THE ENERGETICS OF *COROPHIUM VOLUTATOR* (Pallas)

by

DAVID EDWIN MOSSMAN

Thesis presented to the University of London

for the degree of Doctor of Philosophy

NOVEMBER 1977

ABSTRACT

An energy budget has been constructed for the mud-dwelling amphipod *Corophium volutator*. Population production was estimated from field samples while metabolism was calculated by applying the results of laboratory measurements to a model of the population.

The measurement of ingestion rates and assimilation efficiency proved difficult and indirect methods were found to be necessary in order to calculate energy intake. Observations and experiments on the feeding biology of *Corophium* are described and the possible effect of the population on algal productivity in the salt marsh is discussed. Some of the difficulties involved in the study of invertebrate feeding energetics are pointed out.

Of the total estimated energy intake, less than 30% was absorbed and assimilated. Gross growth efficiency was in the order of 10% but production was high in relation to assimilation - 40% of assimilated energy went into production and the ratio of production to mean biomass was also high.

The potential importance of *Corophium* as a component of salt marsh energy flow is discussed.

ACKNOWLEDGEMENTS

I am grateful to R.P.Dales, Professor of Zoology at Bedford College, in the first instance for providing the opportunity to carry out this study and, secondly, for much useful advice and constant encouragment throughout.

I should like to thank Chief Technician D.J.S.Field and his staff for material assistance. Thanks are also due to Z. Podhorodecki for the photographs, Mr. S. White for the construction and patient modification of the bomb calorimeter and Mr. C. Funnel for the loan of the Micromat Combustion apparatus.

I would also extend my gratitude to Mr. Les Ellam, for his encouragment during the course of the work, and to my wife for her continued support and patience during the preparation of the manuscript.

This work was supported by an award from The Science Research Council.

THE ENERGETICS OF *COROPHIUM VOLUTATOR* (PALLAS)

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Tidal mudflats and salt marshes provide a habitat for a number of ecologically important mud dwelling animals. The amphipod crustacean *Corophium volutator* (Pallas) is one such animal which is common around the coastlines of Great Britain and North West Europe. The best known of the 32 species which make up the genus (9 have been reported in Britain- Crawford, 1937) *C. volutator*, although patchy in it s distribution, is locally very abundant, favouring intertidal muds with high silt and organic contents where densities in excess of 50,ooo m^{-2} are probably not uncommon.

When present in such numbers *C. volutator* must be an important influence on energy flow in the community. In parts of Benfleet Creek, Essex, for example, it is clearly the dominant species (mean population density 35,000 m^{-2}) and has a visible effect on the ecology of the salt marsh.

In some estuaries *C. volutator* may be an important source of food for wildfowl. Goss-Custard (1969) stated that redshank (*Tringa totanus*) took between 16 and 38% of the autumn population in the Ythan estuary in North East Scotland. The birds appeared to have a preference for *C. volutator* as a prey species. It is undoubtedly also an important food for estuarine fishes like flounder and eels.

In view of the potential impact of *C. volutator* populations on the ecology of estuarine mudflats it was felt that a study of the energetics of one such population in the Thames estuary might be informative. Estimates of production and energy flow can be useful in assessing the importance of animal populations in a given ecosystem. Hughes (1970), for example, estimated the annual energy budget for a population of the bivalve *Scrobicularia plana* on a mudflat in North Wales while Kay & Brafield (1973) used the results of laboratory maintenance experiments to estimate annual energy flow through the population of the worm *Neanthes virens* at Southend-on-Sea.

At the time of writing I am aware of no published information on the productivity of *C. volutator* although the animal has been studied as part of a wider programme to assess production and trophic relationships in the Ythan estuary (Milne & Dunnet, 1972).

Other aspects of it s biology have been well covered in the literature. It s responses to salinity have been studied by McLusky (1967, 1968, 1969, 1970) while Meadows (1964 a, b & c,1967) has discussed substrate selection in some detail. General behaviour has been described by Meadows & Reid (1966) and the reader is also referred to Hart's (1930) informative account of the species. Clay's (1965) exhaustive literature survey contains a good deal of information from a variety of sources.While the animal has been described very thoroughly elsewhere, a brief

outline of it s natural history will, it is hoped, help in the interpretation of later discussions.

Tubicolous in habit *C. volutator* excavates a U shaped burrow (seldom more than 5 cm in depth) in the upper layers of soft intertidal muds. Most of it s time is spent in the burrow where it feeds, using enlarged antennules (a diagnostic feature of the genus) to rake surface sediment and organic detritus towards the opening. Here material is actively sorted by the gnathopods and maxillipeds. It is a selective deposit feeder (Hart, 1930, Fenchel *et al* 1975) discarding the larger particles of sediment and detritus. Newell (1965) suggested that deposit feeders probably get the major part of their energy supply from the microorganisms associated with sand grains and organic detritus. Epibenthic algae undoubtedly form part of *C. volutator*'s diet- I have observed the animal feeding actively upon a variety of diatomaceous material. Early reports from Bate & Westwood (1863) of it feeding as a predator,while charming and evocative, appear to have no foundation. Life span is rather less than a year (Watkin, 1941) with reproductive animals present in the population throughout the spring and summer months. A single female may give rise to two or more broods and the eggs (mean no. 26) are held in the brood pouch until the young hatch and undergo their first moult (Hart, 1930). The offspring reach sexual maturity (around 5 mm in length) after about 2 months. The life cycle of the Thames population will be discussed

more fully in section 2.

Ecological energetics is concerned with the fate of energy entering an ecosystem or population (energy flow). Since the now classic studies of Teal (1957) and Odum (1957) there has been considerable interest in the energetics of population and this has led to the development of an equation which describes, in simplified form, the major components of energy flow. Following I.B.P. terminology (Ricker, 1968, Holme & McIntyre, 1971) the energy budget for a population or organism is as follows:

$$C = P + R + F + U$$

where C = *Consumption* - the total energy consumed as food.

P = *Production* - energy assimilated and used for growth and reproduction.

R = *Respiration* - energy assimilated and converted into heat of metabolism.

F = *Egesta* - the energy not absorbed but voided in the faeces.

U = *Excreta* - the energy released as the waste products of metabolism and exudates such as mucus.

The equation above is in it's simplest form. Production can be subdivided into growth and gonad output,

although the distinction has not been drawn in this work since production could only be estimated from fluctuations in standing stock biomass in a natural population. A separate estimate of gonad output would be inappropriate since part of this component, the energy derived from the egg by the developing embryo, contributes to population production. *Growth or Production efficiency* ($^{P}/_{C}$) may be as high as 70% in a developing embryo (Holme & McIntyre, 1971). The energy content of sperm and undeveloped eggs, on the other hand, cannot be taken into account so that production may be slightly underestimated.

Assimilation has been defined as that part of the consumption (C) which is utilized for physiological purposes, that is to say, for respiration and production (A= P + R). Holme & McIntyre have discussed the distinction between energy absorbed from food and that which is actually assimilated and converted into physiologically useful energy. The I.B.P. definition of *Assimilation efficiency* ($\frac{C - F}{C}$) is clearly not strictly consistent with this earlier definition of assimilation and would be more accurately termed *Absorption efficiency*. In order to avoid confusion the term assimilation efficiency will be used in the accepted sense as the proportion of energy consumption which is absorbed into the organism - $\frac{C - F}{C}$.

It has been the aim of this work to use the equation to construct a model of energy flow through a population of *C. volutator* . Ideally the construction of an energy

budget along these lines should be based upon independant measurment of all the major components. This may not be possible in all cases so that one or more of the terms in the equation must be found by difference or by indirect means. Such was the case in Hughes' (1970) budget for *S. plana* where consumption was calculated from rates of faeces production and the calorific values of food and faeces.

Many of the techniques used in energetics rely upon laboratory measurments. Feeding and metabolism studies can rarely be carried out in the field and conditions in the laboratory may not accurately reflect those in nature. To the purist this is perhaps seen as the major limitation of ecological energetics and yet there is no reason why, provided that adequate attention is paid to the design of experiments and interpretation of reults, laboratory techniques should not provide valid data. Indeed it is on this precise basis that most energy budgets have been evaluated. Hargrave (1971) for example, like Hughes, has estimated production alone from field data while all other perameters were calculated from laboratory experiments. Kay & Brafield (1973) took this a step further and applied the results of a series of laboratory maintenance experiments to a model of a *Neanthes virens* population.

In the present study laboratory data has been used to calculate Consumption (C), Respiration (R)and Excreta (U) while Production (P) has been estimated from field

studies. Direct measurement of Egesta (F) was deemed to be impracticable and this component has been calculated as the difference between consumption and absorption. (Absorption is equivalent to assimilation - A = P + R + U).

To comply with the recommendation of the Royal Society (1972) the energy unit adopted in this work is the joule (J). Since most previous studies have used the calorie as the unit of measurement conversions are given throughout to facilitate comparisons.

2. PRODUCTION

2.1. Introduction

The calculation of the term P in an energy budget can be approached in two ways. The growth of individuals can be monitored under controlled laboratory conditions (Kay & Brafield, 1973, Nilsson, 1974) or alternatively, production can be estimated for a population in the field (Hughes, 1970, Hargrave, 1971). The two methods are not strictly comparable since growth rates may differ considerably depending, for example, upon the nature of the food supply (Swiss & Johnson, 1976), so that rates determined in the laboratory may not accurately reflect those achieved in nature.

Attempts to measure growth of *Corophium* in the laboratory met with little success. While size frequency analysis showed an apparent increase in the size of the animals in stock tanks mortality was such that the shifts in modal size classes could not, with certainty, be attributed to growth. Production was, therefore, estimated from a study of the dynamics of a population inhabiting a tidal creek in the lower Thames estuary. The calorific value of *Corophium* was determined so that production and standing stocks could be expressed in terms of energy units rather than biomass.

