THE ENERGETICS OF THE BRACKISH WATER SERPULID POLYCHAETE *MERCIERELLA ENIGMATICA* FAUVEL

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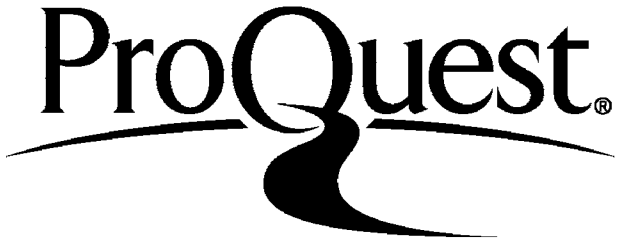

ABSTRACT

Investigations of the distribution of Mercierella enigmatica and the physical environment in the Thames estuary form the basis for a detailed laboratory study of the energy relations of this fouling organism. It was found that Mercierella's intertidal distribution is positively correlated with that of the surface water at Greenhithe, which effectively buffers the animal against extremes in oxygen tension, salinity, and temperature, whilst allowing unlimited feeding.

The following components of the energy budget under winter and summer conditions were investigated: particulate feeding; the integumentary uptake of dissolved organic compounds; egestion; respiration; nitrogenous excretion; somatic growth; reproduction; tube production; and mucus production. The flow of energy through the various channels was quantified using microbomb calorimetry, biochemical techniques, respirometry (a volumetric micro-respirometer was designed specifically for this purpose), and radioactive tracers.

A comprehensive investigation of the uptake kinetics, effects of endogenous and exogenous factors, and the fate of absorbed organic molecules, shows that these are apparently of little value as a supplementary nutritional source. An alternative hypothesis is proposed for the role of exogenous DOM* in Mercierella's energy budget.

The oxygenational properties of the blood pigment were investigated, and the relative significance of the potential respiratory surfaces during the different phases of activity are

* DOM = dissolved organic molecules

estimated based on histological evidence. In common with the majority of chlorocruorins, the blood pigment has a high P_{50} and is incompletely saturated when in equilibrium with air. The branchial crown is the major surface for gaseous exchange when it is extended, whereas the crown and rest of the body function as separate systems when the worm withdraws inside its tube.

The changes that occur in the gonadial tissues, coelomic cells, and population during the annual reproductive cycle were investigated, and it was found that the Greenhithe population is composed of males, females, and protandrous hermaphrodite individuals. Although the latter are a means by which the energy expended in reproduction by young individuals is reduced, whilst maximising their potential contribution to the population's reproductive output, it is shown that these are of no apparent value in temperate localities.

An important outcome of this study is the discovery that mucus production and respiration are the two major pathways through which energy leaves the body of the worm. These are followed by tube production, somatic growth, reproduction, and nitrogenous excretion, in decreasing order of magnitude. These results are compared and discussed in relation to published values for other aquatic invertebrates.

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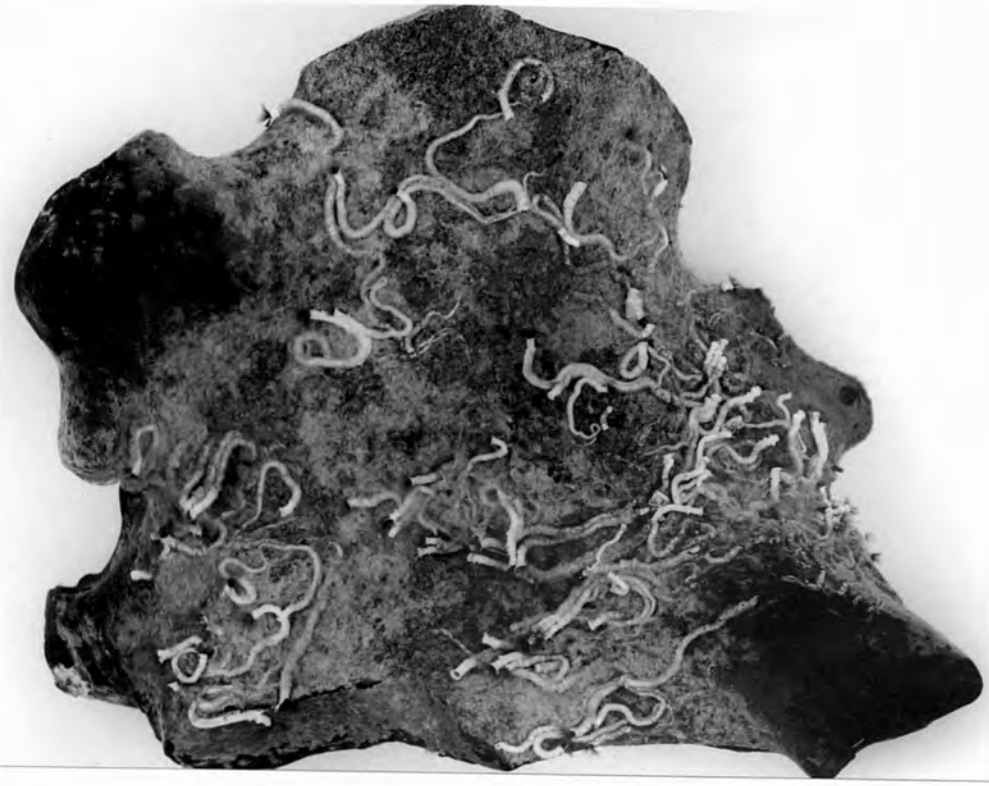
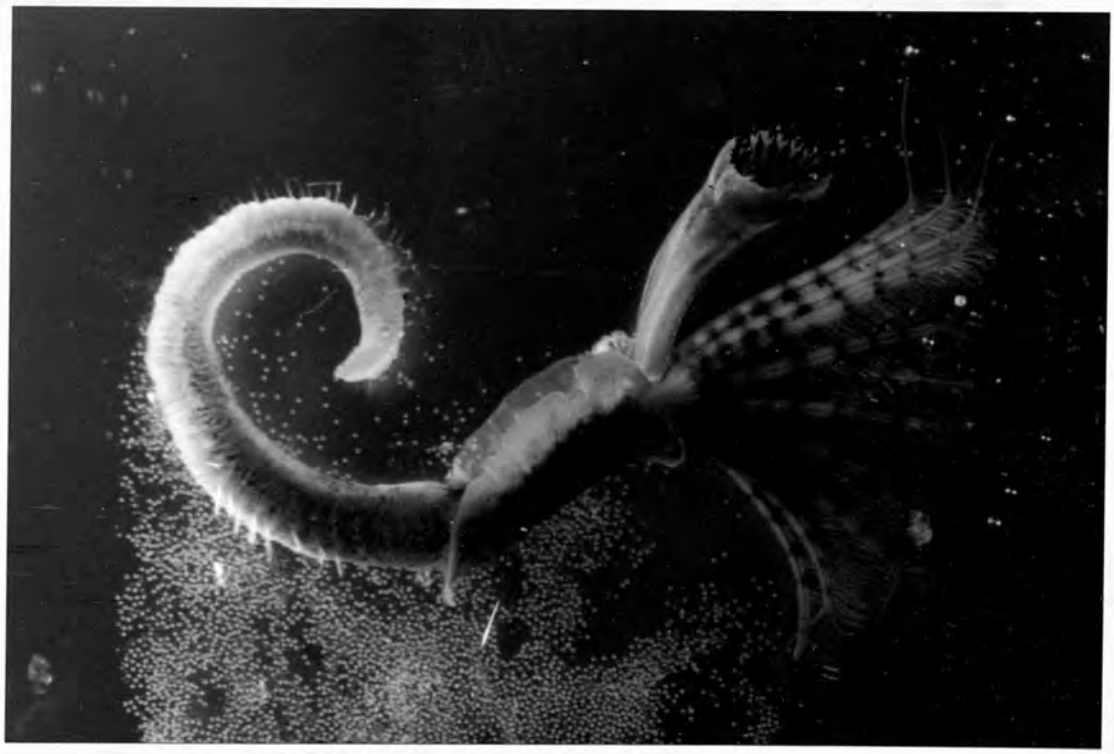




1. GENERAL INTRODUCTION

Mercierella enigmatica Fauvel is a brackish water serpulid polychaete. Serpulids are a group of filter-feeding, sedentary, worms which are typified by a usually white, calcareous tube (Fig. 1). Although there is no permanent anatomical attachment with the tube the worm never leaves its protection under normal circumstances.

Anatomically, the adult Mercierella is divided into three distinct regions : the branchial crown, thorax, and abdomen (Fig. 2). The branchial crown is composed of a number of filaments arising from two, semi-circular, outgrowths of the prostomium, on opposite sides of the mouth. The average number of filaments on the specimens examined is 18, but this varies and depends to some extent on the age of the animal; younger worms have fewer filaments. Each filament bears many, paired, finger-like projections, the pinnules, which are absent from the distal, tactile-sensory, portion. The most dorsal filament on the left side of the crown lacks pinnules and has become greatly modified to form the chitinised operculum, which is somewhat reminiscent of a cat's paw.

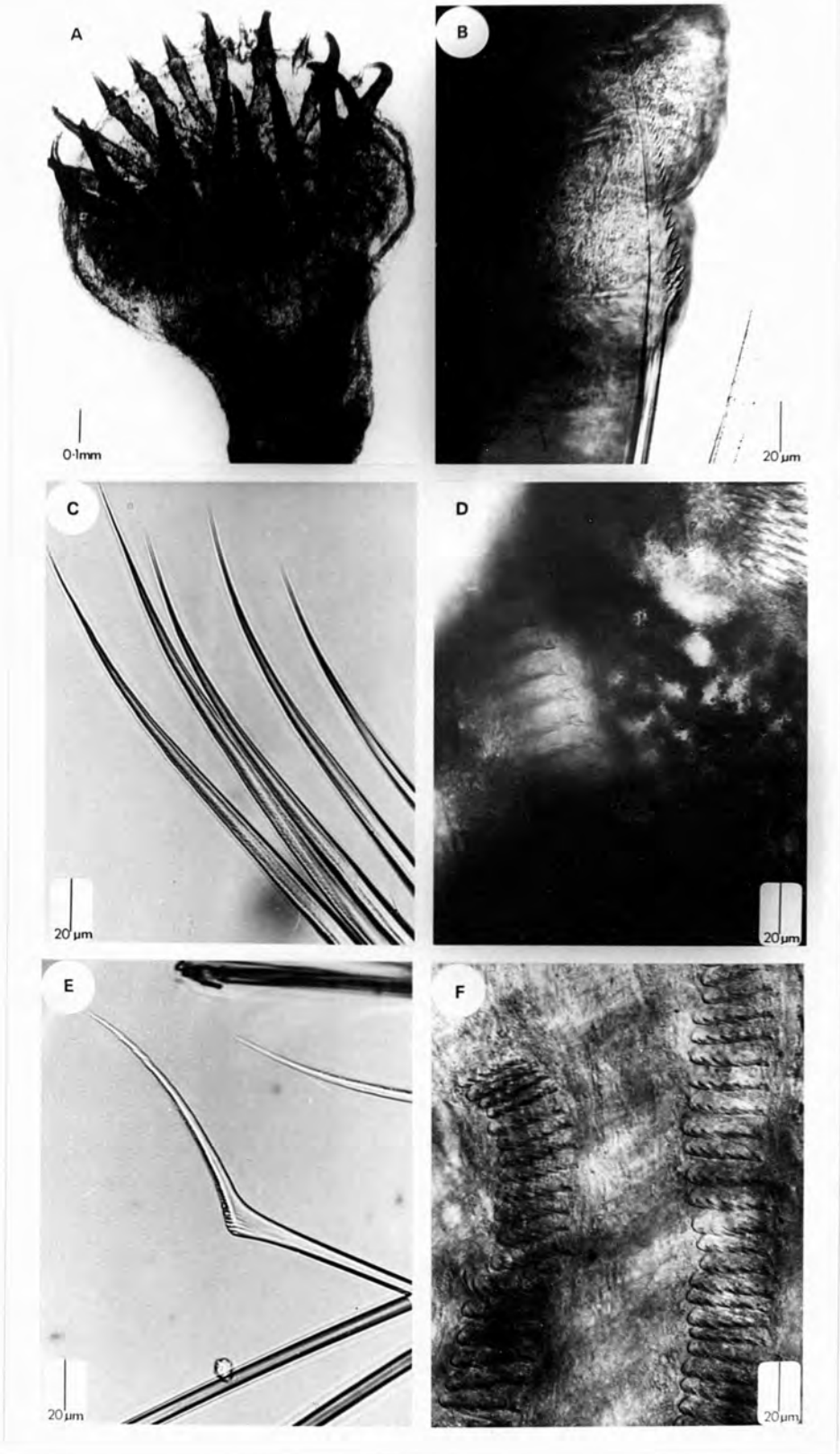
The thorax comprises seven chaetigerous segments. The anterior margin of the first thoracic segment extends to form a thin membrane or collar, which is divided dorsally where it is continuous with the lateral extensions of the body wall, the thoracic membranes. Posteriorly, the margin of the last thoracic segment extends as a thin membrane over the first abdominal segment. The glands which secrete the tube material are situated beneath the collar, bilaterally on the first thoracic segment.

Movement within the tube is effected by the action of the paired bundles of capillarychaetae which are present on all the thoracic and abdominal segments apart from the last. Anchorage is achieved by means of uncini which are highly modifiedchaetae. Ventral to each bundle of capillarychaetae on the thorax is a row (torus) of uncini which is arranged transversely. Each uncinus is a comb-like structure in which the teeth point anteriorly. There are no uncini on the first thoracic segment, whose capillarychaetae are of two distinct types : simple straight ones similar to those on the rest of the body, and elaborate collarchaetae. In contrast to the arrangement in the thorax, the positions of thechaetae and uncini are reversed on the abdomen, with the uncini being dorsal to thechaetae.

In the relaxed state the abdomen is of a smaller diameter than the thorax and is normally longer than the combined lengths of the other two regions. It consists of numerous metameric segments, the more anterior ones being longer than those behind. The number of abdominal segments ranged from about 20 to more than 100 in the specimens examined. The anus is situated postero-ventrally on the last, achaetous, abdominal segment. When removed from the tube, the abdomen curls towards the dorsal side.

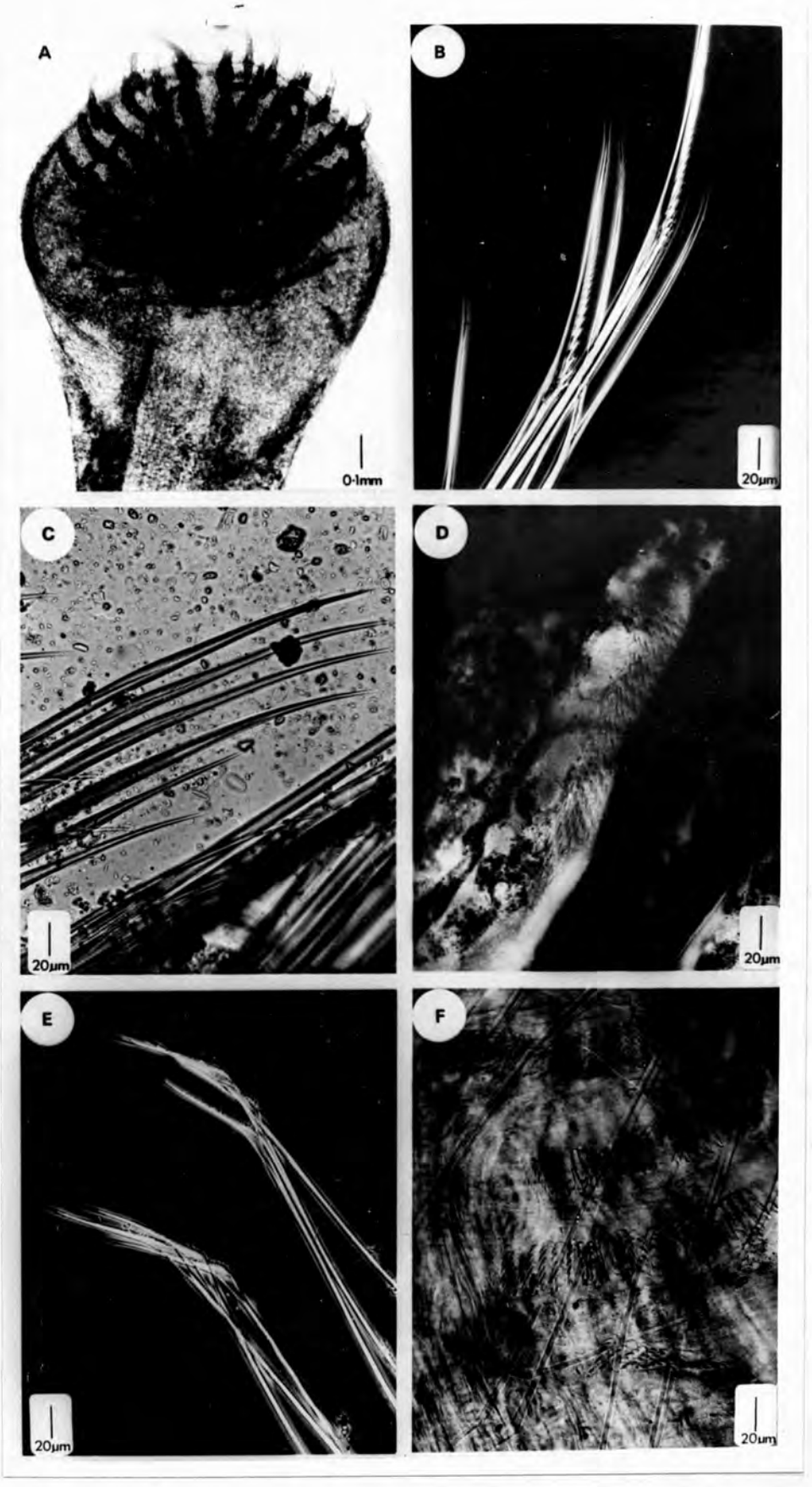
Since the time that Mercierella enigmatica was first described as a new genus and species by Fauvel (1922), from the Caen Canal in N.W. France, it has been recorded from numerous brackish water localities throughout warm temperate and tropical regions (for references see Vuillemin, 1965; Hartmann-Schröder, 1967; Harris, 1970; Straughan, 1972). Tebble (1953) recorded M. enigmatica from localities south of the 16°C isotherm in July in the northern

Fig. 3. Taxonomic features of a Mercierella enigmatica from Greenhithe: A, operculum; B, chaeta from 1st. thoracic segment (collarchaeta) side view; C, chaetae from 3rd. thoracic segment; D, thoracic uncini seen from above, showing the gouge-shaped anterior tooth; E, abdominal chaeta, side view showing finely toothed blade; F, abdominal uncini, oblique view showing antero-posterior arrangement in the torus.


A
0·1mm
B
20 µm
C
20 µm
D
20 µm
E
20 µm
F
20 µm

hemisphere to the north of the 21°C isotherm in July in the southern hemisphere. It is believed to have been spread largely by ships, both as adults attached to hulls and as planktonic larvae in ballast tanks (e.g. Fauvel, 1931, 1933). In recent years, however, there have been a number of conflicting accounts concerning the identification of the tropical form (see Pillai, 1960; Hill, 1967; Hartmann-Schröder, 1967; Straughan, 1972), and ten Hove and Weerdenburg (personal communication, 1976) are currently revising the genera Ficopomatus, Mercierella, Mercierellopsis, Neopomatus, and Sphaeropomatus. ten Hove informs me that Straughan's (loc. cit.) assertion that M. enigmatica and Neopomatus uschakovi Pillai are synonyms "is definitely incorrect", which casts some doubt on the identity of the animal she worked on, and consequently that of Hill's (loc. cit.) animal. Where the results of these works are referred to in this thesis the animal is described as M. enigmatica (= Neopomatus ?). Whilse ten Hove and Weerdenburg's revision may perhaps go some way to explain the differences between the growth rates (Section 8.1.4), reproduction cycles (Section 8.2.4), and salinity tolerances of the tropical and temperate populations, which tempted Harris (1970) to make exaggerated claims concerning the physiology of the species, this still leaves a number of conflicting reports concerning the reproduction and salinity tolerances of the temperate form (e.g. Seurat, 1927; Fischer-Piette, 1937; Heldt, 1944; Tebble, 1953, 1956; Mathias & Izac, 1963) which in the absence of anatomical evidence suggests at least the existence of physiological races. In view of these complications and in anticipation of further revision photographic evidence is provided of the main taxonomic features of the serpulid

Fig. 4. Taxonomic features of a Mercierella enigmatica from Weymouth Harbour backwater: A, operculum, showing concentric rows of inwardly directed spines; B, chaetae from the 1st. thoracic segment, showing the large, proximate, blunt teeth (phase contrast); C, thoracic setae from segments three and four, showing the finely-serrate blades; D, thoracic uncini; E, abdominal chaetae (phase contrast); F, abdominal uncini, posterior is at the top.


A
0·1mm
B
20µm
C
20µm
D
20µm
E
20µm
F
20µm

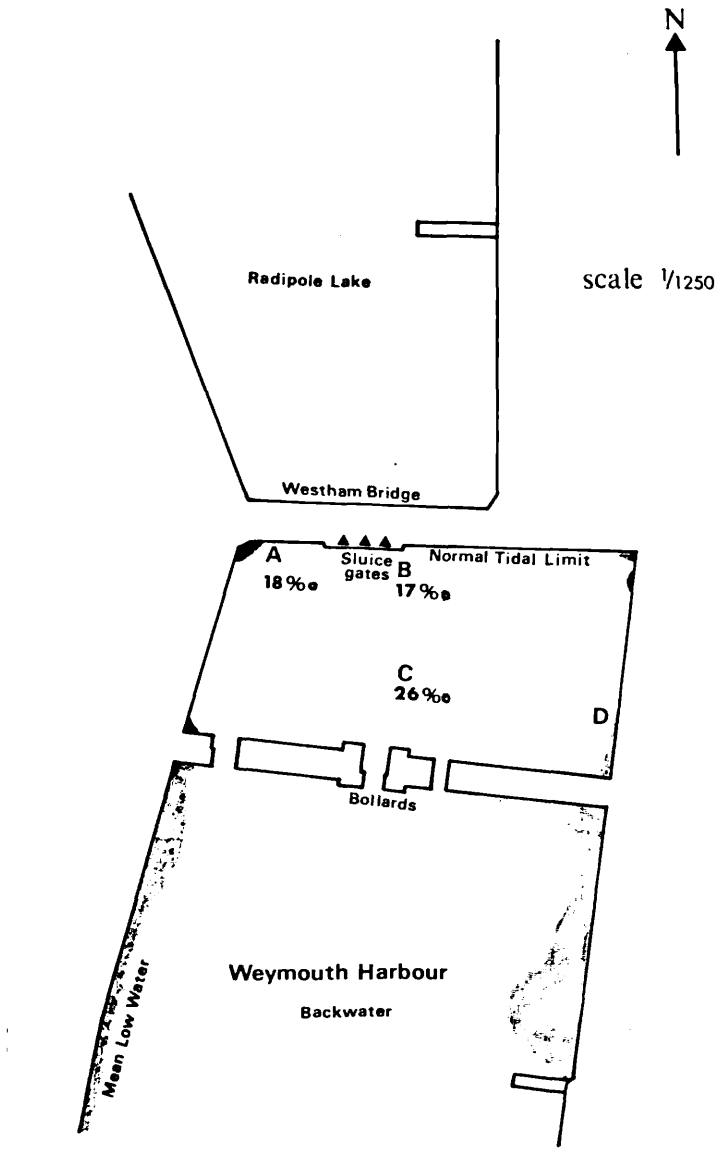
used in the present study, to support the assertion that it complies with Fauvel's original description of Mercierella enigmatica (Figs. 3 and 4).

M. enigmatica is of considerable economic importance as a fouling organism on ship's hulls (e.g. Monro, 1924; McIntosh, 1924; Hartmann-Schröder, 1967), power station conduits and screens, pier pilings (e.g. Wolff, 1969; Hove, 1974), and a variety of other submerged structures (e.g. Fischer-Piette, 1937; Tebble, 1953, 1956). Its introduction into cooler latitudes has undoubtedly been favoured by the heated discharges of power stations, e.g. the Kanaal door Walcheren, S. W. Netherlands (Wolff, 1969), and other water-side industry, e.g. Ramsden/Cavendish Dock, Barrow-in-Furness (Dock Engineer, personal communication, 1973). The best documented case of fouling by M. enigmatica in the British Isles occurred at Radipole Lake, Dorset, in the autumn of 1952 (Tebble, 1953, 1956).

Radipole Lake is a freshwater lake adjacent to Weymouth Harbour backwater, from which it is separated by sluice gates which are normally kept closed (Fig. 5). A population of M. enigmatica was first reported from the vicinity of the sluices in November 1937 (British Museum (Natural History) records), when it caused a limited fouling problem which was soon controlled by the local application of anti-fouling paint, and no more was mentioned of it until 1952.

As a means of destroying the large numbers of midge larvae which were present in the lake, it was decided to open the sluice gates during the period March to September, thereby increasing the salinity from that of fresh water to about 20 $^{o}/_{oo}$. This treatment effectively rid the town of the midge problem so the gates were

Fig. 5. Map of Weymouth Harbour backwater and the southern end of Radipole Lake, showing the sites of collection of M. enigmatica on 4th. October 1974. Salinities were measured at the following times: A, 14 51; B, 15 00; C, 15 50. Low water (0.3 m) was at 13 51. Water temperature at the times of sampling was 12°C.

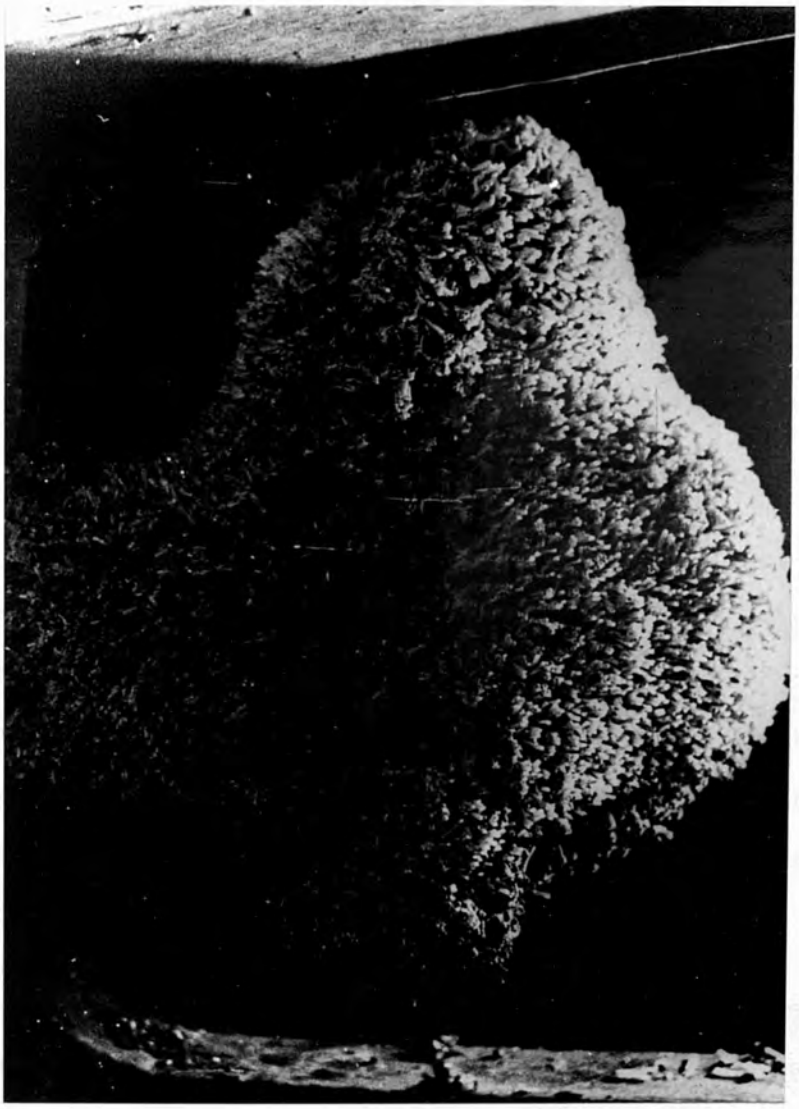

N
Radipole Lake
scale 1/1250
Westham Bridge
A
18‰
Sluice gates
B
17‰
Normal Tidal Limit
C
26‰
D
Bollards
Weymouth Harbour
Backwater
Mean Low Water

closed and the salinity of the water in the lake gradually returned to about 0.1 °/oo. About six weeks after the gates were closed an extensive growth of Mercierella tubes was found to be covering all the solid substrata within the lake. Growth had been extremely rapid resulting in a maximum thickness of about 15 cm in less than 3 months. With the return to freshwater, however, the worms died but their calcareous tubes persisted for a number of years.

M. enigmatica is still present in Weymouth Harbour backwater (personal observation, October 1974). Although it has never again reached the pest proportions described above, it does produce limited but troublesome growths each summer on vessels moored in the region of the sluices (Fig. 6) where the salinity range is the most suitable for its reproduction.

There are over 150 papers relating to M. enigmatica, undoubtedly more than for any other serpulid species. The majority are concerned with the geographical distribution, particularly its apparent rapid spread throughout Europe and N. Africa following its discovery in N.W. France in the autumn of 1922 by Prof. M. Mercier (Fauvel, 1922, 1931, 1933). For references relating to its distribution see Tebble, 1953; Vuillemin, 1965 (for an extensive bibliography); Hartmann-Schröder, 1967; Harris, 1970; Hove, 1974). Fauvel gave it its specific name because of its puzzling appearance and origin; in the light of the recent taxonomic controversy this seems even more applicable now. A number of studies have been concerned with its anatomy (Fauvel, 1922, 1925; McIntosh, 1924, 1926; Rioja, 1924; Swan, 1950; Hall, 1954; Rullier, 1955; Hartmann-Schröder, 1967; Di Grande and Sabelli, 1972), reproductive biology

Fig. 6. A clump of M. enigmatica growing on the phosphor-bronze screw of a wooden boat moored in Weymouth Harbour backwater near a sewer outfall. The growth appeared at the end of summer, 1975, and at the time that the photograph was taken, in the same year, worms were seen attached to the hull.
Photograph reproduced by courtesy of Weymouth Youth Activities Centre.



(e.g. Fischer-Piette, 1937; Rullier, 1955; Straughan, 1970, 1972; Di Grande & Sabelli, 1972), and physiology (e.g. Swan, 1950; Soldatova & Turpaeva, 1960; Turpaeva, 1961; Mathias & Izac, 1963; Skaer, 1974a,b,c,d).

The physiological investigations have been concerned largely with its osmotic relations since it is able to survive in an unusually wide range of salinities (e.g. Seurat, 1927; Heldt, 1944). There has been no previous investigation of the energy relations of M. enigmatica, or for that matter of any other sedentary polychaete. It is the aim of the present study to attempt to fill this gap in our knowledge of this fascinating and successful species.

The only previous, detailed, investigation of the energy relations of a polychaetous annelid is of Neanthes (= Nereis) virens (Sars) by Kay and Brafield (1973), who studied a population in the mud flats at the mouth of the Thames estuary at Southend-on-Sea. Coincidentally, the population of M. enigmatica used in the present study is at Greenhithe in the middle reaches of the same estuary. A single collection was made, however, from Weymouth Harbour backwater in October 1974.

In contrast to the filter-feeding Mercierella, N. virens is a largely carnivorous species. It is of interest, therefore, to compare the energy utilisation of these dissimilar polychaetes which are representatives of the 1^{o} consumer and detritivore levels, and 2^{o} and 3^{o} consumer levels respectively, in the estuarine food chain.

Mercierella is particularly suited to a study of this type because of its small size, and the ease with which it can be maintained in the laboratory. An attempt was made to quantify all known channels of energy flow into and out of its body, including those which in

previous budget studies have been largely ignored, dissolved organic molecules (DOM), mucus production, and shell (tube) production. Of necessity the major part of this work was conducted under controlled laboratory conditions, although where it is considered valid to do so the results are discussed in relation to what is known of the field situation.

Due to the fragmentary and sometimes contradictory nature of the accounts relating to Mercierella's natural environment, a detailed investigation was made of the Mercierella population and field conditions both at Greenhithe and in the estuary in general. The annual cycle of the various environmental parameters was determined, on which the conditions in the laboratory were based. The results of these investigations are presented in Chapter 2.

The subsequent chapters correspond to the primary components of the energy budget : feeding (Chapter 3); egestion (Chapter 5); respiration (Chapter 6); nitrogenous excretion (Chapter 7); and production (Chapter 8). The results of a preliminary investigation showed that, in common with the other soft-bodied, marine invertebrates that have been investigated, Mercierella enigmatica is able to absorb appreciable quantities of dissolved organic molecules (DOM) from the surrounding water by means of an integumentary transport mechanism, without the participation of the gut. Since dissolved organic uptake has been treated extensively in the literature as at least a potential source of nutrients, the results of the detailed investigation of the uptake kinetics, the influence of environmental parameters, and the fate of dissolved organic compounds, are presented after the chapter on ingestion, in Chapter 4.

Formerly, it had been intended that were DOM found to be a supplementary source of nutrients, this discovery could be tested on the basis of the results of what is the most comprehensive study of the energy relations of any marine invertebrate, which has been carried out to date. It will be demonstrated, however, that DOM are apparently of little value to the animal as a form of nourishment and an alternative hypothesis is presented for their role in the energy budget.

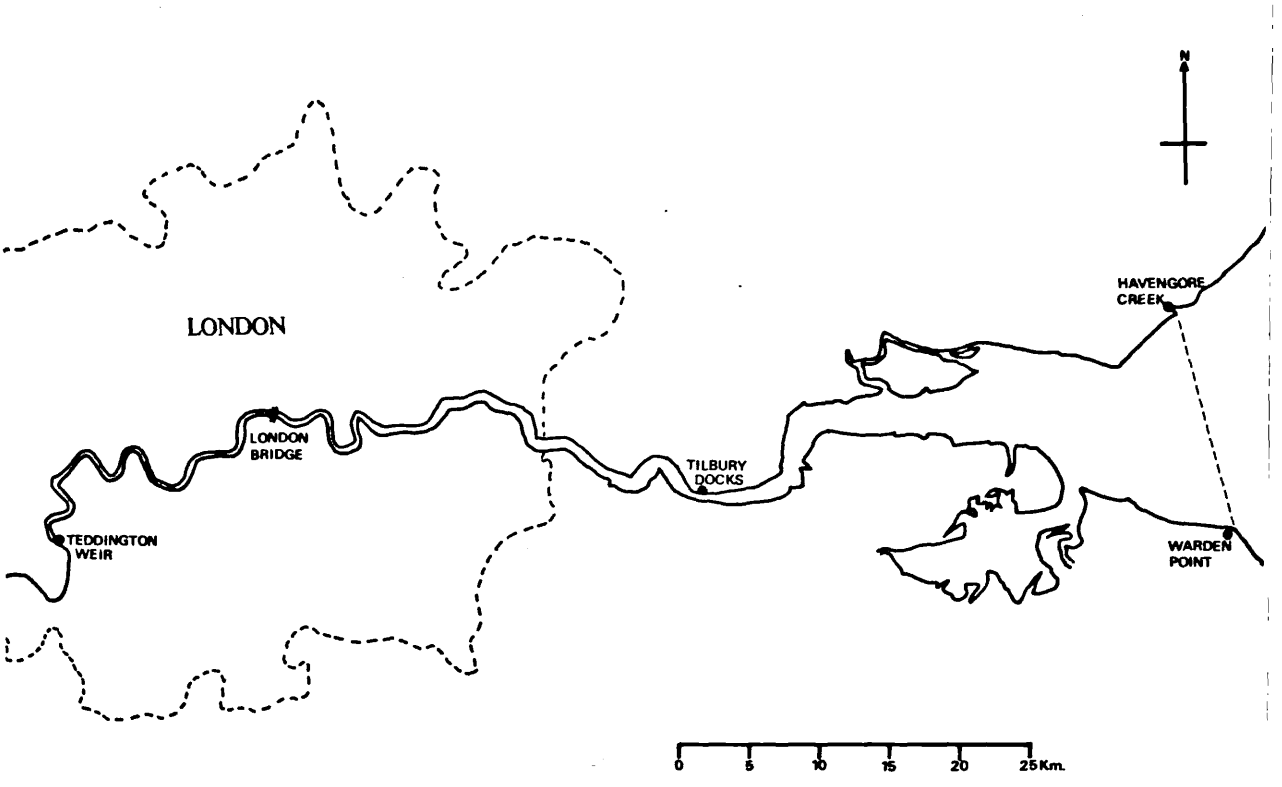
Whilst the energy theme connects each chapter, investigations have also been carried out on various aspects of Mercierella's general biology and the results of these are presented in the relevant chapters. These studies were made both out of general interest, and to provide the essential background information for the energy budget.

Each chapter or section is complete in so far as it opens with a specific introduction, leading on via a description of materials and methods, to a results section and a discussion of the specific topic in question. Each chapter ends with the derivation of the energy values for the respective component of the winter and summer energy budgets for a standard Mercierella. These values are brought together in the final discussion (Chapter 9) where they are discussed in relation to M. enigmatica's general biology, and the energy relations of selected aquatic invertebrates representing the different trophic levels.

S.I. units have been used with c.g.s. units or calorific equivalents in brackets, thereby aiding comparison with published works.

Fig. 7. Map of the Thames estuary showing the upper limit of tidal influence, Teddington Weir; normal landward limit of salt water, London Bridge; and the statutory seaward limit of the estuary.

Based upon the Ordnance Survey Map No. 17. Crown Copyright reserved.


N
LONDON
HAVENGORE CREEK
LONDON BRIDGE
TILBURY DOCKS
TEDDINGTON WEIR
WARDEN POINT
0
5
10
15
20
25 Km.

2. ECOLOGY

2.1 General description of the Thames estuary

The Thames estuary (Fig. 7) is situated in south-east England (51°29'N.,0°40'E.), and opens into the North Sea, 80 km below London Bridge. The statutory seaward limit of the estuary is represented by a line joining Havengore Creek on the north side and Warden Point on the south side. However, the influence of fresh water extends beyond this limit. The tidal Thames extends approximately 109 km upriver to Teddington Weir, but the normal landward limit of salt water is London Bridge, which serves as a useful reference point.

The Thames estuary is shaped like a funnel with the more extensive shores nearer the mouth (Huddart, 1971). The tides are semidiurnal, and the tidal range at London Bridge is almost twice that at Southend (P.L.A. Handbook of Tide Tables, 1976). There is approximately a 25% difference in vertical range between the spring and neap tides. The spring tide range at Tilbury (40 km below London Bridge) is about 6.1 m and the neap tide range is about 3.8 m. However, tidal levels, especially at low water, vary considerably with the fresh water discharge volumes. Another modifying factor on tidal level is the effect of wind; strong winds blowing from offshore into the estuary have a pronounced effect of driving sea water into the mouth and markedly raising the water level. Similarly, strong winds blowing from offshore will retard its emptying.

The single most important source of fresh water in the estuary is the River Thames. The quantity of fresh water entering the estuary

from the Thames is regulated at Teddington Weir. The estuary also receives water from a number of lesser rivers. These are the rivers Crane (+ 24.5 km above London Bridge), Brent (+ 21.5), Lea (- 11 km below London Bridge), Roding (- 18.5), Beam (- 23), Ingrebourne (- 24) and Mardyke (- 29), on the north shore and Beverley Brook (+ 13), Wandle (+ 10.5), Ravensbourne (- 7), Cray and Darent (- 29) and Medway (- 70.5), on the south shore. The flow of water from the River Thames is normally continuous, although the flow rate is variable as a result of natural fluctuations in the amount of precipitation falling in the catchment area. During the summer drought of 1976 the flow over Teddington Weir was stopped in an attempt to conserve water.

A considerable, but discontinuous, source of fresh water is the sudden run-off from the paved metropolis after a storm (Renn, 1956), which enters the estuary via the storm-drains. Apart from the major sources of fresh water mentioned above, there are numerous minor points throughout the whole length of the estuary where fresh water enters either continuously or discontinuously. All the muddy shores for example, have run-off rills formed by rain water. These minor sources may not be sufficient to significantly alter the salinity in the estuary as a whole, but they are sufficient to have an effect on the local conditions.

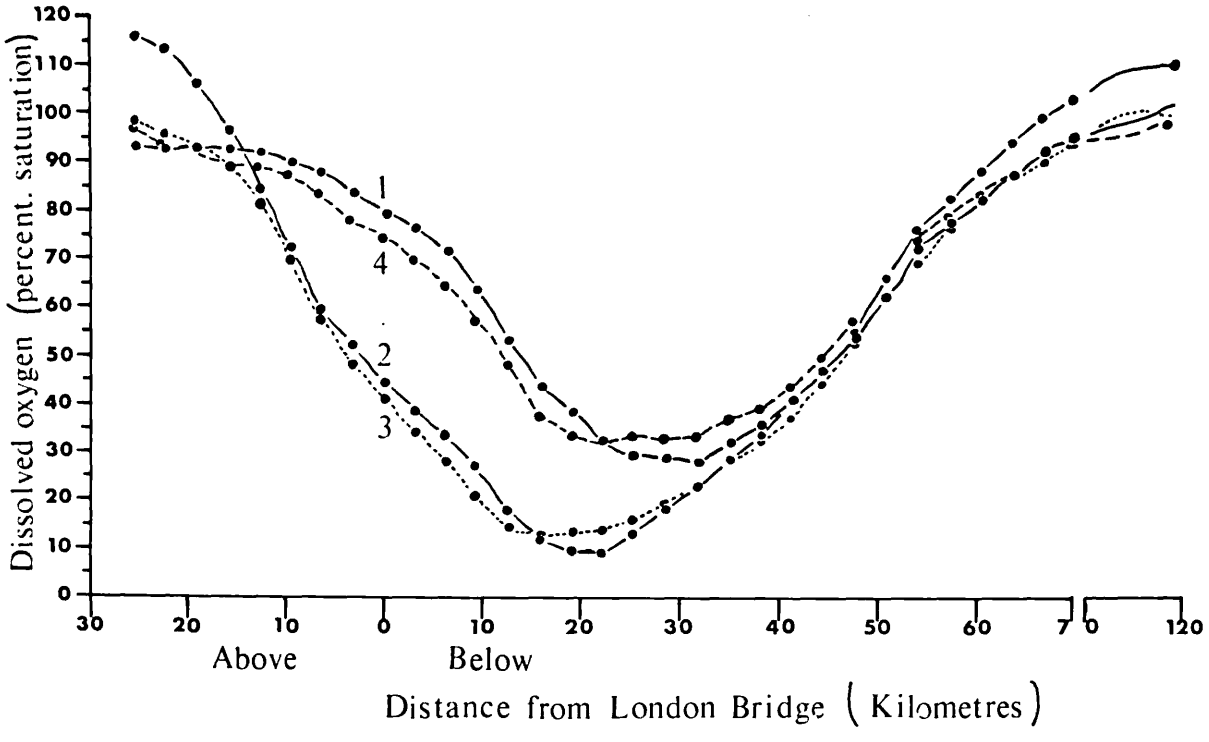
The volume of river discharge is important in determining the amount of dilution of the sea water at different stages of the tidal cycle. As the sea water moves up the estuary it becomes progressively diluted by the fresh water. As a result of the action of the strong tidal currents in the Thames estuary almost complete mixing of the

fresh water and sea water takes place, resulting in a condition approximating a vertically-homogeneous estuary (Barnes, 1974).

The shores within the estuary are very, if not extremely, sheltered and those at the mouth are exposed to waves with a maximum fetch of only 150 km. As a result, deposit shores predominate. However, a variety of hard substrata are present at most points in the estuary. At the upper limit of sea water influence, approximately at London Bridge, the intertidal zone consists of gravel and small boulders, for the most part overlain by shallow mud. A range of artificial hard substrata are also present, such as concrete and stone embankments, bridge supports, concrete and wooden jetties, pilings, buoys and a variety of metallic, plastic and wooden debris. In the middle reaches, around Greenhithe, there is a natural chalk exposure with associated flints. Below this region are the mud reaches of the estuary. By Allhallows the marine influence is very much in evidence with expanses of bound shingle, which contains large numbers of marine bivalve shells. Apart from the fixed, artificial, hard substrata there is an enormous number and variety of the mobile kind ranging from small pieces of wood to large cargo vessels.

The Thames estuary has on its banks the metropolis of London; an industrial and commercial centre of immense proportions. Into 32 km of the tidal river is discharged the waste of approximately eleven million people and their industries, and a significant amount is added further down the estuary. Since the middle ages there has been a gradual deterioration in the condition of the estuary with the worst conditions recorded between 1949 and 1959, when approximately 26 km of the estuary below the outfalls of London's sewage treatment works were, "foul, fetid, and lifeless", (Potter, 1971). For comprehensive historical accounts of pollution in the Thames, see H.M.S.O. (1964), and Potter

Fig. 8. Dissolved oxygen sag curves for the four quarters of 1974. Based upon data supplied by the Thames Water Authority.

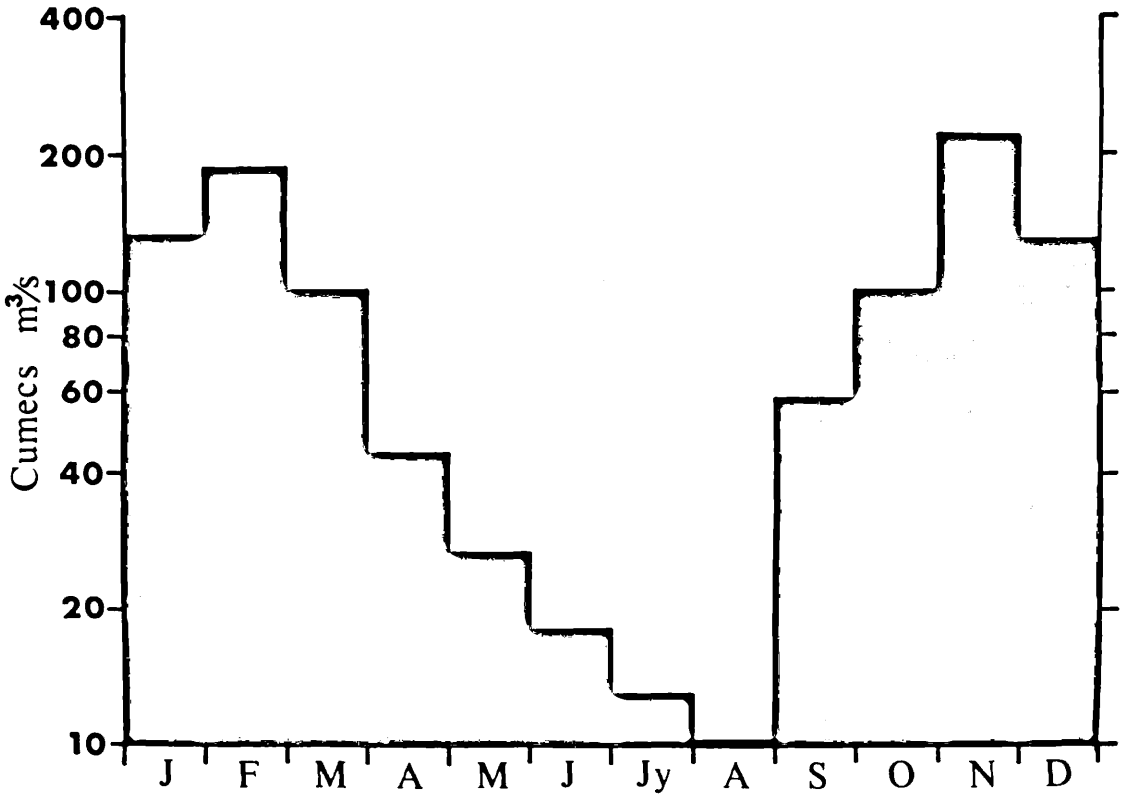

Dissolved oxygen (percent. saturation)
120
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
30
20
10
0
10
20
30
40
50
60
70
120
Above
Below
Distance from London Bridge (Kilometres)

(1971:1973).

By far the greatest polluting load, 79%, arises from the effluents of sewage works (Water Pollution Research Laboratory, quoted in Potter (1971)). A consequence of organic pollution is the depletion of dissolved oxygen resulting from the activities of bacteria which break down the organic matter. Polluting discharges after their release into the estuary are not carried immediately out into the North Sea, instead they oscillate backwards and forwards with the tides. There is, however, an overall downriver movement which can be described in the following gross simplification of the existing regimen; which is accepted by the water authorities concerned as a useful 'rule-of-thumb'. A particle of matter liberated into the tidal water at London Bridge, at high water, will travel 16 km downriver on the ebb-tide and will return 15 km on the flood, and oscillate in this manner for between 6 and 12 weeks before reaching the North Sea. The extended presence of such particles in the estuarine system with the associated aerobic breakdown results in a rapid removal of dissolved oxygen from the system. Unless this oxygen is just as rapidly replaced there will be an overall reduction in the amounts of dissolved oxygen as compared to the amounts present in the unpolluted headwaters and in the sea water. Such a situation exists in the Thames estuary.

The dissolved oxygen content of the water in the estuary is generally expressed as a percentage of oxygen content of water of a similar salinity and temperature in equilibrium with air at atmospheric pressure (% saturation). Longitudinal configuration of the dissolved oxygen content shows a form which is high at the upper and lower ends of the estuary, and depressed in the middle reaches. Fig. 8 demonstrates

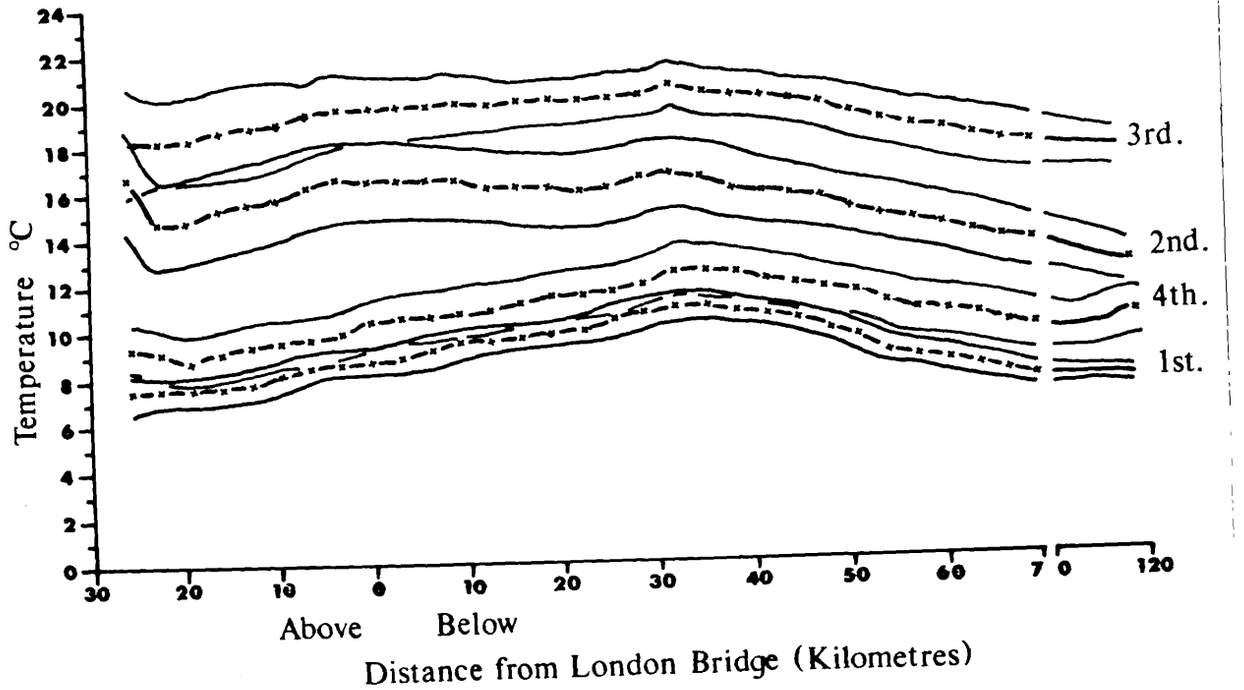
Fig. 9. Monthly mean fresh water flow rates of the River Thames gauged at Teddington Weir, for the year 1974. Based upon data supplied by the Thames Water Authority.


400
200
100
80
60
40
20
10
Cumecs m³/s
J
F
M
A
M
J
Jy
A
S
O
N
D

the average 'dissolved oxygen sag curves' for the four quarters of the year 1974. All the sag curves have the same general form although the position and magnitude of the nadir displays some variation. The curves were calculated from data supplied by the Thames Water Authority. The data were collected by Cremer and Warner (Chemical Engineers), who at that time were responsible for monitoring the water quality of the tidal Thames for the Greater London Council. The measurements were carried out from a sludge boat, or a launch, and the positions at which they were taken were corrected geographically, using a computer, to the equivalent longitudinal position had the sample been taken at half-tide. Hereon this will be described as the 'half-tide correction', permitting direct comparison to be made between the oxygen levels at different stations on the estuary.

The dissolved oxygen content of the Thames estuary varies both in distance and in time. The main factors determining this are: the biological oxygen demand of the polluting discharges, upland flow (the fresh water flow from the Upper Thames over Teddington Weir), wind effects, temperature, tidal mixing, biological activity, and chemical equilibria in relation to bacterial activity. The monthly mean fresh water flow rates of the River Thames gauged at Teddington Weir (approximately + 30 km), for the year 1974, are shown diagramatically in Fig. 9. Generally the flow of fresh water over Teddington Weir is small in comparison with the volume of water in the estuary (Potter, 1973), although periods of high upland flow such as occurred in the first and last quarters, have the effect of moving the centre of the dissolved oxygen sag curve further down the estuary. Conversely, during periods of low upland flow (second and third quarters) the centre of the sag curve moves further up the estuary. The Thames estuary is a dynamic system with a mean tidal excursion of approximately

Fig. 10. Half-tide corrected mean water temperature profiles for the four quarters of 1974. The 95% confidence limits about each profile are represented by the solid lines. These data were supplied by the Thames Water Authority.

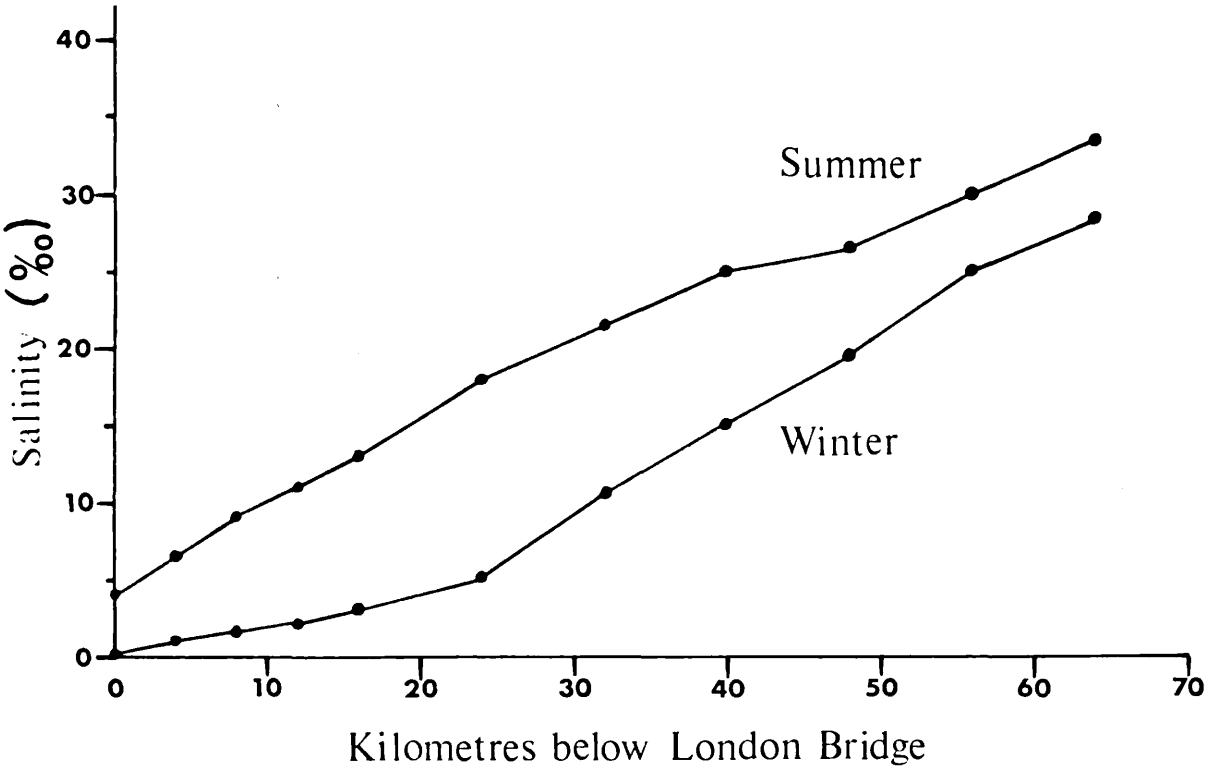

Temperature °C
24
22
20
18
16
14
12
10
8
6
4
2
0
3rd.
2nd.
4th.
1st.
30
20
10
0
10
20
30
40
50
60
70
120
Above
Below
Distance from London Bridge (Kilometres)

14 km. Thus, the positions of the nadirs shown in Fig. 8 apply only to the half-tide situation. As a result of the tidal movement, benthic organisms in the estuary are exposed to diurnal and seasonal changes in dissolved oxygen levels.

Half-tide corrected mean temperature profiles for the four quarters of the year 1974, are presented in Fig. 10. The 95% confidence limits about each profile are represented by the solid lines. Water temperatures in the Thames estuary are governed by climate, resulting in high mean water temperatures in the summer quarters and low mean water temperatures in the winter quarters. An important, secondary, factor is the influence of the heated discharges from the 13 electricity generating stations located on the tidal Thames, from Fulham to Tilbury. All of these stations are cooled directly by river water, removing enormous volumesof water from the estuary for this purpose, e.g. West Thurrock (- 33.5 km) has a total pumping rate of over 136 million litres per hour, which is returned to the estuary several degrees above the ambient water temperature. The general effect of the heated discharges is to raise the temperature of the middle reaches of the estuary above that existing upstream and downstream. In 1974 the half-tide mean temperature differential between the middle reaches (c. - 35 km) and Sea Reach was as much as 3°C.

Raising the water temperature reduces the quantity of dissolved oxygen in the estuary directly by altering the amount of oxygen which can be dissolved in the water and its rate of absorption, and indirectly through its influence on the bacterial and general biological activity. In an attempt to reduce the effects of its heated effluents on the estuarine system, the Central Electricity Generating Board has adopted a policy of oxygenating the cooling

Fig. 11. Variation in salinity in the Thames estuary below London Bridge during the summer (low fresh water flows) and winter (high fresh water flows) periods for a typical year. The points are mean half-tide corrected values. Data supplied by the Thames Water Authority.


40
30
20
10
0
Salinity (‰)
Summer
Winter
0
10
20
30
40
50
60
70
Kilometres below London Bridge

water on its return to the estuary, to endeavour to compensate for its raised temperature. Due to the mean tidal excursion of c. 14 km, benthic organisms in the estuary are also exposed to diurnal and seasonal changes in water temperature.

By definition an estuary (Latin: aestus, tide) is the tidal mouth of a river. Fluctuations in salinity are probably the most characteristic feature of an estuarine environment. The Thames estuary is well mixed vertically (Huddart, 1971; Barnes, 1974) so the salinity does not at any point vary significantly from surface to the bottom. However, there is a horizontal salinity gradient stretching from the mouth to the head. The average summer and winter salinity values for a typical year are presented in Fig. 11. These data were calculated from measurements made by the Thames Water Authority of the specific gravity, assuming the specific gravity of sea water is 1.028 at 40°F. Except when conditions are very unstable, the variation in salinity with distance at half-tide is almost linear in the salinity range 5 to 20 $^{o}/_{oo}$, the salinity changing by c. 0.53 $^{o}/_{oo}$ km^{-1}.

Table 1. The locations of some of the specimens referred to in the text .

Locality	Location		
London Docks	B.M.N.H.	1923.9.30.	1/40
	"	1923.9.30.	41/45
Crossness, S.E. London	"	1948.5.3.	14
Woolwich - Gravesend	"	1970.1.	(dry)

2.2 The recorded distribution of M. enigmatica in the Thames estuary

Mercierella enigmatica was first described in the British Isles from a barge in the Royal Albert Dock (c. -14 km), in the summer of 1923 (Monro, 1924; McIntosh, 1926). Monro (loc. cit.) described how on at least two occasions during the period 1922 - 1923, similar serpulids were sent for identification to the British Museum (Natural History), which had been collected from the sides of ships in the London Docks. The absence of further records for this station indicates that despite favourable conditions for settlement and growth, the worm subsequently failed to become established in the London Docks.

Approximately 20 years later, in September 1942, white calcareous tubes were observed in a cooling water pond at the Southern Outfall Sewage Works at Crossness, in S.E. London (-21.5 km). The pond filled with water from the estuary at high tide. Worms were still thriving in September, 1943, when specimens were sent to the British Museum (Natural History) for identification. Salinity and temperature measurements made at the time the worms were collected were equivalent to 55% sea water (c. 19 °/oo), and 22°C, respectively. No worms were seen when Crossness was visited in October, 1976.

In August, 1966 when the dredger, 'India' docked at Gravesend (-41.5 km), fairly large quantities of tubes were found attached to the hull below the water-line. Since the previous overhaul, in June 1965, the 'India' had plied between Woolwich (c. -16 km) and Gravesend, so the exact time and place of settlement cannot be determined more accurately within these limits.

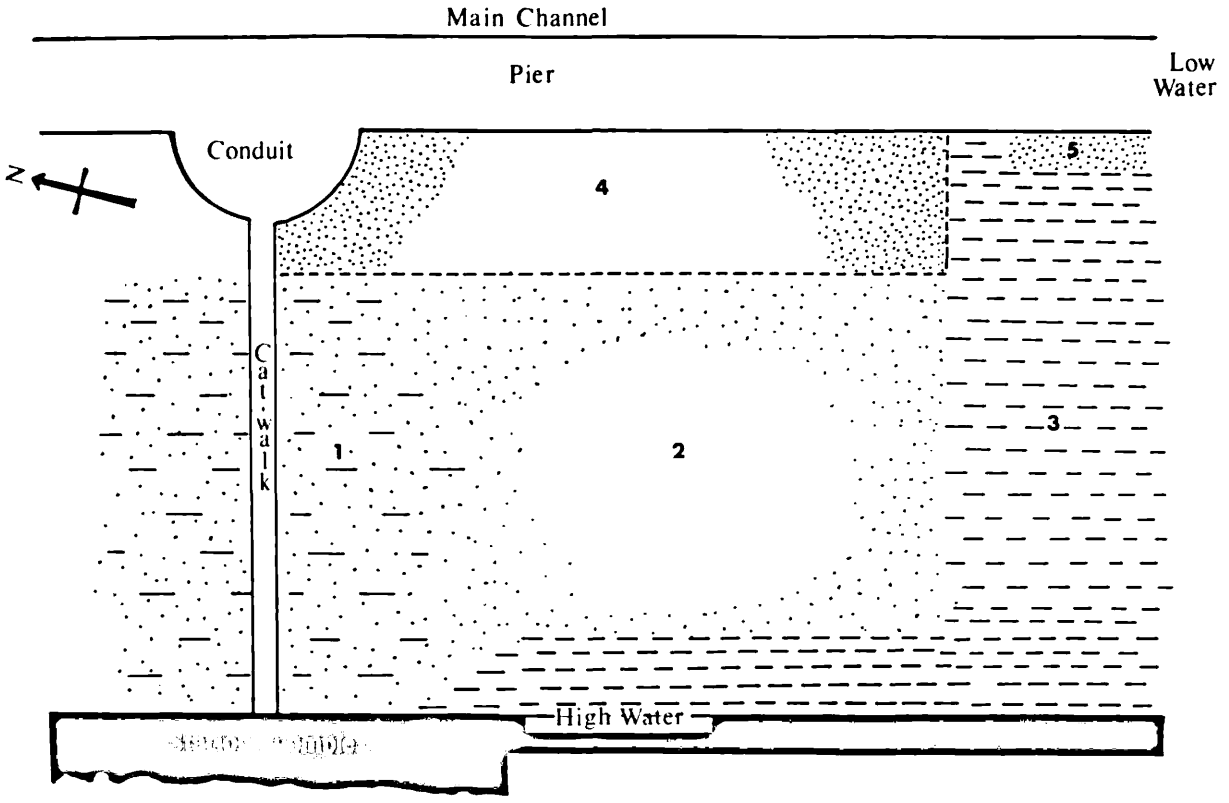
Fig. 12. West Thurrock Power Station (Installed capacity 1 300 Mega Watts) seen from across the Thames estuary. This station is on the northern shore, about 35 km below London Bridge, and has a significant influence on the biota in Fiddler's Reach.

Subsequent records have been divided into four categories : boulder shores, the pilings of jetties and piers, power station cooling-water systems, and the hulls of vessels. In 1971, Mr. J. Hunter of King's College, London, reported seeing tubeworms growing on the pilings of a wooden jetty owned by Empire Paper Mills, at Greenhithe, Kent, on the south side of the estuary (-33.8 km). I first visited this station in August, 1972 and discovered the worm to be M. enigmatica. Contrary to Mr. Hunter's observation it occurred not on the pilings but on the undersides of chalk boulders and flints, and on the undersurfaces of metallic and other debris which abounds in this region. The worm occupied a zone which had vertical limits 2 m on each side of the mid-tide line.

On the 27th October, 1972 a visit was made to West Thurrock Power Station, which is across the estuary from Greenhithe (Fig. 12). Twenty complete but empty tubes were found on the underside of a boulder at c. mid-tide level on the foreshore, opposite the cooling water outfall.

Another record of worms living on the undersides of boulders was made at Belvedere Power Station on the south side of the estuary (-24 km), on the 11th March, 1974. The worms were situated at c. the mid-tide level on the foreshore and were immersed in standing-water during the low-tide period (Fig. 13). There were up to 12 animals per 25 cm square boulder. The majority were adults with tube lengths greater than 50 mm, and all showed signs of recent tube deposition. The water temperature at Belvedere, measured at mid-tide, was 10.2°C. Approximately 6% of the population was composed of juveniles, the result of the previous year's settlement. These had tubes about 10 mm long and a few had a dorsal, longitudinal juvenile ridge.

Fig. 13. Map of the foreshore below Belvedere Power Station. Approximately 150 m of shore are shown. Mud cushions on metal grills in the tower over the entrance to the conduit contained numerous tubificids, and the sabellid, Manayunkia aestuarina (Bourne).
Zone 1, scattered boulders in shallow mud, bearing M. enigmatica and scattered clumps of an orange hydroid, Clava sp., with fruiting bodies; Zone 2, mud < 0.3 m deep containing tubificids, and occasional boulders covering Nereis diversicolor Müller and Hydrobia sp.; Zone 3, fixed rocks; Zone 4, raised mud with tubificids; Zone 5, deep mud with tubificids, and concrete pilings bearing filamentous green algae, brown diatoms, and hydroids at the base.


Main Channel
Pier
Low Water
Conduit
N
4
5
Catwalk
1
2
3
High Water

In mid November, 1972, a visit was made to the Blue Circle Cement Company, on the south side of the estuary (c. -34.5 km), which resulted in the discovery of an isolated but dense growth of about one hundred M. enigmatica growing around the opening of a drainage pipe that protruded several centimetres from one of the concrete pilings. A trickle of water was observed flowing over the worms despite the time being 2 hours after low water. The worms were about midway between the mid-tide and high water levels.

In the autumn of 1972 a temporary but dense clump of worms was seen on a corroded bolt protruding from a transverse wooden beam, at the mid-tide level on the Greenhithe shore, at a point where run-off water dripped off the beam (Fig. 14). The only other substantiated record of M. enigmatica growing on wooden pilings at Greenhithe was made in January, 1974, when two adult worms were found on a vertical shoreward face at about the mean low water neap tide level. Mr. P. Board (Biologist for the C.E.G.B.) observed tube worms growing on the lower parts of wooden piles of the jetty belonging to Proctor and Gamble Ltd., adjacent to West Thurrock Power Station (personal communication, 1973). The worms were only exposed during the extreme low water of spring tides.

Isolated tubes were seen by the Engineer on the vertical walls of a 2.4 m^2 inlet conduit and on the condenser case at Tilbury Power Station (- 44 km), when it was closed down for inspection in November, 1972. I observed M. enigmatica of c. 30 mm tube length on one section of No.9 band-screen on the 8th November, 1973. The band-screens remove debris and nekton from the cooling-water before it enters the condensers. There were c. 20 worms m^{-2} of mesh and they were growing in the direction of the water flow and close to the wire. Their distribution

Fig. 14. A temporary growth of M. enigmatica on the rusty excrescence on a corroded bolt protruding from a transverse, wooden beam, which forms part of the jetty at Greenhithe. Run-off water trickled over the clump of worms throughout the intertidal period. The clump was about 15 cm across in the autumn of 1972.

was limited to an area of screen equivalent to that which was submerged at any one moment in time, which suggested that they had settled during a period when the screen was not operational. From the good condition of the tubes, lack of discolouration, and the presence of a juvenile ridge in some cases, it was evident that they had settled that year. These worms survived daily anti-fouling measures in the form of 254 kg of chlorine added to the cooling-water, producing a residual level of 0.5 ppm. Mr. Board has observed tube worms on the walls of the inlet conduits of Belvedere and West Thurrock power stations.

In mid February, 1973, a 3.6 m long glass-fibre dinghy was moored at the M.H.W.N. tide level, alongside the Empire Paper Mill jetty at Greenhithe. An examination of the hull, on the 29th March 1973, showed it to be covered (1600 m^{-2}) below the water line with worm tubes c. 20 mm long, a result of the previous year's settlement. The tubes contained the remains of dead M. enigmatica indicating that they were probably alive when the dinghy was first moored, but had subsequently perished as a result of prolonged exposure to the air.

A similar settlement of worms was recorded on the 24th March, 1973 on a large cargo vessel, M.V. 'Carrier', which was beached for repairs alongside the paper mill jetty. The worms occupied a distinct zone extending from 1.5 m below the water line to the base of the hull, whilst the area between the top of the M. enigmatica zone and the water line was densely covered with the barnacle Balanus improvisus Darwin. Mercierella and Balanus were also spatially separated on the rudder and screw. The barnacle was confined to the rudder and the serpulid to the screw, where it was surviving despite the high water pressures and velocities to which it was exposed when the vessel was under

power. This observation supports Nelson-Smith's statement that once established on a ship's screw serpulids, as a result of their lower profile, are less disturbed by its rotation than are barnacles (Nelson-Smith, 1971). Enquiries disclosed that the 'Carrier' had recently berthed at Gravesend, and was used for transporting cargo within the estuary.

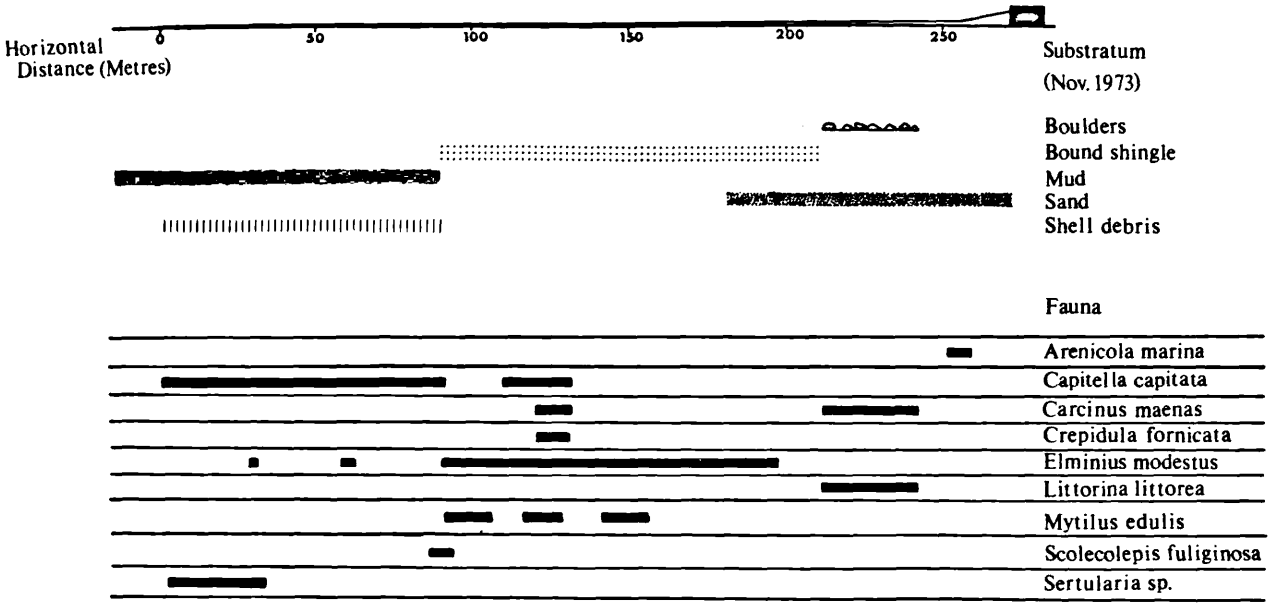
M. enigmatica has been recorded from non-brackish localities on several occasions. For example, Seurat (1927) discovered it in a river in Tunisia where the water was supposedly fresh, and Fischer-Piette (1937) and Harris (1970) found it in sea water of normal salinity (34 - 35 °/oo). It was decided therefore to check M. enigmatica's recorded distribution in the Thames estuary by visiting some shores on the upper and lower limits.

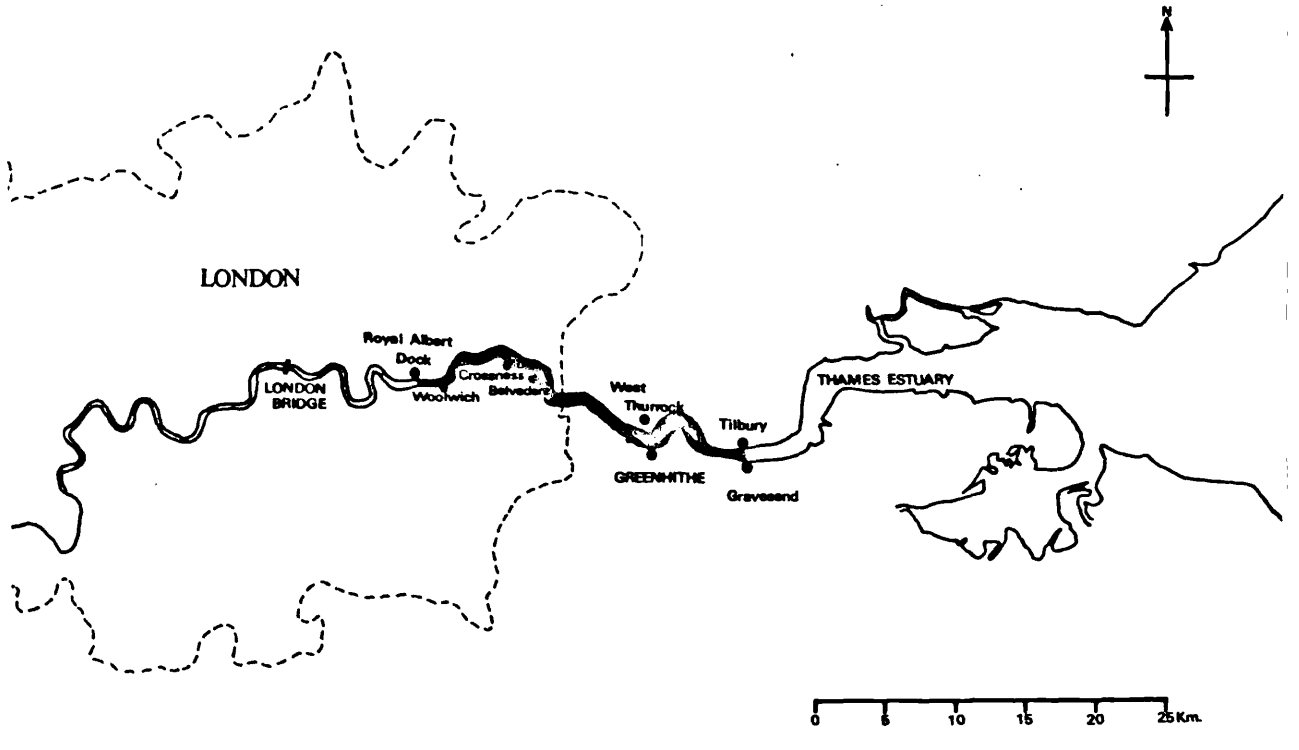
Adult worms are able to survive exposure to fresh water (Tebble, 1953, 1956; Mathias & Izac, 1963), and I have kept animals in running tap-water for as long as 18 days before any mortalities occurred. Straughan (1970) found, however, that tubes disintegrate if exposed for prolonged periods to rapidly flowing fresh water. It was decided therefore, to investigate the shore at London Bridge which is the upper limit of the sea water influence in the Thames. The half-tide salinities range from an average value of 4 °/oo in the summer months to fresh water in the winter (Fig. 11). A visit was made to the south shore at London Bridge in the Autumn of 1974.

Part of this shore was surveyed by Huddart (1971). The hard substrata in this region are gravel and large stones, multifarious debris, stone embankments and bridge supports. Careful searching failed to reveal any signs of Mercierella's presence at this station.

Fig. 15. The horizontal distribution of animals on the shore at Allhallows, at the mouth of the Thames estuary (- 66 km).

Fig. 16. The recorded distribution of M. enigmatica in the Thames estuary (shaded region), showing some of the stations described in the text.


Horizontal Distance (Metres)
0
50
100
150
200
250
Substratum (Nov. 1973)
Boulders
Bound shingle
Mud
Sand
Shell debris
Fauna
Arenicola marina
Capitella capitata
Carcinus maenas
Crepidula fornicata
Elminius modestus
Littorina littorea
Mytilus edulis
Scolecolepis fuliginosa
Sertularia sp.


N
LONDON
Royal Albert Dock
LONDON BRIDGE
Woolwich
Crossness
Belvedere
West Thurrock
Tilbury
GREENHITHE
Gravesend
THAMES ESTUARY
0
5
10
15
20
25Km.

The following animals were recorded from the debris: the freshwater louse, Asellus aquaticus (L.), the wandering snail, Limnaea pereger (L.), and two leech species that were identified by Huddart as Erpobdella (= Herpobdella) octoculata and Helobdella stagnalis, indicating that the benthos was made up of fresh water species.

The two seaward stations were chosen because of their accessibility. Fig. 15 shows the distribution of animals at Allhallows (-66 km) in November, 1973, according to a transect taken down the shore. The presence or absence of each species was recorded at 1 m intervals. The following animals are also known to be present at this station: (=Cardium) Cerastoderma edule L., Gammarus locusta (L.), Macoma balthica (L.), Nephtys hombergii L., and Neanthes (= Nereis) virens (Sars). Despite the presence of a variety of hard substrata, including wooden groynes (not shown in Fig. 15), Mercierella was not found at Allhallows. Similarly, several visits to Whitstable (c. -89 km) between October 1973 and October 1975, failed to reveal evidence of its presence in the outer estuary. Thus the map shown in Fig. 16 is probably an accurate representation of M. enigmatica's distribution in the Thames estuary.

2.2.1 Factors determining M. enigmatica's distribution

Mercierella's appearance in the London Docks in the early 1920's coincided with its discovery in the Caen Canal in N.W. France (Fauvel, 1922). Fauvel made an extensive study of its distribution and concluded that Mercierella had an exotic origin, namely in the Province of Madras, India (13° 2' N., 80° 22' E.), from where he believed it was transported to the Thames estuary on the hulls of ships plying between the colony and London (Fauvel, 1931, 1933). More recently, however, specimens from the coast of Madras, now residing in the British Museum (Natural

History), Location: 1938. 5.7. 92/94, have been redetermined as Neopomatus sp. (Pillai, 1960) by Hartman-Schröder in 1966, and also by Zibrowius in 1972, which suggests that the European specimens may have a more local origin. Irrespective of where M. enigmatica originated from, it is generally accepted that shipping is an important vector, introducing the worm into localities where it was previously absent, for example, the appearance in the Kanaal door Walcheren, S.W. Netherlands, in 1967 was attributed by Wolff (1969) to a particular ship.

Since the adult phase is sedentary, M. enigmatica can only spread within the estuary as a larva. The length of time spent in the plankton between fertilisation of the egg soon after release from the female worm and settlement is not known exactly (see Section 8.2.4), but published values range from one week (Vuillemin, 1965) to at least one month (Mathias & Izac, 1963). What is certain is that the time spent in the plankton will depend on several factors, including food supply and temperature. The larva has a strong positive phototactic response, at least up to four days old and can swim by means of the equatorial ciliated prototroch. However, unless the larva has some behavioural adaptation enabling it to maintain its horizontal position in the estuary, or is carried by water currents into a backwater or eddy, it will ultimately be carried downstream by the overall seaward movement of water, described in Section 2.1, if it remains in the water column. A larva released into the estuary in the region of the London Docks (c. -14 km), assuming a planktonic life of 1 - 4 weeks, will be carried between 14 and 56 km downstream and will settle somewhere between Dartford Creek and Southend-on-Sea.

It is possible that developing larvae could be transported in the ballast tanks of ships, enabling them to settle on their release

at stations further up the estuary than they would naturally reach. This could explain the temporary appearance in the London Docks (loc. cit.). It is tempting to suggest that the downstream populations depend for their recruitment on larvae produced by the populations living further upstream. A more likely explanation, however, is that the downstream populations release larvae which remain in the vicinity of the parents as a result of their becoming trapped in the considerable eddies created by the power stations and the natural water swirls, for example off Greenhithe.

Although larval settlement has been recorded in relatively high water velocities (e.g. Vuillemin, 1965, 1.6 - 2.2 km h^{-1}), much higher water speeds occur in power station conduits when they are in operation. For example, the maximum water velocity at Tilbury Power Station is 6.9 km h^{-1}. Settlement in these units therefore undoubtedly takes place when these are closed down as a result of a decreased demand for power, which incidentally occurs most often during the warmer months, or for regular maintenance and repair. This is demonstrated by the discrete settlement of M. enigmatica on the band-screen at Tilbury Power Station (Section 2.2), which could only have occurred when the screen was not operational.

The published values for the time taken to reach maturity after settlement range from a few weeks (Fischer-Piette, 1937) to 4 months (Vuillemin, 1965). It is almost certain, however, that in the Thames estuary, the new individuals do not spawn until the following summer (see Sections 8.2.3 and 8.2.4). Although 18°C may be essential for natural spawning (Hartmann-Schröder, 1967), larval development and settlement have been observed at lower temperatures. Mathias and Izac (1963) reported that the development rate in the laboratory is

temperature dependent, with the trochophore stage being reached in 24 h at 16°C. Vuillemin (1965) recorded settlement in water temperatures as low as 10°C. Furthermore, I have found sexually mature females in December (Section 8.2.3) when the mid-tide water temperature was approximately 10°C, and a few males containing mature sperms are always present outside of the breeding season. When a few of these individuals were stimulated to spawn by breaking open their tubes, fertilisation took place at room temperature giving rise to trochophore larvae in about 3 days, thus showing that the gametes were viable. It is possible therefore, that fertilisation might be effected in the estuary outside of the recognised breeding season, as a result of the cleaning of ships hulls. During which process the tubes would be smashed and the gametes mixed together.

Where M. enigmatica occurs in the intertidal zone of the Thames estuary it is normally covered by running or standing water during periods of low tide. In non-tidal localities the upper limit of Mercierella's vertical range is commonly only a few centimetres below the water surface (e.g. Fauvel, 1933; Hall, 1954; Hove, 1974). In contrast, in tidal localities the worm is usually restricted to the lower parts of the shore (Purchon, 1948; Harris, 1970). Consequently, Mercierella is not exposed to desiccation or extremes in temperature, and is potentially capable of feeding throughout the tidal cycle.

M. enigmatica also occurs subtidally, as is demonstrated by its presence in the cooling-water systems of power stations. An important limiting factor of the subtidal distribution is the availability of suitable substrata for settlement. In the Thames estuary most of the hard substrata are on the upper part of the shore (Huddart, 1971); the bed of the middle reaches is covered by a layer of semi-liquid mud

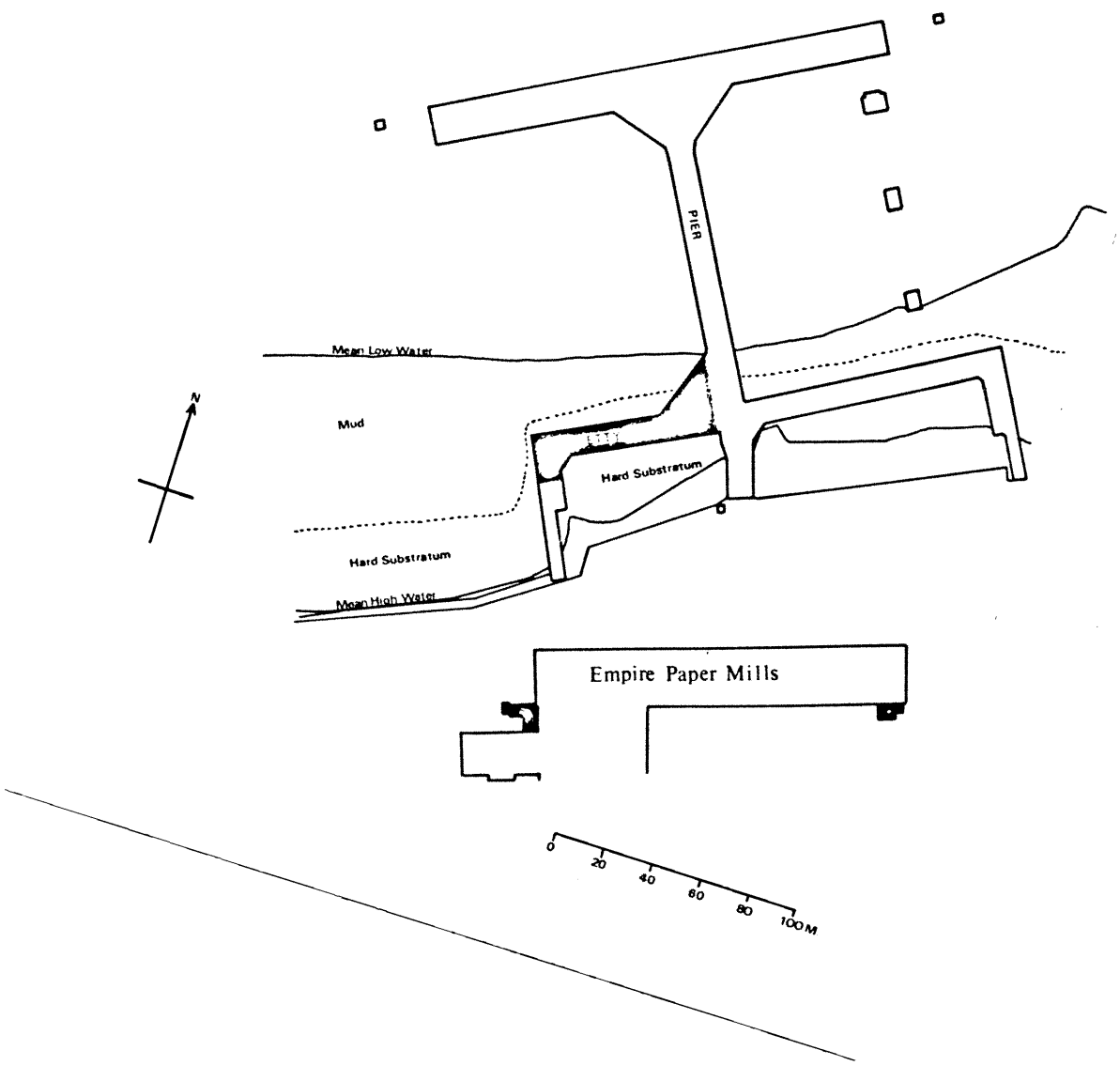
(H.M.S.O., 1964). Consequently, artificial hard substrata, such as the submerged hulls of ships and the cooling-water systems of power stations, must represent important sites for larval settlement.

