the chromactivating substance found in impure samples of ecdyson obtained from silk worms, *Bombyx mori*, is not ecdyson itself but some impurity. Insect ecdyson has itself no action on colour change of prawns but stimulates their moulting activity. It is not, however, as potent as the similar material obtained from *Crangon*. Conversely, the material isolated from *Crangon* is less active on insects than insect ecdyson. Dr Carlisle has published an account in *Nature, Lond.*, Vol. 182, pp. 32–4, on the inactive precursor of a chromactivating substance of the sinus gland of *Leander* and on the ways in which this inactive substance may be converted into the active hormone.

Dr Carlisle has investigated further the endocrine basis of sex reversal in *Pandalus*. He has found evidence that the vas deferens gland participates in the control of this phenomenon. His account of neurosecretory transport in the pituitary stalk of *Lophius piscatorius* has been published in *Zweites Internationales Symposium über Neurosekretion* (Springer–Verlag, Berlin), 1958.

Dr Carlisle is now Recorder of the Protochordate Section of the *Zoological Record*, and for this section the Record for 1955 and 1956 has been published in the course of the year.

Dr Carlisle's work with Mr L. G. Hummerstone on niobium in ascidians and in the sea has continued and two papers on the subject have appeared in *Nature, Lond.*, Vol. 181, pp. 933 and 102–3.

In collaboration with Dr P. C. J. Brunet of Oxford, Dr Carlisle has been investigating the exuvia, cuticle or tube of a number of species of marine animals to determine their composition. The most worthwhile result to date is that the tube of four species of Pogonophora consists primarily of chitin and protein, contrary to the statement of A. V. Ivanov who reports that these tubes consist of cellulose or tunicin. A preliminary account of this work has

appeared in *Nature, Lond.*, Vol. 182, p. 1689, and it is hoped to publish a fuller account in the *Journal*.

While Dr Carlisle was at the Kristineberg Laboratory in Sweden, pursuing his investigations of *Pandalus*, he observed moulting to take place in *Priapulus* and found that the exuvia of this species consists of chitin and tanned protein while the new cuticle of the post-moult individual contains only protein, the chitin portion appearing as the cuticle hardens and thickens. An account of this investigation is in the press in *Arkiv för Zoologi, Stockholm*.

Dr Carlisle has continued employing diving equipment to study the biology of crabs, prawns and ascidians in their natural habitats.

Dr E. D. S. Corner has continued his studies of the poisoning of *Maia squinado* by mercury. ^{203}Hg-labelled $HgCl_2$ and n-$C_5H_{11}HgCl$ have been used in order to compare the distributions of mercury compounds of different lipoid solubilities throughout the test animals, and it has been found that whereas the organomercury compound is readily assimilated by various internal organs, notably the antennary glands, most of the inorganic compound remains attached to proteins in the blood. Paper chromatography has been used in attempts to identify amino acids present in the blood and urine of *Maia*, and although no marked differences have been found in the relative quantities of amino acids in the body fluids of poisoned and control animals, further quantitative experiments have shown that *Maia* treated with mercury poisons excretes greatly increased amounts of urinary amino-N, usually without any corresponding increase in the blood level. It has also been found that quantities of $HgCl_2$ that cause a marked increase in urinary amino-N have little effect on the excretion of total sulphate. These findings are consistent with the view that mercury compounds impair the function of the antennary glands by inhibiting the process by which amino acids are re-absorbed from the urinary filtrate into the blood. A paper describing these results has been accepted for publication in *Biochemical Pharmacology*.

In collaboration with Dr R. D. Bulbrook, of the Royal College of Surgeons, an attempt is being made by Dr Corner to extract from marine invertebrates an enzyme catalyzing the hydrolysis of androsterone sulphate. This sulphatase would be of considerable use in analyses of androsterone in conjugated form. So far the digestive glands of *Maia squinado*, *Cancer pagurus* and *Buccinum* have been examined, but the preparations obtained from these organs possessed only small activity. It is hoped that continued search will eventually disclose a source of suitably active material.