2.2. Materials & Methods

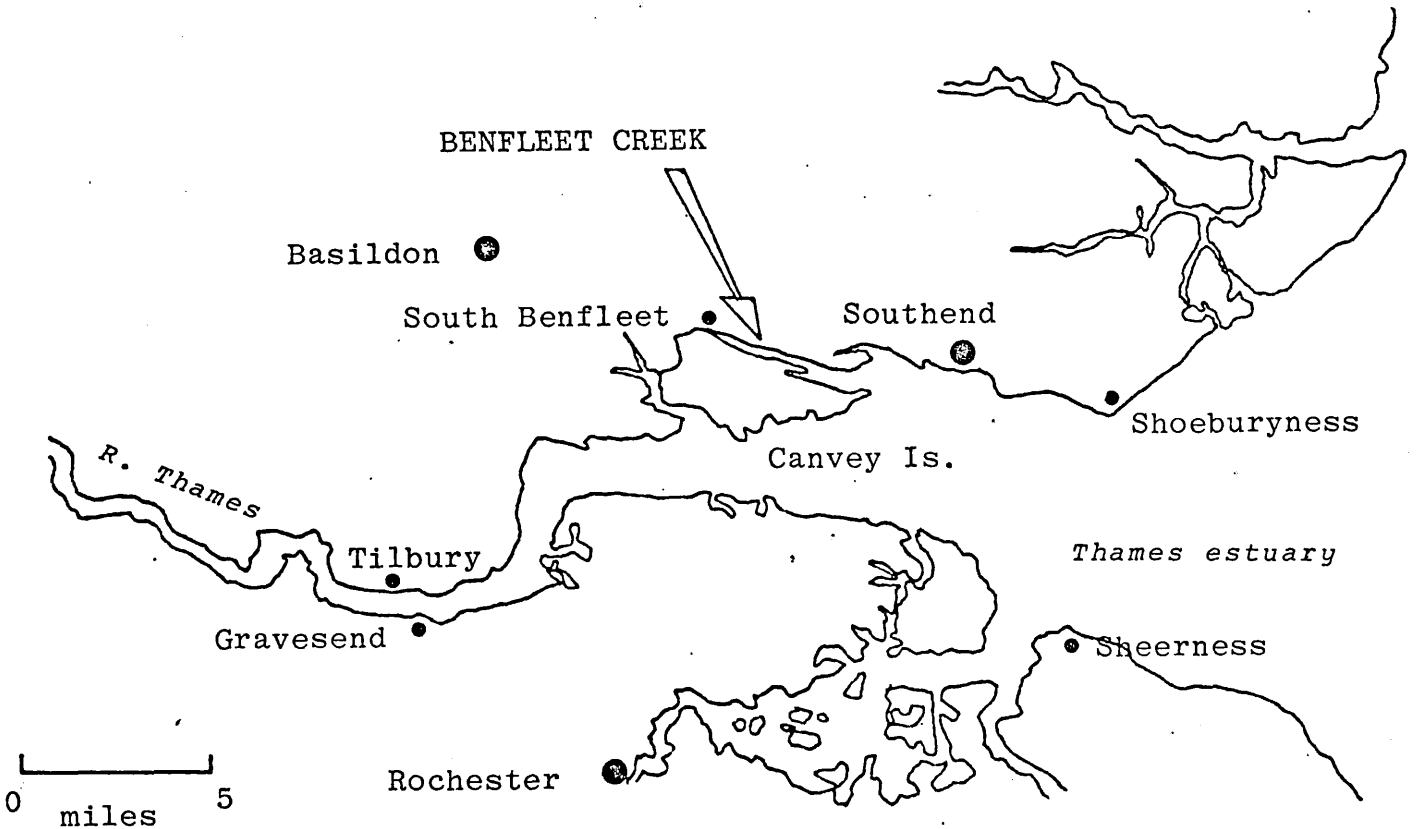
2.2.1. Sampling locality - Benfleet Creek, Essex.

The population studied inhabits a belt of mud along the upper shores of a tidal creek at South Benfleet, Essex. The creek, which separates Canvey Island from the mainland, lies on the north bank of the Thames estuary some 11 km from it's mouth at Shoeburyness and a little over 60 km by road from Central London (fig. 1).

Because of it's seaward location the area is fairly free from pollution and is subjected only to minor fluctuations of salinity (Gee, 1961). Estuarine water enters at both ends of the creek and mixes at a point just west of the Canvey Island road bridge. There is little in the way of fresh water drainage into the creek so that changes in salinity over the tidal cycle are small while the normal range lies between about 27 and 31% .

The creek supports a particularly large population of *Corophium volutator* in a well defined zone along it's north bank. The substratum of the *Corophium* beds is characterized by a layer of soft mud (about 7 - 8 cm deep) with high silt and water content, overlying firmer, anoxic London Clay. The zone extends eastwards from the road bridge for about 0.75 km after which *Corophium* quickly disappears as the substrate becomes firmer and it's drainage characteristics better. Gee felt that drainage was one

Fig. 1. Map of the Thames estuary showing the location of Benfleet Creek



Plates 1 & 2. Benfleet Creek - looking towards Cancey Island.

Plates 3 & 4. Benfleet Creek - the sampling site.

of the most important factors affecting the distribution of *Corophium* in Benfleet creek. My own observations have indicated that although the *Corophium* zone is only under water for between 2 and 3h in the tidal cycle, the substrate does not dry out appreciably at low water. This means that the animals are able to move about and feed throughout the day.

2.2.2. Sampling method and analysis

Monthly samples of the Benfleet population were obtained from a site near the seaward limit of the *Corophium* zone. Sampling was over a period of 12 months from November of 1975 and on each collection date replicate samples of mud were taken using a rectangular corer with an area of 0.01 m^{-2}. The corer was a simple metal frame which was pushed into the sediment to a depth of 7 - 8 cm (below which the animal did not burrow). Sediment blocks obtained in this way were taken back to the laboratory for analysis. The use of larger samples, while reducing potential sampling error, was found to be impracticable owing to the large numbers of animals present and the sheer volume of mud to be processed.

Once in the laboratory cores were washed in a sieve of mesh size 250 μm. The greater part of the sediment material was thus discarded so that animals could be separated, visually, from the remainder. Individuals were measured and assigned to size classes based upon their

length from rostrum to telson (Watkin, 1940). Finally the individuals from each sample were pooled, dried at 60° C. for 24 h and weighed so that estimates of total biomass m^{-2} could be made.

2.2.3. Calorimetry

Two methods were employed to measure the calorific value of *Corophium* tissue. Mean values for adult animals were determined by bomb calorimetry. Analysis of the various size classes by this method proved impracticable and so these values were obtained indirectly by wet-oxidation. 1) A non-adiabatic microbomb calorimeter of the type that was described by Phillipson (1964) was used to measure heats of combustion of samples of adult male and female tissue. Owing to rather high ash contents (37 - 46%) due in part, to salt from the medium and to sediment material in the gut, *Corophium* burned incompletely and somewhat unpredictably. To reduce this problem and to obtain consistent results, only starved animals* were used and their tissues were dialysed for 24 h against distilled water and then dried at 60 C for a similar period. Dried tissue was then ground up in a small glass tissue homogenizer and pressed into pellets weighing between 5 and 12 mg dry wt.

Sample combustion was carried out in a constant temperature room at 10 C. with the bomb linked to a chart

*n = 200

recorder. Complete equilibration was allowed between the bomb and ambient air temperature before ignition - thus obviating the need for a pre-fire correction. The system was calibrated in the normal way using pellets of benzoic acid. Corrections for acid formed and fuse-wire burnt (Golley, 1961) were not applied since preliminary tests showed their values to be insignificant.

Ash weights were determined by burning pre-weighed samples in a muffle furnace for 4 - 6 h at 550 C (Crisp. 1971). Results are expressed in joules (J) or kilojoules (kJ) g^{-1} ash free dry wt. (AFDW). Equivalents in calories are also given to aid comparisons with previous studies.

2) To determine the calorific values of the different size classes a wet-oxidation method was used. Samples were digested in a mixture of 0.1 N potassium dichromate in conc. sulphuric acid. The use of wet-oxidation serves to reduce the problems associated with the prescence of inorganic material in the sample. Specific interferences can be minimized by simple chemical modifications to the procedure, meaning that sample preparation can be much less tedious than in bomb calorimetry - an important consideration when a large number of samples is to be run. The major drawback of the method lies in the fact that oxidation is seldom complete but by the use of an appropriate factor the values obtained can be simply corrected.

The procedure adopted was essentially that described

by Winberg (1971). Oven dried samples (5 - 8 mg) were digested for 15 min in 10 mls of the dichromate/sulphuric acid mixture. To eliminate interference by chlorides which can act as reducing agents (Southward, 1952) 100 mg of silver sulphate was added to each sample flask. After cooling, the neck of each flask was washed down with 15 mls of distilled water followed by the addition of 2 mls of 70% phosphoric acid. Excess dichromate was then titrated against 0.02 N ferrous ammonium sulphate in the prescence of acidified diphenylamine. The end-point of the titration is denoted by a change from the red/blue colour due to dichromate ions to the characteristic green of chromic oxide. The addition of phosphoric acid serves to precipitate ferric ions which would otherwise interfere with the colour change at the titration end-point. The chemistry of both digestion and titration has been described, in some detail, by Maciolek (1962).

The amount of oxygen consumed in the digestion of a sample (the sample oxygen demand) is simply calculated from the volume of dichromate used (1 ml of 0.1 N dichromate is equivalent to 0.8 mg O_2). The application of an oxycalorific coefficient to the sample oxygen demand will give an indirect measure of the energy content of the sample. Thus:

$$J\ mg^{-1} = \frac{V \times 0.8 \times c}{W}$$

where

V = the volume of dichromate used (mls)

w = the weight of sample (mg dry wt. Ash free)

c = an oxycalorific coefficient -

14.2 J mg O_2^{-1} (3.4 cals mg^{-1} - Winberg, 1971)

Values can now be expressed in J g^{-1} (AFDW).

2.3. Results & Discussion

2.3.1. Field sampling - population structure, density and Biomass

The results of the sampling programme in Benfleet Creek are best expressed in a series of histograms and graphs. The structure of the population at the beginning of each month is illustrated by the size frequency histograms which make up figs. 2 & 3.Frequencies of the different size classes are expressed in absolute numbers rather than percentages. It was felt that by doing this the histograms would be more informative and would give a visual impression of the changes in population density as well as changes in it's size structure. Monthly figures for density and biomass are given in table 1 and fig. 4.