Straughan (1972) found that the intertidal vertical range of Mercierella (= Neopomatus?) in the Brisbane River estuary, E. Australia, decreased to zero metres above the low water level at the upstream and downstream limits of its horizontal distribution. It is not known whether a similar phenomenon exists in the Thames estuary. The greatest depth from which it has been recorded is 19 m in the Bay of Gelenjick, in the Black Sea (Annenkova, 1929).

Since M. enigmatica was first recorded in the Thames estuary, thirteen power stations have been built between Fulham (+12 km) and Tilbury (-44 km). All these stations use water from the Thames to cool their condensers, and have a combined installed capacity of 6434 MW. Their combined effects on the middle reaches of the estuary is to increase the water temperature by as much as 3°C (Fig. 10). During the third quarter the average mid-tide water temperature in this region ranges from 18.5°C to about 21°C. In view of M. enigmatica's requirement for a water temperature of at least 18°C for its natural reproduction (Hartmann-Schröder, loc. cit.) it seems plausible that the electricity generating stations have a major influence on the worm's distribution within the Thames estuary both from the point of view of providing suitable sites for settlement and by creating suitable conditions for its reproduction. Wolff (1969) believed that Mercierella's introduction into the Netherlands had been favoured by the warm water produced by a power station, which raised the water temperature to a level 3 - 5°C higher than comparable natural waters (Hove, 1974).

Fig. 17. Map of the Empire Paper Mill jetty and pier at Greenhithe, showing the study site (shaded region).
Based upon Ordnance Survey Maps TQ 5975 NW and SW.

River Thames
St.Clement's or Fiddler's Reach



2.3 Field study of the Greenhithepopulation

2.3.1 Introduction

A field study of the M. enigmatica population and the physical environment at Greenhithe, Kent (-33.8 km), was carried out between 1973 and 1975, to supply information concerning the worm's ecology to serve as a basis for the energetics study.

The population is restricted intertidally to the shore beneath a wooden jetty which is contiguous with the Empire Paper Mill pier (O.S., TQ59367548) (Fig. 17). The shore at this station faces N, 25° W, and is sheltered from the prevailing East wind by the pier and jetty. Similarly, the pier attenuates the strong water currents that are characteristic of this part of the estuary.

Greenhithe stands on St. Clement's or Fiddler's Reach, and is immediately across the estuary from West Thurrock Power Station (Fig. 12). Fiddler's Reach is renowned for its unusual water currents (H.M.S.O., 1964), and the name is believed to be derived from 'fiddling' a term used by seamen to describe an irregular water swell (Murray's Handbook for the County of Kent, quoted in Linney, 1932). However, some authorities believe that it arose from a tradition of the death by drowning of a trio of fiddlers (Linney, 1932).

Huddart (1971), mentions an extensive semi-permanent anticlockwise waterswirl off Greenhithe, where, "water on the North bank usually moves upstream and water on the South bank usually moves downstream whatever the direction of the main tidal stream". This observation was in agreement with the results of a study made by Inglis and Allen (1957) approximately 3 km upstream from Greenhithe, which showed a net landward

Fig. 18. Paper mill effluent being released into the estuary immediately offshore from the study site at Greenhithe. At the time the photograph was taken the tide was on the ebb. The seaward limit of Long Reach can be seen in the distance.

transport of suspended solids on the North side and the reverse effect on the South side of the estuary. Furthermore, Mr. M. Andrews (T.W.A.[1] Biologist) found that the benthic organisms are not evenly distributed in this part of the estuary, which he believes may be attributable to the anticlockwise waterswirl (personal communication, 1976). In contrast, my own observations of the direction taken by the Paper Mill effluent (Fig. 18), which is discharged continuously from a pipe several metres out from the Mean Low Water mark on the upstream side of the pier, have always been in agreement with the expected normal direction of water flow at different stages of the tide, which suggests that the pier has a corrective influence on the direction of water flow in the vicinity of the M. enigmatica population.

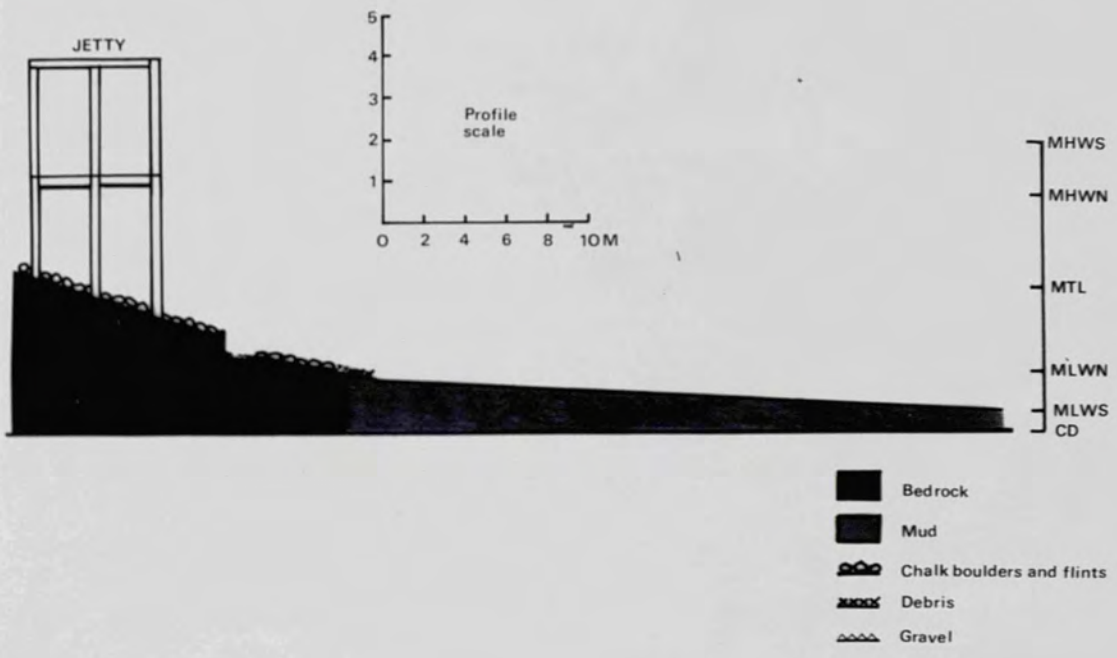
2.3.2 Materials and Methods

There is little variation in profile along the shore, so a single transect was surveyed approximately mid-way along its length and extending from the mean low water of spring tides (M.L.W.S.) to the landward side of the wooden jetty. A 'T-shaped' levelling device, incorporating a spirit level in the cross-piece, was used to measure vertical heights at intervals along the transect line, referring to the local Chart Datum as the zero level. The height of the local Chart Datum, c. 3.19 m below Ordnance Datum (Newlyn), was calculated from the levels given in the P.L.A.[2] Tide Tables for Tilbury and North Woolwich. M.L.W.S. at Greenhithe is 0.5 m above the local Chart Datum (P.L.A. Tide Tables, 1976), and M.L.W.N., M.H.W.N., and M.H.W.S. are respectively 1.4, 5.6, and 6.9 m above the local C.D. (calculated from Tilbury and North Woolwich values). The estimated position of M.T.L. was marked on the transect, and good agreement was obtained between the calculated time at which the tide would reach the

1. Thames Water Authority 2. Port of London Authority

Fig. 19. Profile of the Greenhithe shore based on a transect surveyed mid-way along the study site. Note that different scales are used in the horizontal and vertical directions.

Fig. 20. Part of the study site taken from the position of the profile transect looking upstream towards the village of Greenhithe, showing the middle zone (see text) with its multifarious substrata, and the downshore side of the jetty.


JETTY
5
4
3
2
1
Profile scale
0 2 4 6 8 10M
MHWS
MHWN
MTL
MLWN
MLWS
CD
Bedrock
Mud
Chalk boulders and flints
Debris
Gravel

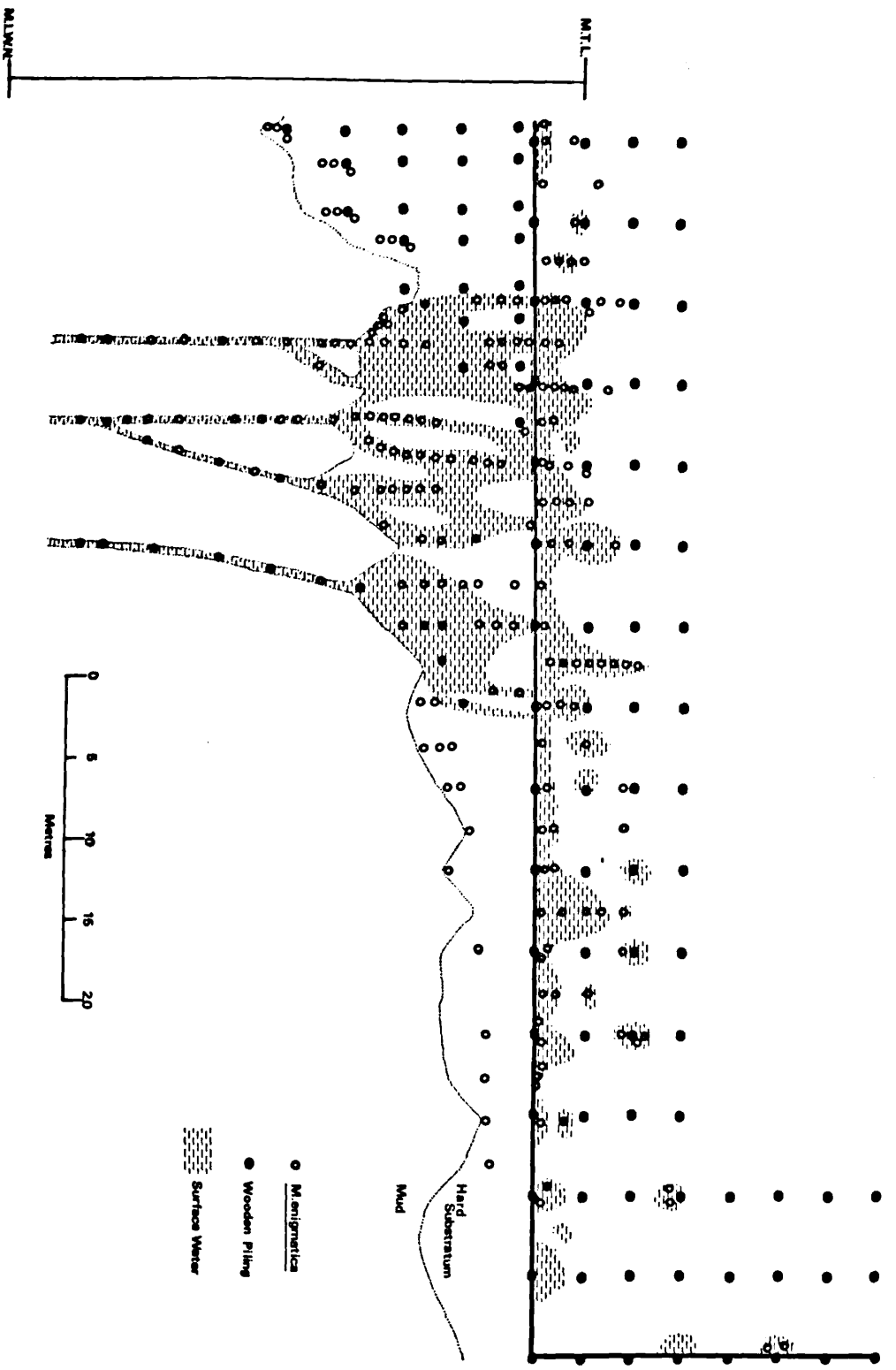
mark and the observed time on numerous occasions, allowing for variations in water level associated with changes in barometric pressure *etc*.

An investigation of the distribution and abundance of *M. enigmatica* on the upstream side of the Empire Paper Mill pier was carried out using 32 parallel transect lines placed at intervals of *c.* 2.5 m across the shore, stretching from the landward side of the jetty down to the hard substratum/mud interface or the low water mark, whichever was applicable. A 625 cm^2 quadrat was placed at intervals of *c.* 1 m on each of the transect lines, and a record was made of the type of substratum, the presence of surface water, and the number of worms within the area encompassed by the sides. Tubes containing living worms can be distinguished from empty tubes by the presence of a white collar of recently secreted calcareous material at the anterior end. Furthermore, empty tubes soon become blocked with mud and detritus and the anterior portion becomes discoloured as a result of algal growth; empty tubes soon become 'chalky' in flowing water. Nevertheless as a check to avoid error, all the worms in a sample were removed from their tubes and good correspondence was found between the estimated number and the actual number of worms present.

2.3.3 Results

The Greenhithe shore profile presented in Fig. 19 accurately depicts the topography of the beach at the time that the survey was carried out in 1973. Vertically, the shore is divided into three distinct zones. An upper relatively steep zone comprised of chalk boulders and flints, the downshore movement of which is prevented by a transverse wooden beam that traverses the riverward side of the jetty. The middle zone consists of successive belts of gravel, mixed chalk

Fig. 21. The intertidal distribution of M. enigmatica in the region of the disused jetty at Greenhithe, also showing the distribution of surface water on the shore.


M.L.W.N.
M.T.L.
0
5
10
15
20
Metres
Surface Water
Wooden Piling
M.enigmatica
Mud
Hard Substratum

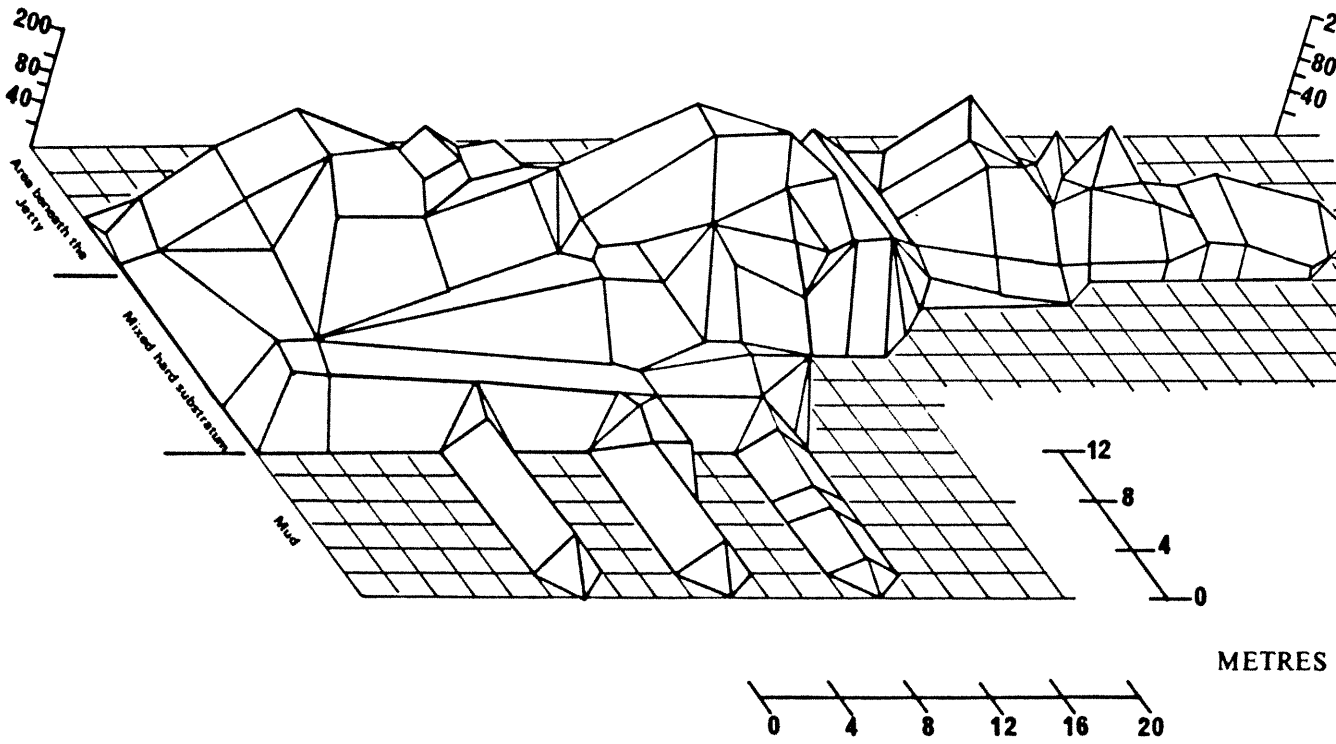
boulders and flints, and metallic and other types of debris (Fig. 20). At its riverward edge the debris overlies soft mud that represents the lowest zone, and extends to the low water mark and beyond.

Fig. 21 shows the distribution of M. enigmatica on the shore at Greenhithe. The worm occupies a distinct zone with vertical limits 2 m each side of the mid tide level. The majority (81%) of the records were from parts of the shore that are covered by pools of standing water and surface run-off, throughout the intertidal period. The remainder are in places that as a result of position or topography, stay damp throughout the period of emersion.

M. enigmatica is confined to the underhanging and sheltered upper surfaces of chalk boulders, flints, and debris (metallic, glass, and clinker). No worms were recorded from the wooden piles, mud or gravel. Only 14% of the records were from chalk boulders despite these being the most common type of hard substratum on the shore. In contrast, the remaining 86% were from the various types of debris and flints. This is undoubtedly due to the soft nature of the chalk, the surface of which becomes water-logged and therefore of no value for tube attachment. Furthermore, the rounded profile offers little in the way of suitable underhangs and cavities for colonisation.

The numerical distribution of M. enigmatica at Greenhithe is presented diagramatically in Fig. 22, in which density is indicated as numbers in four square metres of beach surface. The greatest number of worms recorded at any one station was approximately 75 m^{-2}, growing on a 0.2 m length of corroded iron bar. The usual number however, was less than 5 m^{-2}. The highest density occurred in the pools of comparatively still water at the downshore limit of the upper zone.

Fig. 22. Diagrammatic representation of the numerical distribution of M. enigmatica in the region of the disused jetty at Greenhithe.


200
80
40
Area beneath the jetty
Mixed hard substratum
Mud
200
80
40
Number of individuals per 4 square metres.
12
8
4
0
METRES
0
4
8
12
16
20

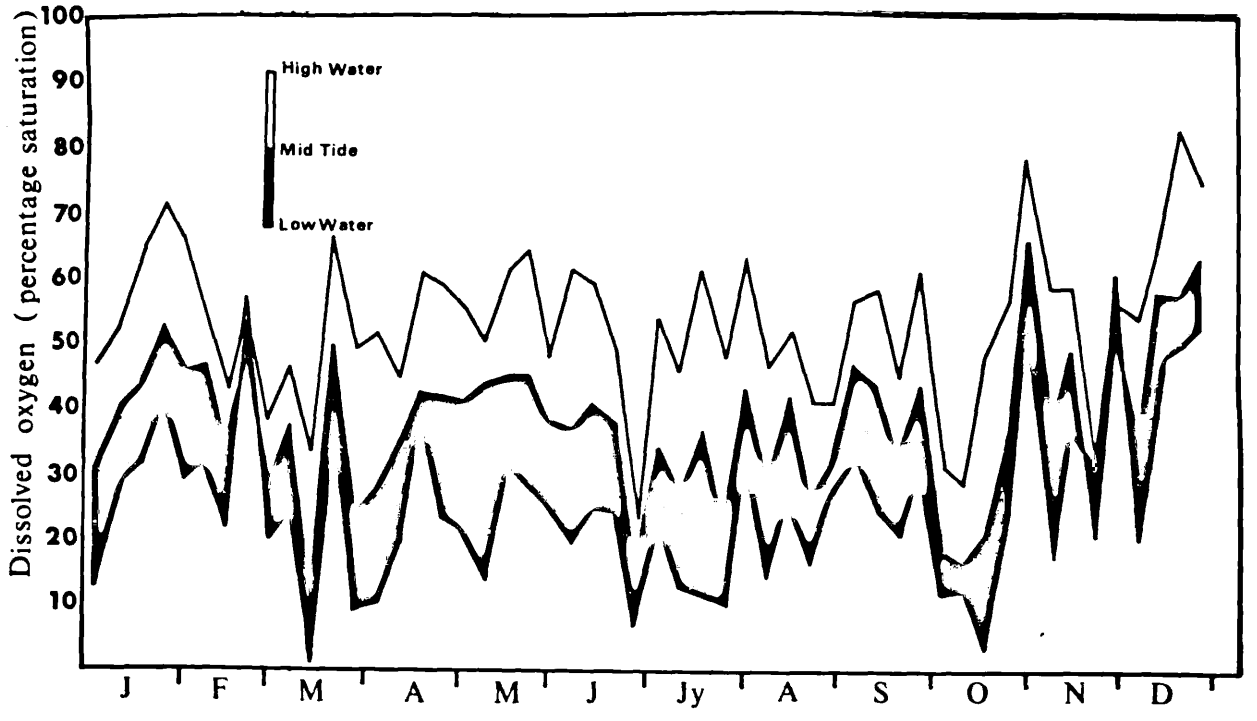
Although the stations at which Mercierella was found on the shore have certain environmental conditions in common, it was not always present where these prevailed. In addition, when present it was never evenly distributed. This is due to a larval settlement requirement for the microbial surface-film on the substratum to be at a particular stage in its development, and to a gregarious response during settlement (Straughan, 1972). Consequently it would be incorrect to apply a factor, based on the 'suitable' surrounding area, to the recorded number of worms to derive an estimate of the total number of M. enigmatica present on this part of the shore at Greenhithe. It was decided to assume, therefore, that the total number of worms recorded represents a conservative estimate of the total number of animals present. Similarly, although the total area of the shore is over 3.3×10^3 m^2, most of it is not suitable for colonisation. It was decided therefore, to treat the area enclosed within the total number of 1/16th m^2 quadrats placed on the beach during the survey, as the 'effective area' of the shore as far as M. enigmatica is concerned. Therefore, since a total of 592 quadrats were placed on the shore, the effective area is $\frac{592}{16} = 37$ m^2. Since the number of living worms recorded within this 37 m^2 is c. 8140, the average number of Mercierella on the shore at Greenhithe in the winter of 1973 was 220 m^{-2}.

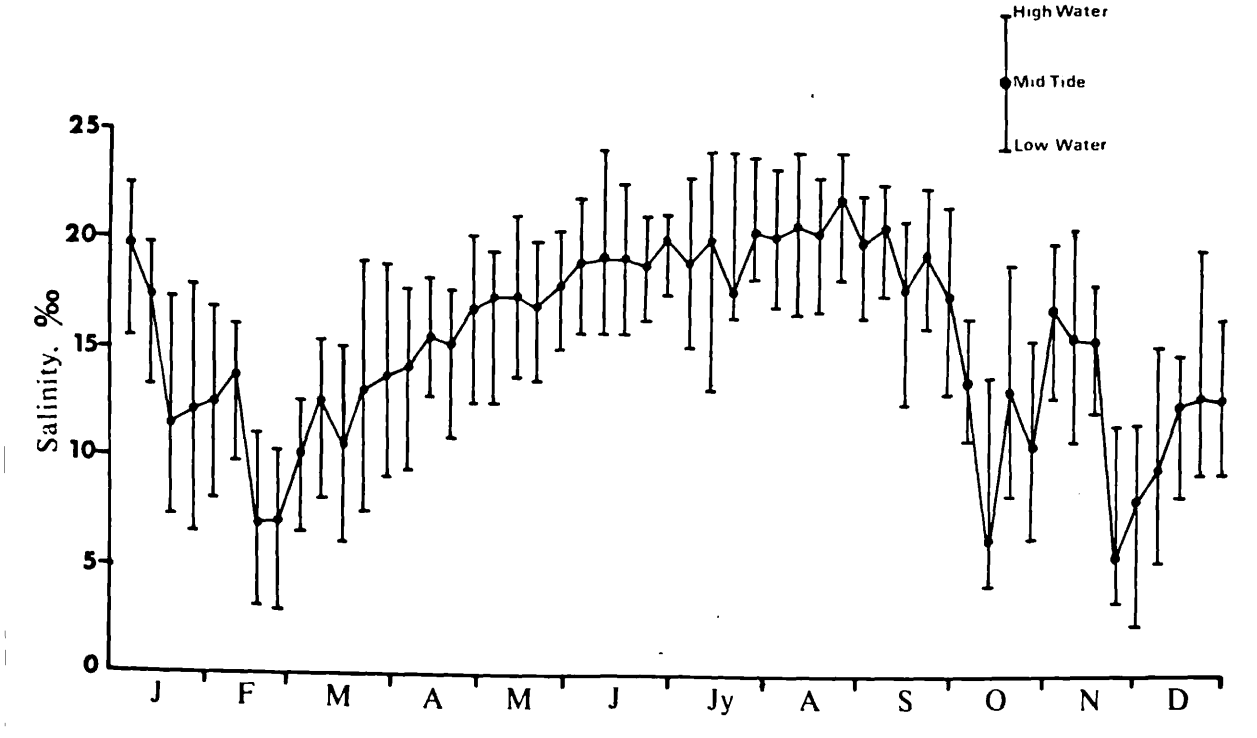
2.3.4 The physical environment

Weekly measurements of dissolved oxygen in the estuary at Greenhithe, at three different stages of the tide: high, mid, and low water, for the year 1974 are presented in Fig. 23. The basic data were supplied by the Thames Water Authority, who have a weekly monitoring programme of the water quality in the tidal Thames. All

Fig. 23. The weekly levels of dissolved oxygen for three stages of the tide at Greenhithe during 1974. Basic data supplied by the Thames Water Authority.

Fig. 24. Weekly salinity values for three stages of the tide at Greenhithe during 1974. Basic data supplied by the Thames Water Authority.


Dissolved oxygen (percentage saturation)
100
90
80
70
60
50
40
30
20
10
High Water
Mid Tide
Low Water
J
F
M
A
M
J
Jy
A
S
O
N
D


Salinity, ‰
25
20
15
10
5
0
High Water
Mid Tide
Low Water
J
F
M
A
M
J
Jy
A
S
O
N
D

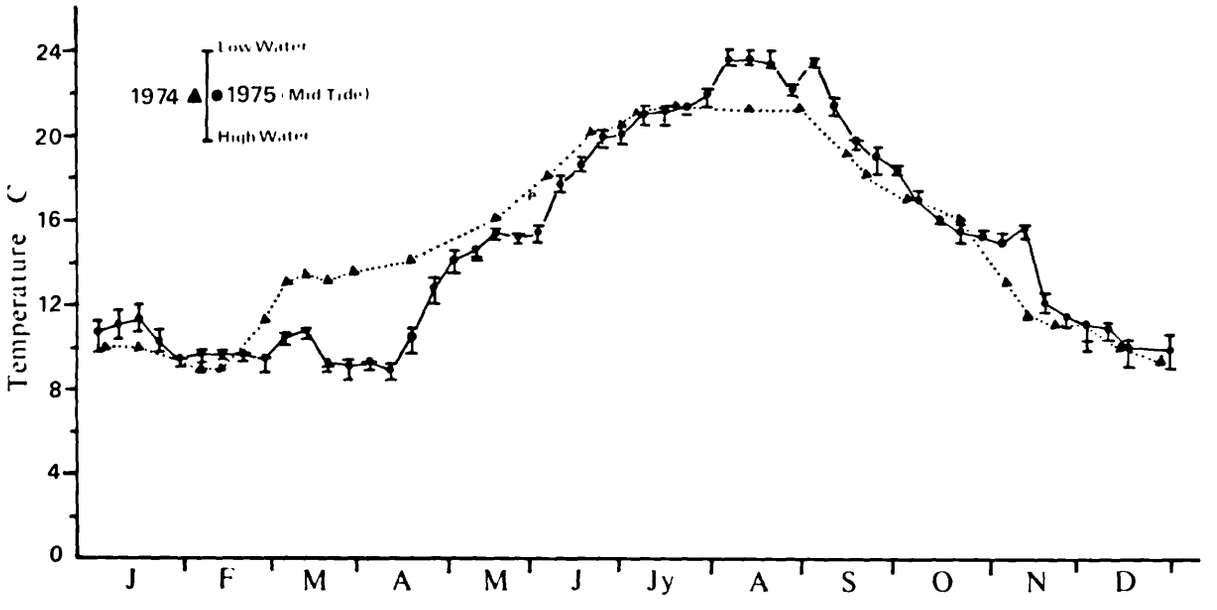
the measurements are corrected by computer to the equivalent longitudinal position had the samples been taken at half-tide (mid tide). Since the mean tidal excursion is c. 14 km, the low and high water levels of dissolved oxygen at Greenhithe correspond to the mid tide values at Erith and Gravesend, c. 7 km on each side of the study population.

There are considerable tidal and seasonal fluctuations in the level of dissolved oxygen, and the factors influencing this are described and discussed in Section 2.1. As a result of its intertidal distribution, M. enigmatica is not exposed to the extremely low dissolved oxygen levels that occur generally during the low tide period.

Weekly salinity values for high, mid, and low water periods for 1974 are shown in Fig. 24. These data were calculated from the T.W.A's half-tide corrected percentage sea water measurements for Erith, Greenhithe, and Gravesend, assuming that average sea water has a salinity of 34 °/oo (Prosser & Brown, 1961). In contrast to the dissolved oxygen regimen the highest salinities were recorded during the summer months. Factors effecting salinity are described and discussed in relation to the total estuary in Section 2.1. The average salinities for the winter and summer periods are 10 °/oo and 20 °/oo respectively, and the maximum recorded tidal fluctuation is 11.4 °/oo. Due to their intertidal position on the Greenhithe shore, however, Mercierella are not exposed to the low salinities that prevail during periods of low water.

Water temperature data for 1974 and 1975 are presented in Fig. 25. The 1974 values were measured at the mid tide level on the Greenhithe shore. The high, mid, and low water temperature values for 1975, were

Fig. 25. Water temperatures for Greenhithe in 1974 and 1975.


Low Water
1974 ▲ ● 1975 (Mid Tide)
High Water
Temperature C
24
20
16
12
8
4
0
J
F
M
A
M
J
Jy
A
S
O
N
D

calculated from the T.W.A's weekly, half-tide corrected, measurements at Erith, Greenhithe, and Gravesend, and are presented for comparison.

There is good agreement between the temperature data for the two successive years. Higher water temperatures occur during the summer months, with a maximum water temperature of 23.8°C recorded in three successive weeks in 1975. It should be noted that during the two winter periods, the water temperature never went below 8.4°C. Although there was a relatively small differential between the high and low water temperatures when compared with the situation for dissolved oxygen and salinity, the relationship was reversed when compared with these two other parameters with the highest water temperatures occurring during the low tide period. This is due to the reduction in the dilution of the heated discharges from the power stations by sea water during the low tide period. Other factors influencing water temperature in the estuary are described and discussed in Section 2.1.

Hydrogen ion concentration in samples of water collected at low, mid, and high tide was measured in mid March 1975, and values obtained ranged from pH 7.2 - 7.5, with the lowest value pertaining to low tide, when the river water has its greatest influence, and the highest value to the high water period, when the sea has its greatest effect. T.W.A. pH measurements for Greenhithe, taken in 1974, ranged from 7.2 - 7.6, and once again the lowest values are associated with low water periods and other times of increased fresh water influence. Huddart (1971) reported that the pH of the water off West Thurrock Power Station is approximately neutral.

The concentration of suspended solids in this part of the estuary is extremely variable and depends both on the weather conditions and on the state of the tide. As water moves up the shore,

it carries with it quantities of fine particulate matter from the lower part of the beach. The water at the advancing edge of the tide is extremely turbid, with zero visibility.

Measurements of the amount of suspended matter showed considerable variation with an average value of 16% by weight (160 x 10^3 ppm). The suspended matter has a very high organic content, with a loss on ignition (400°C) of c. 1% of the dry weight (10 x 10^3 ppm). The off shore surface water is less turbid, however, and Huddart (1971) reported ignition values, for the suspended solids in the water off West Thurrock, of up to 200 ppm. Much higher levels of suspended solids are known to occur nearer to the bottom in the off shore waters. In the Thames estuary values (total suspended solids) greater than 1 x 10^3 ppm are normal for samples of bottom water, and the concentration in winter may exceed 50 x 10^3 ppm because of increased run-off and fresh water flow (H.M.S.O., 1964).

2.3.5 The local environment

M. enigmatica is found largely in areas on the shore that are covered by pools of standing water or run-off throughout the intertidal period (Section 2.3.3). It was of interest therefore to investigate the local environment during the periods when the rest of the shore is exposed to the air.

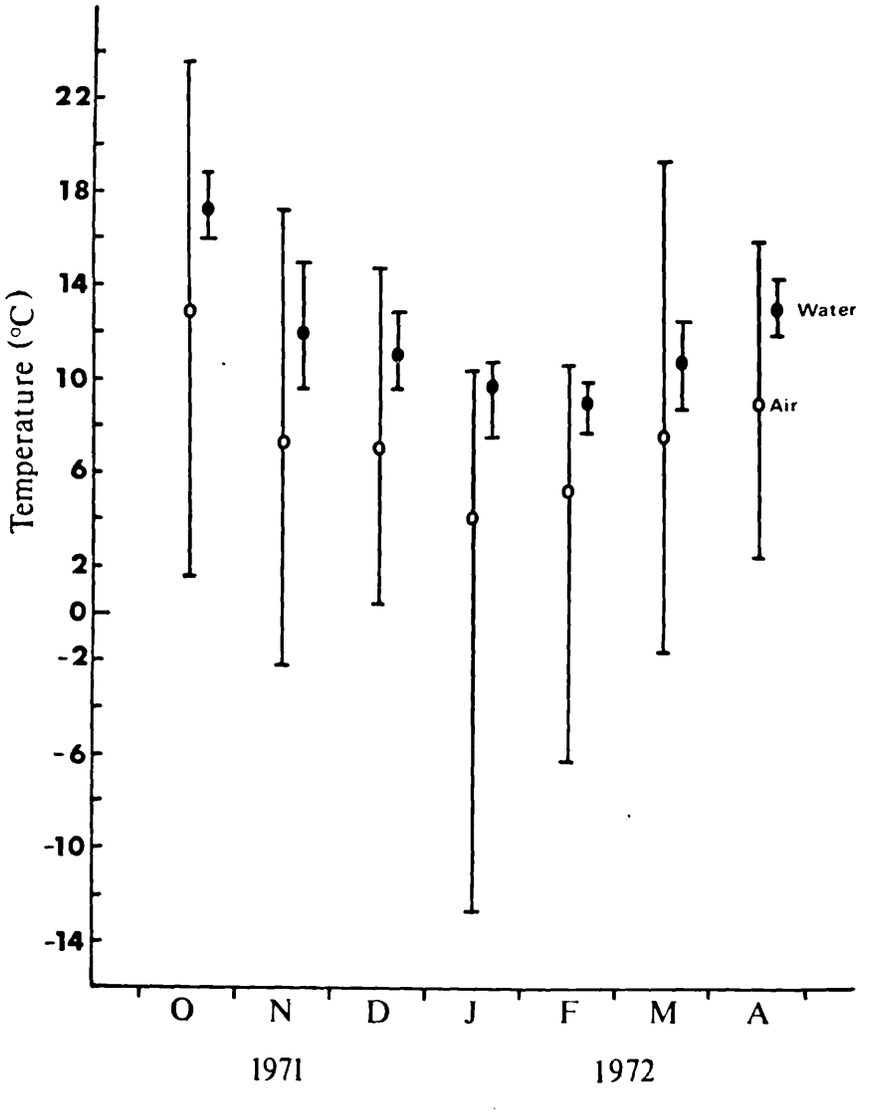
In addition to the water that drains away across the surface of the beach as the tide recedes, there are discharges from clefts in the substatum beneath the jetty that feed the pools and rills throughout the intertidal period. In dry weather, the salinity of the constant run-off is approximately equal to the high tide value, indicating that it originates in the extensive areas of slag further up the shore, into which it permeates during high tide.

In addition to the large network of interconnected pools and rills (Fig. 21) there are numerous pools of standing water, that range in size from small hollows beneath single rocks to large pools several metres across. The salinity in these pools is higher than the low tide value unless there is a prolonged and heavy rainfall during the intertidal period. However, the jetty, which is covered at the top along most of its length, offers some protection from precipitation, whilst attenuating winds and providing shade, and in the case of the smaller pools some protection is also afforded by the boulders.

Water samples from the estuary, surface run-off, and pools of standing water were taken during a low tide period in October, 1973. Surface water samples were collected with a siphon, and the low tide samples in a glass bottle suspended from the pier. The samples were allowed to equilibrate for 30 min to ensure that a steady state had been achieved between the oxygen level in the bottle and in the pool, rill or estuary. Careful manipulation was necessary to avoid trapping air bubbles in the run-off water samples. The levels of dissolved oxygen in the water samples were determined using the Winkler method (Mackereth, 1963), which revealed that the surface run-off and standing pools were saturated with oxygen, whereas the sample taken at the same time from about 3 m beneath the surface of the estuary was only 10% saturated.

Temperature measurements, using themistor probes ($\pm$ 0.5°C), showed a considerable temperature differential between the lower surfaces of the rocks that remained immersed in surface water, and the surrounding air. The temperature of the run-off water was found to be about the same as the mid tide value. Fig. 26 shows mean, maximum, and minimum monthly air temperatures for the period October 1971 to April

Fig. 26. Monthly mean, maximum and minimum air (open circles), and water (closed circles) temperatures at Greenhithe, for the period October 1971 - April 1972.
Data provided by the Meteorological Office, Bracknell, and the Thames Water Authority.


Temperature (°C)
22
18
14
10
6
2
0
-2
-6
-10
-14
Water
Air
O
N
D
J
F
M
A
1971
1972

1972 (Meterological Office data for Greenwich), together with the monthly mean, maximum, and minimum water temperatures for Greenhithe during the same period (Thames Water Authority data for Greenhithe and Gravesend).

2.3.6 Discussion

M. enigmatica's intertidal distribution at Greenhitherepresents an intermediate situation between the high level distribution in non-tidal localities, and the low level distributions on tidal shores lacking surface water (for references see Section 2.2.1). Assuming the interval between high tides averages 12 h 25 min, more at neaps and less at springs (Lewis, 1964) the zone occupied by Mercierella has its temporal limits c. 0.5 h and 4.75 h above the time of low water. During an average tidal cycle, the upper limit will be emersed for 9.5 h and the lower limit for 1 h. However, this investigation demonstrates the close association of Mercierella with run-off and standing water in which extremes of temperature, and the effects of precipitation and insolation during emersion would be minimised, whilst allowing unlimited feeding. Furthermore, where the run-off water flows across the mud on the lower part of the shore, it has cut permanent channels (see Fig. 21), exposing the underlying substratum that represents suitable sites for colonisation. Thus the surface water is also responsible for extending and maintaining the lower limits of M. enigmatica's vertical distribution at Greenhithe.

By exploiting the intertidal zone by means of this relationship with surface water, Mercierella avoids exposure to the extreme fluctuations in the levels of dissolved oxygen, salinity, and temperature in this polluted estuary.

3. PARTICULATE FEEDING

3.1 Introduction

The water in the field situation contains a variety of inorganic and organic particulate matter suitable for ingestion by M. enigmatica. The organic component represents c. 1% of the suspended solids at Greenhithe (Section 2.3.4) and is comprised largely of detritus, together with its associated bacterial flora. There are however, commonly, large numbers of ciliate protozoans, unicellular algae especially diatoms, yeasts, and the reproductive cells of sessile animals and plants, although their numbers tend to fluctuate both spatially and temporally. Examination of the gut contents of worms recently collected from the field population revealed considerable quantities of inorganic material, thus indicating that M. enigmatica does not feed selectively on the suspended organic particles.

Dales (1957) observed that fan worms were more efficient at filtering inert particles of detritus than motile algae, which he thought was a possible explanation of their abundance in the mouths of estuaries. Although Mercierella can trap a number of motile algal species, detritus, because of the vast quantities present in the estuary, undoubtedly features largely in its diet. In the laboratory energetics study however, it was decided to use Brachiomonas submarina Bohlin, a unicellular alga, as the food organism, thus avoiding problems associated with the heterogeneous composition of detritus, and leaching of soluble organic matter before the particles could be ingested by the experimental animals (see Tenore, 1975).

Ingestion rates were measured in the present study using ^{14}C-labelled algal cells. Since the method involves the measurement of accumulated radioactivity per unit time, it is necessary to know how

long food takes to pass through the gut (passage time), to avoid underestimating the rate of ingestion due to some of the labelled material being egested during the feeding period. It was decided therefore to carry out a preliminary investigation of passage time before the tracer experiments commenced.

3.2 The time taken for food to pass through the gut of *M. enigmatica*

3.2.1 Materials and Methods

M. enigmatica used in the feeding experiments were collected at Greenhithe in January 1976. In the laboratory, 6-litre stock tanks were maintained in a constant temperature room at 10°C, and at room temperature (*c.* 20°C). These temperatures approximated the winter and summer values in the field (Fig. 25). Worms were acclimated to the experimental temperatures for a minimum of 28 d and fed twice weekly with a suspension of washed *B. submarina* cells. The stock tanks contained Plymouth sea water diluted with tap water to a salinity of 15 °/oo, an average value based on the field data (Fig. 24). Artificial aeration was provided to prevent stagnation and to reduce algal sedimentation, that occurred particularly at the lower temperature.

About 1 week before the experiments commenced, the tubes were removed intact from the chalk boulders, that had been especially selected for this purpose, and scraped clean of all encrusting matter, after which they were placed in 500 ml crystallising dishes containing water at the appropriate acclimation temperature. The water in the tanks and dishes was changed before each second feed. It was found necessary to move the tubes at frequent intervals to prevent the worms secreting fresh calcareous material onto the bottom on the dishes, or becoming fused to adjacent tubes.

Feeding experiments were carried out using tubed animals because once removed from its tube M. enigmatica is no longer able to ingest particulate matter that is trapped by the branchial crown, since the rim of the tube is required to support the filaments in correct alignment with the mouth. Otherwise, the mucous strings leaving the bases of the filaments become entangled in the centre of the crown, effectively ligaturing the anterior end (Hall, 1954).

14 groups, each of 10 worms representing the population size range, were selected from each acclimation temperature and placed in 200 ml finger bowls containing dilute suspensions of B. submarina cells. The algal suspensions were changed every 12 h over a 3 d period, to ensure that the worms were in a fed condition when the experiment commenced. At the end of 3 d the pure algal suspensions were replaced with mixed suspensions of Brachiomonas cells and Congo red stained yeast cells (for method of preparation see Hall, loc. cit.). The groups of worms were left to feed on the mixed suspensions for 12 h, during which time they produced faecal pellets containing stained yeast cells. At the end of this period they were transferred to pure algal suspensions and normal feeding behaviour was observed within a few minutes of their introduction. Finally, at intervals of 30 min, groups of worms were successively transferred to fresh algal suspension, in which they remained for 30 min before these were filtered through 4.7 cm diameter Whatman GF/C filter pads, and the trapped faecal pellets examined for stained yeast cells. Great care was taken to avoid contaminating the fresh suspensions with stained yeasts or pellets adhering to the tubes or animals. This was avoided by rinsing the tubes thoroughly in several changes of clean water, and allowing sufficient time for the worms to extend their crowns in the clean water before they were transferred to a fresh algal suspension.

Table 2. The presence of Congo red stained yeast cells (+) in the faecal pellets released by M. enigmatica acclimated to 10 °C and 20 °C, following transfer to pure algal suspensions .

Time (min)	Temperature (°C)			
	10		20	
30	+		+	
60	+		+	
90	+		+	
120	+		+	
150	+		+	
180	+		+	
210	+		+	
240	+		+	
270	+		+	
300	+	Pure algal pellets present	+	Pure algal pellets present
330	+		+	
360	+	"	+	"
390	+	"	+	"
420	+	"	+	"

3.2.2 Results

Table 2 shows the results of the microscopic examination of the faecal pellets released by worms that had been removed from the B. submarina/yeast suspension for progressively longer periods of time. Groups of worms at both temperatures had ingested considerable quantities of yeast cells, since all the faecal pellets released during the first 4.5 h contained signs of the stain. After this time, pellets containing only algal cells began to be released in successively greater proportions, until by the seventh hour the vast majority of faecal pellets lacked visible signs of the stained yeasts.

3.2.3 Discussion

It takes a minimum of 4.5 h for food to pass through the gut of fed M. enigmatica at 10°C and 20°C. The presence of a few yeast cells in some of the pellets released after 6.5 h shows that the food undergoes considerable mixing during its passage through the gut. This is effected by the combined activities of the cilia lining the gut which beat in an antero-posterior direction (Hanson, 1948), and the muscles surrounding the gut blood-sinus, that move the blood in a postero-anterior direction and which therefore work in opposition to the cilia with regard to the direction of movement of the food. Body size certainly has some influence on the rate of passage since the residual yeast cells were confined to the largest pellets, indicating that they had been released by the larger worms. It is interesting to note that there is no apparent effect of temperature on passage time. It was noticed, however, that the worms at the lower temperature produced appreciably fewer faecal pellets per unit time which shows there is a quantitative effect on the ingestion rate.

Hall, (1954) reported that it takes c. 20 min for food to pass through the gut of M. enigmatica. Although he gave no indication of the temperature at which his observation was made, it seems likely that his experiment was carried out at room temperature. Hall's observation is in complete contradiction with the results of the present study, and can be explained if he used fasted or starving animals. He gave no information, however, as to their pre-experiment treatment apart from mentioning that the colonies require "a fresh supply of plankton-rich water at least once a week".

A preliminary experiment using Greenhithe worms that had been deprived of particulate food for 7 days, showed that faecal pellets were released within a few minutes (10 - 15 min) of their being presented with a B. submarina suspension, but these differed from those which were released after the first hour. The earlier pellets were green in colour and were comprised largely of algal cells that appeared completely unchanged by their journey through the gut. In contrast, the pellets that were released after 60 min were a dark brown colour indicating that chlorophyll degradation had taken place, and were more representative of the pellets produced by fed animals. These results indicate therefore that when fasted M. enigmatica are first presented with particulate food, the particles are not digested to the same extent as they are when the worms have been actively feeding for some time. Furthermore, they offer a possible explanation for the discrepancy between the results of the present study and those of Hall (loc. cit.), and show that great care must be taken to ensure that animals used in feeding experiments are in the desired nutritional condition.

3.3 The effect of food concentration on ingestion rate

3.3.1 Introduction

In the Thames estuary, Mercierella is exposed to considerable fluctuations in the quantity of suspended matter that is present in the surrounding water. During the intertidal period, M. enigmatica is immersed in water that contains relatively low concentrations of suspended material either as a result of it having recently percolated through large banks of slag (Section 2.3.5), or being fairly static, resulting in much of the suspended matter settling out onto the bottom. In both cases the amounts of suspended solids is usually very small unless particulate materials are washed into the pools and run-off rills during heavy precipitation. This contrasts with the situation that exists when the rising tide passes across the shore, carrying with it large amounts of suspended materials (Section 2.3.4). Finally, during the high tide period the water generally contains less suspended solids than it did during the flood (Section 2.3.4), although bottom water samples may contain more than 5% by weight of suspended solids during periods of high water flow (H.M.S.O., 1964).

In addition to the large scale fluctuations in suspended solids that are typical of its general habitat, M. enigmatica's preference for the undersurfaces of the rocks on which it lives, introduces yet another variable into any calculation of the concentrations of suspended matter to which it is exposed. In view of the complexity of the field situation with regard to particulate feeding, it was decided to investigate the worm's feeding behaviour over a range of cell concentrations.

3.3.2 Materials and Methods

Preliminary investigations of the removal of Phaeodactylum tricornutum Bohlin by M. enigmatica from dilute suspensions, showed

that it is possible to measure feeding rates colorimetrically, using a spectrophotometer calibrated with cell counts made with a haemacytometer slide (Appendix 1.1). However, since filter-feeding rates of bivalve molluscs and planktonic copepods are known to be influenced by the ambient concentration, it was decided to measure M. enigmatica's feeding rate using ^{14}C-labelled B. submarina cells. This method allowed relatively unlimited volumes of cell suspension to be used, on which the feeding worms had an insignificant effect with regard to cell concentration.

1 litre of actively growing B. submarina culture was inoculated with 50 µl of sodium hydrogen(^{14}C)carbonate (Radiochemical Centre, Amersham), that had a specific activity of 59.1 µCi µmol^{-1}. The labelled cells were harvested 2 d later as 10 ml samples, by slow centrifugation (1 000 r.p.m. for 5 min). After rinsing once with clean water to remove label not attached to the cells together with the culture medium, that may have altered the worm's feeding behaviour, followed by a second centrifugation, the cells were resuspended in clean water. From this labelled stock solution a range of cell concentrations was made up as 500 ml volumes. Cell numbers ml^{-1} were estimated from a previously constructed Beer's Law plot (see Bender, 1972) of absorbance at 660 nm, the red absorption maximum for chlorophyll a (Holt,1965) expressed as a function of cell concentration as measured with a haemacytometer slide (10 0.1 µl counts per concentration) (Appendix 1.2). Percentage transmission was measured with a Unicam SP 600 spectrophotometer.

From 2 to 5 10 ml samples from each of the experimental cell suspensions were accurately pipetted onto individual 2 cm diameter discs

cut from ashed Whatman GF/C glass fibre filters (overnight at 500°C) mounted in a sintered funnel, and the water was drawn off slowly to avoid disrupting the cells. The GF/C discs were then transferred to an oven at 60°C and dried to constant weight. After weighing, the cells were ashed at 500°C for 5 h, and after cooling were reweighed to the nearest 1 µg on a Cahn 4100 Electrobalance. Weight loss caused by ashing was equivalent to the ash-free dry weight of organic matter in 10 ml of cell suspension.

36 feeding M. enigmatica acclimated to 20°C were separated into 4 groups of 8 or 10 individuals, with similar size distributions. A group of worms was placed into each of the aerated, 500 ml B. submarina suspensions and left to feed for 2 h. This time was chosen based on the results of the investigation described in Section 3.2, and it was assumed that none of the label, should it have been assimilated, was respired during this period.

At the end of the feeding period, the worms were removed from their tubes and rinsed individually in several changes of clean water to remove any cells adhering to the crown and general body surface. This was followed by a 5 s rinse with distilled water to remove salt contamination. After blotting dry with paper tissue, the worms were individually weighed to the nearest 0.1 mg on a Cahn RTL Electrobalance before transferring to individual liquid scintillation vials, each containing 0.2 ml of 1.0 M Hyamine hydroxide (Koch-Light), which were then tightly sealed with polythene lined screw-tops to prevent the loss of volatile radioactivity during the digestion process, that took normally 2 days at 60°C.

Five 1 ml samples of each experimental suspension were taken before the groups of worms were introduced, and 2 similar samples were removed

Table 3. The ash-free dry weight of organic matter ml^{-1} in a range of Brachiomonas cell concentrations.

Estimated algal cell concentration (ml^{-1})	Number of determinations	Arithmetic mean ash-free dry wt. ml^{-1} (μg)
7.1×10^3	2	7.1
1.14×10^4	5	11.4
2.75×10^4	2	27.5
3.98×10^4	2	39.8

Table 4. Mean activity values for the cell suspensions and background counts at the beginning and end of the feeding experiments.

(The figures in parentheses represent the background activity expressed as a percentage of the initial activity in the cells)

Cell concentration (μg ash-free dry wt. ml^{-1})	Arithmetic mean activity ml^{-1} (d.p.m.)	Arithmetic mean initial background (d.p.m.)	Arithmetic mean final background (d.p.m.)
7.1	9 417	343 (3.8 %)	350 (3.9 %)
11.4	15 121	334 (2.3 %)	798 (5.4 %)
27.5	36 476	343 (0.9 %)	830 (2.3 %)
39.8	52 857	1 022 (2.0 %)	1 242 (2.4 %)

at the end of the 2 h feeding period. The latter and two of the former were filtered without pressure, to avoid damaging the cells, through 13 mm diameter, 1.2 μm pore size Sartorius membrane filters. The filtrates, together with the unfiltered cell samples were placed in individual liquid scintillation vials.

From 5 - 8 ml of toluene/Triton X-100 (Koch-Light) liquid scintillation cocktail (see Cooksey, 1972; and Section 4.2) was added to the soma digests, suspension filtrates, and the cell suspension samples, together with sufficient (1 - 2 ml) 80% ethanol in the case of those samples containing volumes of sea water, to redissolve the white precipitate that forms on contact with neat cocktail. After the samples containing Hyamine hydroxide had been dark equilibrated for 48 h to prevent errors arising from chemiluminescence, ^{14}C activity was counted in a Packard model 2003 liquid scintillation spectrometer for 1 or 5 min, depending on the level of activity in the sample. Those samples with low activity, namely the filtrates, were counted for the longer time to obtain a significant count with respect to normal background activity. The samples were counted twice over the course of 1 h as a check against chemiluminescence, but because the majority had high levels of activity, a single count was sufficient to obtain a representative measurement of the contained radioactivity. The results were corrected by the channels ratio method (see Dyer, 1974).

3.3.3 Results

Table 3 shows the ash-free dry weight of organic matter present ml^{-1} in each of the 4 B. submarina suspensions used in this experiment. There is excellent agreement between the estimated cell number and the mean ash-free dry weight of organic matter at all concentrations.

Table 5. Ingestion rate at a range of cell concentrations .

(The figures in parentheses represent the number of animals on which each value is based)

Cell concentration (µg ash-free dry wt. ml^{-1})	Arithmetic mean of total (wet) weight (mg)	Extreme (wet) weights (mg)	Arithmetic mean ingestion rate per animal (µg ash-free dry weight h^{-1})	S.D.
7.1	7.46 (10)	5.14 - 11.8	12.14	7.43
11.4	7.26 (8)	4.29 - 11.0	12.46	8.8
27.5	7.38 (8)	4.04 - 10.1	17.16	5.62
39.8	7.50 (10)	4.80 - 10.7	22.22	16.08

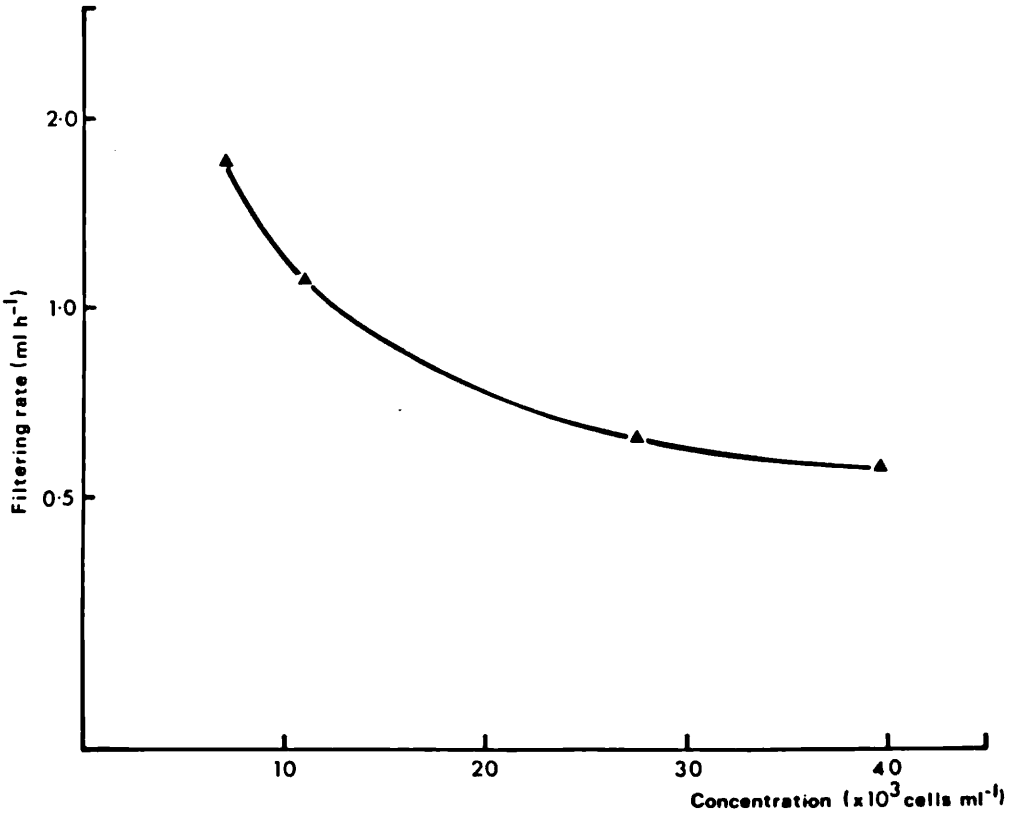
Furthermore, these results agree favourably with the estimated dry weight of an individual B. submarina cell, 1.11×10^{-6} (Section 3.6.2).

Ingested radioactivity was not corrected for label leakage during the feeding period, because the amount of leakage was never more than 3.1% of the activity initially present in the cells, and was therefore considered to be insignificant (Table 4). A summary of the results of the ingestion rate measurements for a range of ambient cell concentrations is presented in Table 5.

3.3.4 Discussion

There is positive relationship between the rate of ingestion and ambient cell concentration, over the range of concentrations used in the experiment. Ingestion rate, however, does not increase in direct proportion with the increase in cell numbers, e.g. a 5.6 fold increase in cell concentration is accompanied by only a 1.8 times increase in ingestion rate, which indicates that some factor(s) other than cell availability is influencing ingestion rate. The rate of ingestion is determined, at least partly, by the 'handling time', i.e the time taken for the trapped food to reach the mouth, which is directly under the control of biological factors such as the rate of mucus production, ciliary activity and the length of the branchial filaments, some of which are influenced by environmental parameters, e.g. temperature. This is supported by the observation that the worms in the most concentrated suspension showed erratic feeding behaviour, spending significantly greater amounts of time withdrawn inside their tubes, and when they were feeding the branchial filaments were continually undergoing individual movements, indicating that they were being disturbed by the dense B. submarina suspension.

Fig. 27. The relationship between filtering rate of a standard *M. enigmatica* (c. 7.0 mg wet weight) and *B. submarina* cell concentration. It should be noted that a semi-logarithmic transformation has been carried out on these data.


2·0
1·0
0·5
Filtering rate (ml h⁻¹)
10
20
30
40
Concentration (x10³ cells ml⁻¹)

In addition to the erratic feeding behaviour that was displayed at the highest cell density, which is reflected in the greater variability in the ingestion rates at that concentration (see Table 5), this group was seen to produce much greater quantities of pseudo-faeces, which indicates that when feeding they were unable to cope with the cells trapped by the crown. No pseudo-faeces were produced, however, at the two lower densities.

Fig. 27 shows filtering rate expressed as the volume of water effectively cleared of B. submarina cells h^{-1} by a standard worm plotted as a function of cell concentration. The highest mean filtering rate corresponds with the lowest ambient cell concentration. However, these values refer only to the effective filtering rates and take no account of the water volumes represented by those algae that escaped the branchial crown or were ejected as pseudo-faeces, particularly at the higher concentrations.

The only published values concerning the filtering rates of serpulid polychaetes are those of Dales (1957), who measured the volumes of water cleared of colloidal graphite indirectly by means of a colorimeter. Dales reported mean filtering rates of 11.16 and 27 ml $animal^{-1}h^{-1}$ for Pomatoceros triqueter (L.) and Hydroides norvegica (Gunnerus) at 16 - 17°C, which are significantly higher than the values obtained for Mercierella, even when their greater size is taken into account. In fact, M. enigmatica's filtering rate per unit total fresh weight is more in accordance with those reported for the much larger sabellids, Myxicola infundibulum (Rénier) and Sabella pavonina Savigny (Dales, loc. cit.). It is possible that the difference between Mercierella and the other serpulids may be a result of it being adapted to life in turbid water. Foster-Smith (1975) reported

examples of bivalve molluscs that appear to have relatively low filtering rates as adaptations to life in water with a high silt content.

3.4 The effect of temperature on ingestion rate

3.4.1 Materials and Methods

Eighteen and fifteen M. enigmatica, selected as representing the sample size range, were removed from the 10°C and 20° acclimation tanks respectively, and placed as groups of 7, 8, or 9 worms in 500 ml suspensions of B. submarina, with an estimated initial cell concentration of 1.1×10^4 cells ml^{-1}. The previous experiment having shown that feeding was normal at this concentration, the suspensions were changed at 12 h intervals during the subsequent 2 days, to ensure that the worms were in a fed condition at the time of the experiment. Finally, the suspensions were replaced with ^{14}C-labelled B. submarina cells (Section 3.3.2) at a similar concentration, in which the worms were allowed to feed for 2 h (Section 3.2.3). The algal suspensions were artifically aerated to ensure that the cells remained available for ingestion. Without continuous aeration the cells settled onto the walls and bottom of the container, especially at the lower temperature.

After 2 h, the worms were detubed and avoiding holding them in a way as might have caused any of the gut contents to be extruded, rinsed in several changes of clean water to remove any cells sticking to the general body surface. After a quick rinse with distilled water to reduce salt contamination which was followed by careful blotting of excess moisture with a paper tissue, the worms were weighed to the nearest 0.01 mg on a Cahn RTL Electrobalance. The worms were then placed in individual liquid scintillation vials containing 0.2 ml of 1.0 M Hyamine hydroxide for digestion at 60°C (Section 3.3.2).

Table 6. Results of the measurements of radioactivity in the algal suspension used in the feeding experiment, and the background levels of activity immediately before and after the feeding period .

Sample	Arithmetic mean radioactivity (d.p.m. ml^{-1})	Number of determinations
B. submarina suspension	15 237	3
Background		
Time = 0	450	2
Time = 2 h	831	2
Corrected cell radioactivity	14 787	

Five 1 ml samples of labelled algal suspension were taken before the worms were introduced, and two 1 ml samples from one of the experimental suspensions after the worms had been removed. Two of the former plus the latter samples, were filtered without pressure through 13 mm diameter, 1.2 µm pore size, Sartorius filters. Finally, the soma digests, filtrates, and the remainder of the algal suspension samples were counted in the scintillation counter for 1 or 5 min (Section 3.3.2).

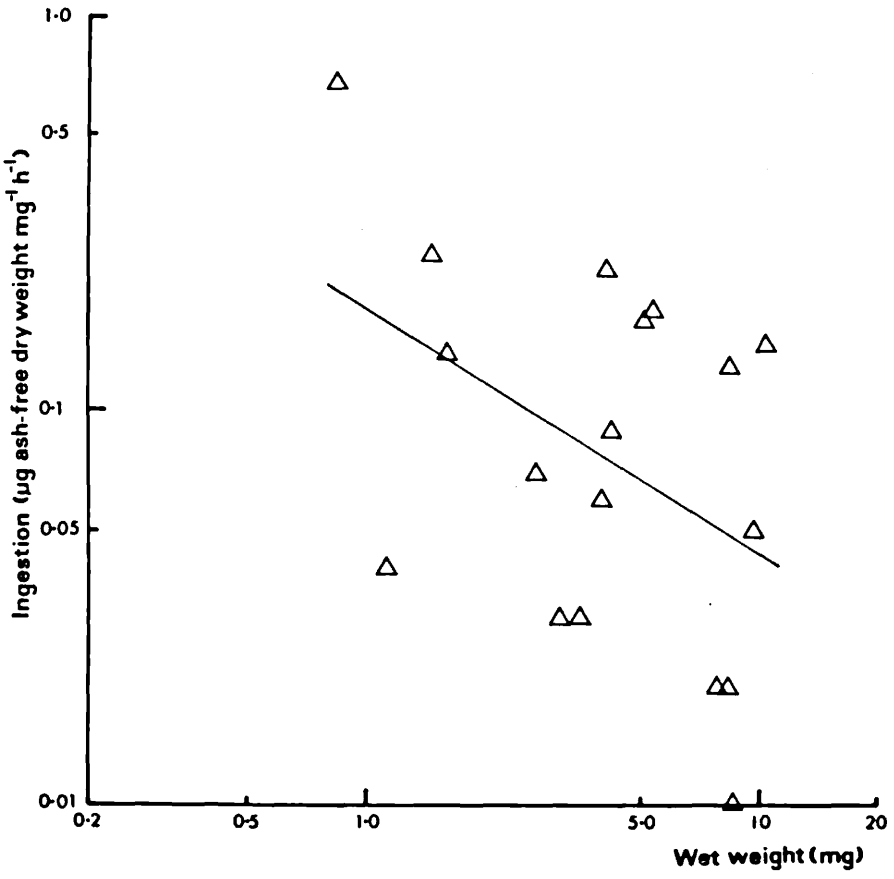
3.4.2 Results

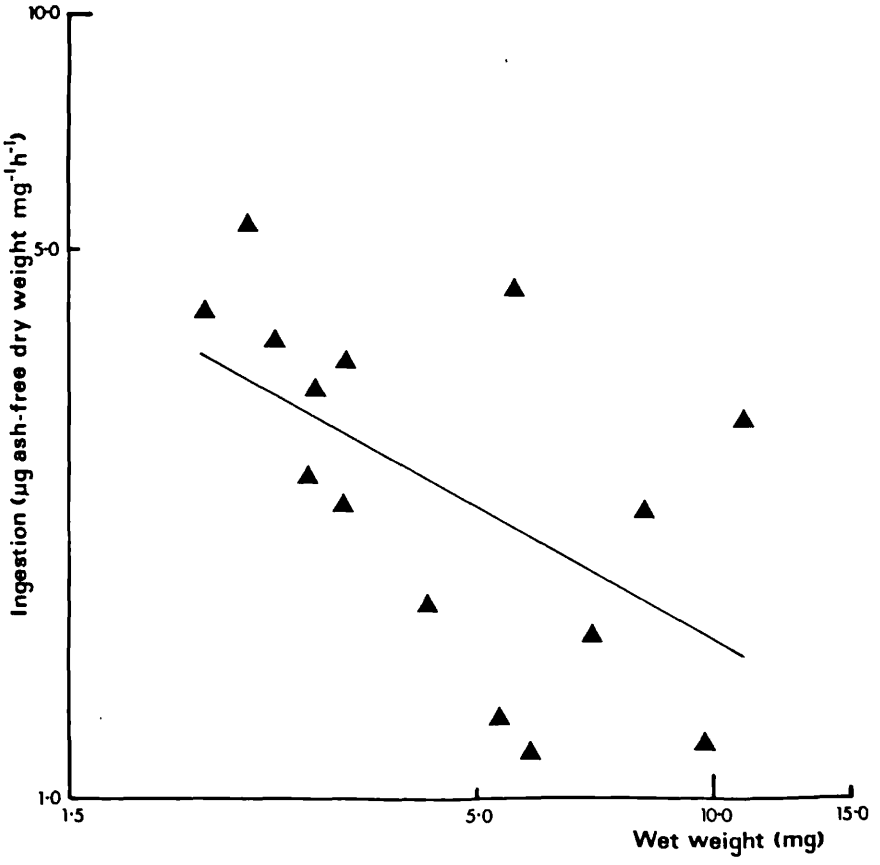
Table 6 shows the levels of radioactivity recorded in the cell suspension, and the background activity immediately before and after the 2 h feeding period. The cell suspension activity was corrected for the initial background count to give the radioactivity present in the cells. The individual amounts of radioactivity recorded in the worms were converted to ash-free dry weight of organic matter, assuming 14 787 d.p.m. was equivalent to 11.4 µg ash-free dry weight. (1.14×10^4 *B. submarina* cells, cultured under similar conditions to those in the present experiment, having been found to contain 11.4 µg of dry, ash-free, organic matter (Section 3.3.3).) The individual ingestion values were converted to rate per hour and are presented in Appendix 1.3. The results were not corrected for label leakage since during the 2 h feeding period this amounted to less than 2.6% of the radioactivity initially present in the cells, and was therefore considered to be insignificant.

The individual ingestion values were finally converted to µg ash-free dry weight of organic matter ingested mg^{-1} wet weight of worm h^{-1}, which are shown expressed as a function of total weight of

Fig. 28. Relationship between specific ingestion rate (ingestion rate mg^{-1}) and weight of animal for M. enigmatica acclimated to 10°C and feeding on a B. submarina suspension with a concentration of 1.14×10^4 cells ml^{-1}. The regression line was fitted by the method of least squares, assuming a linear relationship exists between the two parameters after a logarithmic transformation of the data. The regression coefficient (b) is -0.62.

Fig. 29. Relationship between specific ingestion rate and wet weight of M. enigmatica acclimated to 20°C and feeding on a suspension of B. submarina with an initial concentration of 1.14×10^4 cells ml^{-1}. The regression line was fitted to these data by the method of least squares, assuming a linear relationship after logarithmic transformation; b is -0.56.


1·0
0·5
0·1
0·05
0·01
Ingestion (µg ash-free dry weight mg⁻¹ h⁻¹)
0·2
0·5
1·0
5·0
10
20
Wet weight (mg)


10·0
5·0
1·0
Ingestion (µg ash-free dry weight mg⁻¹h⁻¹)
1·5
5·0
10·0
15·0
Wet weight (mg)

animal, for the winter and summer temperatures in Fig. 28 and Fig. 29. The regression lines were fitted by the method of least squares, and the correlation coefficients, r, are -0.44 and -0.57 respectively for the 10°C and 20°C results, with only the 20°C relationship being significant at the 5% level of significance ($P = < 0.05$). Counting efficiencies were 58 - 65% for the tissue digests, and 51-60% for the filtrates and cell suspension samples. Natural background amounted to 23 - 32 d.p.m. Pugh (1973) evaluated several methods for the liquid scintillation counting of radioactivity in algal suspensions and concluded that a Triton X-100 : toluene fluor is very effective giving relatively high counting efficiencies. Replicate preliminary tests with 1 ml aliquots of the suspension used in this feeding experiment failed to show a significant alteration in the counting efficiency with the internal standards method. The great advantage that the radioactive label technique has over the other methods in common use for measuring feeding rates, is that the animals are presented with a relatively infinite amount of food suspension which in this respect closely resembles the situation in the field. After 2 h feeding at the higher temperature, the group of worms with the greatest combined wet weight had reduced the total number of cells present in the 500 ml suspension by less than 4%.

3.4.3 Discussion

A cell concentration of 1.14×10^4 cells ml^{-1} was used in the ingestion study because the worms appeared to feed normally at this density (Table 5) and produced no visible amounts of pseudo-faeces, thus indicating that they were able to ingest at least the majority of the cells trapped by the branchial filaments. Furthermore, sufficient quantities of faecal matter could be harvested at this cell

concentration within a relatively short time for gravimetric and biochemical determination.

The ash-free dry weight of organic matter (11.4 $\mu g\ ml^{-1}$) was considerably in excess of the range of values quoted by Jørgensen (1966) for the standing stock of organic matter, as phytoplankton, taken on a global scale. However, the organic content of 11.4 mg l^{-1} is within the range he quotes for the total suspended matter that has been reported for inshore waters; although it should be noted that this refers to a combination of the organic and inorganic fractions. The experimental concentration is also somewhat less than the average values of 16% ($^{w}/v$) recorded for the total suspended matter in the flood tide waters at Greenhithe (Section 2.3.4). Thus since M. enigmatica will readily ingest both inorganic and organic particles as long as these are of the right order of size, the experimental situation, on a dry weight basis, is similar to the field. However, since organic matter makes up only c. 1% of the suspended solids in the estuary water (Section 2.3.4), the laboratory situation also represents about a 100-fold increase in the amount of available organic material.

Ingestion rates of smaller worms per unit body weight (specific ingestion rate) are higher than those of the larger animals at both experimental temperatures, so the rate of ingestion complies with the general relationship between the rates of physiological processes and body size. This must however, be considered as unproven for the lower temperature, 10°C, where the calculated correlation coefficient is just below the tabulated value at the 5% level of significance. It is assumed that a logarithmic relationship exists between these two parameters at both temperatures.

The relationship between the specific ingestion rate (I) and body size (W) can be described by the equation $I = aW^b$ where a and b are constants. If the decline in specific ingestion rate with increasing body weight is related to the relatively smaller size of the branchial crown in larger worms, the exponent b might be expected to approximate 0.67, which is the power law relating surface area to volume. The slopes (b) of the regression lines relating I at winter and summer temperatures to wet weight, -0.62 and -0.56 respectively, are in fair agreement with the ideal surface relationship considering the amount of individual variation that there is in these data and would seem to support this hypothesis.

Comparison of the average specific ingestion rates of standard (7.0 mg) worms under winter$_{(0.06)}$ and summer$_{(2.0)}$ conditions shows that the 10°C reduction in temperature is accompanied by a 97.15% decrease in I; a significantly greater reduction than was recorded for oxygen consumption (Section 6.7.4), somatic growth (Section 8.1.3), and tube production (Section 8.3.5). Walne (1972) reported particularly marked reductions in the filtering rates of the lamellibranchs, Venerupis decussata (L.) and Mercenaria mercenaria (L.) over the same temperature range, from which he concluded that feeding activities could be reduced for long periods during the winter period.

It is not possible to deduce from these data whether the reduction in feeding rate at the lower temperature is due to reduced ciliary activity and mucus production, or an alteration in the feeding behaviour, or some other component of the feeding mechanism. It was decided therefore, to investigate the feeding behaviour at the two experimental temperatures to see whether this would provide an explanation for the considerable difference between the ingestion rates under winter and summer conditions of temperature.

3.5 The effect of temperature on feeding behaviour

3.5.1 Materials and Methods

Fifteen and fourteen M. enigmatica were transferred from the 10°C and 20°C acclimation tanks respectively, to 500 ml suspensions of B. submarina cells with a concentration of 1.14×10^4 cells ml^{-1}. The worms, selected as representing the sample size range, were fed every 12 h for 2 - 3 days with fresh algal suspensions, to ensure that they were in an identical nutritional state to those used in the previous experiment.

Following the feeding period, the worms were separated into 3 size classes on the basis of their tube dimensions, $\leq$ 4.0 mg, 4.0 - 8.0 mg, and $>$ 8.0 mg. A preliminary investigation having shown that an exponential relationship exists between tube length and body weight (Section 8.1.3), the worms were then placed as groups of 4 or 5 similar sized individuals in a petri-dish containing a suspension of B. submarina cells at the appropriate acclimation temperature, which was kept circulating by a stream of air bubbles from a wide-bore hypodermic needle attached to an air line.

After a 30 min recovery period the worms were observed as groups of 4 or 5 animals at a time, for two 1 h periods that were separated by c. 1 h. At 60 s intervals throughout the observation periods, a record was made of the position of the crown of each animal. An extended crown means that the worm is actively feeding, and a withdrawn crown indicates a lack of feeding activity.

Since M. enigmatica is extremely sensitive to shadows and vibration, and responds by withdrawing rapidly into its tube, it was

Table 7. The percentage of total time spent feeding at 10°C and 20°C by a) the combined groups of worms, b) worms <4.0 mg and >8.0 mg wet wt. .

a)

Behaviour	10°C Mean ± S.D.	Number of determinations	20°C Mean ± S.D.	Number of determinations
Feeding	74.53 ± 35.86	15	98.07 ± 2.03	14
Withdrawn	25.47 ± 35.86		1.93 ± 2.03	

b)

	Size class <4.0 mg	Number of determinations	Size class >8.0 mg	Number of determinations
10°C	89.2 ± 5.35	5	59.0 ± 84.13	5
20°C	97.88 ± 2.66	4	98.7 ± 1.99	5

necessary to design an observation set-up which allowed the worms to be observed whilst shielding them from the movements of the observer. This was achieved by means of a screen and a mirror. The screen was positioned between the observer and the worms thus concealing his movements, whilst allowing the animals to be observed in the mirror mounted at an angle of 45° above the water bath. The system was improved by having only the water bath illuminated. Great care was taken to avoid causing vibrations during the observation period.

3.5.2 Results

The results of the feeding behaviour observations at 10°C and 20°C are presented in Table 7, and the results of the statistical analyses are performed on these data are given in Table 8. Since it was not possible to predict the exact size of the experimental animals from the size of their tubes because these were often incomplete, the results have been treated in the following manner: as combined groups at the two acclimation temperatures; and small and large individuals namely <4.0 mg and >8.0 mg wet weight, thus avoiding the possibility of overlap.

The mean values for the 10°C and 20°C data are based on 30 and 28 worm-hours respectively, and the individual values used in the calculations are the means of the combined two 1 h periods. The crown retractions associated with faeces ejection are not included in these data, so the percentage time spent withdrawn refers only to those periods that lasted for more than a few seconds duration.

The behaviour of M. enigmatica acclimated to 10°C is considerably more variable than is that of worms acclimated to 20°C. This difference, however, is not constant throughout the different size classes but is largely localised to the worms with the greatest weights,

Table 8. The results of statistical analyses performed on the feeding behaviour data for acclimation temperatures of 10°C and 20°C .

% time spent feeding at/by	F-test	Statistical test	Result
10°C and 20°C	P = <0.01	Modified t (Bailey, 1959)	Significant (P =< 0.05)
<4.0 mg and >8.0 mg at 10°C	P =<0.01	"	Not significant (P =>0.05)
<4.0 mg and >8.0 mg at 20°C	P =>0.05	Student's t	Not significant (P =>0.05)
<4.0 mg at 10°C and 20°C	P =>0.05	Student's t	Significant (P = <0.05)
>8.0 mg at 10°C and 20°C	P = <0.01	Modified t	Not significant (P =>0.05)

which displayed particularly erratic behaviour since some remained extended throughout most of the 2 h period whereas others spent the majority of the time fully withdrawn.

3.5.3 Discussion

The 20°C acclimated M. enigmatica spent a significantly greater amount of time feeding, than did the 10°C acclimated worms, so these results are in accordance with the results of the ingestion rate investigation (Section 3.4). However, the recorded difference of 24% does not fully explain the difference between I at the two acclimation temperatures, thus indicating that another component of the feeding mechanism is also being influenced by temperature. The most likely factors are ciliary activity and mucus production. A decrease in ciliary activity and mucus production would be accompanied by an increase in the handling time (Section 3.3.4). This is supported by the observation of erratic feeding behaviour in the case of the larger worms, which it will be remembered have got lower specific ingestion rates (Section 3.4.3), that could have been caused by their becoming swamped with more cells (a similar situation to that described in Section 3.3.4) than the ciliated tracts could handle at one time. The smaller worms, despite a significant reduction in their ingestion rates, had not reached the threshold concentration where they were no longer able to function effectively with regard to particulate feeding, although they did show what could be interpreted as the beginnings of this effect, namely greater variability in their behaviour at the lower temperature together with a tendency to spend greater amounts of time withdrawn inside their tubes.

3.6 Brachiomonas submarina : The biochemical composition, calorific content, and dry weight

3.6.1 Materials and Methods

Branchiomonas submarina (The Culture Centre of Algae and Protozoa, Cambridge) is a spherical cell about 20 µm in diameter. This chlorophycean was particularly suited to the present study because it is euryhaline, and swims actively through the water column by the action of flagella , thus making it readily available for ingestion by the experimental animals. Since the chemical composition, dry weight, and therefore the calorific content of algal cells depends on the nutrient status, light intensity, temperature and the age of the culture (Pugh, 1975), it was necessary to standardise the conditions under which B. submarina was cultured.

Unialgal cultures of B. submarina cells were maintained in 5 litre aspirators containing Miquel-Allen medium (Allen & Nelson, 1910) made up in 0.2 µm Sartorius filtered diluted sea water at a salinity of 15 °/oo. The culture medium was sterilised prior to inoculation by heating to 70°C for 20 min. All glassware was sterilised by autoclaving for a similar length of time at 2 atmospheres. Constant illumination was provided by a combination of natural sunlight from a North-facing window and a fluorescent strip-light that was mounted 10 cm above the culture vessels. Cultures were aerated with compressed air that had first passed through a glass wool filter and a solution of antibiotics (Section 6.5.2). Under these conditions the cells multiplied rapidly giving rise to dense suspensions within 14 days. Since centrifugation revealed a progressive increase in the proportion of cell ghosts once the culture had left the logarithmic growth phase, it was decided to discard the cultures at the end of the period of rapid growth.

Protein. Protein was estimated by total nitrogen determination using a Coleman Model 29 Nitrogen Analyser, and multiplying the amount of nitrogen by the factor 6.25 to give the quantity of protein (Brody, 1945). (16% by weight of protein is nitrogen.) Algal cells were separated from the culture medium by centrifugation. 10 ml samples of medium were centrifuged at 1 000 r.p.m. for 5 min. Following resuspension in 0.2 µm Sartorius membrane-filtered, diluted, artificial sea water (SeAquariums Ltd., Sheffield), the Brachiomonas cells were filtered onto weighed 20 mm diameter discs cut from ashed Whatman GF/C glass-fibre filters. The GF/C filters were ashed by heating overnight at 500°C before weighing on a Cahn 4100 Electrobalance. Cells retained by the filters were given a quick rinse with glass-distilled water to remove excess surface salt before drying to constant weight at 60°C. Nitrogen readings were corrected for background using GF/C filter blanks that had been previously rinsed with distilled water.

Lipid. Lipid was extracted from pre-weighed cell samples on GF/C filters with di-ethyl ether in a micro-Soxhlet apparatus. Following 1.5 h extraction, which a preliminary experiment had shown to be sufficient to achieve a constant weight, the filters were dried to constant weight, and the lipid content was estimated by difference. The samples received a similar pre-determination treatment to those used for the protein estimation, and dry weights were measured to the nearest 0.01 mg on a Cahn 4100 Electrobalance.

Ash. The inorganic component was determined by ashing pre-weighed algal cell samples to constant weight on ashed GF/C filter discs. Ashing was done in a muffle furnace at 500°C (Paine, 1964).

Carbohydrate. No carbohydrate analyses were carried out. The percentage carbohydrate content of Brachiomonas was estimated by difference.

Calorific content. 10 ml samples from an actively growing culture were separated from the medium by centrifugation and resuspended in filtered diluted sea water. Following a second centrifugation the cells were resuspended in glass-distilled water and transferred to glass crystallising dishes, which were then placed in a drying oven at 60°C, after they had been loosely covered with aluminium foil lids to exclude dust particles. When dry, the cells were scraped off the bottom of the dishes and forced through a 125 µm steel meshed sieve to ensure that the samples were completely homogeneous. The powdered cells were stored in a desiccator over anhydrous calcium chloride.

The calorific content of B. submarina was measured in a Scott micro-bomb calorimeter (Scott, 1975) which was calibrated with benzoic acid (British Chemical Standards). A 0.1 mV deflection on the chart recorder was equivalent to c. 0.418 J (0.1 cal).

Ashed and weighed 4 mm diameter GF/C filter discs were placed on a suction device similar to the one described by Scott (loc. cit.) and a drop of distilled water was pipetted onto the filter together with a small quantity of dry B. submarina homogenate. The algal material was mixed into the water droplet with the tip of a seeker. Gentle suction was then applied to the suspension so that the homogenate evenly covered the surface of the filter disc. The samples were then dried to constant weight at 60°C before igniting in the calorimeter. A fuse wire correction was applied to the recorded temperature rise, and the filters and samples were weighed to the nearest 5 µg on a Beckman LM-500 microbalance.

Table 9. The chemical composition of some chlorophycean cells (percentage dry weight).

Species	Protein	Carbohydrate (estimated)	Lipid	Total Pigment	Ash	Reference
			(means and standard deviations)			
Brachiomonas submarina Bohlin	83.16 ± 6.73	3.34	8.99 ± 4.13	—	4.51 ± 0.62	
Dunaliella salina (Dunal) Teodor	57 (68)	31.6	6.4	3.0	7.6	Parsons et al. (1961)
Tetraselmis maculata Butch	52 (72)	15.0	2.9	2.1	23.8	"

Dry weight. An estimate of the number of B. submarina cells per ml 8 day old culture was obtained by haemacytometer counts of twenty 0.1 mm^3 heat-killed sub-samples. Ten 200 ml samples of the culture were then centrifuged at 2.4×10^3 r.p.m. for 30 min before resuspending in glass-distilled water, followed by dialysis against running tap water for 24 h in Visking tubing. After dialysis treatment to remove the compounds of low molecular weight (salts), the samples were transferred to individual weighed crystallising dishes and dried to constant weight at 60°C. Care was taken to avoid losing cells at each stage of the method. After drying, the dishes were reweighed and the dialysed dry weights of the ten 200 ml samples were obtained by subtraction. All weighings were made to the nearest 0.1 mg on a Sartorius semi-micro balance, and the dialysed dry weight of an individual B. submarina cell was calculated by dividing the dry weights of the samples by the estimated number of cells in 200 ml of the original culture.

A similar experiment was carried out in which two 20 min washes in glass-distilled water were substituted for the dialysis treatment.

3.6.2 Results

Table 9 shows the results of the chemical analyses as means and standard deviations expressed as percentages of dry weight (see Appendix 1.4 - 1.6 for experimental values). The results obtained by Parsons, Stephens, and Strickland (1961) for two other planktonic green algae are presented for comparison.

In common with D. salina and T. maculata, protein is the principal organic constituent of B. submarina cells. Brachiomonas, however, appears to have a significantly greater proportion of protein than do the other species. Parsons et al. (loc. cit.) found a discrepancy

between the results they obtained with the Kjeldahl method for total nitrogen using the generally accepted factor of 6.25 to convert their nitrogen values to protein, and the determinations of protein made with a colorimetric method, using 2,5-hexanedione reagent standardised with casein (values in parentheses in Table 9). Although their suggestion that nitrogen containing compounds other than amino acids were present in appreciable quantities does not explain the discrepancy, their results serve to illustrate the potential errors when attempting to estimate protein by an indirect means.