Dr Corner and Mr B. W. P. Sparrow (International Paints Research Laboratory, Newton Ferrers) have extended their studies of the effects of toxic agents on marine organisms to include experiments with the intertidal red alga *Plumaria elegans*. In collaboration with Mr A. D. Boney (Plymouth Technical College) a method has been devised for comparing the effects of heavy metals and other more specific respiratory inhibitors on the growth and

viability of *Plumaria* sporelings. In general, it has been found that the test organisms are very resistant to poisoning by heavy metals and the correlation between lipoid solubilities and toxicities, especially within the homologous series of primary *n*-alkylmercuric chlorides, is less exact than that observed in similar experiments using crustaceans. In a complementary investigation, carried out primarily by Mr Boney, a comparison has been made of the abilities of various species of red algae to withstand harsh environmental conditions and the resistances of these plants to poisoning by certain heavy metals. Results obtained so far have been encouraging in that ecological and toxicological data show a close correlation. An account of this work is being published in *Biochemical Pharmacology*.

During his year at Plymouth, Mr B. R. Jewell was engaged in investigations of the mechanical and thermal properties of the anterior byssal retractor muscle (ABRM) of *Mytilus*. Initially, a good deal of time was devoted to making improvements in the apparatus and methods that are used in experiments on the ABRM; these included developing a method of isolating intact preparations of the muscle (demonstrated to the Physiological Society in March, 1958) and designing a new type of multi-electrode assembly, which was constructed by Mr F. G. C. Ryder; the improvement in the standard of the experimental work amply justified the overall expenditure of time and effort.

The experiments revealed striking differences in the mechanical state of the ABRM during phasic and tonic responses. During the former, the ability of the muscle to do work and to re-develop tension after it has been allowed to shorten is much greater than it is during the latter. Such findings cannot easily be explained by the 'tetanus' hypothesis, for they suggest that the phasic and tonic responses are not based on the same fundamental unit, the muscle twitch.

Some preliminary studies of the thermal properties of the ABRM were made with Mr J. V. Howarth, in Prof. A. V. Hill's laboratory at University College, London. It was shown that, in the presence of oxygen, the tonic response is accompanied by a sustained increase in the heat production of the muscle. Other results suggested that, in the absence of oxygen, a reversible state may be reached in which the muscle can maintain tension without the expenditure of any energy.

Library

During the year the librarian, Miss L. M. Serpell, visited a number of libraries at other marine laboratories and institutions. Visits were also made to the library of the Plymouth laboratory by other librarians. Such meetings contribute towards closer liaison between the libraries.

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.

Published Memoirs

Vol. 37, No. 2, of the *Journal* was published in June, Vol. 37, No. 3, in October 1958, and Vol. 38, No. 1 in February 1959.

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MEMBERSHIP OF THE ASSOCIATION

The total number of members on 31 March 1959 was 1035, being 41 more than on 31 March 1958; of these the number of life members was 121 and of annual members 914. The number of associate members is five, Mr G. M. Graham, C.M.G., O.B.E., having been elected during the year.

GRANT FOR AQUARIUM RECONSTRUCTION

The Council wishes to record their grateful thanks for the following donation towards the cost of reconstructing the aquarium:

Messrs Pilkington Brothers Ltd. £50

MORLEY NEALE STAFF FUND

Mr Morley H. Neale, C.B.E., a Vice-President of the Association, has made a generous gift of £1300 to be used by the Director of the Plymouth laboratory at his sole discretion for the benefit and pleasure of the Association's staff. £1000 of this is to be invested and the annual interest used for the purposes of the Fund.

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.

Private Income. The Council gratefully acknowledge the following generous grants received during the year:

Fishmongers' Company (£400), The Royal Society (£100), British Association (£50), Physiological Society (£50), 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*.), Southampton (£15. 15*s*.), Reading (£10. 10*s*.), Nottingham (£10. 10*s*.), Hull (£10. 10*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), The Imperial College of Science and Technology (£10), Gonville and Caius College, Cambridge (£5), and the Zoological Society of London (£10. 10*s*.).

PRESIDENT, VICE-PRESIDENTS, OFFICERS AND COUNCIL:

The following is the list of those proposed by the Council for election for the year 1959–60:

President

Prof. A. V. HILL, C.H., O.B.E., Sc.D., LL.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., F.R.S.
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.
G. M. GRAHAM, C.M.G., O.B.E.

COUNCIL

To retire in 1960

Prof. E. BALDWIN, Ph.D.
C. H. MORTIMER, Dr.phil., D.Sc., F.R.S.
S. SMITH, Ph.D.
Prof. J. E. SMITH, Sc.D., F.R.S.
H. G. VEVERS, M.B.E., D.Phil.