By combining this information it is possible to construct a fairly detailed picture of the changes which occur in the population throughout the year.

A description of the *Corophium* population in Benfleet Creek

Watkin (1941) has described the yearly life cycle of *Corophium volutator* in the Dovey estuary in North Wales and in general his description seems to fit the Thames population. The present study suggests fewer broods and a shorter breeding season but in many respects the two groups are comparable.

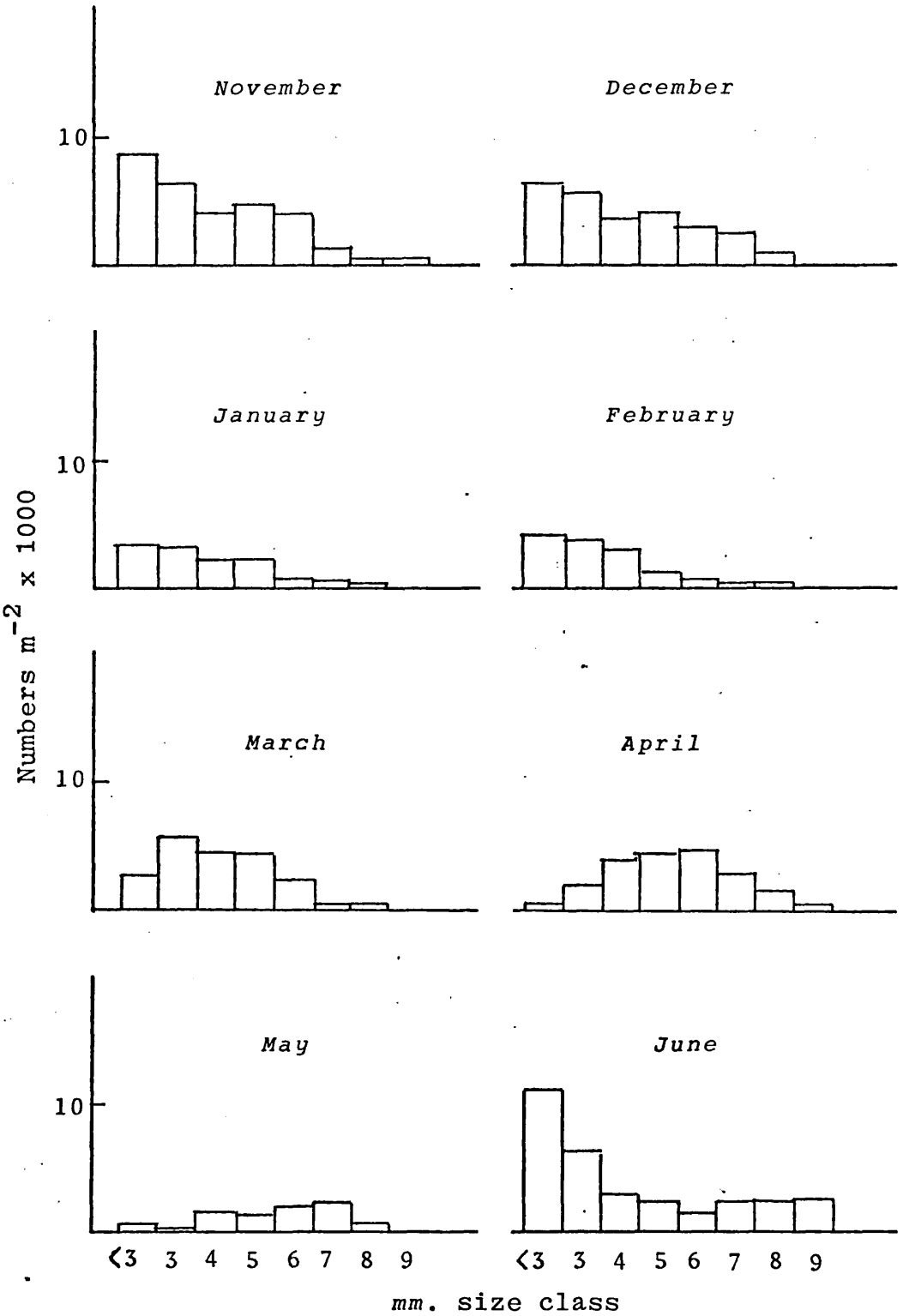


Fig. 2. Size frequency analysis of the Benfleet population of *Corophium volutator*

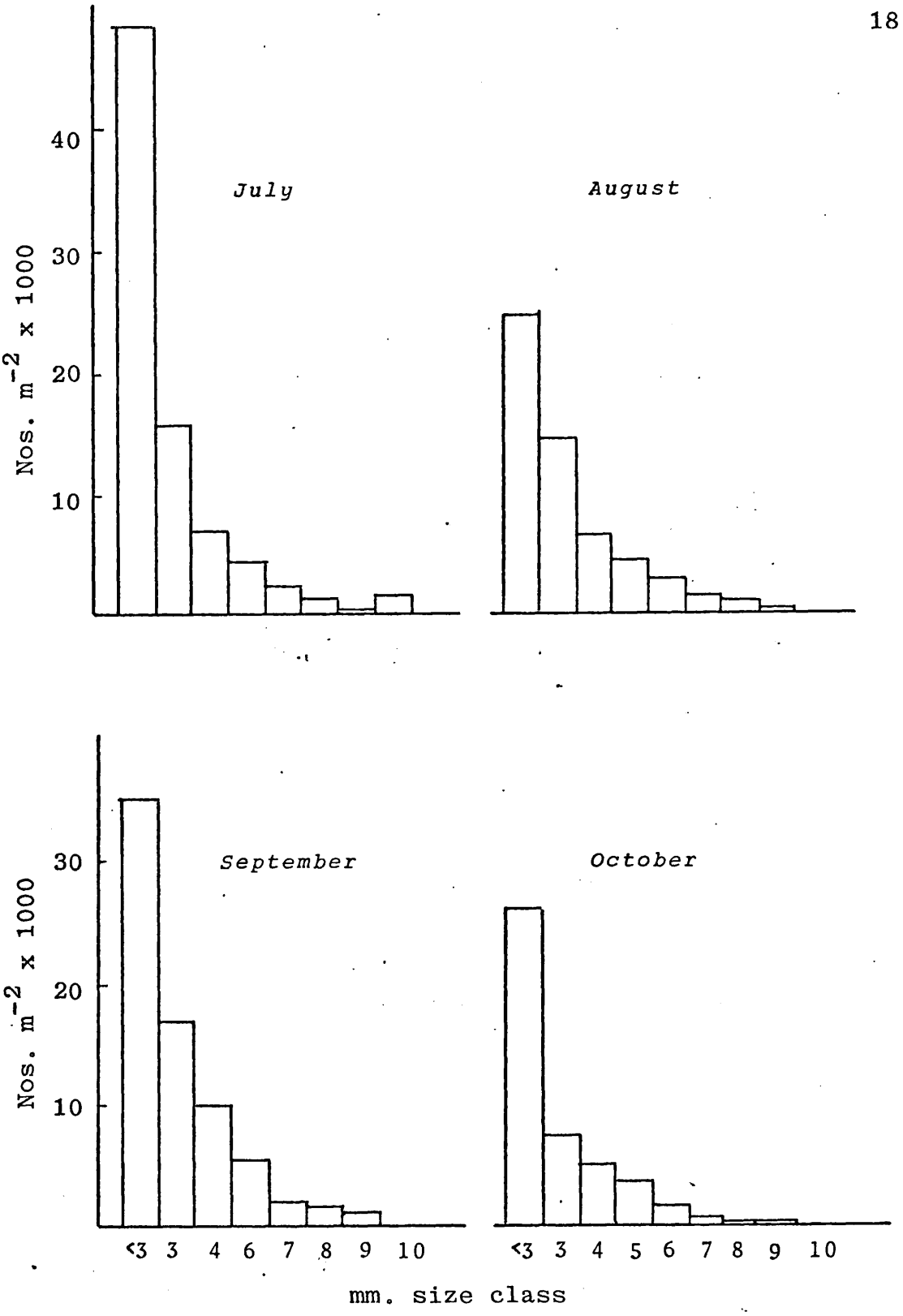


Fig. 3. Size frequency analysis of the Benfleet population of *Corophium volutator*

Table 1. Monthly fluctuations of biomass and density in the Benfleet population*

Sample	Biomass ($g\ m^{-2}$)	Population Density (m^{-2})	Mean individual Dry wt. (mg)
November	14.74	29,300	0.5
December	13.0	26,000	0.5
January	6.6	12,300	0.54
February	3.8	13,100	0.29
March	6.96	20,600	0.34
April	11.5	20,000	0.58
May	16.1	9,300	1.73
June	20.3	32,000	0.63
July	17.8	81,600	0.22
August	12.9	55,000	0.23
September	16.0	71,800	0.22
October	10.0	44,800	0.22

* Data from replicate samples collected at the beginning of each month (from Nov. 1975)