It seems extremely unlikely that the factor of 6.25 that has been extensively used for converting nitrogen values to protein is universally applicable. Furthermore, it is expected that plant cells especially will contain appreciable amounts of inorganic nitrogen, which unless determined separately will lead to overestimations of the amounts of protein in the cells. Since protein was estimated from the total nitrogen in the present study, it is possible therefore that the percentage protein could have been overestimated.

The lipid content of B. submarina compared favourably with the amounts reported in the other species. However, no measurement was made of the total pigment in B. submarina, that leached out during the Soxhlet extraction, which means that the value for lipid content is overestimated. This is unlikely to have seriously affected these results because pigment is known to represent only a relatively minor portion of the total dry weight of algal cells (e.g. Parsons et al. (see Table 9); Pugh, 1975).

The ash content of B. submarina is significantly less than the levels recorded in the planktonic algae investigated by Parsons et al.

Table 10. Cell volumes, dry weights and calorific contents of some chlorophycean species.

Species	Approximate cell volume (μm^3)	Dry weight per cell ($\times 10^{-6}$ mg) (mean and S.D.)	Calorific content joules mg^{-1} dry wt. (calories in parentheses)	Reference
Brachiomonas submarina Bohlin	400 - 500	1.11 ± 0.05 (dialysed) 0.9 (d. H_2O)	19.02 ± 3.26 (4.55 ± 0.78)	
Chlamydomonas reinhardi Dangeard	—	0.248 ± 0.017	22.11 ± 0.34 (5.289 ± 0.0956)	Richman (1958)
Dunaliella salina (Dunal) Teodor	400	0.13	—	Parsons et al. (1961)
Tetraselmis maculata Butch	310	0.23	—	"
Tetraselmis suecica (Kylin) Butch	—	0.066	23.4 ± 0.38 (5.6 ± 0.09)	Widdows & Bayne (1971)

(loc. cit.). Platt and Irwin (1973) reported ash contents of mixed phytoplankton samples, ranging from 33.8 - 50.5% of the dry weight, although these values were undoubtedly swollen by the presence of diatoms, which have extremely high ash contents (Parsons et al., loc. cit.; Paine & Vadas, 1969). The 4.5% of the dry weight recorded for B. submarina approaches the low ash contents reported for freshwater algae, e.g. 3.94% in Chlamydomonas reinhardi Dangeard (Richman, 1958). It is possible that this reflects B. submarina's euryhalinity, the salts having been rapidly leached out through a highly permeable cell wall during rinsing with distilled water.

Finally, the estimated carbohydrate content of B. submarina is considerably less than the values reported by Parsons et al. (loc. cit.) for 11 phytoplankton species, including 3 diatoms and 1 coccolithophore. Pugh (1975), reported values of 7 - 12% of dry cell weight for the diatom, Coscinodiscus eccentricus Ehrenberg, during the log growth phase, and quotes references from which he calculated an average carbohydrate value for the non-stationary growth phase of 25.3%.

Considering that the cell wall in B. submarina is composed of cellulose, in contrast to the siliceous and calcareous walls of the diatoms and coccolithophores, respectively, and that the cells used in the analyses were actively growing, indicating that the products of photosynthesis were greater than the basic metabolic requirement, it seems likely that the quantity of carbohydrate in B. submarina is underestimated as a result of the protein content being overestimated.

Table 10 shows the results of the cell dry weight and calorific content determinations (see Appendix 1.7 and 1.8 for the experimental values), together with the range of cell volumes based on cell

diameters measured with a graticule eyepiece. Values for several other green algal species are presented for comparison.

The relationship between cell volume and dry weight for B. submarina is in accordance with the values reported by Parsons et al. (loc. cit.). The difference between dialysed dry weight and the distilled water washed dry weight indicates that an appreciable quantity of soluble organic matter was lost from the cells during the washing treatment with distilled water.

The calorific content of the B. submarina cells is less than the values reported for C. reinhardi and T. suecica. When converted to the calorific value per unit ash-free dry weight, 4.76 ± 0.82 cal mg^{-1}, it is similar to the average value given by Paine and Vadas (1969) for 10 species of green algae.

There is considerable variation in the calorific values for B. submarina. As a check for incomplete combustion, the GF/C filters were examined after ignition for traces of organic residue. In each case only white ash remained. As a further check, the filters were weighed before they were heated in a muffle-furnace at 500°C for several hours, after which they were reweighed. There was no appreciable difference between the first and second weighings of all the samples, indicating that complete combustion of the organic material on the filters had occurred in the calorimeter. It is possible that some material could have been lost from the filters at the time of firing, which did not undergo combustion resulting in the observed variability. Examination of the inside of the calorimeter revealed no signs of a deposit but in view of the small quantities involved, it is possible that this could have been overlooked. Unfortunately, it

was not possible to check for weight loss from the sample using the expected weight of ash left after complete ignition, since the fuse wire invariably became fused with the glass-fibre filter during ignition. Another possible explanation for the recorded variability is that some soluble organic matter may have leached out from the samples during the treatment with distilled water that is necessary to attach the sample to the GF/C filter.

3.6.3 Discussion

Further evidence for an overestimation of the amount of protein in B. submarina and the resulting underestimate of the percentage carbohydrate, is the considerable discrepancy between the calorific content as measured with a micro-bomb calorimeter and the value calculated from the percentage composition according to the results of the biochemical determinations. The heats of combustion of glucose, lipid, and protein, according to Brody (1945) are 15.88 J mg^{-1} (3.8 cal mg^{-1}), 39.5 J mg^{-1} (9.45 cal mg^{-1}), and 23.62 J mg^{-1} (5.65 cal mg^{-1}) respectively. The estimated calorific content of B. submarina is 23.7 J mg^{-1} dry weight (5.67 cal mg^{-1}), more than 4.5 joules higher than the value obtained by calorimetry.

The carbohydrate basic unit of B. submarina is assumed to be glucose since Parsons et al. (loc. cit.) reported that 80% of the carbohydrate in T. maculata is comprised of glucose. Furthermore, since glucose is the major constituent of cellulose (Street, 1963) the assumption is believed justified.

Since there is considerable evidence to show that the protein component has been overestimated, it was decided to calculate an average protein content based on published values for other phytoplankton

Table 11. Estimated average composition of phytoplankton with respect to protein, carbohydrate and lipid, based on published values.

Species	Protein (% ash-free dry weight)	Carbohydrate (% ash-free dry weight)	Lipid (% ash-free dry weight)	Reference
Tetraselmis maculata	73.8	19.65	3.8	Parsons et al. (1961)
Dunaliella salina	55.72	34.13	6.91	"
Mixed phytoplankton	33.37	44.69	21.94	Platt & Irwin (1973)
Average % composition	54.3	32.82	10.88	

species, for use in subsequent computations in place of the experimentally derived value. In view of the discrepancy between the protein values obtained by Parsons et al. using two different methods, it was decided to derive the percentage protein for B. submarina indirectly from carbohydrate, lipid, and total pigment values when expressed as percentages of ash-free dry weight. Platt and Irwin (1973) analysed the biochemical composition of mixed phytoplankton samples collected during a spring bloom (their data shows poor agreement between %N and % protein). Correcting their carbohydrate and protein values for ash content gives the following respective percentages, 44.69 $\pm$ 15.23 (S.D.)% and 33.37 $\pm$ 4.89% of ash-free dry weight (N = 10). These values plus percentage lipid, together with those derived from Parsons et al.'s data were used in the calculation of the average values, and are presented in Table 11.

3.7 The energetic aspect of ingestion

It was decided to restrict the energy study to one size of animal, namely a worm of 7.0 mg wet weight (1.0 mg dry weight) (from now on referred to as a standard animal). Average values for the ingestion rates of M. enigmatica weighing 7.0 mg at winter and summer temperatures were derived from the regression lines relating specific ingestion rate and body size (Fig. 28 and Fig. 29). When correcting for weight these are 0.385 µg and 13.51 µg ash-free dry weight h^{-1} respectively for the 10°C and 20°C acclimated animals. Since the calorific value mg^{-1} ash-free dry weight of B. submarina is 19.02 x $\frac{100}{95.49}$ = 19.92 J (4.76 cal), the ingestion rates of a standard worm at 10°C and 20°C are equivalent to 7.67 x 10^{-3} J h^{-1} (1.83 x 10^{-3} cal) and 2.69 x 10^{-1} J h^{-1} (6.44 x 10^{-2} cal) respectively.

4. DISSOLVED NUTRIENTS

4.1 General Introduction

August Pütter was the first to suggest that dissolved organic matter is used as a source of energy and materials by aquatic animals for their catabolic and anabolic processes, from the results of a series of investigations carried out during the first quarter of this century which showed an insufficiency in the amount of particulate food in the surrounding water to satisfy the minimum energy requirements of a number of planktonic and benthic organisms including several filter-feeding species. Pütter concluded that the utilisation of DOM must represent an important nutritional source.

Since Pütter first published his hypothesis, there have been a number of accounts refuting his argument on the basis that he underestimated the available food supply and overestimated the concentrations of DOM in the environments with which he was dealing. The main reasons for the discrepancy between Pütter's computations and those of subsequent workers are his lack of knowledge concerning : the existence of nannoplankton; the importance of detritus in the energy budgets of suspension feeders; the irregular dispersion patterns of the food organisms; sensory, tactic, and feeding mechanisms; digestive mechanisms; and his use of faulty analytical techniques. However, despite his hypothesis being based on erroneous data, Pütter made a valuable contribution to animal physiology by focusing attention on the immense quantity of DOM in the aquatic environment as a whole from the point of view of its potential as a nutritional source for metazoans.

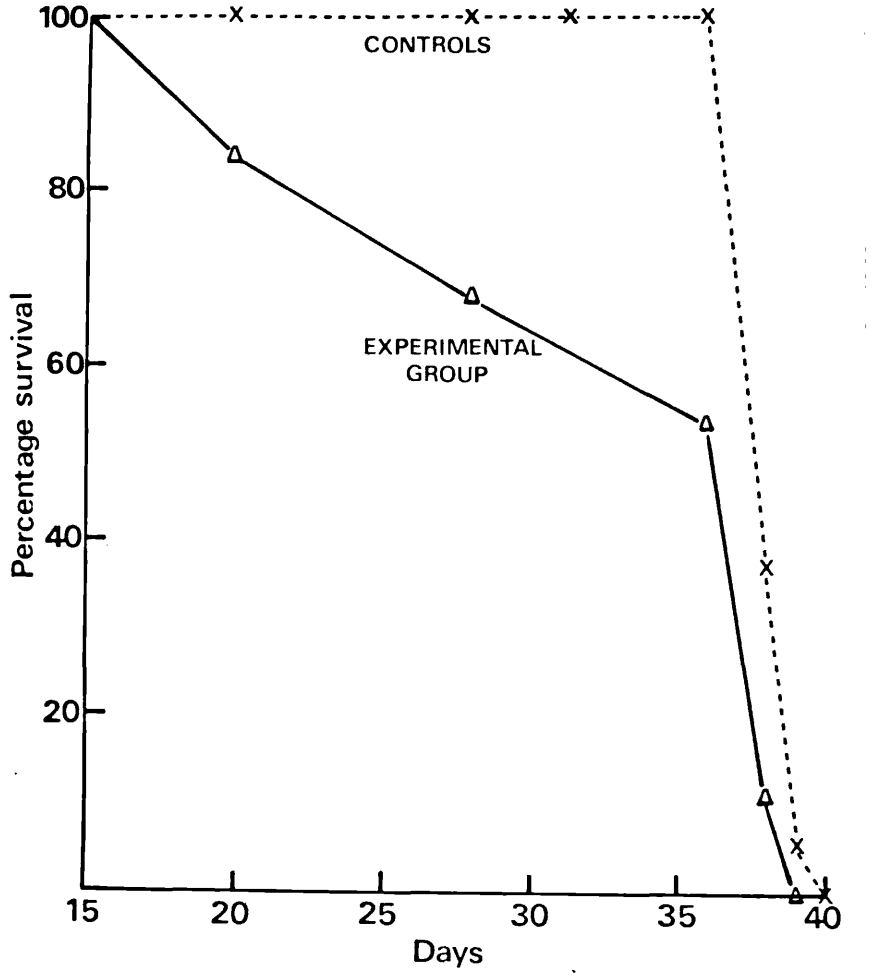
For a comprehensive and critical review of Pütter's work and that of the other early investigators see Krogh (1931); the most recent review of this subject is that by Jørgensen (1976).

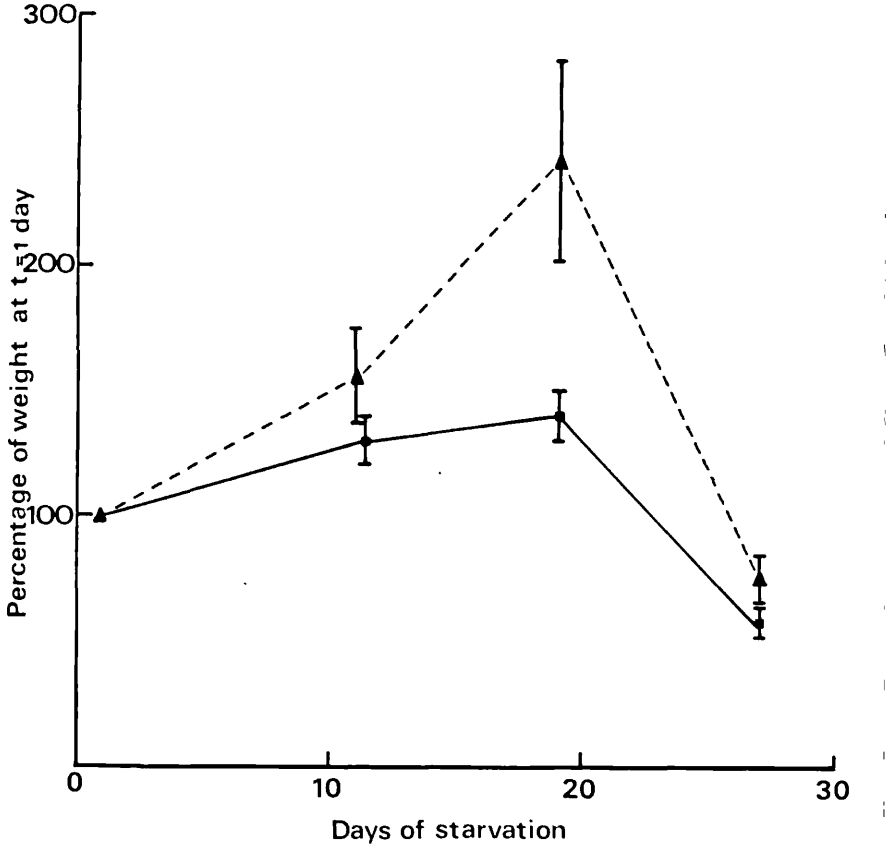
Due to the enormous amount of potential energy that is represented by the DOM in the sea (Jørgensen, 1966, 1976) repeated attempts have been made to evaluate its contribution to the nutrition of marine invertebrates. These studies have used either a direct approach, in which the effects of DOM on animals deprived of a particulate source of energy are investigated (e.g. Gillespie et al., 1964, 1966), or an indirect method where the potential energy contribution of the measured uptake of DOM is compared with the metabolic requirement (e.g. Stephens, 1962a; Southward & Southward, 1972).

The contribution that those investigations in the former category have made to our knowledge of the nutritional value of DOM uptake is minimal. Firstly, by their frequent use of non-sterile conditions these investigators have failed to take into account the ability of mucus-secreting filter-feeding animals to utilise bacteria as food (see Zobell et al., 1937; Sorokin, 1973). Secondly, these workers have used only a single compound, usually glucose, which cannot be expected to perform as a complete nutritional substitute, since it makes no allowance for the animal's nitrogen requirement. Thirdly, since some naturally occurring dissolved organic compounds, e.g. glucose and glycine, have been shown to elicit behavioural response in marine invertebrates (Collier et al., 1953; Lavandowsky & Hodgson, 1965; Carr, 1967; Loeb & Blanquet, 1973), the enhanced metabolic rates reported for organisms following exposure to DOM (e.g. Shick, 1975) do not necessarily imply that this is acting as a

Fig. 30. Survival curves for detubed, Weymouth Harbour, M. enigmatica in artificial sea water (SeAquariums Ltd., Sheffield), with and without a 10^{-4} M glucose supplement. The water was replaced with fresh (30 °/oo, pH 8.16) containing benzylpenicillin (30 mg l^{-1}) and streptomycin sulphate (50 mg l^{-1}) at 5 - 7 day intervals. Size ranges were 1.1 - 8.6 mg (N = 16) and 0.9 - 11.2 mg wet weight (N = 19) respectively for the control and experimental groups.

Fig. 31. Time course of wet weight changes in M. enigmatica during starvation. ▲ mean values for worms with initial weights < 4 mg, and ■ animals weighing 4 - 8 mg. The vertical lines represent one standard error above and below the mean. N values: ▲ = 4; ■ = 10.


100
80
60
40
20
Percentage survival
CONTROLS
EXPERIMENTAL GROUP
15
20
25
30
35
40
Days


300
200
100
Percentage of weight at t=1 day
0
10
20
30
Days of starvation

nutritional source. In an investigation of the effect of dissolved glucose on the survival of detubed (starving) Mercierella (see Section 4.3.4), it was the worms that received the glucose supplement that were the first to die (Fig. 30). This was probably due to the glucose enhancing their metabolic rate through its effect on their behaviour, thus causing them to oxidise their storage products at a faster rate than those in artificial sea water containing only antibiotics. Gillespie et al. (1966) reported that oysters maintained in a mixture of glucose and amino acids died sooner than those without DOM supplements, from which they incorrectly concluded that the compounds were having a toxic effect. Finally, Stephens (1968) incorrectly reported Gillespie et al. (1966) as having demonstrated that additions of glucose supported some growth in Crassostrea virginica (Gmelin). However, Gillespie et al.'s data refers to wet weight which my preliminary experiments with Mercierella showed to increase during the initial stages of starvation (Fig. 31) whereas dry weight decreased throughout the experimental period.

Indirect studies of the role of DOM in the nutrition of marine invertebrates (for references see Jørgensen, 1976) have generally paid little attention to the non-catabolic functions of these compounds. It is misleading to consider the total uptake in terms of its potential contribution to the organism's immediate metabolic energy requirement, when there is every reason to expect them to enter anabolic and, in the case of certain amino acids, osmoregulatory processes, in addition to whatever involvement they might have in the catabolic reactions. Thus the published estimates for the possible contribution that DOM uptake may make to the metabolic energy requirements of various soft-bodied marine invertebrates, (these range from 6% to 150% (Stephens,

1963; Shick, 1975)), are of little value when one is attempting to determine their role in those organisms' energy budgets, unless substantiated by data concerning the recovery of the label in the form of $^{14}CO_2$. It is only that fraction of the accumulated compound that becomes oxidised to carbon dioxide which should be considered in terms of a metabolic energy contribution. This method was used in this study of the uptake of DOM by Mercierella enigmatica.

4.2 Materials and Methods

The Mercierella used in the DOM experiments were collected from the Thames population at Greenhithe, Kent, in January 1976, and were maintained in the laboratory at the appropriate acclimation temperature for a minimum of 21 days (maximum 5 months) before the experiments commenced. The worms were fed twice weekly with a suspension of washed Brachiomonas cells, however, their diet was supplemented with particles of organic matter that became detached from the boulders, stones, and various debris, on which they were growing. The water in the tanks was changed before each second, sometimes third, feed, and small amounts of tap water were added at intervals to replace evaporative losses.

The worms were maintained in artificially aerated, diluted Plymouth sea water, at a salinity of 15 °/oo. Aeration prevented stagnation and the particles of food from settling out of suspension. A temperature of 10°C was achieved using a constant temperature room, and the 20°C animals were acclimated at room temperature. Since the room temperature was found to display an appreciable amount of diurnal fluctuation, the 20°C animals were kept for several days prior to the experiments in a water bath at 20 $\pm$ 0.2°C, in an attempt to counteract the variation. A third experimental temperature of 15°C was achieved by the use of a water bath and dip cooler (see Section 6.4.4).

Since it was not possible to remove entire tubes from flint which comprised the most common type of substratum, and as the worms are usually in close association with other tubes, resulting in their being fused together and closely intertwined, it was necessary to use detubed Mercierella in the majority of the experiments. In these instances the pre-experimental treatment was as follows.

After acclimation the worms were carefully removed from their calcareous tubes and given several rinses in clean water, to remove adhering tube fragments and particles of food remaining in the branchial crown, before transferring them to crystallising dishes containing 0.2 μm Sartorius membrane filtered water at the appropriate acclimation temperature. The dishes were then loosely covered to exclude dust particles and set aside for 24 h, after which the worms were examined for signs of damage, and any damaged individuals discarded. Healthy Mercierella, characterised by extended crowns and responses to shadow and touch stimuli, were transferred to a solution of antibiotics, streptomycin sulphate (50 mg l^{-1}) and benzylpenicillin (30 mg l^{-1}), in which they remained for 24 h to reduce bacterial contamination, a preliminary experiment (Section 6.5) having shown that these antibiotics are effective in reducing bacterial activity without modifying the worm's respiration rate. Following the decontamination treatment, and several rinses in filtered water to remove traces of antibiotics adhering to the body surfaces, the worms were ready for experimentation. To avoid damaging their external surfaces, the worms were manipulated in drops of water between blunt forceps.

Glass, screw-top, liquid scintillation vials were used as experimental vessels. Working strength solutions of radioactive glucose, or glycine, were made up no more than 1 h before the experiments commenced, to reduce the possibility of error resulting

from bacterial activity. Measured aliquots of the labelled compounds were added to precisely determined volumes of filtered, diluted sea water, and thoroughly mixed before being dispensed into the experimental vials. The experiments were carried out at the appropriate acclimation temperature, and sufficient time was allowed for the radioactively labelled solution to equilibrate with the temperature of the water bath, before the worms were introduced.

Single worms were incubated in 2 ml aliquots of the experimental medium, and when small groups were used 2 ml of solution was allowed per individual. No antibiotics were used in the incubation media to minimise the number of variables, and controls for bacterial catabolism were used.

At the end of the incubation period, the worms were rinsed free of surface contamination in several changes of filtered water, blotted dry on a paper tissue and weighed to the nearest 0.01 mg on a Cahn RTL Electrobalance. 0.1 ml samples of the experimental media were removed for counting immediately before and after the incubation period. The radioactivity which entered the worms was treated as three separate fractions of the total uptake: the ethanol soluble fraction; the ethanol insoluble fraction; and the fraction recovered as $^{14}CO_2$, which represents that part of total uptake that was catabolised, presumably to supply energy for the worm's immediate metabolic needs.

The ethanol soluble fraction was extracted from the individual somas during one or two 24 h periods spent in separate 0.1 ml volumes of 80% ethanol. The extracted somas were given a 5 s rinse in clean ethanol before they were transferred to individual vials containing 0.2 ml of 1.0 M Hyamine hydroxide (Koch-Light). The aluminium cap liners were replaced with polythene to prevent the loss of volatile

label during the digestion of the tissues; a process that normally took from 48 to 72 h at 50°C. In some instances, the ethanol extraction was omitted and the somas were transferred directly to Hyamine hydroxide.

The radioactivity released as $^{14}CO_2$ during the incubation periods was collected for counting in the following way. The worms were incubated in vials with polythene cap liners to prevent the loss of label. After the worms were removed from the vials, a small glass tube, sealed at one end and containing a Whatman no. 40 filter paper wick impregnated with 0.2 ml of Hyamine hydroxide, was suspended by a length of cotton thread over the surface of the medium, to which sufficient 30% sulphuric acid was added to make a 3% solution. The vials were then sealed and set aside for 24 h, sufficient time for the respired $^{14}CO_2$ to be absorbed by the alkali on the wick, which was then removed for counting. The 0.1 ml samples of the medium were removed after the acidification treatment to avoid counting the hydrated $^{14}CO_2$ together with the residual compound. The medium samples were corrected for the volume of the acid. Control vials, lacking Mercierella were incubated and then acidified, and the measured radioactivity used to correct the experimental values for bacterial catabolism.

The scintillation fluor used was toluene and Triton X-100 (Koch-Light) 3 : 1 by volume, containing 4 g of 2,5 Diphenyloxazole (PPO) and 0.3 g of 1,4-Di-(2-(5-phenyloxazolyl))-benzene (POPOP) per litre (Cooksey, 1972). From 5 to 8 ml of this solution was added to each sample, plus 2 ml of 80% ethanol in the case of the medium samples to produce a clear solution. Samples containing Hyamine hydroxide were dark equilibrated for 48 h before counting to avoid chemiluminescence error.

^{14}C labelled glucose and glycine were purchased from the Radiochemical Centre, Amersham. The ^{14}C-D-glucose(U) had a specific activity of 317 mCi $mmol^{-1}$, and the ^{14}C-glycine(U) 112 mCi $mmol^{-1}$.

^{14}C activity was counted in a Packard model 2003 liquid scintillation spectrometer for 1 min. The samples containing Hyamine hydroxide were counted twice at an interval of about 1 h as a check for chemiluminescence. Due to the high levels of radioactivity in the samples, a single count was sufficient to give an accurate indication of the amount of radioactivity present. The results were corrected by the channels ratio method (e.g. Dyer, 1974).

4.3 The influence of antibiotics and the calcareous tube on the uptake rate of ^{14}C-glycine

4.3.1 Introduction

The outside of the tube is commonly colonised by a variety of organisms including bryozoans and algae, that enrich the organic matter and silt trapped in the surface irregularities with the particulate by-products of their metabolism, and in time with their decaying tissues. The organic component supports a considerable bacterial flora which represents a potential source of error in the DOM investigations, since heterotrophic micro-organisms are of major importance in metabolising and recycling the DOM in sea water (see Jørgensen, 1976; Williams et al., 1976).

The inner wall of an intact tube is unlikely to support a large population of organisms because the mucous secretion that lines its surface contains protease enzymes (Zottoli & Carriker, 1974). Should the tube become damaged however, as a result of abrasion or erosion by water action, as commonly happens to the older portions (Straughan, 1972)

detrital material soon accumulates thus producing a suitable site for microbial colonisation. In addition, the chitinous opercular spines trap quantities of organic debris (Fig. 3A) whose thriving bacterial populations attract considerable numbers of ciliates. McIntosh (1926) observed this phenomenon and suggested that many of the organisms which live amongst the debris may be carried into the mouth by the ciliary currents of the branchial crown, thereby inferring that the complex defense structures on the distal surfaces of serpulid opercula serve a feeding function. Whereas McIntosh exaggerated their nutritional role, these do harbour an appreciable number of organisms which could seriously affect the results of DOM studies.

In addition to its endo and epi-fauna and flora, Mercierella's calcareous tube is potentially a barrier to the uptake of DOM, due to its impermeability and because only the anterior end of the worm extends beyond its confines. Little and Gupta (1969) have reported a significant increase in the rate of phenylalanine uptake by the pogonophore, Siboglinum ekmani Jägersten, following detubing, even though in this case the tube proved to be permeable to this amino acid.

It can be appreciated that since numerous organisms are commonly associated with the tube, it is desirable to use detubed animals in the DOM investigations. However, to help transfer the data to environmental conditions it is essential to determine the influence of the tube itself on the accumulation of dissolved organic compounds, and also the effect of pretreatment with antibiotics on the uptake mechanism. For without this information it would be impossible to assess whether the subsequent results are physiologically and ecologically valid.

4.3.2 Materials and Methods

Thirty-six Mercierella, acclimated to 15°C, were removed intact from chalk boulders. The outer surfaces of the tubes were scraped clean of encrusting organisms, detrital material, and fragments of substratum, followed by several rinses in clean water, before they were transferred to finger bowls containing clean water. All specimens exhibited normal activity within a few minutes, indicating that they had suffered no ill effects as a result of the cleansing treatment.

After 24 h, the worms were separated by eye into four groups with similar size distributions. Two groups were detubed, and one of these together with a group of tubed animals were placed in a bath of filtered water containing the antibiotics, streptomycin sulphate (50 mg l^{-1}) and benzylpenicillin (30 mg l^{-1}) (Marshall & Orr, 1955). The two groups of worms were treated in the solution of antibiotics for 24 h; the remaining groups were incubated for 24 h in filtered water lacking antibiotics.

To investigate the effect of antibiotics on the uptake mechanism, it was necessary to ensure that the body surfaces of the worms were first free of all micro-organisms. It was therefore necessary to assume that the rinsing treatment was sufficient to remove any debris adhering to the crown and operculum, and any organisms associated with the integument. It is unlikely that the general body surface would support a large microbial population, because it is normally covered with a mucous secretion which apart from being of low nutritional value, has been shown in some serpulid species to contain protease enzymes (Zottoli & Carriker, 1974). It is possible, however, that some of the organic matter associated with the operculum may not have been removed

by the rinsing treatment, so this has to be considered as a potential source of error in this investigation.

Following the antibiotic treatment, the worms were rinsed in several changes of filtered water, after which they were transferred as groups of 3 individuals to 6 ml aliquots of filtered water containing ^{14}C-glycine, at a concentration of 1.0 µM l^{-1}, in which they were incubated for 1 h. The experimental vials were shrouded with black polythene to exclude light and possible disturbances were avoided during the incubation period.

At the end of the incubation period, the worms were rinsed free of radioactivity adhering to their body surfaces and the tubedspecimens were carefully detubed, taking care to retain the tube fragments for counting. After blotting with paper tissue, the worms were individually weighed, before extracting for 24 h in 80% ethanol, followed by digestion in Hyamine hydroxide. The tube fragments were rinsed for 5 s in distilled water to reduce salt error, before they were air dried to constant weight on tared aluminium foil trays. After weighing to the nearest 0.1 mg on a Cahn RTL Electro-balance, the radioactivity adhering to the individual tubes was extracted in 0.2 ml of Hyamine hydroxide, at 50°C for 48 h.

All samples were corrected for background activity, 23 - 32 disintegrations per minute (d.p.m.). Counting efficiencies were 54 - 67% for the medium samples; 66 - 68% for the ethanol extracts; and 63 - 70% for the Hyamine digests and extracts.

Table 12. The uptake rates of ^{14}C-glycine from a 1.0 µM l^{-1} solution after the four experimental treatments.

Treatment	Number of determinations	Mean uptake rate ($\times 10^4$ d.p.m. indv.$^{-1}$ h^{-1})	± S.D.
Tubed with antibiotics	9	3.86	± 2.28
Tubed without antibiotics	9	3.62	± 2.44
Tubeless with antibiotics	9	3.59	± 1.47
Tubeless without antibiotics	9	3.72	± 1.07

4.3.3 Results

The estimated initial concentrations of labelled glycine for the four treatments : tubed with antibiotics; tubedwithout antibiotics; detubed with antibiotics; and detubed without antibiotics, were respectively, 1.15 $\pm$ 0.15 (S.D.), 1.24 $\pm$ 0.19, 1.02 $\pm$ 0.1, and 1.07 $\pm$ 0.07 $\mu M\ l^{-1}$(each value is based on 3 separate determinations). At the end of the 1 h incubation period, the levels of radioactivity in the experimental vials were reduced by the following amounts: 20 $\pm$ 9.9%; 24.4 $\pm$ 9.3%; 19.4 $\pm$ 12.8%; and 19.1 $\pm$ 10.5%, respectively. Since there is no significant difference between the 4 treatments as to the amounts of radioactivity removed, no correction was made to the uptake rates for the change in the ambient ^{14}C-glycine concentration during the incubation period.

The radioactivity in the ethanol extract and Hyamine digest were combined to give the net uptake of radioactivity $individual^{-1}\ h^{-1}$, i.e. minus the amount catabolised during the incubation period, and the individual uptake values were corrected for weight, using the formula: $\frac{d.p.m.\ indv.^{-1}\ h^{-1}}{weight^{0.67}}$, assuming uptake and weight are related in the form, uptake = kW^a, where k is a constant, W is weight, and a is the slope of the line relating uptake and weight, namely 0.67 (see Section 4.5.4).

Statistical comparison of the mean uptake rates for the four experimental treatments (Table 12), using Student's t-test, showed no significant difference between the rates of ^{14}C-glycine accumulation by the tubed and detubed Mercierella, with or without the pretreatment with antibiotics ($P = > 0.05$). The standard deviations indicate that although there is considerable variability within each treatment, there is, however, significantly less variation in the results for tubeless worms (F-test : $P = < 0.05$).

The amounts of radiation recorded from the decontaminated and non-decontaminated tubes are 8 $\pm$ 3 d.p.m. mg^{-1} dry weight, and 1 216 $\pm$ 369 d.p.m. mg^{-1} dry weight, respectively. This indicates that the vast majority of the radioactivity recorded from the tubes was due to microbial activity and only a relatively small quantity was due to adsorption and capillarity.

4.3.4 Discussion

Pretreatment with antibiotics appears to have no effect on the DOM uptake mechanism. Furthermore, there is no evidence to suggest the presence of appreciable numbers of micro-organisms on the external surfaces of the cleansed worms. However, in view of the organic matter commonly associated with the operculum, and the possibility of contamination with gut flora, it was decided in subsequent experiments to pretreat the worms with antibiotics.

In contrast to the findings of Little and Gupta (1969), detubing has no significant effect on the mean rate of amino acid accumulation. This suggests that the branchial crown is the major uptake site. The crown is structurally suited for this purpose due to its relatively large surface area, rich vascular supply (Section 6.3.3), and abundant cilia which promote a rapid flow of medium across its surface. Although there is a circulation of water around the body of the worm at all times, except when the operculum is tightly closed, this volume is relatively small compared with the amount coming into contact with the extended crown.

Although there is no significant difference between the mean uptake rates for the four groups, the individual uptake rates for the tubed worms exhibit far greater variation when compared with the values

for detubed animals. There is proabably a behavioural explanation for this apparent anomaly.

The results of the activity studies (Section 3.5) show that feeding is a discontinuous process. During periods of inactivity when the crown is withdrawn inside the tube, the latter forms a barrier to the accumulation of DOM, which is further reduced by the concomitant cessation of blood flow to the crown (Section 6.1). This may provide an explanation for the low uptake values obtained for some of the tubed worms. Furthermore, since detubed animals are deprived of this behavioural versatility it would, in part, account for the smaller variation in the values obtained for these animals.

The higher rates of glycine accumulation by some of the tubed Mercierella may have been due to their ability to ingest the mucus produced by the branchial crown. In contrast, detubed worms are incapable of ingesting the mucous strings produced by the crown (Hall, 1954). These subsequently build up as a plug in the centre of the crown, effectively ligaturing the anterior of the worm. The adsorption of DOM to mucus was suggested by Collier et al. (1953) in connection with glucose accumulation by bivalve molluscs, and Stephens and Schinske (1961) described it as a possible mechanism of amino acid uptake in marine invertebrates. Stephens (1962a) demonstrated that ^{14}C-glucose will bind to mucus secreted by the solitary coral, Fungia scutaria. Tubed Mercierella therefore have a mechanism for the accumulation of DOM that is not available to detubed specimens.

In the absence of evidence to suggest that pretreatment with antibiotics, or removal from the calcareous tube, have a significant effect on the mean uptake rate of ^{14}C-glycine by Mercierella, these manipulations need not be regarded as potential sources of error in subsequent DOM uptake experiments.

4.4 The effect of time on ^{14}C-glycine uptake

4.4.1 Introduction

The results of investigations carried out on a variety of marine invertebrates have shown a linear relationship between the accumulation of DOM and the incubation time, during experimental time periods ranging from 180 seconds (Wright et al. 1975), to 30 minutes or longer (Stephens, 1963; Southward & Southward, 1970; Shick, 1975). It is important to establish what relationship exists between the accumulation of dissolved nutrients and time, since this has a bearing on their potential contribution to Mercierella's energy budget. Furthermore, the results of this investigation will determine the length of the incubation periods used in subsequent experiments.

4.4.2 Materials and Methods

Six groups, composed of 4 - 5 worms acclimated to 20°C, selected by eye as having similar size distributions, were exposed to a ^{14}C-glycine solution (c. 2.0 µM l^{-1}) for the following periods of time : 5, 15, 30, 60, 90 and 120 min. Following incubation, the somas were digested with Hyamine hydroxide, and the medium was acidified to drive off the $^{14}CO_2$ which was then collected on alkali wicks. The $^{14}CO_2$ results were corrected for bacterial catabolism.

Background amounted to 23 - 35 d.p.m. Counting efficiencies were 53 - 57% for the medium samples, 48 - 50% for the Hyamine digests, and 57 - 58% for the respired radioactivity.

4.4.3 Results

The initial ^{14}C-glycine concentration in the incubation medium was 2.19 µM l^{-1} (the mean of two determinations that differed by less

Table 13. Summary of the results of the ^{14}C-glycine uptake as a function of time investigation.

Mean wet weight of group	Standard deviation	Number of worms	Exposure time (min)	Animal activity (x 10^5 d.p.m.)	$^{14}CO_2$
5.46	± 3.44	5	5	4.95	0.09
5.72	± 3.07	5	15	8.15	0.25
5.43	± 2.45	5	30	13.47	0.51
5.56	± 2.47	5	60	21.96	1.45
*6.64	± 5.57	4	90	26.01	3.41
5.19	± 2.24	5	120	31.91	4.68

*The 90 min group had a somewhat different size distribution to the rest but a similar total wet weight of tissue.

than 5%). The bacterial controls amounted to less than 1% of the animal respiration after 1 h.

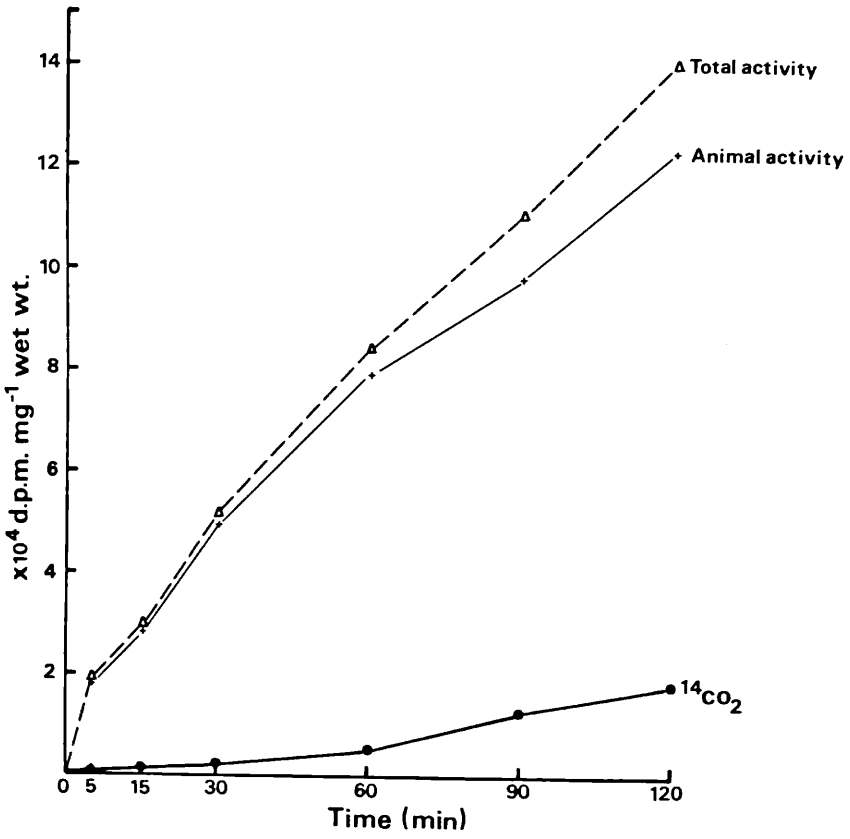
The experimental results are summarised in Table 13. Both the amount of radioactivity accumulated in the body and the respired activity increased as functions of the incubation time. After 120 min, the animals contained 6x the amount of radioactivity accumulated in the first 5 min, and during the same period they respired almost 49x the amount catabolised during the initial period.

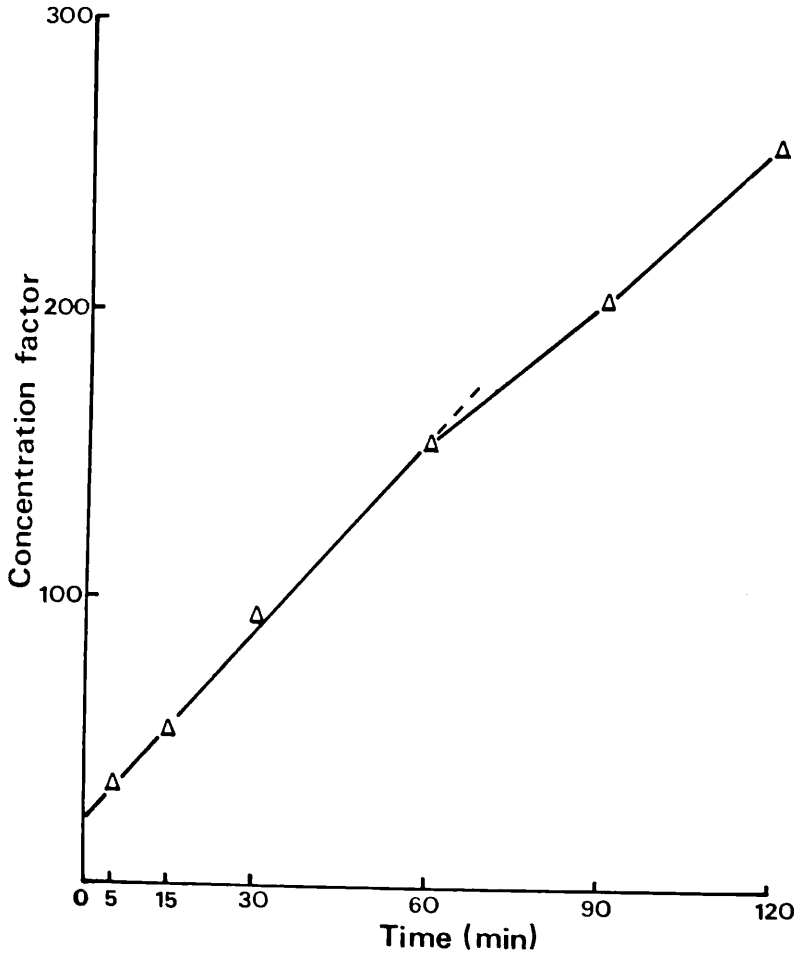
Fig. 32 shows the time course of ^{14}C-glycine incorporation into the animal (radioactivity in the Hyamine hydroxide digest) and respired activity fractions. Although the total activity (Hyamine hydroxide digest plus respired fractions) continued to increase throughout the 120 min exposure period, there was a reduction in its rate of incorporation into the animal fraction after 30 min, resulting in this fraction containing 87% of the total uptake after 120 min, compared with 98% after 5 min. In contrast, the respired activity showed a marked increase after 30 min. This probably reflects the time lag between absorption of ^{14}C-glycine and its catabolism to $^{14}CO_2$.

Fig. 33 shows the concentration factor, expressed as a fraction of the incubation time. The concentration factor is the ratio of the d.p.m. mg^{-1} of worm to d.p.m. μl^{-1} of medium. Total uptake (animal activity plus respired activity) was used in the calculation. There is a linear relationship between the total uptake and time for at least 60 min. However, this was followed by an apparent reduction in the rate of ^{14}C-glycine accumulation, which resulted in the concentration factor after 120 min being only 84% of the value predicted from the results for the first 60 min.

Fig. 32. Time course of ^{14}C-glycine incorporation into the animal (radioactivity recovered from the worm tissues) and respired activity fractions. The initial ambient concentration was 2 μM l^{-1}, and each point is based upon the total uptake of four or five animals.

Fig. 33. Relationship of total uptake (animal activity plus respired activity), expressed as a concentration factor (the ratio of the d.p.m. mg^{-1} of worm to d.p.m. μl^{-1} of medium), to time of exposure. The regression line was fitted by eye estimation.


Δ Total activity
+ Animal activity
$^{14}CO_2$
x10⁴ d.p.m. mg⁻¹ wet wt.
Time (min)
0 5 15 30 60 90 120
2 4 6 8 10 12 14


Concentration factor
Time (min)
0 5 15 30 60 90 120
100 200 300

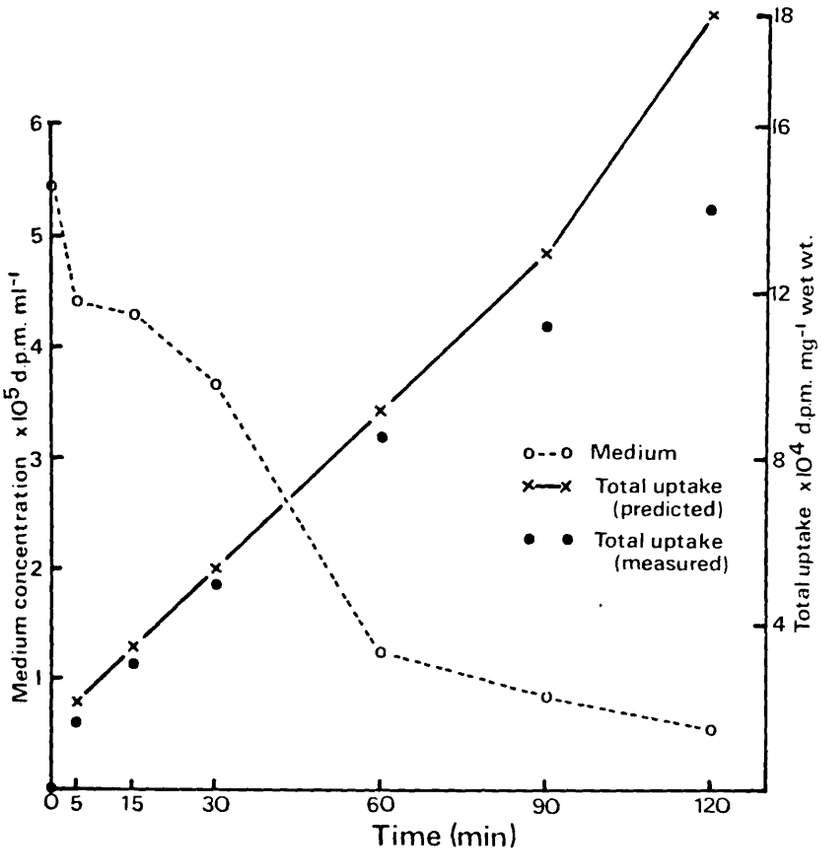
During the 120 min incubation period, the radioactivity in the medium was rapidly depleted (Fig. 34), so the apparent reduction in the rate of uptake could have resulted from the effect of a declining ambient ^{14}C-glycine concentration on the accumulation processes (Section 4.6). However, when a correction is applied to the total uptake values for each of the incubation periods, based on the percentage recovery of radioactivity from the animals, the respired fraction and the ambient medium, the calculated uptake values remained linear for at least 90 min (Fig. 34, predicted values). This indicates that the apparent reduction could have resulted from a progressively greater proportion of the accumulated label entering a fraction that was less effectively determined. Since a progressively greater proportion of the label was entering the $^{14}CO_2$ fraction, which because of its volatile nature is the most likely component to be underestimated, this has to be considered as at least a partial explanation for the apparent reduction in ^{14}C-glycine accumulation after 60 min.

4.4.4 Discussion

The results of this experiment show that the accumulation of ^{14}C-glycine by Mercierella is linear against time for a minimum of 60 min, and is therefore in accordance with the reported relationship for a variety of other soft-bodied marine invertebrates, including bivalve mollusc tissues, cnidaria, and pogonophores. This suggests that the uptake of dissolved nutrients by Mercierella is continuous, thereby conferring the process with the potential for being a supplementary nutritional mechanism.

The positive shift of the extrapolated line (Fig. 33) away from the origin toward a positive intercept on the ordinate, may have been due to a more rapid initial uptake rate. This phenomenon

Fig. 34. The depletion of radioactivity in the incubation medium by five M. enigmatica. The left ordinate is the amount of radioactivity remaining in the medium; the right ordinate is the amount of radioactivity absorbed by the worms.


Medium concentration x10^5 d.p.m. ml^-1
Total uptake x10^4 d.p.m. mg^-1 wet wt.
Time (min)
0 5 15 30 60 90 120
o--o Medium
x—x Total uptake (predicted)
• • Total uptake (measured)

was also observed in a preliminary experiment using groups of smaller animals (2.3 $\pm$ 1.4 mg wetweight).

Shick (1975) showed a linear uptake of ^{14}C-glycine by Aurelia aurita (L.) syphistomae, for at least 120 min, in which there was an initially higher rate of accumulation during the first 5 min. Stephens (1963) presented ^{14}C-glycine uptake data for the bamboo worm, Clymenella torquata, to support his claim that accumulation was linear against time for the first 30 min. However, Stephens' data are more suggestive of a curvilinear relationship with a positive intercept with the ordinate, thus implying a higher initial rate of uptake. In contrast, Wright et al.(1975) showed a linear relationship for ^{14}C-cycloleucine uptake by the isolated gill tissues of the mussel, Mytilus californianus Conrad, in which the extrapolated line intercepted the origin. Apart from their use of isolated organs, rather than whole animals, Wright et al.'s experiments were carried out using much shorter exposure times (15 - 180 s).

This difference between the results of the short-term and long-term experiments may be due to some intrinsic property of the uptake mechanism. However, an alternative explanation for the failure of the extrapolated line to intercept with the ordinate, is the adsorption of some of the labelled compound to the mucous secretions on the external surfaces of the body, which is supported by the results of the previous experiment (Section 4.3.4), and the findings of Stephens (1962a).

4.5 The effect of body size on ^{14}C-glycine uptake

4.5.1 Introduction

Due to the natural size variation within the population (Section 8.1), it was necessary to use worms of a range of sizes in the experiments. Attempts at selecting animals of a similar size were usually prevented by the difficulty in obtaining accurate measurements of the lengths of tubes that were intertwined with others (Section 8.1.3). Furthermore, the detubing of a large number of individuals in order to obtain a sufficient quantity of similar sized animals for experimental purposes would have resulted in a considerable wastage, due to their subsequent inability to feed on particulate matter.

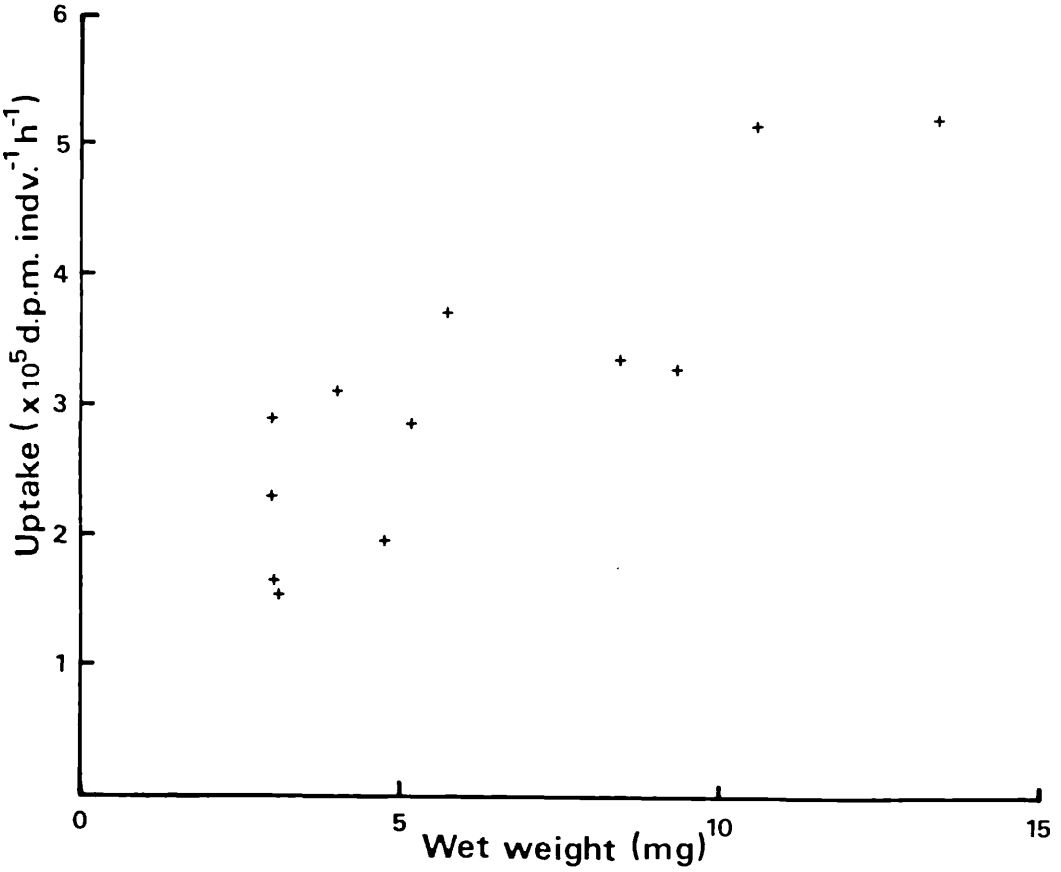
It has been generally observed that body size has a significant effect on a variety of physiological processes (e.g. Zeuthen, 1953; Hemmingsen, 1950, 1960; Dejours, 1975; Schmidt-Nielsen, 1975), including the accumulation of amino acids by polychaete worms (e.g. Stephens, 1963, 1964; Reish & Stephens, 1969). It was therefore important to determine the relationship between body size and uptake of DOM by Mercierella, so that this could be taken into account when analysing accumulation data with respect to other variables.

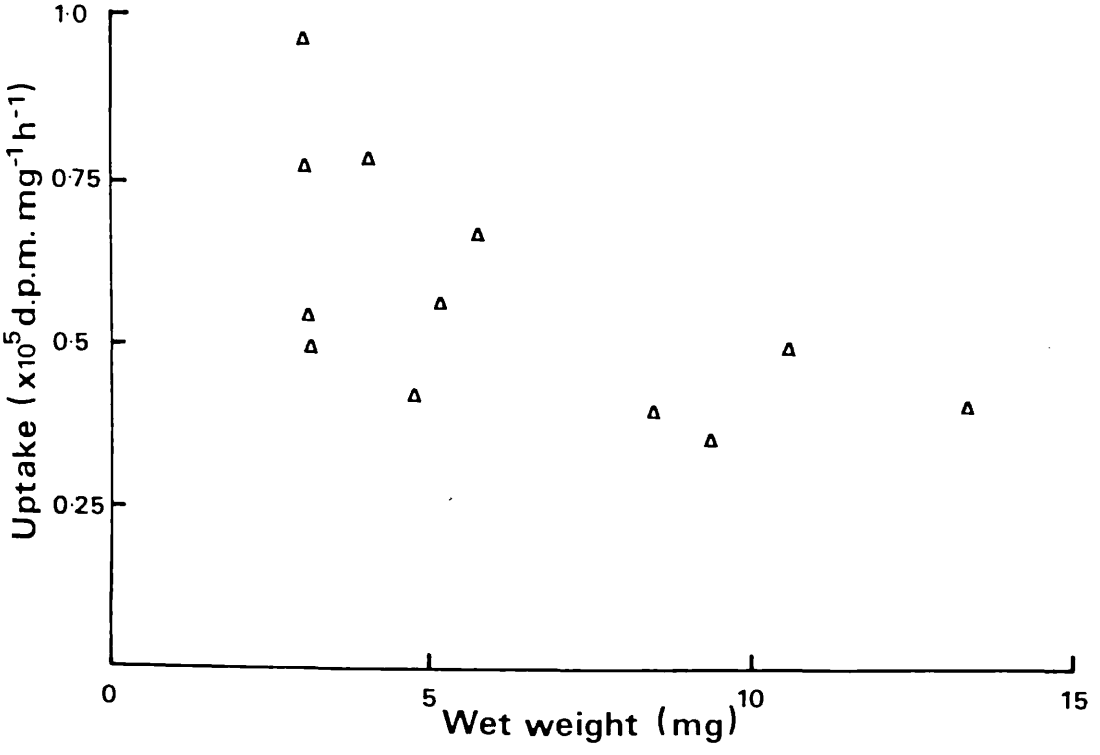
4.5.2 Materials and Methods

Mercierella, acclimated to 15°C and representing the full sample size range, were incubated for 1 h, in a ^{14}C-glycine solution with an initial concentration of 1.88 µM l^{-1} (mean of two determinations). A 1 h incubation period was chosen because ^{14}C-glycine uptake is linear for a minimum of 60 min (Section 4.4.3).

Fig. 35. The rate of uptake of ^{14}C-glycine expressed as a function of wet weight, from an initial labelled concentration of *c.* 1.9 μM l^{-1}.

Fig. 36. The specific rate of ^{14}C-glycine uptake (mg^{-1}) expressed as a function of wet weight. Experiment carried out at 15°C.


Uptake (x 10^5 d.p.m. indv.^-1 h^-1)
6
5
4
3
2
1
0
5
10
15
Wet weight (mg)


Uptake (x10^5 d.p.m. mg^-1 h^-1)
1·0
0·75
0·5
0·25
0
5
10
15
Wet weight (mg)

Following the incubation period, the worms were extracted individually in 0.1 ml volumes of 80% ethanol for 24 h, before the somas were digested with 0.2 ml of Hyamine hydroxide. These values were combined to give the net uptake of radioactivity; no attempt was made to measure the radioactivity that was lost from the worms as $^{14}CO_2$.

Counting efficiencies were : 64 - 69% for the ethanol extracts; 62 - 66% for the Hyamine digests; and 55 - 67% for the medium samples. Background amounted to 23 - 35 d.p.m.

4.5.3 Results

Fig. 35 shows the individual net uptake values expressed as a function of wet weight. Despite the considerable variability in these data, it is evident that there is a positive relationship between the rate of ^{14}C-glycine uptake and body size, with the largest worms accumulating c. 2.5x the amount absorbed by the smallest individuals. There is however a reduction in the specific uptake rate (per unit weight) with increasing size. Fig. 36, in which the specific rate of ^{14}C-glycine accumulation is expressed as a function of wet weight, demonstrates this more clearly. The smaller worms have specific uptake rates more than two times greater than those of the larger animals.

4.5.4 Discussion

The results show that ^{14}C-glycine uptake by Mercierella complies with the generally observed relationship between the rates of physiological processes and body size. The smaller worms having higher specific accumulation rates when compared with larger animals.

Table 14. The slopes of the regression lines for the log of rate of ^{14}C-glycine uptake expressed as a function of the log of wet weight, at three ambient concentrations.

Initial concentration ($\mu M\ l^{-1}$)	Number of measurements	Slope a	Correlation coefficient r	P
0.91	8	0.6	0.84	<0.01
1.88	12	0.59	0.81	<0.01
9.94	8	0.59	0.92	<0.01

Stephens (1963) showed that the relationship between the rate of amino acid accumulation and wet weight of the bamboo worm, Clymenella torquata, could be described by the equation, uptake = kW^a, where k and a are constants, and W is the wet weight. The values of a and k are obtained from a regression line calculated for the logarithm of the accumulated radioactivity expressed as a function of the logarithm of wet weight. The slope of this line is equivalent to the value of a, and the intercept to that of k.

Table 14 shows the values of a at three different medium concentrations; for further details of the method see Section 4.6. There is a highly significant correlation between the ^{14}C-glycine accumulation rate and body size at all three ambient concentrations. These experimentally derived values for a are in fair agreement with the expected 0.67, should there be a surface relationship between the two parameters (see any general textbook of animal physiology, e.g. Schmidt-Nielsen, 1975). This seems to be a reasonable assumption in view of the unequivocal evidence supporting an integumentary transport mechanism (Section 4.3.4).

For ease of calculation, an exponent of 0.67 was used in the formula, weight corrected ^{14}C-glycine uptake rate = $\frac{\text{uptake indv.}^{-1}}{\text{weight}^{0.67}}$. Reish and Stephens (1969), reported a similar surface relationship between the rate of ^{14}C-glycine accumulation and wet weight of the polychaete, Neanthes arenaceodentata (Moore).

4.6 The effect of the ambient concentration on ^{14}C-glycine uptake

4.6.1 Introduction

This investigation was carried out to determine the effect of the ambient ^{14}C-glycine concentration on the accumulation rate. Since

Stephens (1962a) suggested that the relationship between the uptake rate of ^{14}C-glucose by Fungia scutaria and the surrounding concentration could be formally treated like an enzyme-catalyzed reaction, it has been general practice to describe the uptake process in terms of the Michaelis-Menten, or Briggs-Haldane equation. Although the generally observed relationship has saturation kinetics similar to enzyme catalyzed reactions, it remains to be demonstrated that the process is enzyme mediated. With this reservation, the expression of this relationship in Michaelis-Menten terms, Vmax, the maximum attainable rate of reaction (uptake), and Km, the Michaelis-Menten constant or the substrate concentration at which the rate of uptake is half the maximum, provides a useful means of comparison with the uptake processes of other organisms.

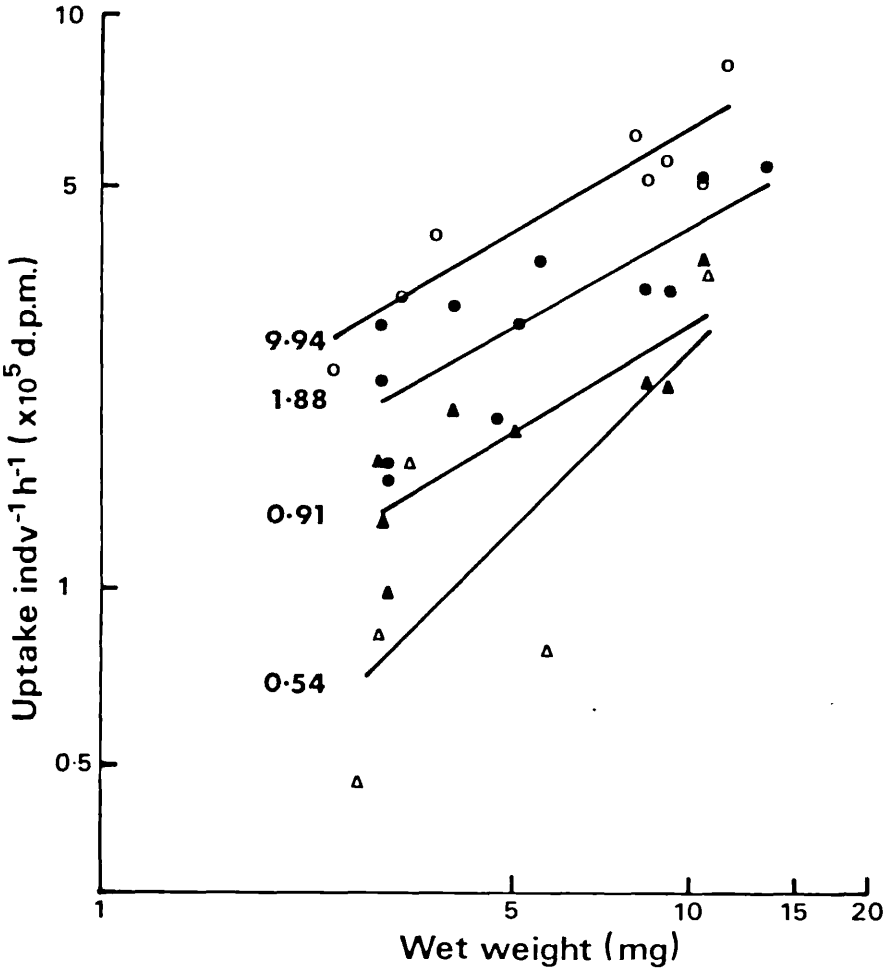
4.6.2 Materials and Methods

Groups of 4 - 5 Mercierella, acclimated to 15°C, were incubated for 1 h in 10 ml aliquots of the following ^{14}C-glycine concentrations, 0.54, 0.91, 1.88, and 9.94 μM l^{-1}.

After the incubation period, the worms were extracted individually in 0.1 ml volumes of 80% ethanol for 24 h, before digesting with 0.2 ml volumes of Hyamine hydroxide. No measurements were made of the radioactivity in respired CO_2, consequently the individual values represent the net uptake of ^{14}C-glycine.

Counting efficiencies were 64 - 69% for the ethanol extracts, 62 - 66% (with a single value of 73%) for the Hyamine hydroxide digests, and 53 - 67% for the medium samples. Background amounted to 23 - 35 d.p.m.

Fig. 37. The relationship between the rate of ^{14}C-glycine uptake and wet weight at four medium concentrations. The figures refer to the initial, ambient, labelled concentration, in $\mu M\ l^{-1}$.


10
5
1
0·5
Uptake indv⁻¹ h⁻¹ (x10⁵ d.p.m.)
9·94
1·88
0·91
0·54
1
5
10
15
20
Wet weight (mg)

4.6.3 Results

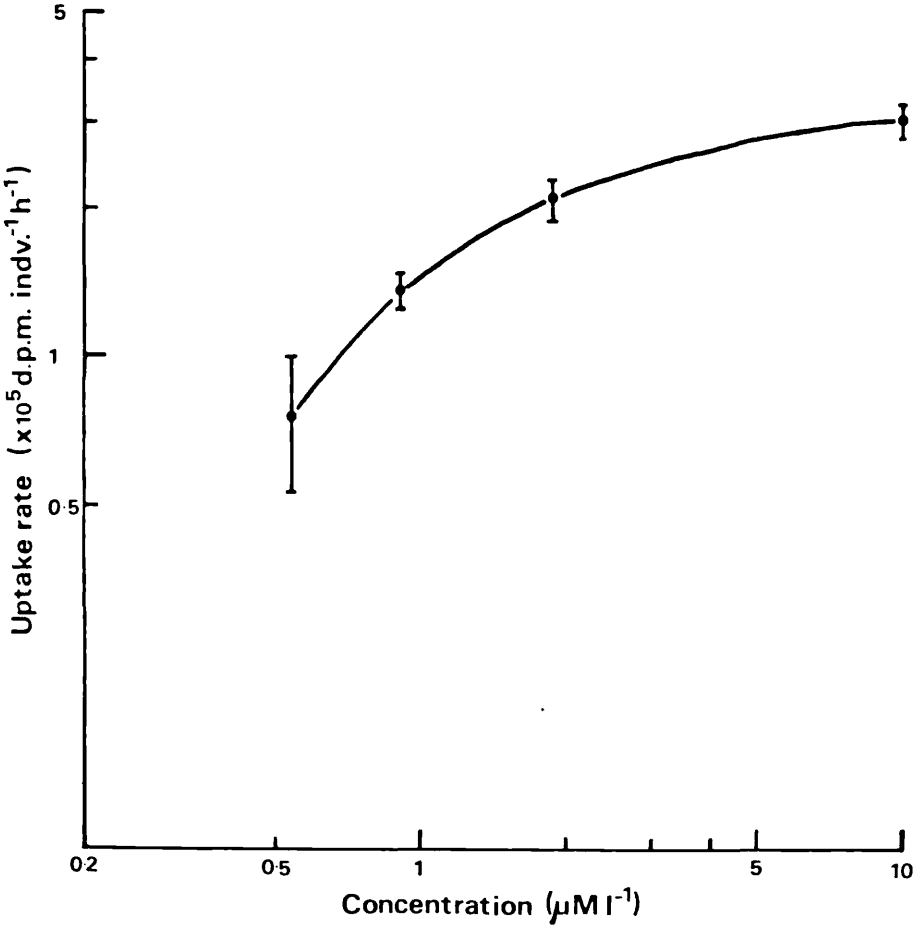
Fig. 37 shows the relationship between the rate of ^{14}C-glycine uptake and weight at four medium concentrations. With the exception of the lowest concentration, there is a marked similarity in the relationships between the two parameters. However, since the correlation coefficient for the lowest concentration was not significant (r = 0.75; P => 0.1) for an N value of just 5 worms, which contrasts with the highly significant relationships at the three higher concentrations (Table 14), the difference in slope probably has no biological significance.

Whilst the effect of raising the ambient ^{14}C-glycine concentration is to increase the rate of accumulation, the uptake mechanism exhibits a tendency to become saturated at the highest ambient concentration. To make this clearer, the accumulation of radioactivity by a worm of 3.0 mg wet weight was plotted as a function of the initial ambient concentration (Fig.38). It is evident that a limit to the accumulation rate is approached at the highest concentration, 9.94 µM l^{-1}. This is in accordance with the relationship between the uptake of DOM and the ambient concentration, reported for a variety of other marine invertebrates, (e.g. Stephens, 1964; Southward & Southward, 1970; Wright, Johnson & Crowe, 1975; for further references see the comprehensive review by Jørgensen, 1976), and indicates that the transport mechanism has a restricted transport capacity.

4.6.4 Discussion

The relationship between ^{14}C-glycine uptake by Mercierella and the concentration in the medium takes the form of a rectangular hyperbola, since the uptake rate does not increase linearly with

Fig. 38. Relationship of the rate of uptake of ^{14}C-glycine to ambient concentration. Each point is the mean value for an individual M. enigmatica of 3.0 mg wet weight. The vertical lines show one standard deviation on each side of the mean.


5
1
0·5
Uptake rate (x10⁵d.p.m. indv.⁻¹h⁻¹)
0·2
0·5
1
5
10
Concentration (μM l⁻¹)

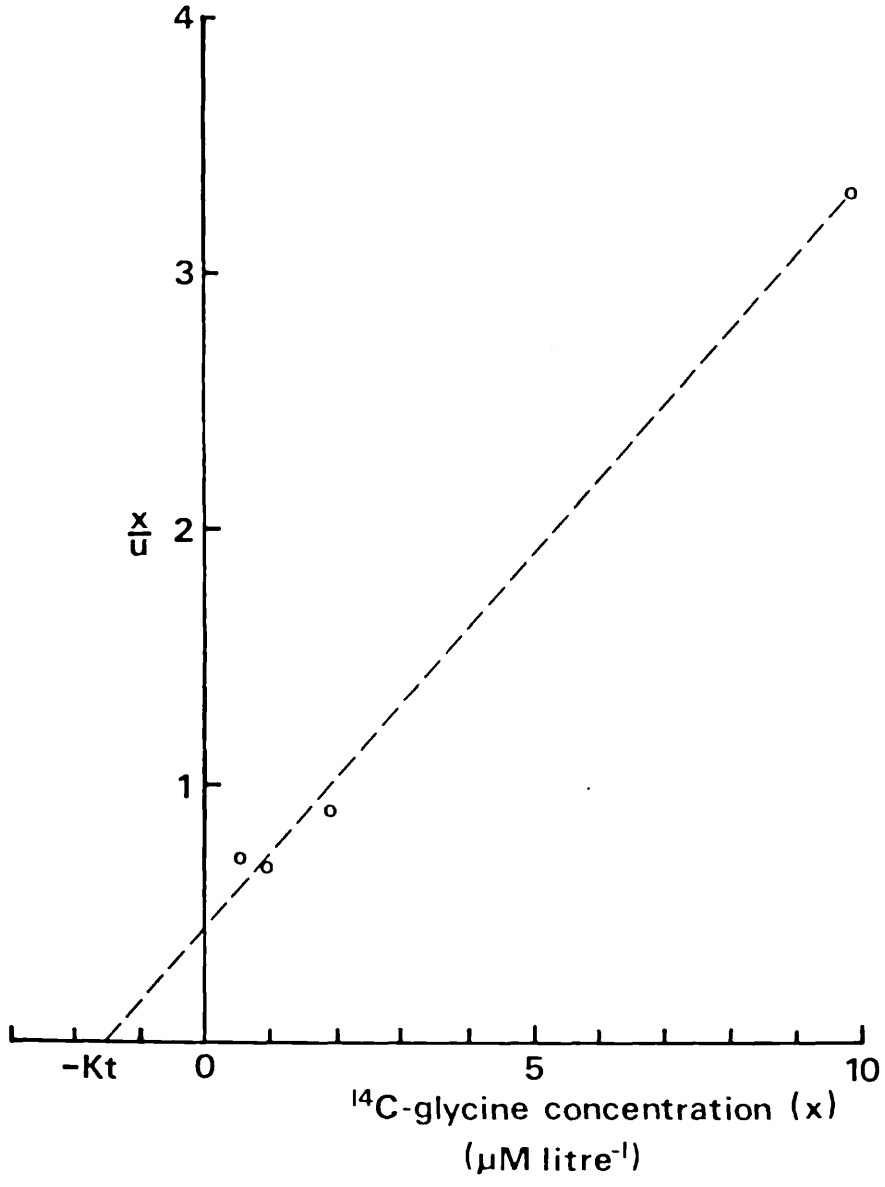
increases in the ambient concentration, rising sharply at low concentrations, followed by a progressive decrease in slope to the highest concentration.

For ease of comparison with the data from other species, the relationship is expressed in terms of the Michaelis-Menten equation: $U = \frac{Vmax.\ x}{x + Kt}$, where U is the initial uptake rate, Vmax is the maximum attainable uptake rate, x is the concentration of the compound in solution, and Kt is a modified form of the constant, Km, and refers to the value of x where $U = \frac{Vmax}{2}$. The Michaelis-Menten constant Km should be reserved for enzyme reactions (see Southward & Southward, 1972).

It has been general practice in DOM uptake studies, _ _ graphically to determine the values of Vmax and Kt from a reciprocal transformation (Lineweaver-Burke plot) of the uptake versus concentration data. This involves transposing the equation to the form $\frac{1}{Vmax} = \frac{x + Kt}{Vmax.x} = \frac{Kt}{Vmax - x}$, and plotting $\frac{1}{U}$ against $\frac{1}{x}$ which gives a straight line of slope $\frac{Kt}{Vmax}$ and intercept $\frac{1}{Vmax}$. Although the equation in this form provides a simple method for the determination of the constants, there is the disadvantage that disproportionate emphasis is placed on the rate of uptake at the lower medium concentrations, thus yielding unreliable kinetic parameters (Neame & Richards, 1972).

Following the example set by Neame and Richards, a linear transformation of the Michaelis-Menten equation was applied to the uptake data for Mercierella. This method does not distort the relationship in any way. Fig. 39 shows how the Vmax and Kt values were determined by plotting $\frac{x}{U}$ against x (Woolf plot). The relationship between the two parameters is highly significant ($r = 0.99$, $P = < 0.01$).

Fig. 39. ^{14}C-glycine accumulation data presented in a linear transformation of the Michaelis-Menten equation (Woolf plot). The abscissal intercept is equivalent to -Kt, and the slope of the line to $\frac{1}{Vmax}$. The regression line was fitted by the method of least squares, $\underline{r} = 0.99$, $\hat{Y} = 0.44 + 0.29X$. Kt = 1.55 µM l^{-1}, Vmax = 3.4×10^5 d.p.m. indv.$^{-1}$ h^{-1}.


4
3
2
1
x/u
-Kt
0
5
10
^{14}C-glycine concentration (x)
(μM litre^{-1})

The slope of the regression line, calculated by the method of least squares, is equivalent to $\frac{1}{Vmax}$, and the abscissal intercept to -Kt.

Table 15 shows the amino acid transport constants for several polychaete species, including errant and sedentary forms. Although it is evident that there is considerable variation in the Vmax and Kt values, in general the systems appear to have low transport capacities.

Mercierella appears to have the lowest Kt value, which is only approached by the values reported for Nereis diversicolor, which is commonly found in association with Mercierella clumps at Greenhithe. In contrast, Clymenella, the only strictly burrowing species mentioned, has the highest transport constants, whereas the nereids which divide their time between a sedentary and errant existence have intermediate Kt values.

The reported Kt values are high, when compared with the amino acid levels in sea water (for references see Southward & Southward, 1972; and the critical review by Jørgensen, 1976). The amino acid concentration in unpolluted oceanic sea water seldom exceeds 1.0 μM l^{-1}, although the usual amount present in coastal waters is several times greater. Stephens (1962b, 1975a,b, and personal communication, 1975) recognised the apparent discrepancy between the transport constants of marine invertebrates, and the natural levels of DOM, and has carried out a series of investigations of the naturally occurring concentrations in the interstitial water surrounding the burrows of tubicolous polychaetes. Stephens has found that these are considerably more in accordance with the measured transport constants. This suggests that the Kt values for the polychaete examples quoted above, may be indicative of the natural environmental concentrations to which the worms are normally exposed.

Table 15. Amino acid transport constants for several polychaetes.

Species	Compound	Vmax ($\mu M\ gh^{-1}$)	Kt ($\mu M\ l^{-1}$)	Reference
Clymenella torquata	Lysine	3.64	120	Stephens (1963)
	Valine	4.35	270	
	Glycine	10.35	200	
	Phenyl-alanine	3.53	50	
Mercierella enigmatica	Glycine	0.46	1.55	
Nereis diversicolor Müller	Mixed amino acids	0.08 - 0.21	1.6 - 14	Southward & Southward (1972)
Nereis limnicola Johnson	Glycine	0.12	37.5	Stephens (1964)
Nereis succinea Frey & Leuckart	Glycine	1.44	168.5	" "
Nereis virens Sars	Glutamic acid	0.2	43.5	Taylor (1969)

Stephens (quoted in Wright et al. 1975) has suggested that there is a selective advantage for the transport mechanism to be synchronised with the naturally occurring levels of DOM. This is supported by Southward and Southward's (1972) observation that a variety of transport systems, including those of pogonophores and the vertebrate gut, are adapted to operate at the naturally occurring concentrations of dissolved nutrients. Mercierella, whose transport system appears to be adapted to function at the lowest ambient concentration, which incidentally is in accordance with the expected glycine level in the organically polluted estuary, is the only member of the group of worms presented in Table 15, in which the body is not exposed for at least part of the time to direct contact with the interstitial water surrounding the particles of sediment.

Further evidence for the postulated relationship between the transport kinetics and the natural environmental concentration is provided by the results of Stephens' (1975b) study of primary amine accumulation by the sedentary Capitella capitata (Fabricius) and Nereis diversicolor. Stephens found that in contrast to the uptake of glycine which is similar in both worms (expressed as $\mu M\ gh^{-1}$), the rates of accumulation of glutamate and aspartate by Capitella are an order of magnitude higher than those of Nereis from an ambient concentration of 50 $\mu M\ l^{-1}$. Furthermore, the average hourly influx of total primary amines is about a third higher in the case of Capitella. The similar values for glycine accumulation by these two worms show that the differences in uptake are not due to size, the Capitella used in these experiments were less than one tenth the weight of the Nereis, but instead are related to the natural environmental concentrations to which these animals are normally exposed.

In addition to the effect of concentration on the uptake of DOM, it is interesting to note that Mercierella appears to have a higher rate of accumulation than is reported for N. diversicolor, which could be related to their dissimilar body forms. Mercierella with its finely divided branchial crown, has a relatively greater surface area, than has the more vermiform Nereis. However, since these investigations were carried out with different compounds and under different experimental conditions, the cause of the observed difference awaits unequivocal verification.

4.7 The effect of temperature on ^{14}C-glycine uptake

4.7.1 Introduction

Since the uptake process has saturation kinetics similar to an enzyme catalyzed reaction (Section 4.6.4), an investigation of the effect of temperature on the accumulation rate seems a natural sequel. Compared with what is known about the effect of concentration, there are surprisingly few accounts dealing with temperature (Stephens, 1962a, 1962c; Reish & Stephens, 1969; Stephens, 1975a; Shick, 1975), especially since the majority of the DOM investigations have concerned eurythermal organisms.

In the Thames estuary, Mercierella experiences diurnal and annual fluctuations in temperature (Section 2.3.4), which have been shown to have a major influence on its particulate feeding rate (Section 3.4), growth rate (Section 8.1.3), reproduction (Section 8.2.4), and respiration rate (Section 6.7). It is important, therefore, to determine the effect of temperature on the uptake process, because a proper evaluation of its potential nutritional contribution cannot be made until an investigation of the effects of the various environmental factors has been carried out.

4.7.2 Materials and Methods

Two groups of 10 Mercierella (0.67 - 14.1 mg wet wt.), acclimated to winter and summer water temperatures for Greenhithe (10°C and 20°C), were exposed for 1 h to a 6.4 µM l^{-1} ^{14}C-glycine solution at the appropriate acclimation temperature.

After the incubation period, the worms were extracted individually in 0.1 ml of 80% ethanol for 24 h, before digesting in 0.2 ml of Hyamine hydroxide. No attempt was made to determine the amounts of radioactivity in respired CO_2. The radioactivity measurements for each worm were combined to give the net uptake of ^{14}C-glycine per hour.

Counting efficiencies were 64 - 67% for the ethanol extracts; 55 - 63% for the Hyamine hydroxide digests; and 57 - 58% for the medium samples. Background amounted to 24 - 35 d.p.m.

4.7.3 Results

The initial ^{14}C-glycine concentration in the medium was 6.45 µM l^{-1}, the mean value of two determinations that differed by less than 0.4% of this amount. Only one measurement of the final medium concentration, for a 20°C sample was made. The percentage recovery of radioactivity, based on this final concentration of 4.22 µM l^{-1}, plus the animal (combined radioactivity in the ethanol extract and Hyamine digest) activities, was 87%, indicating that an appreciable proportion of the radioactivity had been respired as $^{14}CO_2$.

The individual results were corrected for weight using the method described in Section 4.5.4. The mean ^{14}C-glycine accumulation rates at 10°C and 20°C, are presented in Table 16. The large standard deviations indicate considerable variation in individual uptake rates

Table 16. Weight-corrected ^{14}C-glycine uptake rates for M. enigmatica acclimated to winter and summer temperatures, from an initial ambient concentration of 6.4 µM l^{-1}.

Acclimation temperature	Number of worms	Mean uptake rate (x 10^5 d.p.m. $individual^{-1}$ h^{-1})	Standard deviation
10°C	10	0.96	± 0.5
20°C	10	1.32	± 0.58

at the two temperatures. A comparison of these values using Student's t-test, showed that there is no significant difference between the individual hourly uptake rates of ^{14}C-glycine by Mercierella at the winter and summer temperatures. The calculated value of t, 1.5, is somewhat less than the tabulated value at the P = 0.1 level of significance. However, there is a tendency for the uptake rates at 10°C, to be lower than those at the higher temperature.

4.7.4 Discussion

A temperature coefficient of 1.38 for the temperature range, 10 - 20°C, demonstrates that the uptake of ^{14}C-glycine by Mercierella is insensitive to temperature change over the natural environmental range. Furthermore, the lack of a significant difference between the uptake rates at the winter and summer temperatures, indicates that in some instances thermal compensation was complete. This deduction may be false, however, for only one ^{14}C-glycine concentration was used. It is possible that there may be an alteration of the slope in the relationship relating uptake and ambient concentration (Fig. 38) in response to a change in temperature, that could be overlooked using a single relatively high $(6.4\ \mu M\ l^{-1})$ glycine concentration. Recognising this potential source of error (which seems to have been overlooked by other authors) it was decided to limit any discussion to the reported concentrations.

The results of the present investigation are in accordance with the responses to temperature change reported for the DOM uptake mechanisms of several other marine invertebrates. Stephens (1962a) reported a temperature coefficient of between 1.19 and 1.36 for the uptake of glucose by the solitary coral, Fungia scutaria, from an

initial concentration of about 2.0 μM l^{-1}, for the abnormally high temperature range, 20 - 35°C. He also calculated a Q_{10} of 1.7 (5 - 25°C) for phenylalanine uptake by the maldanid polychaete, Clymenella (Stephens, 1962c), and temperature coefficients ranging from 1.5 - 2.0 for other amino acids (unpublished data referred to by Reish & Stephens, 1969). Stephens (1975a) also showed that Arenicola marina (L.) exhibited little difference in amino acid uptake over a temperature range of 10°C.

The most detailed study to date of temperature effects on the rate of DOM uptake, is that of Shick (1975) on the accumulation of ^{14}C-glycine by Aurelia aurita scyphistomae. Shick reported temperature coefficients ranging from 0.8 for the temperature interval, 30 - 35°C, to 15.9 and 5.4 for the intervals, 12 - 15°C and 15 - 20°C, respectively. Over the intermediate range, 20 - 30°C, the Q_{10} was about 2.0. From these results, Shick concluded that the scyphistomae were showing extreme thermal sensitivity above the lower lethal limit, reduced sensitivity at intermediate temperatures, and insensitivity as the incipient high lethal level was approached. Furthermore, he found no difference in this relationship for starved animals.

Although there is a need for further investigation into the effects of acute temperature fluctuations on the uptake process, it is evident that the thermal insensitivity over Mercierella's natural environmental range, may reflect a regulatory mechanism, analogous to that described for the standard respiration rates of some intertidal marine invertebrates (see Newell, 1973; Pye & Newell, 1973).

Apparent thermal insensitivity does not exclude the possibility that the transport of DOM across Mercierella's integument is enzyme mediated, because a number of pure isolated enzyme systems are known

to exhibit temperature insensitive rates of catalysis at physiological substrate concentrations (for reviews see Hochachka & Somero, 1968; Somero & Hochachka, 1969; Somero, 1969, quoted in Pye & Newell, 1973; Hochachka & Somero, 1973, quoted in Shick, 1975).

Although the exact nature of the transport mechanism is unknown, there is evidence which suggests that it is not a decrease in 'enzyme'-substrate affinity that is causing the effect. Stephens (1962a) reported no apparent change in the temperature coefficient when, in an attempt to demonstrate that glucose uptake by Fungia was not limited by diffusion, he increased the ambient concentration by about 270-fold. This appears to indicate that it is some other factor, possibly the amount of carrier substance or the supply of energy, that appears to be limiting the reaction.

It is believed that a low temperature coefficient for the standard metabolic rate of intertidal marine invertebrates has a considerable selective advantage, since it prevents the rapid depletion of the metabolic energy reserves during the period that these organisms are exposed to high air temperatures. However, whilst the similarity of DOM uptake with respiratory phenomena is reassuring, the role of the low temperature coefficient cannot be similarly explained.

Whilst it remains to be shown whether the uptake of DOM has any nutritional value to Mercierella, an obvious result of the mechanism's relative insensitivity to temperature, in the light of the measurements of respiration rate (Section 6.7), is its potentially greater contribution to the animal's energy requirement during the winter period, when the metabolic rate is considerably reduced.

4.8 The distribution of the radioactivity following ^{14}C-glycine uptake

4.8.1. Introduction

In the experiments just described, the distribution of the accumulated radioactivity within Mercierella's body was not considered. In addition to their involvement in the anabolic and catabolic processes, amino acids are known to play an important role in cellular osmoregulation in brackish water and marine invertebrates (Clark, 1968a, b; Oglesby, 1969 (a general review of polychaete osmoregulation); Pierce & Greenberg, 1973). It is important therefore, to determine the fate of ^{14}C-glycine following its absorption, before an attempt is made to evaluate its possible nutritional contribution.