To retire in 1961

H. A. COLE, D.Sc.
G. E. FOGG, Ph.D.
Prof. J. E. HARRIS, Ph.D., F.R.S.
C. E. LUCAS, C.M.G., D.Sc.
Prof. C. M. YONGE, C.B.E., D.Sc., F.R.S.

To retire in 1962

J. N. CARRUTHERS, D.Sc.
Prof. A. L. HODGKIN, F.R.S.
Prof. O. E. LOWENSTEIN, D.Sc., F.R.S.
Prof. G. E. NEWELL, Ph.D.
Prof. W. F. WHITTARD, D.Sc., F.R.S.

Hon. Treasurer

HARRISON S. EDWARDS, Westhumble Lacey, Near Dorking, Surrey

Secretary

F. S. RUSSELL, C.B.E., D.S.C., D.F.C., LL.D., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. WALL (Ministry of Agriculture, Fisheries and Food)
The Worshipful Company of Fishmongers:
The Prime Warden
Major E. G. CHRISTIE-MILLER
HARRISON S. EDWARDS
Prof. Sir Alister HARDY, Kt., D.Sc., F.R.S. (Oxford University)
C. F. A. PANTIN, Sc.D., F.R.S. (Cambridge University)
EDWARD HINDLE, Sc.D., F.R.S. (British Association)
N. B. MARSHALL (Zoological Society)
Prof. Sir JAMES GRAY, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1958–9

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET

31 MARCH 1959

	£	£
CAPITAL RESERVE ACCOUNT:		
As at 31 March 1958	174,054	
Add: Expenditure on fixed assets recovered	625	
	174,679	
Less: Transfer to surplus account being an amount equivalent to the depreciation provided on assets acquired out of Development Fund grants	3,674	171,005
SURPLUS ACCOUNT:		
As at 31 March 1958	13,522	
Add: Transfer from Capital Reserve Account	3,674	
Transfer from 'Plymouth Marine Fauna' Fund	1,100	
Decrease in provision for diminution in value of General Fund investments	70	
	18,366	
Deduct: Excess of expenditure over income for the year	8,888	
		9,478
		180,483
BALANCES ON SPECIAL FUNDS (see annexed statement)		5,461
CURRENT LIABILITIES:		
Sundry creditors and accrued expenses	2,179	
Subscriptions and grants received in advance	327	
		2,506

Note: Capital commitments outstanding amount to £8,695 (1958 £1,200) of which approximately £7,550 (1958–nil) is recoverable

O. D. HUNT
F. S. RUSSELL } *Members of the Council*

£188,450

	Cost or Valuation	Depreciation	£
FIXED ASSETS:	£	£	£
Boats and equipment:			
At cost:			
R.V. 'Sarsia'	137,761	6,539	131,222
M.F.V. 'Sula'	12,500	815	11,685
R.L. 'Gammarus'	200	20	180
	150,461	7,374	143,087
Laboratory apparatus, equipment and machinery:			
At cost	17,070	5,320	11,750
Library at valuation in 1941 plus additions as valued by the Director	23,850	—	23,850
	£191,381	£12,694	
			178,687
INVESTMENTS AT MARKET VALUE:			
General Fund (including Composition Fees) at book amount (Market value £1,370; last year £1,220)		1,751	
E. T. Browne Bequest Funds at cost (Market value £3,431; last year £3,086)		4,689	
		6,440	
Less: Provision for diminution in value of investments		1,639	
			4,801
CURRENT ASSETS:			
Stocks on hand as valued by the Director		3,614	
Sundry debtors and prepayments		1,051	
Balances at bankers and cash in hand		297	
			4,962
			£188,450

AUDITORS' REPORT 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 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 31 March 1959 and of its excess of expenditure over income for the year ended on that date.