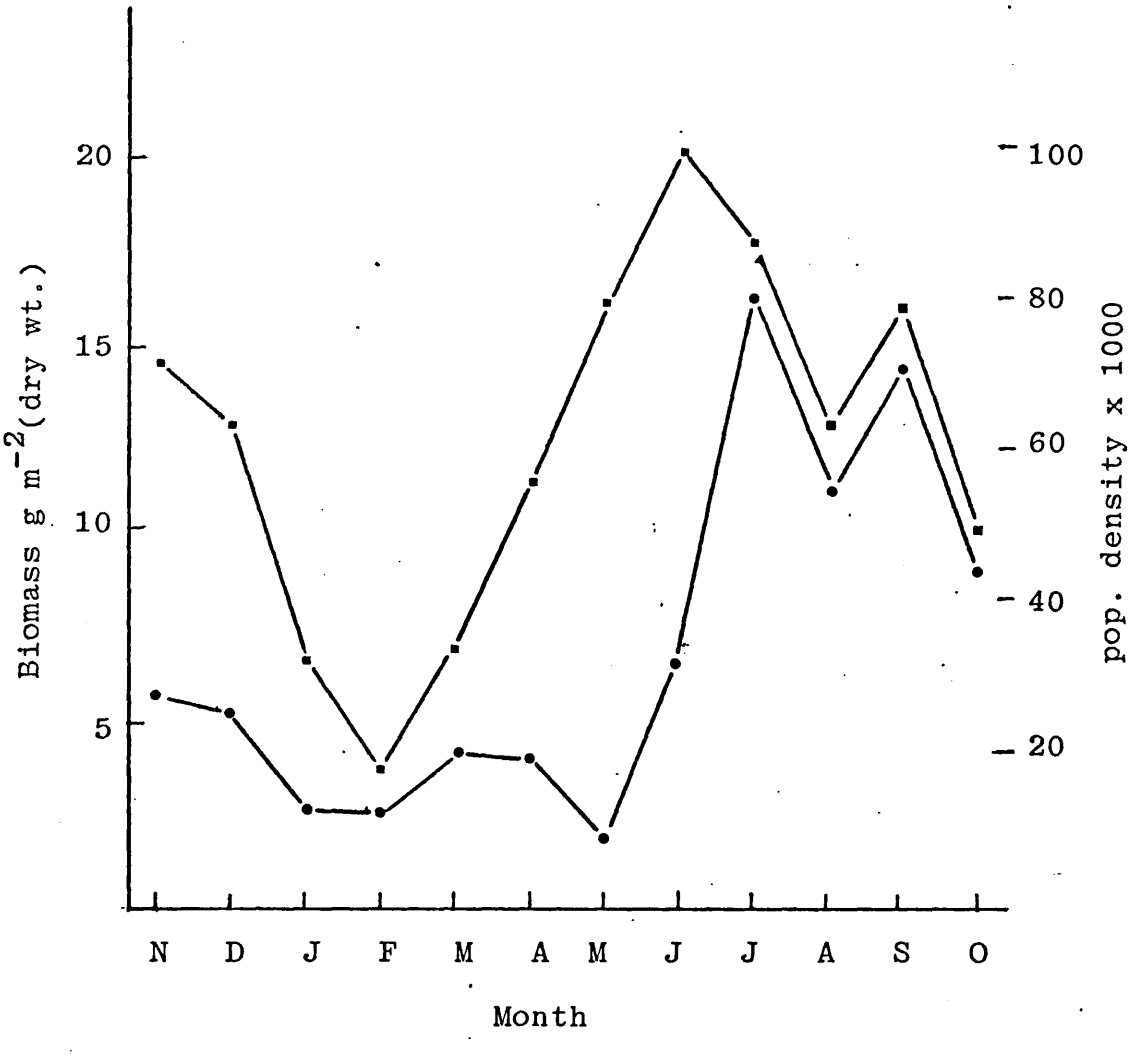


Fig. 4. Monthly biomass and population density in the Benfleet population of *Corophium volutator*.

Squares denote biomass, circles denote population density.

Like Watkin's population *Corophium* in Benfleet Creek has a complex population structure. The life cycle is rather short with animals seldom living as long as a year and 9 months appearing to be the average duration of a generation. Recruitment occurs in early spring and throughout the summer while mortality occurs all year round. Growth is simultaneous with recruitment and mortality with considerable overlap of generations. This makes it rather difficult to follow a particular generation through size frequency diagrams. At certain times of the year modes can be identified and followed through subsequent samples but at other times overlap is such that descriptions of population growth must be speculative. Nevertheless, the analysis of the Benfleet population has been informative and a number of distinct phases in it s annual cycle can be identified.

Phase 1. The overwintering population.

Throughout November, December and January the population is made up of overwintering animals derived from the previous summer's broods. Growth during this period is negligable and there is little change in the size frequency distribution for these months. The interesting feature of the overwintering population is that it is made up of both adults and juveniles so that histograms have a bi-modal form. Watkin noted this same feature in the Dovey animals. This is a period characterized only by mortality, particularly heavy in December when over 50%

of the population disappears. The decline of the population is clearly demonstrated in fig. 4 where biomass drops to an annual low by the end of January.

Phase 2. Spring growth

Towards the end of January overwintering animals give rise to the first brood of the year. Females begin to appear with eggs in their brood pouches and the offspring are released in February. Biomass and population numbers at this time of the year are low and February heralds a period of rapid growth. Through March and April the newly hatched corophiids along with the overwintering juveniles increase in size so that by the early May sample 90% of the survivors have reached maturity, the 7 and 8 mm size classes being the most numerous. A progressive increase in the modal size can be seen in the histograms for February to May (fig. 2).

Recruitment appears to be unimportant during March and April and by the May sample there are only a few 3 and 4 mm juveniles and none less than 3 mm. Although there is quite heavy mortality in April biomass is quadrupled in the three months following the first brood.

Phase 3. Summer recruitment

In May summer recruitment begins. Overwintered animals and those hatched in early spring give rise to the first of the summer broods. By the beginning of June the numbers have increased from 9300 to 32,000 m^{-2}, while by early July population density reaches it's maximum with

over 81,000 m^{-2}. In June there is again a distinct bimodal frequency distribution with large reproductive adults of 7 - 9 mm and the newly hatched young up to 4 mm. In July this trend, although now less obvious, continues but the numbers of large adults are in decline. July sees a slump in the overall population density suggesting that recruitment no longer keeps pace with mortality which seems to be particularly high in the young. By the August sample numbers have dropped to 55, ooo m^{-2} while the largest size classes have all but disappeared from the population. The larger reproductive animals which grew up during the spring are thus dying out and giving way to their fast growing offspring which, by now, are already reaching sexual maturity. A second recruitment peak in August is probably due in the main to these young animals although the survivors from the parent generation may also contribute.

Females in breeding condition are present in the population throughout the summer and into September so that even though two recruitment peaks have been identified it does not necessarily follow that there are only two broods. It seems likely that an individual female is able to produce two or more broods since both small 5 - 6 mm and larger 7 - 9 mm animals are found with eggs in the brood pouch.

The complexity of the summer population is such that growth of modal size classes cannot be followed. The

combination of recruitment, disappearance of older generations and the inevitable mortality of young in their early developmental stages means that there is little change in the form of the size frequency distributions. The basic pattern is the same from July to October, the only real differences being in terms of overall numbers. We can only speculate on rates of development although it has already been suggested that the young hatched in May and June form the breeding stock in August. This would mean a development time, from hatching at 1 mm to sexual maturity at 5 - 6 mm, of two months or less.

Recruitment ceases by October and the survivors from the summer broods grow to make up the new overwintering population. It seems likely that the animals which will overwinter as adults are derived from the early summer broods and are, in fact, the parents of the overwintering juveniles.

2.3.2. Calorific values for *Corophium volutator*

All animals used in calorimetry were freshly collected in spring and early summer. Calorific values for adults determined by bomb calorimetry, fell within the range 16.1 - 21.8 kJ g^{-1} AFDW (3.85 - 5.20 kcals g^{-1}AFDW). The values for males had a mean of 18.4 $\pm$ 1.1 kJ g^{-1} (4.4 $\pm$ 0.27 kcals) while the mean for females was slightly higher at 20.6 $\pm$ 1.1 kJ g^{-1} (4.93 $\pm$ 0.25 kcals). (Table 2)

As stated earlier, values obtained by wet-oxidation tend to be underestimated due to the incomplete oxidation of biological material. By using sample material of a known calorific content (maltose and *Corophium* tissue used previously in bomb calorimetry) it was possible to determine an appropriate correction factor. Oxidation was estimated to be approximately 85% efficient and the figure 1.18 has been used to correct all values obtained by the procedure. Table 3 summarizes the results of the size class analysis. Young animals appeared to have lower energy contents than did adults with the highest values seen in 7 - 8 mm adults (those at the peak of reproductive activity). There appeared to be no overall significant difference between values for males and females. The overall mean value calculated over the range of size classes was 17.85 $\pm$ 2.1 kJ g^{-1} AFDW (4.26 $\pm$ 0.49 kcals). It is this value which will be used in production calculations.

Table 2. Calorific values of adult *Corophium volutator.*

	kJ g^{-1} AFDW *	kcals g^{-1} AFDW *
Males	18.8	4.50
	19.6	4.68
	18.7	4.47
	18.1	4.32
	17.8	4.26
	16.1	3.85
	19.7	4.72
Mean	18.4	4.40
S.D.	± 1.1	± 0.27
Females	19.9	4.77
	19.3	4.62
	21.7	5.20
	21.2	5.06
	20.9	5.01
Mean	20.6	4.93
S.D.	± 1.1	± 0.25

All values determined by bomb calorimetry using pellets of between 5 and 12 mg dry wt.

Values are given in both joules and calories (most of the values given in the literature are in cals.).

* Ash free dry wt.

Table 3. Calorific values of *Corophium volutator*: the analysis of size classes from 3 to 9 mm.

Length (mm)	kJ g^{-1} AFDW*	kcals g^{-1} AFDW*
9 mm Male	17.4±0.26	4.16±0.063
Female	19.1±1.2	4.57±0.29
8 mm Male	19.4±0.66	4.65±0.16
Female	19.9±1.5	4.75±0.36
7 mm Male	20.7±0.35	4.95±0.08
Female	20.1±0.55	4.80±0.13
6 mm Male	18.1±0.74	4.33±0.18
Female	15.6±0.53	3.74±0.13
5 mm	16.2±0.60	3.87±0.16
4 mm	15.6±0.69	3.73±0.16
3 mm	13.7±0.70	3.27±0.17
Overall mean	17.8 ± 2.04	4.26±0.49

* Means and standard deviations (n = 3 in each)

All values determined by wet-oxidation.