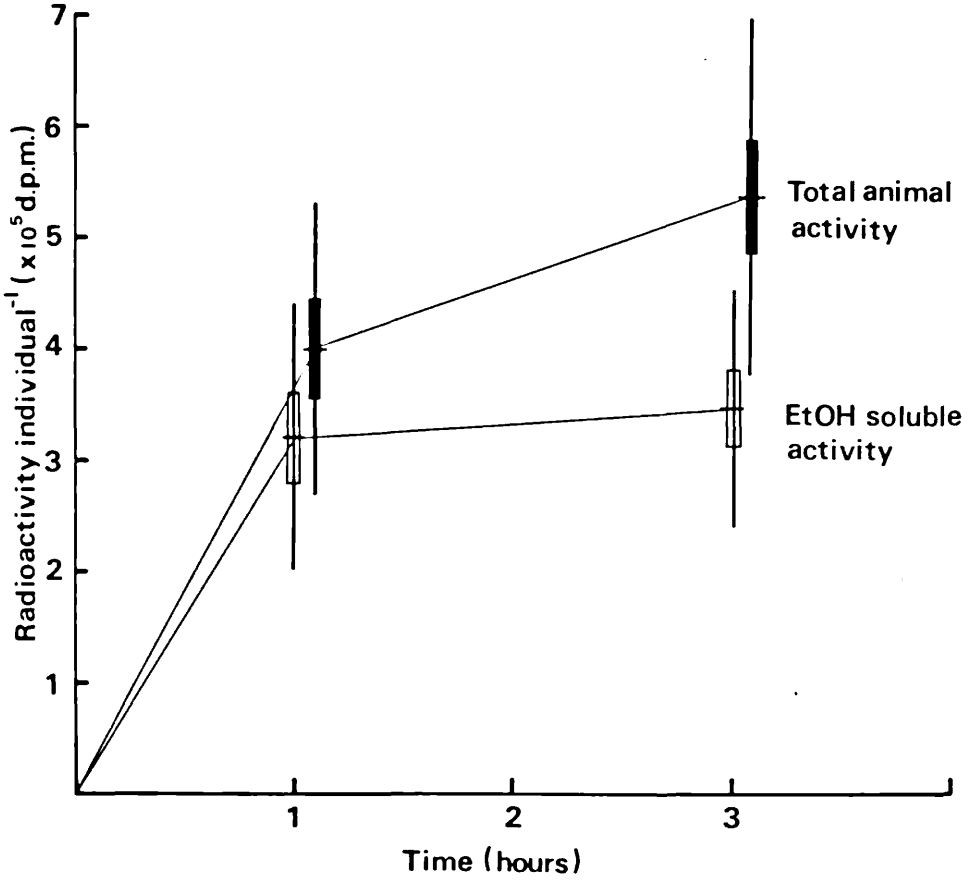
4.8.2. Materials and Methods

Two groups composed of 9 and 10 worms, acclimated to 15°C, were incubated individually for 1 and 3 h respectively, in 2 ml of filtered and diluted sea water containing ^{14}C-glycine, at an initial concentration of about 5.0 µM l^{-1}. At the end of the incubation period, the worms were extracted for two 24 h periods in 0.1 ml volumes of 80% ethanol, before digesting in 0.2 ml volumes of Hyamine hydroxide. The medium was acidified following the incubation period, and the respired $^{14}CO_2$ was collected for counting.

Counting efficiences were 63 - 67% for the ethanol extracts; 61 - 66% for the Hyamine digests; 57 - 65% for the Hyamine impregnated wicks; and 57% for the medium samples.

Since no measurements were made of the amounts of radioactivity remaining in the media after acidification, the quantities were calculated by difference.

Fig. 40. Uptake of ^{14}C-glycine into the ethanol soluble fraction (EtOH soluble) from a 5.0 µM l^{-1} solution, compared with the total animal activity after 1 and 3 h exposures. The points represent the mean values of nine 1 h, and ten 3 h measurements, vertical bars $\pm$ 1 standard error of the mean and vertical lines $\pm$ 1 standard deviation.


Radioactivity individual^{-1} (x 10^5 d.p.m.)
7
6
5
4
3
2
1
1
2
3
Time (hours)
Total animal activity
EtOH soluble activity

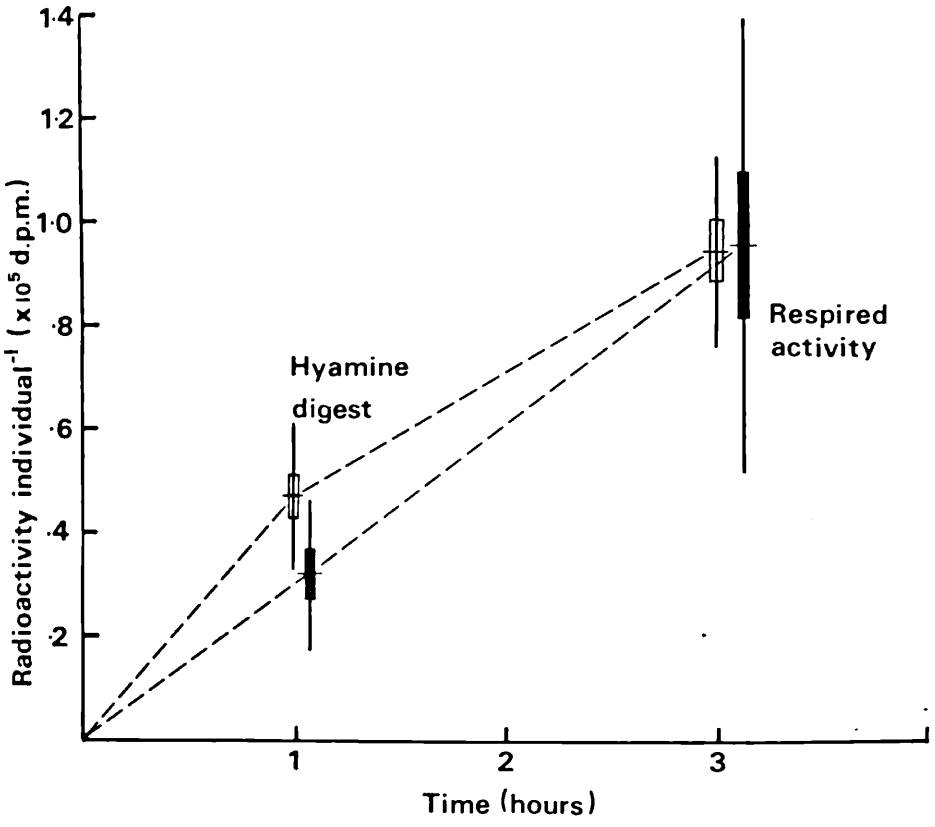
4.8.3. Results

The initial level of radioactivity in the medium was equivalent to a ^{14}C-glycine concentration of 5.21 µM l^{-1}. The amount of radioactivity remaining in the medium at the end of the incubation period depended on the size of the worm and the length of the exposure. The Mercierella that were incubated for 1 h, removed on average 50% of the label, and the 3 h worms about 75%. In no instance did an animal remove all the ^{14}C- glycine from the medium.

The individual results were corrected for weight using the method described in Section 4.5.4. Fig. 40 shows the level of radioactivity in the ethanol soluble fraction, compared with the total animal activity (including the respired component), after the 1 h and 3 h exposures. The large standard deviations indicate that there is considerable variation in the individual results. In contrast to the total animal activity, which continued to increase throughout the 3 h incubation period, the ethanol soluble component achieved a steady-state condition after an initial rapid increase during the first hour, which accounted for 80% of the total animal activity. The reduction in the total uptake rate after 1 h is probably due to the effect of the declining ambient ^{14}C-glycine concentration (Section 4.6.4).

Whilst the amounts of radioactivity incorporated into the Hyamine digest and respired fractions continued to increase throughout the 3 h incubation period (Fig. 41), there was a decrease in the rate at which ^{14}C-glycine entered the bound fraction (presumably where it is incorporated into large non-diffusible compounds, e.g. proteins). This decrease was accompanied by an increase in the amount of radioactivity released as $^{14}CO_2$, which probably reflects a delay between the absorption of ^{14}C-glycine and its catabolic breakdown (see Section 4.4.3).

Fig. 41. Levels of radioactivity in the Hyamine digest and respired activity ($^{14}CO_2$) fractions after 1 and 3 h exposures to a ^{14}C-glycine concentration of 5.0 µM l^{-1}. The points represent the mean values of nine 1 h and ten 3 h measurements, vertical bars ± 1 standard error of the mean and vertical lines ± 1 standard deviation.


1·4
1·2
1·0
·8
·6
·4
·2
Radioactivity individual^{-1} (x 10^5 d.p.m.)
Hyamine
digest
Respired
activity
1
2
3
Time (hours)

After 1 h, the Hyamine digest and respired activity fractions contributed about 12% and 8% respectively, to the total animal activity, whereas after 3 h, when the ethanol soluble fraction had become reduced to 65%, these each represented 17.5%. These results show that it was largely the incorporation of radioactivity into the bound and respired fractions, that accounted for the continued increase in total animal activity after the first hour.

4.8.4 Discussion

It is evident that the ^{14}C-glycine becomes involved in several distinct processes within Mercierella. In addition to the anabolic and catabolic pathways, indicated by the Hyamine digest and respired fractions respectively, a considerable quantity must have entered Mercierella's free amino acid (FAA) pool if uptake follows a similar pattern to that demonstrated by Ahearn and Townsley (1975).

Since glycine is a major component in the intracellular FAA pool of polychaetes (see review by Oglesby, 1969), and amino acids are largely responsible for intracellular osmoregulation, it seems likely that the ethanol soluble fraction has a largely osmoregulatory function.

This is supported by the observed steady-state relationship for this fraction, and by the findings of Ahearn and Townsley (loc. cit.) who showed that the majority of the free labelled amino acid in the apodous sea cucumber, Chiridota rigida, remained in the intracellular compartment. Glycine is also present in the blood of Mercierella (Skaer, 1974b) where the concentration is directly related to ambient salinity, which agrees with Mercierella being an osmoconformer (Skaer, 1974a).

In an energetic sense it is desirable for an organism to use a non-metabolic substance(s) for osmoregulation, or otherwise it will have to expend energy in repumping fresh compound each time that some is used for anabolic or catabolic purposes. Since some of the accumulated ^{14}C-glycine was catabolised to $^{14}CO_2$ this indicates that the osmotic and nutritional processes are interrelated in Mercierella.

4.9 The effects of starvation on the uptake and subsequent utilisation of ^{14}C-D-glucose and ^{14}C-glycine

4.9.1 Introduction

In the preceding investigations of ^{14}C-glycine uptake by Mercierella, the worms were fed with a regular supply of Brachiomonas cells, prior to the experiment. Whilst Krogh's suggestion, "Death from starvation is no doubt of very frequent occurrence in the sea as on land" (Krogh, 1931), may not apply to Mercierella in the Thames estuary, it seems likely that under certain conditions particulate food is not readily available. Worms in the intakes of power stations, and on the hulls and propellors of ships, probably experience extended periods when particulate feeding is not possible as a result of fast water currents, although throughout these periods the animals remain in contact with water containing DOM. It is of interest therefore, to determine the effects of food deprivation on the uptake and subsequent utilisation of DOM, since it is under these conditions that dissolved organic compounds might contribute significantly to Mercierella's nutrition.

4.9.2 Materials and Methods

Mercierella acclimated to 20°C, were removed from their tubes and separated into six groups with similar size distributions, each

comprised of 10 or 14 individuals. After the 48 h decontamination procedure, three groups were set aside for 14 days in darkened containers of filtered, diluted sea water plus antibiotics. No food was offered during this period, and the water was replaced with fresh at 4 day intervals. The remaining groups were used for immediate experimentation.

The first group (1) of recently fed worms (2 days fasted), was incubated individually in 2 ml of a 2.0 µM l^{-1} ^{14}C-glycine solution for 1 h. The second group (2) was incubated individually for 1 h, in 2 ml of a 2 µM l^{-1} ^{14}C-D-glucose solution, and the third group (3) was used individually in a volumetric micro-respirometer (Section 6.4) for 5 h, before they were incubated for 1 h in 2 ml of a 2.0 µM l^{-1} ^{14}C-glycine solution.

At the end of the incubation period the worms were weighed, and groups 1 and 2 were extracted for 48 h in two 0.1 ml changes of 80% ethanol, followed by digestion in 2 ml of Hyamine hydroxide. Meanwhile, group 3 were placed into individual vials containing 2 ml of filtered water and transferred to fresh water at the following time intervals : 1, 2, 4, and 6 h. At 8 h, the worms were given a 5 s rinse in distilled water to reduce salt contamination, before they were dried to constant weight on tared aluminium foil trays, and 60°C.

The incubation media and the water which contained the group 3 worms following their exposure to ^{14}C-glycine, were acidified and the $^{14}CO_2$ was collected on Hyamine impregnated wicks. After 14 days, these treatments were repeated with the three groups of starved worms (4, 5, and 6, respectively), which were deprived of particulate food for a total of 16 days.

Table 17. The weight-corrected components of total ^{14}C-glycine uptake by fasted and starved M. enigmatica, from an initial concentration of about 2.0 µM l^{-1}.

($\times 10^5$ d.p.m. individual^{-1} h^{-1})

Component of total uptake	Fasted worms: No. of determinations	Mean	S.D.	S.E.	Starved worms: No. of determinations	Mean	S.D.	S.E.	P
EtOH soluble	10	1.64	0.55	0.17	9	2.01	0.33	0.11	$<0.1>0.05$
EtOH insoluble (Hyamine digest)	10	0.067	0.04	0.013	9	0.062	0.02	0.007	>0.1
Respired activity	9	0.061	0.05	0.017	9	0.14	0.036	0.012	<0.002
Total uptake	9	1.77	0.6	0.2	9	2.21	0.36	0.12	$<0.1>0.05$

Counting efficiencies were 60 - 66% for the ethanol extracts; 58 - 60% for the Hyamine digests; 57 - 60% for the Hyamine impregnated wicks; and 55 - 58% for the medium samples. Background activity amounted to 29 - 33 d.p.m. The respired radioactivity was corrected for bacterial catabolism, whose hourly rate was 34x and 130x the background in the fasted and starved uptake studies using ^{14}C-glycine, and 73x and 87x for the respective ^{14}C-D-glucose investigations.

4.9.3 Results

Since a preliminary investigation showed that wet weight fluctuates during starvation (Fig. 31), it was necessary to use dry weight in this study. In the case of the Mercierella digested in Hyamine hydroxide, their dry weights were estimated from the relationship between wet weight and dry weight of other worms in a similar nutritional state (Appendix 1.9). The individual results were corrected for weight, using the method described in Section 4.5.4, since the relationship between ^{14}C-glycine uptake and weight of starved Mercierella is similar (r = 0.7, P = < 0.05) to that of fed animals.

The mean, standard deviation, and standard error for each fraction of total ^{14}C-glycine uptake by fed and starved Mercierella are presented in Table 17, together with the results of the comparisons made with Student's t-test. Despite considerable variation, there is a tendency for the results for the starved worms to be less variable, although these differences are not significant (F-test, P = > 0.05). Furthermore, there is no significant difference between the total uptake of ^{14}C-glycine by fed and starved Mercierella during the 1 h exposure, although there is a suggestion that starved worms have a higher total uptake rate.

Table 18. The weight-corrected components of total ^{14}C-D-glucose uptake by fasted and starved M. enigmatica, from an initial concentration of about 2.0 µM l^{-1}.

($\times 10^{4}$ d.p.m. individual^{-1} h^{-1})

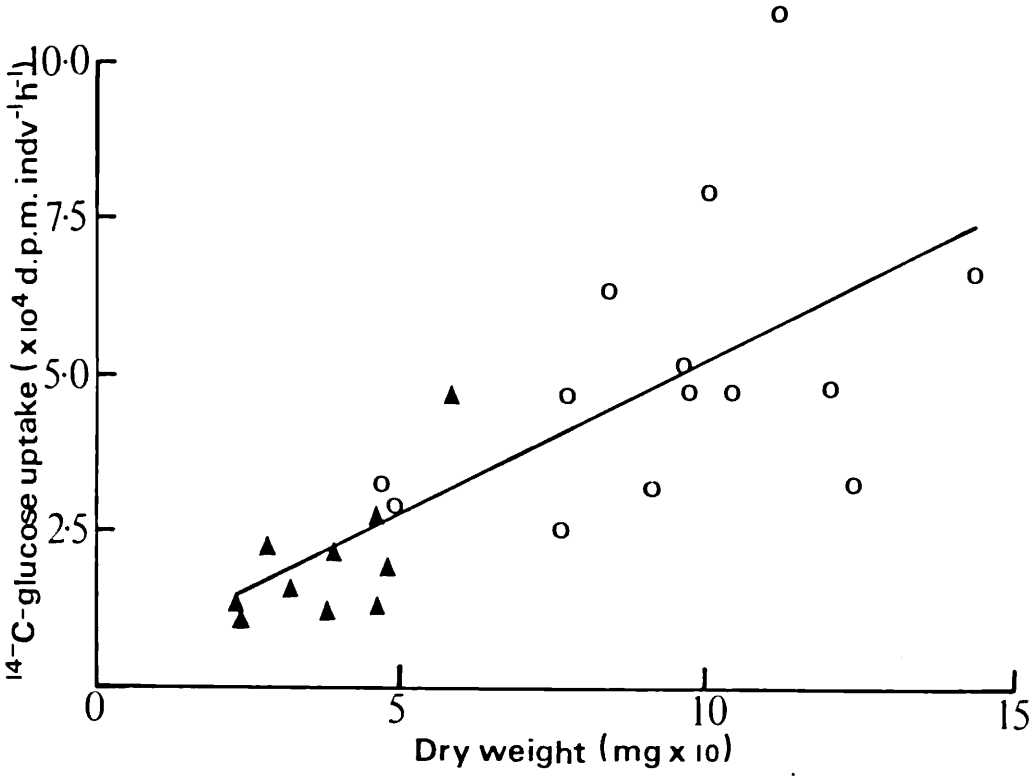
Component of total uptake	No. of determinations	Fasted worms Mean	S.D.	No. of determinations	Starved worms Mean	S.D.	P
EtOH soluble	14	2.61	1.18	10	1.85	0.83	<0.1 > 0.05
EtOH insoluble (Hyamine digest)	14	2.49	0.93	10	0.98	0.44	<0.001
Respired activity	14	0.10	0.09	10	0.27	0.15	<0.01
Total uptake	14	5.2	2.02	10	3.1	1.31	<0.01

Whilst there is no change in the amount of radioactivity incorporated into the bound fraction after 16 days starvation, there is a significant increase in the rate of $^{14}CO_2$ production. The ethanol soluble fraction represents about 93% and 91% respectively, of the total ^{14}C-glycine uptake by fed and starved Mercierella, compared with the 4% and 3% in the Hyamine digests, and 3% and 6% in the respired activity fractions. Thus starvation appears to increase the rate of $^{14}CO_2$ production by a factor of two, on both an absolute and relative basis.

The means and standard deviations for the components of total ^{14}C-D-glucose uptake by fed and starved Mercierella are shown in Table 18, together with the results of comparisons made using Student's t-test. In contrast to the results for ^{14}C-glycine uptake by starved animals, there is a significant reduction in the rate of ^{14}C-D-glucose accumulation after food deprivation. Furthermore, the ethanol soluble fraction in both fed and starved worms represents a considerably smaller proportion, about 55% of the total glucose uptake, which is in accordance with polychaetes having relatively small amounts of free sugars in their body fluids (see the review of polychaete carbohydrate metabolism by Scheer, 1969).

Whilst starvation has no significant effect on the levels of free glucose, there is a tendency for the starved Mercierella to have somewhat lower values than were found in the fed animals. This is accompanied by a marked decrease in the amount of radioactivity that becomes bound to the tissues (glycogen?), together with a smaller, though still significant increase in the rate of $^{14}CO_2$ production. $^{14}CO_2$ production by starved Mercierella is greater by a factor of about three, and represents an increase from 2% to 9% of the total ^{14}C-D-glucose uptake in relative terms ($P =< 0.001$).

Fig. 42. Uptake of ^{14}C-D-glucose from an initial concentration of about 2.0 µM l^{-1} by ○, recently fed (2 days fasted) and ▲, 16 days starved M. enigmatica, expressed as a function of estimated dry weight. The regression line was fitted by the method of least squares, $r = 0.73$, $\hat{Y} = 0.22 + 0.5X$.


^{14}C-glucose uptake (x 10^4 d.p.m. indv^{-1}h^{-1})
10·0
7·5
5·0
2·5
0
5
10
15
Dry weight (mg x 10)

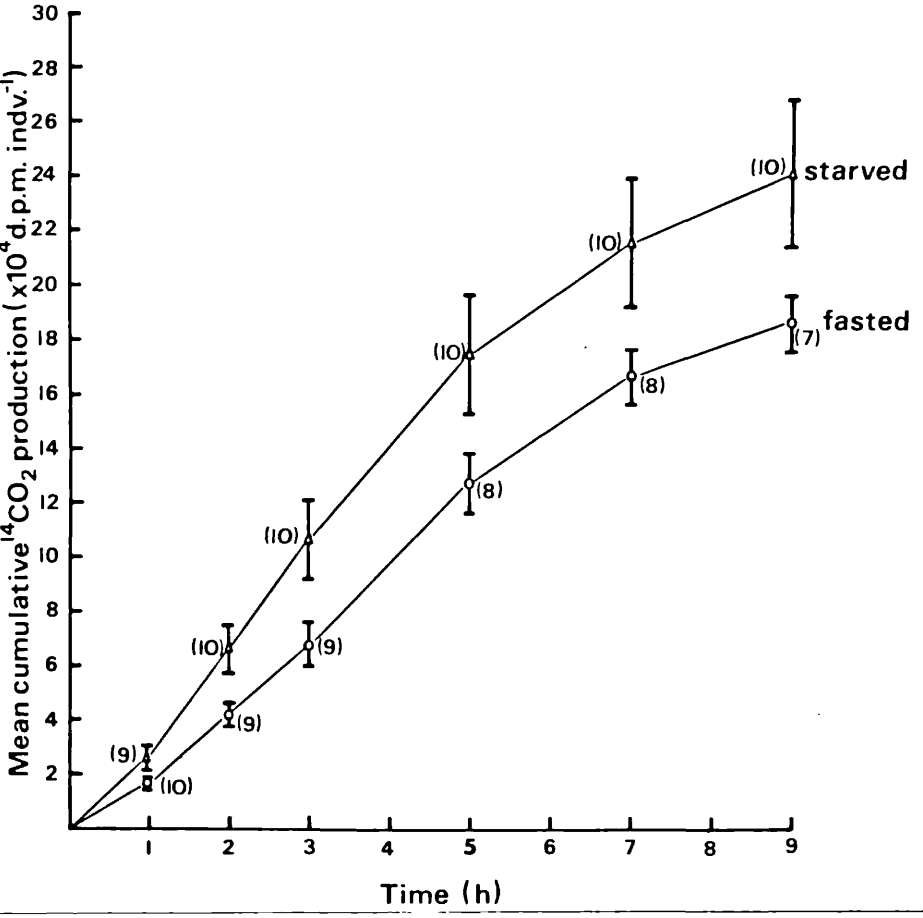
The apparent relationship between the ethanol soluble fraction and the insoluble fraction of total ^{14}C-D-glucose uptake is in accordance with observations for other species, including pogonophores (Southward & Southward, 1970, A.J. Southward, personal communication, 1977).

As in the ^{14}C-glycine experiment, the majority of the starved worms weighed less than the fed individuals. Fig. 42 shows the invididual ^{14}C-D-glucose uptake data for fed and starved Mercierella, expressed as a function of the estimated dry weight. The correlation between these two parameters is highly significant (r = 0.73, P = < 0.001). The regression line was fitted to these data by the method of least squares.

The slope of the regression line, 0.5, departs markedly from the expected 0.67 (Section 4.5.4), therefore an exponent of 0.5 was substituted for a in the equation: uptake = kW^a, when the individual results were corrected for weight. It is of interest to note that Stephens (1963) reported a similar slope for the relationship between the uptake of the amino acids, phenylalanine, lysine, and glycine, and weight of the polychaete, Clymenella. The significance of this value is not understood.

Fig. 43 shows the mean cumulative $^{14}CO_2$ production by fed and starved Mercierella following a 1 h exposure to a 2.0 µM l^{-1} ^{14}C-glycine solution. As was demonstrated previously, the rate of $^{14}CO_2$ production by the starved worms during the incubation period, was about twice that of the fed animals. A higher $^{14}CO_2$ production rate was maintained throughout the 9 h period, with the maximum rate of production by starved worms being reached by the end of the third hour, which contrasted with the fed animals, that did not achieve their maximum rate of $^{14}CO_2$

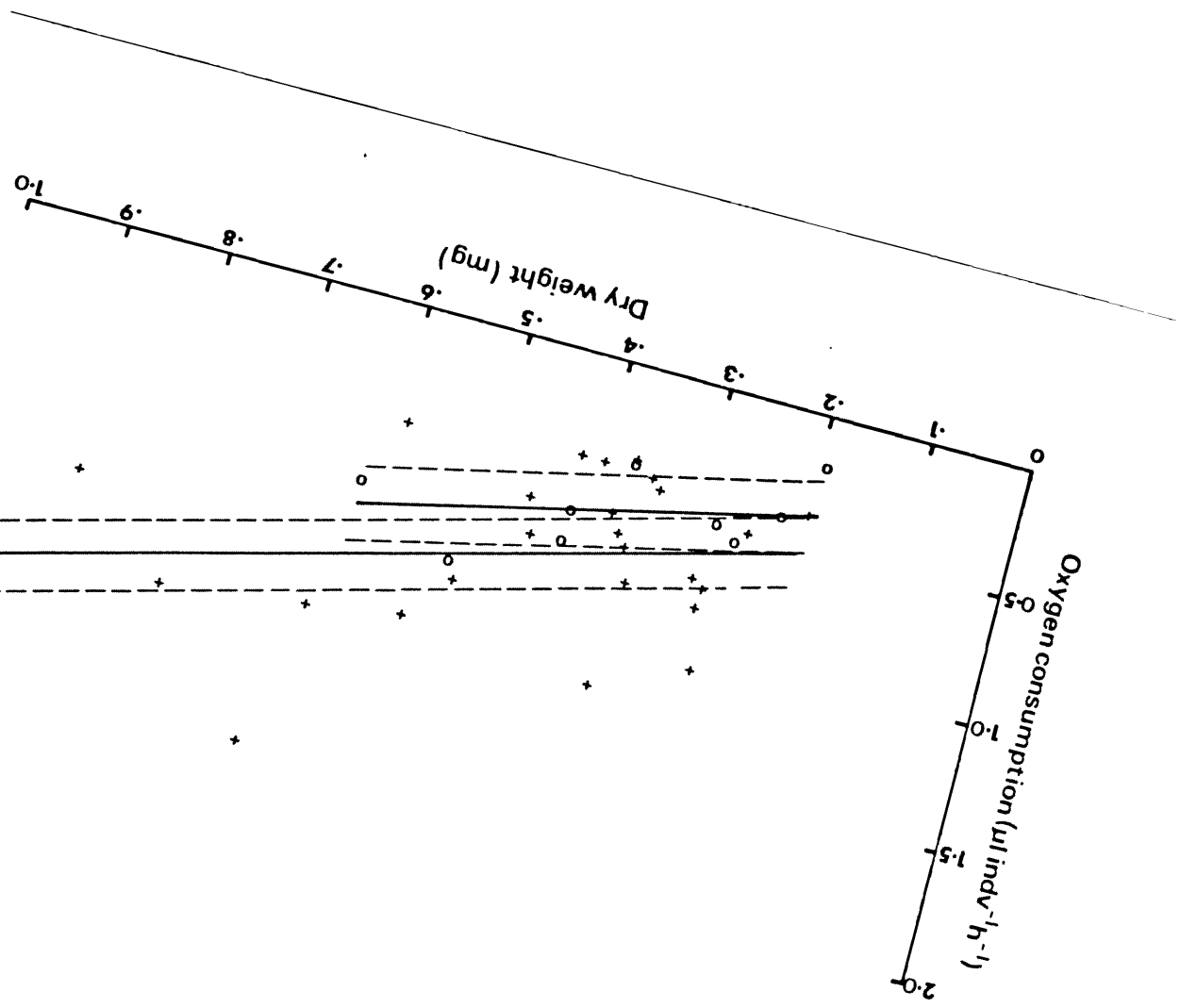
Fig. 43. Cumulative $^{14}CO_2$ production by recently fed (2 days fasted) and starved (16 days) M. enigmatica, following a 1-h exposure to ^{14}C-glycine at an initial concentration of about 2.0 µM l^{-1}. Each point represents the mean of n individual values (shown in parentheses), and the vertical lines ± 1 standard error on each side of the mean.


Mean cumulative $^{14}CO_2$ production ($x10^4$ d.p.m. indv.$^{-1}$)
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
1
2
3
4
5
6
7
8
9
Time (h)
(10)
starved
(7)
fasted
(9)
(10)
(10)
(9)
(10)
(9)
(8)
(10)
(8)

release until nearly two hours later. After reaching the maxima, the rates of $^{14}CO_2$ production steadily declined until the end of the ninth hour, when most of the accumulated compound had been respired (see Table 17). These results show that there are both quantitative and temporal differences in the catabolism of ^{14}C-glycine by fed and starved Mercierella. Compared with the results for the fed worms, there is greater variation in the individual $^{14}CO_2$ production rates by the starved animals (for standard deviations see Appendix 1.10). This probably reflects the differing degrees of starvation reached by worms of different sizes after a given time, due to the smaller animals having higher specific metabolic rates (see Section 6.7.4). A related problem associated with starvation experiments of this type, is the possibility that the experimental animals, although acclimated to the experimental conditions, may differ with regard to the amounts of storage materials they contain. This may be determined by a variety of factors apart from size, such as sex, genetic constitution and pre-collection experience.

Fig. 44 shows the oxygen consumption rates of fed (2 days fasted) and starved (16 days) Mercierella plotted against dry weight. The respiration rates of the starved Mercierella are less variable (S.D. = 0.17), compared to the rates for the fed worms (S.D. = 0.31) (F-test, $P = < 0.05$). This reduction in variability could be due to starved animals metabolising the same respiratory substrate, namely glycogen, whereas fed worms probably respire a mixture of compounds. Thus the oxygen consumption rates of the starved animals probably represent the minimum expenditure of energy compatible with body-maintenance.

Fig. 44. Oxygen consumption rate related to dry weight of recently fed (crosses) and starved (circles) M. enigmatica. The regression lines were fitted by the method of least squares and the dashed lines represent the 95% confidence limits about each line; +, n = 25, $\hat{Y} = 0.32 + 1.07X$; ○, n = 9, $\hat{Y} = 0.2 + 0.94X$.


Oxygen consumption (µl indv^{-1} h^{-1})
2·0
1·5
1·0
0·5
0
·1
·2
·3
·4
·5
·6
·7
·8
·9
1·0
Dry weight (mg)

In contrast to the relationship between oxygen consumption and dry weight of starved Mercierella (r = 0.67, P = < 0.05), the correlation between these parameters for fed worms in this experiment is not significant (r = 0.55, P = > 0.05). Since there is no difference between the treatment and method used in this investigation and in the respiration experiment described in Section 6.7, it was possible to supplement the data with values obtained previously for animals of a similar size range. Due to the increased n value, the correlation between the two parameters is highly significant (r = 0.56, P =< 0.01).

Due to the variability in the data, there is no statistically significant difference between the respiration rates of fed and starved Mercierella (P = > 0.05), although the lower average respiration rates of the starved animals, are in accordance with the generally observed effect of nutritional stress on the oxygen consumption rates of marine invertebrates (e.g. Thompson & Bayne, 1972; Bayne, 1973a, b; Newell, 1973).

4.9.4 Discussion

The results of the ^{14}C-glycine uptake experiment give further support to the proposed major osmoregulatory role of this amino acid (Section 4.8.4), and are in accordance with the reported effects of starvation on the uptake processes of other marine invertebrates (Reish & Stephens, 1969; Shick, 1973, 1975).

The suggested increase in the amount of radioactivity in the ethanol soluble fraction of starved Mercierella, agrees with the increased levels of glycine and taurine in the FAA pool of starved Aurelia aurita scyphistomae (Shick, 1975). Shick suggested that the presence of these compounds would compensate for the reduced

concentration of other amino acids that were catabolised during starvation. Similarly in Mercierella, glycine may be taken up to maintain osmotic equilibrium between the FAA pool and the external medium.

There is also evidence which suggests that when glycine becomes incorporated into the synthetic pathways (Stephens, 1964; Stephens & Virkar, 1966; Shick, 1973) its function is osmoregulatory rather than simply anabolic. These authors have demonstrated an inverse relationship between ambient salinity and the amount of bound ^{14}C-glycine, indicating that some marine invertebrates, including polychaete examples, may synthesise protein as a means of removing amino acids from the intracellular pool during periods of hypo-osmotic stress. Bedford (1971) suggested this as a possible mechanism for cell volume regulation by the gastropod, Melanopsis trifasciata. Since the size of the bound fraction is dependent on the ambient salinity rather than on nutritional status, this would account for the similarity between the amounts of bound activity in the fed and starved Mercierella that were maintained at a constant salinity of 15 $^{o}/_{oo}$.

In contrast to glycine's major involvement in the osmoregulatory processes, glucose is known to perform a nutritional role, both as a respiratory substrate and as an energy reserve (glycogen) (see the review by Scheer, 1969). However, some of the accumulated ^{14}C-glycine was catabolised to $^{14}CO_2$ by fed and starved Mercierella, showing that it too is able to serve as a respiratory substrate.

Although it is tempting to suggest that the decrease in the level of bound ^{14}C-D- glucose that accompanied the increase in amount of $^{14}CO_2$ released by starved Mercierella, was due to their catabolising

the accumulated compound at the expense of the glycogen store, since the increased $^{14}CO_2$ production accounted for only 18% of the reduction in the amount of radioactivity which entered the bound fraction of starved animals, this appears to be only a partial explanation for the observed relationship. Furthermore, Barry and Munday (1959) have shown that in Patella vulgata L., the blood glucose level also falls when glycogen is used as the energy reserve, which suggests that this compound is also catabolised when in the free form. Thus an alternative explanation is required for the observed decrease in the bound fraction and the overall reduction in ^{14}C-D-glucose uptake following starvation.

An extremely interesting outcome of this investigation is the discovery of an apparently direct relationship between the amounts of radioactive glucose that become incorporated into the body fractions of fed and starved Mercierella, and the quantities of unlabelled compound already present in each of the compartments. The possible reduction in the amount of radioactivity that was recovered from the ethanol soluble fraction of starved worms is in accordance with the decrease in free sugars reported for Littorina littorea (L.) during starvation (Holland et al., 1975), whilst the reduction in the bound fraction after starvation reflects the expected decrease in glycogen reserves. This seems to suggest that the uptake of dissolved glucose by Mercierella is somehow determined by the amount of the compound present inside the worm at the time of exposure, and could be interpreted as indicating that the apparent accumulation of this compound into these fractions results from homo-exchange diffusion. Furthermore, a concommitant decrease in ^{14}C-D-glucose uptake in response to depleted glucose levels in the starved animal appears to contradict what might be expected were

DOM acting as an important supplementary nutritional source during periods of particulate food deprivation.

There is evidence, however, of enhanced catabolism of exogenous glycine and glucose by starved Mercierella which tends to support an increased contribution to the worm's metabolic energy requirement during starvation, especially as the maintenance energy requirement appears to be reduced after a period of starvation. It must be pointed out that no measurement was made of Mercierella's oxygen consumption rate following exposure to labelled DOM, which may have increased due to the possible effect of DOM on feeding activity (Section 4.1).

Shick (1973) suggested that the increased quantity of $^{14}CO_2$ produced by starved Aurelia scyphistomae could have resulted from the labelled compound representing a greater proportion of the total amount of that compound present inside the animal, rather than being due to an enhanced rate of oxidation. Whilst this cannot be discounted as the cause of some of the increased $^{14}CO_2$ production following starvation, particularly in the case of glucose which probably shows an overall decrease as a result of food deprivation, the reduced delay between ^{14}C-glycine uptake and the maximum rate of $^{14}CO_2$ production following exposure, shows conclusively that there is enhanced catabolism in Mercierella. It is evident, therefore, that when Mercierella is deprived of particulate food there is an increased participation of exogenous DOM in its catabolic processes resulting in an increase in their potential contribution to its nutrition.

4.10 The estimated contributions of dissolved glucose and glycine to Mercierella's energy budget

The classical approach when considering the nutritional role of food materials is to compare the energy content of the food intake with

the metabolic energy requirement (Lavoisier & Laplace, 1780, in Stephens, 1968; Brody, 1945). Due to the practical difficulties associated with direct measurement of heat production by aquatic invertebrates, an estimate of the energetic cost of metabolism is generally derived indirectly from respiration rate (Section 6.8).

Pütter (1909, quoted in Krogh, 1931) was the first to apply the indirect approach to dissolved organic compounds, and it has subsequently been general practice to compare the amount accumulated with the maintenance energy requirement (e.g. Stephens, 1962a, 1963, 1964, 1968; Southward & Southward, 1972; Anderson & Bedford, 1973; Wright et al, 1975). It is misleading to consider total uptake in terms of the potential contribution to an organism's catabolic energy requirement because only a small proportion of the accumulated compound is oxidised during the exposure period (Section 4.8). The majority is incorporated into the anabolic pathways or used in the osmoregulatory processes. The amount of $^{14}CO_2$ produced during the exposure period is therefore a more realistic indication of the accumulated compound's contribution to the animal's respiratory energy requirement, and this approach is adopted in the present study.

The weight-corrected oxygen consumption rates of fed and starved Mercierella at 20°C, are 1.53 $\pm$ 0.68 mm^3 $indv.^{-1}$ h^{-1}, and 1.06 $\pm$ 0.28 mm^3 $indv.^{-1}$ h^{-1}, respectively. The individual respiration rates were corrected for weight using the surface law exponent 0.67 (Section 4.5.4). The energetic cost of metabolism for fed Mercierella was calculated using the oxycalorific coefficient (13.93 J mg^{-1} O_2) 3.33 cal mg^{-1} O_2, for worms feeding on Brachiomonas cells (Section 6.8), and a coefficient of (14.76 J mg^{-1} O_2) 3.53 cal mg^{-1} O_2 was used for the starved animals,

assuming that these were respiring glycogen (3.53 cal mg^{-1} O_2 is the generally quoted Q_{ox} for glucose).

The estimated hourly cost of metabolism for fed and starved Mercierella is 3.04×10^{-2} J (7.28×10^{-3} cal) $indv.^{-1}$, and 2.23×10^{-2} J (5.34×10^{-3} cal) $indv.^{-1}$ respectively. (Specimen calculation: $\frac{1.53}{1000} \times \frac{32}{22.4} \times 13.93 = 0.0304$).

Brody (1945) quoted 3.11 kcal g^{-1} as the calorific value of glycine, which is equivalent to 9.75×10^5 J $mole^{-1}$ (2.33×10^5 cal $mole^{-1}$). Since Brody's value refers to the heat of combustion, which is greater than the amount of potential energy available to Mercierella, in which the degradation of protein is incomplete (Section 7.1), it is necessary to correct this value for the energy lost in the form of ammonia.

The biological oxidation of 1 mole of glycine by an ammoniotele results in the formation of 1 mole of ammonia. Since aqueous ammonia has a calorific value of 3.48×10^5 J $mole^{-1}$ (8.33×10^4 cal $mole^{-1}$) (Section 6.8), assuming efficient deamination and aerobic oxidation, the maximum amount of energy that Mercierella can obtain from the catabolism of 1 mole of glycine is 6.27×10^5 J (1.5×10^5 cal).

The amount of radioactivity respired by fed Mercierella in a 2 µM l^{-1} labelled glycine solution (Section 4.9.3) is equivalent to 2.41×10^{-5} µM h^{-1}, or 1.51×10^{-5} J h^{-1} (3.61×10^{-6} cal h^{-1}), whereas the starved worms respired 5.63×10^{-5} µM h^{-1}, or 3.53×10^{-5} J h^{-1} (8.44×10^{-6} cal h^{-1}) which represent just 0.05% and 0.16% respectively of the maintenance energy requirement. Thus the energy derived from the integumentary uptake of glycine, accounts for only a very small proportion of the estimated energetic cost of metabolism, and contrasts

markedly with the reported range of values (6% - 150%) based on the potential contribution of the total accumulated amount of amino acid (e.g. Stephens, 1963; Shick, 1975; for other references see Jørgensen, 1976).

Similar calculations were carried out using the results of the glucose uptake investigation (Section 4.9). Glucose, in contrast to glycine, is not used in osmoregulation (Section 4.9.4), and should therefore be a better indicator of the value of DOM in Mercierella's energy budget.

Although the natural levels of glucose in unpolluted oceanic sea water are generally as low (e.g. 1.9×10^{-7} - 3.9×10^{-6} M l^{-1}, Josefsson, 1970), as the concentrations of amino acids (3.33×10^{-8} M l^{-1}, Degens et al., 1964; 3×10^{-9} - 1×10^{-6} M l^{-1}, references quoted by Southward & Southward, 1972), the glucose concentration in coastal waters, in common with amino acids, is several times greater (see Jørgensen, 1976, for references). Thus the concentration of about 2 μM l^{-1} used in the present study, is probably of the correct order of magnitude for the Thames estuary, the organically polluted waters of which undoubtedly contain a high concentration of free sugars, resulting from bacterial decomposition of particulate organic matter both in the water column and organically rich sediments that are characteristic of the middle reaches (Section 2.1 and 2.3.4).

In addition to the sugars produced bybacterial activity, it is likely that a significant amount of soluble carbohydrate is released by phytoplankton, benthic diatoms and filamentous algae, during their photosynthetic activities (e.g. Fogg, 1966). Whereas it is extremely unlikely that benthic algae are able to photosynthesise during periods

of high water because of the turbidity of the water (Section 2.3.4), rapid photosynthesis takes place during intertidal periods which probably results in localised high concentrations of free sugars in the pools of standing water. Since Mercierella lives in close proximity to these plants these compounds should be readily available to the worms, that retain a feeding posture throughout the intertidal period as long as they are covered by water. Wright et al., (1975) reported high concentrations of DOM in the region of algal mats growing on wharf piles, and suggested that these could be important with regard to mussel nutrition.

Brody (1945) reported an energy content for glucose of 3.75 kcal g^{-1}, which is equivalent to 2.82×10^{6} J $mole^{-1}$ (6.75×10^{5} cal $mole^{-1}$). Since all the energy contained within the glucose molecule is available to Mercierella (Section 6.8), the radioactivity respired by the fed worms in the 2 µM l^{-1} ^{14}C-D-glucose solution is equivalent to 1.4×10^{-6} µM h^{-1}, or 3.95×10^{-6} J h^{-1} (9.45×10^{-7} cal h^{-1}) whereas the starved animals oxidised the equivalent of 3.8×10^{-6} µM h^{-1}, or 1.07×10^{-5} J h^{-1} (2.56×10^{-6} cal h^{-1}). These amounts of energy represent just 0.013% and 0.048% respectively of the estimated average hourly energetic cost of metabolism for fed and starved Mercierella.

Thus the catabolism of exogenous glucose and glycine by Mercierella, from natural environmental concentrations, accounts for only a small part of the maintenance energy requirements of fed and starved worms. The percentages are conservative estimates, however, since the delay between uptake and catabolism, as is demonstrated in the long term $^{14}CO_2$ production experiment (Section 4.9.3), was not taken into account. Neither was the DOM already present in the medium (natural sea water was used in most cases, unless otherwise stated). However, since maximum $^{14}Co_2$ production rates by fed and starved Mercierella

(Appendix 1.10) exceeded the values used in the calculations by only 1.6x and 1.7x respectively, and as the water is unlikely to have contained a greater concentration of DOM than was used in the experiments, the estimated contributions are unlikely to be out by more than a factor of two, and are certainly of the correct order of magnitude.

In contrast to glycine uptake, a considerable proportion of the accumulated glucose became bound to the tissues. It is of interest, therefore, to calculate the percentage contribution this represents to the worm's production. The radioactivity recovered as the bound fraction of fed Mercierella, following exposure to a 2.0 μM l^{-1} ^{14}C-D-glucose solution (Section 4.9.3) is equivalent to 9.84×10^{-5} J h^{-1} (2.35×10^{-5} cal h^{-1}). Since a worm with a respiration rate equivalent to 2.78×10^{-2} J h^{-1} (6.64×10^{-3} cal h^{-1}), has a production rate of 3.93×10^{-2} J h^{-1} (9.4×10^{-3} cal h^{-1}) (Section 9.0), the radioactivity incorporated into the synthetic pathways accounts for only 0.25% of the estimated total production. Thus exogenous glucose plays a minor role in Mercierella's anabolic activities.

DOM accumulation studies have been criticised for the general lack of information concerning the loss of material through leakage (e.g. Johannes, Coward, and Webb, 1969). It is only when complete data are available for the influx and efflux of dissolved organic compounds across the integument that the absolute contribution to the energy budget can be determined (see Stephens, 1975b). Despite the lack of information concerning the efflux of materials in the present study, it is evident that exogenous dissolved organic compounds do participate in Mercierella's anabolic and catabolic activities, although it is unlikely that DOM could possibly represent an important nutritional

supplement, even if there is no loss of materials through leakage. If there is no loss of materials through leakage, the total uptake of ^{14}C-D-glucose and ^{14}C-glycine account for only 0.68% (2.07×10^{-4} J h^{-1}; 4.95×10^{-5} cal h^{-1}) and 0.55% (1.23×10^{-4} J h^{-1}; 2.94×10^{-5} cal h^{-1}) and 1.46% (4.45×10^{-4} J h^{-1}; 1.06×10^{-4} cal h^{-1}) and 2.5% (5.57×10^{-4} J h^{-1}; 1.33×10^{-4} cal h^{-1}) respectively of the estimated metabolic energy requirements of fed and starved worms. Thus in relative terms, the individual contributions are appreciably less than the reported values for a variety of other marine invertebrates (see Section 4.1; and Jørgensen, 1976).

Further evidence for the lack of importance of exogenous glycine in Mercierella's energy budget is provided by the saturation kinetics of the uptake process (Section 4.6.4). To satisfy the maintenance energy requirement of a fed worm, the trans-integumentary uptake of glycine would have to be 4.85×10^{-2} µM h^{-1}, whereas, the Vmax value for glycine (the maximum attainable rate of uptake) is only 1.34×10^{-3} µM h^{-1} (8.44×10^{-4} J h^{-1}; 2.02×10^{-4} cal h^{-1}) or just 2.77% of the maintenance energy requirement. It is therefore impossible for the trans-integumentary uptake of this particular compound to exceed this amount (temperature remaining constant).

Thus in the absence of evidence to suggest that the uptake of DOM is a significant nutritional supplement it remains to explain why Mercierella should accumulate these compounds.

As one might expect as a result of the evolutionary process, there is certainly no single explanation that applies to all animals and all compounds. In contrast to some amino acids, including glycine, that undoubtedly enter the animal as part of the osmoregulatory process

(Section 4.8.4), and whose subsequent oxidation is incidental rather than essential, some DOM may enter the body of the organism through the same channels as the osmoregulatory materials but itself performs no role in osmoregulation, e.g. sterols (Saliot & Barbier, 1973). This uptake mechanism could explain those observations that the accumulation process requires energy (e.g. Schlichter, 1973, 1975; Emig & Thouveny, 1976). The metabolic inhibitors used in these studies, could be blocking the osmoregulatory mechanism, rather than a transport process specifically adapted to the accumulation of DOM. The existence of this channel is supported by Stephens' observation that osmoregulation and uptake of DOM are incompatible (Stephens, 1964). Several references have also been made to the presence of microvilli on the outer surfaces of soft-bodied marine invertebrates (e.g. Schlichter, 1973, 1975), that have been referred to in connection with the accumulation of DOM. Nott (1973) however, has postulated that the micro-villi of larval serpulids have an important function in the absorption of inorganic ions.

Due to the highly permeable integuments of soft-bodied marine invertebrates it is not surprising that there is a trans-integumentary movement of dissolved organic compounds (it should be noted that Crustacea are the only group of marine invertebrates that do not accumulate significant quantities of DOM (Anderson & Stephens, 1969)). It has long been known that marine invertebrates contain high internal concentrations of dissolved organic compounds (e.g. Fredericq, 1901; Awapara, 1962; Skaer, 1974b), which would tend to leach out of their bodies as a result of diffusion processes. If, as is suggested in Section 4.9.4, the uptake mechanism is operating by homo-exchange diffusion (see Neame & Richards, 1972), the observed accumulation rates

could result from an exchange with material present in the animal giving rise to no net change in the internal concentration. It will be remembered that the rate of diffusion depends on the size of the differential between the internal and external compartments. Thus the observed uptake phenomenon could be a mechanism for maintaining the status quo in spite of the necessary permeability for the worm's survival in rapidly fluctuating salinities (see Skaer, 1974a).

In common with the majority of marine invertebrates, Mercierella is capable of feeding on particulate food (Section 3.0). Since this process undoubtedly requires a great deal more energy than does the transport of DOM across the body wall (see Stephens, 1968; and Schlichter, 1973, for estimates of the energetic cost of trans-integumentary uptake), it is paradoxical in view of evolutionary parsimony, why such organisms should have evolved complex feeding organs and digestive systems, if they are capable of obtaining sufficient amounts of energy from solution. This supports the findings of the present study, and suggests that in most instances, the accumulation of DOM is at most a supplementary feeding mechanism.

Finally, the uptake of DOM may be a vestigial mechanism. It will be remembered that accumulation of dissolved organic compounds is the process by which heterotrophic aquatic micro-organisms obtain their nourishment (for references see Jørgensen, 1976). This could have been retained by soft-bodied marine invertebrates, when due to their increased body size and complexity, in association with the invasion of different niches, they evolved alternative methods for obtaining their food.

The only group of free living marine invertebrates to which DOM is of major importance as a source of energy is the Pogonophora (e.g. Southward & Southward, 1968, 1970, 1972). Since these are believed to have arisen from primitive annelid stock (George & Southward, 1973) this supports the theory that DOM uptake is a primitive characteristic of this group. The pogonophores have retained this mode of nutrition because of the high ambient DOM levels in the homogenous environment in which they live, and have secondarily lost the mouth and gut.

It should be pointed out that DOM may be an important source of energy for the epidermal structures of echinoderms which have no other apparent means of obtaining their nourishment apart from by direct uptake (see Burgh, 1975), and it is perhaps at the organ level that further studies of the role of DOM in the lives of marine invertebrates, should be pursued.

5. EGESTION

Introduction

Mercierella produces discrete, mucus-bound faecal pellets which are carried to the anterior of the tube by the action of cilia in the ventral abdominal groove, at the junction of the abdomen with the posterior lobe of the thoracic membrane, and on the dorsal surface of the thorax. When a number of faecal pellets have collected at the base of the branchial crown, they are expelled from the tube into the strong rejection current by a rapid, longitudinal, contraction of the animal's body. The faeces appear from the tube as an amorphous mass since they adhere together by their mucous coats. This facilitates rapid sinking out of the feeding zone, whilst the mucous covering prevents re-suspension and re-ingestion of essentially non-nutritive material.

The worms used in the egestion investigations were collected at Greenhithe, in November 1975 and January 1976. In the laboratory they were maintained in 6-l stock tanks in a constant temperature room at 10°C, and at room temperature (*c.* 20°C). The worms were acclimated to the experimental temperatures for a minimum of 28 days, during which time they were fed twice weekly with a suspension of *B. submarina* cells. The tanks contained Bay of Biscay, or Plymouth, sea water diluted to a salinity of 15 °/oo with tap water. The water in the stock tanks was artificially aerated, and changed before each second feed.

About 1 week before the experiments commenced, the tubes were removed intact from the chalk boulders and cleaned of encrusting

matter, before they were placed in 500 ml crystallising dishes containing water at the appropriate acclimation temperature. A similar feeding régime was observed to that in the stock tanks.

5.2 The effect of food deprivation on the rate of egestion

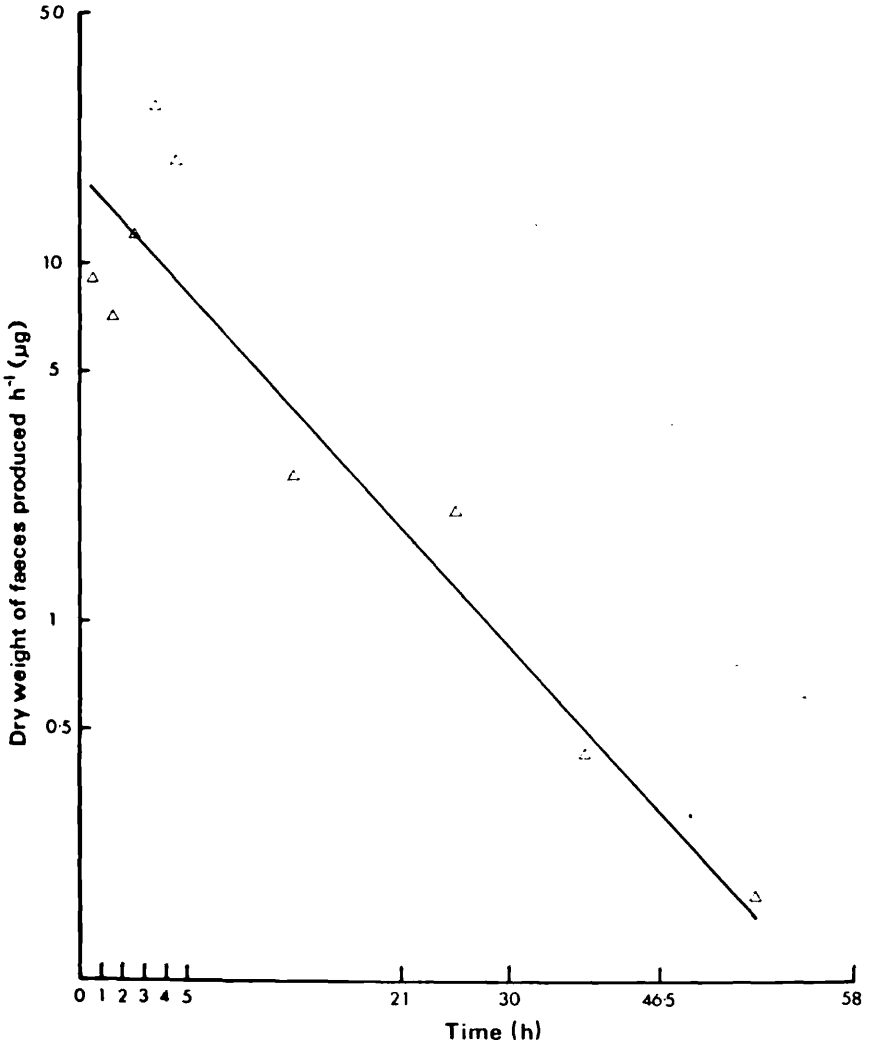
5.2.1 Introduction

An experiment is described in which the effect of particulate food deprivation on egestion rate was investigated. It is necessary to understand this relationship, since in subsequent experiments it was intended to harvest faeces for weighing, and calorific and biochemical determinations. The results of this experiment will show whether it is feasible to harvest faecal pellets in the absence of a food suspension, since the algal cells have a tendency to adhere to the mucus surrounding the pellets and thereby represent a potential source of error in the analyses. This can only be done, however, if there is no significant change in passage time (the time taken for food to pass through the gut) during the harvest period.

5.2.2 Materials and Methods

Ten large Mercierella (> 8.0 mg wet weight) acclimated to 20°C, were transferred to 250 ml of dilute algal suspension, which was replaced with fresh suspension at 12 h intervals during a 2 d preliminary feeding period. After this feeding period, the worms were transferred to a petri dish containing 0.2 µm Sartorius membrane filtered water, via several rinses with filtered water to remove any adhering particulate matter that would otherwise have represented a source of error in the subsequent weighings. After a few minutes the worms commenced normal filtering activity, and began to release amorphous masses of faeces from the anterior ends of their tubes.

Fig. 45. Relationship between rate of faeces production and time following removal from the food suspension. The points represent the mean hourly faeces production values, by ten large M. enigmatica, for the time intervals shown on the abscissa. The regression line was fitted by eye estimation after a semi-logarithmic transformation of the data.


50
10
5
1
0·5
Dry weight of faeces produced h⁻¹ (µg)
0 1 2 3 4 5
21
30
46·5
58
Time (h)

After 1 h, the worms were transferred to a similar dish for a further 60 min. This process was repeated at intervals during a total of 58 h.

The contents of the dishes were filtered through ashed (500°C for 12 h), and pre-weighed 20 mm discs of Whatman GF/C glass fibre filters mounted in a sintered funnel of c. 1 mm pore diameter. After salt contamination had been reduced with a 3 s rinse in glass-distilled water, the filters plus faecal material were dried to constant weight at 60°C. Weighings were made to the nearest 1µg on a Cahn 4100 Electrobalance. The dry weights of faeces released during the various time intervals following the worms removal from the food suspension were obtained by subtraction.

5.2.3 Results

Fig. 45 shows the mean hourly egestion rates for the 10 large Mercierella during various intervals of time following their removal from the algal suspension. Egestion rate did not remain constant throughout the post-feeding period. Instead, after an initial period of at least 5 h duration when there was considerable variation in the rate of faeces release, there was a marked decline resulting in the mean egestion rate for the final 11.5 h being less than 1% of the initial rate.

5.2.4 Discussion

A preliminary investigation revealed that the guts of fasted Mercierella (7 d without food in filtered water plus antibiotics) contained appreciable amounts of food material. This observation together with the results of the present experiment contrast with the findings of the feeding experiment (Section 3.2) which show that under normal feeding conditions food has a passage time of between 4.5 h and c. 6.5 h. It would appear from the results of these investigations

that incoming food has a significant influence on the passage of food material through Mercierella's gut. This could be achieved in two interrelated ways. First, due to physical pressure where the incoming food forces the gut contents towards the posterior end of the worm. Second by maintaining the appropriate level of food in the gut, so that the cilia lining the alimentary canal (see Hanson, 1948) can move it effectively in a posterior direction.

The presence of residual material in the gut of Mercierella during extended periods of food deprivation has some survival value since it may act as an 'external' energy store, steadily releasing nutrients whilst the digestive enzymes continue to act on it. In addition, soluble materials could be released into the gut lumen as a result of bacterial activity.

These observations raise a question mark concerning the results of those experiments in which marine invertebrates have been left for a few hours only in clean water "to allow time for them to clear their guts". Obviously, a general investigation is required into the relationship between food deprivation and passage time in marine invertebrates.

Since there is no significant reduction in egestion rate during the first 5 h following removal from an algal suspension, it was decided to adopt the method which has been generally employed in energetics studies, which is to transfer fed animals from the food supply to containers of clean water. The faecal material is then harvested after a predetermined period of time has elapsed. The published time periods range from a few hours to 2 or 3 days (e.g. Hughes, 1970, 1971a; Kofoed, 1975a; Johnson, 1976). It is recognised,

however, that the longer the time period, the greater is the possibility that some of the faeces has undergone a greater amount of digestion, and the more chance there is of significant changes taking place in its chemical composition (see Johannes & Satomi, 1966) and hence in its calorific content.

Investigation showed that faecal pellets released during the initial 4 h following removal from the algal suspension were brown in colour and contained appreciable amounts of chlorophyll (this leached from the faeces when these were placed on wet filter paper). In contrast, the faeces that were released after 4 h were dark brown - black and contained relatively less chlorophyll and more phaeo-pigments, which are products of its degradation (See Shuman & Lorenzen, 1975). To reduce the possibility of error resulting from leaching and increased digestion, it was decided to harvest the faeces in subsequent experiments at the end of two hours.

5.3 The effect of temperature on egestion rate

5.3.1 Materials and Methods

One hundred and seventy five and one hundred and eighty five Mercierella acclimated to 10°C and 20°C respectively, were placed into 500 ml volumes of B. submarina suspension with an estimated cell density of 1.1×10^4 cells ml^{-1} and left to feed for 3 days, the suspension being replaced with fresh at 12 h intervals. At the end of the feeding period, both groups of worms were separated on the basis of their tube dimensions into groups of small (< 4.0 mg wet weight), medium (4.0 - 8.0 mg wet weight), and large (8.0 - 21.0 mg wet weight), composed of 2 - 16 individuals. Following several rinses with clean water to remove any particulate material, the groups were transferred to

Table 19. The egestion rates of individual M. enigmatica at 10°C and 20°C, when fed on a suspension of B. submarina at a cell density of 1.1 x 10^4 cells ml^{-1}.

(Numbers in parentheses are the number of weighings on which the mean values are based)

-------- ash-free dry weight indv.$^{-1}$ h^{-1}--------

Size class (mg wet wt.)	10°C Mean egestion rate (µg h^{-1})	± S.D.	20°C Mean egestion rate (µg h^{-1})	± S.D.
<4.0	2.56	± 0.7 (4)	2.31	± 1.41 (5)
4.0 - 8.0	3.34	± 1.06 (11)	3.73	± 2.83 (12)
8.0 - 21.0	5.66	± 2.74 (7)	11.93	± 6.25 (16)

individual petri dishes containing filtered water. The antibiotics streptomycin sulphate and benzylpenicillin (see Section 6.5) were added to the water 1 h before it was used, to reduce bacterial activity. The groups of worms were left undisturbed for 2 h, before they were returned to the food suspensions for a further 12 h, after which the process was repeated until sufficient faecal material had been harvested.

The contents of the petri dishes were filtered through individual, ashed, 5 mm diameter discs of Whatman GF/C glass fibre filter. Filtration was carried out under negative pressure on a suction apparatus similar to that described by Scott (1975). The faeces were given a 3 s rinse with glass-distilled water to reduce salt contamination, before the filter discs were dried to constant weight at 60°C. After weighing, the discs plus faeces were ashed in a muffle furnace at 500°C for 4 h, and when cool were reweighed. Weight loss during ashing is equivalent to the amount of ash-free organic material egested by a group of worms during the 2 h post-feeding period. Weighings were made to the nearest 1 µg on a Cahn 4100 Electrobalance.

5.3.2 Results

The average egestion rates of individual worms in the 3 size classes at the 2 temperatures are presented in Table 19. The values for individual worms were obtained by dividing the total ash-free dry weight of faeces produced by each group in 2 h by the number of individuals. These values were then divided by 2 to give the hourly egestion rates. Despite the considerable variation in these data, due to a combination of natural variability and the range of sizes comprising each class, there is reasonable agreement between the amounts of faeces produced and body size.

Table 20. Results of the statistical tests performed on the egestion data for the 'small','medium' and 'large' M. enigmatica at 10°C and 20°C.

Mean ash-free dry wt. of faeces produced by:	F-test	Method of comparison	Result
1) 10°C small and 10°C large	P = <0.05	Modified t-test (Bailey, 1959)	Significant (P = <0.05)
2) 20°C small and 20°C large	P = <0.01	"	Highly significant (P = <0.001)
3) 10°C small and 20°C small	P = >0.05	Student's t	Not significant (P = >0.05)
4) 10°C medium and 20°C medium	P = <0.01	Modified t-test	Not significant (P = >0.05)
5) 10°C large and 20°C large	P = <0.05	"	Significant (P = <0.01)

The results of the statistical tests that were performed on the data in Table 19 are summarised in Table 20. There is a significant size effect at both experimental temperatures, with the large worms producing a greater amount of faeces than did the small animals per unit time. It is not possible to compare the results for adjacent size classes due to the unavoidable overlap between them, since it was impossible to predict the exact size of an animal from its tube dimensions due to the natural variation in the relationship between body size and tube length (see Section 8.1.3). Furthermore, in most instances the tubes were incomplete.

In contrast, there is no significant effect of temperature on the egestion rates of small and medium size Mercierella, although there is a significant effect on the rate of egestion of large worms. Worms belonging to the large size class produced on average about twice the amount of faeces at the higher temperature. It seems likely, however, that any temperature effects on the other size classes are concealed by the increased variation.

5.3.3 Discussion

The results of this experiment indicate the anomalous nature of the results described in Section 3.4 concerning the effect of temperature on ingestion rate. This experiment was conducted under similar conditions to the present one, namely the same food concentration and temperatures, yet there is a complete lack of agreement between the amount of material ingested at the lower temperature, 10°C (see Fig. 28) and the quantity of faeces produced. In contrast there is good agreement between the rates of ingestion and egestion at the higher temperature, 20°C.

Table 21. Faeces expulsion behaviour of 'small', 'medium' and 'large' *M. enigmatica*, at 10°C and 20°C, when fed on a suspension of *B. submarina* cells, at a cell concentration of 1.1×10^4 cells ml^{-1}.

(Numbers in parentheses are the number of worm-hours on which the adjacent value is based)

Size class (mg wet wt.)	10°C Contraction rate (h^{-1})	± S.D.	20°C Contraction rate (h^{-1})	± S.D.
<4.0	1.9	± 0.89 (10)	3.5	± 1.78 (8)
4.0 - 8.0	1.3	± 1.2 (10)	2.4	± 0.89 (10)
8.0 - 21.0	0.9	± 1.24 (10)	1.6	± 0.96 (10)
Grand means	1.37	± 1.13 (30)	2.43	± 1.37 (28)

It is not clear why the 10°C acclimated Mercierella used in the experiment in Section 3.4 should have behaved differently than those used in the experiment described here, although it must be noted that the faeces were harvested in clean water. It is possible that 10°C acclimated animals spend extended periods of time withdrawn inside their tubes, as demonstrated by a few of the worms used in the feeding behaviour investigation (see Section 3.5.2), in which periods of withdrawal were interspersed by periods of more rapid feeding than is suggested by the results of Section 3.4. The results of the present experiment suggest that Mercierella's feeding rate at 10°C is more in accordance with the usual relationship between the rate of a physiological process and temperature which has been recorded on several separate occasions for this animal (see Sections 6.7.3 and 8.1.3), namely between a half and one third of the rate recorded at 20°C. For some unknown reason all the worms used in the ingestion experiment at 10°C appear to have been retracted for most of the time.

5.4 The effects of size and temperature on faeces expulsion behaviour

5.4.1 Introduction

Throughout the feeding behaviour studies (Section 3.5), a simultaneous record was kept of the number of faeces ejection movements made by worms in the 3 size classes, at the 2 acclimation temperatures, 10°C and 20°C. Worms belonging to the 'small' size class, < 4.0 mg wet weight, that were acclimated to 20°C, were observed for a total of 8 worm-hours, whereas the behaviour of the other groups was recorded for 10 worm-hours each.

5.4.2 Results

The results of the faeces ejection behaviour study are summarised in Table 21. The mean numbers of body contractions h^{-1} associated

Table 22. Summary of the results of the statistical analyses performed on the faeces expulsion behaviour data for the 'small', 'medium' and 'large' M. enigmatica, at 10°C and 20°C, feeding on a suspension of B. submarina cells, at a concentration of 1.1×10^4 cells ml^{-1}.

Comparison of the mean contraction rate of:	F-test	Method of comparison	Result
1) 10°C small with 10°C large	$P \geq 0.05$	Student's t-test	Not significant ($P \geq 0.05$)
2) 20°C small with 20°C large	$P \geq 0.05$	"	Not significant ($P \geq 0.05$)
3) 10°C small with 20°C small	$P \geq 0.05$	"	Not significant ($P \geq 0.05$)
4) 10°C medium with 20°C medium	$P \geq 0.05$	"	Not significant ($P \geq 0.05$)
5) 10°C large with 20°C large	$P \geq 0.05$	"	Not significant ($P \geq 0.05$)
6) 10°C sample with 20°C sample	$P \geq 0.05$	"	Significant ($P < 0.05$)

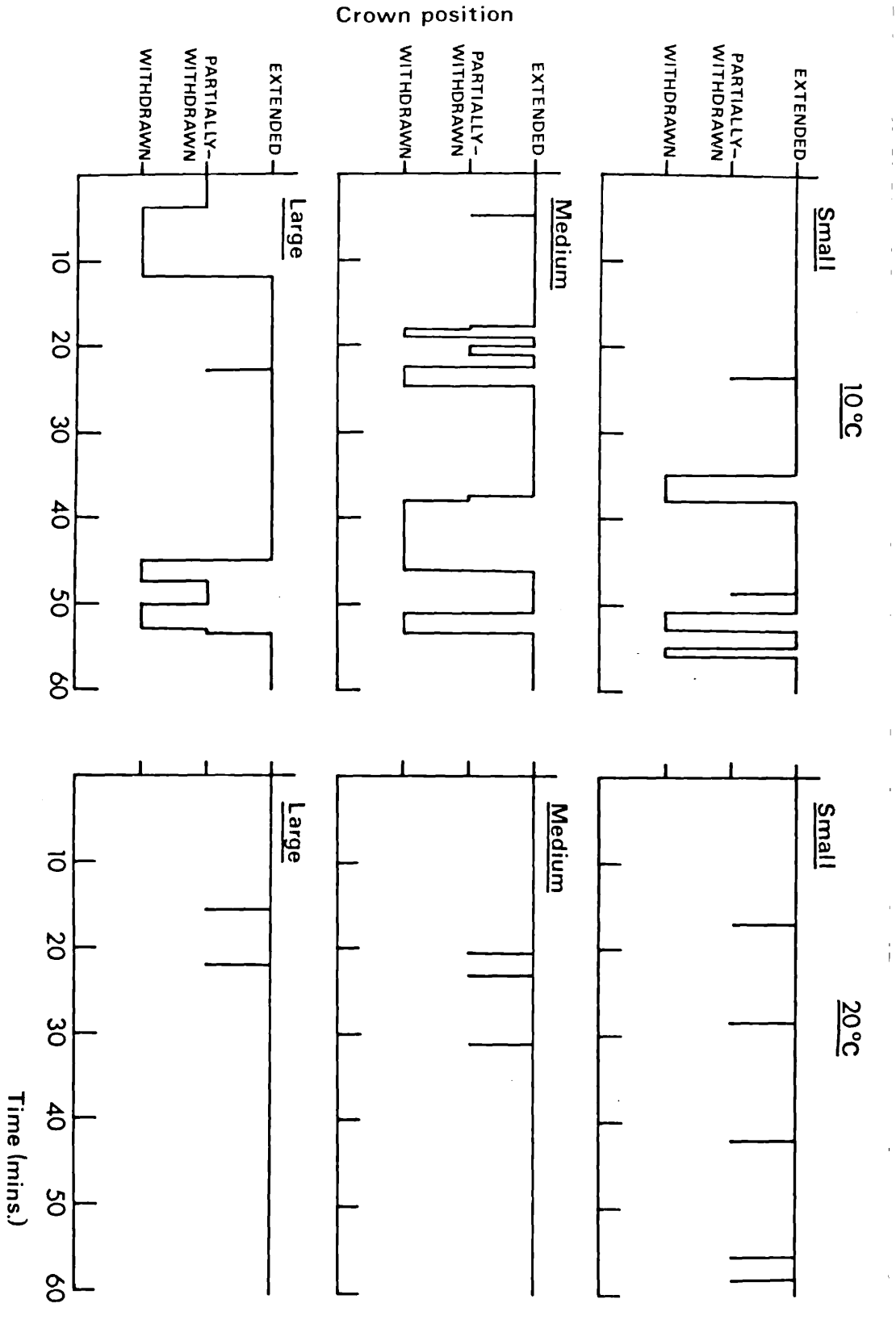
with the release of faeces from the tubes of worms belonging to the three size classes, at 10°C and 20°C, are based on the combined means of two 1 h observation periods, which in each case were separated by c. 1 h. The results of the statistical tests that were performed on these data are summarised in Table 22. Since it was not possible to predict the exact size of a worm from its tube dimensions (see Section 5.3.2), only the 'small' and 'large' groups at any one temperature have been compared.

It is apparent from the results that there is a tendency for smaller Mercierella to expel their faeces more often than do larger worms, at both experimental temperatures. There is also a tendency for worms in any one size class to expel their faeces more often at the higher temperature. In common with the majority of the other physiological processes that have been investigated in the present study, the effect of a 10°C increase in acclimation temperature is to increase the average rate of the reaction by a factor of about 2. However, due to the considerable variation in these data, as indicated by the standard deviations about each of the means in Table 21, the apparent size effect on faeces expulsion behaviour is not significant, and only the combined data for each temperature show a significant temperature effect.

5.4.3 Discussion

Preliminary investigation of Mercierella's egestion mechanism was carried out using worms with the posterior ends of their tubes removed to expose the last few segments of the abdomen. Although the faecal pellets are released singly from the anus (see Section 8.4.5 for details of the rate at which these are released in relation to temperature), they are accumulated at the anterior end of the thorax

Fig. 46. Ethograms for representative worms from each of the size classes; 'small' (<4.0 mg wet weight), 'medium' (4.0 - 8.0 mg), and 'large' (8.0 - 21.0 mg), at the two temperatures. Faeces expulsion activity, when the branchial crown is partially withdrawn inside the tube for < 2 s, is depicted by the short spikes.


Crown position
EXTENDED
PARTIALLY-WITHDRAWN
WITHDRAWN
Small
Medium
Large
10 °C
20 °C
10
20
30
40
50
60
Time (mins.)

before being ejected from the tube as an amorphous mass. Faeces expulsion is effected by a rapid, longitudinal contraction of the worm's body, which forces water out of the tube, carrying with it the faecal material. The body contraction generally lasts for less than 2 s, during which time the branchial crown is partially withdrawn inside the tube. Fig. 46 shows ethograms for representative worms from each of the 3 size classes, at the two experimental temperatures. Faeces expulsion behaviour is depicted by the short spikes. Occasionally, faeces ejection contractions are followed by the complete withdrawal of the crown, and a mass of faecal pellets is sometimes expelled from the tube before a worm recommences feeding activity.

5.5 The biochemical composition and calorific content of faeces produced by Mercierella acclimated to 10°C and 20°C

5.5.1 Materials and Methods

Two groups of 30 Mercierella acclimated to the two experimental temperatures, 10°C and 20°C, were placed into 500 ml volumes of B. submarina suspension, which had a cell density of 1.1×10^4 cells ml^{-1}. Each of the groups was selected as representing the sample size range. At the end of a 3 d preliminary feeding period, during which the algal suspension was replaced with fresh at 12 h intervals, the worms were transferred to petri dishes containing filtered water plus antibiotics, via several rinses with clean water to remove adhering cells. Each petri dish contained about 5 worms chosen to represent the sample size range to counteract possible size effects on the biochemical composition and calorific content of faecal material. The faeces produced by the groups of worms were harvested after 2 h (see Section 5.2), and the worms returned to the algal suspensions for a further 12 h, after

which the process was repeated until sufficient faecal material was obtained for analysis.

For biochemical analysis, the contents of the petri dishes were filtered through ashed and weighed, 20 mm diameter discs of Whatman GF/C glass fibre filter. After a 3 s rinse with glass-distilled water to reduce salt contamination, the discs were dried to constant weight at 60°C. The faeces used for the calorific determinations were concentrated by slow centrifugation (1 000 r.p.m.) and the supernatant discarded. The faecal matter was then resuspended in glass-distilled water, and after a second centrifugation, transferred to a clean crystallising dish. The dish was loosely covered to exclude dust particles before it was placed in a drying oven at 60°C. When the faecal material was dry, it was scraped off the bottom of the dish, and forced through a 125 μm steel-meshed sieve to ensure complete homogeneity. The dry faeces samples were stored in a desiccator, over anhydrous calcium carbonate.

Protein. This was estimated from total nitrogen content, determined with a Coleman Model 29 Nitrogen Analyser. The measurements were multiplied by a factor of 6.25 to give the amounts of protein in the faeces samples. (It was assumed that protein contains 16% N by weight.) Six faeces samples collected at 10°C (size range: 1.26 - 1.93 mg dry weight), and fifteen 20°C samples (size range: 1.01 - 2.97 mg dry weight) were analysed for nitrogen content, and the readings corrected for background using glass fibre filter blanks. Weighings were made to the nearest 0.1 mg on a Sartorius semi-microbalance.

Lipid. Lipid content of the faeces samples was determined gravimetrically after extraction with diethyl ether (1.5 h at 60°C) in a micro-Soxhlet apparatus. Twelve 10°C faeces samples (size range:

0.22 - 2.27 mg dry weight), and sixteen 20°C samples (size range: 0.79 - 2.11 mg dry weight) were analysed for lipid content. Dry weights were measured to the nearest 10 µg on a Cahn 4100 Electrobalance.

Ash. The ash content found depended on the method, rather than being a characteristic of the organism. Due to the repeated harvestings that were necessary from the 10°C acclimated worms, to obtain sufficient faecal material for a single sample, these had higher ash contents (caused by the accretion of salts during the repeated dryings), than the samples taken from the 20°C worms. Recognising these differences as being non-biological in origin, all computations have been carried out using ash-free dry weights.

Since N determination involved the complete destruction of the sample, it was necessary to estimate the percentage ash content of the protein analysis samples by using duplicate samples. Eight 10°C faeces samples (size range: 0.22 - 2.27 mg dry weight) and nine 20°C samples (size range: 1.03 - 5.4 mg dry weight) were oxidised in a muffle furnace at 500°C for 4 h (Paine, 1964). In contrast, it was possible to recover the lipid analysis samples after extraction in the Soxhlet apparatus, consequently ash content was determined using the same samples. The ash contents of twelve 10°C faeces samples (initial size range: 0.22 - 2.27 mg dry weight) and sixteen 20°C samples (initial size range: 0.79 - 2.11 mg dry weight) were measured. Weighings were made to the nearest 10 µg on a Cahn 4100 Electrobalance.

Carbohydrate. No carbohydrate analyses were carried out. The percentage carbohydrate contents of the faeces samples were estimated by difference.

Calorific content. The calorific contents of the faeces samples were measured in a Scott micro-bomb calorimeter (Scott, 1975). The calorimeter was calibrated with 14 separate determinations of benzoic acid (British Chemical Standards), the samples ranging in size from 10 - 95 µg. There is a linear relationship between the calorific values of the samples (0.26 - 2.51 J) and the recorded galvanometer deflections (0.11 - 0.57 mV).

Small amounts of dry faeces were placed onto dampened, ashed and weighed, 4 mm diameter discs of GF/C glass fibre filter, mounted on a suction device similar to that described by Scott (1975). Excess water was then drawn through the filter by gentle negative pressure, and the faeces were dried onto the discs in an oven at 60°C. When they were dry, the filter discs were re-weighed before the adhering faeces were ignited in the calorimeter.

In contrast to the B. submarina cells (Section 3.6) the faeces samples failed to combust completely in the calorimeter, and examination of the discs revealed considerable quantities of salt, particularly in the case of the 10°C samples. Previous experiments, using somatic tissues (Section 8.1.2), and gametes (Section 8.2.2) had shown that their combustibility is improved by dialysis treatment. It was decided therefore to resuspend the faecal material in glass-distilled water for 5 min to remove some of the salts. The faeces were then recovered by centrifugation (1 000 r.p.m. for 30 s) and transferred to fresh glass fibre discs. As this calorimetry was carried out at the Scottish Marine Biological Association's Laboratory at Dunstaffnage, Oban, a distilled water wash was used instead of dialysis with Visking tubing. This treatment improved their combustibility, but 50% of the samples still failed to ignite completely, so in these cases, the recorded

Table 23. The chemical composition of the food and faeces of *M. enigmatica* acclimated to 10°C and 20°C, feeding on a cell suspension with a concentration of 1.1×10^4 cells ml^{-1}.

-----% ash-free dry weight-----

(Numbers in parentheses are the number of analyses on which the adjacent value is based)

	Protein ± S.D. (N x 6.25)	Carbohydrate (by difference)	Lipid ± S.D.
Brachiomonas submarina	87.09 ± 7.05 (6)	3.5	9.41 ± 4.33 (6)
	54.3*	36.29	9.41 ± 4.33 (6)
10°C faeces	48.42 ± 9.41 (6)	28.39	23.19 ± 2.57 (2)
20°C faeces	54.46 ± 14.05 (15)	28.35	17.19 ± 3.94 (16)

*average value for protein content of phytoplankton, based on the results of Parsons *et al.* (1961), and Platt & Irwin (1973) (see Section 3.6.3 and Table 11).

temperature rise was corrected for the weight of the uncombusted but combustible material. This was determined by ashing the weighed residue in a muffle furnace for about 4 h, and re-weighing the discs plus ash.

The ash-free dry weights of the faeces samples which completely combusted in the calorimeter, were estimated from the percentage ash contents of 3 replicates for each temperature. Weighings were made to the nearest 5 µg on a Beckman LM-500 microbalance. Four 10°C faeces samples (size range: 10 - 113.7 µg ash-free dry weight, and five 20°C samples (size range: 42.6 - 127.8 µg ash-free dry weight), gave galvanometer deflections of 0.073 - 0.536 mV, and 0.15 - 0.475 mV respectively, after a fuse wire correction was applied.

5.5.2 Results

Table 23 shows a summary of the results of the biochemical analyses performed on the faecal material produced by *Mercierella*, at 10°C and 20°C, together with the results of the *B. submarina* analyses (Section 3.6).

The faeces produced at 10°C contained on average 5.46 $\pm$ 1.06% N, and the 20°C faeces 6.63 $\pm$ 1.71% N. These samples were estimated as having protein contents of 34.13 $\pm$ 6.63%, and 41.44 $\pm$ 10.69% of the dry weight respectively. There is no significant difference between the mean ash contents of faeces samples used for the nitrogen determinations (29.51 $\pm$ 9.8% of the 10°C samples, and 23.91 $\pm$ 13.62% of the 20°C samples). After an *F*-test had shown there to be no significant difference between the variances about the mean ash-free dry weights of protein in the 10°C and 20°C faeces samples ($P = > 0.05$),

Table 24. Statistical analyses performed on the estimated lipid contents of the B. submarina cells, and faeces produced at 10°C and 20°C

Lipid content of:	F-test	Method of comparison	Result
1) 10°C faeces with 20°C faeces	P => 0.05	Student's t	Not significant (P => 0.05)
2) B. submarina with 10°C faeces	P => 0.05	"	Significant (P = <0.01)
3) B. submarina with 20°C faeces	P => 0.05	"	Highly significant (P = <0.001)

the means were compared using Student's t-test, which showed there is no significant difference between them ($P = > 0.05$).

Excluding an anomalous lipid value of 61.7% of the dry weight recorded for a faeces sample with an initial dry weight of 2.27 mg and ash content of 16.3%, the majority (10) of the 10°C samples failed to show a significant loss of weight in the micro-Soxhlet apparatus. In contrast, the remaining two 10°C samples had lipid contents of 21.37% and 25.01% ash-free dry weight. Those samples which showed zero weight loss had very high ash contents, ranging from 42.3 - 68.5% of the dry weight, whereas the two samples which showed significant losses of weight had ash contents of only 7.95% and 6.98% dry weight. It is not known why the presence of relatively large amounts of salts apparently prevented the release of lipids in the Soxhlet apparatus. Thus only two 10°C analyses were used in the calculation of the value shown in Table 24.

The results of the statistical tests performed on the mean values for lipid content of B. submarina cells, and the faeces produced at 10°C and 20°C, are presented in Table 24. It should be noted that the values for lipid content are slightly overestimated because no correction is made for the amount of pigment lost from the samples during the Soxhlet extraction (see Section 3.6.2).

The 10°C and 20°C faeces samples that were combusted in the micro-bomb calorimeter, had estimated ash contents of 61.96% and 34.33% of their dry weight respectively. The calorific values of the faeces samples, together with the calorific content of B. submarina cells (see Section 3.6.2), are presented in Table 25a. The value for the food alga is based on an ash-content of 4.51% dry weight (Table 9). Compared with the results obtained for the B. submarina cells, and the

Table 25. A summary of the results of a) the calorific content measurements of the food, and faeces at 10°C and 20°C, and b) the statistical analyses performed on them.

-----% ash-free dry weight-----

a)

Sample	Mean calorific content (joules mg^{-1}) ± S.D.	Mean calorific content (calories mg^{-1}) ± S.D.
Brachiomonas submarina	19.9 ± 3.43	4.76 ± 0.82
10°C faeces	27.21 ± 7.86	6.51 ± 1.88
20°C faeces	14.46 ± 1.30	3.46 ± 0.31

b)

Calorific content of:	F-test	Method of comparison	Result
1) 10°C faeces with 20°C faeces	$P = <0.01$	Modified t-test (Bailey, 1959)	Significant ($P = <0.02$)
2) B. submarina with 10°C faeces	$P = >0.05$	Student's t	Not significant ($P = >0.05$)
3) B. submarina with 20°C faeces	$P = <0.05$	Modified t-test	Significant ($P = <0.05$)

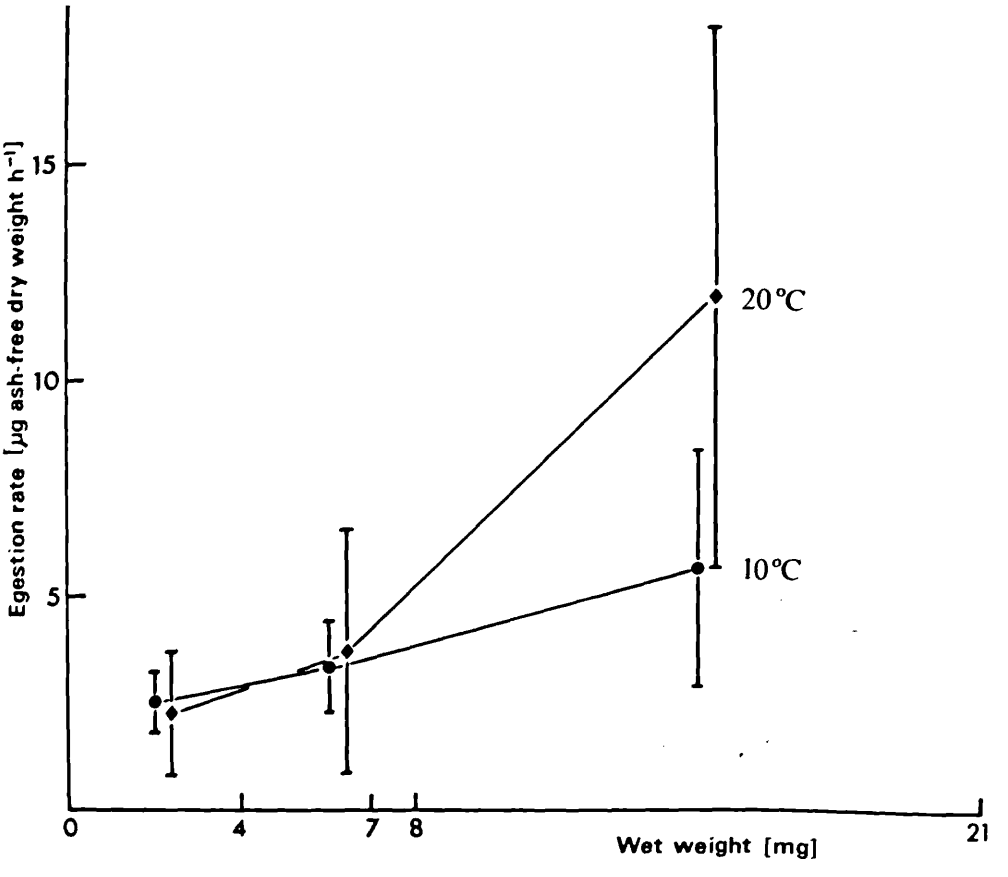
20°C faeces samples, the 10°C values show considerable variation. This is probably due to the difficulty that was experienced in achieving combustion of these samples in the calorimeter. It is likely that appreciable amounts of soluble organic matter were lost from the faeces during the washing treatment to reduce the salt content. The results of the statistical tests that were carried out on the calorific data for the food and faeces are summarised in Table 25b.