We have obtained all the information and explanations which we considered necessary. In our opinion the Association has kept proper books and the said accounts which are in agreement with them and with the said information and explanations, give in the prescribed manner the information required by the Companies Act 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
14 May 1959

PRICE WATERHOUSE & CO.
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT

FOR THE YEAR ENDED 31 MARCH 1959

	£	£
SALARIES (including additional for previous year) NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND SUPPLEMENTARY PENSIONS		34,846
LABORATORY AND BOATS' CREWS' WAGES (including additional for previous year), NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS, PENSIONS AND EMPLOYERS' LIABILITY INSURANCE ...		38,482
UPKEEP OF LIBRARY		670
SCIENTIFIC PUBLICATIONS, *less* SALES		1,907
UPKEEP OF LABORATORIES:		
Buildings and machinery	1,409	
Electricity, gas, coal and water	1,513	
Chemicals and apparatus	3,283	
Depreciation of laboratory apparatus, equipment and machinery	1,302	
Rents and insurance	401	
Travelling expenses	831	
Audit fee	149	
Stationery, postage, telephone and sundries	1,381	
Specimens	200	
Collecting expenses and upkeep of truck	245	
		10,714
MAINTENANCE AND OPERATION OF BOATS:		
Petrol, oil, paraffin, etc.	2,189	
Maintenance and repairs	6,882	
Depreciation	3,674	
Insurances	2,356	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
		15,496
ENTERTAINMENT EXPENSES		148
		£102,263

	£	£
GRANTS AND TABLE RENTS:		
Ministry of Agriculture, Fisheries and Food—Grant from Development Fund	83,871	
Fishmongers' Company	400	
Miscellaneous (including Royal Society £100, British Association £50, Physiological Society £50, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s.*, Leeds £20, Southampton £15. 15*s.*, Durham £10. 10*s.*, Exeter £10. 10*s.*, Leicester £10. 10*s.*, Manchester £10. 10*s.*, Nottingham £10*s.* 10*s.*, Hull £10. 10*s.*, Reading £10. 10*s.*, and Sheffield £10. 10*s.*, Imperial College £10, Zoological Society of London £10. 10*s.*, Ministry of Works £96, Imperial Chemical Industries Ltd., £52. 10*s.*, International Paints Ltd. £39. 7*s.* 6*d.*, Gonville and Caius College, Cambridge £5) ...	1,344	
		85,615
SUBSCRIPTIONS		876
SALES:		
Specimens	2,937	
Fish	622	
	£	
Nets, gear and hydrographical equipment	2,307	
Less: Cost of materials...	1,037	
	1,270	
		4,829
INCOME FROM INVESTMENTS		58
INTEREST ON BANK DEPOSITS, *less* CHARGES		154
AQUARIUM:		
Admission fees	2,072	
Sale of guides	40	
	2,112	
Less: Maintenance, printing and advertising	269	
		1,843
BALANCE being excess of expenditure over income for the year ...		8,888
		£102,263

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31 MARCH 1959

	E. T. Browne Bequest										
	Library	Special Apparatus	Scientific Publications	Aquarium Reconstruction Fund	Rockefeller Foundation Fund	Library Extension and Dogfish House Fund	'Plymouth Marine Fauna' Fund	Reservoir and Sea Water Tanks Fund	Algal Culture Fund	Research Funds*	TOTAL
	£	£	£	£	£	£	£	£	£	£	£
BALANCES AT 31 MARCH 1958 (after providing £1,496 for diminution in value of investments)	975	1,835	528	1,172	14	679	1,100	834	178	354	7,669
Add: Income during year											
Grants (including amounts paid direct to contractor by the Nuffield Foundation)	—	—	—	4,128	1,383	—	—	—	—	3,362	8,873
Income from investments	40	83	26	—	—	—	—	—	—	—	149
Bank deposit interest	—	—	—	39	—	18	—	36	2	—	95
Other income	—	—	69	—	—	—	—	—	—	—	69
Reduction in provision for diminution in value of investments	53	152	33	—	—	—	—	—	—	—	238
	1,068	2,070	656	5,339	1,397	697	1,100	870	180	3,716	17,093
Deduct: Expenditure during year	10	—	—	4,198	896	—	—	1,775	180	3,473	10,532
Transfer to General Fund	—	—	—	—	—	—	1,100	—	—	—	1,100
BALANCES AT 31 MARCH 1959	£1,058	£2,070	£656	£1,141	£501	£697	—	£(905)	—	£243	£5,461

* Including International Paints Ltd. 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. 27 (p. 761) and Vol. 31 (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 have they access to the books in the library at Plymouth.

The Commissioners of Inland Revenue have approved the Association for the purposes of Section 16, Finance Act, 1958, and that the whole of the annual subscription paid by a member who qualifies for relief under the section will be allowable as a deduction from his emoluments assessable to income tax under Schedule E.

All correspondence should be addressed to the Director, The Laboratory, Citadel Hill, Plymouth.

CONTENTS

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