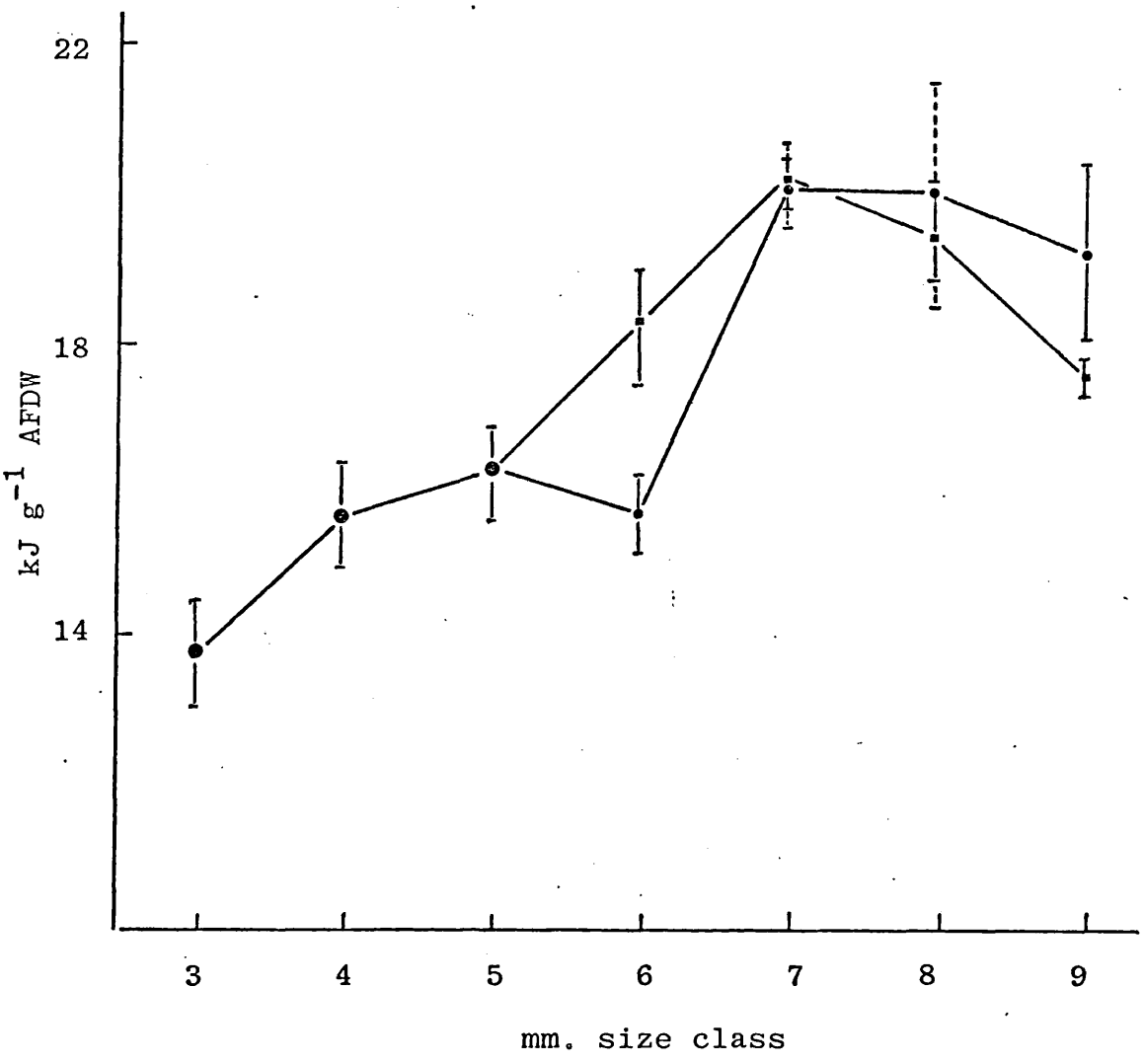


Fig. 5. The effect of size on calorific values of *Corophium volutator*.

Squares denote male values, circles denote female. Large circles represent combined male and female. Vertical bars are standard deviations about each mean.

The range of values determined for *Corophium* tissue is broadly comparable to data on other amphipods. For *Hyalella azteca* , for instance, Hargrave (1971) reported a range of 3.5 - 4.2 kcals g^{-1}AFDW while Nilsson (1974) gave rather higher values for *Gammarus pulex*. He found that male values fell between 4.11 and 5.4 kcals g^{-1}AFDW and that females were between 4.92 and 5.96. Goss-Custard (1977) quotes T.Wood's values of 4.17 - 4.54 kcals g^{-1}AFDW for *C. volutator*.

The calorific value of eggs was determined in the microbomb calorimeter. Only three pellets were produced and there was insufficient material for ash weight determinations so that the value obtained - 5.57 $\pm$ 0.25 kcals g^{-1} or 23.3 $\pm$ 1.1 kJ g^{-1} - is expressed in terms of dry wt. rather than AFDW.

2.3.3. Calculation of production*

In the abscence of reliable data on individual growth rates population production must be estimated on the basis ofloss of biomass due to mortality and fluctuations in the standing stock. Since the population is considered to be stable the annual increase due to production must equal the biomass eliminated by mortality agents (B_e).

Therefore $P = B_e$

For each month of the year P has been calculated from the expression

$$P = B_e + (B_t - B_o)$$

where B_e = biomass eliminated from the population (mortality due to all causes)

B_o = biomass at the beginning of the month

B_t = biomass at the end of the month

For those months when recruitment did not occur (or could be assumed to be negligable) the number of animals eliminated was simply determined by

$$N_o - N_t$$

By multiplying this number by the estimated mean dry wt. of individuals during the month (w) an estimate of B_e can be obtained thus:

$$B_e = (N_o - N_t)\ \tfrac{1}{2}\ (w_o + w_t)$$

where w_o = mean dry wt of animals at the beginning of the month

*See Crisp (1971)

and w_t = mean dry wt. of animals at the end of the month

In the month of April 1976, for example, recruitment did not occur and 10,700 animals m^{-2} were lost from the population

$$B_e = 10,700 \times \tfrac{1}{2}(0.58 + 1.73)$$
$$= 12.33 \text{ g } m^{-2}$$

and production was

$$P = 12.33 + 4.6$$
$$= 16.93 \text{ g } m^{-2}$$

For those months when recruitment did occur population density showed a net increase and no direct measure of mortality was available. Here it was necessary to estimate the numbers eliminated. In September and October following the summer recruitment peaks, mortality was respectively 37 and 35% of standing stock, while the mean for those months where direct estimates were available was 32%. A figure of 40% has been adopted for these calculations. Even this rather arbitrary figure may be an underestimate since the bulk of the population at these times is made up of recently hatched animals and mortality among the young can be expected to be high. In stock laboratory tanks monthly mortality of a newly recruited summer population was between 60 and 90% depending upon maintenance temperature. Johnson (1976) reported that only 24% of newly hatched *Cirolana harfordi* (isopoda)

survive the first month of development.

Assuming 40% mortality it is possible to estimate the expected population density at the end of a month in which recruitment operates and from that the probable mortality. Thus for June 1976, for example, the population density by the end of the month was 81,600 m^{-2} and the expected density would be 136,000 m^{-2} (assuming that the actual figure represented 60% survival). Mortality would then be 136,000 - 81,600 = 54,400 m^{-2}. This method has been used to estimate mortality for the period from May to August and for the spring recruitment in February (Table 4).

To test the validity of this approach a comparison can be made with expected recruitment based upon estimates of egg production. The mean number of eggs per female has been estimated as 26 $\pm$ 9. Of the standing stock at the beginning of May about 5,700 animals m^{-2} were sexually mature females. It follows that these animals could produce about 148,000 young m^{-2}. Reference to table 4 shows that mortality during this early summer recruitment peak has been estimated as 75,700 m^{-2}(combined figures for May and June) which when summed with the number surviving (81,000 m^{-2}) gives a total expected density of 157,300 m^{-2}. As the survivors include some of the parent generation it can be seen that the two estimates are broadly similar.

It is felt that figures calculated by this procedure give a fairly realistic though approximate estimate of the productivity of the Benfleet *Corophium* population.

Calculations are summarized in table 4 - the final column giving monthly production in g m^{-2}. Table 5 gives the calorific equivalents of standing stocks and production for each month. Production in g m^{-2} was converted to g AFDW and multiplied by 17.85 J (the mean calorific value for *Corophium* tissue from 2.3.2).

The description of the annual cycle in the Benfleet population is reflected in the monthly production figures calculated above. Productivity shows marked variations throughout the year (fig. 6) with the lowest values in the winter months when ambient temperatures and food supplies are also low. This is the period of the overwintering population when any growth of individuals is masked by heavy mortality.

A steep rise in February is a result of the first brood of the year and thereafter production continues to increase up to a peak in early summer. Most of the spring production can be attributed to the rapid growth of the newly hatched brood. Throughout the period, environmental temperatures are rising and in response to the more favourable conditions, production, both primary and secondary, in the creek is on the increase. The conditions must be favourable to the *Corophium* population too since the mean dry weight of the individuals increases from 0.34 to 1.73

Month	N_e ($N_0 - N_t$)	$\bar{w}$ $\frac{1}{2}(w_o + w_t)$	B_e (g m^{-2})	$B_t - B_o$ (g m^{-2})	P (g m^{-2})
November	3,300	0.5	1.65	$^{-}$1.74	$^{-}$0.09
December	13,700	0.54	7.4	0.7	0.7
January	1,600 *	0.42	0.67	$^{-}$2.1	$^{-}$2.1
February	13,700 *	0.32	4.38	3.16	7.54
March	600	0.46	0.28	4.54	4.82
April	10,700	1.16	12.33	4.6	16.93
May	21,300 *	0.63	13.51	4.2	17.7
June	54,400 *	0.43	23.17	$^{-}$2.55	20.62
July	36,700 *	0.23	8.29	$^{-}$4.87	3.42
August	47,900 *	0.23	10.93	3.12	14.07
September	27,000	0.22	6.02	$^{-}$6.0	0.02
October	15,500	0.36	5.63	4.74	10.37
				Total	95.6

Table 4. The calculation of production of *Corophium volutator* at Benfleet.

* Estimated. For full explanation see text.