5.5.3 Discussion

In common with the results of biochemical analyses of B. submarina (Section 3.6.2 and Section 3.6.3), protein predominates among the organic components of the faeces. Assuming that the estimated protein contents are correct, there is no difference between the percentage protein composition of the food and faeces at the two experimental temperatures. In contrast, the faeces appear to contain appreciably more lipid than do the algal cells (on a unit weight basis), indicating that relatively less lipid assimilation had occurred compared with protein. In addition, whereas there is no difference between the levels of carbohydrate in the 10°C and 20°C faeces samples, these contained less carbohydrate than do the B. submarina cells, indicating that relatively more carbohydrate than protein was assimilated.

The results of the statistical analyses performed on the calorimetry data (Table 25b) show that there is no significant difference between the calorific contents of B. Submarina and the faeces produced at the lower temperature, whereas the 20°C faeces have a significantly lower calorific value per unit ash-free dry weight. Calculation of the energy content of B. submarina, 10°C faeces and 20°C faeces, from their proximate biochemical composition, using values given by Brody (1945), for the heats of combustion of glucose, lipid

Fig. 47. Egestion rate expressed as a function of body size of M. enigmatica acclimated to winter and summer temperatures, and feeding on a B. submarina suspension with a concentration of 1.1×10^4 cells ml^{-1}. The points represent the mean rate of faeces production per individual and the vertical lines $\pm$ 1 standard deviation.


Egestion rate [µg ash-free dry weight h⁻¹]
15
10
5
0
4
7
8
21
Wet weight [mg]
20°C
10°C

and protein (see Section 3.6.3) gave the following values: 22.3 J mg^{-1} ash-free dry weight, 25.11 J mg^{-1} ash-free dry weight and 24.15 J mg^{-1} ash-free dry weight respectively. Thus, whereas there is reasonable agreement between the estimated and measured values in both the B. submarina and 10°C faeces samples, there is a considerable discrepancy in the case of the faeces produced at the higher temperature. Possible reasons for this discrepancy have been discussed already in Section 3.6.2.

5.6 The loss of energy through defaecation

The average hourly egestion rates at 10°C and 20°C by Mercierella weighing 7.0 mg wet weight, when feeding on a B. submarina suspension with a density of 1.1×10^4 cells ml^{-1}, were obtained from lines fitted to the average rates of egestion by the 3 size classes (Table 19) when plotted against body size (Fig. 47). The estimated rates of egestion by a standard worm when acclimated to winter and summer temperatures are 3.6 and 4.7 µg ash-free dry weight $indiv.^{-1}$ h^{-1} respectively. From the calorific measurements made using the Scott micro-bomb calorimeter (Table 25a), it was calculated that these values represent the following energy losses: 9.8×10^{-2} J h^{-1} (2.34×10^{-2} cal h^{-1}) at 10°C, and 6.8×10^{-2} J h^{-1} (1.63×10^{-2} cal h^{-1}) at 20°C.

6. RESPIRATION

6.1 Introduction

When Mercierella is covered by water the branchial crown extends from the end of the tube, and the large collar is folded back over the anterior margin. The expanded branchial crown forms a funnel through which water is drawn from the sides to be forced out of the centre as a strong rejection current. Dales (1957) demonstrated that serpulid tube-worms filter relatively considerable volumes of water in terms of body weight during the course of their feeding activities. Pomatoceros triqueter strains 1.4 l h^{-1} g^{-1} fresh weight, and Hydroides norvegica, 0.9 l h^{-1} g^{-1} fresh weight. Behavioural investigations showed (Section 3.5.2) that Mercierella filters water for the majority of the time, and unless it is disturbed, the worm's feeding activities are only interrupted by the sporadic, rapid, longitudinal contractions of the body that are associated with the ejection of faecal pellets into the rejection current.

In contrast to the branchial crown, the rest of the body does not leave the confines of the hard, impermeable calcareous tube. However, a flow of water inside the tube has been described for several serpulid species: Filograna implexa Berkeley (Faulkner, 1929 in Thomas, 1940); Pomatoceros triqueter (Thomas, 1940); and Mercierella enigmatica (Hall, 1954). In contrast to Filograna's tube which is open at each end, Mercierella's tube is usually closed at the narrow, posterior end, and unless recently damaged there are no holes in the walls. For the worm will repair any small holes and fractures that can be reached with the calcium carbonate secreting glands situated beneath the collar. There is also evidence which suggests that serpulids may be able to secrete tube material from other parts of their bodies (see Hedley, 1958).

Since under normal conditions the tube is complete, water can only enter through the anterior opening. Hall (1954) reported that water is drawn into the tube between the divided dorsal lobes of the collar and circulates in the chamber formed by the folds of the thoracic membrane. Initially the water travels in a posterior direction against the dorsal (inner) surface of the thoracic membrane before turning back, after several segments, on the dorsal surface of the thorax. A similar anteriorly directed current occurs in Pomatoceros.

In addition to these thoracic currents, there is a strong ciliary generated movement of water along the median ventral groove on the abdomen, which was demonstrated in Mercierella living in clear polythene tubes, by introducing carmine and graphite particles into the open posterior end. Within a few seconds of their introduction, the particles were swept in this current to the anterior part of the abdomen, from where they were directed by the ventral lobe of the thoracic membrane onto the adjacent, dorsally-directed, lateral ciliary tracts, which carried them to the dorsal surface of the thorax and the anteriorly directed water currents that carried them to the outside.

Apart from the inwardly directed water current on the dorsal surface of the collar, which moves water towards the tube entrance, there are no other posteriorly directed ciliary generated currents. Instead, there is a passive movement of water along the dorsal and lateral walls of the abdomen to replace that which is displaced in an anterior direction, by cilia beating in the median ventral groove.

When disturbed, Mercierella withdraws the branchial crown into the safety of the tube entrance, whilst leaving the sensory-tactile tips of the branchial filaments protruding from the end of the tube. The cilia

on the branchial filaments remain active throughout this partially withdrawn phase, and if no further disturbance follows, the worm will return to the normal feeding position within a few min. However, if the tips of the branchiae are touched, as by a potential predator, the worm quickly retreats into the posterior portion of the tube, blocking the entrance with the defensive operculum.

When Mercierella is fully withdrawn inside its tube, ciliary activity on the crown ceases altogether, but faecal pellets continue to be released from the anus and are carried to the base of the crown by the ciliated tracts on the body which indicates that water continues to circulate around the general body surface. The operculum of a fully withdrawn worm is not held tightly in place, unless it has been recently disturbed, and a narrow gap usually remains enabling an exchange with oxygenated water from the outside to take place.

When the crown is partially withdrawn, the flow of blood along the gut-sinus becomes reduced, and when the worm is fully withdrawn inside the tube the blood flow is further reduced. Measurements of the rate at which the antiperistaltic waves of muscular contraction passed a fixed point on the gut of a worm growing on a microscope slide (this enabled the gut to be viewed through the body wall since the tube is incomplete on the lower side), showed that the rate of blood flow is 16 contractions min^{-1}, at 17°C, 12 min^{-1} in a partially withdrawn animal, and 3 min^{-1} in a fully withdrawn specimen. When Mercierella is fully withdrawn, the flow of blood to the operculum and branchial filaments ceases.

Up till now, the observations have dealt with animals that are immersed in water. However, on occasion the worm is exposed to the air. When Mercierella becomes emersed, it withdraws fully inside its tube and

closes the entrance with the operculum. Unless the inside of the tube begins to dry out, a narrow gap remains around the edge of the operculum which allows gas exchange to take place between the chamber enclosing the animal and the surrounding air, a situation that is analogous to the function of the micropyle of the barnacle, Chthamalus stellatus (Poli). Under damp conditions Mercierella will sometimes protrude the collapsed branchial crown from the tube entrance, a behavioural phenomenon that parallels a condition observed during the animal's exposure to anaerobic conditions resulting from the breakdown of an air pump. The tank in question contained a considerable amount of detrital material whose decomposition hastened the depletion of oxygen. In contrast, if the inside of the tube begins to dry out, the worm will tightly seal itself in by means of the operculum, remaining in an inactive state for as long as 2 d at room temperature (c. 20°C).

In view of the behavioural complexity associated with a tubicolous habit, it is of interest to determine the relative significance of the various parts of the body as respiratory surfaces during the different behavioural phases. However, before this was carried out, it was necessary to know the extent of the blood system and the nature and properties of the blood pigment.

6.2 The blood-system and blood pigment

6.2.1 Introduction

The anatomy of the blood-system in the Serpulidae is described in detail by Hanson (1950). Apart from giving a detailed account of the blood-system in Pomatoceros triqueter and the variations found in 11 other serpulid species, Hanson also reviewed the previous work that had been carried out on this group.

Basically, the blood-system consists of two parts: a central system consisting of large vessels, and a peripheral system comprised of blind-ending vessels of smaller diameter. The dorsal blood vessel has lost its identity, except in the oesophageal region; along the rest of its length it envelopes the gut, forming the gut blood-sinus (Fig. 56). The outer wall of the blood-sinus contains muscle fibres, in common with all other serpulid blood vessels (Hanson, 1950), which force the blood along it in an anterior direction, by a series of antiperistaltic waves of contraction, each of which is several segments in length. Anteriorly, the blood enters the dorsal blood vessel, that lies above the oesophagus. From the dorsal vessel the blood flows into the branchial crown and operculum, which form part of the peripheral blood-system and/or is directed through the transverse and circum-oesophageal vessels to the ventral vessel (Fig.56), where it travels in a posterior direction, finally making its way back into the gut-sinus through the segmentally arranged ring vessels. In the anterior part of the dorsal vessel there is a valve that prevents blood leaving the branchial region from flowing back into the dorsal vessel.

The peripheral blood-system consists of the branchial vessels (Fig. 53), and their branches that supply the crown filaments and the operculum, and the vessels supplying the collar, lips and anterior portion of the alimentary canal, the plexus surrounding the oesophagus, and the trans-septal vessels which supply the body wall, chaetae and the thoracic membrane. The peripheral blood-system consists of blind-ending vessels that are alternately full and empty.

6.2.2 Materials and Methods

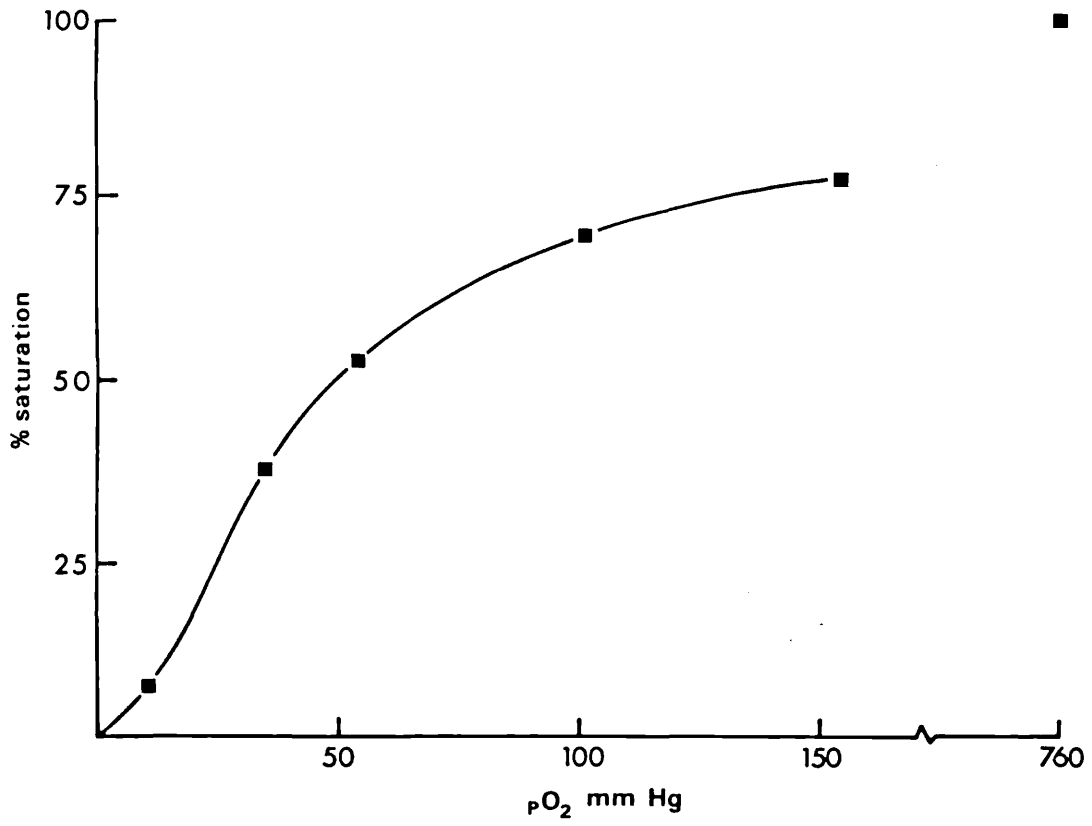
Mercierella were collected from the pools of standing water at about the mid-tide level at Greenhithe, Kent.

The worms were bled soon after return to the laboratory. Blood was collected from the gut-sinus in glass micro-pipettes, after the body cavity had been opened along its dorsal surface and the viscous coelomic fluid blotted off with a paper tissue. The micro-pipettes were inserted at an oblique angle to prevent rupturing the gut. The operation was performed on a moistened glass slide under a binocular microscope, whilst the worm was restrained with thin sheets of rubber placed across its anterior and posterior ends. Blood was also collected from the operculum in a micropipette inserted through the peduncle. Approximately 0.2 µl of blood was obtained from each animal. The blood samples were stored(after the ends of the pipettes had been sealed in a flame) packed in ice for no longer than 3 days.

A pooled 30 µl sample of whole blood was diluted with distilled water, to give a 1% solution of pH 7.0, and centrifuged at 20 000x g for 5 min to remove debris. The resulting clear supernatant had a faint green tinge, indicating that the respiratory protein had not been spun down by this treatment.

Three 1 ml samples of the 1% blood solution were equilibrated for about 10 min with different oxygen tensions, carbon monoxide (coal gas), and reduced with sodium hydrosulphite, in a tonometer that incorporated a glass spectrophotometer cuvette of 1 ml internal capacity. Gases were selected from pressurised cylinders of oxygen-free nitrogen, pure oxygen, and compressed air, and mixed in a Gallenkamp gas mixer. The experiments were carried out at room temperature (c. 20°C). The approximate positions of the absorption maxima were first located with a Beckman DB-G spectrophotometer, before their wavelengths and percentage transmission were measured exactly with a Unicam SP 500 spectrophotometer.

Fig. 48. Sigmoid-shaped oxygen equilibrium curve for the diluted blood of M. enigmatica (1% solution) at pH 7.0 and 20°C, showing its failure to reach full saturation at an atmospheric oxygen tension of 152 mm Hg (pO_2).


100
75
50
25
% saturation
50
100
150
760
$_{P}O_2$ mm Hg

6.2.3 Results

The blood pigment in Mercierella is dichroic, being red in the large blood vessels and green in the smaller ones, and is in free solution in the blood. The 1% solution had α and β absorption maxima at 604 and 554 nm, and a prominent Sorêt band at 437 nm in the oxygenated state. In the reduced condition, the Sorêt peak is shifted towards the red end of the spectrum at 447 nm. Moreover, the α and β maxima become depressed into a broad weak band, with traces of the two peaks being recognisable at 600 and 571 nm, showing that there is a concomitant decrease in the span between them. These results are in agreement with the data of Fox (1926, 1949), Antonini et al., (1962), and Wells and Dales (1975) for the iron-containing protein, chlorocruorin, that is found in the other members of this group.

Compared with oxychlorocruorin, the α peak of carboxychlorocruorin is shifted towards the violet end of the spectrum at 600 nm, and the β peak is depressed. The span between the α peaks of oxy- and deoxychlorocruorin differs from one chlorocruorin to another (Fox, 1926). Similarly, different genera and families show differences in the exact positions of the absorption maxima.

6.2.4 Discussion

Fig. 48 shows the oxygen equilibrium curve for the diluted blood of Mercierella, at pH 7.0 and 20°C. Like other chlorocruorins, with the exception of that of Myxicola infundibulum (Wells & Dales, 1975) Mercierella's blood is not fully saturated with oxygen when it is in equilibrium with air (pO_2 152 mm Hg). It is only 78% saturated in air, complete saturation being achieved by exposing the solution to pure oxygen, pO_2 760 mm Hg.

A P_{50} of 50 mm Hg suggests that Mercierella's chlorocruorin functions at high external oxygen concentrations, although Wells and Dales (1975) pointed out that despite their relatively low oxygen affinities, chlorocruorins appear to have high oxygen capacities, which could enable them to transport oxygen under lower ambient oxygen levels than the equilibrium curves suggest is possible. However, due to its intertidal distribution, Mercierella is not generally exposed to the extremely low levels of dissolved oxygen that prevail during the low tide periods at Greenhithe (Fig. 23). The recorded distribution is thus in agreement with the experimentally determined properties of the blood.

Occasionally, worms in the Greenhithe population are exposed to low external dissolved oxygen levels (< 20% > 10% saturation), although for only a few hours at a time. Straughan (1970) described a Mercierella (= Neopomatus ?) population in the North Pine River, S. Queensland, that survived exposure to completely deoxygenated water for about 3 h each tide, after which the oxygen content of the water gradually rose to the normal surface level (D. Straughan, personal communication, 1974). Another reported sighting of serpulids surviving exposure to reduced levels of dissolved oxygen is that by T.C. Nelson (in Tebble, 1956), in Barnegat Bay, New Jersey. These specimens were not positively identified as Mercierella enigmatica, but the environmental conditions were appropriate for this species. Whilst these observations seem to refute the generally held view that fan worms are restricted to well aerated waters because their blood is unable to transport oxygen under low external concentrations, the possible importance of the intermittent periods of high external oxygen levels should not be overlooked.

6.3 The relative significance of the potential respiratory surfaces during the different phases of activity

6.3.1 Introduction

There are conflicting reports in the literature concerning the sites of gaseous exchange in serpulid tubeworms. Thomas (1940) stated respiratory exchange takes place through the branchial crown and the general surface of the body, whereas Straughan (1968) reported that the branchiae are used mainly for the collection of food, "and not, as their name suggests for respiration". Straughan stated that gas exchange occurs through the surface of the collar and thoracic membrane, although she provided no data to support this statement.

The essential criteria for a respiratory organ are : a large surface area; tissues that are permeable to gases; and a rich blood supply. This is complicated, however, in the case of Mercierella since only the anterior portion is extended into the surrounding water when the worm is feeding, and generally the whole body is withdrawn inside the calcareous tube when it is emersed. It is unlikely therefore, that the same organs will function as the major gas exchange sites during all phases of activity.

The possible respiratory surfaces are: the branchial filaments and pinnules; the operculum; the lips; the oesophagus; the collar; the thoracic membrane; the general body surface; and the rectum. An histological investigation has been made of each of these, and their relative significance as respiratory surfaces during the three phases of activity: feeding, partially withdrawn, and withdrawn, has been assessed based on this and observations of the worm's behaviour.

6.3.2 Materials and Methods

Adult Mercierella with a total body length of about 10 mm were removed from their tubes and fixed in glass tubes, that prevented the abdomens curling during fixation. For the general anatomical investigation the specimens were fixed in 70% alcohol, whereas those used in the study of the blood-system were fixed in 10% formalin. Longitudinal and transverse serial sections were cut of whole worms at a thickness of 8 µm. For general anatomy, the sections were stained with haemotoxylin and eosin, whereas the blood-system was stained using the benzidine method for peroxidases (Pfaff & Williams, 1942 in Gray, 1954; Ziegler, 1945).

The permeability of an organ to oxygen depends on the thickness of the cuticle, and the nature and thickness of the underlying tissues. Measurements of cuticle thickness, and the minimum and maximum distances between the external surface and the nearest blood vessel were carried out using a calibrated graticule eyepiece. Each cuticle value is the mean of 10 separate measurements. Since the benzidine method results in the production of appreciable volumes of oxygen gas, that has a tendency to damage and distort sections, all the measurements were made using haemotoxylin and eosin stained material.

Observations of the blood flow in the branchial crown, and ciliary activity, were made using worms living in clear polythene tubes.

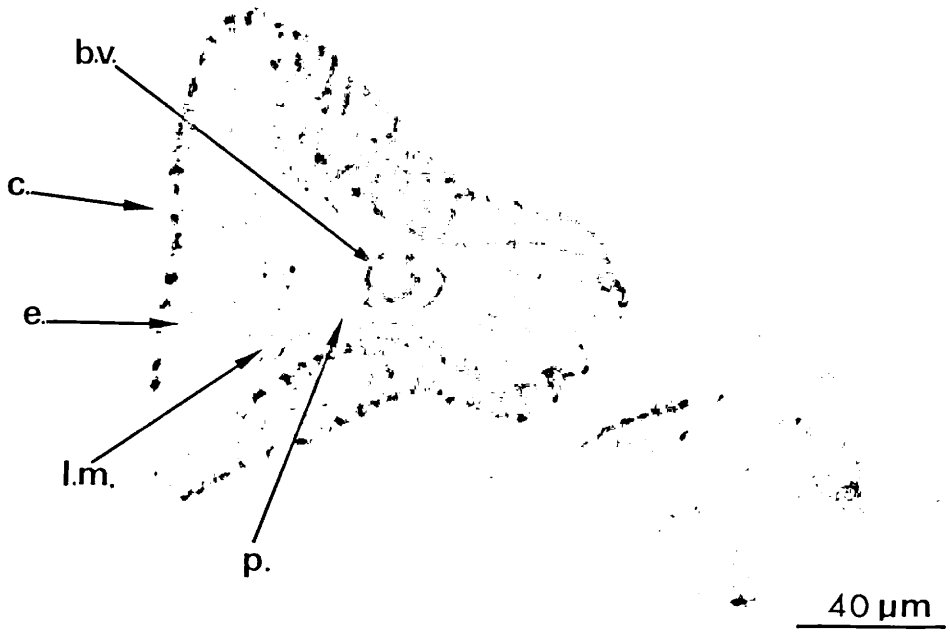
6.3.3 Results

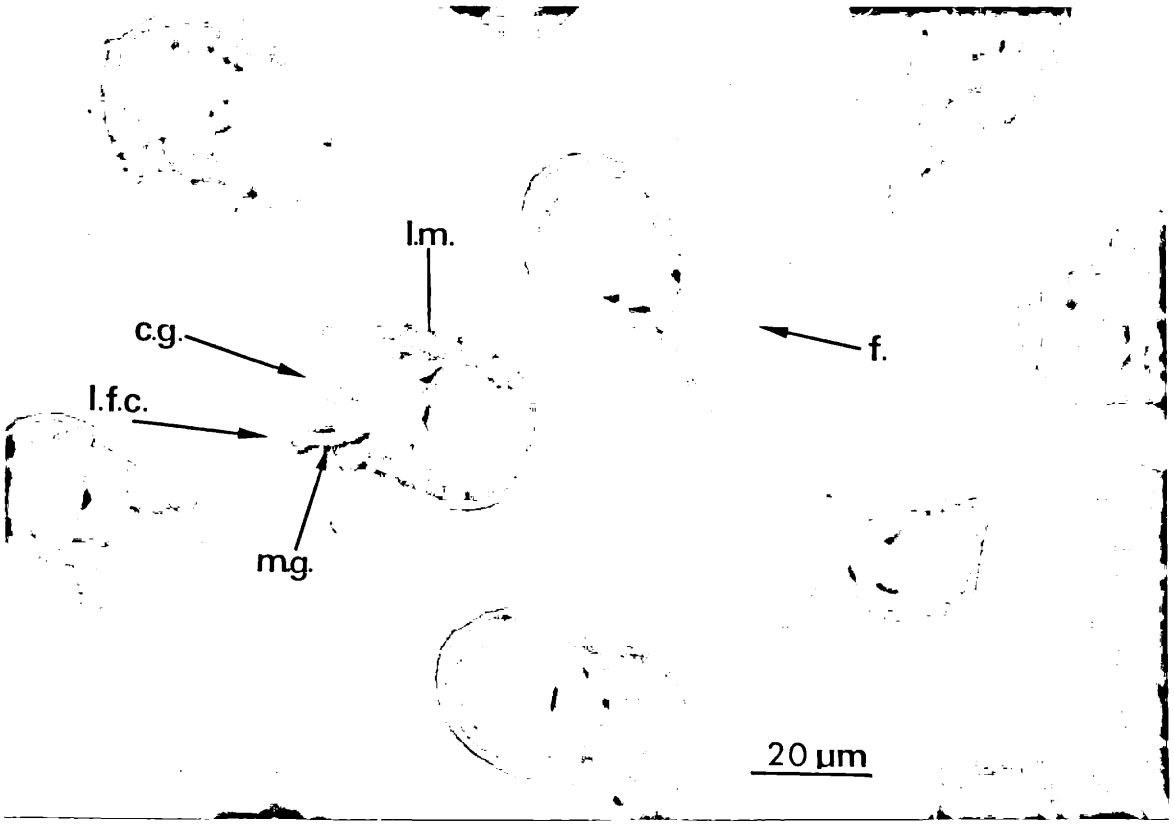
The branchial filaments

In transverse section a branchial filament is quadrilateral, with the shortest side innermost (Fig. 49). The innermost surface is concave, ciliated, and lacks a cuticle. In contrast, the three

Fig. 49. Transverse section of a branchial filament stained with haemotoxylin and eosin. The innermost surface (R.H.S.) is damaged in this specimen. b.v., blood vessel; c., cuticle; e., epithelium; l.m., longitudinal muscle fibres; p., parenchymatous tissue.

Fig. 50. Transverse section of branchial pinnules stained with haemotoxylin and eosin, showing the approximately ovate form. The blunt, innermost surface lacks a cuticle and bears a ciliated groove, c.g., which carries the food laden mucous secretions, produced by mucous glands, m.g., to the filament. f., frontal cilia; l.f.c., latero-frontal cilia; l.m., ring of longitudinal muscle fibres.


b.v.
c.
e.
l.m.
p.
40 µm


l.m.
c.g.
l.f.c.
m.g.
f.
20 µm

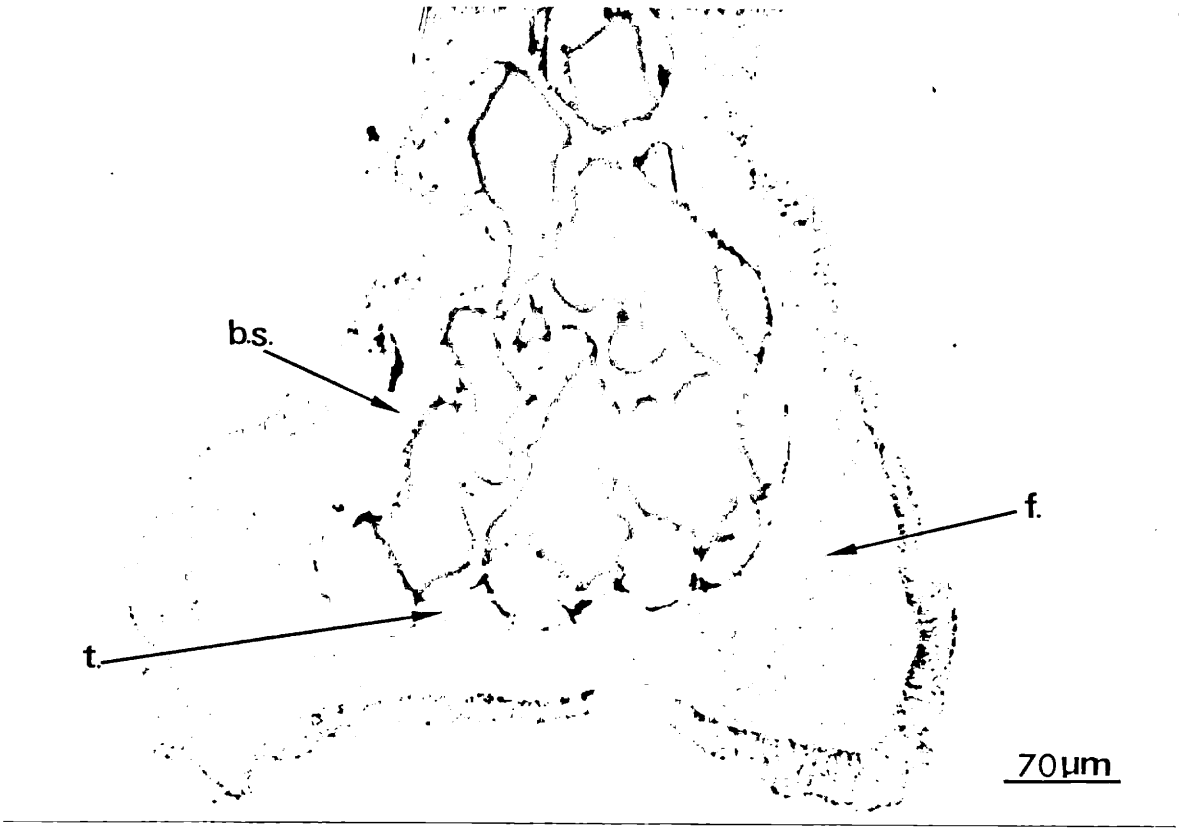
unciliated sides are covered with a thin cuticle. The wall of the filament is composed of a single layer of columnar epithelium, beneath which is a region of parenchyma, that contains discrete bundles of longitudinal muscle fibres. Beneath the parenchymatous region is a small coelomic space that surrounds a central, thin-walled, blood vessel.

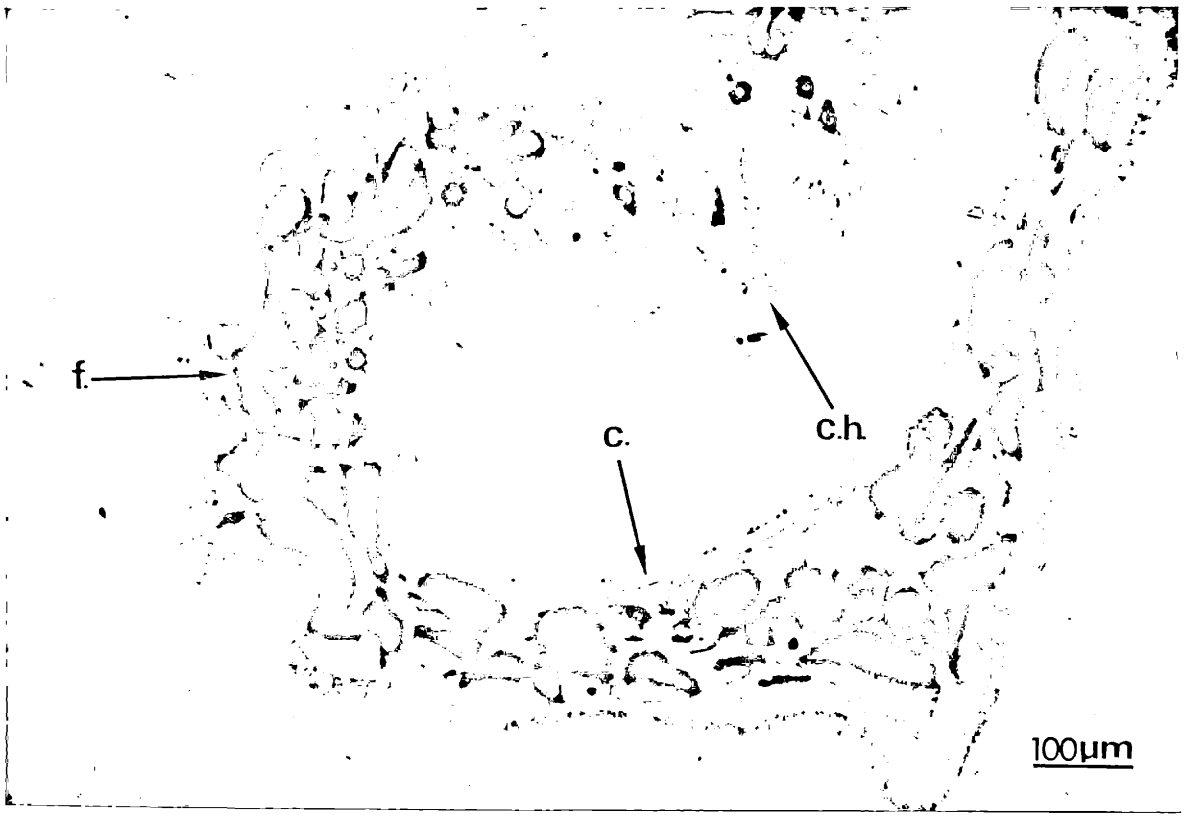
The paired pinnules are borne either side of the ciliated groove on the innermost face of the filament. Each pinnule is approximately oval in transverse section (Fig. 50), although the innermost face is slightly concave. The concave surface, which is continuous with the groove on the filament, is ciliated. The cilia are shortest in the middle (frontal cilia), and extremely long at the sides (latero-frontal cilia). Like the filament, the three unciliated sides of the pinnule are covered with a thin cuticle. Anatomically, the pinnules are simpler than the filaments, since they lack the layer of parenchymatous tissue, and in contrast to the filament musculature, which consists of four discrete bundles of muscle fibres, there is a ring of longitudinal muscle fibres beneath the layer of columnar epithelial cells.

The ciliary pattern on Mercierella's branchial crown is similar to that of Sabella pavonina (Nicol, 1930), and Pomatoceros triqueter (Thomas, 1940). The latero-frontal cilia on the pinnules cause the water to move centripetally, with respect to the funnel formed by the extended branchial filaments, and trap particles of food from the passing water which are then transferred to the intermediate ciliated groove. Here the food particles become entangled in a mucous secretion produced by special gland cells (Fig. 50), which is wafted into the ciliated groove on the filament by the frontal cilia. Once in the filament groove the

Fig. 51. Transverse section of a peduncle (operculum stalk), stained using the benzidine method and counter stained with eosin; the thin dorsal wall is damaged in this section. b.s., large central blood-space traversed by trabeculae, t.; f., fibrous tissue.

Fig. 52. Transverse section through the oblique, distal end of an operculum, stained using the benzidine method and counter stained with eosin. c., thick layer of cuticle; c.h., insertion of chitinous hook; f., relatively thin fibrous layer.


b.s.
f.
t.
70µm


f.
c.
c.h.
100µm

food particles are carried to the mouth in the mucous strings produced by the filament gland cells. Mercierella enigmatica's feeding mechanism has been described by Hall (1954).

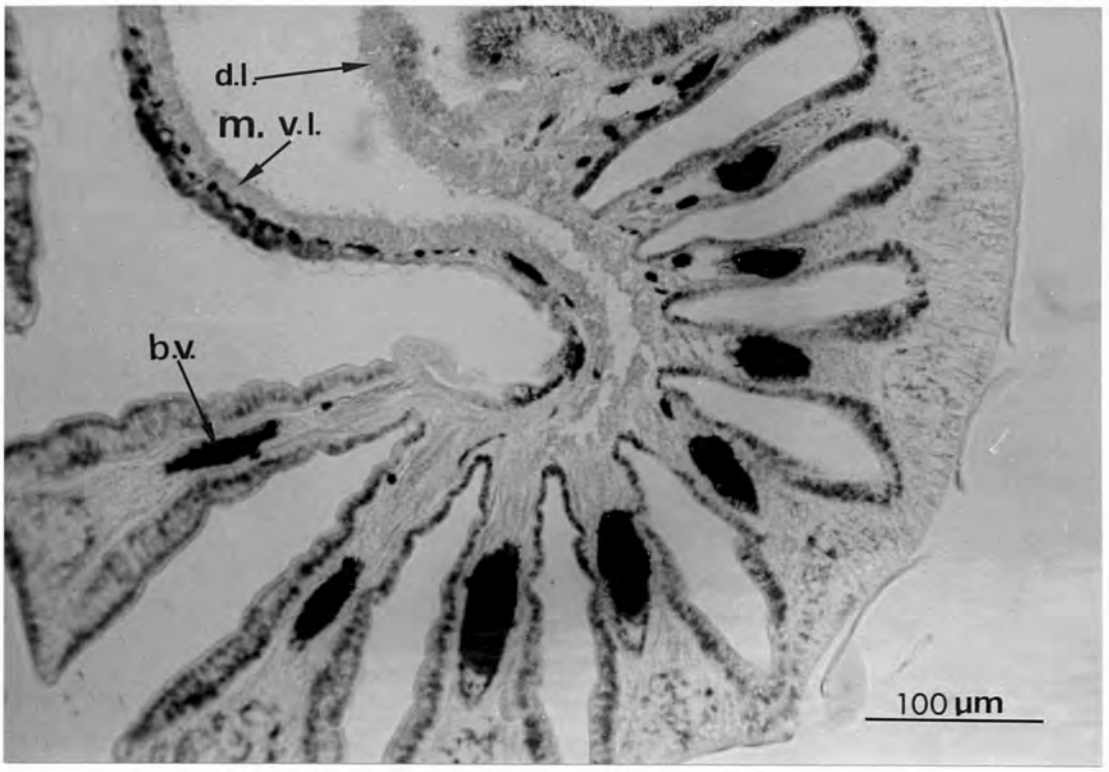
The operculum

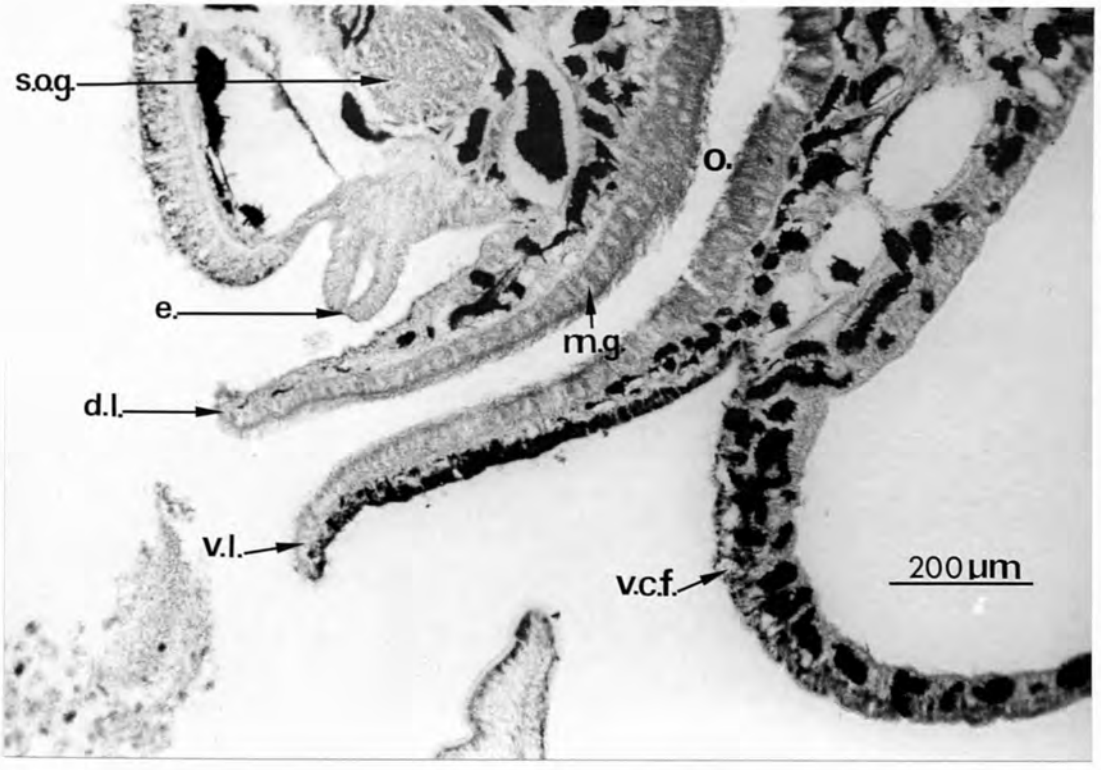
The operculum is a fig-shaped structure with a stalk, or peduncle, at the narrow, proximal end. The oblique distal end has a concave surface, which bears several concentric rows of dark, inwardly directed, chitinous hooks (see Fig. 3A). The number of hooks is variable, and depends to some extent on age, younger Mercierella tend to have fewer hooks than do older ones. Recently regenerated opercula ,however,have fewer hooks than were present on the original. In contrast to the roughly circular distal end of the operculum, the peduncle is triangular in transverse section, and has a pronounced dorsal groove. The morphological variations in the operculum of Mercierella enigmatica have been described by Hartman-Schröder (1967).

A transverse section of a peduncle is shown in Fig. 51. Externally it is covered with a thick cuticle. The outer wall is composed of a single layer of columnar epithelial cells which encloses a layer of fibrous tissue. The fibrous zone is of variable thickness , being thicker at the three corners, especially on the ventral side, and has a structural supporting function. The central region consists of a large blood-space that is traversed by fine strands of trabecular tissue. McIntosh (1926) postulated that the trabeculae might play an important role in the circulation and efficient replenishment of the blood that enters through a single, median blood vessel and Hanson (1950) suggested that Mercierella has a spiral vessel similar to the one she described in P. triqueter. However, the results of this

Fig. 53. Part of a transverse section through the base of the branchial filaments in the region of the mouth. Stained using the benzidine method and counter stained with eosin, showing the dorsal and ventral lips, d.l. and v.l., mouth, m.,and the blood vessels supplying the individual filaments, b.v..

Fig. 54. Longitudinal section through the anterior end of the thorax, stained with the benzidine method and counter stained with eosin. The internally ciliated lips, d.l. and v.l., and oesophagus, o.,the ventral collar fold, v.c.f., and the body wall contain numerous blood vessels. e., excretory pore; m.g., mucous gland; supra-oesophageal ganglion, s.o.g..


d.l.
m. v.l.
b.v.
100 µm


s.o.g.
o.
e.
m.g.
d.l.
v.l.
v.c.f.
200 µm

investigation failed to reveal conclusive evidence that the trabeculae are arranged in anything other than a random way. The central blood-space appears to be nothing more than an irregular shaped cavity. The operculum of an average sized adult worm (c. 10 mm) contains about 0.1 µl of blood.

Fig. 52 shows a transverse section through the oblique, distal end of an operculum. In addition to the insertions of the chitinous hooks, the concave surface is covered by a very thick layer of cuticle. In contrast to the peduncle, the distal end of the operculum contains a relatively small amount of fibrous tissue, which results in the blood-space being generally closer to the surface.

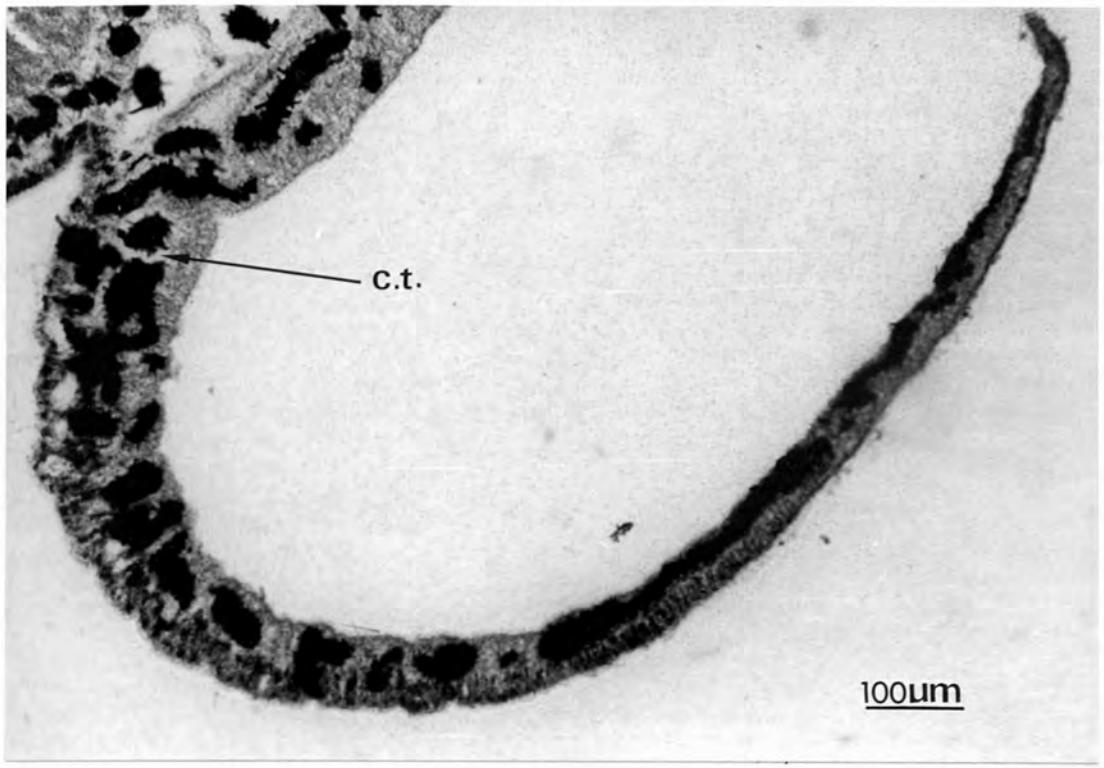
Lips and oesophagus

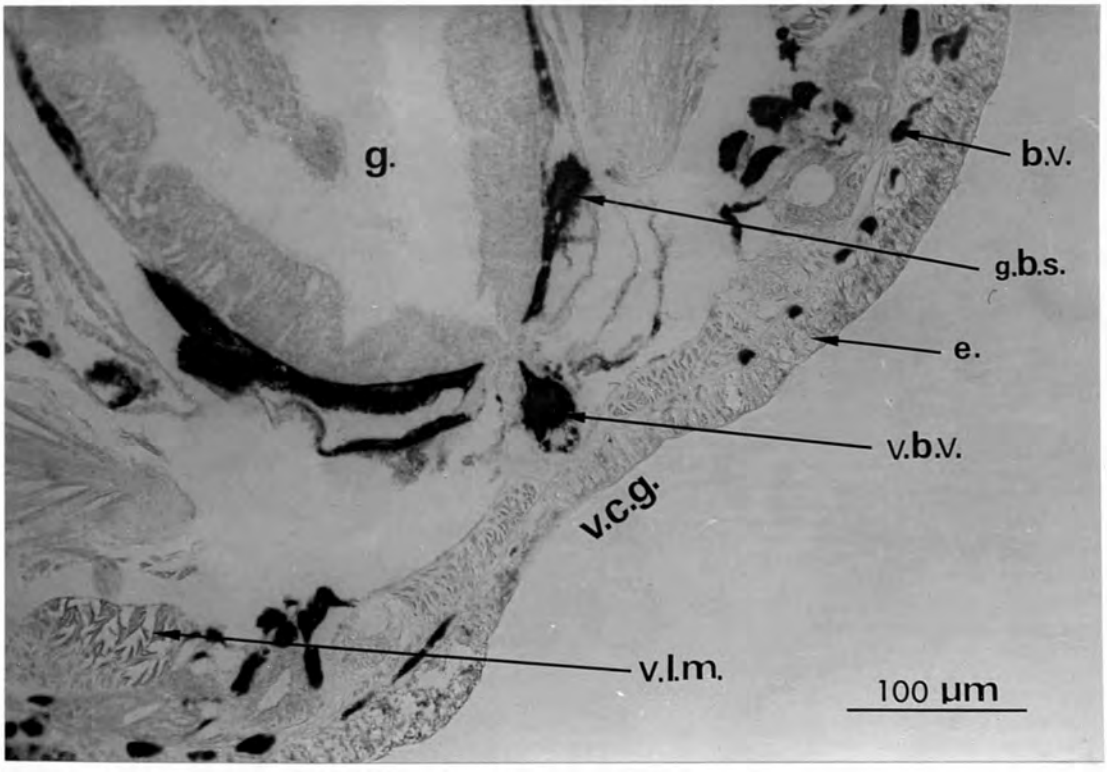
In the centre of the branchial crown at the base of the filaments is the slit-like mouth, bordered by crescent-shaped, dorsal and ventral lips (Fig. 53). The lips are bounded internally and externally by a single layer of columnar epithelium (Fig. 54). The external epithelium is covered with a thin cuticle, whereas the internal surface is richly ciliated and contains numerous mucous cells. A component of the external epithelial cells stained darkly with the benzidine stain. Thomas (1940) reported a similar reaction for P. trigueter when stained with iron haemotoxylin. The internal and external epithelia are separated by connective tissue which contains numerous blood vessels. Thomas also reported circular muscle fibres beneath the internal epithelium, but these could not be distinguished in the Mercierella preparations.

The mouth opens into the oesophagus, which is lined internally with a layer of ciliated columnar epithelium which contains numerous

Fig. 55. Longitudinal section through the large, ventral collar fold, stained using the benzidine method and counter stained with eosin. Note the rich blood supply (dark masses) and proximal wedge of connective tissue, c.t., separating the dorsal and ventral layers of columnar epithelium.

Fig. 56. Part of a transverse section through the abdomen, showing the gut, g., surrounded by the gut blood-sinus, g.b.s., the ventral blood vessel, v.b.v., ventral longitudinal muscle, v.l.m., and peripheral blind-ending blood vessels, b.v., beneath the cuticularised layer of epithelium, e., which in the mid-ventral region is richly ciliated and contains numerous mucous glands, forming the ventral ciliated groove, v.c.g.. The section was stained using the benzidine method and counter stained with eosin.


c.t.
100µm


g.
b.v.
g.b.s.
e.
v.b.v.
v.c.g.
v.l.m.
100 µm

mucous cells. The epithelium is surrounded internally by a layer of circular muscle, and in this respect differs from the rest of the gut which lacks a muscular wall. Externally the muscle layer is covered with connective tissue, in which run the blood vessels that constitute the peri-oesophageal plexus. Lying above the oesophagus and joined to it by the connective tissue is the large dorsal blood vessel.

The collar

Fig. 55 shows a longitudinal section through the large ventral collar fold. The dorsal and ventral surfaces are covered with a thin cuticle, beneath which is a layer of columnar epithelium. The cells on the dorsal surface are ciliated. Proximally, the dorsal and ventral epithelia are separated by connective tissue, containing muscle fibres and numerous blind-ending blood vessels, whereas distally connective tissue is absent.

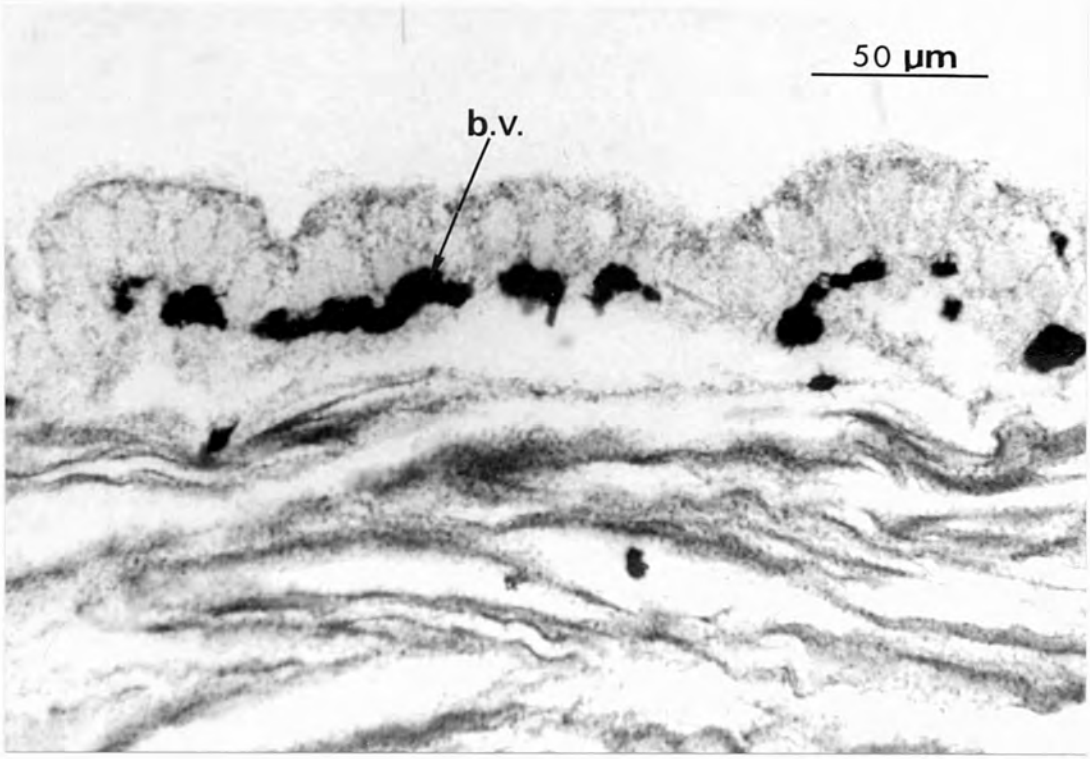
The thoracic membrane

The lateral lobes of the thoracic membrane consist of two layers of cuticularised epithelium, that are separated by numerous blood vessels. The dorsal surface is ciliated. In contrast, the posterior lobe of the thoracic membrane is similar in structure to the ventral collar lobe with a proximal wedge of connective tissue.

The thorax and abdomen

These are covered with a thin cuticle that overlies a layer of epithelium (Fig. 56), which in certain areas, e.g. the abdominal ventral groove, contains numerous mucous glands. The dorsal surface of the thorax, and the abdominal ventral groove are richly ciliated. Beneath the epithelium are numerous, blind-ending, blood vessels (Fig. 57).

Fig. 57. Peripheral, blind-ending, blood vessels, b.v., underlying the outer layer of epithelium, in the region separating the epidermis from the muscle layers. Longitudinal section of the abdominal body wall, stained using the benzidine method and counter stained with eosin.


50 µm
b.v.

The rectum

This is lined with a layer of ciliated columnar epithelium which contains numerous mucous cells. Externally, the rectum is surrounded by the gut blood-sinus.

6.3.4 Discussion

The results of this investigation are summarised in Table 26. Since the cuticle is thicker on the outermost side of the branchial filaments and distal end of the operculum, and thinner in the peduncle's dorsal groove, than it is on the rest of these organs, two values are given for cuticle thickness. The relative areas of the potential repiratory surfaces, and their estimated respiratory contributions during the three phases of activity are expressed as star ratings from one to five, five star rating representing the highest proportional value in each case.

During the feeding periods, and when the worm is partially withdrawn, the branchial crown and collar are the major sites of respiratory exchange. Conversely, during the withdrawn phase, when blood flow to the crown ceases (Section 6.1), the thoracic appendages and the general body surface are largely responsible for this function.

Several authors have suggested that the large blood-spaces in serpulid opercula may play an important role in respiration, especially when the worms are withdrawn inside their tubes (Orley, 1884, and Loye, 1908, in Hanson, 1950; McIntosh, 1926). This seems unlikely, however, because of the thick cuticle, and large amount of fibrous tissue separating the epithelium from the central blood-space. In

Table 26. Summary of results of the investigation into the relative contributions of the potential respiratory surfaces.

Respiratory surface	Cuticle thickness (μm)		Minimum and maximum diffusion distances (μm)		Relative surface area	Relative respiratory contribution when:		
						Feeding	Partially withdrawn	Withdrawn
Branchial filaments	2.7	4.2	30.5	66.2	****	****	****	*
Pinnules	1.3		4.3	13.7	*****	*****	*****	*
Operculum	4.4	11.9	17.3	69.6	**	*	*	*
Peduncle	2.6	3.5	17.4	174.0	**	**	**	*
Lips	4.0		9.0	13.0	*	*	*	**
Oesophagus	—		43.5	74.0	*	*	*	*
Collar	1.7		2.6	14.8	****	****	****	*****
Thoracic membrane	1.3		4.3	15.7	***	***	***	*****
posterior lobe	1.7		2.6	14.8	***	***	***	****
Body wall	1.3		15.0	43.5	****	***	***	*****
Rectum	—		11.3	60.9	*	*	*	*

addition, where the fibrous tissue is thinnest, at the distal end, the chitinous hooks are generally covered in a thick coat of debris, thus making efficient gas exchange extremely unlikely. The thin region on the dorsal surface of the peduncle probably acts as a respiratory surface but because of its limited area, and since blood flow in the operculum ceases when the crown is fully withdrawn, it is unlikely to serve more than a minor role.

Tubed Mercierella that have been dissected midway along the thorax will usually form a new, though initially miniature, crown within 3 weeks at room temperature (c. 20°C). Wells (1952) reported that predation results in about half the Sabella population suffering mutilation of the anterior region at one time or another. Although no data are available for Mercierella populations, there is a record of Nereis diversicolor supposedly browsing on the extended branchiae (Mathias & Izac, 1963). Mercierella that have suffered crown damage as a result of dehydration are able to autotomise the whole crown and grow a new one. This ability to regenerate a lost crown shows that Mercierella can obtain sufficient oxygen while in its tube, to supply both the basal metabolism of the thorax and abdomen, and the regenerative processes. There is no anatomical evidence, however, for supposing that the branchial crown and the rest of the body function as separate systems with regard to the supply of oxygen when the worm is feeding, as was suggested by Wells (1952) for Sabella pavonina, since there is an intercommunicating, circulating blood-system. Skaer (1974c) reported that there was a rapid transfer of fluorescein dye, following its injection into Mercierella's opercular blood-space, throughout the vascular system. The branchial crown therefore contributes to the oxygen supply of the rest of the body during the periods that the worm

is active. However, when the worm is withdrawn inside its tube the crown and the rest of the body function as separate systems.

The oesophagus is not a respiratory surface of general importance because apart from being relatively small, it contains an appreciable amount of mucus that will reduce the rate of oxygen diffusion in the lumen. In addition, Mercierella feeds on algae and detrital material that contains large numbers of micro-organisms, which will compete with the epithelium for available oxygen. Thomas (1940) found no histological evidence of enzyme-secreting cells in the oesophagus of P. triqueter, which suggests that the food organisms will continue to respire until they reach the stomach. Any oxygen that does diffuse into the cells lining the wall of the oesophagus is unlikely to reach the blood vessels because it is probably utilised by the cilia or the muscle cells.

Stephenson's (1913) claim that ascending water currents are present in the guts of several polychaete species, including serpulids, was criticised by Lindroth (1938, in Hanson, 1948) for failing to discriminate between the metachronal waves, that travel in a posterio-anterior direction, and the effective ciliary stroke which Hanson (1948) demonstrated conclusively as travelling in an anterio-posterior direction in P. triqueter. From the behaviour of carmine particles near the anus of Mercierella, I could find no evidence to support the existence of an ascending current in Mercierella, and should one be present it is of little respiratory value since the rectum is occluded by descending faecal pellets.

6.4 A differential volumetric micro-respirometer

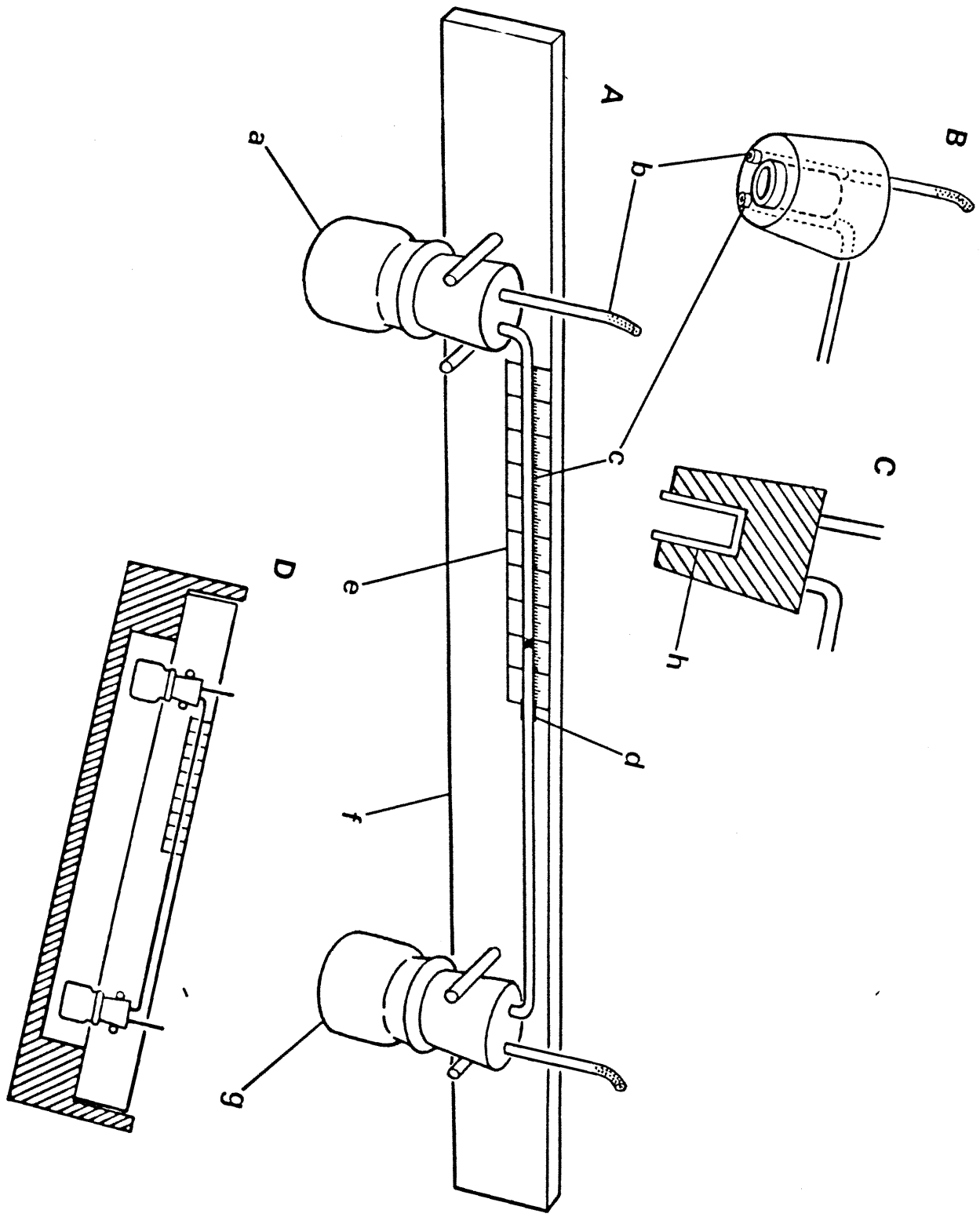
6.4.1 Introduction

A simple and inexpensive volumetric respirometer is described in which respiration rates of less than 5 μl O_2 h^{-1} have been measured. It is of the differential type in which gas pressure and volume change simultaneously, and consists essentially of 2 vessels connected by a horizontal capillary tube containing an index droplet. The history and theory of this and other volumetric methods are reviewed by Dixon (1951), Umbreit *et al.* (1964), and Holter and Zeuthen (1966).

The sensitivity of a volumetric respirometer can be increased by decreasing the volume of the vessels and the diameter of the capillary tube (Dixon, 1951; Umbreit *et al.*, 1964). In practice however, there is a lower limit to the size of vessel determined by the size of the organism, or the amount of tissue required to produce a detectable change in gas volume. Furthermore, Kok *et al.* (1953) demonstrated that there is a minimum capillary diameter (1.0 mm) below which a proportional decrease in sensitivity results from an increase in the pressure required to induce movement of the index fluid. The major improvement in design that this apparatus has over existing models (*e.g.* Fenn, 1927) is the relatively large bore of the capillary. This complies with the findings of Kok *et al.* (*loc. cit.*) who suggested the use of isovaleric acid as the index fluid. The latter however produces nauseous fumes and in this apparatus has been replaced with Apiezon oil, which due to its low vapour pressure and good wetting properties, provides a satisfactory alternative.

A further improvement is the transfer of the alkali wick to the roof of the respiration chamber, which facilitates the manipulation of

Fig. 58A : Volumetric micro-respirometer. B and C : The bung assembly. D : Vertical section through the slotted rack showing a single respirometer unit in position. a, respiration vessel; b, valve; c, capillary; d, connecting tube; e, graduated scale; f, 'Perspex' holder; g, compensation vessel; h, alkali well. As an indication of scale, the 'Perspex' holder is about 30 cm long. (Drawing by A.J. Lee).


A
B
C
D
a
b
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d
e
f
g
h

the experimental material. In addition, the design of the valves is such that fluids can be dispensed into or removed from the respiration and compensation vessels by means of a syringe after the apparatus has been assembled. This allowed worms to be fed with an algal suspension whilst in the respirometer, permitting the measurement of their standard and feeding oxygen consumption rates, with a minimum of disturbance during the experimental procedure.

6.4.2 Description and construction

The apparatus (Fig. 58A) consists of a respiration vessel with an alkali well, valve and graduated capillary containing a drop of index fluid connected by a short length of thin-walled polythene tubing to a compensation system. This is mounted on a 'Perspex' holder, and supported by a frame in a water bath.

The wide-mouthed respiration and compensation vessels, internal capacities of about 5.5 ml, are supplied by F.B.G. Trident Ltd., London (1 dram vials). The capillary tubing is manufactured by Clay Adams, U.S.A., as 100 µl micro-pipettes (Yankee, Micropet). A satisfactory scale was constructed with lacquered, 1 mm squared graph paper threaded onto the horizontal arm of the capillary tube by means of two, short, transverse slits at either end. A finer scale can be produced by photographic reduction.

No. 17 rubber bungs were drilled to accommodate a glass well and capillary tubing (Fig. 58 B & C). The wells comprise 10 mm lengths of glass tubing (internal diameter c. 5 mm), sealed at one end. The micro-pipettes were bent into a right-angle approximately 30 mm from one end, in a cool flame. Valves were constructed from 40 mm lengths of

capillary tubing inserted through the rubber bungs, with 20 mm lengths of polythene tubing (internal diameter 2 mm) pushed over their free ends so that 10 mm extended beyond the end of the capillaries.

The holder consists of a strip of 3 mm 'Perspex' drilled to accommodate four 25 mm long pegs which hold the bungs in position. A slotted rack (Fig. 58D) was constructed to accommodate 6 respirometers (5 experimental and 1 control) approximately 50 mm apart in a water bath. The rack was supported in the water bath by four ⌠-shaped hooks.

6.4.3 Calibration and preliminary test

For the successful operation of the apparatus it is essential that the gas volumes in the respiration and compensation sections of the respirometer are identical. Since the combined internal volumes of the capillary and valve are the same in the two sections, it remains to determine the internal capacities of the respiration and compensation vessels when attached to the bungs. This was achieved using a gravimetric method, in which mercury was run into the vessel up to the level of the bung. The mercury was then weighed after its temperature had been measured. The weight of the mercury in grammes, divided by its density at that temperature (see Dixon, 1951, p. 141) gave the required volume in ml. It is essential that the bung is inserted to the same depth in the vessel each time, and this is facilitated by the thick rim in the vessels described above, the lower edge of which acts as a convenient marker.

To ensure that the index droplet moves freely the tube wall must be, ideally, wetted with Apiezon oil, and this is achieved by running a drop of oil through the capillary under gravity. The capillary is

allowed to stand upright until all the excess oil has drained through. Excess oil should then be removed with paper tissue. A 3 mm long index drop of Apiezon oil is then introduced into the long arm of the graduated capillary which should be tilted to allow the drop to travel down into the graduated section. The respirometer is then assembled and all the joints greased with silicone high vacuum grease (supplied by Edwards High Vacuum Ltd., Sussex, England.

The respirometer is tested for leaks in the following way. The valves should be sealed with 3 mm of silicone grease, this will cause some movement of the index droplet and it is better if the compensation side of the apparatus is closed first, since this will prevent the drop from being pushed into the interconnecting polythene tube. After the valves are closed, the compensation vessel should be warmed slightly with the hand, which will cause the index droplet to move towards the respiration vessel. When the hand is removed the drop will move back towards its original position. The test should be repeated using the respiration vessel. If the index droplet fails to respond when a vessel is warmed this indicates that there is a leak on that side of the system which should be treated by the application of more grease to the joints. After the valves have been opened by snipping off the blocked sections with a pair of sharp scissors the respirometer is ready for use.

6.4.4 Operation

It is essential that the respiration vessels are kept scrupulously clean and free from all traces of algal or bacterial contamination. This is achieved by washing the vessels in sterile water after use, and autoclaving them prior to each experiment. The

bung bases should be wiped clean with a paper tissue wetted with a dilute solution of antibiotics (streptomycin sulphate (50 mg l^{-1}) and benzylpenicillin (30 mg l^{-1})). As a further precaution against contamination water for use in the respirometer was passed through a sterile 0.2 μm Sartorius membrane filter and treated with antibiotics.

A sufficient volume of water is added to the respiration vessel to cover the animal completely. In the case of Mercierella 1.5 ml was used. To ensure that there is a rapid exchange of gas between the gaseous and liquid phases in the vessel it is important that the surface area to volume ratio of the liquid is as large as possible. Under these conditions shaking of the apparatus was found to be unnecessary. Sufficient sterile water should be added to the compensation vessel to balance exactly the gas volumes on the two sides of the system. After the lip of the glass well in the respiration chamber has been lightly greased to prevent alkali creep, a fluted wick made from Whatman No. 42 (starch-free) filter paper, and wetted with a 5% solution of KOH is inserted, taking care to blot off excess alkali. The organism should now be placed in the respiration vessel and the vessels connected to the rest of the respirometer. The respirometers should be arranged in the water bath so that the water level reaches the 'Perspex' pegs.

Once sufficient time has been allowed for complete temperature equilibration, the valves should be closed with silicone grease, and after a few minutes the initial reading can be made. The scale can be moved so that a convenient mark can be aligned with the meniscus on the leading edge of the index droplet. A lens may prove useful when measuring very small changes in gas volume, and care must be taken

when reading the position of the lowest point on the meniscus to avoid errors resulting from parallax.

The readings in mm of oxygen are converted to µl at S.T.P. by substituting the in equation:

$$x = d\left[2A \cdot \frac{P}{P_o} \cdot \frac{273}{T} \right]$$

where: x = the amount of gas absorbed at S.T.P.

d = the distance in mm through which the oil moves in the capillary.

A = 1.316 mm^2, the cross-sectional area of the capillary.

P = the barometric pressure at the time the valves were closed (in mm of Hg).

P_o = standard pressure (760 mm Hg).

T = the absolute temperature of the water bath

It should be noted that this is a simplification of the equation given by Dixon (1951 p. 47). The omission of the volume, solubility, and additional pressure terms has an insignificant effect on the constant when the apparatus is used in the present context. Readers are referred to Dixon's excellent book (pp. 24 - 33, and 46 - 47) should they decide to use the respirometer for other purposes.

The average respiratory run has been 6 h but respirometers have been left for 12 h without a detectable reduction in the apparent rate of oxygen consumption. It is advisable, however, to recharge the wicks with fresh alkali if longer periods are intended, and the build up of waste products should be taken into consideration. The index droplet can be re-set without dismantling the respirometer, by opening the valves and attaching a hypodermic syringe with a wide bore needle to the

compensation valve. The position of the index droplet in the capillary can then be adjusted by manipulating the plunger.

For the successful operation of the respirometer it should be maintained at a constant temperature. Satisfactory thermal stability of $\pm$ 0.1°C was achieved by opposing the cooling effect of a dip cooler (Cambridge Instruments Ltd.) by the more powerful, thermostat-controlled heater of a large Grant water bath.

6.5 The effect of antibiotics on respiration rate

6.5.1 Introduction

It was desirable to use tubed animals in the respiration investigations since the feeding behaviour study (Section 3.5) showed that Mercierella acclimated to 10°C spend significantly less time feeding than do those at 20°C. Detubed Mercierella adopt a permanent feeding position. Due to the appreciable microbial population that is associated with the calcareous tube (Section 4.3.3), it was necessary to use antibiotics in the respirometer to prevent errors resulting from microbial respiration.

Simultaneously, an experiment was conducted to determine the effect of antibiotics on the oxygen content of the water in the respirometer vessels.

6.5.2 Materials and Methods

Mercierella enigmatica were collected from the seaward side of the sluice gates beneath Westham Bridge, in Weymouth Harbour backwater, Dorset, in October 1974 when the water temperature at low tide was 12°C. In the laboratory the worms were maintained at 13°C in artificially

aerated sea water of salinity 30 °/oo, the high tide values given by Tebble (1953) for the site of permanent growth of Mercierella at Weymouth. The worms were fed weekly with a suspension of Phaeodactylum tricornutum (Bohlin), and the water was changed before every third feed. The worms were acclimated to these conditions for 6 weeks before the experiments commenced.

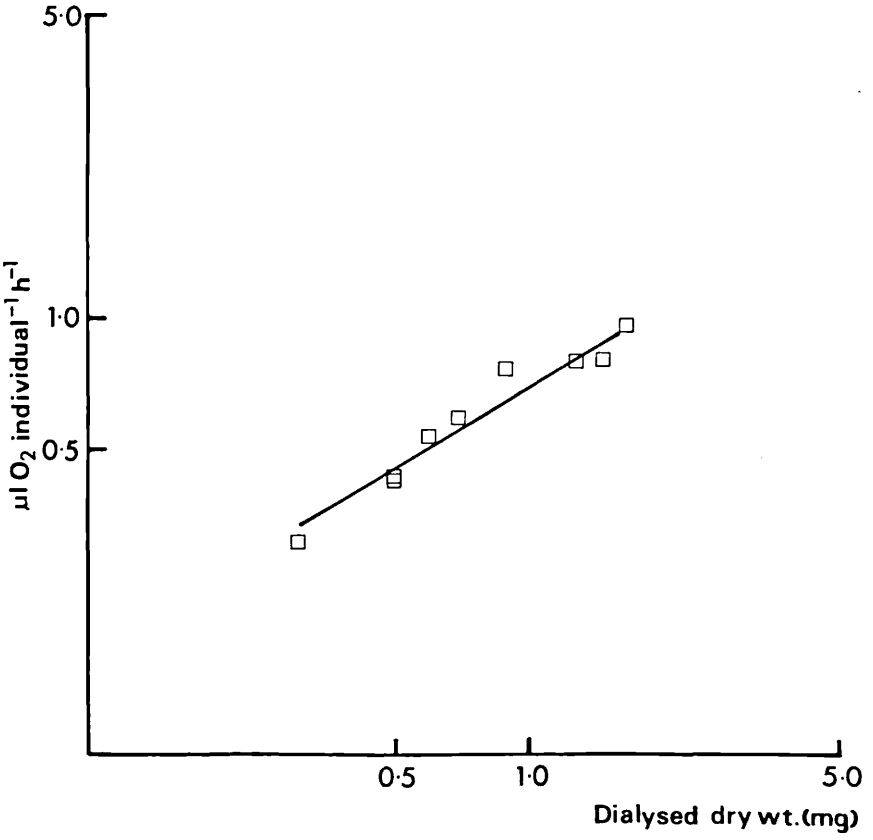
The sea water used in the respirometers was filtered through a sterile 0.2 µm Sartorius membrane filter not longer than 3 d before it was used, and stored in the dark at 4°C. Respirometer vessels and glassware were autoclaved at 2 atmospheres for 20 min before these were used. To reduce microbial contamination detubed animals were used in these experiments. The worms were removed from their tubes 24 h before the experiments commenced and were given several rinses with filtered sea water to remove any debris adhering to the branchial crown. Immediately before the experiments began, they were examined for signs of injury, and any damaged animals were discarded. The experimental salinity was 30 °/oo and temperature was 13°C.

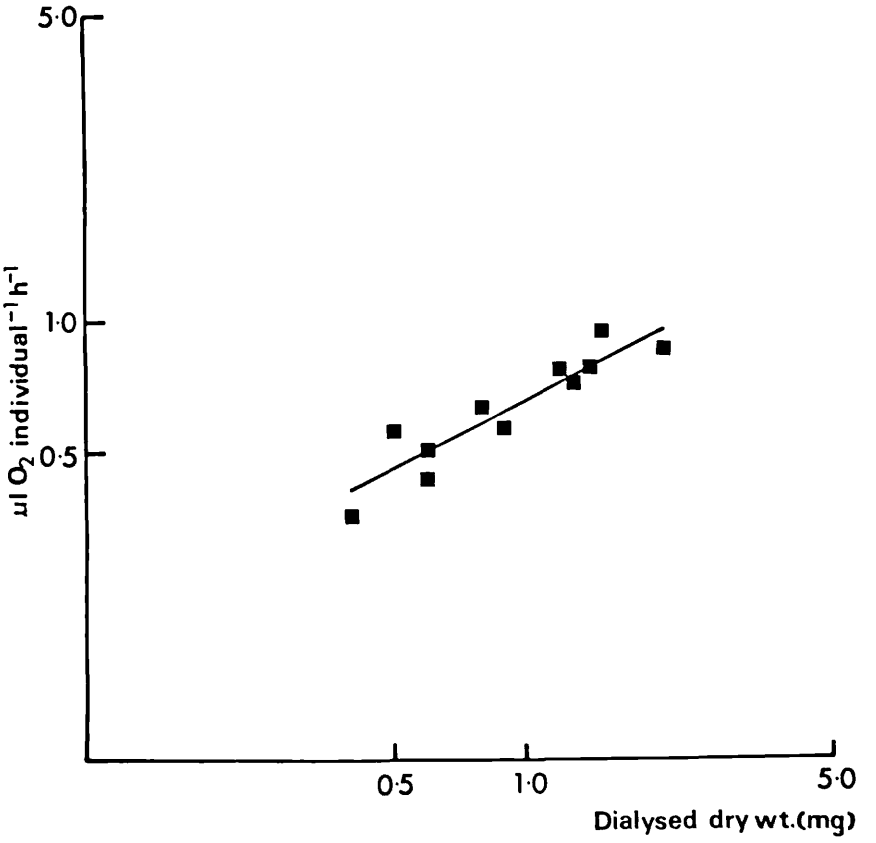
Respirometers were set up containing: individual Mercierella with and without antibiotics (streptomycin sulphate (50 mg l^{-1}), and benzylpenicillin (30 mg l^{-1})); filtered water without animals or antibiotics; and water with antibiotics. The antibiotics were added to the water 1 h before the experiments commenced.

The individual respiration rates of the worms with and without antibiotics were measured at hourly intervals for 6 h. Two control respirometers (lacking worms and antibiotics) were run simultaneously, and the mean hourly changes in gas volume were used to correct the individual readings for the group lacking antibiotics. The size range

Fig. 59. Oxygen consumption rate of detubed M. enigmatica in sea water of salinity, 30 °/oo, at 13°C, plus antibiotics, expressed as a function of dialysed dry weight. Each point represents the mean value of six hourly measurements, and the regression line was fitted by the method of least squares, assuming a linear relationship after logarithmic transformation of the data. Regression coefficient (b) = 0.61; intercept (ordinate) = 0.17.

Fig. 60. Oxygen consumption rate as related to dialysed dry weight of detubed M. enigmatica in sea water of salinity, 30 °/oo, at 13°C, without antibiotics. Regression coefficient (b) = 0.52; intercept (ordinate) = 0.2.


5·0
1·0
0·5
µl O2 individual⁻¹ h⁻¹
0·5
1·0
5·0
Dialysed dry wt.(mg)


5·0
1·0
0·5
µl O2 individual⁻¹ h⁻¹
0·5
1·0
5·0
Dialysed dry wt.(mg)

of each group was chosen to be representative of the original sample. The mean control value (based on two determinations) was about 0.1 µl h^{-1}. At the end of the experiment the worms were dialysed against distilled water for approximately 3 h, before drying to constant weight at 60°C on tared aluminium foil trays. Weighings were made to the nearest 0.1 mg on a Sartorius semi-micro balance.

The gas volume changes in those respirometers used for determining the effect of antibiotics on the oxygen content of filtered sea water were recorded at 1 d intervals. The mean hourly respiration rate of each worm was obtained from a regression line fitted to the individual readings by the method of least squares; the mean hourly rate is equal to the slope of the line. The individual respiration rates and the gas volume changes in the respirometers lacking animals were converted to S.T.P. (Section 6.4.4).

6.5.3 Results

Figs. 59 and 60 show the individual mean hourly respiration rates of detubed Mercierella with and without the presence of antibiotics, expressed as a function of dialysed dry weight. Regression lines were fitted by the method of least squares. There are high correlations between the two parameters for both groups, 0.97 and 0.91 respectively for the animals with and without antibiotics ($P = < 0.001$).

The low variances about the lines, 0.1 and 0.25 respectively for those with and without antibiotics, indicate that behaviour was uniform throughout the experimental period, and agrees with what is known about the behaviour of detubed worms. Since an F-test carried out on the two variances failed to reveal a significant difference between them ($P = > 0.05$), the regression coefficients (b) were compared using a

Table 27. The cumulative reductions in gas volume in the respirometers containing 1.5 ml of water, with and without antibiotics. (µl O_2 at S.T.P.)

A. With antibiotics

Respirometer No.	Time (days) 1	2	3	4	5	6	7
1	0	0.5	1.0				
2	1.5	1.75	2.0		3.5	4.0	4.5
3	0	0	0		0.5	0.5	0.5
4	0	0.5	0.6		1.0	1.0	1.0
Mean	0.38	0.69	0.9		1.68	1.83	2.0
Standard deviation	0.75	0.76	0.84		1.61	1.89	2.18

B. Without antibiotics

5	0	12.0	30.0	
6	37.0	56.0	90.0	
7	5.5	6.0		
Mean	14.2	24.7	60.0	
Standard deviation	19.9	27.3	42.4	

t-test, similar to that described by Bailey (1959) for samples smaller than 30. The calculated value for t, 1.01, is smaller than the tabulated value for 18 degrees of freedom at the P = 0.05 level, so the antibiotics had no significant effect on the respiration rates of detubed Mercierella.

Table 27 shows the individual results of the investigation into the effect of antibiotics on the oxygen content of filtered sea water. It is evident that the oxygen content of the water containing streptomycin sulphate and benzylpenicillin remained fairly constant throughout the 7 d period. In contrast there was a significant fall in the oxygen level in the respirometers lacking antibiotics due to microbial activity, despite the cleansing and sterilising treatments that the glassware and sea water had received.

6.5.4 Discussion

Some workers (e.g. Richman, 1958) avoided the use of antibiotics in respirometery experiments by the use of control respirometers (lacking animals), whereas others (e.g. Atkinson & Smith, 1973) flushed out the respiration chamber with a solution of antibiotics but did not expose the animals to them. This investigation shows that streptomycin sulphate (50 mg l^{-1}) and benzylpenicillin (30 mg l^{-1}) reduce microbial activity without having a significant effect on Mercierella's respiration rate. These antibiotics were used by Marshall and Orr (1955) in similar experiments on Calanus, and are reported to have little or no effect on feeding activity when used at the above concentrations. Since these antibiotics also have no detectable effect on the integumentary uptake of ^{14}C-glycine (Section 4.3) it is considered safe to use them in the experimental programme.

These results also show that when small gas volume changes are being measured, the effect of microbial activity can be significant, particularly when respiration rate is monitored for an extended period. Furthermore, there can be considerable variation in control values under similar conditions.

6.6 The influence of feeding on respiration rate

6.6.1 Introduction

It is general practice in energetics studies to derive the cost of metabolism from the rate of oxygen consumption due to the practical difficulties associated with the direct measurement of heat production by aquatic invertebrates. Energy budgets in general take no account of activity and are therefore inaccurate in this respect since marine invertebrates exhibit different levels of oxygen consumption depending on their level of activity (see review by Newell, 1973). In most instances this omission is understandable because the organisms either do not behave naturally in the respirometer, or their behaviour patterns are not easily defined. This does not apply, however, in the case of Mercierella since, in common with other sedentary filter-feeders, it has a limited behavioural repertoire which it is able to perform within the confines of the respirometer. Whilst the worm is covered by water the branchial crown is extended for the majority of the time because it is used for both feeding and respiration. It is therefore possible to predict the animal's behaviour when it is in the respirometer, which allows the energy expended during activity, namely pumping water, to be included in the energy budget.

Since Mercierella filters water for the majority of the time it would not be possible to measure the standard (or quiescent) respiration

rate without recourse to the use of narcotics, which is totally unnatural and for this reason unacceptable in the present context. Recently, Bayne and co-workers (e.g. Thompson & Bayne, 1972; Bayne, 1973a, b) have shown that the mussel Mytilus edulis L. reduces its filtration rate and level of oxygen consumption in the absence of food. Having thus decided to incorporate the energy cost of activity in Mercierella's energy budget, it is necessary to determine whether there is a similar difference between the rates of oxygen consumption before and during feeding, since the failure to incorporate this difference, should one exist, would lead to an underestimation in the estimated amount of energy expended in activity.

6.6.2 Materials and Methods

Mercierella were collected from the intertidal zone at Greenhithe, Kent, on the southern shore of the Thames estuary. Worms that were attached to chalk boulders were selected since their tubes could be removed intact with the aid of a scalpel blade. In the laboratory the worms were maintained in 6 l containers of artificially aerated, diluted sea water with a salinity of 15 $^{o}/_{oo}$, at 20°C, and fed twice a week with a suspension of Brachiomonas submarina cells. The water was changed before every third feed, and the worms were acclimated to these conditions for 21 d before the experiment commenced.

Two d after the last feed, solitary tubes were selected on the basis that they would fit into the respiration vessels, and carefully removed from boulders. After these had been cleaned of algae and polyzoans, they were given several rinses in filtered water before being placed in a dilute solution of antibiotics, that the previous experiment (Section 6.5) had shown was effective in reducing microbial activity without affecting Mercierella's respiration rate.