Table 5. The calorific equivalents of production and standing stock of *Corophium volutator* in Benfleet Creek

Month	Calorific value of standing stock (kJ m^{-2})	Production (kJ m^{-2})
November	155.2 (37.0)	-0.88 (0.21)
December	137.0 (32.7)	7.37 (1.76)
January	69.6 (16.6)	-22.12 (5.28)
February	40.2 (9.6)	79.40 (19.0)
March	73.3 (17.5)	50.70 (12.1)
April	121.1 (28.9)	178.20 (42.6)
May	170.1 (40.6)	186.40 (44.5)
June	213.7 (51.0)	217.20 (51.8)
July	186.9 (44.6)	36.00 (8.6)
August	135.8 (32.4)	148.20 (35.4)
September	168.4 (40.2)	0.21 (0.05)
October	105.2 (25.1)	109.20 (26.1)

Total - 1007 kJ

(240 kcals)

Figures in brackets are kcals m^{-2}.

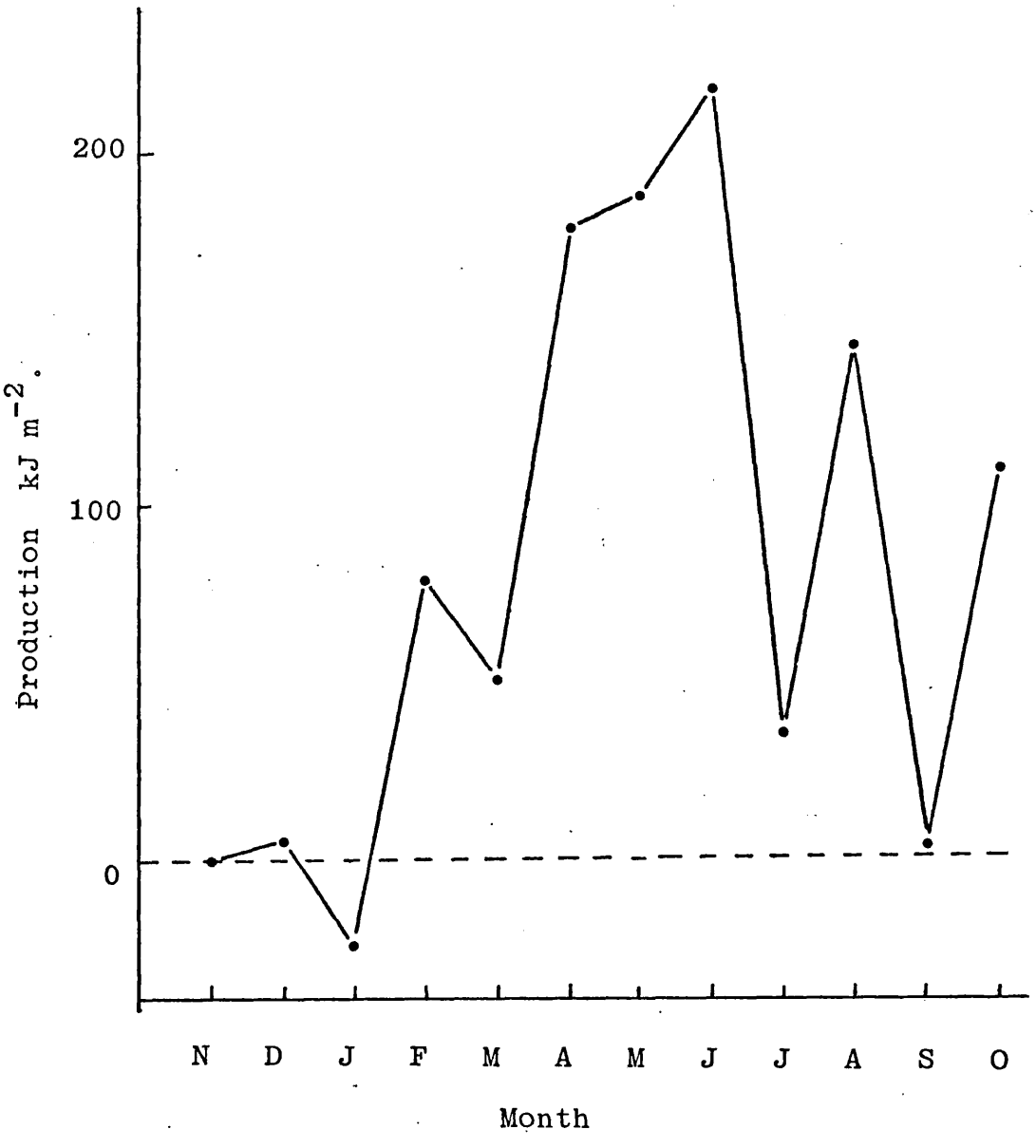


Fig. 6. The monthly population production of *Corophium volutator* in Benfleet Creek (kJ m^{-2})

mg in only two months from March to May.

By June, with the appearance of large numbers of young animals, production has reached a peak of 217 kJ m^{-2} month $^{-1}$ (51.8 kcals). For the remaining months of the year production alternates between high and low values. In July and September figures are low. These months follow the two summer recruitment peaks and the figures would seem to suggest that mortality is the main feature of the population. As well as heavy losses of juveniles, adults from the early spring brood will also be dying out. Production figures for August and October are high but for different reasons. The August figure is due to the second summer recruitment peak. In October recruitment no longer occurs and the relatively high figure reflects growth of animals surviving from the summer broods. Mean animal dry wt. increases from 0.22 to 0.5 mg as animals grow to form the new overwintering stock.

Annual production has been estimated to be 1007 kJ m^{-2} yr^{-1} (240 kcals) while the mean monthly standing stock had a calorific value of 131.2 kJ m^{-2} (31.3 kcals).This gives a ratio of production to biomass ($^{P}/_{B}$) of 7.7. Winberg (1971) reviewed a number of amphipod production studies and quotes annual $^{P}/_{B}$ coefficients in a range from 1.6 (*Gammarus fasciatus* in lake Baikal) to 3.8 (for *Hyalella azteca*). The rather high value derived from the present study is probably a result of rapid growth rates and the fact that at least two separate generations are

contributing to the annual production total.

No account has been taken of egg production and energy lost as moults. To this extent production may be slightly underestimated.

In later sections these results will be discussed further in the context of an energy flow model for the Benfleet population.

3. Metabolism

3.1. Introduction

A proportion of the energy absorbed from ingested food is used in the maintenance of the normal metabolic processes of the organism - processes like enzyme synthesis, osmoregulation and muscular activity. Energy is released as heat when food substrates are oxidized. In some cases metabolic heat production can be measured directly. This approach is, however, generally regarded as being impracticable for small animals and more often, heat production is calculated indirectly from rates of O_2 uptake. The ammount of heat liberated per unit weight or volume of O_2 consumed when a given food substrate is oxidized (the oxycalorific coefficient for that substrate) is fairly accurately known (Winberg, 1971, Crisp, 1971) and the application of an appropriate coefficient to the rate of O_2 uptake gives an indirect measure of metabolic rate - the heat energy released by respiration (R).

The indirect method is not without it's disadvantages and certain assumptions must be made regarding the nature of the food being oxidized. Fortunately, though, the energy equivalents for the major classes of food, protein, fat and carbohydrate, differ only slightly and a mean value can generally be assumed without incurring an error

of more than about 5%.

A certain ammount of absorbed energy is released as the waste products of protein catabolism. This energy, U, is not assimilated in the strict sense (it is not used for physiological purposes) but is merely rejected as toxic nitrogen compounds. It should, however, be considered as part of the animals energy requirments since it is an end-product of normal metabolism.

The aim of this section has been, primarily, to estimate the energy required for metabolism (R + U) by the Benfleet population.

3.2. Laboratory measurments of O_2 uptake

3.2.1. Introduction

In order to calculate R, the heat energy liberated by respiration, a study of O_2 uptake rates was required. One disadvantage of the indirect method has already been outlined in the general introduction but the real limitations are likely to lie in the measurment of O_2 uptake itself. Crisp (1971) suggested that the accuracy of the recognized techniques for respiratory measurments is limited by the degree of constraint put upon the behaviour of the animals. In other words, if the animal is not behaving normally in the respirometer the values obtained must be regarded with caution. This is especially true in energetics where the magnitude of R affects the computation of energy budgets and energy flow models.

The differences between the metabolic rates of active and non-active animals may be considerable (Newell & Northcroft, 1967) and care must be taken to ensure that O_2 uptake reflects, as far as is possible, the normal activity levels of animals in their natural surroundings. With this in mind, O_2 uptake was measured in animals which were left undisturbed and which displayed only sporadic swimming activity. Fry (1957) termed this state "routine" metabolism and defined it as the normal state with the animal displaying bursts of spontaneous activity.

3.2.2. Materials & methods

The O_2 consumption of small groups of animals (between 8 and 30 depending on size) was measured at 10, 14 and 20 C. Animals were acclimated to the experimental temperatures for two weeks. Groups of equally sized *Corophium* were placed in small glass specimen tubes (4 x 10 cm) in 20 mls of seawater (30‰) which had been filtered through millipore membrane of pore size 0.2 µm, to remove all other organisms which might affect O_2 measurments. The animals themselves were rinsed, twice, in filtered seawater containing antibiotics . Finally a 1 cm layer of liquid paraffin was laid over the surface of the seawater and the whole immersed in a water bath held at the required temperature.

After an initial period of about 20 min, during which the animals became accustomed to their new surruondings, changes in the O_2 content of the experimental water were measured electrically using a Uniprobe galvanic O_2 electrode and an O_2 meter (North West Scientific Instruments). The meter was linked to a Servoscribe chart recorder so that a continuous record of reductions in O_2 levels could be obtained. Experiments were stopped when O_2 levels in the water fell below about 70% saturation. Probe and meter were calibrated daily, in nitrogen purged and air saturated seawater. For calculations of O_2 uptake % saturation was converted to µl O_2 ml^{-1} at N.T.P.

The use of galvanic electrodes offers a simple and relatively reliable means of O_2 measurement provided that certain of their properties are recognized. Firstly, they are prone to drift as the current they produce falls off with the age and condition of the electrodes and electrolyte. This was the reason for the daily calibrations mentioned above. Secondly, the probe itself does consume a certain ammount of O_2 as reduction occurs at the cathode. This must be taken into account in a continuous recording system such as is described here. Probe consumption was measured in control experiments and was subtracted from overall consumption in all subsequent runs. Finally, and resulting from probe consumption, localized depolarization can occur near the probe membrane as O_2 in the adjacent water is reduced. This can result in an underestimate of O_2 levels in the water body as a whole and, in turn, an overestimate of O_2 uptake. Many authorities favour the use of a stirring device to ensure even distribution of oxygenated water. It was felt that, in these experiments, the respiratory and swimming movements of the animals in a small body of water were enough to prevent depolarization. Moreover the production of artificial currents might well have induced the animals to increased activity.