Sixteen Mercierella were placed into individual respirometers. The respiration vessels contained 1.5 ml of filtered water, plus antibiotics, at a temperature of 20 ± 0.1°C. Four control respirometers were run simultaneously. Individual respiration rates were measured at 60 min intervals for 4 h, before the valves were opened and 100 µl of a concentrated, axenic, suspension of Brachiomonas cells was introduced into the respiration vessels of 11 of the respirometers containing worms and three of the controls. After a similar volume of sterile water was added to the compensation vessels to balance the gas volumes on either side of the apparatus, the valves were closed and the oxygen consumption measured for a further 4 h. A 100 µl glass syringe with a long, wide-bore needle was used to inject the cells and water into the vessels via the valves. The respirometers were covered between readings with a sheet of black polythene to exclude light, preventing the worms being disturbed and errors resulting from photosynthesis. At the end of the experiment the worms were removed from their tubes and, after a 5 s rinse with glass-distilled water to reduce salt contamination, dried to constant weight at 60°C on tared aluminium foil trays. The worms were weighed to the nearest 10 µg on a Cahn 4100 Electrobalance.

Brachiomonas submarina was cultured in diluted sea water (15 °/oo) in Miguel-Allen solution, at room temperature, under a mixed light régime of natural and artificial light (Section 3.6.1). Axenic cultures were obtained by sub-culturing in 1 l flasks containing a solution of antibiotics. The medium was replaced with fresh twice a week during the period of rapid growth, after which the cells were separated by slow centrifugation (1 000 r.p.m) from the medium and resuspended in sterile water.

The density of the final suspension was measured in a Beckman DB-G spectrophotometer that had been calibrated with counts made using a haemacytometer slide (Section 3.3.2). It was decided to use a dense Brachiomonas suspension in the respirometers, 4.0×10^5 cells ml^{-1}, to avoid complications associated with the density-dependent range (Section 3.3.3). A preliminary investigation showed that Mercierella continues to feed in this concentration of cells.

6.6.3 Results

Initially there were $4.0 \times 10^5 \times 1.5 = 6.0 \times 10^5$ Branchiomonas cells in each respirometer, and these had a mean total respiration rate of 2.7 µl h^{-1} (mean of 3 determinations). As the experiment was conducted in the dark each Branchiomonas cell had a respiration rate of 4.5×10^{-6} µl h^{-1}.

Assuming the ingestion rate of an average Mercierella in the experimental concentration is similar to that in a cell density equivalent to 39.8 µg ash-free dry weight of cells ml^{-1}(3.98×10^4 cells ml^{-1}), namely 22.22 µg ash-free dry weight of cells h^{-1} (Table 5), it is possible to calculate the rate of cell removal due to feeding activity. Since an average Brachiomonas cell weighs 1.1×10^{-6} mg dry weight and has an ash content of 4.5% (Tables 9 & 10), at the end of the first hour the ambient cell density was reduced by $\frac{22.22 \times 10^{-3}}{1.15 \times 10^{-6}}$ $= 1.93 \times 10^4$ cells h^{-1}. After 4 h the ambient cell concentration will have been reduced by only 12.9% of the initial value.

At the end of each successive hour that the combined respiration rates of worm plus algal suspension were measured, the algal component was reduced by an average of $1.93 \times 10^4 \times 4.5 \times 10^{-6} = 8.69 \times 10^{-2}$ µl.

Table 28. Individual results of the experiment on the effect of feeding on respiration rate of M. enigmatica.

Experimental Group (fed B. submarina)				Control Group ('fed' sterile H_2O)			
Dry weight (mg)	Initial respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)	Feeding respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)	Percentage of the initial rate	Dry weight (mg)	Initial respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)	Final respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)	Percentage of the initial rate
0.53	0.8	0.6	75	1.6	1.9	1.8	95
0.85	1.1	1.1	100	2.3	2.5	2.1	84
1.3	1.8	1.6	89	3.1	3.1	3.2	103
1.3	1.5	0.8	53	3.4	3.3	3.6	109
2.4	2.2	3.1	141	5.6	4.7	3.7	79
2.6	2.7	2.6	96				
2.7	2.6	2.1	81				
2.8	2.8	3.1	111				
3.0	3.3	2.4	73				
3.5	3.0	1.9	63				
4.8	4.0	3.1	77				

Experimental group: $\bar{x}$ % of initial rate = 87 ± 24.5 (S.D.)%

Control group : " " " " = 94 ± 12.6%

Thus, the algal components for each of the 4 successive hours are: 2.61, 2.53, 2.44, and 2.35 $\mu l\ h^{-1}$ respectively. A mean algal respiration rate of 2.48 $\mu l\ h^{-1}$ was calculated from these values, and used to correct each of the combined values for algal oxygen consumption.

Table 28 shows the individual respiration rates of the experimental group (equivalent to slopes of regression lines fitted to the individual data) before and during the 4 h period that they were feeding on the suspension of algal cells, together with the results for the control group which were 'fed' a similar volume of sterile water. The total gas volume change in the blank respirometer was less than 8% of the total oxygen consumption of the smallest worm and was considered to be negligible.

The feeding and control respiration rates (after the introduction of sterile water) are expressed as percentages of the initial rates of oxygen consumption (columns 4 and 8), and the mean percentage values were compared using Student's *t*-test, after an *F*-test had shown there is no difference between the variances of the two samples ($P = > 0.05$). The calculated value for *t*, 0.6, was smaller than the tabulated value, for 14 degrees of freedom, at the 0.05 level of significance. There is, therefore, no significant difference between *Mercierella*'s respiratory response to *Brachiomonas* cells and sterile water.

A regression line fitted by the method of least squares to the individual oxygen consumption rates of worms acclimated to 20°C, expressed as a function of dry weight, following a logarithmic transformation of the data (Fig. 61), has a slope of 0.71. The slope of the line is the exponent *a* in the relation: individual oxygen consumption = kW^a where *k* is a constant and *W* is dry weight. 0.71 is

Table 29. Weight-corrected initial and feeding oxygen consumption rates of the experimental worms.

Dry weight (mg)	$\underline{W}^{0.67}$	Corrected initial respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)	Corrected feeding respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)
0.53	0.6542	1.22	0.92
0.85	0.8962	1.23	1.23
1.3	1.191	1.51	1.34
1.3	1.191	1.26	0.67
2.4	1.793	1.23	1.73
2.6	1.914	1.41	1.36
2.7	1.939	1.34	1.10
2.8	1.986	1.41	1.56
3.0	2.08	1.59	1.15
3.5	2.305	1.30	0.82
4.8	2.845	1.41	1.10

Activity	Mean ± weight-corrected respiration rate ($\mu l\ O_2\ indv.^{-1}\ h^{-1}$)	Standard deviation	Variance
Initial	1.36 ±	0.123	0.015
Feeding	1.18 ±	0.31	0.098

in good agreement with the expected value of 0.67 should the rate of oxygen consumption be related to body surface (Section 6.7.4). The initial and feeding respiration rates of the experimental worms were corrected for weight by dividing the individual oxygen consumption values by $W^{0.67}$, the more convenient exponent of 0.67 was substituted for the experimentally derived figure. The weight-corrected values are presented in Table 29.

The mean corrected respiration rates were calculated and compared using a modified form of Student's t-test, as described by Bailey (1959), after an F-test had shown the variances to be significantly different from each other at the P = 0.01 level. Since the calculated value for t, 1.74, does not exceed the tabulated figure for 14 degrees of freedom at the P = 0.05 level of significance, it is concluded that there is no significant difference between the mean respiration rates of feeding and fasting Mercierella.

6.6.4 Discussion

Dissolved and particulate matter is known to elicit behavioural responses in members of several marine invertebrate groups (for DOM references see Section 4.1; particulate matter e.g. Loosanoff, 1962) resulting in increased rates of oxygen consumption (see references quoted in Section 6.6.1). In contrast, the results of the present study show that there is a single level of oxygen consumption associated with the active state in recently fed Mercierella (see Section 4.9.3) and indicate that the majority of the energy expended during feeding activity is used in moving water across the crown. With only a relatively small (undetectable) amount being used during the trapping and processing of the food particles.

The reduced filtering rate in the absence of particulate food, as is demonstrated for a number of bivalve molluscs, has obvious survival value, since it results in a decrease in the rate at which the energy stores are depleted, and subsequently increases the chances of the organism's survival until food is once more available. Since the dissolved uptake studies (Section 4.10) show that DOM is of little value to Mercierella as a nutrient source it remains to explain why this organism can afford to maximise its filtering rate in the absence of food.

The most logical explanation is that in the natural environment particulate food is never in short supply, which is certainly the case in the Thames estuary since Mercierella does not depend on algae for its major source of energy. From what is known of natural populations of Mytilus edulis (B.L. Bayne, personal communication, 1977) it would appear that those in coastal habitats experience regular periods of nutrient stress when there is insufficient algal growth to supply their maintenance energy requirements. So it is as a survival adaptation that the "routine" rate of filtration (metabolism) has evolved.

6.7 The effect of temperature on respiration rate

6.7.1 Introduction

Mercierella's intertidal distribution at Greenhithe is restricted largely to parts of the shore that remain covered by water throughout the low tide period (Section 2.3.3, and Fig. 21), so it is only necessary to consider aquatic respiration in the energy budget.

Since the temperature of the surface water is about the same as the mid tide value (Section 2.3.5), the mid and high tide temperature data presented in Fig.25 give a reasonable indication of the range to

which the worm is exposed annually. An experiment is described where Mercierella's respiration rate was measured at winter and summer temperatures. For experimental purposes, the winter and summer values are simplified into 10°C and 20°C respectively.

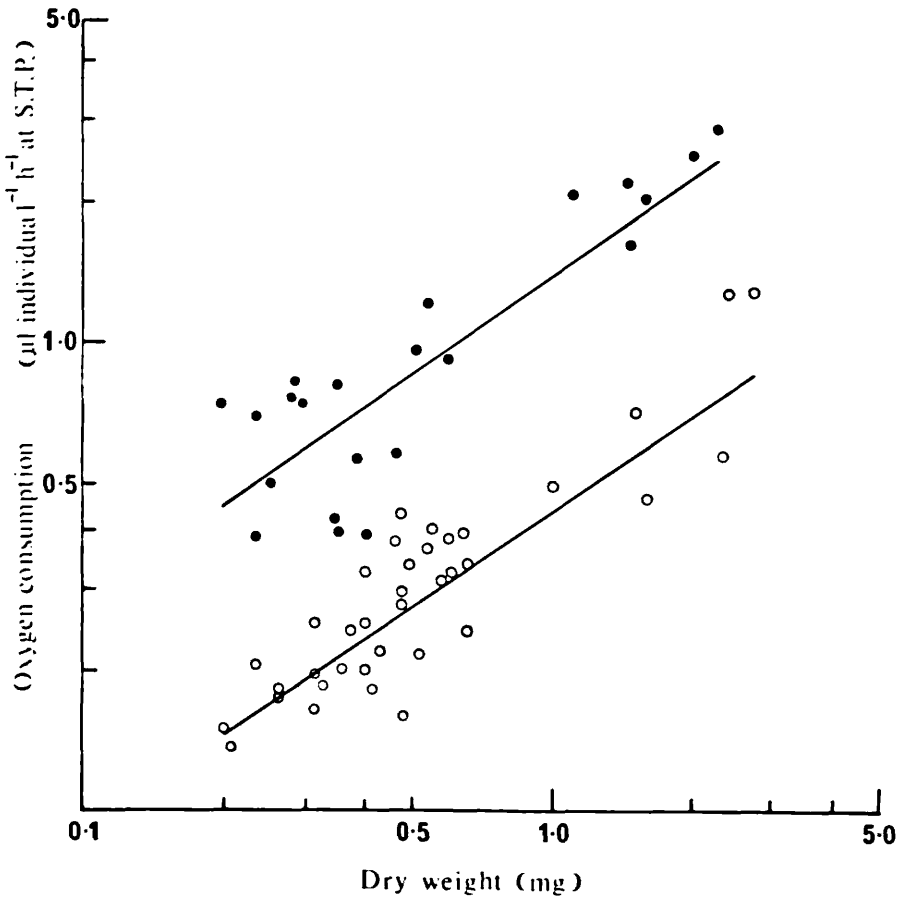
6.7.2 Materials and Methods

Worms were collected from pools of standing water at approximately the mid tide level on the shore at Greenhithe, in July and November 1975. In the laboratory the worms were maintained in 6-l containers of artificially aerated, diluted sea water of salinity 15 °/oo an average value based on the field data (Section 2.3.4, and Fig. 24). The winter animals were kept at 10°C in a constant temperature room, and the summer worms at room temperature (c. 20°C); temperatures that are similar to the field conditions at the times of collection (Fig. 25). The worms were fed twice a week with a suspension of Brachiomonas cells, and the water in the tanks was changed before every third feed. At other times the tanks were covered with opaque 'Perspex' lids to exclude light and reduce evaporation. The worms were acclimated to these conditions for three weeks before the commencement of the experiment.

At the end of the acclimation period, the tubes were carefully removed from the chalk boulders followed by a decontamination treatment similar to that described in Section 6.6.2. Those worms that displayed filtering behaviour were selected for the experiment.

After the decontamination treatment the respiration rates of individual animals were measured in a respirometer similar to that described in Section 6.4. Since preliminary investigation showed that it is possible to estimate a worm's size from the length of the tube (Fig. 64), each of the experimental groups was selected as representative of the sample size range.

Fig. 61. Oxygen consumption rate plotted as a function of dry weight of M. enigmatica acclimated to 10°C (open circles) and 20°C (closed circles). The regression lines were fitted by the method of least squares, assuming linear relationships after logarithmic transformation of the data. The regression coefficient (b) for the 10°C relationship is 0.74, and for the 20°C relationship, b = 0.71. The respective ordinate intercepts are 0.09 and 0.28.


5·0
1·0
0·5
0·1
0·5
1·0
5·0
Oxygen consumption (µl individual^{-1} h^{-1} at S.T.P.)
Dry weight (mg)

Oxygen consumption was measured at the acclimation temperatures, $\pm$ 0.1°C, at 1 h intervals for 6 h. The respiration vessels contained 1.5 ml of filtered water plus antibiotics (Section 6.5), and the respirometers were covered with a black polythene sheet between readings, to prevent the worms being disturbed since they are sensitive to changes in light intensity, and simulated the natural condition since the estuary water is extremely turbid (Section 2.3.4). The worms were not fed since there is no significant difference between the respiration rates of feeding and fasting animals (Section 6.6).

The average hourly respiration rates were obtained by calculating the slope of the regression line fitted to the individual data for the 6 h period, using the formula given by Crowe and Crowe (1969):

$$\text{slope } m = \frac{\sum xy - n\,\bar{x}\,\bar{y}}{\sum x^2 - n\,\bar{x}^2}\,.$$

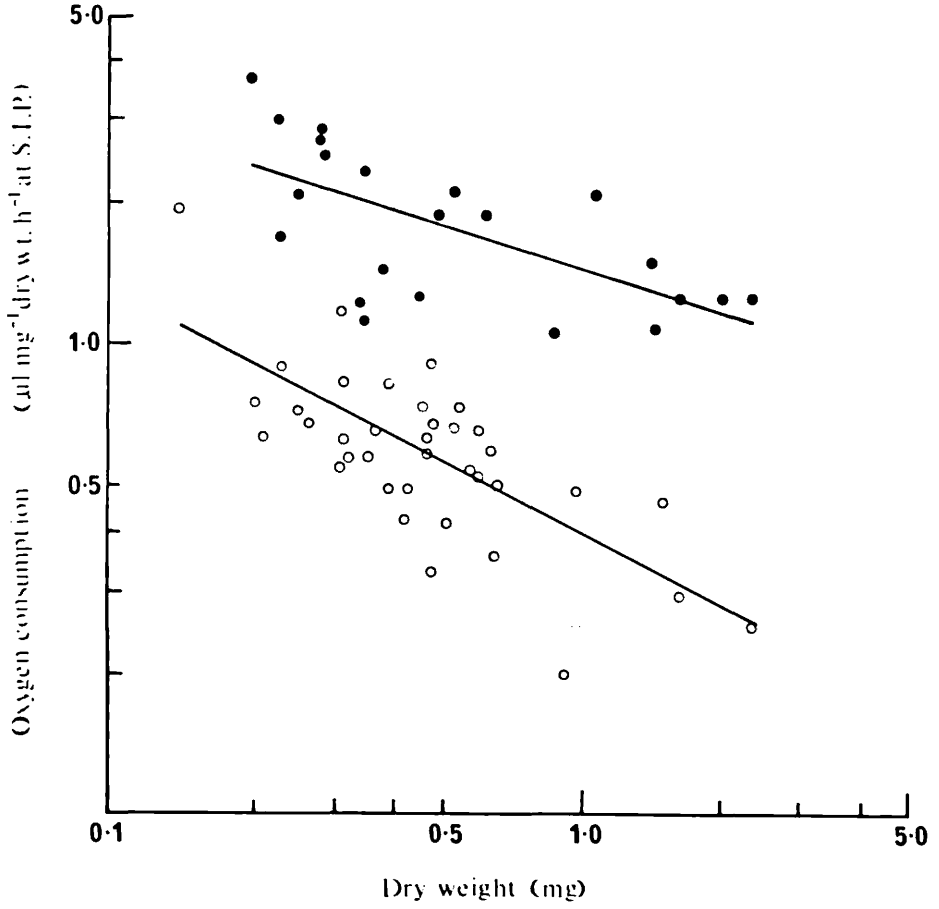
The individual average respiration rates in mm h^{-1} were converted to µl h^{-1} at S.T.P. (Section 6.4.4). Control respirometers showed no significant gas volume changes during the 6 h period. Furthermore, measurements of the oxygen consumption rates of isolated tubes in water containing antibiotics were negligible.

At the end of the respiration run, the worms were detubed and after a 5 s rinse with glass-distilled water to reduce salt error they were dried to constant weight at 60°C on tared aluminium foil trays. Weighings were made to the nearest 10 µg on a Cahn 4100 Electrobalance.

6.7.3 Results

Fig. 61 shows the rates of oxygen consumption of individual Mercierella acclimated to 10°C and 20°C, expressed as functions of dry

Fig. 62. The weight-specific rate of oxygen consumption of M. enigmatica acclimated to 10°C (open circles) and 20°C (closed circles), plotted as a function of dry weight. $\underline{b}_{10°C} = -0.52$; $\underline{b}_{20°C} = -0.3$. The ordinate intercepts of the regression lines, fitted by the method of least squares after logarithmic transformation of the data, are 1.3 at 10°C and 3.0 at 20°C.


5·0
1·0
0·5
0·1
0·5
1·0
5·0
Oxygen consumption (µl mg⁻¹dry wt. h⁻¹at S.T.P)
Dry weight (mg)

weight. Assuming a linear relationship after a logarithmic transformation, regression lines were fitted to these data by the method of least squares. Good correlation exists between the two parameters at both acclimation temperatures, r is 0.8 at 10°C, and r is 0.75 at 20°C. Both values considerably exceed the tabulated figures at the $P = 0.05$ level of significance.

In Fig. 62 the same data are presented as the rate of oxygen consumption per unit dry weight, expressed as a function of dry weight. Once again regression lines were fitted after logarithmic transformation. The correlation coefficients are significant at the $P = 0.05$ level (r is 0.52 at 10°C; r is 0.4 at 20°C), but there is a great deal more scatter.

6.7.4 Discussion

Both groups of worms show an increase in oxygen consumption with increasing size. Since an F-test failed to show a significant difference ($P = > 0.05$) between the variances about the regression lines shown in Fig. 61, the regression coefficients b were compared using a t-test, similar to that described by Bailey (1959) for small samples. This showed no significant difference between them at the $P = 0.05$ level of significance.

Von Bertalanffy (1957) grouped organisms into three classes based upon the exponent b in the equation $V_{O_2} = aW^b$ relating oxygen consumption directly to body weight: 1) those whose metabolic rate followed the surface law, $b = 0.67$; 2) those whose metabolic rate was directly related to body weight, $b = 1$; and 3) those whose metabolic rate was intermediate between types 1 and 2. From the results of these investigations conducted at winter and summer temperatures, the exponents

0.74 and 0.71 respectively place Mercierella in the intermediate class $0.67 < b < 1.0$. It is possible, however, that the small discrepancy between the experimentally derived exponents and a value of 0.67 may be due to natural variation. Further support for Mercierella's respiration rate being related to body weight by the surface law exponent is provided by the results of a previous experiment (Section 6.5, Figs. 59 & 60) which show lower values, 0.52 and 0.61, for worms at an intermediate temperature. Dales (1969) in his review of respiration and energy metabolism in annelids quotes values for b ranging from 0.5 to 1.0 for different annelids.

A Q_{10} of 3.0 for the temperature range 10°C to 20°C, was calculated using the oxygen consumption values for the median dry weight in Fig. 61. Thus there is a threefold increase in Mercierella's respiration rate over the natural temperature range. It should be noted, however, that worms maintained at 10°C spend significant periods of time withdrawn inside their tubes and therefore not filtering, whereas worms at the higher temperature actively circulate water for the majority of the time (Section 3.5). Thus it is necessary to consider the oxygen consumption rates at 10°C as representing a combination of active and standard metabolism, whereas those at 20°C are active rates. Although this difference is of no importance in an intra-specific sense (assuming that activity has no effect on the type of respiratory substrate) it does prevent direct comparison with other organisms unless their behaviour is defined. Furthermore, since Q_{10}'s should be calculated from the standard metabolic rate, the above mentioned value of 3 should not be taken as meaning that there is an absence of a plateau in the relationship between metabolic rate and temperature over the animal's natural temperature range, which has been

reported for a number of marine invertebrates including several annelids (e.g. Newell & Northcroft, 1965, 1967; Dales, 1969; Pye & Newell, 1973).

Finally, the data presented in Fig. 62 show that the rate of oxygen consumption per unit weight of smaller Mercierella is higher than that of larger worms, at both experimental temperatures. These observations are in accordance with the generally observed relationship between metabolic rate and body size (see any general textbook of animal physiology, e.g. Prosser & Brown, 1961; Schmidt-Nielsen, 1975). It is apparent that there are both endogenous and exogenous factors influencing Mercierella's respiration rate.

6.8 The energetic cost of metabolism

The heat produced as a result of Mercierella's metabolic activity was measured indirectly from the rate of oxygen consumption. In the previous experiments, the measurement of the respiration rates of individual animals was preferred to the use of groups since the experimental conditions are more easily defined. The metabolic capacity of individual organisms with a weight-dependent metabolism cannot be accurately derived from the rate determined for a group, because the latter is affected by the size distribution within the group.

Since the heat produced per volume of oxygen consumed varies to some extent with the material respired, it is necessary to determine the appropriate oxycalorific coefficient for Mercierella when it is feeding on B. submarina cells. The oxycalorific coefficient, Q_{ox}, is usually defined as the ratio of the heat produced in kilo-calories to the oxygen consumed in grammes. In the simple case of a hexose sugar, the complete oxidation of 1 mole produces 678 kcal of heat, and consumes

6 moles of oxygen (192 g), thus giving an oxycalorific coefficient of 3.53. This value has been generally quoted for the aerobic respiration of carbohydrate (for references see Brafield & Solomon, 1972; Elliott & Davison, 1975).

Due to the different types and combinations of fats that have been used in the determinations, there is a range of published values for the Q_{ox} of fat. Elliott and Davison (loc. cit.) reported values ranging from 3.22 - 3.32 kcal g^{-1} (references cited), and quote 3.28 kcal g^{-1} as the modal figure. Since the lowest and highest values differ by only about 3% of this amount, the modal estimate appears to be an acceptable coefficient for fat.

In contrast to the oxidation of carbohydrate or fat, the oxidation of protein is incomplete, and results in the formation of nitrogen compounds which are excreted as ammonia, urea or uric acid. The composition of the nitrogenous excreta depends upon the nature of the organism's environment, and is discussed in general textbooks of animal physiology (e.g. Schmidt-Nielsen, 1975). Each of the excreted nitrogen containing compounds has a different energy content, thus making the situation for protein catabolism more complex than the oxidation of carbohydrate and fat, since the oxycalorific coefficient depends upon both the composition of the substrate and the nitrogenous excreta.

The generally accepted calorific value for protein of 23.62 J mg^{-1} (5.65 cal mg^{-1}) is used in the following calculations since the amino acid composition of Brachiomonas submarina cells is not known. It should be noted that some amino acids have widely different energy contents, e.g. glycine, 13.0 J mg^{-1} (3.11 cal mg^{-1}), and tyrosine, 24.72 J mg^{-1} (5.92 cal mg^{-1}) (Brody, 1945), so there is a possibility that the true value for B. submarina may differ somewhat from this average value.

There is some evidence, however, which supports the use of this average value, namely the findings of Parsons et al.'s (1961) investigations of the amino acid composition of two other green alga species. Tetraselmis maculata and Dunaliella salina. Their results show that no individual amino acid accounts for more than 26% of the total amino acid nitrogen, and therefore do not contradict the adoption of an average value.

The few studies that have been made of the nitrogenous excretion products of polychaete worms have shown that ammonia predominates, although small quantities of urea have been detected in some instances. Since nitrogenous excretion is generally recognised as being a labile character (e.g. Prosser & Brown, 1961) with the composition of the excretory product often changing as a result of an alteration in nutrition, salinity, temperature, etc. (see Bayne & Scullard, 1977) it is assumed that in the case of Mercierella the end product of protein catabolism is ammonia.

Making the above assumption, the oxidation of protein by Mercierella can be described in the following way:

Protein (A grammes) + O_2 (B grammes) $\longrightarrow$ CO_2 (C grammes) + H_2O (D grammes) + NH_3 (E grammes).

Brody (1945) gave the elemental composition of mixed protein, with a calorific value of 23.62 J mg^{-1}, as 52% C, 7% H, 23% O, and 16% N. Presumably the remaining 2% is sulphur.

Calculation of the Q_{ox} for the protein catabolised by Mercierella requires knowledge of the components A, B, and E in the above equation, together with the heats of combustion of the protein and ammonia (in the aqueous state, ΔH_{aq}). Converting Brody's (loc. cit.) elemental composition percentage values to grammes, and a knowledge of the

proportions by weight which the respective elements combine to form the compounds on the right-hand side of the equation enables B to be calculated in the following way: Oxygen combines with carbon and some of the hydrogen to form carbon dioxide and water. Since carbon combines with oxygen in the ratio 12 : 32 by weight, the amount of oxygen which combines with carbon is given by $\frac{52}{12} \times 32 = 138.67$ g.

Before the quantity of oxygen which combines with hydrogen is calculated, it is necessary to determine what proportion of the hydrogen will combine with nitrogen to produce ammonia. Since nitrogen combines with hydrogen in the ratio 14 : 3 by weight, the amount of hydrogen which remains to combine with oxygen to form water is $7 - (\frac{16}{14} \times 3) = 3.57$ g. Hydrogen combines with oxygen in the ratio 2 : 16 by weight, so the quantity of oxygen which combines with the remainder of the hydrogen is $\frac{3.57}{2} \times 16 = 28.56$ g.

Thus the total quantity of oxygen required to oxidise 98 g of mixed protein to CO_2, H_2O, and NH_3 is 138.67 + 28.56 = 167.23. Since the protein contains 23 g of oxygen, the amount of additional oxygen needed for the oxidation is 144.23 g (B). Balancing the equation gives:

Protein	+ O_2	⟶	CO_2	+ H_2O	+ NH_3
(98 g)	(144.23)		(190.67 g)	(32.13 g)	(19.43 g)

The calorific value of ammonia (ΔH_{aq}) can be calculated using the standard heats of formation of aqueous ammonia (-19.19 kcal $mole^{-1}$) and water (-68.31 kcal $mole^{-1}$), from the equation:

$$NH_3 + \frac{3}{2} O_2 \longrightarrow \frac{3}{2} H_2O + \frac{1}{2} N_2.$$

Substituting,

$$\Delta H_{aq} = \frac{3}{2} \times -68.31 + 0 - (-19.19) + 0$$

$$= \frac{-3}{2} \times 68.31 + 19.19$$

$$= -83.27 \text{ kcal mole}^{-1}.$$

Since the catabolism of 98 g of protein produces 19.43 g of NH_3, which represents $\frac{19.43}{17.0} \times -83.27 = -95.17$ kcal _____ that will not appear as such from the body of the worm, but is instead lost as excreta, the Q_{ox} for protein, $\frac{553.7}{144.23} = 3.84$ kcal $g^{-1}O_2$, has to be corrected for this amount of energy which is not available to the worm when it is excreting ammonia. This is done by first calculating the Q_{ex}, the excreta-calorific coefficient, which is the ratio of the heat lost as excreta (kcal) to the oxygen consumed in grammes. The Q_{ex} in the above case is $\frac{-95.17}{144.23} = 0.66$ kcal $g^{-1}O_2$ consumed. Thus correcting the oxycalorific coefficient with this value for ammonia excretion gives $Q_{ox} - Q_{ex} = 3.84 - 0.66 = 3.18$ kcal g^{-1} O_2 consumed.

The appropriate oxycalorific coefficient for Mercierella when feeding on B. submarina cells was calculated using 3.53 as the Q_{ox} for carbohydrate, 3.28 for fat, and 3.18 for protein when ammonia is being excreted. The following values refer to a standard worm of 7 mg wet weight (1 mg dry weight), and it is assumed that the 3 classes of food are respired in the proportions that they are assimilated.

The average rates of ingestion at the two experimental temperatures, 10°C and 20°C, were calculated from values obtained from the regression lines fitted to the ingestion data shown in Figs. 28 & 29, and respective egestion rates were derived from the curves fitted to the average rates of egestion for each of the experimental size classes shown in Fig. 47. Mercierella's ingestion rates when acclimated to 10°C and 20°C are

0.385 and 13.51 µg ash-free dry weight h^{-1}, and the respective egestion rates are 3.6 and 4.7 µg ash-free dry weight h^{-1}. Due to the discrepancy between the ingestion and egestion values for 10°C it was only possible to obtain a Q_{ox} value from the data for 20°C animals.

It was calculated, using the estimated chemical composition of B. submarina cells (Table 11), that the hourly intake of this alga comprises 7.34 µg of protein, 4.9 µg of carbohydrate, and 1.27 µg of fat. In addition, it is estimated that the worm ingests 0.93 µg dry weight of crown mucus every hour (Section 8.4.3). Assuming that mucus is comprised of equal amount of protein and carbohydrate (Section 8.4.4) this represents an additional 0.465 µg of each of these components, bringing the hourly ingestion values up to 7.81 µg of protein, 5.36 µg of carbohydrate, with fat remaining at 1.27 µg. Thus the total dry weight of matter ingested h^{-1} is 14.44 µg ash-free dry weight.

Similarly, from the chemical analyses performed on faeces produced by Mercierella acclimated to 20°C (Table 23) it was calculated that 4.7 µg ash-free dry weight of faecal matter is composed of 2.56 µg of protein, 1.33 µg of carbohydrate, and 0.81 µg of fat. In addition to the residual unassimilated algal material, the egested matter also contains a small quantity of mucus which forms the mucous coat surrounding the individual faecal pellets. The hourly production of faeces contains about 0.027 µg dry weight of mucus (Section 8.4.5) which, assuming this is composed of equal amount of carbohydrate and protein, results in the above egestion values for these two materials being overestimated by 0.0135 µg in each case. Since the mucous coat surrounding each faecal pellet forms part of the production component

in the energy budget, faeces composition was corrected for mucus production. The corrected values are 2.547 µg of protein, 1.316 µg of carbohydrate, with fat remaining unchanged at 0.81 µg, thus giving a total corrected weight of egested algal material of 4.673 µg ash-free dry weight h^{-1}.

From the corrected amounts of ingested and egested food materials, the quantities of assimilated protein, carbohydrate, and fat were calculated. These are 5.263 µg, 4.044 µg, and 0.46µg respectively, giving a total of 9.767 µg ash-free dry weight h^{-1}. Converting these values to percentages of the total amount assimilated gives, 53.89% for protein, 41.4% for carbohydrate, and 4.71% for fat. Thus the appropriate oxycalorific coefficient for Mercierella acclimated to 20°C, and feeding on B. submarina cells is 3.33 kcal g^{-1} O_2.

Average values for oxygen consumption by 7 mg worms (1 mg dry weight) acclimated to 10°C and 20°C were obtained from the regression lines fitted to oxygen consumption and size shown in Fig. 61. These values are 0.435 µl h^{-1}, and 1.4 µl h^{-1} respectively. Assuming the Q_{ox} is the same at both temperatures, the values for metabolic heat loss were obtained by converting these values for oxygen consumption at 10°C and 20°C to mg (1 µl of oxygen gas at N.T.P. weighs 1.429×10^{-3} mg), and multiplying by the oxycalorific coefficient, 3.33. Thus the heat loss resulting from metabolism (the energetic cost of metabolism) is 8.65×10^{-3} J h^{-1} (2.07×10^{-3} cal h^{-1}) at 10°C, and 2.78×10^{-2} J h^{-1} (6.65×10^{-3} cal h^{-1}) at 20°C. Over three times more heat is lost at the higher temperature due to increased metabolism.

7. NITROGENOUS EXCRETION

7.1 The energy lost as a result of nitrogenous excretion

The Q_{ex} value of 0.66 kcal g^{-1} O_2 consumed for Mercierella excreting ammonia (Section 6.8) was used to convert the rates of oxygen consumption by a 7 mg worm (1 mg dry weight) at 10°C and 20°C, into the rates at which energy was lost as excreta. Since it is assumed that Mercierella respires food materials in the proportions in which they are assimilated, it is necessary to convert the Q_{ex} value of 0.66 kcal g^{-1} O_2 for worms respiring protein, to an appropriate value for Mercierella respiring only 53.89% protein. The appropriate Q_{ex} of 0.356 kcal g^{-1} O_2 when multiplied by the oxygen consumption values in mg, 0.622×10^{-3} mg at 10°C, and 2.0×10^{-3} mg at 20°C (hourly rates) gives the amounts of energy lost h^{-1} as ammonia at the two acclimation temperatures. These amounts are 9.2×10^{-4} J h^{-1} (2.2×10^{-4} cal h^{-1}) at 10°C, and 2.97×10^{-3} J h^{-1} (7.1×10^{-4} cal h^{-1}) at 20°C.

There are only a few published studies of the end products of nitrogen metabolism in polychaete worms. In common with other aquatic invertebrates (see Schmidt-Nielsen, 1975), ammonia is the main nitrogen compound in the excreta. Aphrodite aculeata L. releases 80% of the total urinary nitrogen as ammonia, and only 0.2% and 0.8% respectively as urea and uric acid (Baldwin, 1959, quoted in Prosser & Brown, 1961). Hult (1969) reported that Cirriformia spirabrancha (Moore, 1904) releases at least 96.8% of its excreted nitrogen as ammonia, and the rest as urea with traces of taurine. Thus it appears from these results that although polychaetes are primarily ammonotelic, at least some species are capable of a limited amount of ureogenesis.

Table 30. The rates of ammonia excretion by several polychaete species.

Species	Rate of ammonia excretion (moles g^{-1} wet wt. h^{-1})	Reference
(Numbers in parentheses are the number of individual values on which the adjacent figure is based)		
	($\bar{x}$) (S.D.)	
Cirriformia spirabrancha (Moore, 1904)	$0.151 \pm 0.035 \times 10^{-6}$ (13)	Hult (1969)
	10°C 20°C	
Mercierella enigmatica Fauvel	0.38×10^{-6}, 1.2×10^{-6}	
Neanthes (=Nereis) virens (Sars)	$0.22 \pm 0.04 \times 10^{-6}$ (11)	Kay & Brafield (1973)
Nereis virens	$0.14 - 0.5 \times 10^{-6}$	Haberfield et al. (1975)
Spirographis (=Sabella) spallanzanii Viviani	$1.4 - 4.89 \times 10^{-3}$ (12)	Fox (1938)

The rates of ammonia excretion by several polychaete species, including both errant and sedentary forms, are presented in Table 30. The rates of ammonia release are expressed in moles g^{-1} wet weight h^{-1}, and where it was necessary these have been calculated from the reported data. Fox (1938) presented the nitrogenous excretion rates of tubed Spirographis spallanzanii, in mg of ammonia nitrogen per c.c. of worm h^{-1}. These data were converted to moles of ammonia g^{-1} wet weight by assuming the worms had the same density as sea water, 1.023 g ml^{-1} (30 °/oo at 10°C), and multiplying the nitrogen values by 1.2143 to give the weight of ammonia. Kay and Brafield (1973) presented the excretion values for Neanthes (= Nereis) virens in calories. Since they used an incorrect calorific value for ammonia (4.05 cal mg^{-1}), the excretion results for the 35 day super-maintenance feeding experiments were recalculated using the correct calorific value of 4.9 cal mg^{-1} (see Section 6.8), and then converted to moles g^{-1} wet weight h^{-1}, using a mean wet weight of 2.75 g.

Considering the differences between the nutritional states and organic contents of the worms used in the ammonia excretion determinations, plus the different salinities and temperatures at which the measurements were made, there is in general a remarkable degree of concordance between rates of ammonia excretion shown in Table 30. The exception being the values derived from Fox's data for the sabellid Spirographis, which are about 1 000 x higher than the rest. In view of the similar mode of life to that of Mercierella, it is not possible to explain this discrepancy in biological terms. Since Fox (loc. cit.) included oxygen consumption figures for Spirographis (= Sabella), albeit for just 3 tubed individuals, this allows the rate of ammonia excretion at 21°C to be calculated, assuming that this tubicolous,

*Brafield ('75, pers. comm.) soon corrected this error.

filter-feeder has a similar Q_{ex} to Mercierella, namely 0.356 kcal g^{-1} O_2. The mean rate of oxygen consumption is 96.7 µl $c.c.^{-1}$ of worm h^{-1}, or 1.38×10^{-1} mg O_2 $c.c.^{-1}$ of worm h^{-1}. This is equivalent to $\frac{1.38 \times 10^{-1}}{1.023} = 1.35 \times 10^{-1}$ mg O_2 g^{-1} wet weight h^{-1}. Thus the amount of energy lost as ammonia is estimated to be $0.356 \times 1.53 \times 10^{-1} = 0.48 \times 10^{-1}$ cal g^{-1} h^{-1}, which is equivalent to 0.58 µmoles of ammonia g^{-1} wet weight h^{-1}.

In contrast to Fox's values obtained using Nessler's method, the estimated value of 0.58×10^{-6} g^{-1} h^{-1} is in agreement with the values for the other polychaetes presented in Table 30. The rate of ammonia excretion by Spirographis at 21°C is lower than the rate of excretion by Mercierella at a similar temperature, but this difference can be explained by the generally observed relationship between metabolic rate and body size.

8. PRODUCTION

The assimilated energy which is in excess of Mercierella's immediate metabolic requirement is available for incorporation into synthetic pathways. In the present study four components of production are investigated: somatic growth; reproduction; tube production; and mucus production.

8.1 Somatic growth

8.1.1 Introduction

An experiment is described in which the rates of somatic production of Mercierella in the Greenhithe population were investigated in the field and laboratory, under winter and summer conditions. Growth rates of individual worms were measured, after a preliminary investigation showed that it is not possible to estimate growth rate from monthly weight-frequency histograms, because the considerable variation in this parameter prevents identification of the older year groups.

8.1.2 Materials and Methods

Growth studies in the laboratory. Mercierella were collected from pools of standing water on the shore at Greenhithe, in January and July 1975. The winter worms were maintained in the laboratory at 10°C, and the summer animals at 20°C, in 6-l containers of dilute sea water, with a salinity of 15 °/oo (an average value based on the field situation, see Fig. 24), under a 12 h day - 12 h night light régime. These temperatures approximate to the field conditions at the times of collection.

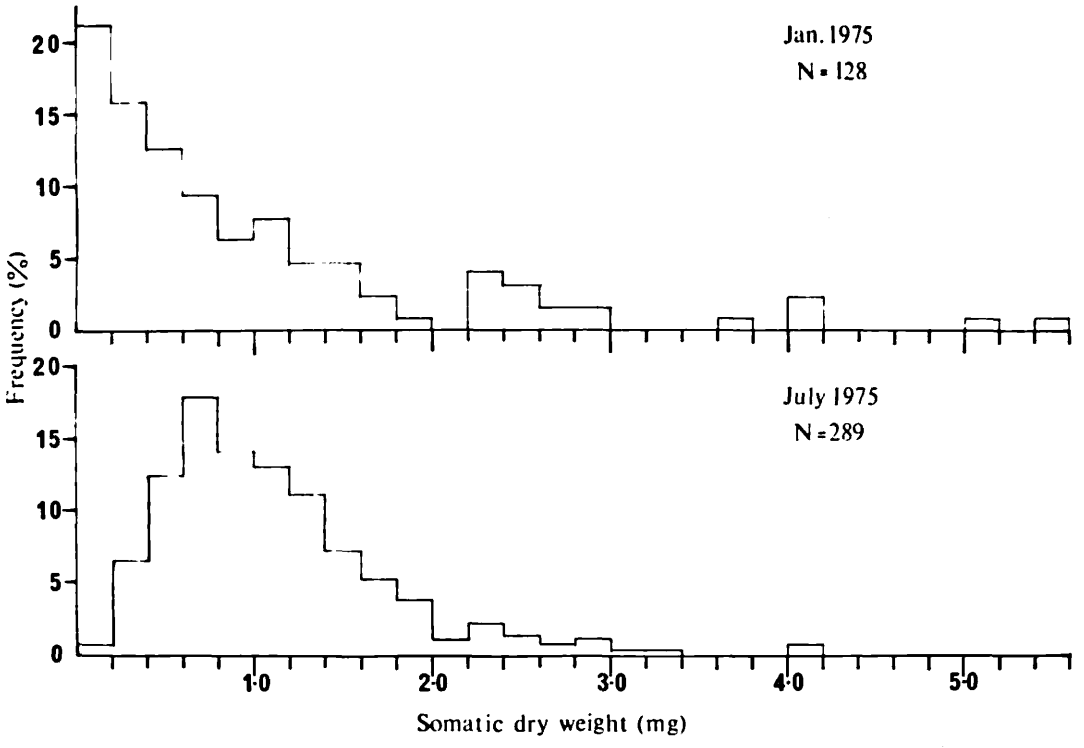
The worms were fed daily with a suspension of B. submarina, at a maximum density of $3.8 \pm 0.77 \times 10^4$ cells ml^{-1}. Colorimetric measurements of cell numbers in the tanks between consecutive feeds (Appendix 1.11) showed there was a steady decline in concentration mostly due to settlement, which additional artificial aeration failed to prevent. However, this mirrored to some extent the field situation, where the concentration of suspended solids varies throughout the tidal cycle (see Section 2.3.4), and on a number of occasions, the density of algal cells mid way between consecutive feeds, was roughly similar to that used in the rest of this study.

Within a few days of their collection, one hundred and twenty eight winter worms and two hundred and eighty nine summer worms were detubed, and their somatic tissues prepared for weighing. Since the summer worms, and some of those collected in January, contained gametes, two laterally positioned longitudinal slits were made in the abdominal wall through which the coelomic contents were carefully teased and washed out. Attempts were also made to remove food materials from their guts to minimise this potential source of error, without rupturing the gut blood-sinus. After two 10 s rinses in glass-distilled water to reduce salt contamination, the somas were transferred to individual, tared, aluminium foil trays, and dried to constant weight at 60°C. Weighings were made to the nearest 0.1 mg on a Sartorius semimicro balance. Somatic dry weight-frequency histograms were constructed from these data, after Chi-square tests for goodness-of-fit showed no significant differences in population structure of Mercierella living under separate rocks which were immersed in surface water during intertidal periods, sampled at the same time.

Forty eight winter animals and forty six summer worms, representing the size ranges of the samples, were used in the laboratory growth study. Due to Mercierella's calcareous tube, it was not possible to weigh the worms before the end of the experiment, so somatic growth was measured indirectly taking advantage of the relationship between tube length and somatic dry weight. Since the majority of the tubes curled in three-dimensions, their initial lengths were measured by winding dampened cotton thread along their lengths and cutting it to size. The individual cotton threads were measured with a ruler to the nearest 1 mm. Before the worms were replaced in the experimental tanks, the anterior ends of the tubes were painted with Indian ink, which was allowed to dry. At weekly intervals, the new tube growth, identifiable by its white colour, was measured to the nearest 0.05 mm with vernier calipers. The growth experiments lasted for 35 d, sufficient time for significant growth to be recorded at the two temperatures.

A non-adiabatic micro-bomb calorimeter basically similar to the one described by Phillipson (1964) was used to measure the calorific value of the somatic tissues. Samples of Mercierella, collected from the field population in July, were sexed on the basis of the types of gametes they contained, and their somatic tissues were then dialysed in Visking tubing for 24 h against glass-distilled water, to reduce their salt content. A high salt content made samples difficult to ignite in the calorimeter. This method is based upon that described by Kay and Brafield (1973). After dialysis treatment, the samples were dried in an oven at 60°C. To ensure that the pellets were completely homogeneous, the dried somatic tissues were forced through a 125 μm steel meshed sieve. Bombing was carried out in a constant temperature

Fig. 63. Percentage weight-frequency histograms for shore samples of M. enigmatica collected at Greenhithe immediately after larval settlement (January 1975) and immediately before the breeding season (July 1975). The N values refer to the number of animals on which each histogram is based.


Jan. 1975
N = 128
July 1975
N = 289
Frequency (%)
20
15
10
5
0
1·0
2·0
3·0
4·0
5·0
Somatic dry weight (mg)

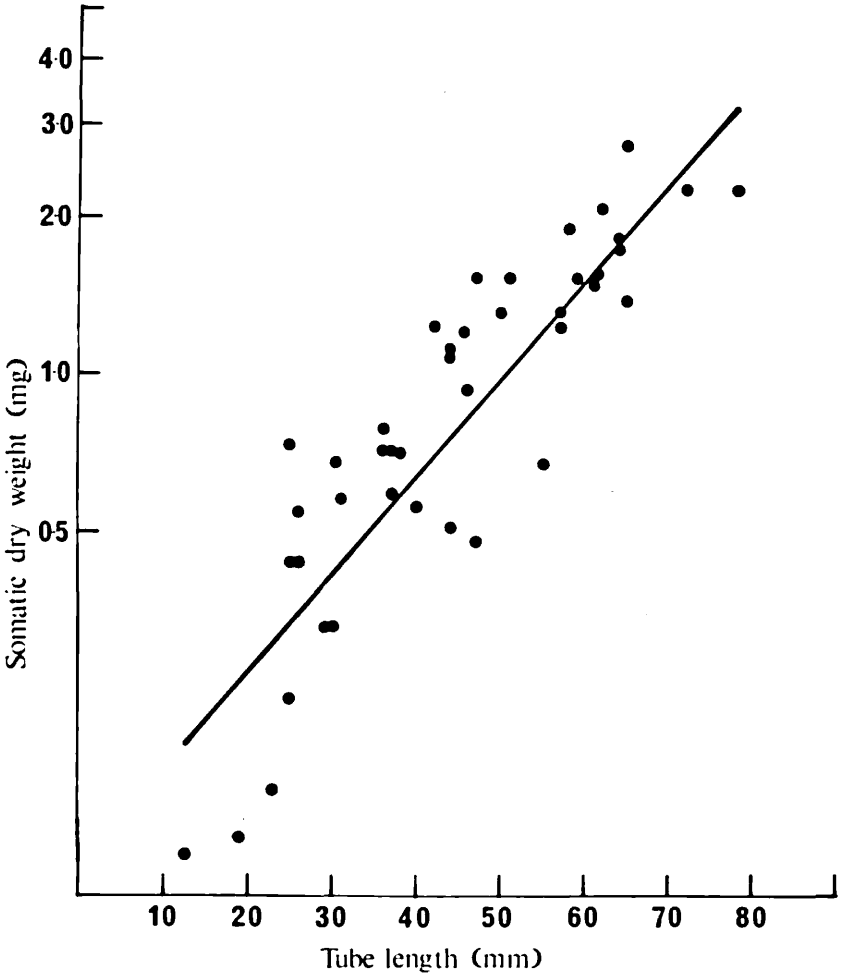
room at 10°C. Complete temperature equilibration between the calorimeter and the ambient air was allowed before igniting the samples, thereby avoiding the need for a pre-fire correction. A post-fire correction was made to the recorded temperature rise. The constant ambient temperature meant that all the post-fire corrections were positive, thus avoiding the potential error associated with having to estimate the position of maximum temperature on the chart trace. Fuse wire and acid corrections were not applied to these measurements because preliminary investigations showed them to be negligible. Ash content was determined by oxidising pre-weighed samples in a muffle furnace at 500°C, for 4 h (Paine, 1964).

Growth studies in the field. Approximately sixty Mercierella, attached to the underside of a c. 0.5 m^2 piece of asbestos, were collected from a pool of surface water mid-way between the low water and mid tide levels on the Greenhithe shore, on the 9th of February 1974. Since the tubes were growing in close association with the asbestos sheet, their lengths were recorded photographically and measured planimetrically. The worms were maintained overnight in water of salinity 15 °/oo at 10°C. The following day, they were returned to their original position on the shore, and left undisturbed for about 3 months (9th May), before transporting them back to the laboratory where the tubes were measured as before.

8.1.3 Results

The somatic dry weight-frequency distributions of the population samples for winter and summer are shown in Fig. 63. Apart from the most recent '0' group, it is not possible to identify any other year-groups due to the considerable variation in growth rate. The January

Fig. 64. The relationship between tube length and somatic dry weight of M. enigmatica. The regression line was fitted by the method of least squares, assuming a linear relationship exists between these parameters after semi-logarithmic transformation. b = 1.17.

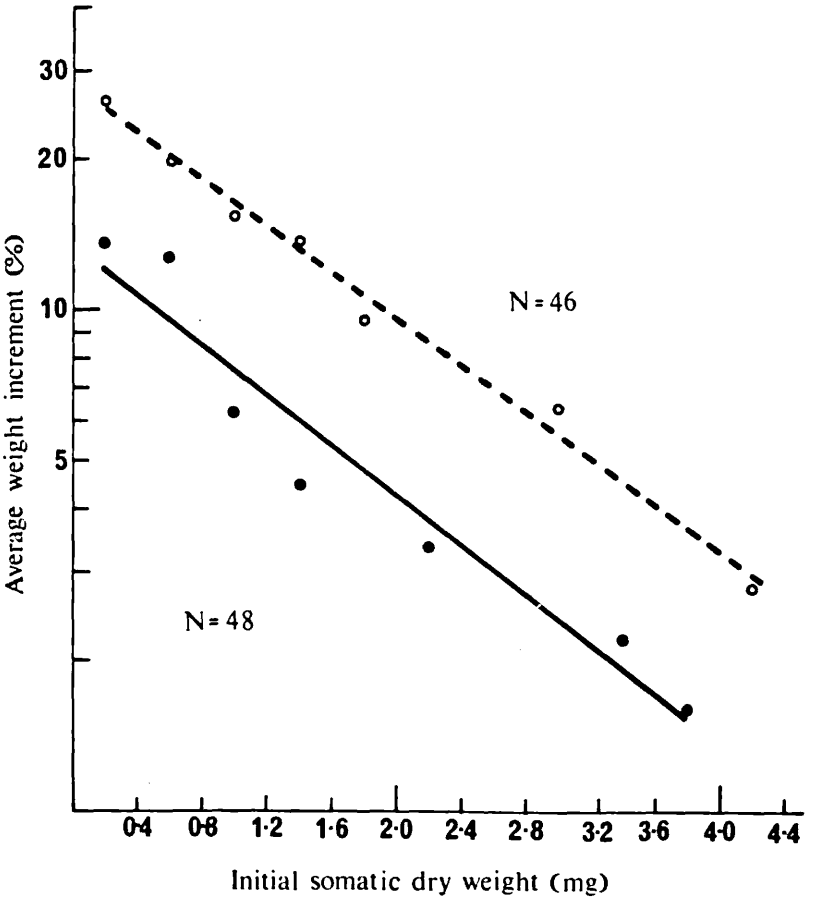

4·0
3·0
2·0
1·0
0·5
Somatic dry weight (mg)
10
20
30
40
50
60
70
80
Tube length (mm)

sample is considerably more skewed, in comparison to the more normal distribution in July, as a result of the large proportion of small individuals (< 4.0 mg), the majority of which grew to between 6 and 8 mg by the following summer.

Fig. 64 shows somatic dry weight expressed as a function of tube length for a sample consisting of forty two worms. The regression line was fitted by the method of least squares, assuming a linear relationship exists between these two parameters after semi-logarithmic transformation. The correlation coefficient, r = 0.88, is highly significant at the P = 0.05 level of significance. There is a non linear relationship between somatic dry weight and tube length, over the range 12 - 78 mm, since smaller worms have relatively longer tubes than do larger animals. Part of the reason for the scatter about the regression line is the change in the direction of tube growth which results in the formation of a peristome (see Section 8.3.1). No correction was necessary for peristome formation since all the adult size classes, except juveniles which sometimes have a longitudinal (juvenile) ridge, produce peristomes at irregular intervals and are therefore affected equally.

Weekly weight increments were combined to give the individual total weight increments during the 35 days, at 10°C and 20°C. After these values were converted to percentages of the initial somatic dry weights, the average percentage weight increment was calculated for each 0.4 mg size class (Fig. 65). The regression lines were fitted by the method of least squares after semi-logarithmic transformation. Both groups of data have a similar correlation coefficient, r = -0.99, which is highly significant at the P = 0.05 level. When the slopes of the lines, b = -0.78 (10°C) and b = -0.73(20°C) were compared using a t-test

Fig. 65. The relationship between initial somatic dry weight and average weight increment of M. enigmatica in 35 days at 10°C (solid line) and 20°C (broken line); regression lines fitted by the method of least squares, assuming linear relationships after semi-logarithmic transformation of the data. $b_{10°C} = -0.78$; $b_{20°C} = -0.73$; and the respective ordinate intercepts are 13 and 28. N values refer to the total number of individual values at each temperature, on which the average values used in the calculations are based.


30
20
10
5
Average weight increment (%)
N = 46
N = 48
0·4
0·8
1·2
1·6
2·0
2·4
2·8
3·2
3·6
4·0
4·4
Initial somatic dry weight (mg)

for small samples (Bailey, 1959), after a F-test showed there is no significant difference between the variances about the regression lines ($P = > 0.05$), there was found to be no significant difference between them ($P = > 0.05$). Thus the relationship between growth and body size is the same under winter and summer conditions. In common with other organisms Mercierella's relative growth rate is highest for young animals (see Thompson, 1917). A Q_{10}, based on the percentage weight increments of the median size class at 10°C and 20°C, has a value of 2.0.

Three pellets (3.4 - 7.6 mg dry weight) for each of dialysed male and female somatic tissues had calorific values of 17.56 ± 0.52 J mg^{-1} (4.2 ± 0.125 cal mg^{-1}), and 18.39 ± 1.17 J mg^{-1} (4.4 ± 0.28 cal mg^{-1}) respectively. After a F-test had shown there is no significant difference between the variances about the means ($P = > 0.05$), these were compared using a t-test, similar to that described by Bailey (1959) for small samples. There is no significant difference between the calorific contents of male and female somatic tissues ($P = > 0.05$). These had a mean ash content of 22.6% of the dialysed dry weight (based on three determinations).

Some of the Mercierella used in the field growth study are shown in Fig. 66., at the beginning and end of the experiment. It is evident that appreciable amounts of growth occurred during the intervening three months. On the 10th of February 1974 the tubes were approximately the same size, 43.6 ± 4.2 mm ($N = 38$), which indicates that the worms were of the same year-group, and therefore had similar histories. Their tube lengths increased by an average of 10.3 ± 4.5 mm, during the three months. From the relationship between tube length and somatic dry weight

Fig. 66. Some of the M. enigmatica, attached to the underside of a c. 0.5 m^2 piece of asbestos, collected from a pool of surface water mid-way between the low water and mid tide levels on the Greenhithe shore, that were used in the field growth study. A, shows the worms on the 10th February 1974 and B, the same animals three months later (9th May), when new tube growth was clearly visible. It is also apparent that a considerable amount of polyzoan growth had taken place during the intervening three months. The small divisions on the rule are millimetres.


A


B

it was deduced that at the beginning of the experiment, the individual somatic dry weights averaged 0.74 mg, and three months later, 1.16 mg, representing an average increment of 56.8%. The water temperature for the same period increased from an initial 10°C to 14°C (Fig. 25).

The average amount of growth during the three months was converted to the percentage weight increment in 35 days, 22.6%, and compared with the average weight increments for worms of initial weight, 0.74 mg, at 10°C and 20°C in the laboratory. Values of 8.8% at 10°C, and 19.9% at 20°C, were derived from the regression lines in Fig.65. A modified t-test (Bailey, 1959) for small samples where the variances are not assumed equal (F-test, $P = < 0.05$) showed that the average rate of growth in the field during the period February to May is not significantly different from the growth rate measured in the laboratory at 20°C ($P = > 0.05$).

8.1.4 Discussion

The Greenhithe population of Mercierella enigmatica contains individuals which range in somatic dry weight from less than 0.2 mg to more than 5.4 mg. It is possible to identify the post settlement 0 group (< 0.4 mg), which comprised 37% of the January sample (Fig. 63). In addition to their small size, the young worms had not commenced gametogenesis at the time the sample was taken (see Section 8.2.3), and some tubes had a distinct juvenile ridge (see Hartmann-Schröder (1967) for a description of the form of the tube in this species).

A stable population structure of the kind described for Mercierella appears to be characteristic of all polychaete species which are known to breed several times during their lives (for references see Olive, 1975). There is no evidence to suggest that

Table 31. Estimated somatic production of *M. enigmatica* m^{-2} in winter and summer at Greenhithe.

Winter					Summer				
Weight class (mg)	Estimated standing crop (mg)	Estimated standing crop (cal)	Estimated production (mg)	Estimated production (cal)	Weight class (mg)	Estimated standing crop (mg)	Estimated standing crop (cal)	Estimated production (mg)	Estimated production (cal)
0 - 0.2	4.62	19.96	2.97	12.8	0 - 0.2	0.15	0.67	0.21	0.9
0.2 - 0.4	10.34	44.67	6.1	26.4	0.2 - 0.4	4.33	18.7	5.4	23.3
0.4 - 0.6	13.75	59.4	7.1	30.7	0.4 - 0.6	13.7	59.18	15.25	65.9
0.6 - 0.8	14.52	62.73	6.82	29.5	0.6 - 0.8	27.72	119.75	27.5	118.8
0.8 - 1.0	12.32	53.22	5.2	22.5	0.8 - 1.0	28.07	121.26	25.3	109.3
1.0 - 1.2	18.92	81.73	7.0	30.2	1.0 - 1.2	31.81	137.42	25.74	111.2
1.2 - 1.4	13.42	57.97	4.5	19.4	1.2 - 1.4	31.7	136.94	22.95	99.1
1.4 - 1.6	15.4	66.53	4.5	19.4	1.4 - 1.6	24.0	103.68	15.62	67.5
1.6 - 1.8	8.8	38.0	2.3	9.9	1.6 - 1.8	19.36	83.64	11.24	48.6
1.8 - 2.0	3.3	14.26	0.81	3.5	1.8 - 2.0	15.84	68.43	8.3	35.9
					2.0 - 2.2	4.84	20.91	2.3	9.9
2.2 - 2.4	19.8	85.54	3.7	16.0	2.2 - 2.4	10.63	45.92	4.62	19.9
2.4 - 2.6	17.16	74.13	2.86	12.4	2.4 - 2.6	7.6	32.83	2.86	12.3
2.6 - 2.8	9.46	40.87	1.4	6.0	2.6 - 2.8	4.1	17.71	1.38	6.0
2.8 - 3.0	10.12	43.72	1.4	6.0	2.8 - 3.0	6.64	28.68	2.1	9.1
					3.0 - 3.2	2.4	10.37	0.68	2.9
					3.2 - 3.4	2.55	11.02	0.68	2.9
3.6 - 3.8	6.6	28.51	0.55	2.4					
4.0 - 4.2	21.12	91.24	1.4	6.0	4.0 - 4.2	6.23	26.91	1.03	4.4
5.0 - 5.2	9.02	38.97	0	0					
5.4 - 5.6	9.68	41.82	0	0					
Totals	218.35	943.27	58.61	253.1		241.67	1044.02	173.16	747.9

mortality is particularly localised to any one fraction of the population, as occurs in those polychaetes which die shortly after spawning, e.g. Nereis virens Sars (Brafield & Chapman, 1967), and whose population structure is characteristically unstable.

It is not possible to compare directly the calorific values obtained for Mercierella's somatic tissues with the values reported by other authors, since the present study is the only one in which growth and gamete production are treated separately. However, by 'reconstructing' a worm of 1.5 mg somatic dry weight, the median weight in Fig. 79, values of 17.14 J mg^{-1} dry weight (4.1 cal mg^{-1} dry wt.) for a sexually mature male, and 18.35 J mg dry weight (4.39 cal mg^{-1} dry wt.) for a mature female, were obtained. Converting these amounts to ash-free dry weight gave 23.83 J mg^{-1} (5.7 cal mg^{-1}) for a male, and 24.66 J mg^{-1} (5.9 cal mg^{-1}) for a female. These values compare favourably with the average value of 23.91 J mg^{-1} ash-free dry weight (5.72 cal mg^{-1} ash-free dry wt.) for Neanthes (= Nereis) virens (Sars), which was calculated from the results of Kay and Brafield (1973).

The standing crops of somatic tissue m^{-2} in the "effective area" of shore (see Section 2.3.3) were calculated from the somatic dry weight-frequency distributions for January and July, assuming a stable population density of 220 Mercierella m^{-2}, using the median weight for each 0.2 mg size class (Table 31, columns 2, 3, 7, and 8). Somatic standing crop averaged 4.15×10^3 J m^{-2} (993.64 cal m^{-2} yr^{-1}), and was slightly lower in the winter, 3.94×10^3 J m^{-2} (943.27 cal m^{-2}), than in the summer, 4.36×10^3 J m^{-2} (1 044.02 cal m^{-2}). Since January and July are the post-spawning and pre-spawning periods respectively (Section 8.2.3), the similarity between the winter and summer values illustrates the stability of the population structure.

All size classes underwent some growth during the 35 days, at both the winter and summer temperatures. Somatic production m^{-2} on the Greenhithe shore during the winter and summer, assuming a stable population density of 220 worms m^{-2}, was calculated by multiplying the average somatic dry weight increments in 35 days (Fig. 65) of the median values of the 0.2 mg size classes in Fig. 63, by 5.214 to give the estimated somatic production during the 6 month winter and summer periods (Table 31, columns 4, 5, 9, and 10). For the purposes of the calculations, the annual cycle of mid tide water temperatures (Fig. 25) was simplified into two 6 month periods of 10°C and 20°C. The estimated somatic production during the winter period is 1.06×10^3 J m^{-2} (253.1 cal m^{-2}), and for summer, 3.13×10^3 J m^{-2} (747.9 cal m^{-2}), making a total of 4.19×10^3 J m^{-2} yr^{-1} (1.001 kcal m^{-2} yr^{-1}). Thus the annual somatic production : standing crop ratio for Mercierella at Greenhithe is approximately 1 : 1.

Under the controlled laboratory conditions, somatic growth rate was directly related to temperature, other factors being equal. The Q_{10} of 2 for the temperature range, 10°C - 20°C, shows that the rate of growth conforms with temperature over the natural environmental range. Although there are no published recordings of somatic growth rates for Mercierella from other localities, there are a number of accounts which include information concerning tube growth and body size (see Table 32). Several of these reports support the findings of the present investigation concerning the relationship between growth rate and temperature (see Hartman, 1969; Straughan, 1972; Hove, 1974), e.g. Straughan (loc. cit.) reported that the rate of tube growth of Mercierella (= Neopomatus ?) in the Brisbane River estuary, S.E. Australia, increases with temperature, resulting in growth being faster in the summer months.

Table 32 cont.

Locality	Form of colonies	Tube length (mm)	Total body length (mm)	Growth rate	Salinity (°/oo)	Temperature	Reference
*Lagos Harbour Nigeria (6 20N 3·20E)	< 60 mm across	30 (max. 40)	—	4.4 mm month^{-1} (tube)	< 5 - 30	25 - 31°C	Hill (1967)
*Brisbane River estuary, Australia (27 0s 152 30E)	small	32	—	5.3 mm week^{-1} 1.1 mm week^{-1} (tube)	Brackish	26 - 27°C 16 - 20°C	Straughan (1972)
Lake Merrit N. America (37 40N 122 25W)	"great masses"	80	—	—	Brackish	20	Fauvel (1933) Hall (1954) Hartmann-Schröder (1967)

*Mercierella enigmatica (= Neopomatus ?)

Table 32 cont.

Locality	Form of colonies	Tube length (mm)	Total body length (mm)	Growth rate	Salinity (°/oo)	Temperature	Reference
The Rance France (48 10N 1 41W)	> 100 mm thick	35 - 40	10 - 13	35 - 40 mm in 4 months (tube)	1.5 - 16	late summer	Fischer (1925) Fischer-Piette (1937)
Roussillon (Fr. mediterranean) (42 40N 2 40E)	.3 - .8 m diam. x .2 - .5 m high	40 - 50	—	—	30.2 - 35.4 23	—	Petit & Rullier (1956)
Kanaal door Walcheren Netherlands (51 27N 3 35E)	150 mm thick	—	—	—	brackish	3 - 5°C higher than natural waters	Hove (1974)
Marstal Denmark	—	60	14	—	—	—	Hartmann-Schröder (1967)
Port of Gandia Spain	large colonies	20 - 40	20 - 22	—	18.1	—	Rioja (1924)
Lake of Tunis Tunisia (36 48N 10 13E)	large colonies	—	6 - 25	—	38 - 55	—	Heldt (1944)

Table 32. Growth of *M. enigmatica* in different localities.

Locality	Form of colonies	Tube length (mm)	Total body length (mm)	Growth rate	Salinity (°/oo)	Temperature	Reference
London Docks (51 28N 0 0)	"masses of interlacing tubes"	—	10	1 mm week^{-1} (body length)	brackish	19°C	Monro (1924) McIntosh (1926)
Radipole Lake, adjacent Weymouth Harbour (56 36N 2 25W)	150 - 200 mm thick	—	—	150 mm in 8 weeks (colony)	16.88 - 24.7	summer	Tebble (1953) " (1956)
Weymouth (56 36N 2 27W)	—	50 - 60	22	—	6.56 - 30.81	—	Tebble (1953) " Hartmann-Schroder (1967)
Caen Canal France (49 15N 0 27W)	large	—	6 - 12	.3 - 3 mm in 15 days (juveniles, body length)	1.81 - 2.45	summer	Fauvel (1922) Rullier (1955)
St. Malo and St. Servan France (48 42N 2 2 W)	Abundant	30 - 60	10	30 mm in <2 months (tube)	35	summer	Fischer-Piette (1937)

Straughan also reported that the main factors affecting tube growth rate, other than temperature, are age, salinity, and inter- and intra-specific competition. She found (in common with the findings of the present investigation) that tube growth decreased with age, However, the Brisbane River animals reached an apparent maximum length of 32 mm in just 6 weeks in summer and 28 weeks in winter (see Table 32). In contrast to Thames population (Section 8.2.3) there is an extended period of reproduction in the Brisbane River. Straughan's results do not contradict those of the present study, since the apparent maximum tube length was not due to a cessation of tube secretion, but resulted from this becoming "approximately equal" to the dissolution rate of the posterior end. This phenomenon has not been observed in either the Thames or Weymouth populations, in which the remains of the juvenile tubes are usually present (e.g. see Fig. 66).

Several authors have described the effects of salinity on the growth of Mercierella tubes. There is general agreement concerning the existence of an optimum range over which tube growth is insensitive to salinity fluctuations, whilst sudden changes in salinity (Soldatova & Turpaeva, 1960), and exposure to extreme levels (Mathias & Izac, 1963; Hill, 1967 (= Neopomatus ?)) cause a reduction or cessation of tube growth. Straughan (loc. cit.) reported that worms in the middle region of the Brisbane River estuary had longer tubes than those at either the seaward or landward limits of the range. Hill (loc. cit.) found that in Lagos Harbour, Nigeria, tube growth was relatively constant over the range 5 - 30 $^{o}/_{oo}$, and Mathias and Izac (loc. cit.) reported that whereas no tube growth occurred in fresh water, growth was constant up to a specific gravity of 1.028 (no temperatures given). It seems unlikely therefore that salinity is a major factor influencing the rate

of tube growth in the Thames population (see Fig. 24), and supports the use of a single salinity of 15 °/oo in the laboratory experiments.

Straughan (loc. cit.) demonstrated that tube growth was inversely related to population density, as a result of what she believed was interference between feeding animals. Similarly, cirral activity of the barnacle, Balanus balanoides (L.) also disturbed their feeding behaviour, and therefore tube building activity, since additions to the anterior of the tube can only be made when the branchial crown is extended (see Section 8.3.4). Furthermore, when Balanus was present in large numbers the Mercierella were "rapidly overgrown, crushed, or smothered". At Greenhithe, however, Mercierella is rarely present at a sufficient density for there to be even the possibility of intra-specific competition, with regards to feeding space (this has never been observed in the laboratory, even under extremely crowded conditions). In addition, inter-specific competition with barnacles does not take place, because at Greenhithe these organisms have entirely different niche preferences. Whilst Mercierella actively selects the undersides of rocks, barnacles are found most commonly on the wooden pier pilings (see Section 2.2).

The reason for the discrepancy between the field and laboratory growth rates is not known for certain. It is unlikely, however, that any of the above mentioned factors are responsible since: the field temperature range was intermediate to the laboratory temperatures; age (initial size) was allowed for in the calculation; the extreme salinities in the field for the 3 month period, 7 - 21 °/oo (Fig. 24), are within the range of salinity insensitivity (see above for references); and competitive influences on tube growth, in both the field and laboratory situations, were minimal.

The faster growth rate in the field is probably related to the quality of the food in the two situations. (It will be remembered that the ash-free dry weight of organic matter ml^{-1} in the laboratory experiments was considerably in excess of the estimated concentrations in the field (Section 3.4.3)). First, in the laboratory experiments, Mercierella was fed on a single algal species, B. submarina, whereas in the field there is a variety of food types available (Section 3.1). It is unlikely that B. submarina is a complete and balanced food for Mercierella, since it is generally recognised that most animals do better when offered a variety of food types. Second, Mercierella possibly requires some silt in its diet, to act as a mechanical stimulant to the feeding and digestive processes. Winter (1976) has demonstrated that the filtering and growth rates of Mytilus edulis L. are considerably increased when offered a suspension of silt in addition to an optimal concentration of algal cells, when compared with mussels fed only algae. In one experiment an increase in growth rate of 32% was recorded after silt was added to the diet. Since Mercierella is found largely in estuarine situations, which are characterised by the presence of large quantities of silt (Section 2.3.4), it is possible that this worm may be adapted to function most efficiently where there is a proportion of inorganic particles in suspension.

8.1.5 The hourly somatic production rates by standard worms

The average hourly somatic production rates at 10°C and 20°C by Mercierella weighing 7.0 mg wet weight (1.0 mg dry weight) were obtained by dividing the average weight increments derived from the regression lines in Fig. 65 by 35 x 24 = 840 h. The hourly production values in mg are 9.05×10^{-5} mg at 10°C, and 1.94×10^{-4} mg at 20°C. Assuming an ash content of 22.6% of the dialysed dry weight these values are

equivalent to 7.0×10^{-5} mg ash-free dry weight at 10°C, and 1.5×10^{-4} mg ash-free dry weight at 20°C. Expressed in energy units, assuming somatic tissue has an average calorific value of $\frac{17.98 \times 100}{77.4} = 23.23$ J mg^{-1} ash-free dry weight (5.55 cal mg^{-1} ash-free dry wt.), these values are equivalent to 1.63×10^{-3} J h^{-1} (3.9×10^{-4} cal h^{-1}) at 10°C, and 3.49×10^{-3} J h^{-1} (8.35×10^{-4} cal h^{-1}) at 20°C.

8.2 Reproduction

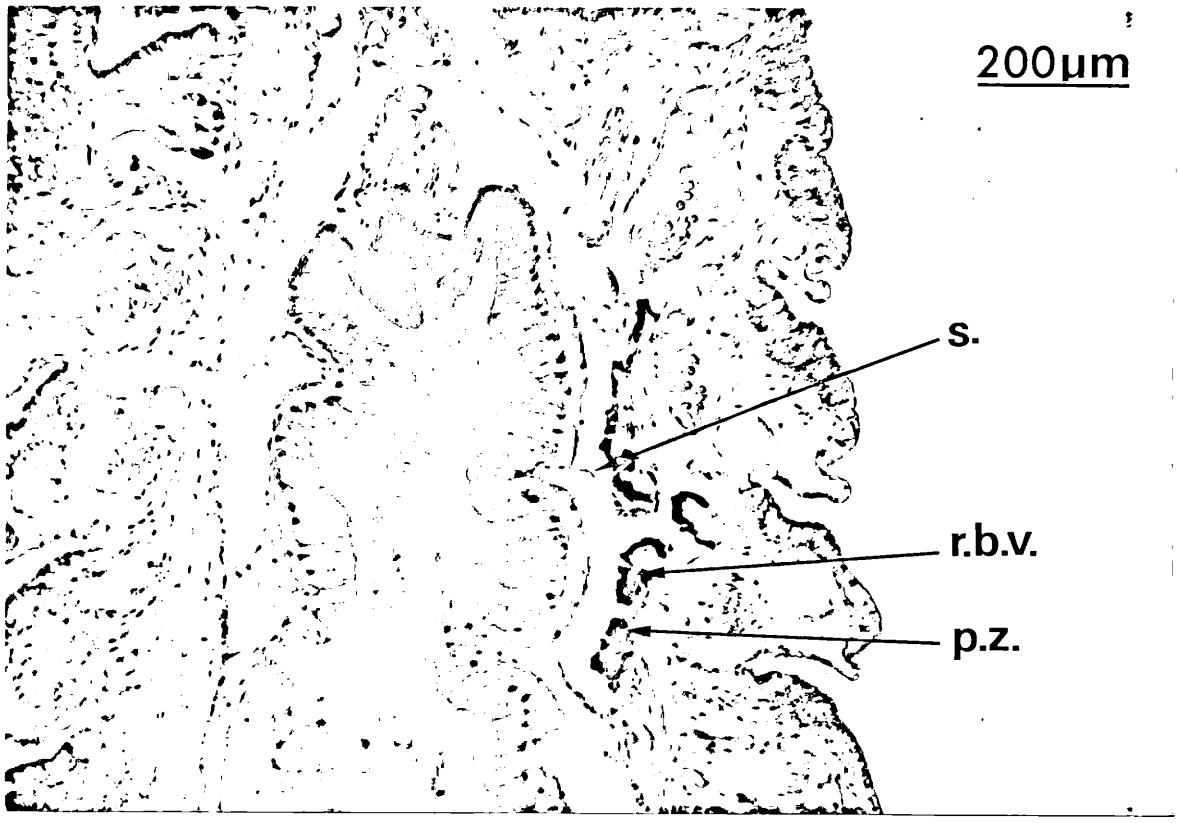
8.2.1 Introduction

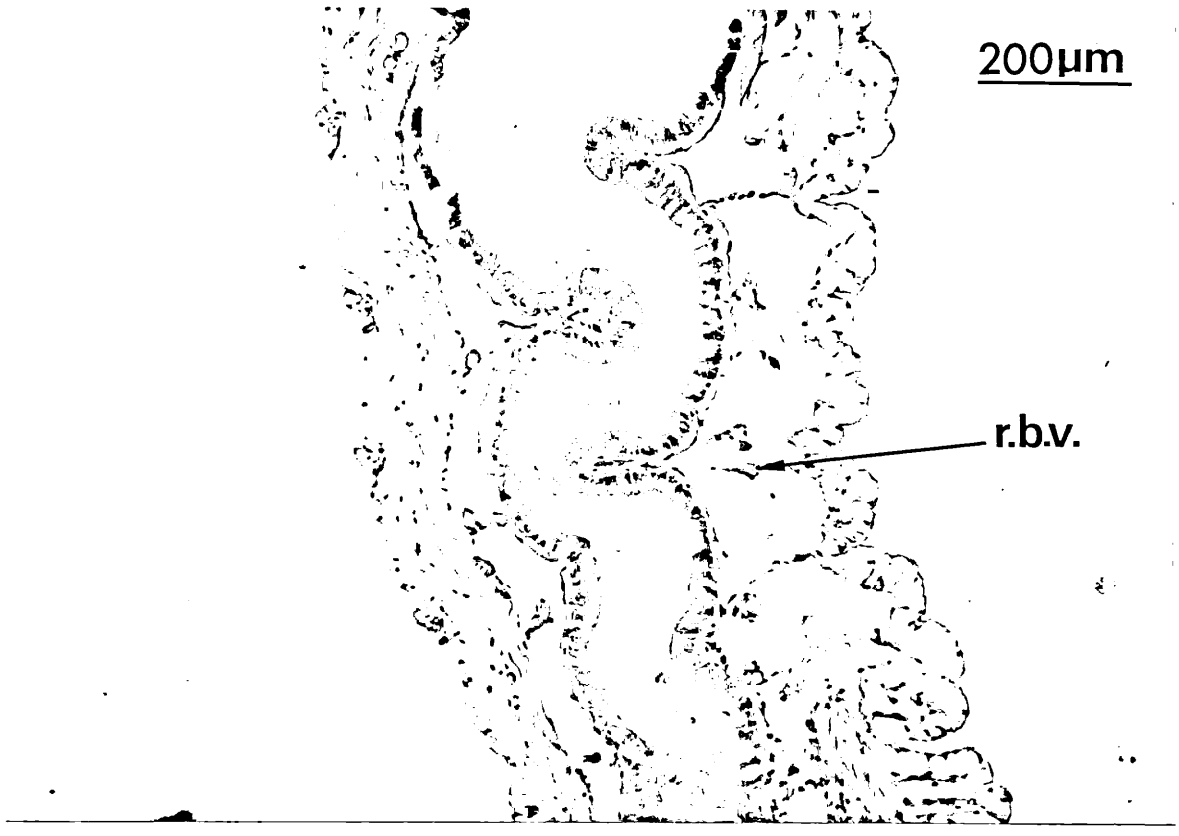
Since the adult worm is sedentary, Mercierella depends largely for dispersal and entirely for colonisation on the planktonic larva. Despite the enormous potential wastage which is inherent in this reproductive regimen, the worm is extremely prolific under favourable conditions, producing large colonies (see Table 32 column 2) within a very few months of settlement (e.g. Tebble, 1953).

The results of an histological investigation of the cellular changes in the gonadal tissues and coelom, which occur during gametogenesis are presented. It was found that the colour of the abdomen is indicative of reproductive condition. The annual reproductive cycle at Greenhithe was investigated by means of monthly samples, and is discussed with regard to the phenomenon of protandrous hermaphroditism. Finally, the energetic cost of reproduction for the Greenhithe population was determined using microbomb calorimetry, and the relationships between reproductive effort and somatic production in temperate and tropical populations are compared.

Fig. 67. Median horizontal section through thoracic segments 1 - 5 of a young worm with a total body length of 4.9 mm, fixed in March, showing the proliferative zones (gonadial tissue), p.z., associated with the ring blood vessels, r.b.v., in the posterior septa, s., of segments 3 and 4.

Fig. 68. Horizontal section through abdominal segments 8 - 17 of the same animal, showing the undeveloped proliferative zones, which contrast with the active gonadial tissue in the thoracic region (see above).


200µm
s.
r.b.v.
p.z.


200µm
r.b.v.

8.2.2 Materials and Methods

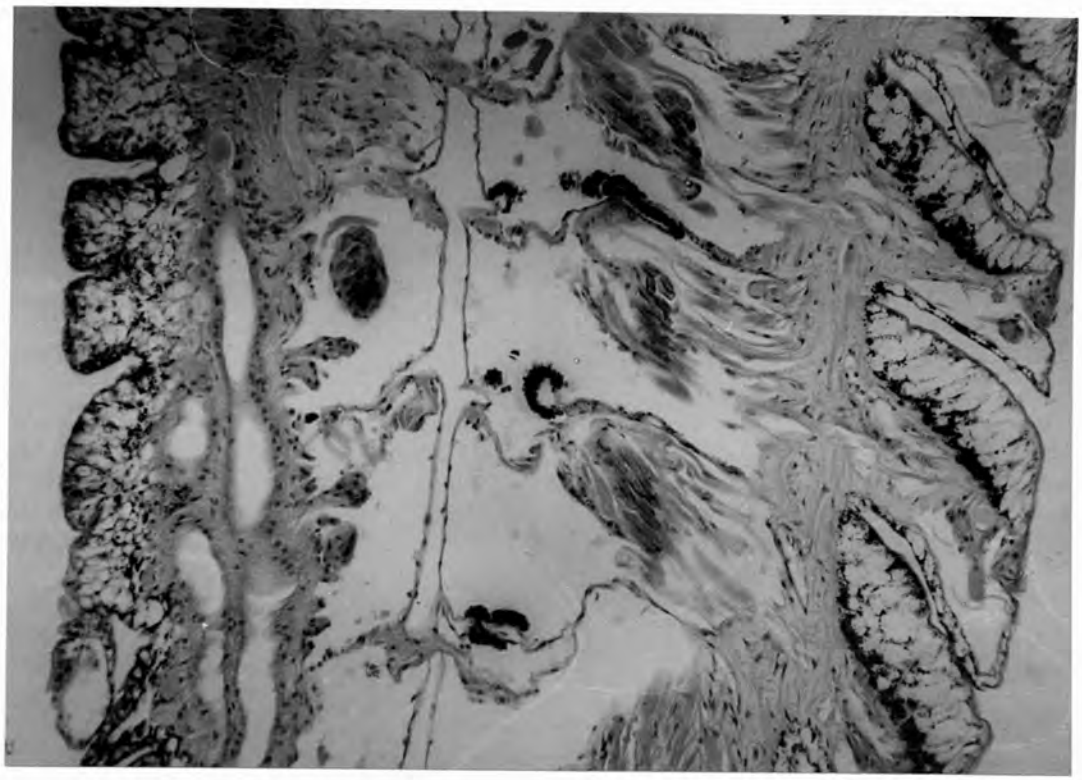
The Mercierella used in the reproduction studies were collected at Greenhithe during 1974 and 1975, and a single collection was made at Weymouth Harbour (Fig. 5) on the 4th October 1974. In the laboratory the worms were maintained in six-l containers of artificially aerated diluted sea water at salinities and temperatures similar to those in the field at the times of collection. Greenhithe worms were fed daily with a suspension of Brachiomonas submarina, and the Weymouth animals on Phaeodactylum tricornutum. Worms were removed from their tubes by breaking open the anterior end with a pair of stout forceps, and then gently pushing the animal out of the tube in a posterior direction with a blunt seeker, which was applied to the middle of the operculum. Using this method the majority of worms were extracted in an undamaged condition.

Smears made from the coelomic contents of Mercierella belonging to the same sample, and those collected at different times during the year, showed that the colour of the abdomen, when viewed with over-head illumination, is a useful indicator of reproductive condition, thus enabling the animals to be separated into reproductive classes.

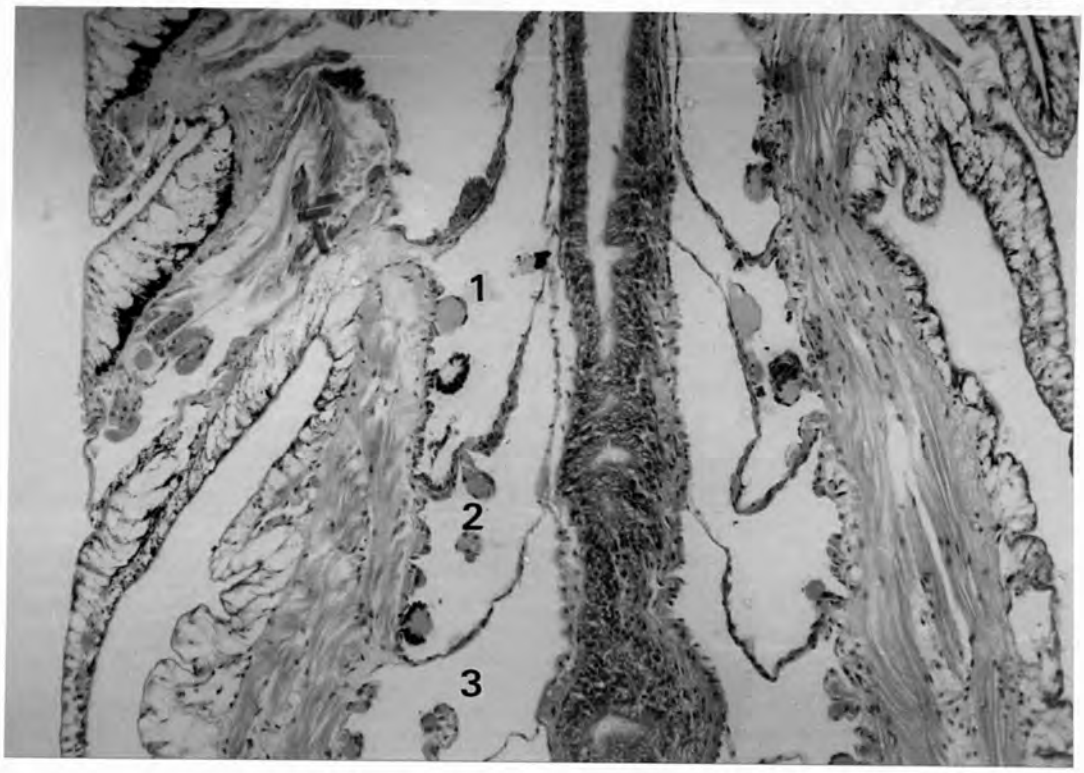
Several worms from each reproductive class were prepared for histological investigation, after they had been fixed in 70% alcohol (Section 6.3.2). Longitudinal serial sections were cut of whole animals, at a thickness of 7 μm, and the sections were stained with haemotoxylin and eosin. Measurements were made of the maximum diameters of representative cells in the proliferative tissues, and gametes free in the coelom, with a calibrated graticule eyepiece.

Fig. 69. Horizontal section through thoracic segments 2 - 6 of a male M. enigmatica fixed in March, with a total body length of 10.4 mm. The proliferative zones contain spermatogonia, 2 µm diameter, and spermatocytes, 3 µm diameter.

Fig. 70. Horizontal section through the junction of the thorax and abdomen of the same specimen, showing the early stages of spermatogenesis, in the first three segments, 1, 2, 3, of the abdomen.