Measurments were also made of "active" uptake in animals induced to constant activity using respirometers of the Warburg constant volume type. Groups of 5 - 20

aniamls were placed in standard reaction flasks containing 2 mls of filtered seawater. CO_2 was absorbed in 20% KOH solution. Flasks were held in a constant temperature water bath and were shaken continuously to ensure equilibration between liguid and gas phases. The movements of the flasks also stimulated swimming activity in the animals. After an initial period of equilibration, experiments were run for 1 h at 10 and 20 C.

3.2.3. Results

"Routine" O_2 uptake at three temperatures, calculated from O_2 probe data, has been plotted against the mean individual dry wt. of the animals in each experiment (figs 7, 8 & 9). All measurments were made on groups of animals of similar size (i.e. in the same mm size class) and each point on the three graphs represents the mean O_2 uptake of the animals in a group.

When first placed in the respiration vessels, animals exhibited a period of hyperactivity. After 10 - 15 min, however, they settled down to what might be termed a "standard" or normal level of activity characteristic of undisturbed animals in the laboratory. This type of behaviour has been described by Meadows & Reid (1966). Animals would spend most of their time lying quiescent with their pleopods beating while individuals would crawl over the bottom of the vessel or exhibit short bursts of swimming activity before sinking again. This pattern is consistent with the observed activity of animals in stock tanks or in the field and, as such, was considered a reasonable basis for measurment of O_2 uptake rates and the subsequent calculation of energy used un respiration.

At all temperatures a logarithmic relationship between O_2 uptake and body weight has been confirmed. This relationship was first demonstrated by Zeuthen (1947) and has since been described for "active" *Corophium* (McLusky, 1969).

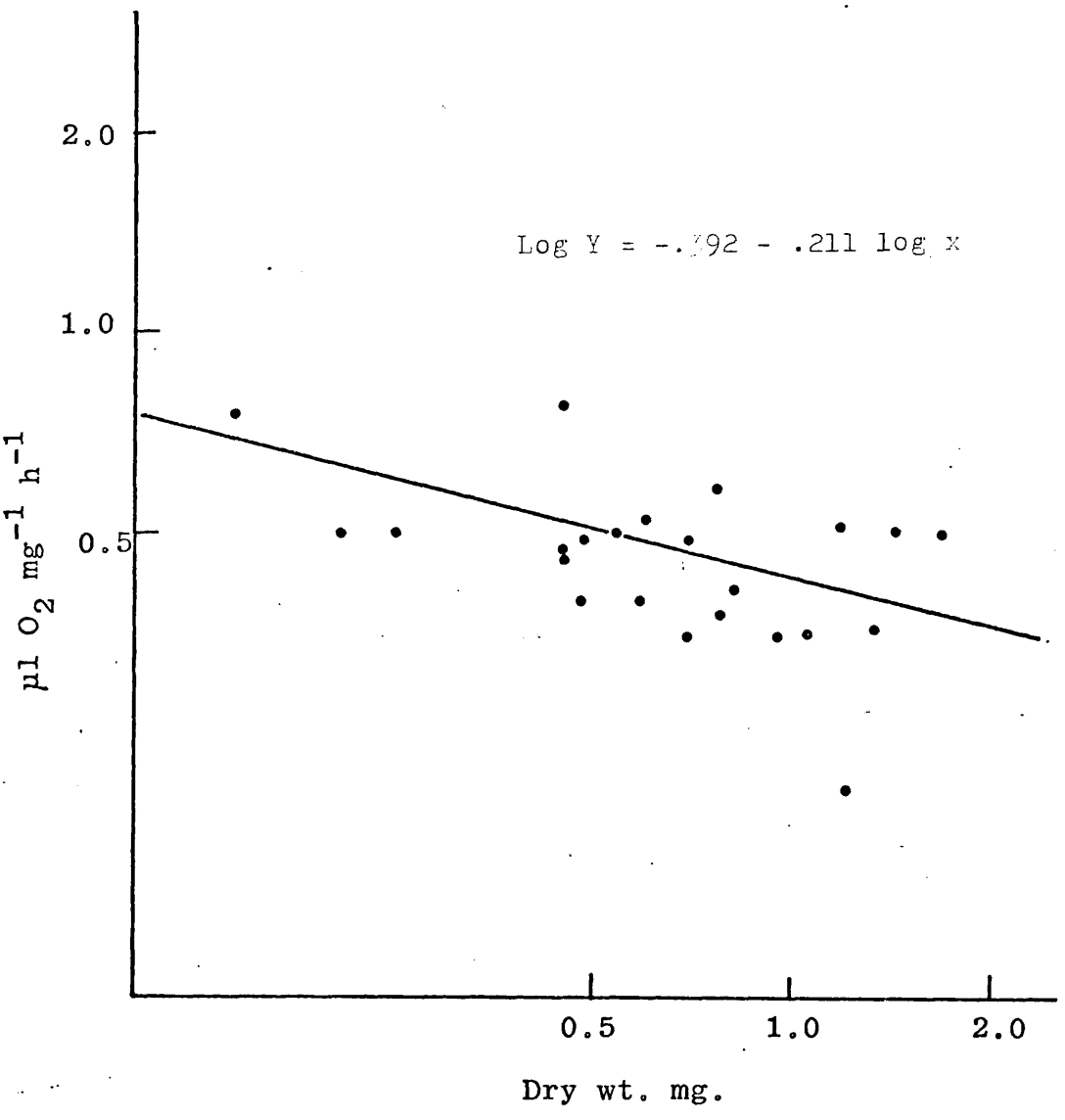


Fig. 7. The oxygen consumption of *Corophium volutator* at 10 C.

"Routine"- animals exhibiting spontaneous activity. Each point represents a group of animals

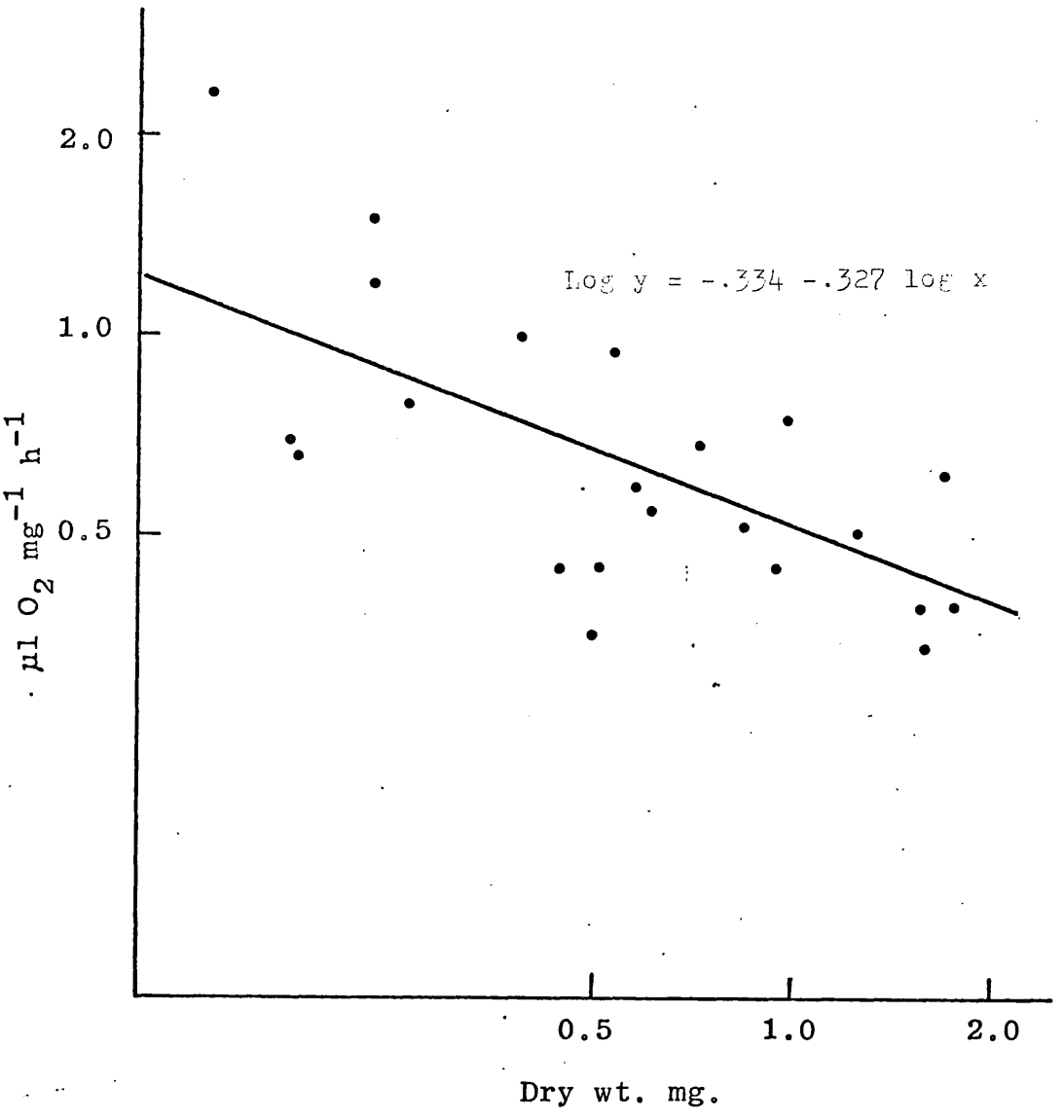


Fig. 8. The oxygen consumption of *Corophium volutator* at 14 C.