200µm



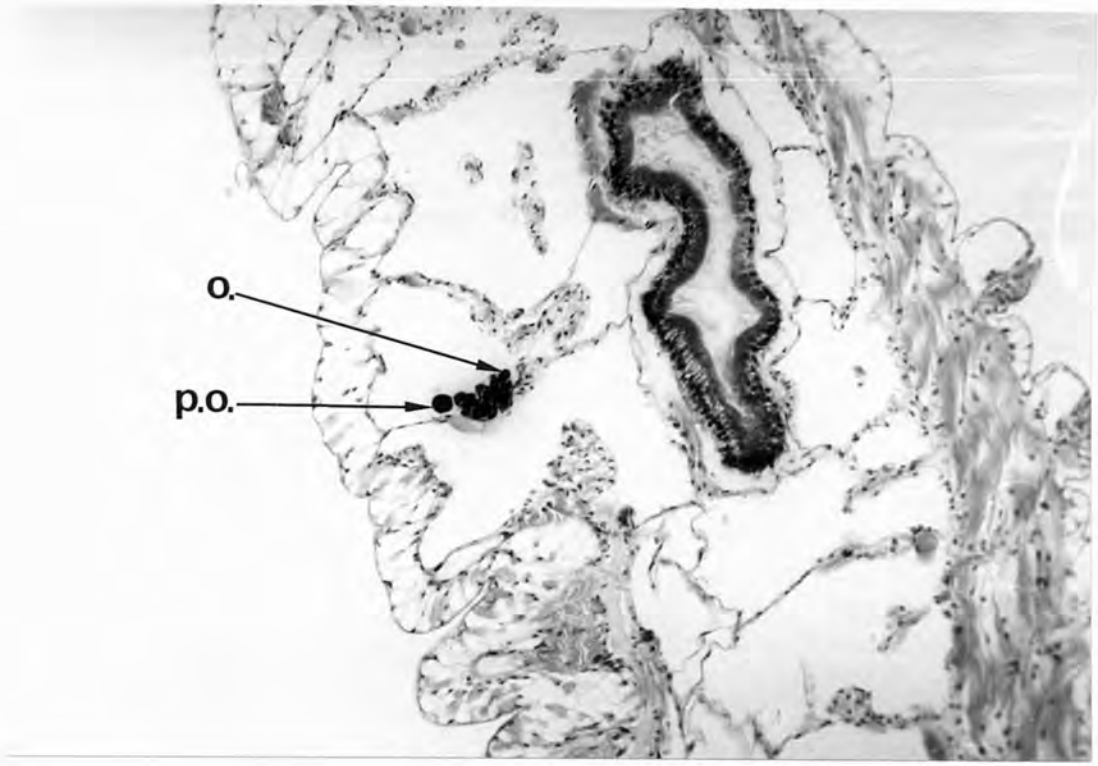
Twelve monthly samples were collected from the pools of standing water at c. the mid-tide level on the shore at Greenhithe, in 1975. Within 3 days of collection, the worms were assigned to one of six classes on the basis of their reproductive condition, as determined by coelomic smears and/or their abdomen colour, and body size.

The sexually mature Mercierella used in the energetics investigation were collected in mid July 1975, and are the same animals as were used for part of the somatic production investigation (Section 8.1), and some of the details concerning method are presented in Section 8.1.2. The gametes from ten males and nine females were collected, taking care to recover any that were released during detubing, and air dried onto individual, tared, aluminium foil trays. After several rinses with glass-distilled water, to reduce salt contamination, the eggs and sperm were dried to constant weight at 60°C, and then weighed to the nearest 10 µg on a Cahn 4100 Electrobalance. The somas were given two 10 s rinses in glass-distilled water, and then dried to constant weight at 60°C on individual, tared, foil trays, before weighing to the nearest 0.1 mg on a Sartorius semimicro balance.

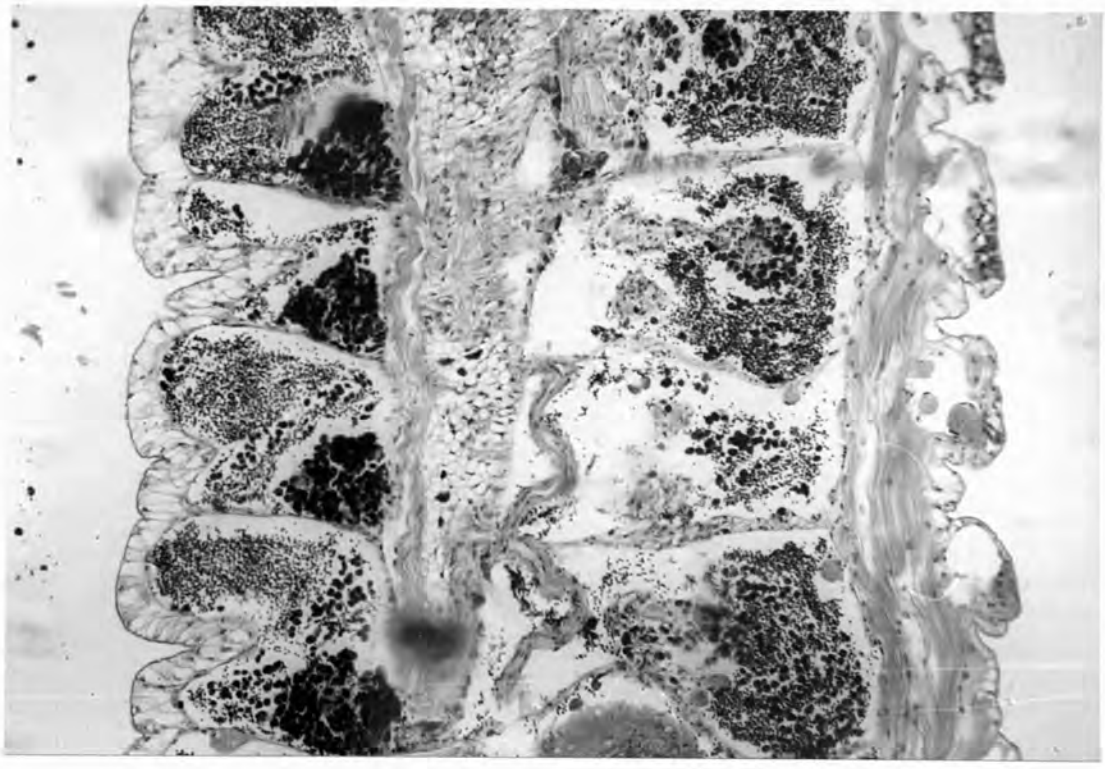
Since a preliminary investigation showed that a high salt content makes samples difficult to ignite in the calorimeter, gametes were first dialysed in Visking tubing against distilled water for 24 h. Three pellets (3.4 - 4.7 mg dry weight) each of dialysed eggs and spermatozoa were ignited in the calorimeter, and their ash contents determined by oxidising pre-weighed samples in a muffle furnace at 500°C, for 4 h (Paine, 1964). Although dialysis improved combustion, the eggs still failed to combust completely so the measurements made with the calorimeter were corrected for the amounts of uncombusted but combustible material, which were calculated from the weights lost by

Fig. 71. Oblique longitudinal section through part of the abdomen of an adult female worm (11 mm long) undergoing the early stages of oogenesis. The proliferarive zone contains oogonia, o., and larger oocytes. A primary oocyte, p.o., is on the point of being released into the coelomic cavity. Animal fixed in April.

Fig. 72. Horizontal section through abdominal segments 13 - 16 of an adult male (7.2 mm total body length), showing all stages of spermatogenesis. The proliferative zones are extremely active, containing spermatogonia and primary spermatocytes, and the coelomic cavity is filled with spermatocytes, quartets of spermatids, and differentiated spermatozoa. Animal fixed in April.



100 µm



weighed samples of residue in the muffle furnace.

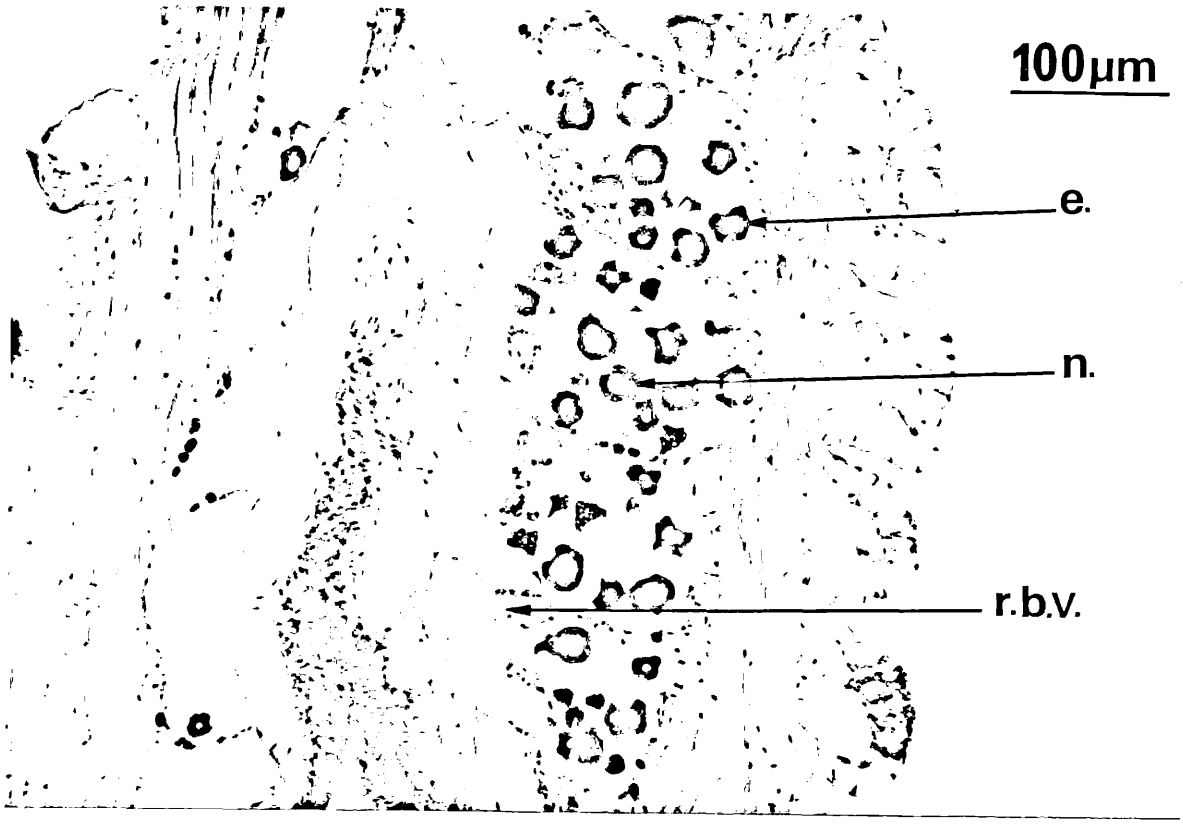
8.2.3 Results

Fig. 67 shows a median horizontal section through thoracic segments 1 - 5 of a young Mercierella which had a total body length of 4.9 mm when fixed in March. The proliferative zones (gonadal tissue) are associated with the ring blood vessels in the posterior septa of segments 3 and 4. A horizontal section through abdominal segments 8 - 17 of the same animal is presented in Fig. 68. Whereas cells in the proliferative zones of thoracic segments 3 and 4 are undergoing early stages of gametogenesis, and have individual cell diameters ranging from 3 - 6 μm, the gonadal tissues in the abdomen are undeveloped.

A horizontal section through thoracic segments 2 - 6 of an adult male Mercierella fixed in March, with a total body length of 10.4 mm, is shown in Fig. 69. The proliferative zones contain numbers of small cells with diameters of 2 and 3 μm, the spermatogonia and spermatocytes, respectively. Newly released (into the coelom) primary spermatocytes are roughly 4 μm in diameter, about twice the size of a spermatogonium. Fig. 70 shows the beginnings of spermatogenesis in the anterior three abdominal segments of the same specimen.

Fig. 71 shows an oblique longitudinal section through part of the abdomen of an adult female worm (11 mm long) undergoing the early stages of oogenesis. The proliferative zone in segment 15 contains oogonia (c. 8 μm in diameter) and oocytes (c. 12 μm). A primary oocyte is on the point of being released into the coelomic cavity.

Fig. 73. Horizontal section through abdominal segments 14 - 18 of a sexually mature female worm (total body length 7.1 mm), fixed in June. The coelomic cavity contains ripe oocytes, e., which have a large nucleus, n., with a prominent nucleolus. The eggs swell and lose the indentations, shortly after they are released from the parent's body. Note the lack of proliferative zone activity; r.b.v., ring blood vessel.


100µm
e.
n.
r.b.v.

A horizontal section through abdominal segments 13 - 16 of an adult male (7.2 mm total body length), fixed in April, showing all stages of spermatogenesis, is presented in Fig. 72. The gonadal tissues are extremely active and contain numerous spermatogonia and primary spermatocytes. The coelomic cavity is filled with spermatocytes, quartets of spermatids (3 - 5 μm in diameter), and differentiated spermatozoa (1.5 - 2 μm head length). During removal from its tube, this animal released a considerable quantity of motile spermatozoa from the openings of the gonoducts, on the ventral surface of the abdomen.

Fig. 73 shows a horizontal section through abdominal segments 14 - 18 of a sexually mature female Mercierella (total body length, 7.1 mm), fixed in June, which released ripe eggs when it was detubed. Its coelomic cavity contains ripe oocytes (c. 40 μm in diameter), each of which has a large nucleus with a prominent nucleolus. The oocytes are irregular in outline whilst they are in the worm's body. This observation has been made previously by Fischer-Piette (1937), and Rullier (1955). There are no signs of proliferative zone activity in mature animals.

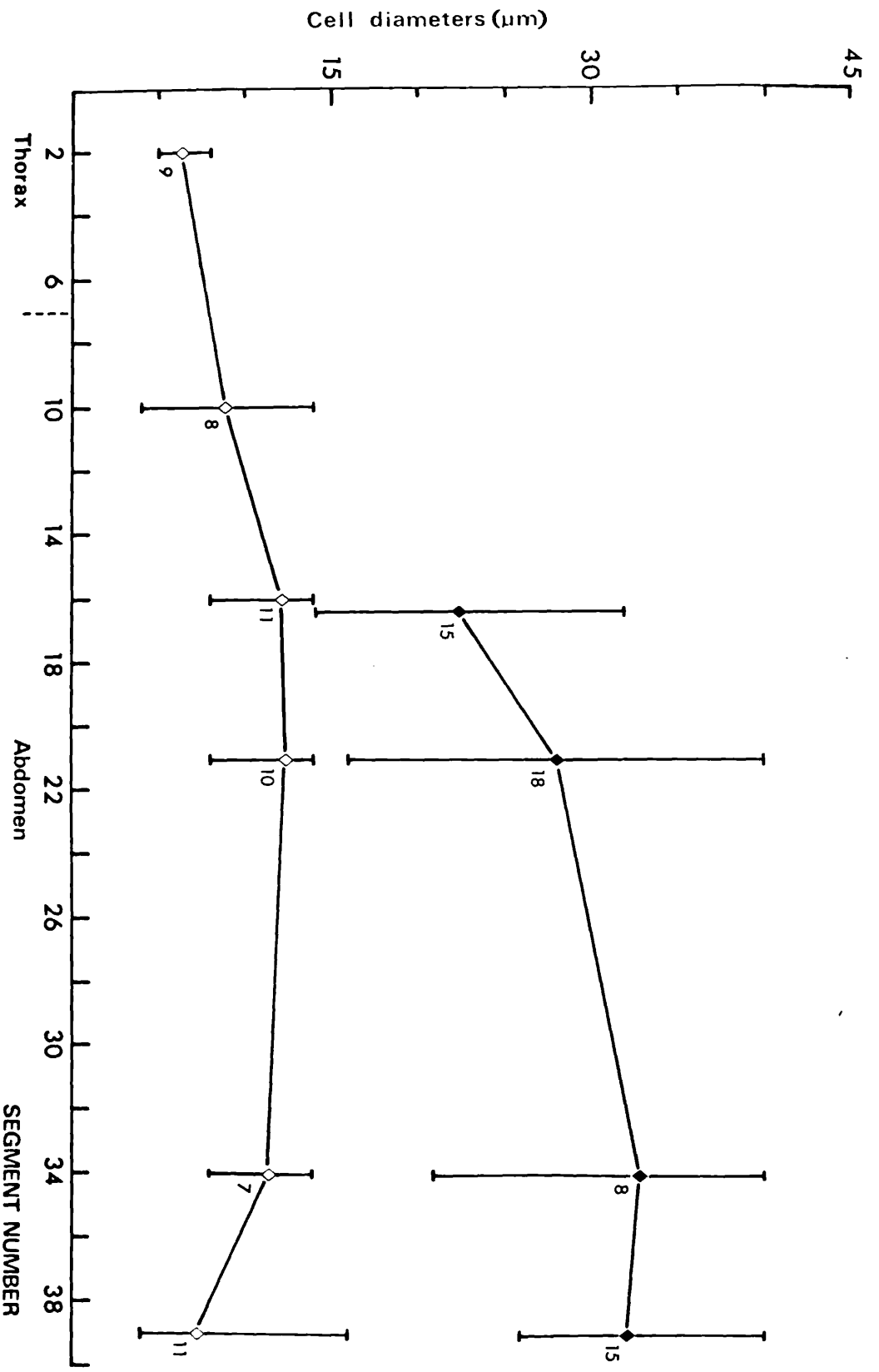
Fig. 74 and 75 show oblique sections through the posterior region of the abdomen of an adult Mercierella (total body length 8.3 mm), fixed in March, which released motile spermatozoa when removed from its tube. The coelomic cavity is filled with spermatids associated in quartets and pairs, individual spermatids, and differentiated spermatozoa. In contrast, however, to the male worm shown in Fig. 72, the proliferative zones contain relatively large cells, ranging from 8 - 14 μm in diameter, which indicate that this individual is a protandrous hermaphrodite

Fig. 74. Oblique section through the posterior region of the abdomen of a protandrous hermaphrodite, of total body length 8.3 mm, fixed in March. Cells in the proliferative zone are undergoing oogenesis, whereas the coelomic cavity is filled with spermatids and spermatozoa.

Fig. 75. Oblique section through the posterior region of the abdomen of the same protandrous hermaphrodite, showing a small group of developing oocytes, e., free in the coelomic cavity.

100 µm

Fig. 76. Mean diameters and extreme size ranges of cells in the proliferative zones, and free in the coelomic cavity, at intervals along the body of a female M. enigmatica to segment 40. Total body length 10.06 mm, and fixed in April. The numbers in parentheses refer to the number of measurements on which the adjacent mean is based.


Cell diameters (µm)
15
30
45
Thorax
Abdomen
SEGMENT NUMBER
2
6
10
14
18
22
26
30
34
38
9
8
11
10
7
11
15
18
8
15

undergoing oogenesis. Developing oocytes (15 - 16 μm) are visible in Fig. 75.

The individual segments of Mercierella that are undergoing gametogenesis do not develop simultaneously; the posterior segments are usually at a more advanced stage of maturity. Fig. 76 shows mean diameters and the extreme size ranges of cells in the proliferative zones, and free in the coelomic cavity, at intervals along the body to segment 40 of a female worm (total body length 10.06 mm) fixed in April. Primordial germ cells are present in the proliferative zones in the thorax, but oocyte production is restricted to the abdominal region. Free developing oocytes are present posterior to abdominal segment 15. The low mean cell diameter in the most posterior proliferative zones is probably due to a reduction in activity as sexual maturity is approached.

Table 33 is a summary of the results of the investigations concerning the relationship between abdomen colour and reproductive condition. The epidermal cells contain a green pigment, which together with the blood pigment in the peripheral vessels (see Fig. 57), gives a green colour to juveniles and recently spawned adults.

At the onset of gametogenesis the reproductive cells become visible through the wall of the abdomen, which appears green-orange. The oocytes contain a rich orange pigment, but the reproductive cells of the male are creamy-white, making the resultant green-orange colour more difficult to explain, except as possibly a light-scattering effect. However, since a proportion of the animals which contained spermatozoa

Table 33. Relationship between abdomen colour and reproductive condition of male and female M. enigmatica.

Male worms			Female worms		
Abdomen colour	Stage of gametogenesis	Proliferative zone activity	Abdomen colour	Stage of gametogenesis	Proliferative zone activity
Green	juvenile	–	Green	juvenile	–
Green-orange	spermatogenesis (free cells)	+	Green-orange	oogenesis (no free cells)	+
Orange	spermatozoa	+	Orange	oocytes	+
Olive green	spermatozoa	–	Cream-orange	oocytes	–
Green	post-spawning	–	Green	post-spawning	–

were protandrous hermaphrodites, in some cases the orange component was due to the presence of female gametes.

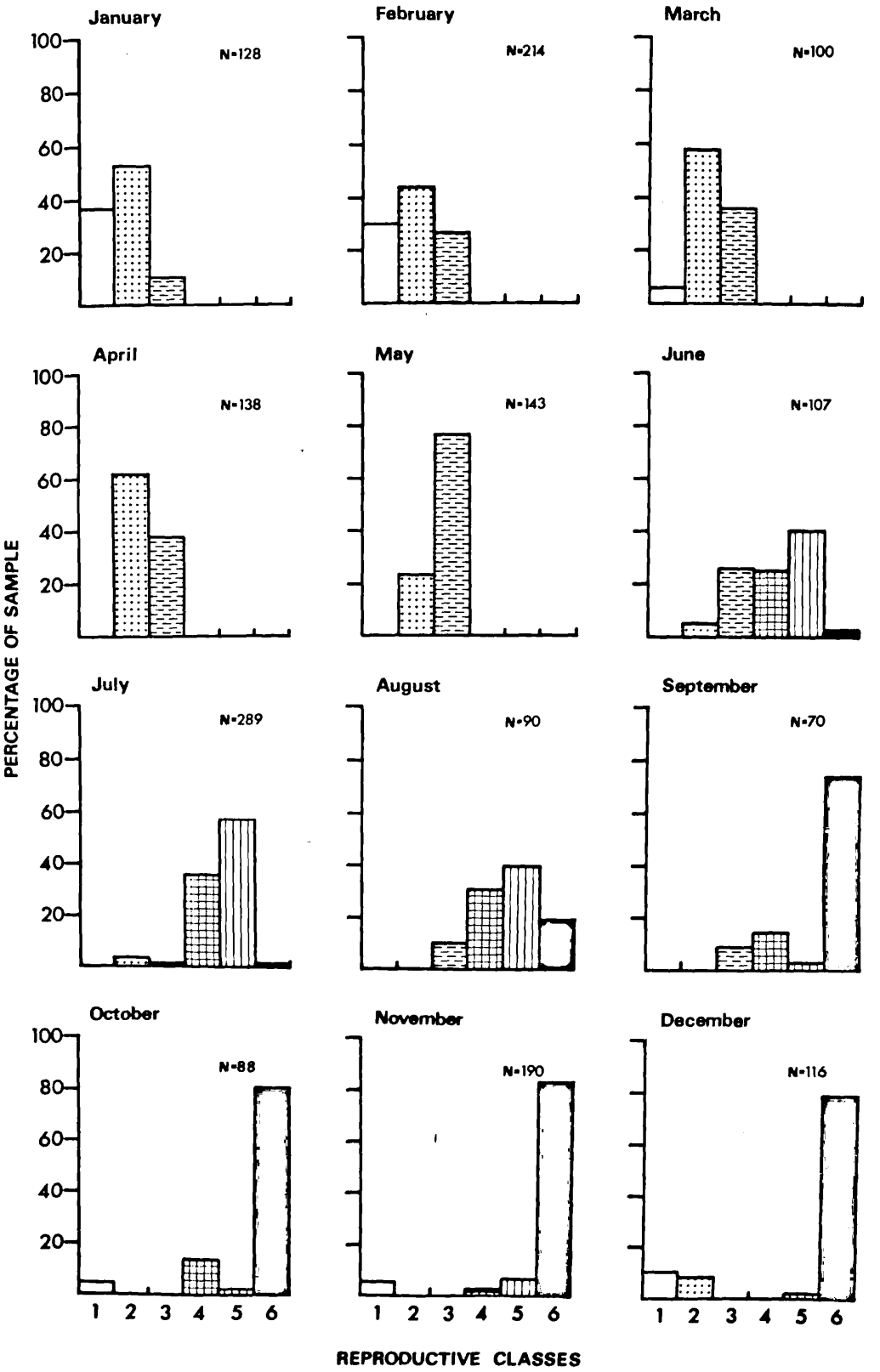
At maturity, males are olive green and females a cream-orange colour. Following spawning the gonadal tissues undergo a short resting phase during which the spent adults resemble juvenile worms, except for their larger size.

The data from monthly sampling of the population in 1975 are presented in Fig. 77, as a series of percentage-frequency histograms based on six reproductive classes. The reproductive classes are: 1) juveniles; 2) worms at early stages of gametogenesis; 3) immature animals containing spermatozoa and/or free oocytes; 4) mature males; 5) mature females; and 6) post-spawning individuals.

In January 37% of the population was comprised of juveniles (< 0.4 mg dry weight), and adult worms were already undergoing gametogenesis, a few of which released motile spermatozoa when removed from their tubes. By February some of the juveniles had commenced gametogenesis; by March only 6% of the population were in class 1; and by May the majority, 77%, contained free gametes.

A small percentage had spawned by the middle of June, but this proportion remained unchanged in July, when the largest proportion of mature females (56%) was recorded. Spawning had begun by the 11th of August and continued through September. A greater proportion of females than males had spawned by the 25th of September, and some males continued to release spermatozoa through October, when the first of the year's settlement was taking place. A few females released eggs in December, when some of the adult animals had recommenced gametogenesis.

Fig. 77. Annual reproductive cycle of M. enigmatica at Greenhithe, (1975), shown as a series of monthly percentage-frequency histograms based on six reproductive classes. These are: 1) juveniles; 2) worms at early stages of gametogenesis; 3) immature animals containing spermatozoa and/or free oocytes; 4) mature males; 5) mature females; and 6) post-spawning individuals. N values refer to the total number of animals in each sample.


January
N=128
February
N=214
March
N=100
April
N=138
May
N=143
June
N=107
July
N=289
August
N=90
September
N=70
October
N=88
November
N=190
December
N=116
100
80
60
40
20
1 2 3 4 5 6
PERCENTAGE OF SAMPLE
REPRODUCTIVE CLASSES

A considerable proportion of the late spawning individuals were of a small size and probably represented at least part of the previous year's settlement; some large animals still contained ripe gametes in late November.

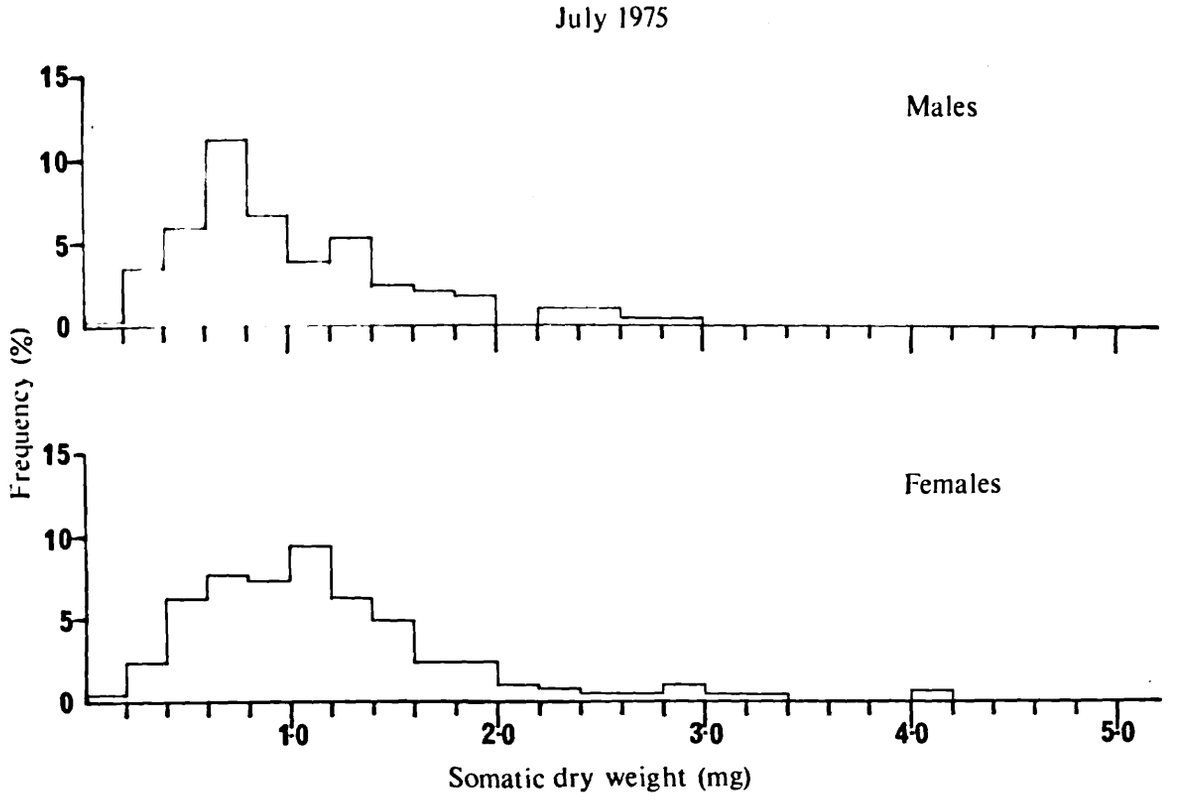
Examination of the coelomic contents of worms that were not fully mature in July showed them to be males; an identical observation was made by Fischer-Piette (1937). This enabled the somatic dry weight-frequency distribution for July (Fig. 63) to be presented as separate histograms for male and female animals (Fig. 78). In July 1975, the percentage of males in the Greenhithe population was 46%.

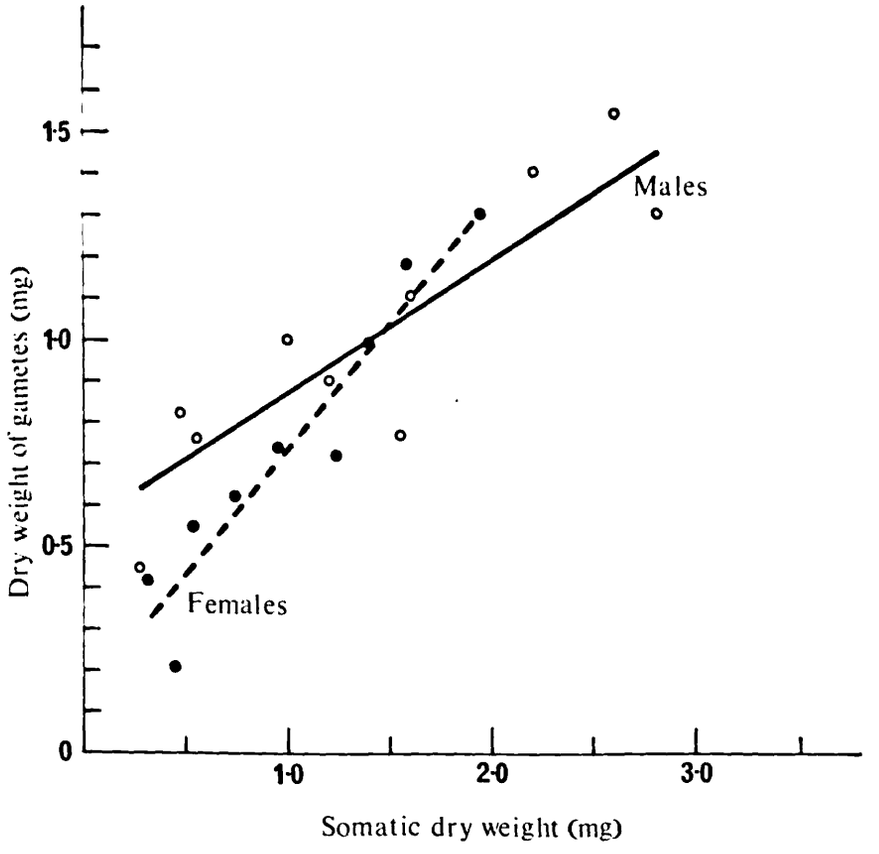
Fig. 79 shows the relationships between somatic dry weight and the dry weight of gametes of sexually mature male and female Mercierella. Regression lines were fitted by the method of least squares, after the correlation coefficients were found to be significant at the $P = 0.05$ level of significance. A better correlation ($r = 0.95$) exists between the two parameters in female worms, than for males ($r = 0.89$), which is partly due to the relative ease with which the eggs were manipulated owing to their relatively large size and orange colour. The regression coefficients were compared using a t-test for small samples (Bailey, 1959), and the calculated value for t is significant at the $P = 0.05$ level ($P = < 0.02$) of significance.

Three pellets for each of dialysed eggs and sperm had calorific values of 18.82 ± 1.09 J mg^{-1} dry weight (4.5 ± 0.26 cal mg^{-1} dry wt.), and 15.89 ± 0.1 J mg^{-1} dry weight (3.8 ± 0.025 cal mg^{-1} dry wt.) respectively. After a F-test had shown that thereis a significant difference in the variances about the means ($P = < 0.05$), these were compared using a modified t-test, similar to that described by Bailey (1959) for small samples. There is a significant difference between

Fig. 78. Percentage weight-frequency distributions of male and female M. enigmatica in a sample of 289 worms, collected immediately before the breeding season (July 1975).

Fig. 79. Relationship between somatic dry weight and dry weight of gametes of male (open circles) and female (closed circles) M. enigmatica, collected immediately before the breeding season (July 1975). The regression lines were fitted by the method of least squares. $\hat{Y}_{males} = 0.54 + 0.33X$; $\hat{Y}_{females} = 0.14 + 0.6X$.


July 1975
Males
Females
Frequency (%)
15
10
5
0
1·0
2·0
3·0
4·0
5·0
Somatic dry weight (mg)


Males
Females
Dry weight of gametes (mg)
1·5
1·0
0·5
0
1·0
2·0
3·0
Somatic dry weight (mg)

the calorific contents of eggs and sperm ($\underline{P} =< 0.05$). The average ash contents of eggs and sperm each based on three samples were respectively 31.12 $\pm$ 0.85 (S.D.) %, and 12.52 $\pm$ 1.8% of the dialysed dry weight. Thus the average energy contents of eggs and sperms mg^{-1} ash-free dry weight are respectively 27.32 J mg^{-1} (6.53 cal mg^{-1}) and 18.16 J mg^{-1} (4.34 cal mg^{-1}).

8.2.4 Discussion

Fauvel's (1922) original description of Mercierella enigmatica makes no reference to the colour of the abdomen in living worms. Monro (1924) reported the Royal Albert Dock specimens (Section 2.2) as having sage green abdomens, which is in accordance with the findings of the present study, since his worms had recently settled at the time of collection, 28th September 1923 (McIntosh, 1926). Mercierella is not unique in this respect since colour dimorphism resulting from the genital products being visible through the abdominal wall has been described in several other serpulids, e.g. Neopomatus uschakovi as M. enigmatica (Hill, 1967); and Pomatoceros triqueter (Thomas, 1940). In each case the ripe ova are described as being pink or orange, contrasting with the creamy-white spermatozoa.

McIntosh (1924) reported that Mercierella brooded its young in a chamber formed by the operculum, a characteristic of some spirorbids, e.g. Janua (Janua) pagenstecheri (Quatrefages), see Knight-Jones and Knight-Jones, (1977) for the most recent description. McIntosh's conclusion was based on a cursory observation of small spherical bodies within the opercula of preserved specimens, which he incorrectly identified as being ova at an early stage in development. Fauvel (1925) showed, however, by measurement and staining properties, that these were not ova, and suggested that they were formed of mucus. This prompted

McIntosh (1926) to re-examine his specimens, and he correctly identified these as being spherules of blood that are produced by the fixation process.

Fauvel (1925) described ripe ova adhering to the mucous secretions on the external surfaces of female Mercierella, and Fischer-Piette (1937) reported that the early developmental stages took place within the tube of the parent worm; the larvae leaving its protection at a ciliated stage. Although this method of brood protection occurs in the Filograninae (Nelson-Smith, 1967), contrary to earlier reports it certainly does not exist in Mercierella, which in common with the majority of other serpulids employs external fertilisation, after the gametes have been shed into the surrounding water. I have commonly observed undisturbed female worms ejecting ripe ova in the crown rejection current, within a few seconds of a cloud of spermatozoa from an adjacent male animal contacting their branchial filaments. This behaviour is certainly identical to the natural situation, and agrees with the observations made by Rullier (1955).

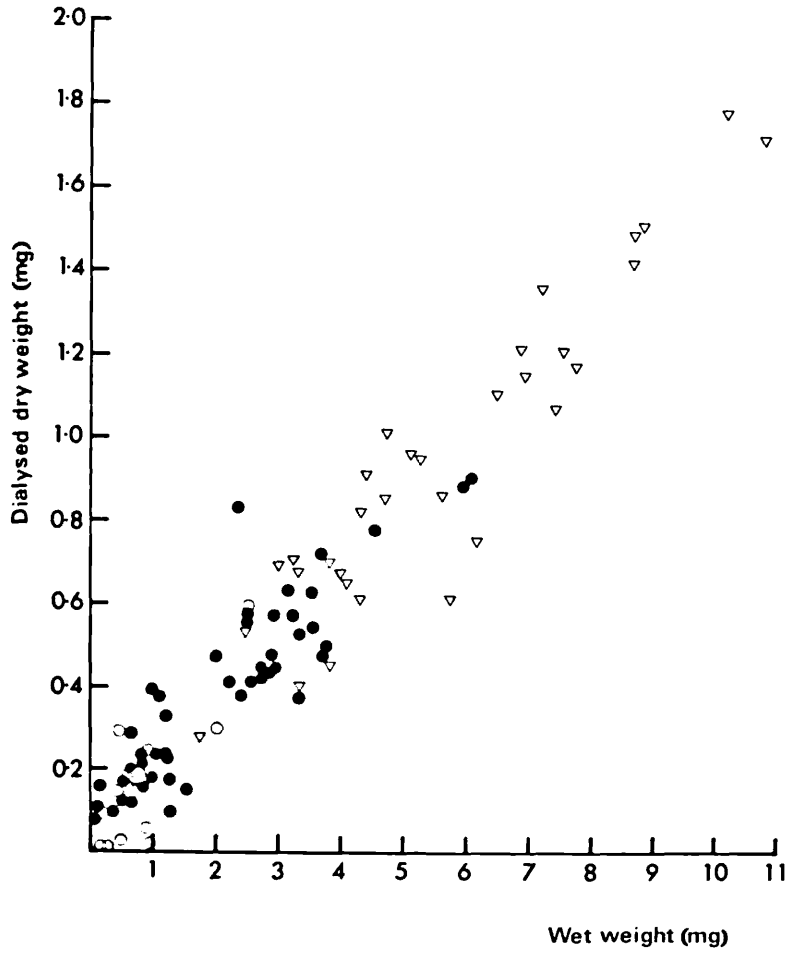
There are no proper gonads in Mercierella, instead the germ cells are produced by division of the germinal epithelia associated with the ring blood vessels in the inter-segmental septa. Rullier (1955) described the gonadal tissues as zones of proliferation, of which there is a pair in each segment apart from the first thoracic and last few abdominal segments. Di Grande and Sabelli (1972) described their structure, and the connections with the intermetameric blood vessels. Similar structures are present in other serpulids, although Mercierella appears to be unique in their septal positioning (see review by Clark & Olive, 1973).

After their release into the coelom the developing gametes derive their nourishment from the surrounding fluid. Since Mercierella has very few coelomocytes, the majority of the nutrients are probably supplied by the storage tissue surrounding the gut (Rullier, 1955).

McIntosh (1926) reported that the posterior of the abdomens of male and female worms are filled with sperms or eggs, and he went on to describe how these sometimes extend far forward, which agree with the progression of gamete maturation found in the Greenhithe animals. This phenomenon was also alluded to by Rullier (loc. cit.), but he believed that as the oocytes grew they were "directed towards the posterior part of the worm". Rullier's hypothesis requires the presence of large pores in the septa to enable the developing oocytes (10 - 40 µm in diameter) to pass between the segments, together with an effector mechanism since they (the oocytes) lack any means of independent locomotion. Whilst neither of these requisites are impossible, since mature eggs are known to pass from the coelom into the opercular brood pouches of several spirorbids, e.g. Janua (Janua) pagenstecheri (although the exact mechanism remains a mystery), the more probable explanation is that demonstrated above.

Eggs in coelomic smears from mature individuals were 40 - 50 µm in diameter, which is significantly smaller than the 60 µm reported by Rullier (loc. cit.). The reason for this discrepancy is that Rullier's measurements were made after the eggs had been shed into the surrounding water. Fischer-Piette (1937) found that the eggs swell after their release from the parent, "regardless of salinity"; Rullier (loc. cit.), and I also observed this phenomenon. Within 20 min of their liberation into water of salinity 15 °/oo at room temperature (c. 20°C), eggs lose

Fig. 80. Relationship between wet weight and dialysed dry weight of M. enigmatica from the Weymouth Harbour population, in mid-February, after c. 4.5 months in the laboratory. The different symbols refer to the reproductive condition as indicated by abdomen colour; ○, juveniles; ◐, early stages of gametogenesis; ▽, immature animals containing spermatozoa and/or free oocytes.


2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
Dialysed dry weight (mg)
1
2
3
4
5
6
7
8
9
10
11
Wet weight (mg)

the concavities which can be seen in Fig. 73. Fischer-Piette (loc. cit.) interpreted this as being an osmotic phenomenon, indicating that the coelomic fluid had a higher osmotic pressure than the surrounding medium, which contradicts Skaer's (1974a, b) findings that Mercierella is an osmo-conformer over the whole of its natural salinity range (1 - 55 °/oo). Skaer, however, made a point of discarding worms that were releasing gametes. It is evident that more work is required on this subject.

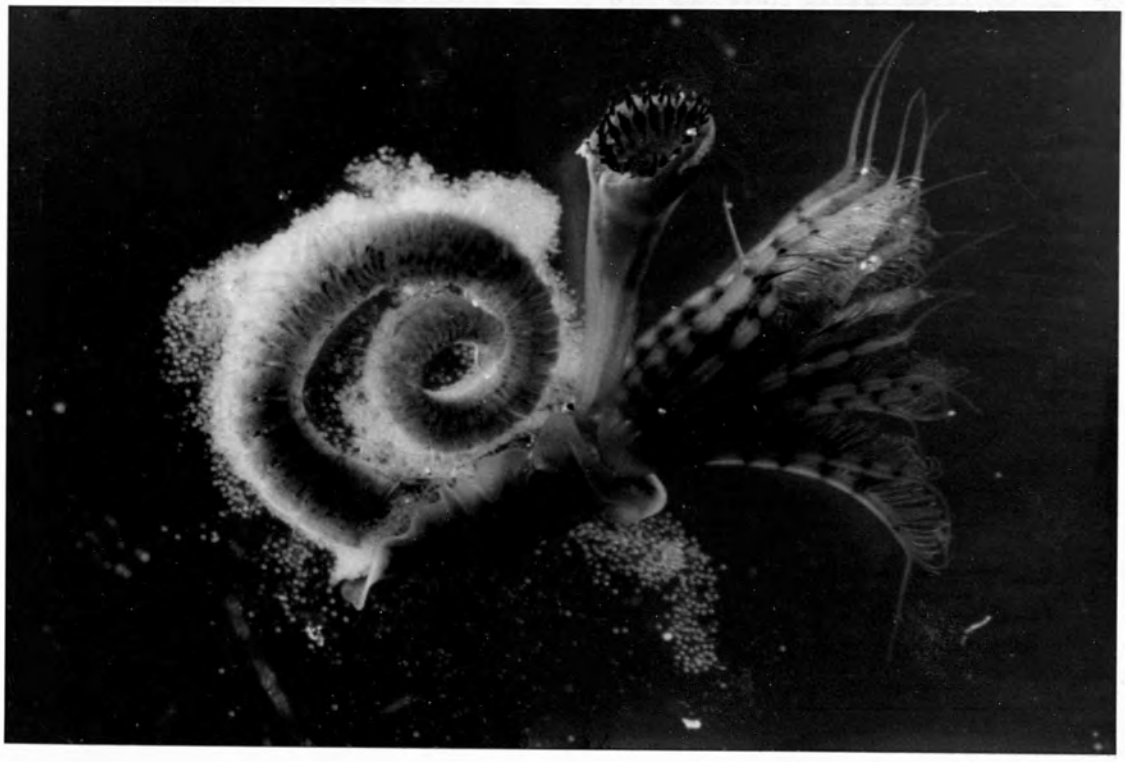
In the Thames estuary gametogenesis takes about six months (January - July). It is initiated during the period of low water temperature, c. 10°C (Fig. 25), and short day length. The young worms from the previous year's settlement do not commence gametogenesis until after those of one year old or more. Fig. 80 shows the relationship between the wet weight and dialysed dry weight of Weymouth animals, which had been maintained in a salinity of 20 °/oo, at 11°C - 12°C, in mid-February when many of the small animals had yet to commence gametogenesis. The pattern of gametogenesis in this laboratory population was similar to that of the Thames population until April, after which no further development took place. In April 1975, the water temperatures in the field began to increase fairly rapidly, which suggests that the later stages of gametogenesis require higher water temperatures.

The majority of the Thames population are sexually mature by mid-July, when the water temperature reaches 20°C, and most spawn during the following ten weeks. Mature female Mercierella release their eggs in response to contact with sperm from nearby males. This response can be elicited at all stages of the tidal cycle by introducing sperm into the feeding currents of gravid females. After a delay of about 5 s,

Fig. 81. Sexually mature male M. enigmatica releasing spermatozoa in response to removal from the tube. The dense mass of sperm on the ventral surface of the abdomen is being carried in an anterior direction by ciliary generated water currents in the ventral groove, and some can be seen leaving the centre of the branchial crown in the rejection current.

Fig. 82. Sexually mature female worm releasing eggs from the pairs of gonoducts that open on the ventral surface of the abdomen. Once the worm has been freed from the tube the branchial crown spreads open into the feeding position and the abdomen curls towards the dorsal surface.

1mm



1mm

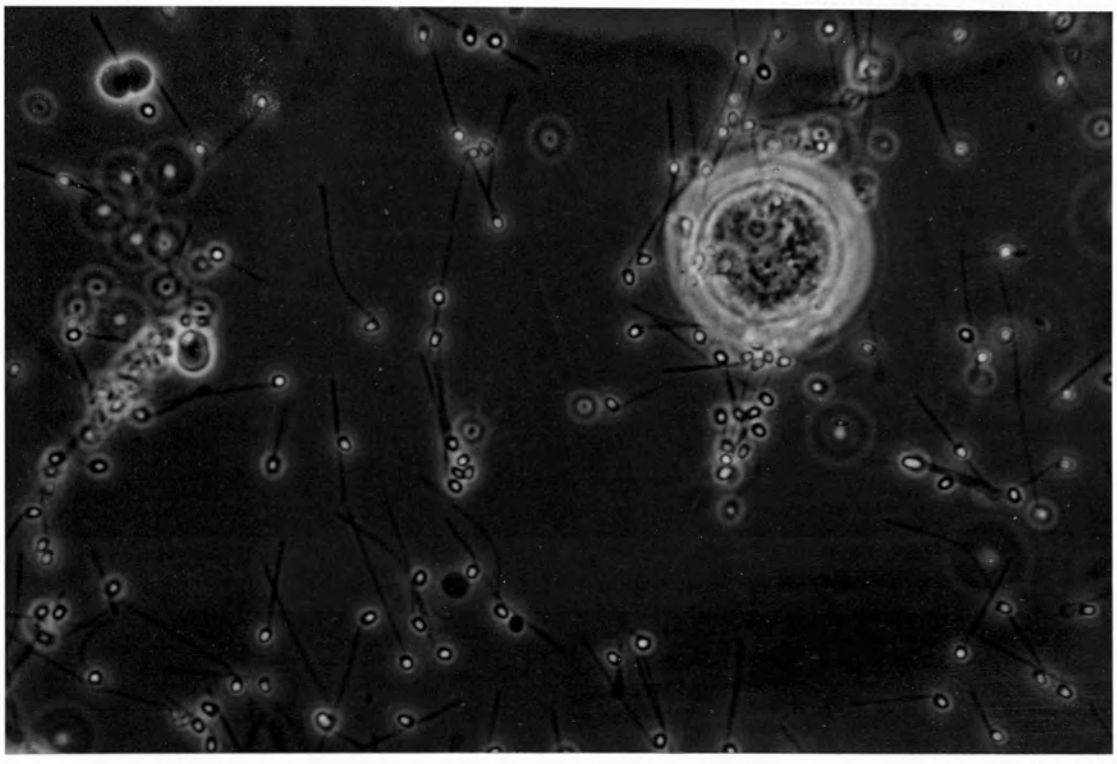
the female worm commences a series of longitudinal contractions, and the eggs are released a few at a time into the rejection current and carried passively away. Thus the exact time of gamete release is determined by factors influencing the male worms. The resultant synchronisation of spawning maximises the chances of the eggs being fertilised and thereby reproductive success. Straughan (1968) states that the eggs and sperm are shed on neap tides, supposedly because there is less water flow at this time so there is more opportunity for the eggs to be fertilised before the gametes are carried away. Figs. 81 and 82 show mature male and female Mercierella releasing their gametes after being removed from their tubes.

Gametes leave the body of the worm through the gonoducts, of which there is a pair in each abdominal segment except the last few which also lack proliferative zones. The gonoducts open on the ventral surface of the abdomen, from where the gametes are carried anteriorly in the ciliated tracts which have already been described in Section 5.1 and Section 6.1 in connection with the transport of faeces, and respiratory currents. When the eggs or sperm reach the base of the crown, their subsequent release into the rejection current is effected by a series of longitudinal body contractions, which force water out of the tube. Thus Mercierella's gametes are transported and ejected in a similar way to its faecal pellets (5.1). The whole process was observed using detubed animals, and sperms can be seen leaving the anterior of the male worm shown in Fig. 81.

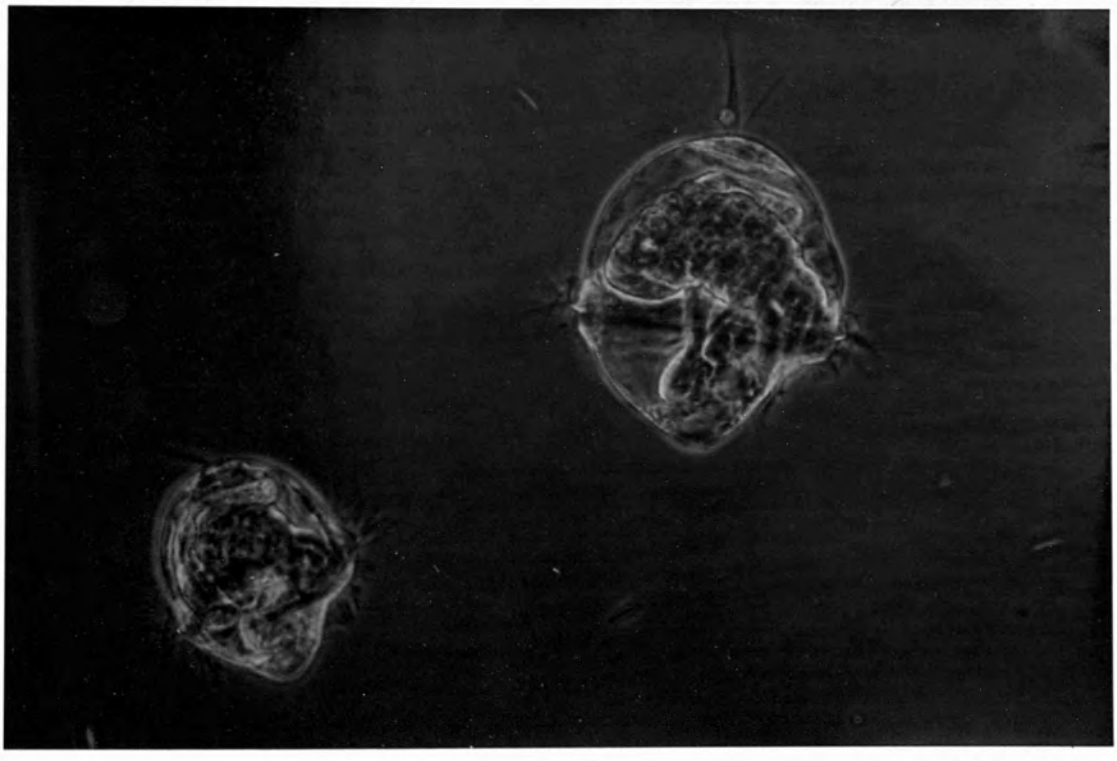
The external opening of the gonoduct (the gonopore) is surrounded by a circular muscle which acts as a sphincter. Although the release of gametes from the body is partly a passive process, since the

Fig. 83. Egg and spermatozoa of M. enigmatica shortly after their release from the parent worms. Eggs have an orange cytoplasm which contains numerous oil droplets. Several sperm heads can be seen in contact with the surface of the egg, whose rapidly vibrating tails cause it to rotate.

Fig. 84. Lateral view of three-day, ciliated, trochophore larvae, that were raised in the laboratory after artificial fertilisation, showing the pigmented eye spot and developing gut.



20 µm



100 µm

abdomens of ripe individuals are considerably distended by the large quantities of gametes they contain, there has to be an additional active (ciliary ?) mechanism. Since otherwise, after the sphincters relax in response to a spawning cue, the gametes would continue to be forced out through the gonoducts, by the pressure exerted by the natural elasticity of the body wall, only until the internal and external pressures have equalised. Spent individuals, however, contain very few residual gametes. In the laboratory, gametes were not all shed at any one time. Instead a stimulated female released eggs for 2 - 3 min, after which it could not be stimulated again for several hours. Tubed animals in general did not release the amounts of eggs and sperm that are typical of detubed worms.

Fertilisation occurs within a few minutes of the genital products being mixed, at 20°C. Fig. 83 shows an egg and spermatozoa shortly after their release from the parent animals. The gametes have already been described by Fischer-Piette (1937), Rullier (1955), and Straughan (1968). The sperm swim actively through the water by lashing their long tails, and on contacting an egg they become attached to the surface of the vitelline membrane. Within a few minutes the eggs are surrounded by numerous superficially attached sperm, whose combined activity causes them to rotate. Fertilisation was not observed, but the two polar bodies were visible after 1 h. Formation of the polar bodies immediately preceded the first mitotic division.

Rullier (loc. cit.) reported that the ciliated morula larvae were formed after about 8 h (no temperature quoted), whereas Straughan (1968) observed them within 3 h of fertilisation at 25°C. The rate of larval development is almost certainly directly related to temperature. At room temperature (c. 20°C) trochophore larvae were present after 24 h. Fig. 84 shows trochophores at 3 days, which have a strong positive

phototactic response. At 4 days, however, their behaviour changes and they disperse throughout the water column. The trochophore has a functional gut, and is the natural dispersal phase in Mercierella's life cycle. Several unsuccessful attempts were made to raise the larvae to maturity in the laboratory, but each time they died when they were 5 days old. The subsequent developmental stages are described by Rullier (loc. cit.), and Vuillemin (1965). Mathias and Izac (1963) reported raising the larvae to the stage at which they settled.

A minimum water temperature of 18°C appears to be essential for Mercierella's reproduction (see Hartman-Schröder, 1967; Straughan, 1972). However, in contrast to its specific temperature requirement, Mercierella is able to reproduce in a wide range of salinities (published range: 7 °/oo - hyper-saline conditions), (e.g. Fauvel, 1931; Fischer-Piette, 1937; Turpaeva, 1961; Vuillemin, 1965; Straughan, 1972). In the tropics Mercierella (= Neopomatus ?) has a much longer breeding season. Straughan (1972) found that in the Brisbane River (27 OS 152 30E) breeding continued from September to late May whilst the water temperatures were above 18°C. This suggests that in the Thames estuary, the breeding season is limited by water temperature.

Settlement commenced at Greenhithe by the 26th October 1975, and was still in progress on the 22nd November when the water temperature had fallen to 11°C. Vuillemin (1965) recorded larval settlement in water temperatures as low as 10°C in Tunisia. These results show that whereas a relatively high water temperature is required for the natural release of gametes, the later stages of development can occur at lower temperatures, a feature which enables Mercierella to breed successfully in the Thames.

The time that the worms spend in the plankton cannot be accurately determined from these results, but it ranges from a minimum of about 2 months to a maximum of 3.5 months. The time spent in the plankton has already been discussed in relation to Mercierella's distribution (Section 2.2.1). Straughan reported that settlement in the Brisbane River occurred at periods of spring tide. It is not known, however, whether a similar relationship exists in the Thames. The newly settled worms have fewer filaments in their branchial crowns (see Rullier, 1955), and in some cases the tubes have a pronounced dorsal longitudinal ridge, the so-called juvenile ridge.

The sexes are separate in the majority of serpulids, but spirorbids are hermaphrodite (Nelson-Smith, 1967). Mercierella is generally believed to be dioecious (Fauvel, 1925; Fischer-Piette, 1937; Rullier, 1955), although Straughan (1968) described the adult population as consisting of "males, females, and hermaphrodites which are at first male and later female". Straughan's observation is supported by the findings of the present investigation; in March 1975 about 3% of the Greenhithe population comprised protandrous hermaphrodites, undergoing the later stages of spermatogenesis and the earlier stages of oogenesis (see Figs. 74 and 75). However, the extensive investigation of smears made from the coelomic fluid of worms during the breeding season failed to produce any evidence of hermaphrodites at that time.

It is not clear what the relationship is between the hermaphrodites and the morphologically dioecious individuals. What is certain is that not all the young worms go through an initial male phase in their first year, which is evident from the low percentage of hermaphrodites compared with the 37% recruitment of the previous autumn (Section 8.1.4). Moreover,

several of the hermaphrodites were too large (in March) to have settled only the previous autumn. It is also evident from the weight-frequency distributions for the two sexes (Fig. 78) that there are both large male and female worms in the population during the summer. It would seem therefore, that protandrous hermaphroditism is restricted to a small percentage of the population, although it is possible that the proportion has been underestimated since only two of the monthly population samples, March and July, were examined with respect to this phenomenon.

Since there were no hermaphrodites in the population during the breeding season, it is evident that they had completed their sex change by the time that the rest were about to spawn. Bacci and Voria (1970) demonstrated that higher temperatures anticipated sex change in the eunicid, Ophryotrocha puerilis Claparède and Mecznikow. It would appear that in Mercierella also the male phase is associated with low temperatures. It would be of interest to know whether the hermaphrodites revert to the male phase when they recommence gametogenesis the following winter.

Not all of the worms which released sperms outside of the breeding season were hermaphrodites. Immature males commonly released motile sperms when they were removed from their tubes. Olive (1975) recorded "sexually mature" males in a Northumberland population of Eulalia viridis (Müller) outside of the breeding season, and he suggested that it may indicate the existence of winter breeding populations elsewhere. A more plausible explanation which is applicable to both these organisms, is that as spermatogenesis proceeds there is a steady production of ripe sperms which are normally stored until the breeding season. There are

probably quantitative differences between the sperms released prior to, and during the breeding season.

Straughan (1972) found that during the summer, Mercierella (= Neopomatus ?) in the Brisbane River reached maturity only 8 weeks after settlement, resulting in 3 - 4 generations in the 8 months that the water temperatures remained above 18°C. In contrast, there is only one generation per year in the Thames estuary. Furthermore, since the majority of the late spawners were of a small size, it is likely that the previous year's settlement contributes little to the successful reproductive output of the population in their first year. These differences between the reproductive capacities of the temperate and tropical populations explain the contrasting growth forms in these localities. It is evident that due to the ecological constraints on its reproductive ability, the Thames population represents a stressed condition, and if it were not for the heating effects of the warm water outfalls from the power stations it seems unlikely that this fouling organism could successfully reproduce itself.

In July 1975 the percentage of males in the Greenhithe population was 46%, which is higher than has been recorded for other populations. Fischer-Piette (1937) found only 28% males in the Rance and Port of St. Malo, in N. W. France (48 42N 2 2W), and the highest percentage of males recorded by Straughan (1972) for any of the Brisbane River populations is 40%.

The relationship between the calorific values of the eggs and sperm is in accordance with their biochemical composition, since the egg yolk contains a high concentration of lipid, which has a higher calorific value than either the protein or nucleic acid of the sperm. Most workers appear to have avoided measuring the energy contents of

gametes, and no other values exist for polychaete worms. Sutherland (1972) reported energy contents of 5.095 kcal g^{-1} and 6.161 kcal g^{-1} respectively for the male and female gonads of the limpet, Acmaea scabra (Gould). Since these values are based on a mixture of gonadial tissues and gametes which would result in them being higher than the value for pure male gametes, and lower than would have been obtained for pure eggs, there is good agreement between the results of this and the present investigation.

It would seem from the data presented in Fig. 79, that on average, male worms of less than 1.5 mg somatic dry weight contain a greater weight of gametes than do females of a similar size. Insufficient data are available on this relationship in larger animals. Since the developing gametes appear to obtain their nourishment from the storage tissue which surrounds the gut (Rullier, 1955), this finite energy store could be the reason for this difference between males and females, in view of the fact that eggs have a higher energy content per unit weight. Assuming similar efficiencies for energy transfer and synthesis in males and females, a smaller quantity of eggs than sperm would be produced from a fixed amount of storage tissue.

It follows, that an obvious benefit of protandry is that it is a means of decreasing the energy expended in reproduction of young individuals, which may not have had sufficient time to accumulate sufficient energy stores to support the production of a large number of energy-rich eggs. Protandry therefore has the effect of maximising the small individual's potential contribution to the population's reproductive output. However, since the male phase of the hermaphrodites in the Thames does not coincide with the normal breeding season, their

Table 34. Estimated gamete production by *M. enigmatica* m^{-2} at Greenhithe.

Weight class (mg)	Estimated production (mg)	Estimated production (cal)	Weight class (mg)	Estimated production (mg)	Estimated production (cal)
0 - 0.2	0.45	1.72	0 - 0.2	0.15	0.67
0.2 - 0.4	5.0	19.1	0.2 - 0.4	1.7	7.65
0.4 - 0.6	9.3	35.5	0.4 - 0.6	5.96	26.82
0.6 - 0.8	18.9	72.2	0.6 - 0.8	9.32	41.94
0.8 - 1.0	12.3	47.0	0.8 - 1.0	10.86	48.87
1.0 - 1.2	7.6	29.0	1.0 - 1.2	16.63	74.83
1.2 - 1.4	11.2	42.8	1.2 - 1.4	12.75	57.37
1.4 - 1.6	5.6	21.4	1.4 - 1.6	11.21	50.44
1.6 - 1.8	5.0	19.1	1.6 - 1.8	6.25	28.12
1.8 - 2.0	4.5	17.2	1.8 - 2.0	6.9	31.05
			2.0 - 2.2	3.2	14.4
2.2 - 2.4	3.0	11.46	2.2 - 2.4	2.34	10.53
2.4 - 2.6	3.1	11.84	2.4 - 2.6	1.26	5.67
2.6 - 2.8	1.1	4.2	2.6 - 2.8	1.35	6.07
2.8 - 3.0	1.1	4.2	2.8 - 3.0	4.34	19.53
			3.0 - 3.2	1.54	6.93
			3.2 - 3.4	1.63	7.34
			4.0 - 4.2	4.0	18.0
Totals	88.15	336.73		101.39	456.24

value seems to be limited to tropical populations which have extended breeding periods and several generations each year. This may have some bearing on the apparent low incidence of hermaphrodites in the Thames population. It is evident that this subject requires further investigation.

Gamete production m^{-2} in the "effective area" of Greenhithe shore (Section 2.3.3), assuming a stable population density of 220 Mercierella m^{-2}, was calculated using the median values of the individual weight classes in the somatic dry weight-frequency distributions for male and female worms, and the relationships between somatic dry weight and the dry weight of gametes for the two sexes. The weights of gametes produced by very small and large individuals were derived by extrapolation of the regression lines in Fig. 79. Values for the total gamete production by males and females in the separate weight classes are presented in Table 34.

The weight classes which made the largest contributions towards the total production of gametes were those which contained the greatest numbers of individuals. Worms of less than 2.0 mg somatic dry weight contributed 90.6% towards the total production of sperm , and 80.6% to the total egg production.

The data for each of the separate size classes were summed to obtain estimates of the energy released as gametes by male and female Mercierella m^{-2} in the summer of 1975. Since smears made from the coelomic fluid of spent individuals contained very few gametes, it is unlikely that significant amounts are absorbed and utilised as maintenance energy, as was shown to be the case in Strongylocentrotus purpuratus by Lasker and Giese (1954). Since no measurement was made

of the amount of sperms produced by hermaphrodites outside of the summer breeding season, the value of 1.41×10^3 J m^{-2} (336.73 cal m^{-2}) for reproductive production by males represents a more conservative estimate than does the 1.91×10^3 J m^{-2} (456.24 cal m^{-2}) for gamete production by females.

The total annual reproductive production of 3.32×10^3 J m^{-2} (792.97 cal m^{-2}) represents nearly half (44.2%) of the combined gamete and somatic production by the Greenhithe population. Since Mercierella employs external fertilisation and depends for its subsequent dispersal on a pelagic larva, it is evident that with such potential wastage inherent in its reproductive regimen, a large proportion of its energy has to be channelled into reproduction to ensure reproductive success.

Straughan (1972) reported that populations in the Brisbane River (27 OS 152 30E) breed more frequently (about 8 times a year) than those in more southern latitudes. Furthermore, at latitudes above 30°, both the colonies and individuals are larger than those at lower latitudes (see Table 32). Hill (1967) made a similar observation for Hydroides uncinata (Phillipi), after comparing the sizes of colonies and individual worms in the tropics (Lagos Lagoon, Nigeria; 6 20N 3 20E) with those described by Fauvel (1927) from the Atlantic. Although measurements of the quantities of gametes produced by the tropical populations are not available, it would seem that the tropical Mercierella channel relatively more energy into reproduction than somatic growth, whilst in temperate regions due to the more restricted breeding season a greater amount of energy is put into growth. Which means that by the following breeding season the animals are larger and therefore produce a greater number of gametes per individual. Thus Mercierella's reproductive system is geared

towards producing the maximum number of gametes, and its reproductive strategy appears to be determined by the environmental temperature régime.

8.2.5 The hourly cost of reproduction to standard male and female worms

For the purpose of the energy budget reproduction is treated as a summer phenomenon, although in the field population it closely approaches being a continuous variable. The average hourly costs of reproduction to male and female Mercierella weighing 7.0 mg wet weight (1.0 mg dry wt.) were obtained by dividing the average weights of sperm or eggs, given by the regression lines relating somatic dry weight and weight of gametes (Fig. 79), by 4380 h, after these had been converted to energy units. The average hourly cost of reproduction to a male is 3.16×10^{-3} J h^{-1} (7.56×10^{-4} cal h^{-1}), and 3.18×10^{-3} J h^{-1} (7.6×10^{-4} cal h^{-1}) to a female.

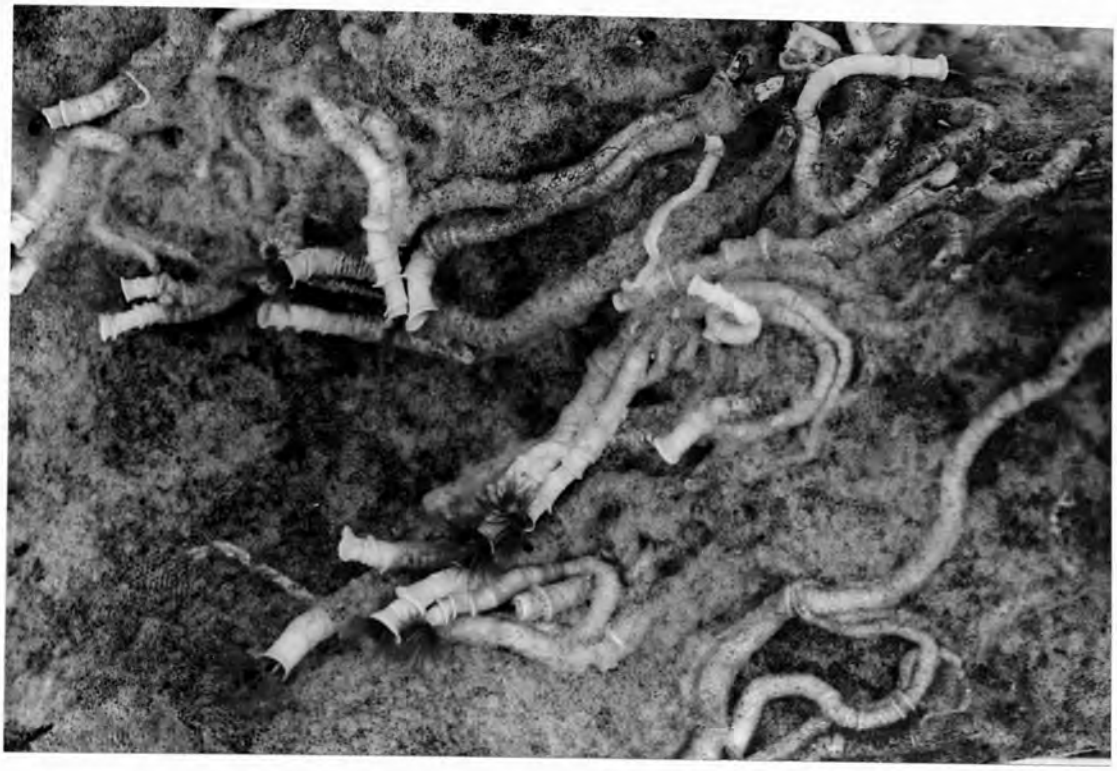
8.3 Tube production

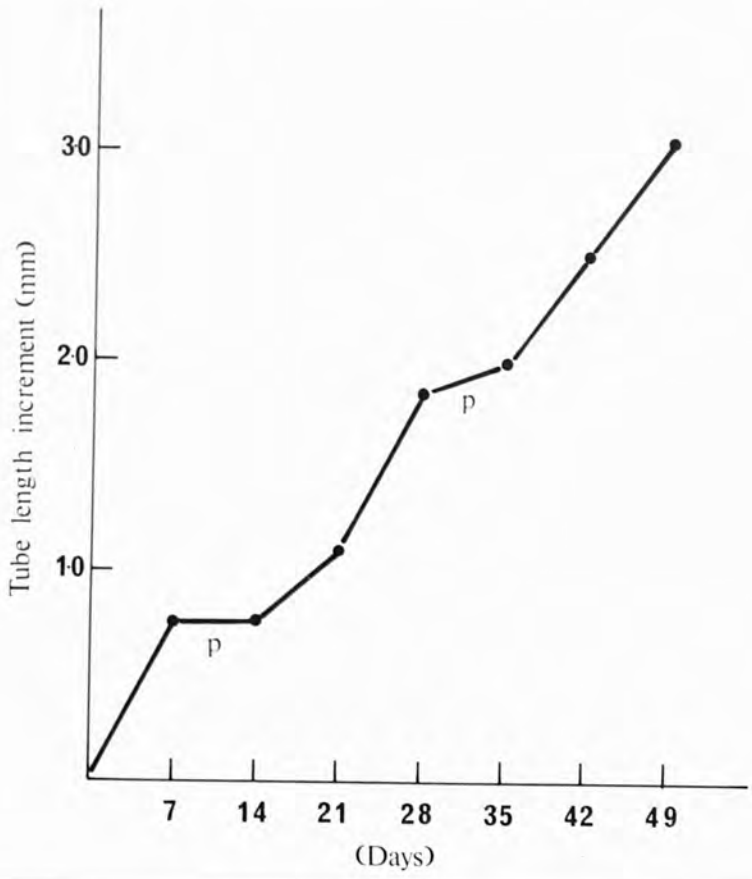
8.3.1 Introduction

Shortly after it settles from the plankton, Mercierella secretes a minute, hard, calcareous tube (Rullier, 1955; Vuillemin, 1965), to which material continues to be added throughout the worm's lifetime, as long as the environmental conditions are suitable. Initially, tube growth is relatively fast, but as the worm increases in weight there is a simultaneous decrease in the rate at which calcareous material is added to the anterior end (see Fig. 64). The tube of the adult animal can be as much as 80 mm long, with an anterior internal diameter of about 2 mm (see Hartmann-Schröder, 1967; Table 32).

Fig. 85. M. enigmatica growing on the underside of a flint boulder. Peristomes and the most recently secreted tube material can be clearly seen. The worms were photographed in water and several have their branchial crowns extended (approximately 3x life size).

Fig. 86. Weekly tube length increments of a 2.5 mg wet weight M. enigmatica maintained in aerated, diluted sea water (15 °/oo), at 10°C, and fed daily with a suspension of B. submarina cells, with a maximum density of 3.8×10^4 cells ml^{-1}. P denotes peristome formation.




3·0
2·0
1·0
Tube length increment (mm)
p
p
7
14
21
28
35
42
49
(Days)

Commonly, the tube wall extends laterally at intervals along its length to form a series of concentr ic, trumpet-like rings or peristomes (Fauvel, 1922), which give it a characteristic appearance (Fig. 85). The tubes of juvenile Mercierella however, lack peristomes, and sometimes have a dorsal, longitudinal ridge which could lead to their being mistaken for small Pomatoceros triqueter (L.), although the distributions of the two worms do not normally overlap due to different tolerances of reduced salinities (Purchon, 1948).

Peristomes are not produced under conditions of high water flow where the tubes adopt a creeping growth form (Section 2.2) contrasting with the erect colonies in situations where the rate of water flow is low (Fig. 6). Although the effect of an absence of peristomes on the streamlining of the tube, and the subsequent survival value in fast water situations are evident, the factors determining their formation are not fully understood. It has been suggested that peristomes are formed as a result of fluctuations in the feeding conditions, with a reduction in tube growth and the formation of a peristome accompanying poor feeding conditions (e.g. Hall, 1954; Mathias & Izac, 1963). However, since the formation of a peristome does not necessarily represent a change in the rate of tube secretion so much as an alteration in the direction of growth, this explanation appears to be inadequate. Peristomes continued to be produced at irregular intervals under the controlled conditions of food concentration, light, salinity, and temperature, in the growth study described in Section 8.1.2 (Fig. 86). It would seem, therefore, that the formation of peristomes is a random phenomenon with regard to their temporal appearance, which can be modified by environmental factors, e.g. the rate of water flow.

As a result of a gregarious settlement response (Straughan, 1972), and the rapid rate of tube growth under favourable conditions Mercierella can form colonies of considerable size within a few months of larval settlement (e.g. Tebble, 1953). Colonies of a metre or more across have been recorded (see Table 32), although 0.1 - 0.2 m is the more usual condition. At the limits of its salinity range conditions do not favour the formation of large colonies, where instead the worms are usually present as stunted, scattered individuals (Fischer-Piette, 1937; Harris, 1970; Straughan, 1972).

Due to the rapidity with which the colonies can develop under a range of fluctuating environmental conditions, and the resistant nature of the calcareous material which may remain for several years after the worms have died, Mercierella is a fouling organism of considerable economic importance (see Nelson-Smith, 1971). Conditions favouring colony growth are therefore of some interest, and also the proportion of the animal's expenditure in terms of energy in tube production. The aim of the present study was to measure this energetic cost of tube production, and compare this with two other components of production, somatic growth and the formation of gametes. No previous study has been made of the calcareous tube-building polychaetes from this point of view (Clark, 1976).

8.3.2 Materials and Methods

Worms attached to flint boulders were collected at Greenhithe in mid November, 1975. In the laboratory, they were maintained in diluted sea water of salinity 15 °/oo, at 10°C, and fed daily with a suspension of Branchiomonas submarina cells, for not more than eight days before they were used in the experiments. A group of animals selected as

representing the sample size range were carefully removed from their tubes, after these had been cleaned of all encrusting matter. To minimise the possibility of error, care was taken to use only undamaged tubes which had the minimum of encrusting organisms. The fragments of the individual tubes were retained, together with the rest of the calcareous material which was scraped off the rock. The hard nature of the flints prevented the tube material becoming contaminated with particles of substratum. It was not possible, however, to collect all of the scar material, so the individual tube weights are slightly low, although since the scar material represented a relatively minute portion of the total tube and there is a direct relationship between the sizes of scar and tube, this error was considered to be negligible. The fragments of the individual tubes were given two 24 h washes in distilled water to remove surface salt before they were dried to constant weight at 60°C on tared aluminium foil trays.

Any worms which were damaged or releasing gametes were discarded. Following a 5 s rinse with distilled water to remove surface salt, the worms were blotted with paper tissue and individually weighed to the nearest 0.1 mg on tared aluminium foil trays. Their dry weights were estimated from the relationship between wet weight and dry weight in winter animals (Appendix 1.12) as measured with a Cahn 4100 Electrobalance to the nearest 10 µg.

Preliminary investigation showed that in contrast to the expected regular relationship between the weight of the tube and that of the organic material remaining after decalcification, there was considerable variation between these two parameters due to the presence of quantities of siliceous particles, namely sand grains and diatom frustules trapped

in the small pits and superficial cracks which are a feature of the older parts of the tube. In contrast to the older portions which in addition to being somewhat eroded are yellow or light brown in colour as a result of ageing (Tebble, 1953; Nelson-Smith, 1967), and usually encrusted with bryozoans and microscopic algae (Fig. 85), the freshly secreted parts are smooth, clean and white. It was decided therefore, to harvest quantities of freshly secreted tube material to use in the determination of the amount of organic material which is present in the tube.

Seventeen tubes with dry weights ranging from 5.2 - 137.7 mg, and five mixed samples of freshly secreted tube with extreme dry weights of 5.2 mg and 22 mg, were decalcified with 5% formic acid, using the method described by Clayden (1952). Decalcification took from 2 - 5 days at room temperature, and the end-point was detected by the absence of precipitation in alkaline solution after the addition of a saturated solution of ammonium oxalate (10% by volume to a sample of the decalcifying solution). Clayden reported that this method had little effect on the histological properties of the organic matrix of bone. It is possible, however, that a small amount of organic material soluble in formic acid was lost. The residues remaining after decalcification were rinsed in distilled water before drying to constant weight at 60°C. Tube materials were weighed to the nearest 0.1 mg on a Sartorius semi-micro balance.

8.3.3 Results

The five samples of freshly secreted tube material had an average organic content of 4.5 $\pm$ 1% (S.D.) of their dry weight. Assuming the proportion of organic material does not change as the worm grows,

Table 35. Proportions of inorganic and organic components of the tube.

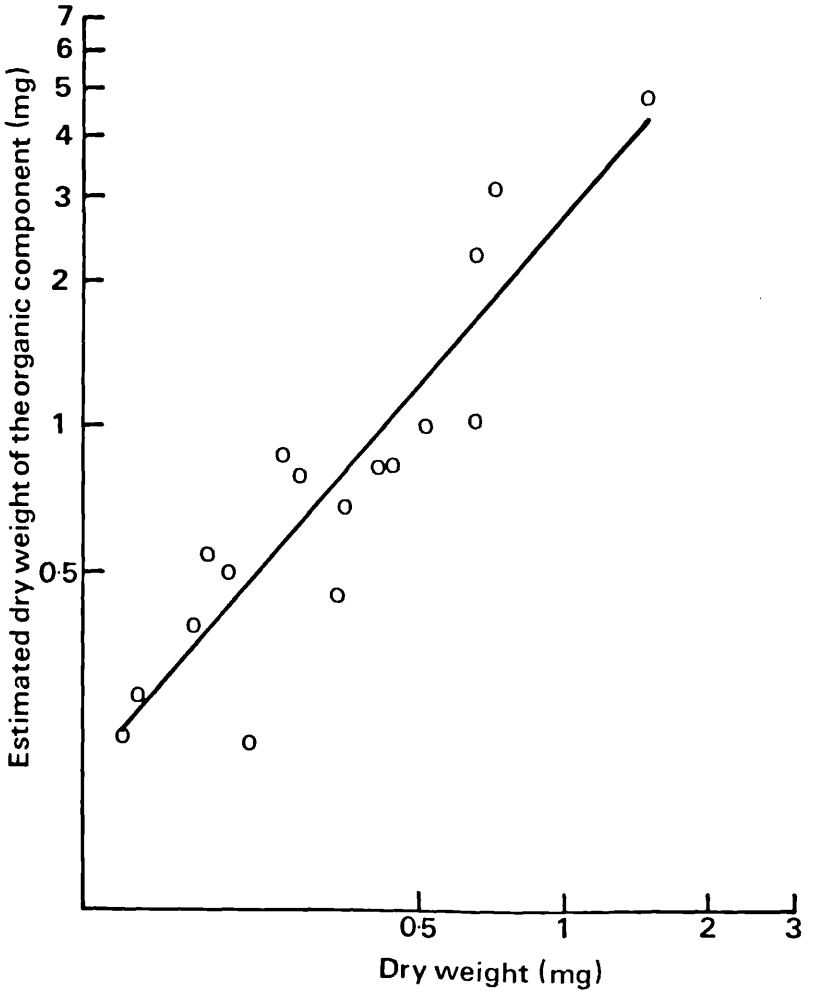
Wet weight (mg)	Estimated dry weight (mg)	Weight loss during decal-cification (mg)	Estimated tube weight (mg)	Estimated weight of organic component (mg)
0.8	0.12	4.9	5.1	0.23
0.9	0.13	6.0	6.3	0.28
1.2	0.17	8.2	8.6	0.39
1.3	0.18	11.4	11.9	0.54
1.4	0.2	10.5	11.0	0.5
1.6	0.22	4.6	4.8	0.22
1.8	0.26	18.4	19.3	0.87
2.0	0.28	16.8	17.6	0.79
2.4	0.34	9.6	10.0	0.45
2.5	0.35	14.4	15.1	0.68
2.9	0.41	17.5	18.3	0.82
3.1	0.44	17.7	18.5	0.83
3.6	0.51	21.2	22.2	1.0
4.6	0.65	47.8	50.0	2.25
4.6	0.65	21.4	22.4	1.01
5.1	0.72	65.3	68.4	3.1
10.5	1.48	103.3	108.2	4.87

the amount of organic matter in each of the decalcified tubes was calculated from the loss of weight of inorganic matter (Table 35). This assumption was supported by the form of the organic tube which remained after decalcification, which is an exact model of the original calcified structure, thus indicating that the inorganic and organic components are thoroughly mixed. Hedley (1956) reported making a similar observation for Pomatoceros triqueter tubes.

Paine (1964) found that the generally accepted temperatures for drying animal tissues are not sufficiently high to remove the relatively large amounts of water of hydration which are loosely bound to the calcium salts in the skeletal structures. He reported that the water of hydration comprised up to 40% of the 'dry' weight in some marine invertebrates. Preliminary investigations showed that when 60°C dry Mercierella tubes were placed in a desiccator at 100°C they underwent a significant weight loss. Because the tubes began to absorb water from the atmosphere when they were taken out of the desiccator, it was not possible to obtain a constant measurement of dry weight until the initial weight was reached. This means that in absolute terms the amount of organic material present in the tubes was underestimated. However, since the same method for drying was used throughout the present study, the error applies to all and the differences are unimportant, although care had to be taken to ensure that the results of other investigations were directly comparable.

Since Barnes et al. (1976) dried their samples at 105°C and Paine (loc. cit.) showed that the water of hydration is removed at temperatures in excess of 140°C, it is possible to compare their results with those of the present study. Barnes et al. (loc. cit.) reported values ranging

Fig. 87. Estimated dry weight of the organic component of the tube plotted as a function of dry weight of worm. The regression line was fitted by the method of least squares assuming a linear relationship between the parameters after logarithmic transformation; ordinate intercept = 0.19; $\underline{b}$ = 1.16.


Estimated dry weight of the organic component (mg)
7
6
5
4
3
2
1
0·5
0·5
1
2
3
Dry weight (mg)

from 0.39 - 2.95% of the dry weight for the organic component of the shells of a range of cirripede species. These values are considerably lower than the 4.5% for Mercierella tubes, and this may account for their greater hardness: Mercierella tubes are appreciably softer than most barnacle shells.

Fig. 87 shows the relationship between the dry weight of the worm and the dry weight of the organic tube component. The regression line was fitted by the method of least squares after a logarithmic transformation of the data, assuming a linear relationship. The correlation coefficient, $r = 0.91$, was highly significant at the $P = 0.05$ level of significance. There is an exponential relationship between the two parameters since larger worms have relatively more massive tubes than smaller animals; the larger tubes have relatively thicker walls and larger numbers of peristomes.

8.3.4 Discussion

The processes involved in serpulid tube formation have been described in a recent review of marginal calcification by Clark (1976). Essentially, small crystals of calcium carbonate are formed in the two subcollar glands and are secreted as a suspension in mucus, which is then applied to the margin of the tube with the aid of the collar. The subsequent hardening of the secreted material is not understood.

The route by which calcium enters the body of the worm for incorporation in the tube is not completely understood and may differ depending on the species or the environmental conditions. Potts (reported by Robertson & Pantin, 1938) found that Pomatoceros utilised calcium from the sea water, whereas Swan (1950) showed that Mercierella contained cells in the anterior part of the gut which absorbed calcium.

He interpreted this as an indication that at least part of the calcium destined for tube formation was absorbed by the gut, presumably from the food, and was transported to the subcollar glands in the blood. More recently, Neff (1971) has pointed out that in view of the relatively minute traces of phosphate which are normally present in sea water, these calcium-rich concretions in serpulid tissues are possibly stores of phosphate for metabolic processes. Nott and Parkes (1975) proposed on the basis of the fine structural similarities of the collar of Spirorbis spirorbis (L.) with the cation pumps described for other animals, that it was directly concerned with extracting calcium from the sea water.

Calcium is present in sea water in high concentrations (0.4 - 0.42 g kg^{-1} H_2O; Culkin, 1965), and most of the surface waters of the oceans are supersaturated with calcium carbonate. However Mercierella is generally found in low salinities in which calcium may not be so readily available as it is in normal sea water. Straughan (1972) reported that the rate of tube growth could be increased by adding calcium salts to the water. Furthermore, the tubes of worms living in low salinities are softer than those living in concentrations approaching normal sea water. It is possible, therefore, that Mercierella may have developed additional means of calcium uptake and storage in association with its invasion of brackish water.

In contrast to the organic component, the calcium carbonate may not require energy expenditure by the worm for it is possible that if the worms were to provide the correct conditions for precipitation the calcium carbonate crystals would form spontaneously (see Clark, 1976). Obviously metabolic energy would have to be expended in providing suitable conditions for the formation of the crystals and in their

subsequent mobilisation until they are finally incorporated in the tube.

Until recently the major interest in invertebrate skeletal structures has been focused on the mechanism of calcium secretion and relatively little attention has been paid to the less conspicuous organic component. The first reference to its chemical properties was made by Cameron (1915, quoted by Swan, 1950), who found that the calcareous tube of Serpula vermicularis L. (as S. columbiana) contained only 0.011 - 0.013% iodine, whereas the residue which remained after the inorganic material had been dissolved away, contained about 0.7% iodine. This observation lead Swan, some 35 years later, to speculate whether this could indicate that the organic and inorganic components might be secreted by different organs (Swan, 1950). He carried out an investigation using radioactive iodine to attempt to answer this question, but the results proved inconclusive. Hedley (1956) demonstrated, using a range of histochemical techniques, that the organic component is produced by at least two separate regions, the major contributor being the ventro-lateral epithelium of the peristomium, the other being the subcollar glands which also produce most of the calcareous material.

Muzii (1968) described an organic membrane coating the insides of the tubes of the serpulids, Hydroides (Eupomatus) dianthus (Verrill), Hydroides norvegica (Gunnerus), and Protula intestinum (Lamarck). The membrane consists of intertwined proteinaceous fibres which are continuous with others randomly interspersed amongst the mineral aggregates, but are regularly arranged and form a distinct layer separating the animal from the mineral zone.

Mitterer (1971) undertook a detailed analysis of the amino acid composition of the organic component in the tubes of Hydroides norvegica and an unidentified serpulid species (? Eupomatus sp.). The tube protein contains a high concentration of acidic amino acids, with aspartic acid and glutamic acid comprising 31% of the total organic residue. There was little variation between the protein composition of the two tubes, and Mitterer postulated that the high acidic amino acid content provides the essential sites for calcification because of the polar side groups. Therefore, part of the production energy which is represented by the organic component of the tube, is intimately linked with the calcification process.

No measurement was made of the calorific value of the organic tube material, but since it is a mucopolysaccharide (Ewer & Hanson, 1945; Hedley, 1956; Muzii, 1968) the value probably lies between 15.88 J mg^{-1} dry weight (3.8 cal mg^{-1} dry wt.) for carbohydrate, and 23.62 J mg^{-1} dry weight (5.65 cal mg^{-1} dry wt.) for protein (Brody, 1945). Taking an average value of 19.75 J mg^{-1} (4.72 cal mg^{-1}), the energy content of the organic material in the tube of a worm with a somatic dry weight of 0.5 mg is 23.7 J (5.67 cal), compared with the 8.99 J (2.15 cal) represented by the somatic tissues (Section 8.1.3). The standing crop of energy in the organic component of the tube is therefore more than twice that in the body of the worm. Furthermore, in one year, based on the results presented elsewhere (Section 8.1.3, and Section 8.2.3), a worm with an initial dry weight of 0.5 mg will produce about 1.0 mg of somatic tissue (17.97 J; 4.3 cal), and 0.52 mg of eggs (9.78 J; 2.34 cal) or 0.75 mg of sperms (11.91 J; 2.85 cal). During the same period, the worm would secrete 3.2 mg of organic tube material, which represents an additional 63.2 J (15.11 cal). Ranking these components in

decreasing order of value gives the following: tube production, at 68%, representing the largest single expenditure of energy, followed by somatic production at about 20%, and gamete production at about 12% (average), of the combined estimated production.

The amounts of organic material in the shells of marine molluscs have been shown to represent a very small part of the total standing crop (Hughes, 1970), and most authors have chosen to ignore this component when evaluating productivity (e.g. Hughes, 1971a, b; Sutherland, 1972). Hughes (1970) reported that the shell protein of Scrobicularia plana (Da Costa) represented 0.64% and 1.14% of the total production of the two populations he investigated, and contrast markedly with the value of 68% obtained in the present study. Although this is partly due to the difference in the proportions of organic material in the shells, 0.4% in Scrobicularia compared with 4.5% in Mercierella, the most important factor is the relative amount of tube, or shell, to animal tissue. An adult Mercierella with a tube 80 mm long, occupies only 15 mm of it, and what is more, the worm's external diameter is also appreciably less than the internal diameter of the tube, which allows water currents to circulate around its body (Section 6.1).

The results of the present study show that tube production, unlike bivalves, represents a considerable portion of the total production in Mercierella enigmatica. Serpulid polychaetes are an extremely successful group with representatives in situations ranging from fresh water to hyper-saline conditions (Hemplemann, 1934; Heldt, 1944). Some species form extensive reefs (e.g. Por & Dor, 1975), and in these situations are a major component of the fauna. Due to their resistance to decay (e.g. Dragastan, 1966), the production of the calcareous tubes represents an energy sink at both the primary consumer and detritivore

levels, which should, therefore, be taken into consideration when measuring the flow of energy through such systems.

8.3.5 The hourly tube production rates by standardworms

The average hourly tube production rates at 10°C and 20°C by Mercierella weighing 7.0 mg wet weight (1.0 mg dry wt.) were obtained by estimating the average somatic growth increments during the six month winter and summer periods, using the hourly values presented in Section 8.1.5, and deriving the amounts of organic tube material produced during these periods from the regression line fitted to the data presented in Fig. 87.

The respective amounts of organic tube material produced in the winter and summer periods are 1.3 mg dry weight and 2.9 mg dry weight. These are equivalent to 25.68 J in six months (6.14 cal in six months), and 57.23 J in six months (13.69 cal in six months), or 5.86×10^{-3} J h^{-1} (1.4×10^{-3} cal h^{-1}) at 10°C, and 1.31×10^{-2} J h^{-1} (3.13×10^{-3} cal h^{-1}) at 20°C.

8.4 Mucus production

8.4.1 Introduction

In addition to the mucous substance which forms part of the tube structure (Clark, 1976), serpulid tubeworms produce mucins which serve a variety of other purposes. These are produced by specialised cells and glands in : the branchial filaments and pinnules; the inner epithelium of the lips; the ventral surface epithelium of the thorax and thoracic membrane; the body wall around the anus and in the ventral ciliated groove; the parapodia; and in the lining of the oesophagus and rectum (Thomas, 1940; Ewer & Hanson, 1945; Section 6.3.3).

Little is known about the chemical composition and structure of the mucous substances which are found in polychaete worms. However, since the composition of mucins is extremely constant because of their specific functions (S. Hunt, personal communication 1974), and in view of the several different types of gland cells which have been reported in some species (for references see Ewer & Hanson, 1945), it is probable that in serpulids the body mucus and tube mucopolysaccharides are not identical. There is also some histo-chemical evidence to support this. Ewer and Hanson (loc. cit.) demonstrated that the mucus-secreting glands on the body of Pomatoceros triqueter, and the sheet of mucus lining the tube had similar staining reactions, which differ from that of the decalcified tube. A similar observation was made during the present investigation. Both the general body surface and the inside of Mercierella's tube give strong positive staining reactions with Alcian blue, which contrast with the weak staining reaction of the tube's organic matrix. Since Alcian blue is a specific stain for acid mucopolysaccharides this would suggest that there is a quantitative difference with respect to these compounds between the body and tube mucins. It is also possible, however, that some material could have leached from the tube before the test was made.

Apart from its role in tube formation, mucus performs an important function in feeding. Particles of food which come into contact with the inner surfaces of the pinnules and branchial filaments become trapped in the mucous secretions and are carried to the mouth in the mucous strings. More mucus is added to the food as it passes over the dorsal and ventral lips (Fig. 54). Any particles which are rejected from the food channels are expelled from the crown as a mucus-bound ball, which because of its greater density quickly falls from the

feeding zone. In the oesophagus yet more mucus is added to the food, before it reaches the stomach and digestion begins (Section 6.3.4). In the gut the mucus protects the gut epithelium from abrasion by silt particles and other sharp materials.

In the hind-gut the undigested material becomes separated into faecal pellets which are bound with a thin mucous membrane (Fig. 88). After their release from the rectum the adhesive pellets are carried to the anterior of the tube by ciliary action, facilitated by the mucous secretions of numerous gland cells (Section 5.1). Once ejected from the tube the faecal pellets, in a similar way to the rejected particles, drop from the feeding zone and become attached to the substratum, or swept away by water currents. Mucus is therefore intimately involved in the efficient operation of the feeding mechanism.

In common with other serpulids, Mercierella lines its tube with a thin sheet of mucus (Ewer & Hanson, 1945; Zottoli & Carriker, 1974), which once again serves several different functions. First, it provides a purchase on the tube's otherwise smooth inner wall, thereby enabling the worm to move within the tube. Second, together with the mucus covering the body it protects the body surface from abrasion, and third, Zottoli and Carriker (1974) have demonstrated that at least one serpulid species, Hydroides dianthus (Verrill), releases protease enzymes into the mucus lining the tube (the lining membrane), which they believe then acts as an anti-fouling mechanism.

Recognising the importance of mucus to Mercierella, it was decided to attempt to determine the energy represented by its production.

8.4.2 Materials and Methods

Mercierella were collected from Weymouth Harbour backwater on the 4th October 1974, and in the laboratory were kept in water of salinity 30 °/oo, at 20°C. They were fed daily with a suspension of Phaeodactylum tricornutum for one week before the experiments commenced. At the end of the acclimation period the worms were detubed, rinsed in several changes of 0.2 µm Sartorius membrane filtered sea water, blotted with paper tissue and weighed individually in tared containers of filtered sea water to the nearest 0.1 mg on a Sartorium semi-micro balance. The worms were then separated into two groups of size range 1.1 - 8.6 mg wet weight.

The first group, comprised of 16 worms were placed into individual, labelled, 5.5 ml wide-mouthed vials (F.B.G. Trident Ltd., London), previously sterilised by autoclaving at 25 p.s.i. for 20 min, which contained filtered, artificial sea water (SeAquariums Ltd., Sheffield) to which the antibiotics benzylpenicillin (30 mg l^{-1}) and streptomycin sulphate (50 mg l^{-1}) were added immediately before the experiment commenced. The vials were then placed (8 to a dish) into 500 ml crystallising dishes containing an antibiotic solution in filtered sea water. This enabled the individual worms, and the mucus they produced, to be identified whilst ensuring that their nitrogenous waste products were sufficiently diluted. After the dishes had been loosely covered to exclude dust particles and reduce evaporative losses, they were placed in a constant temperature cabinet at 20°C. After 5 d the antibiotic solution was replaced with fresh, and after 10 d each worm was examined. Each was found to have produced a ball of mucus from the branchial crown, which was removed with the aid of fine forceps. The animals were then replaced in the constant temperature cabinet and examined at intervals over the next few weeks.

Hall (1954) reported that detubed Mercierella soon produced a mass of mucus which collected in the centre of the branchial crown effectively blocking the mouth. Hall believed that the bases of the branchial filaments, lacking the support of the tube rim, became misaligned with the mouth resulting in the food-bearing mucous secretions not finding their way to the mouth for ingestion. Thus under the above mentioned sterile conditions the build up of mucus at the anterior of the experimental animals represented the mucus which would have normally been produced in order to trap and transport the food particles to the mouth.

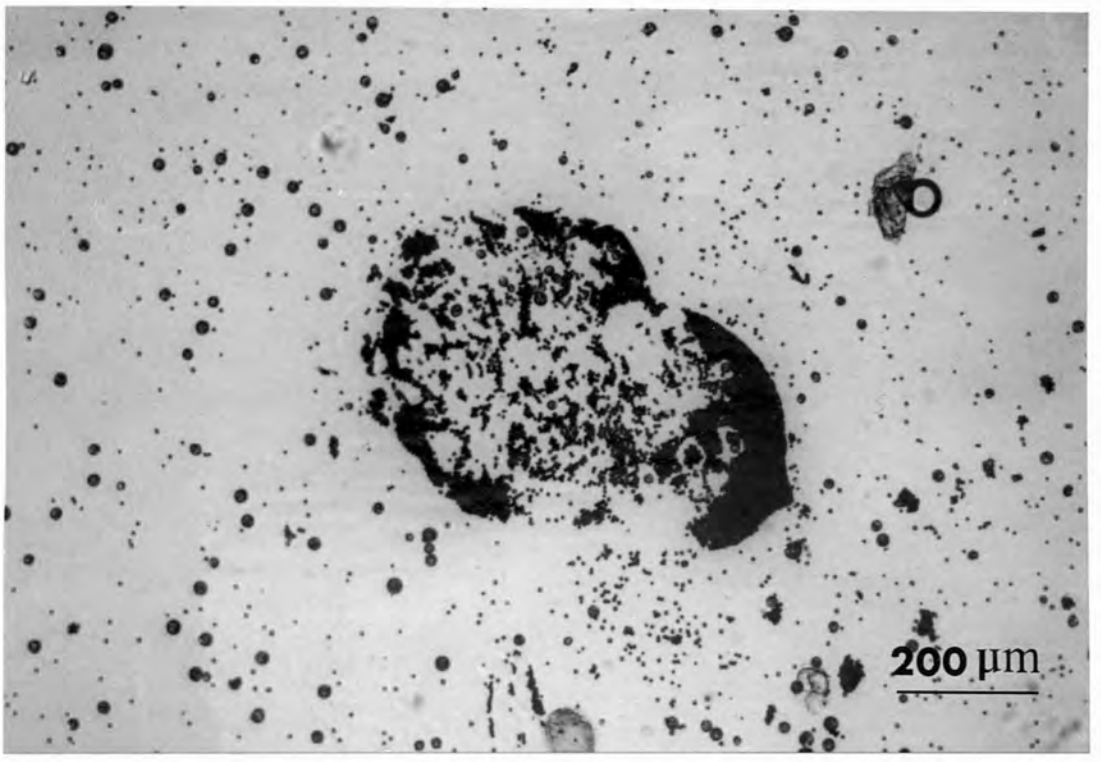
The individual balls of mucus were dialysed against glass-distilled water for 3 h before they were dried to constant weight on tared aluminium foil trays. A check for suspended mucus in both the experimental vials and dialysis tubes, using Alcian blue stain followed by filtration through a Sartorius filter, failed to reveal any evidence of detached mucus flocs. Since dry weighings of the samples revealed that the individual amounts were too small to be weighed with any accuracy on the most sensitive balance available at that time (Sartorius semi-micro), the dry mucus was carefully scraped from each tray and combined to give the total dry weight of mucus produced by the branchial crowns of 16 Mercierella in 10 d.

The branchial crowns of the second group of animals (N = 19) were excised immediately after they were wet weighed, at the junction of the crown and thorax. The worms were kept for 24 h after the operation in sterile sea water, during which time a blastema formed over the wound. The regenerative powers of the sabelliformia are well documented (e.g. Wells, 1952; Berril, 1931; Fitzharris, 1973), so the experimental treatment exploited a natural process. All the worms survived the operation.

After 24 h they were placed into individual 5.5 ml vials, similar to those described above except that a pre-weighed glass cover slip had been placed in the bottom of each, to which it was hoped that the mucous secretions from the thorax and abdomen would adhere. The subsequent experimental treatments were identical to those described above. After 10 d the worms were removed from the vials and several drops of concentrated Alcian blue solution were introduced into the water in each one. At the end of 30 min, sufficient time for any mucus to have stained, the cover slips were removed from the vials and gently rinsed with distilled water. Each cover slip was found to have a small quantity of mucus adhering to it. Following two further rinses with distilled water to remove salt contamination, the cover slips were dried to constant weight at 60°C. Unfortunately, the total dry weight of body mucus adhering to the glass cover slips was below the level of accuracy of the balance (0.1 mg). A check for detached flocs in the vials and water proved negative.

The mucous coat surrounding the faecal pellets was demonstrated by feeding tubed Mercierella with a suspension of Congo red stained yeast cells. Dried brewers yeast was boiled with a spatula full of Congo red for 10 min, centrifuged and washed several times with distilled water. A dilute suspension of the stained yeast cells was made up in some of the tank water, and added about 12 h before the faecal pellets were collected. At the end of the feeding period, the rocks and tubes were rinsed free of stained yeast cells and placed in a tank filled with clean water. The faecal pellets were easily identified by their red colour, collected with a pipette, and placed on a microscope slide for examination. A test was carried out to check whether there is mucus within the pellets. For this some pellets were

Fig. 88. Compressed faecal pellet of a M. enigmatica that had been feeding on yeast cells. The mucous coat has been stained with Alcian blue. The large cells are the alga, Brachiomonas submarina, that was used as a food organism throughout this study.


200 µm

ruptured by pressure applied to the cover slips, and a drop of Alcian blue solution was introduced under the edge of each. After 15 min the stain was replaced with clean water, and the pellets examined.

8.4.3 Results

The total dry weight of mucus produced by the branchial crowns of 16 Mercierella with a combined wet weight of 78.3 mg, was 2.5 mg. Therefore on average, each worm produced 31.9 µg dry weight of mucus mg^{-1} body wet weight in 10 d. All the worms continued to secrete crown mucus for up to 28 d after they were detubed, although the rate of production was appreciably less after 21 d.

Although an accurate measurement of the amount of body mucus secreted during this same period was not obtained, it is possible to deduce from these data that since 19 Mercierella, with a combined body wet weight of 101.6 mg, appear to have only produced less than 0.1 mg dry weight of mucus in 10 d, body mucus production was less than 3.07% of the rate of crown mucus production mg^{-1} body wet weight.

Fig. 88 shows a compressed faecal pellet containing stained yeast cells. The coating of mucus produced by the cells lining the hind-gut can clearly be seen. At the time of release from the worm's body the pellets are cylindrical in shape and have rounded ends. The size of the pellet is directly related to the size of the animal. A worm of 1.0 mg somatic dry weight (7.0 mg wet weight) produces faecal pellets which are about 200 µm long and 60 µm in diameter. The average thickness of the mucous coat of a pellet of this size is about 4.0 µm. The Alcian blue preparations failed to reveal evidence of appreciable amounts of mucus inside the pellets, although the contents did stain weakly, indicating the presence of acid mucopolysaccharides. In contrast, the mucous coating stained darkly with the Alcian blue.

8.4.4 Discussion

Since polychaete mucous secretions have been demonstrated to contain mucopolysaccharides (Ewer & Hanson, 1945; Muzii, 1968), and Defretin (1951) showed that the tube mucins produced by representatives of several tubicolous polychaete families contain considerable quantities of carbohydrates, including a complex polysaccharide resembling hyaluronic acid, it was assumed that Mercierella's mucins have a calorific content of 19.75 J mg^{-1} dry weight (4.72 cal mg^{-1} dry wt.). This value is an average based on the 15.88 J mg^{-1} dry weight (3.8 cal mg^{-1} dry wt.) for carbohydrate, and 23.62 J mg^{-1} dry weight (5.65 cal mg^{-1} dry wt.) for protein (Brody, 1945). Therefore the crown mucus produced by a worm of 7.0 mg wet weight is on average 0.44 J d^{-1} (0.105 cal d^{-1}) over a ten-day period. Under normal feeding conditions, however, most of this energy (mucus) would be ingested together with the food particles, and only a relatively minute amount would be lost with the rejected food.

No study has yet been made of the digestive enzymes in the gut of Mercierella or for that matter of any other serpulid. Carbohydrases and proteases have been detected in the guts of several non-carnivorous polychaetes, including Sabella pavonina Savigny (Nicol, 1930; for a review see Jeuniaux, 1969). Furthermore it is shown in Section 5.5 that Mercierella is capable of digesting the biochemical components of Branchiomonas cells, which include carbohydrate and protein. It seems probable therefore, that Mercierella would be able to digest the crown mucus after this has fulfilled its role in trapping and conveying the food to the mouth. This hypothesis is supported by observations made of the movement of food through the guts of young Mercierella, which lacked the dark epithelial pigmentation and gametes of the older

animals. It was found that the gut in the anterior part of the abdomen contained an amorphous mass of what appeared to be finely divided food material, which lacked the cohesive properties that would have been expected were appreciable amountsof mucus present. Furthermore, although the food appeared to undergo a great deal of mixing the faecal pellets only had significant amounts of mucus on their outsides. These observations discount the possibility of the food being surrounded by a peritrophic membrane of crown mucus throughout its journey along the digestive tract.

Based on the results of the experiment, body mucus production amounted to less than 1.35×10^{-1} J in 10 days (3.23×10^{-2} cal 10 days^{-1}). However, since not all the mucus secreted by the body surface became attached to the cover slip, and because mucus production could be stimulated by the worm moving to and fro within its tube, this estimate has to be regarded as a conservative one. Furthermore, in the case of both crown and body mucins some may have dissolved during the 10 day period.

Mucus has a very high water content of about 99% by weight (mean of three determinations). Assuming that it has a specific gravity similar to that of the surrounding medium, 1.021 at 20°C, the dry weight of mucus surrounding a faecal pellet would occupy 0.979% of the volume occupied by the wet mucus. The volume of mucus surrounding an average faecal pellet, 200 μm long with a diameter of 60 μm, assuming that the outer layer consisted of a mucus coat 4 μm thick, was calculated as being 1.38×10^{5} μm^{3}. Therefore the volume occupied by the dry mucus was 1.35×10^{3} μm^{3}. Correcting this to the dry weight of mucus gives 1.35×10^{-6} mg, which is equivalent to 2.67×10^{-5} J pellet^{-1} (6.39×10^{-6} cal pellet^{-1}).

In their experiments on the external release of proteases by tubicolous polychaetes Zottoli and Carriker (1974) found that the serpulid Hydroides dianthus released proteolytic enzymes which had activities varying from 2.92 - 8.09 µg equivalents of bacterial protease after 1 h (their values refer to groups of 10 individuals). Zottoli and Carriker demonstrated that the protease remained active after 1 month, however reaching a maximum level of activity after 1 week. Their results show that after 1 week activity was 127 - 416% greater than at 1 h, from which they concluded that the enzyme becomes slowly activated, suggesting that very small amounts are needed to provide a long term anti-fouling action.

Since Hydroides is a similar size to Mercierella, it is possible to use their results to derive an estimate of what protease production would represent in terms of energy units. Enzymes are proteins, and Brody (1945) gave a calorific value for protein of 23.63 J mg^{-1} dry weight (5.65 cal mg^{-1} dry wt.). A single worm would produce, on average, a quantity of protease enzyme which had an activity equivalent to 0.55 µg of bacterial protease after 1 h. After 1 week this activity would increase by an average of 272%, which is equivalent to 1.5 µg of bacterial protease. Taking this figure as an estimate of the total amount of enzyme released during the 10 day experiment, this represents a production value of 3.54×10^{-2} J 10 $days^{-1}$ (8.48×10^{-3} cal 10 $days^{-1}$). This value probably represents a conservative estimate, although it seems unlikely that the worm would continue releasing protease at the rate measured at the end of the first hour because first, it would be wasteful in an energetic sense in view of the slow activation, and second, due to the problems which would accompany having a high concentration of protease enzyme in contact with the tube, which is itself composed partly of protein.

8.4.5 The hourly loss of energy as mucus

The energy represented by the hourly production of crown mucus by a 7.0 mg wet weight Mercierella at 20°C, was calculated to be 1.83 x 10^{-2} J (4.38 x 10^{-3} cal). No measurement is available for body mucus production, although as a conservative estimate it is thought to be 5.63 x 10^{-4} J h^{-1} (1.35 x 10^{-4} cal h^{-1}) at 20°C. Since Zottoli and Carriker (loc. cit.) carried out their investigation under similar conditions of salinity and temperature to those described above, it is possible to calculate a value for the energy expenditure as protease enzyme. The conservative estimate of protease production quoted above is equivalent to 1.48 x 10^{-4} J h^{-1} (3.53 x 10^{-5} cal h^{-1}). It is estimated therefore that the secretions from the general body surface (body mucus + protease enzyme) amount to only 7.11 x 10^{-4} J h^{-1} (1.7 x 10^{-4} cal h^{-1}).

Measurements of the rates of release of faecal pellets from the rectum of a 13 mm long Mercierella (c. 7.0 mg wet weight) feeding on a suspension of B. submarina cells at a concentration similar to that used in the rest of this study, namely 1.1 x 10^{4} cells ml^{-1}, had an average value of 20 pellets h^{-1} at 20°C. Egestion therefore represents a loss of 1.35 x 10^{-6} mg x 20 = 2.7 x 10^{-5} mg dry weight of mucus h^{-1}, or 5.34 x 10^{-4} J h^{-1} (1.28 x 10^{-4} cal h^{-1}).

Ranking these components of mucus production in decreasing order of value gives the following : crown mucus production representing the highest proportion at c. 94% of the combined energy, with body mucus and that lost as faeces being roughly equal at c. 3% each. It should be noted, however, that the majority of the energy channelled into mucus is not in fact lost from the animal under normal feeding conditions, although the amount will vary depending on food quality (particle size)

and quantity (Section 3.3.4). Under the most extreme conditions where no food enters the mouth, and all the crown mucus is rejected together with the particles of unsuitable size, the hourly loss of energy as mucus approaches the value for respiration, which explains why under conditions of high particle concentration, Mercierella withdraws inside its tube.

9. DISCUSSION

The flow of energy through an individual or population of animals is generally expressed as an equation with the form:

$$I = P + R + U + F$$

where, I is ingestion; P is production; R is respiration; U is nitrogenous excretion; and F is defaecation. In the present study an attempt was made to measure each component of the budget separately because this enables an internal check to be made of its accuracy, since A, the assimilated energy = $I - F$ and $P + R + U$.

Under the controlled laboratory conditions I consists of two components. The first and larger of the two is the energy represented by the algal cells, whilst the second is in the form of mucous secretions originating from the branchial crown. This is also part of the worm's production and hence is considered twice in the budget both as part of I and as a component of P.

Production is that part of the ingested energy which is channeled into the various synthetic processes which in the present study are considered under the following sub-headings :

$$P = p_{sg} + p_r + p_t + p_m + p_s.$$

p_{sg} is somatic growth; p_r is the energy which is directed into the production of gametes; p_t is the energy represented by the other major component of growth, tube production. The broad term p_m describes the combined energy contents of crown mucus, the mucus secreted by cells lining the hind-gut which appears surrounding the faecal pellets, and the secretions from the general body surface which consist of a mixture of mucus and possibly protease. p_s is the energy of production which is lost from the worm due to the leakage of carbon containing compounds

Table 36. Winter and summer energy budgets for a standard M. enigmatica.

Component		Winter (10°C) J h^{-1}	Winter (10°C) cal h^{-1}	Summer (20°C) J h^{-1}	Summer (20°C) cal h^{-1}
Ingestion: I	Food	1.15×10^{-1}	2.75×10^{-2}	2.69×10^{-1}	6.44×10^{-2}
	Mucus	9.2×10^{-3}	2.2×10^{-3}	1.83×10^{-2}	4.38×10^{-3}
Egestion: F	Faeces	9.76×10^{-4}	2.33×10^{-5}	1.13×10^{-4}	2.7×10^{-2}
	Mucus	4.09×10^{-4}	9.78×10^{-5}	5.34×10^{-4}	1.28×10^{-4}
Respiration R		8.65×10^{-3}	2.07×10^{-3}	2.78×10^{-2}	6.65×10^{-3}
Excretion U		9.2×10^{-4}	2.2×10^{-4}	2.97×10^{-3}	7.1×10^{-4}
Somatic production p_{sg}		1.63×10^{-3}	3.9×10^{-4}	3.49×10^{-3}	8.35×10^{-4}
Gamete production p_r		—	—	3.17×10^{-3}	7.58×10^{-4}
Tube production p_t		5.86×10^{-3}	1.4×10^{-3}	1.31×10^{-2}	3.13×10^{-3}
Mucus production: p_m	Crown	9.2×10^{-3}	2.19×10^{-3}	1.83×10^{-2}	4.38×10^{-3}
	Body	2.82×10^{-4}	6.75×10^{-5}	5.63×10^{-4}	1.35×10^{-4}
	Protease	7.4×10^{-5}	1.77×10^{-5}	1.48×10^{-4}	3.53×10^{-5}
	Faeces	4.09×10^{-4}	9.78×10^{-5}	5.34×10^{-4}	1.28×10^{-4}
		9.97×10^{-3}	2.38×10^{-3}	1.96×10^{-2}	4.67×10^{-3}
Leakage p_s		?	?	?	?
Production P		1.75×10^{-2}	4.18×10^{-3}	3.93×10^{-2}	9.4×10^{-3}
Assimilated energy A		2.7×10^{-2}	6.45×10^{-3}	7.01×10^{-2}	1.68×10^{-2}

via the body wall. The R term describes that portion of the ingested energy which is expended as a consequence of the metabolic processes, whilst the energy which is lost in the form of the nitrogenous excretory product ammonia is represented by U, and F is that which is lost as undigested or unassimilated food materials.

Table 36 shows the winter and summer energy budgets for Mercierella enigmatica under controlled laboratory conditions, based on values calculated for a 'standard' animal of 1 mg dry weight. Unfortunately, due to the anomalous low ingestion rates recorded at 10°C (Section 3.7; Section 5.3.3; and Section 5.6), it was necessary to calculate the energy content of the algal component of I from P + R + U + F - the estimated value for crown mucus, and thereby lose the useful internal check on accuracy described above. The value for gut mucus was obtained by multiplying the summer amount by a factor derived from the relationship between the winter and summer weights of faeces (Section 5.6), namely the rate of faeces production at the lower temperature is 76.6% of the latter. It was assumed on the basis of the effect of a 10°C increase in temperature on the rates of somatic growth (Section 8.1.3) and tube growth (Section 8.3.5), that crown mucus and body secretions are produced at one-half the summer value at the lower temperature. This is supported by the relationship between the estimated winter and measured summer ingestion rates which differ by a factor of about two.

The rate at which energy is lost as undigested or assimilated food material F was, in the case of the summer animal, calculated from the hourly rate of faeces production in milli-grammes (Section 5.6) and the proximate composition of the faeces based on biochemical determinations (Section 5.5.2). This is because the considerable

discrepancy between the calorific value calculated from the biochemical composition and that measured using the microbomb calorimeter (Section 5.5.3) is indicative that a significant amount of soluble organic material had leached from the samples during the emergency distilled water treatment (Section 5.5.1). This value was corrected for the estimated value of the mucus associated with the faecal material (Section 8.4.5).

Despite the use of the corrected value for the energy contained by the faeces produced at the higher temperature, there is still a marked discrepancy between the value for assimilated energy calculated from $\underline{A} = \underline{I} - \underline{F}$, 1.74×10^{-1} J (4.16×10^{-2} cal), and 7.01×10^{-2} J (1.68×10^{-2} cal) derived by the summation of $\underline{P} + \underline{R} + \underline{U}$. The latter is about 40% of the former, making nearly 60% of the apparent assimilated energy unaccounted for. There are a number of possible explanations for this difference and these will be discussed in some detail later in relation to energy losses via excretion, but for now it is sufficient to say that the most likely reason seems to be that relatively large amounts of soluble carbon-containing compounds were lost from the gut, which therefore resulted in the egested energy being underestimated. It was decided therefore to use the compounded assimilation energy in those subsequent calculations requiring an estimate of the assimilated energy.

It is calculated from the estimated hourly ingestion rate at 10°C (correcting for ash) and the calorific value of *B. submarina* (see Section 3.6), that a standard animal under winter conditions ingested 6.07 µg dry weight of algal cells h^{-1}, or 14.6% of its dry weight d^{-1}. Whereas during the summer the same animal ingested 14.15 µg dry weight of cells h^{-1}, or about 34% of its dry weight d^{-1}. The percentages are in agreement with those quoted by Butler *et al.* (1970) for the ingestion

Table 37 cont. Assimilation efficiences for a variety of aquatic invertebrates.

Species	Assimilation efficiency(%)	Laboratory	Field	Sediment	Detritus	Bacteria	Algae	Animal tissues	Fungi	Reference
Gammarus pseudolimnaeus Bousfield	17 - 83	+			17 - 19				68 - 83	Barlocher & Kendrick (1975)
Hydrobia ventrosa	34 - 70	+			34	70				Kofoed (1975b)
Pteronarcys scotti Ricker	11	+			+					McDiffett (1970)
Hyalella azteca Saussure	15 - 18	+		+		+	+			Hargrave (1971)
Scrobicularia plana	61		+	+			+			Hughes (1970)

Table 37. Assimilation efficiences for a variety of aquatic invertebrates.

Species	Assimilation efficiency(%)	Laboratory	Field	Sediment	Detritus	Bacteria	Algae	Animal tissues	Fungi	Reference
Cirolana harfordi (Lockington)	88	+						+		Johnson (1976)
Menippe mercenaria	40 - 85	+						+		Mootz & Epifanio (1974)
Neanthes (= Nereis) virens	85	+						+		Kay & Brafield (1973)
Daphnia magna Straus	<10 ->90	+					+			Schindler (1968)
Fissurella barbadensis	34		+	+			+			Hughes (1971b)
Hydrobia ventrosa (Montagu)	8 - 76	+				70 - 76	8 - 71			Kofoed (1975a)
Littorina irrorata	45		+				+			Odum & Smalley (1959)
Mercierella enigmatica	22 - 24	+					+			
Mytilus edulis	60 - 85	+					+			Widdows & Bayne (1971)
Nerita spp.	39 - 43		+				+			Hughes (1971a)

continued

rates of mixed zooplankton feeding on diatoms, when they were not displaying the phenomenon of "superfluous feeding" which is associated with algal blooms. This supports the findings of the feeding experiment (Section 3.3) which show that Mercierella exhibits normal feeding behaviour in the cell concentration of 1.1×10^4 cells ml^{-1} which was used throughout the rest of the study.

The 2.3 x increase in the amount of ingested energy in the summer is accompanied by a 2.6 x increase in assimilated energy; the assimilation efficiency during the summer $\frac{(P + R + U \times 100)}{I \qquad\qquad 1}$, is 24.4% compared with 21.7% in the winter, which shows that there are both absolute and relative increases in the quantity of energy that is assimilated in the summer period, which is characterised by increased reproductive activity (Section 8.2.3). Hence it appears that reproduction promotes a rise in assimilation efficiency. Schindler (1968) reported similar observations for the planktonic freshwater crustacean, Daphnia magna Straus.

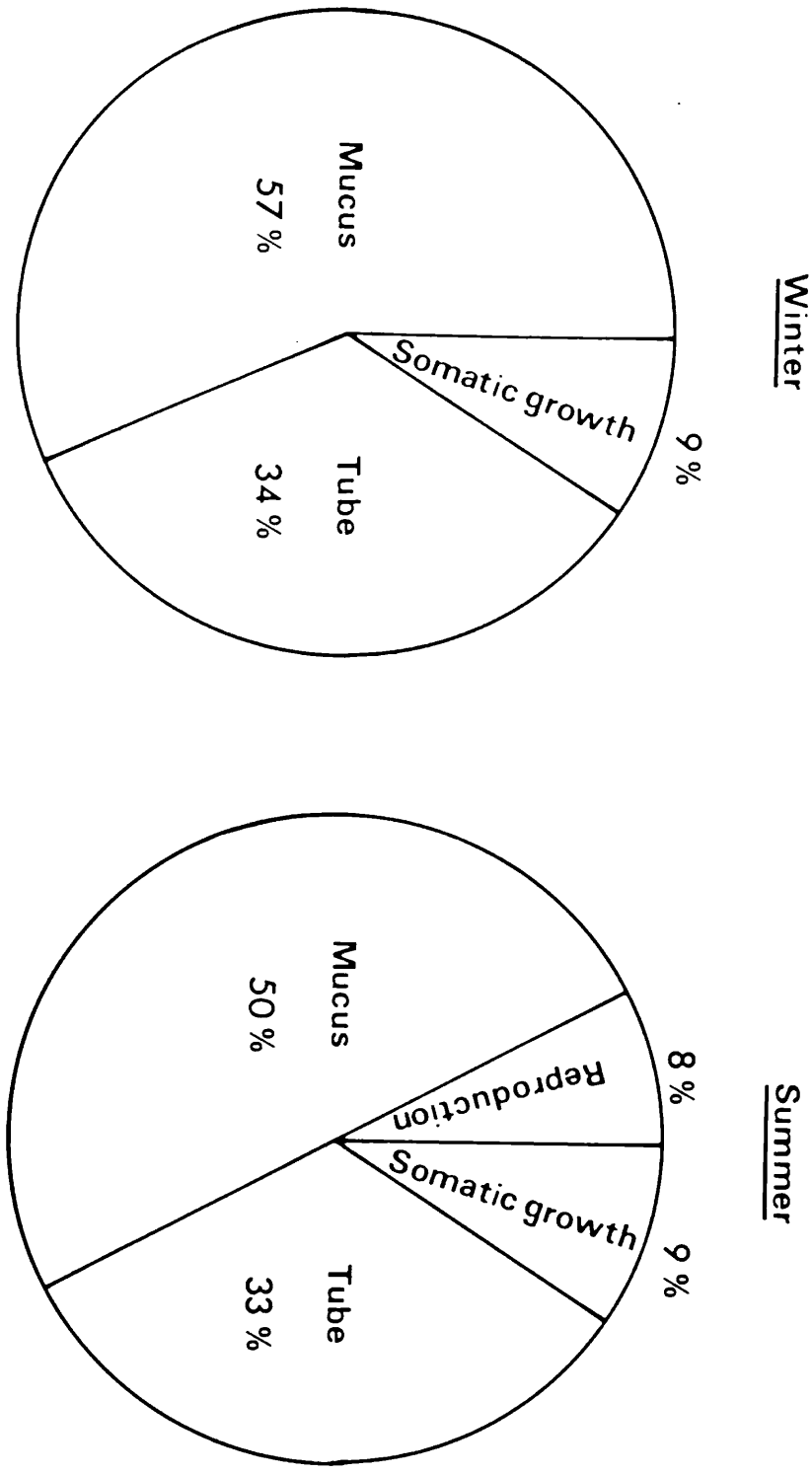
Table 37 shows the assimilation efficiencies for Mercierella, together with some published values for other aquatic invertebrates including detritivores, deposit feeders, herbivores, and carnivores. In general, detritivores have the lowest assimilation efficiencies, carnivores the highest, and herbivores intermediate values. Recent studies have shown, however, that bacteria and fungi are assimilated at high efficiencies (e.g. Bärlocher & Kendrick; and Kofoed, loc. cit.) similar to those recorded for carnivores, which suggests that unless they have been able to feed selectively the values for detritivores may be artificially low. Similarly, Himmelman & Carefoot (1975) in their discussion of the feeding preferences of invertebrate herbivores have

pointed out that the calorific value of the food appears to be an important criterion for food selection.

Factors affecting assimilation efficiency fall into two categories, exogenous and endogenous. Exogenous factors are food quality (e.g. Schindler, 1968; Kofoed, 1975a) and quantity (e.g. Richman, 1958; Butler et al. 1970), environmental factors (e.g. Widdows & Bayne, 1971), experimental method (see above and Table 37), and the method of calculation, i.e. few authors have attempted to measure all channels, and usually U is not measured (e.g. Johnson, 1976) or is considered as negligible (e.g. Hughes, 1971 a,b), resulting in the values for assimilation efficiency generally being underestimated (see Hargrave, 1971). The endogenous factors include the type of organism, its age and body size (e.g. Jørgensen, 1952; Schindler, 1968), reproductive condition (Richman, 1958; and present study), nutritional state (Bayne, 1973b), physiological stress (Widdows & Bayne, 1971); gut passage time (Hubbell et al. 1965; Bärlocher & Kendrick, 1975; present study Section 3.2.3. and Section 5.2.4), and leakage of soluble organic materials (McDiffett, 1970; Bayne, 1973b; Gabbott & Bayne, 1973; Kofoed, 1975b; and present study Section 4.10). The highest assimilation efficiencies tend to be those of carnivores (see Table 37) and larvae. The former due to the greater assimilability of the food, enhanced by selective pressure since these organisms generally have a discontinuous feeding regimen, and the latter due to their rapid growth rate.

The estimated assimilation efficiencies for Mercierella under winter and summer conditions are in the lower part of the range for the herbivores shown in Table 37. This is unusual considering that the measurements were carried out in the laboratory (laboratory assimilation efficiencies have been found to be high compared to those in the field,

Fig. 89. Proportional utilisation of energy expended in production (P) by a standard M. enigmatica during the winter and summer.


Winter
Mucus
57 %
Somatic growth
9 %
Tube
34 %
Summer
Mucus
50 %
Reproduction
8 %
Somatic growth
9 %
Tube
33 %

see Butler et al. (1970)), and more channels than is usual were measured. However, in view of the large number of variables which are known to affect assimilation efficiency it is evident that whilst it is a useful method for comparison on an intraspecific basis, particularly where optimum conditions for growth and reproduction are desired (e.g. mariculture), it is of little value for comparisons of data from different species unless the above mentioned variables can be largely defined.

Production at the higher temperature is 2.25 x the winter level. Fig. 89 shows the compartmentalization of production energy during the winter and summer periods. In each case mucus represents the greatest proportion, followed by tube growth, somatic growth, and finally during the summer period by reproduction. This is extremely interesting because mucus production has largely been ignored in energetics studies, and somatic growth and reproduction are the only components that are usually considered. This indicates that this may be a serious omission particularly in the case of those filter feeders that use a mucous mechanism for trapping their food, e.g. bivalve molluscs, and those organisms in which mucus secretion is important in a locomotary sense, e.g. gastropod molluscs and burrowing annelids; not to mention polychaetes that line their burrows with mucus. A notable exception is the investigation conducted by Teal (1957) of energy flow through a temperate cold spring community, in which he demonstrated that mucus production accounted for about 80% of the productivity by two species of planarians (Phagocata spp.). In contrast, however, to these planarians which apparently lose the vast majority of their mucous secretions, Mercierella enigmatica ingests the largest single component of its mucus, i.e. that which is produced by the crown, which averages

93% of the total. This reduces the actual loss of energy to only c. 7% of this amount under normal feeding conditions (Section 8.4.5). If the energy lost as crown mucus is equal to 10% of the total mucus production this would represent only about 3.5% of the total productivity, and is markedly less than the values for mucus production which have been reported by Paine (1971) for the herbivorous gastropod, Tegula funebralis, and Kofoed (1975b) for the detritivorous gastropod, Hydrobia ventrosa (Montagu). Total mucus production may be underestimated in the present study, however, since the body secretions, mucus and proteases, are conservative estimates (Section 8.4.4).

Unlike mucus the tube does represent a considerable loss of energy from the worm's body, but it does remain with the organism throughout its lifetime (Section 8.1.4 and Section 8.3.1), and should therefore be considered as an external skeletal structure. Somatic growth represents the same proportion of the total productivity in the winter and summer, and reproduction accounts for an almost similar proportion during the summer months.

It has been expressed on a number of occasions (e.g. Hargrave, 1971; Kofoed, 1975b) that appreciable amounts of dissolved organic materials may be lost from the bodies of marine invertebrates because of leakage across the general body surfaces and through excretion. It is evident that were a portion of these leached compounds part of the organism's anabolic component then they need to be considered as representing some of the energy of production p_s. Unfortunately, there has been a tendency to quote the work of Coward, Johannes, and Webb, in this context, in particular the rates of efflux of amino acids from the marine turbellarian, Bdelloura candida (Johannes, Coward & Webb, 1969; Webb, Johannes and Coward, 1971). As has already been pointed

out by Southward and Southward (1972), the experimental results with Bdelloura would be generally more applicable if that species were free-living and not a commensal on the gills of the king crab, Limulus; and if one could be sure that the amino acids liberated into the medium were genuinely leached out through the body wall or excreted, and not merely ejected from the gut lumen as waste products of a high-protein meal ingested some hours earlier. One is forced to agree with Jørgensen (1976) when he stated that there is a general lack of precise knowledge of the rate at which aquatic invertebrates release soluble organic molecules. This is not to say that some dissolved organic materials are not lost across the body wall, although the results of the present investigation have indicated that apart from their being of negligible value in Mercierella's energy budget (Section 4.10) soluble organic molecules appear to be lost as part of an exchange-diffusion process (Section 4.9.4 and Section 4.10), the result of which is no net gain or loss. Thus in the absence of convincing evidence to the contrary it is considered that P_s is negligible in the case of Mercierella.

Commonly used indices of productivity are gross production efficiency $\frac{P}{I} \times 100$, and net production efficiency $\frac{P}{A} \times 100$, the latter indicates what proportion of the assimilated energy is potentially available to the next trophic level. It is acceptable to include mucus and tube production in these calculations because the crab Carcinus maenas (L.) has been observed to eat Mercierella, tube and all, and the older parts of the tubes commonly have a chalky texture presumably as a result of microbial decomposition of the organic matrix (Section 8.3.2). Similarly, a number of organisms have been recorded as ingesting mucus (e.g. Benson & Muscatine, 1974) and faecal pellets containing mucus (e.g. Newell, 1965).

Table 38. Production efficiences for a variety of aquatic invertebrates.

Species	Production efficiency (%) Gross	Net	Habit/Taxon	Reference
Gastropod veligers		60 - 70	larvae	Jorgensen (1952)
Cirolana harfordi	36 - 49	35	sedentary isopod	Johnson (1976)
Menippe mercenaria	12 - 42	28 - 60	crab larva	Mootz & Epifanio (1974)
Neanthes virens	22 - 57	71	burrow-dwelling polychaete	Kay & Brafield (1973)
Phagocata spp.		88	planaria	Teal (1957)
Root Spring: all carnivores		59		" "
Daphnia magna		55 - 73	planktonic crustacean	Schindler (1968)
Fissurella barbadensis	9	27	sedentary gastropod	Hughes (1971b)
Littorina irrorata		14	sedentary gastropod	Odum & Smalley (1959)
Mercierella enigmatica	13.7 - 14.0	56 - 64	filter-feeding polychaete	
Nerita spp.	5 - 8	8 - 13	sedentary gastropods	Hughes (1971a)
Pisidium virginicum (Gmelin)		47	filter-feeding bivalve	Teal (1957)
Root Spring: all herbivores		25		" "
Hydrobia ventrosa	5 - 23	15 - 33	sedentary gastropod	Kofoed (1975b)
Limnodrilus hoffmeisteri		26	tubicolous oligochaete	Teal (1957)
Pteronarcys scotti	4	34	stonefly nymph	McDiffett (1970)
Hyalella azteca	2	15	sedentary amphipod	Hargrave (1971)
Scrobicularia plana		21	sedentary bivalve	Hughes (1970)

Table 38 shows production efficiency values for a range of aquatic organisms, calculated where necessary from the published data. Like assimilation efficiency which has been discussed in some detail earlier, production efficiencies are highly variable and are affected by many of the exogenous and endogenous factors already described above. However, in general there is a direct relationship between food quality and the production efficiencies. Detritivores and deposit feeders tend to have low production efficiencies compared with carnivores because their food is relatively indigestible and more energy is required to process it. Herbivores tend to have intermediate values. In addition, there is an inverse relationship between the energy expended in metabolic activity R and production efficiency. Thus any factor which has an enhancing effect on respiration, e.g. osmotic stress (Thornton & Wilhm, 1974), will concomitantly have a depressing influence on productivity. Obviously, nutritive stress will have a retarding effect on growth (e.g. Dare & Edwards, 1975). Other factors include body size : larvae have very high production indices (e.g. Jørgensen, loc. cit.), as do juveniles (relatively) and younger adults (e.g. Hargrave, 1971); and reproduction : reproductive activity tends to elevate the net production efficiency (e.g. Schindler, 1963; Itoh, 1973).

Mercierella enigmatica's gross production efficiency remained fairly constant under winter and summer conditions, averaging about 13.9%. This figure is in accordance with the published values for herbivores, considering it was feeding on a unialgal suspension which lacked the relatively indigestible detritus and sediment particles (Section 3.6) which had a depressing effect in a number of the other cases (see Hughes, 1971b). The net growth efficiency is lower in the summer, 56%, than in the winter, 64%, because proportionally more energy

Table 39. Respiration rates of some aquatic invertebrates.

Species	Size	Temperature (°C)	Oxygen consumption ($\mu l\ h^{-1}$)	Reference
(where necessary rates have been calculated from the presented data)				
Herbivores				
Ancylus fluviatilis (Müller)	20 mg wet wt.	11	c. 1.9	Berg et al. (1958)
		18	c. 3.8	
Crepidula fornicata L.	160 mg dry wt.	10	c. 75 (s.)	Newell & Kofoed (1977)
			c. 92 (a.)	
		20	c. 123 (s.)	
			c. 209 (a.)	
Gammarus pulex L.	5 mg wet wt.	10	1.9	Wright & Wright (1976)
Littorina littorea	1 mg dry wt.	20	c. 2 (s.)	Newell (1973)
			c. 13 (a.)	
Mercierella enigmatica	1 mg dry wt (7 mg wet wt.)	10	0.435	
		20	1.4	
Mytilus edulis	1 g dry wt.	15	200 (s.)	Widdows (1973)
			600 (a.)	
Carnivores				
Menippe mercenaria	1 mg dry wt.	25	c. 1	Mootz & Epifanio (1974)
Neanthes virens	200 mg dry wt.	10	574	Walsby, 1970 in Kay & Brafield (1973)
Phagocata gracilis woodworthi Hyman	20 mg wet wt.	c. 10	c. 1	Teal (1957)

s. = standard metabolism

a. = active metabolism

is expended in respiration as a result of reproductive activity. These values are in reasonable accordance with those reported for other filter feeders but are higher than is generally found in other herbivores which indicates that filter feeding is an efficient method of obtaining energy for the other life processes (see below).

Table 39 shows the respiration rates of a standard Mercierella at winter and summer temperatures (Section 6.7.3) together with published rates of oxygen consumption for a variety of other aquatic invertebrates. In general, there is good agreement between these values, taking into account the effect of body size and temperature. The only exceptions being the surprisingly low oxygen consumption rate of the stone crab, Menippe mercenaria, larva considering the relatively high temperature, and the high respiration rate associated with activity in the gastropod, Littorina littorea. Assuming that the published oxygen consumption rates refer to the standard level of metabolism (see Section 6.6.1), unless it is stated to the contrary, it is evident that the respiration rates associated with feeding activity of the filter feeders, namely Crepidula, Mercierella, and Mytilus, are in accordance with the rates of oxygen consumption typical of standard metabolism of the more mobile organisms. This indicates that the filter feeding habit is relatively economical in terms of energy expenditure when compared with foraging types, since it obviates the considerable energy expenditure associated with locomotion, as is exemplified by the high oxygen consumption rate for active Littorina littorea.

Widdows and Bayne (1971) described the R component in their energy budget for Mytilus edulis as consisting of the energy equivalent of standard metabolism (the irreducible energy cost of maintenance), the energy equivalent of specific dynamic action, after Brody (1945),

and the energy cost of ventilation and filtration by the gills. Brody (loc. cit.) referred to SDA or calorigenic effect of food, as the heat liberated by the deamination of proteins, and stated that in homeotherms it usually represented about 30% of the metabolisable energy of protein. Recently, Bayne and Scullard (1977) reported an apparent SDA in Mytilus feeding on Tetraselmis suecica equivalent to about 8% of the assimilated energy. However, their respiratory evidence is somewhat less than convincing, and they failed to explain why there is no sign of SDA in Mytilus when feeding on Phaeodactylum tricornutum, which not only has a similar proximate composition of protein, carbohydrate, and fat to Tetraselmis but also amino acid composition.

It remains to determine whether a specific dynamic action exists in Mercierella when the worm is feeding on Brachiomonas submarina. What is certain is that in common with filter-feeding bivalves (see Thompson & Bayne, 1972) the mechanical aspect of filter feeding, namely transporting water through the branchial crown (Section 6.1) consumes a far greater part of Mercierella's metabolic energy than do trapping, ingestion, digestion, and assimilation, or the subsequent catabolic processes including deamination. As is shown by the absence of a significant alteration in the rate of oxygen consumption following the presentation of food (Section 6.6.3 and Section 6.6.4).

A common short-coming of energy budgets in general is that no attempt is made to quantify the energy expenditure associated with activity, which leads to the energy cost of metabolism being under-estimated. In many instances this omission is understandable since it would have been difficult to record activity in the respirometer setup, and many organisms are unable to function normally within the confines

Table 40. Percentage of assimilated energy lost via metabolism.

Species	Percentage	Reference
Carnivores		
Cirolana harfordi	60	Johnson (1976)
Neanthes virens	26	Kay & Brafield (1973)
Phagocata spp.	12 - 14	Teal (1957)
Root Spring: all carnivores	37	" "
Herbivores		
Fissurella barbadensis	73	Hughes (1971b)
Littorina irrorata	86	Odum & Smalley (1959)
Mercierella enigmatica	32, 40	
Nerita spp.	81 - 86	Hughes (1971a)
Pisidium virginicum	53	Teal (1957)
Root Spring: all herbivores	75	" "
Detritivores and deposit feeders		
Hyalella azteca	49	Hargrave (1971)
Hydrobia ventrosa	30 - 53	Kofoed (1975b)
Limnodrilus hoffmeisteri	74	Teal (1957)
Pteronarcys scotti	69	McDiffett (1970)
Scrobicularia plana	79	Hughes (1970)

of the respirometer vessels. In general therefore, the calculations of the calorific equivalent of R are based on what must at least approach the standard level of metabolism.

Table 40 shows the percentages of assimilated energy respired by Mercierella under winter and summer conditions, together with published values for a number of other organisms. Where necessary the values have been calculated from the published data. The higher value for the percentage loss of energy through metabolism by Mercierella at 20°C is due to the increased synthetic activity associated with gamete production.

In general, carnivores have significantly lower values for the percentage assimilated energy lost via metabolism than do herbivores, which reflects the differences in the quality of their foods. Animal tissues require the expenditure of less energy to digest and convert them to a form in which they can be used in catabolic and anabolic pathways, than do plant materials. This relationship can be obscured due to activity, e.g. the carnivorous isopod, Cirolana harfordi is reported as being active in the respirometer (Johnson, loc. cit.); which accounts for the higher proportion of its assimilated energy which was apparently channeled into metabolism.

Compared with the majority of other non-carnivores, Mercierella enigmatica lost a relatively small percentage of its assimilated energy via metabolism. This is partly due to it being a filter feeder, which is shown above to be a relatively efficient method for obtaining food. The other reason is that in the laboratory study it was fed with a pure culture of Brachiomonas cells (Section 3.6.1), whilst the remainder of the primary consumers, deposit feeder, and detritivores shown in Table 40 were on diets which contained at least a modicum of

Table 41. $\frac{P}{R}$ ratios for a variety of aquatic invertebrates.

Species	$\frac{P}{R}$	Reference
Carnivores		
Cirolana harfordi	0.68	Johnson (1976)
Neanthes virens	2.7	Kay & Brafield (1973)
Phagocata spp.	7.3	Teal (1957)
Herbivores		
Fissurella barbadensis	0.37	Hughes (1971b)
Mercierella enigmatica	1.41 - 2.02 ($20^{o}C$) ($10^{o}C$)	
Nerita spp.	0.14 - 0.24	Hughes (1971a)
Pisidium virginicum	0.9	Teal (1957)
Detritivores and deposit feeders		
Hyalella azteca	0.31	Hargrave (1971)
Hydrobia ventrosa	0.28 - 0.85	Kofoed (1975b)
Limnodrilus hoffmeisteri	0.36	Teal (1957)
Pteronarcys scotti	0.52	McDiffett (1970)
Scrobicularia plana	0.27	Hughes (1970)

indigestible material (detritus and/or inorganic sediment). The notable exception is Hydrobia ventrosa which had a similarly low value of 30% when feeding on a pure culture of protein-rich bacteria, which contrasts with the high value of over 50% of its assimilated energy when it was feeding on a mixture of relatively indigestible hay and bacteria (Kofoed, loc. cit.).

It follows, that those organisms which expend less of their assimilated energy in maintenance and activity have potentially more available to channel into production. It is common practice in energetics studies to compare the amounts of energy in the P and R components of the energy budget by means of the P : R ratio. Table 41 shows $\frac{P}{R}$ values for winter and summer Mercierella and those for a variety of other organisms. It is evident from these data that, in general, the converse of the relationship demonstrated in Table 40 applies, i.e. carnivores have higher P : R ratios than do herbivores, detritivores, and deposit feeders. Hence there is a direct relationship between food quality and productivity, other factors being equal. This is particularly well demonstrated by Kofoed's data for Hydrobia ventrosa which show a three-fold increase in this coefficient when the gastropod was feeding on bacteria, compared to when the food was less digestible hay.

Both the filter feeders, Mercierella and Pisidium, have high coefficients compared with the other non-carnivorous members, which supports the hypothesis that they are relatively efficient feeders. Part of the reason for Mercierella's P : R values being higher than that for Pisidium is because more production components were measured. The very high coefficient for the two planarians (Phagocata spp.) studied by Teal (loc. cit.) is due to the inclusion of mucus in the

production value. Whilst this is correct in the case of these organisms since the majority of the mucus is lost from their bodies immediately after it is secreted, it is perhaps misleading to include this in the case of Mercierella because the majority is ingested together with the food. Excluding mucus production from the calculation reduces the P : R ratios for the winter and summer from 2.02 and 1.41 respectively, to 0.87 and 0.71, which are in good agreement with the value of 0.9 for the fingernail clam, Pisidium virginicum, in Root Spring (Teal, loc. cit.).

McNeill and Lawton (1970) examined the relationship between production and respiration in long-lived and short-lived poikilotherms, which they separated in the basis of an arbitrary age of two years. From data for forty two different species they concluded that poikilotherms with short generation times have a tendency to lose less of their assimilated energy via metabolism than do those where a proportion of the population is more than two years old. They explained these phenomena as being due to, on the one hand, short-lived poikilotherms tending to endure non-productive periods as resting and hence low respiratory stages; these are typified by high net production efficiencies. On the other, long-lived poikilotherms experience extended "non-productive periods" with concomitant high respiratory costs, and hence such organisms as these have low net production efficiencies.

Although the Greenhithe population contains a proportion of individuals which are over two years old (Fig. 63), the majority live for only two years. Some however, probably live for as long as four - five years, although these are in the minority. Mercierella therefore

conforms in the older group by definition but it shares greater similarity with the short-lived types since it has low respiratory cost-high production characteristics, with associated high net production efficiencies. However, it can be seen that far from being caused by the display of low R resting stages during inclement periods, Mercierella maintains a high production : respiration ratio throughout the winter period (Table 41) due to the amen..able conditions in the Thames estuary. It should be pointed out that in the present study production includes mucus secretion, whereas McNeil and Lawton (loc. cit.) used only somatic growth plus gamete production, and their calculations of net growth efficiency, $\frac{P}{A} \times \frac{100}{1}$, are based on $R + P = A$. It is evident therefore that, as already suggested by Hargrave (1971), their values for assimilation are underestimated by the fraction of assimilated energy that is excreted, or lost as soluble organic compounds, and likewise their productivity values are underestimated by that proportion of the anabolic component which is lost as a result of leakage or exudates such as mucus. However, when mucus is excluded from the production calculation (see above) this opportunist species still enjoys a relatively high level of productivity throughout the winter period (see field growth studies, Section 8.1.3).

Kay and Brafield (1973) attempted to explain the high production : respiration ratio for Neanthes virens in terms of its apparent inactivity compared with other errant polychaetes. In this manner they suggested that relatively little of its energy is lost in the form of metabolic energy thus leaving a high proportion available for production. Teal (loc. cit.) went so far as to suggest that, in the case of the planarians he studied in Root Spring, these have evolved low oxygen consumption rates (low metabolic cost) as a means of off-

setting the relatively large amounts of energy they lost as mucus. Attempting to demonstrate this Teal incorrectly added the energetic costs of R and mucus production, the sum of which he then compared with the assimilated energy and concluded that the resulting 69% is much more in accordance with the percentage assimilated energy lost via metabolism "by the other animals". It can be seen, however, from Table 39 that there is a surprising degree of similarity between the oxygen consumption rates of these and other organisms. Furthermore, these are all in good agreement with the relations between body weight and metabolic rate for marine animals (Zeuthen, 1953), and also for poikilotherms as demonstrated by Hemmingsen (1960). Therefore, rather than having abnormally low R values, these organisms in fact have relatively high values for P, which in the case of the planarians is due to the inclusion of the energy lost as mucus in the production component.

There has been a tendency in energy budget studies to omit the energy lost via excretion (e.g. Hughes, 1970, 1971a, b; Johnson, 1976) either on the grounds that it was difficult to measure under the experimental conditions and so the value for U has been treated as being negligible, or as in the case of Widdows and Bayne (1971) it was assumed that the energy lost as ammonia represented zero calories to the animal. Apart from the products of deamination namely ammonia and to a lesser extent urea in marine invertebrates (Section 7.0), in recent years it has been recognised that many aquatic invertebrates also lose considerable amounts of soluble organic matter in the form of amino acids, either by excretion (e.g. Bayne, 1973b) or leakage across the body wall (see Jørgensen, 1976).

There appears to be some confusion in the literature as to what is meant by the excretory component U, for example Paine (1971) estimated that Tegula funebralis lost about 10% of its assimilated energy via excretion "probably mostly as mucus"; Hargrave (1971) calculated that Hyatella azteca lost 5% of the ingested energy as soluble excretory products, urine and amino acids across the general body surface; whilst Kay and Brafield (1973) in their study of Neanthes virens, as in the present investigation of Mercierella, treated the U component strictly as ammonia production. It is incorrect to consider mucus as part of excretion since it represents part of production as probably do some of the soluble organic compounds. This results in the production values being in some instances considerably underestimated, for example Kofoed (1975b) demonstrated that Hydrobia ventrosa lost 30% of the assimilated carbon in the form of dissolved organics and mucus. However, it is likely that a considerable part of these materials are unassimilated products of digestion which are lost either directly from the gut lumen through the anus, or rapidly leach out from the faecal pellets, the so-called 'faecal excretion'.

Mercierella enigmatica is estimated to lose 3.4% of its assimilated energy as ammonia in the winter, and 4.2% in the summer. There is a three-fold increase in the absolute amount of nitrogenous excretion in the summer compared with the winter period. These percentages are in good agreement with the value of 3% quoted by Kay and Brafield (loc. cit.), although this is slightly underestimated due to their using an incorrect value of 4.05 cal mg^{-1} for ammonia derived from the erroneous method of calculation presented by Brafield and Solomon (1972). When their value for ammonia excretion by Neanthes virens was corrected using the value of 4.9 cal mg^{-1} (Section 6.8 and

and Section 7.1) this gave 3.5% of the total assimilated energy, which is intermediate to the average values in winter and summer for Mercierella when feeding on a B. submarina suspension.

Whilst the results of the dissolved organic uptake experiments suggest that there may be no net loss of soluble organic materials across Mercierella's general body surface, due to the postulated homo-exchange diffusion mechanism (Section 4.10), it is possible that appreciable amounts of soluble organic materials were lost from the gut either directly via the anus, or due to 'faecal excretion'. Kofoed (1975b) found that 13% of the ingested C leached from the faecal pellets. This would account for the discrepancy between the assimilation efficiency for the summer animal based on $\underline{A} = \frac{\underline{I} - \underline{E} \times 100}{\underline{I} \quad 1}$ and $\underline{A} = \underline{P} + \underline{R} + \underline{U}$. It will be remembered that using the former an assimilation efficiency of about 61% was recorded, whereas by summing the production, metabolic, and excretory components only about 40% of this amount could be accounted for. Although certain components may have been underestimated. e.g. protease and body mucus, (by necessity averages have had to be used, and the errors resulting from these and the estimated Q_{ox} and Q_{ex} values could have been cumulative rather than cancelling each other out as is usually assumed) one might still have expected the two estimations to have differed by no more than 20%, as is suggested by Gabbott and Bayne (1973).

Assuming that the animals were not physiologically stressed, which is known to induce a rapid loss of amino acids in Mytilus (see Gabbott & Bayne, loc. cit.) it is possible that the anomaly between the two equations is due to leakage from the gut or faeces. This is supported by the high digestive efficiency, $\frac{\text{wt. of ingested material} - \text{wt. of faeces}}{\text{wt. of ingested material}}$, of about 65% on an ash-free dry weight basis. Although this discrepancy has no affect on the

calculations used above, it does suggest that Mercierella may have been digesting considerably more food than was needed to supply the energy it required. There are two possible explanations for this. First, in the natural environment this organism ingests a large proportion of inorganic material (Section 3.1) which has the effect of diluting the digestible material. To counteract this the worm may produce a relatively large quantity of digestive enzymes to ensure that it obtains sufficient nutrients for its needs. Zottoli and Carriker (1974), however, found little or no protease in the faecal pellets of Hydroides dianthus which had been freshly removed from the field. Second, it is unlikely that B. submarina represents a balanced diet for Mercierella, in which case it may have to digest an excess of food to obtain its minimum requirement of some essential nutrient (e.g. amino acid), the excess is then lost from the anus in solution. This mechanism has also been proposed by Harvey (1950, in Butler et al. 1969) to explain the superfluous feeding behaviour of zooplankton. Obviously, great care has to be taken in the interpretation of the results of energetics studies conducted in the laboratory.

In summary, the results of this laboratory investigation of the energy relations of Mercierella enigmatica show that under winter conditions of the total energy assimilated 36.9% is accounted for by mucus production, 32% is respired, 21.7% is incorporated into the tube, 6% is laid down in body tissues, and 3.4% is excreted. In the summer 40% is respired, 27.9% is secreted as mucus and 18.7% as tube material, 5% is incorporated into body tissues, reproduction accounts for 4.5%, and nitrogenous excretion 4.2%. The uptake of exogenous dissolved organic molecules via the external body surface appears to be an insignificant source of energy compared with particulate feeding.

It is, however, prudent to draw attention to the following facts. In the field the proportion of organic material in the suspended solids is only 1% by weight and consists largely of detritus (Section 2.3.4 and Section 3.4.3). If Mercierella selects its food purely on the grounds of particle size (Section 3.1), and both types, organic and inorganic, have similar probabilities of being ingested, i.e. of similar size and mass, it follows that to ingest the same weights of organic materials as were ingested under laboratory conditions, a 1 mg worm would have to consume 0.607 mg dry weight of suspended solids h^{-1} in the winter or about 14.6 x its dry weight d^{-1}. A similar animal in the summer would need to ingest 1.415 mg dry weight of suspended solids h^{-1} or nearly 34 x its dry weight d^{-1}. This is assuming that the percentage assimilation of organic materials is the same in the field as it is in the laboratory. Under these conditions it is evident that the worm would have to process relatively enormous quantities of largely indigestible material to obtain similar amounts of energy to those observed in the laboratory.

Since the results of the feeding experiments (Section 3.2 and Section 3.3) show that Mercierella is unable to feed at a sufficiently high rate to meet the above requirements, it seems that if the worm is to obtain sufficient energy for its needs it must assimilate a greater proportion of the ingested organic materials. Detritus, however, is composed largely of apparently indigestible materials, e.g. lignin and cellulose (Kofoed, 1975b; Tenore, 1975), and it has been demonstrated that it is the micro-organisms growing on the surface of the detrital particles which constitute much of the assimilated food of detritivores (e.g. Fenchel, 1970, 1972; Kristensen, 1972; Newell, 1965, 1970). (Although some marine invertebrates are capable of producing their own cellulases, including the sabellid, Sabellastarte indica (Yokoe & Yasumasu, 1964), as yet no serpulid has been shown to have the ability to digest cellulose.) Furthermore, the

presence of inorganic material is known to have a depressing influence on assimilation efficiency and production efficiences through its effect on the percentage of assimilated energy that is lost due to metabolism (see above). Thus there is circumstantial evidence which suggests that unless Mercierella feeds selectively and/or the micro-organisms are especially rich in energy, the worm will not be able to sustain the rate of growth etc. which was observed in the laboratory (Section 8.1.3 and Section 8.1.4).

Since growth may have been faster under field conditions (Section 8.1.3) than it was in the laboratory, it seems likely that some food component may have been overlooked, namely soluble organic compounds!. Fox et al. (1952, 1953) suggested that a considerable proportion of the 'dissolved' organic material in sea water may be in the form of aggregates (micelles) of colloidal dimensions, a result of the molecules becoming adsorbed on to minute inorganic particles (see Stephens, 1962a). These particles are of sufficient size to be trapped by the mucous secretions of filter-feeders and ultimately to be ingested as particulate food (for references see the review by Jørgensen, 1976). In view of the large quantities of fine,particulate, inorganic material which are present in the water column in the Thames estuary, it is feasible that Mercierella enigmatica may derive a major part of its energy from the organic molecules that are associated with the inorganic fraction of its diet.

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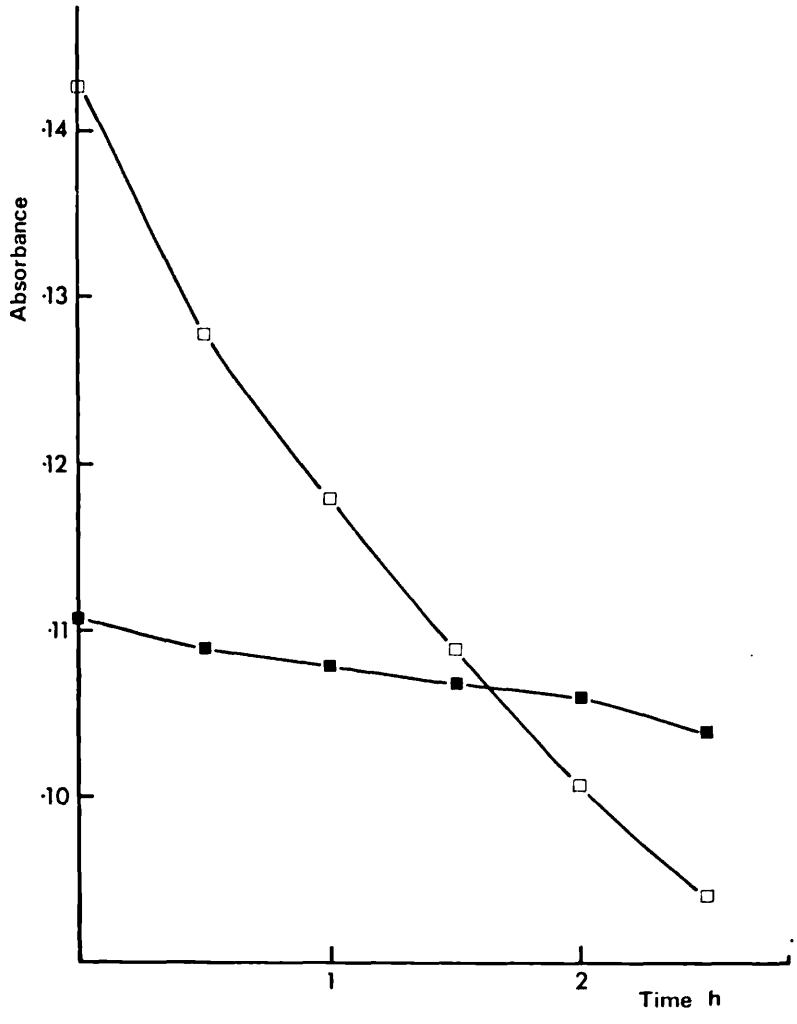
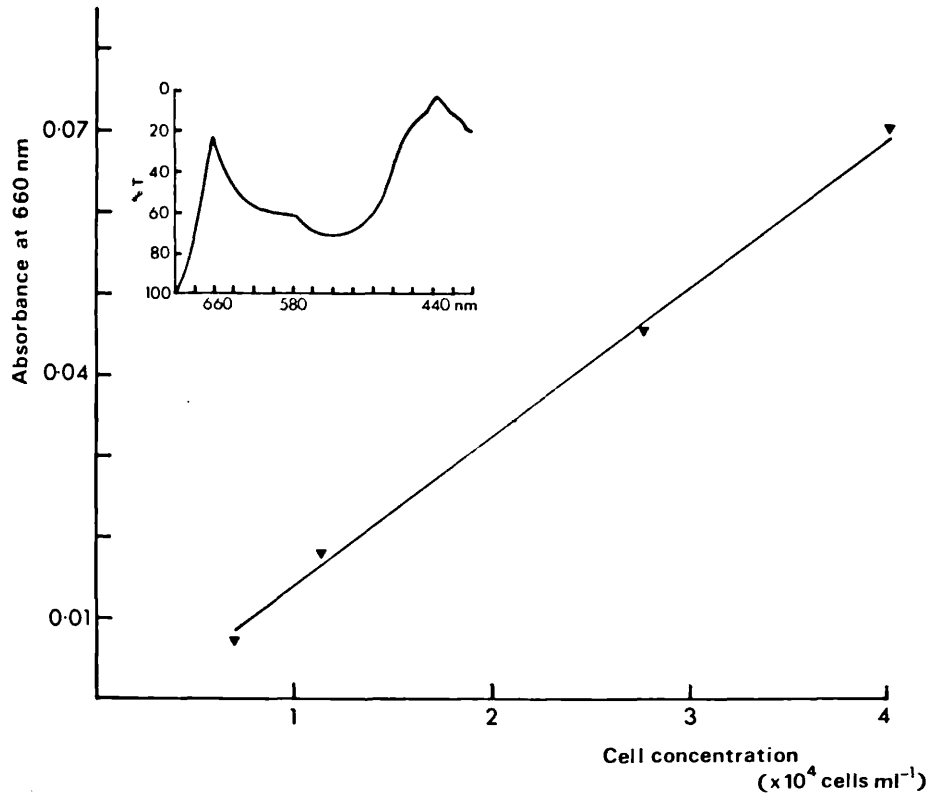
11. APPENDICES

Appendix 1.1. The effects of the filter-feeding of four adult *M. enigmatica* on the *Phaeodactylum* cell concentration in a 4 ml spectrophotometer cuvette (open squares), read as % transmission and converted to absorbance units (see Bender, 1972). Closed squares are the cell concentration changes which occurred simultaneously, due to settlement, in a control cuvette, lacking animals.


Absorbance
·14
·13
·12
·11
·10
1
2
Time h

Appendix 1.2. The relationship between Brachiomonas cell concentration and absorbance, read at 660 nm as % transmission, in a 10 mm square glass cuvette. The inset shows the absorption spectrum for an acetone extract of macerated B. submarina cells (for method see Holden, 1965).


Absorbance at 660 nm
0·07
0·04
0·01
1
2
3
4
Cell concentration
(x10⁴ cells ml⁻¹)
%T
0
20
40
60
80
100
660
580
440 nm

Appendix 1.3. Individual ingestion rates at winter and summer temperatures .

Temperature:	10°C	20°C	
Wet weight (mg)	Ash-free dry wt. of matter ingested h^{-1} (µg)	Wet weight (mg)	Ash-free dry wt. of matter ingested h^{-1} (µg)
0.84	0.57	2.23	9.25
1.12	0.05	2.53	13.44
1.46	0.36	2.73	10.33
1.6	0.22	3.02	7.67
2.68	0.2	3.08	10.14
3.11	0.09	3.35	7.92
3.5	0.09	3.37	12.08
3.96	0.23	4.29	7.54
4.04	0.93	5.28	6.6
4.2	0.36	5.54	24.38
5.06	0.88	5.82	6.56
5.24	0.96	7.0	11.33
7.82	0.18	8.22	19.02
8.3	1.06	9.79	11.56
8.38	0.16	11.0	33.06
8.58	0.08		
9.57	0.52		
10.05	1.53		

Appendix 1.4. Protein analysis of Brachiomonas submarina.

Sample dry wt. (mg)	Total Nitrogen (µg)	% Nitrogen	% protein (calculated)
1.6	215.52	13.47	84.19
2.0	303.41	15.17	94.82
2.2	295.32	13.42	83.9
2.8	342.0	12.21	76.34
3.3	438.73	13.29	83.09
3.7	453.72	12.26	76.64

Appendix 1.5. Lipid analysis of Brachiomonas submarina.

Sample dry wt. (mg)	Estimated dry wt. of lipid (mg)	% lipid	% lipid ash-free dry wt.
0.8	0.1	12.5	13.09
0.95	0.05	5.26	5.51
1.0	0.04	4.0	4.19
1.15	0.15	13.04	13.66
1.2	0.15	12.5	13.09
1.5	0.1	6.67	6.99

Appendix 1.6. Ash content of Brachiomonas submarina.

Sample dry wt. (mg)	Dry wt. of ash (mg)	% ash
0.8	0.03	3.75
0.95	0.05	5.26
1.0	0.05	5.0
1.15	0.05	4.35
1.2	0.05	4.17

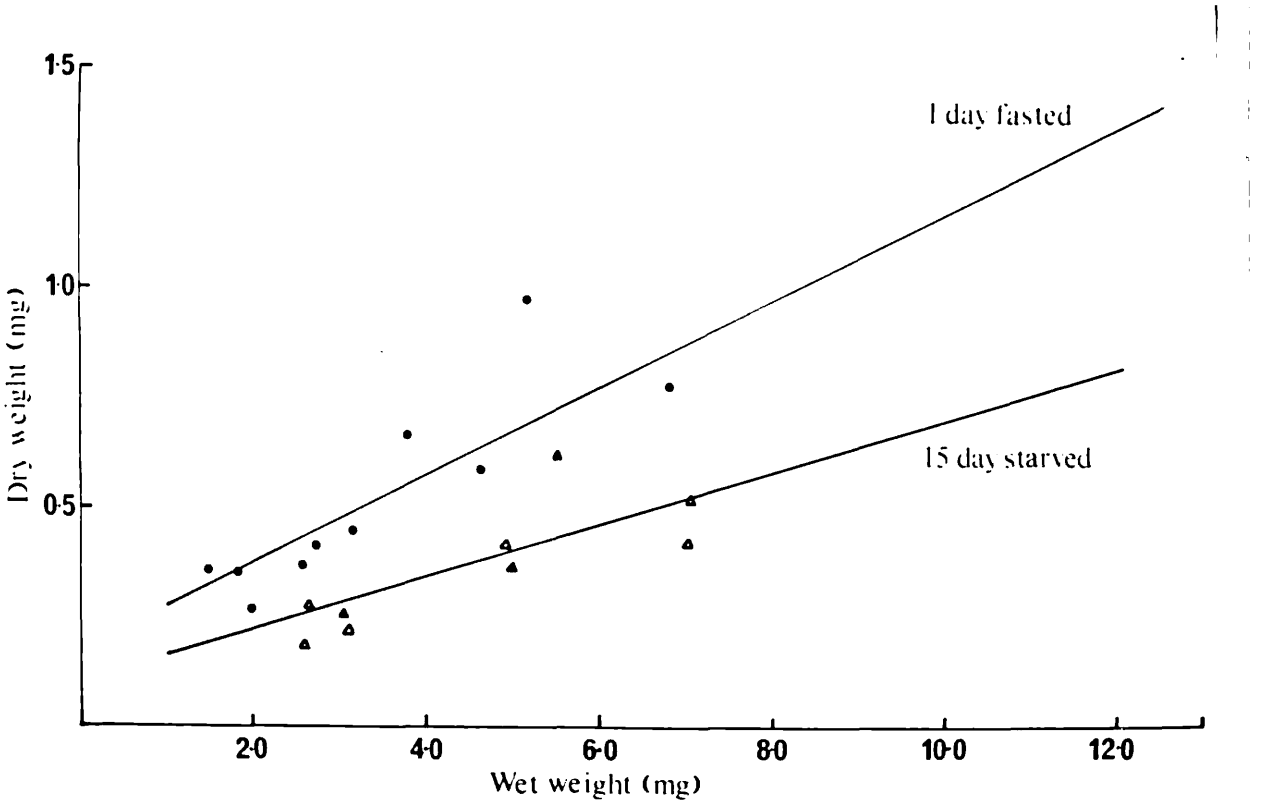
Appendix 1.7. Dialysed dry weight of a B. submarina cell.

Wt. of cells in one ml of 8 d old culture, with an estimated $37.55 \pm 7.74 \times 10^4$ cells ml^{-1} (mg).	Estimated dialysed dry wt. per cell ($\times 10^{-6}$ mg).
0.3957	1.05379
0.3964	1.05565
0.4029	1.07296
0.4029	1.07296
0.4098	1.09134
0.41475	1.10452
0.41935	1.11677
0.4233	1.12729
0.43805	1.16657
0.4553	1.21251

Appendix 1.8. Calorific content of B. submarina cells.

Sample dry wt. (µg)	Calorific value (cal)	cal mg^{-1}	cal mg^{-1} ash-free dry wt.
35	0.1275	3.64	3.81
60	0.33	5.5	5.76
80	0.38	4.75	4.97
80	0.345	4.31	4.51

Appendix 1.9. Relationship between wet weight and dry weight for fasted ⊛, and starved ▲ M. enigmatica. The regression lines were fitted by the method of least squares and extrapolated; ⊛, r = 0.77, P = <0.01, $\hat{Y}$ = 0.18 + 0.1X; ▲, r = 0.78, P = <0.02, $\hat{Y}$ = 0.1 + 0.06X.

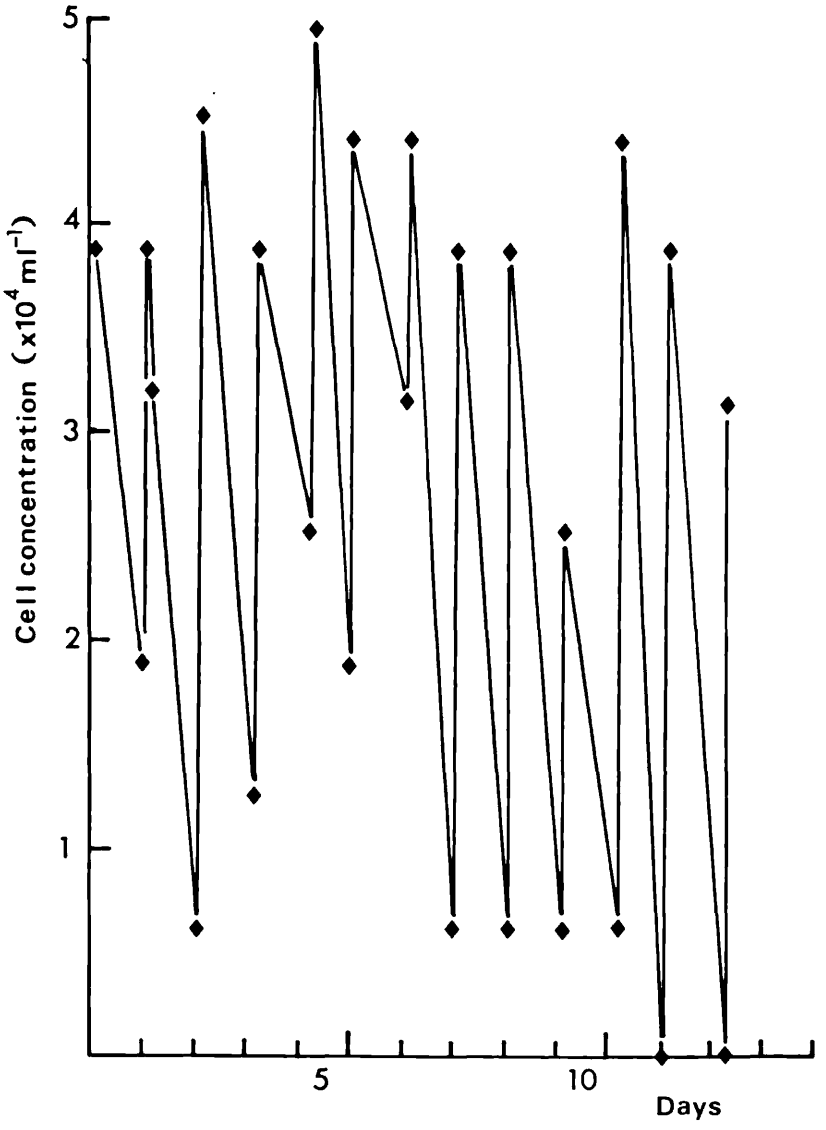

1·5
1·0
0·5
Dry weight (mg)
1 day fasted
15 day starved
2·0
4·0
6·0
8·0
10·0
12·0
Wet weight (mg)

Appendix 1.10. Cumulative $^{14}CO_2$ production by fasted (2 days) and starved (16 days) M. enigmatica, following a 1-h exposure to 2.0 µM l^{-1} ^{14}C-glycine.

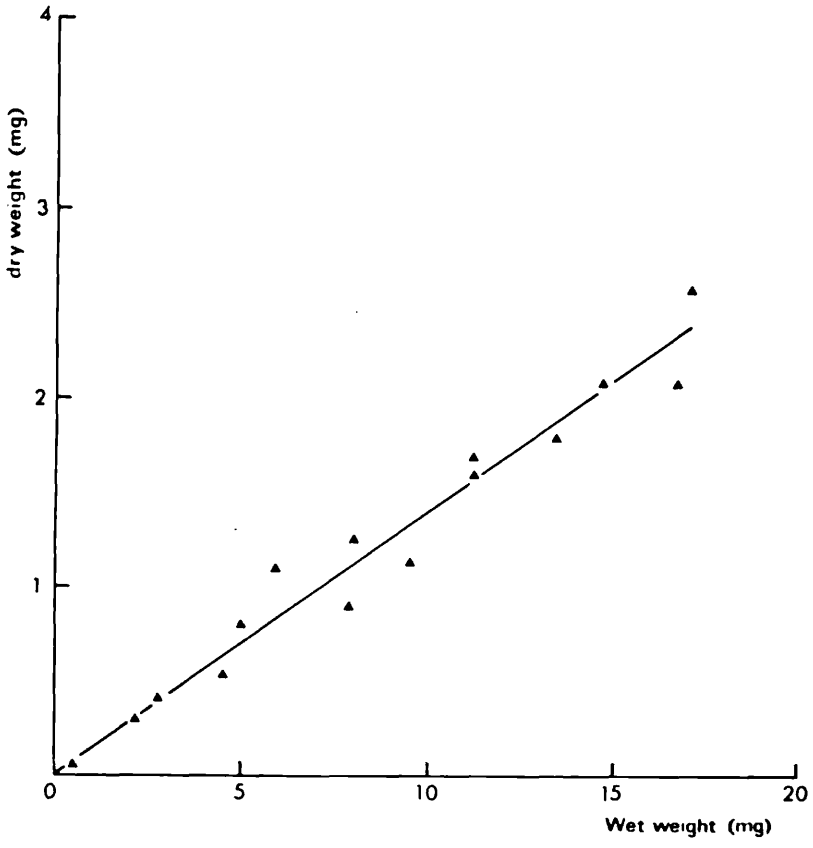
($\times 10^4$ d.p.m. individual h^{-1})

	Fasted worms				Starved worms			
Time (h)	n	Mean	S.D.	S.E.	n	Mean	S.D.	S.E.
1	10	1.69	0.89	0.28	9	2.56	1.17	0.39
2	9	4.13	1.55	0.52	10	6.68	2.76	0.87
3	9	6.77	2.56	0.85	10	10.57	4.67	1.48
5	8	12.74	3.12	1.10	10	17.42	6.91	2.19
7	8	16.58	2.72	0.96	10	21.44	7.94	2.51
9	7	18.62	2.54	0.96	10	24.11	8.73	2.76

Appendix 1.11. Recorded daily fluctuations in Brachiomonas cell number in a 6 l tank containing M. enigmatica used in the laboratory growth study, and maintained at 10°C. The algal cell density was measured in a Gallenkamp Mk 3 Colorimeter that had been calibrated with cell counts made with a haemacytometer slide.


Cell concentration (x10⁴ml⁻¹)
5
4
3
2
1
5
10
Days

Appendix 1.12. Relationship between wet weight and dry weight of M. enigmatica during the post-spawning period (November). The regression line was fitted by eye estimation.


dry weight (mg)
4
3
2
1
0
5
10
15
20
Wet weight (mg)