(as fig. 7)

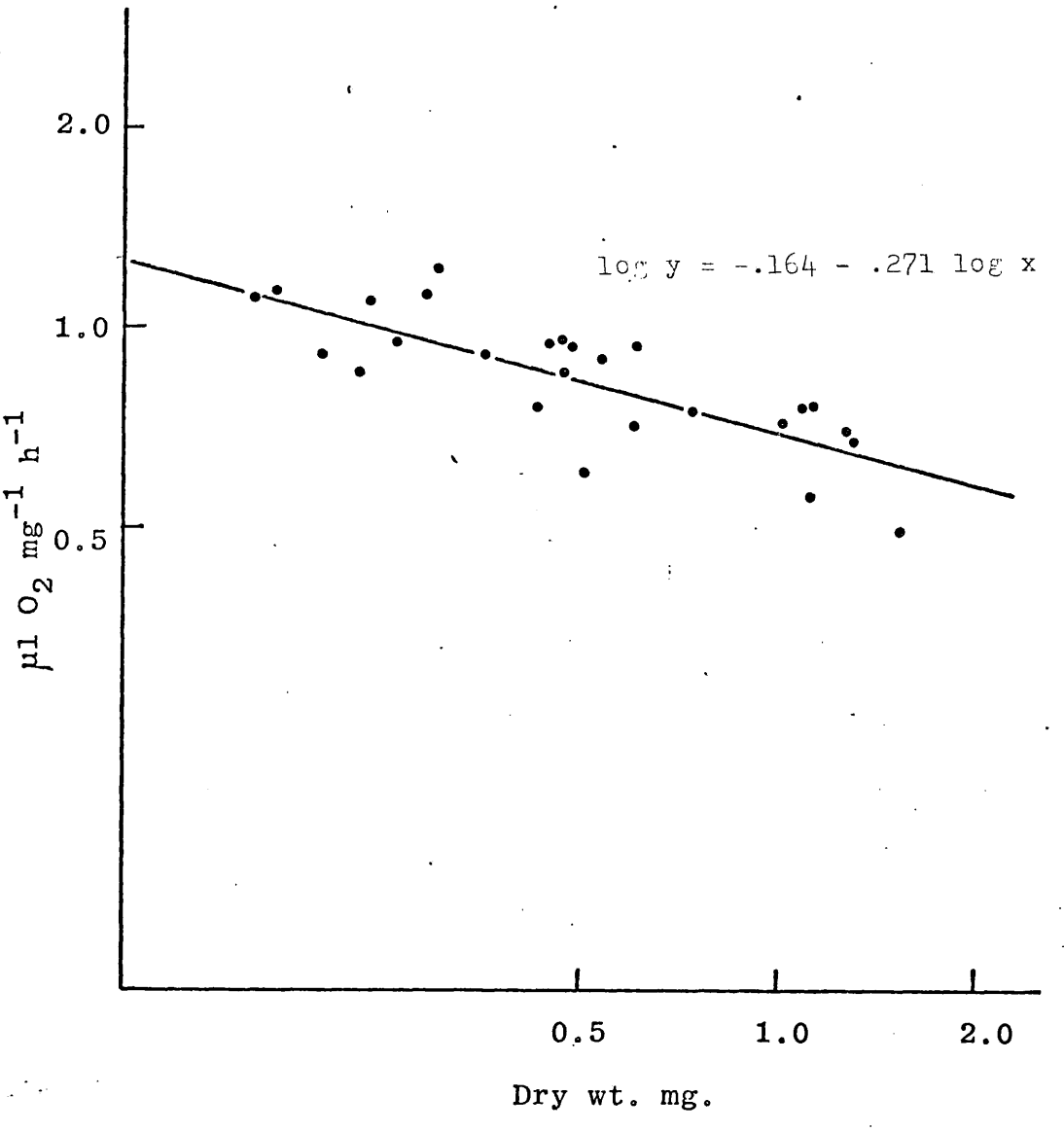


Fig. 9. The oxygen consumption of *Corophium volutator* at 20 C.

(as 7 & 8)

Table 6.1. O_2 consumption of Corophium volutator at different temperatures.

Temp °C	logy = a + b log x	N	Correlation coefficient
Routine			
10	log y = -0.392 - 0.211 log x	23	-0.47
14	log y = -0.334 - 0.327 log x	22	-0.56
20	log y = -0.164 - 0.271 log x	26	-0.79
Active			
10	log y = -0.055 + 0.125 log x	28	+0.14
20	log y = 0.220 + 0.229 log x	19	+0.48

Table 6.2. Significance levels of differences between O_2 consumption at different temperatures

Temperatures compared	Significance levels	
	Slope	Elevation
Routine		
10 - 14 °C	no difference $P > 0.1$	no difference $P > 0.25$
10 - 20 °C	no difference $P > 0.25$	$P < 0.001$
14 - 20 °C	no difference $P > 0.25$	$P < 0.005$ > 0.002
Active		
10 - 20 °C	no difference $P > 0.25$	$P < 0.001$

Table 6.3 O_2 uptake (VO_2) of 1 mg dry wt. *Corophium* at three temperatures

t C.	VO_2 (µl O_2 mg^{-1} h^{-1} *
10	0.43
14	0.53
20	0.70

* from figs. 7, 8 & 9

Rates seemed to be affected by temperature so that O_2 uptake was highest at 20 C (table 6). In general rates were somewhat lower than those reported by McLusky (1969). He found that the mean rate for his "resting" animals was 0.93 $\mu l\ mg^{-1}\ h^{-1}$ at 10 C compared to 0.47 (10 C), 0.73 (14 C) and 0.86 (20 C) from the present study. Such a comparison must be made with caution, however, since the conditions under which each study was carried out were quite different.

Measurments of "active" metabolism were rather misleading, largely, I think, as a result of an unfortunate choice of method. The normal relationship between O_2 uptake and body weight appears to be reversed. The slopes of the regression lines are positive; suggesting that, contrary to the accepted convention, weight specific O_2 uptake is higher in larger animals. McLusky has already demonstrated a normal relationship in active *Corophium* so we must regard thes new results with some suspicion. The effect may simply be the result of a rather limited range of animal size and to the small a mount of data available for small animals. Another explanation, though, lies in the use of the Warburg constant volume respirometers.

Although widely used in both animal and tissue respiration measurments the system has a particular disadvantage when used with an animal like *Corophium*. Quite apart from the unnatural surroundings in which the animal is held (a criticism which can be levelled at most respiro-

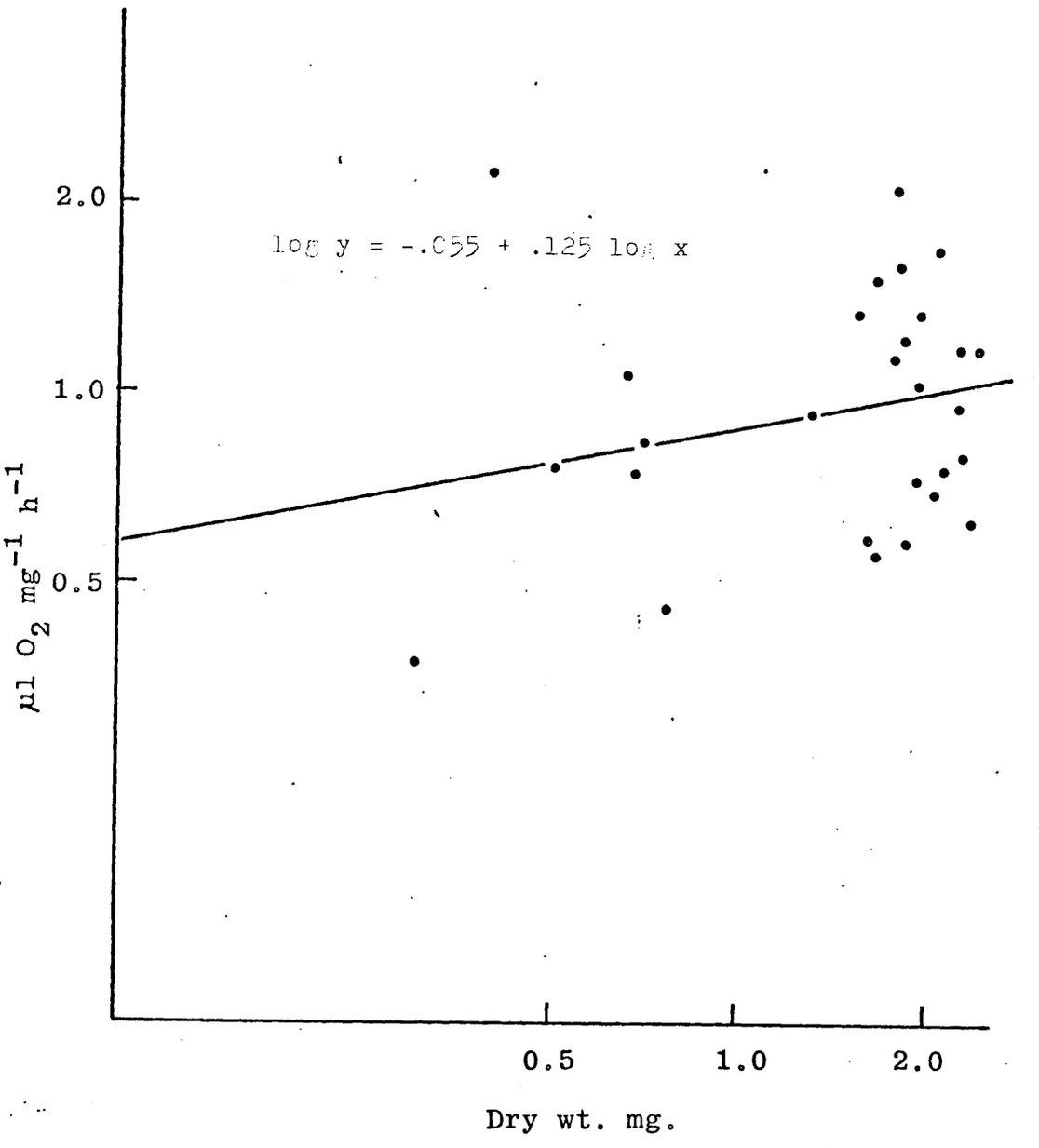


Fig. 10. The oxygen consumption of *Corophium volutator* induced to constant activity at 10 C. "active" animals. Each point represents a group of animals.

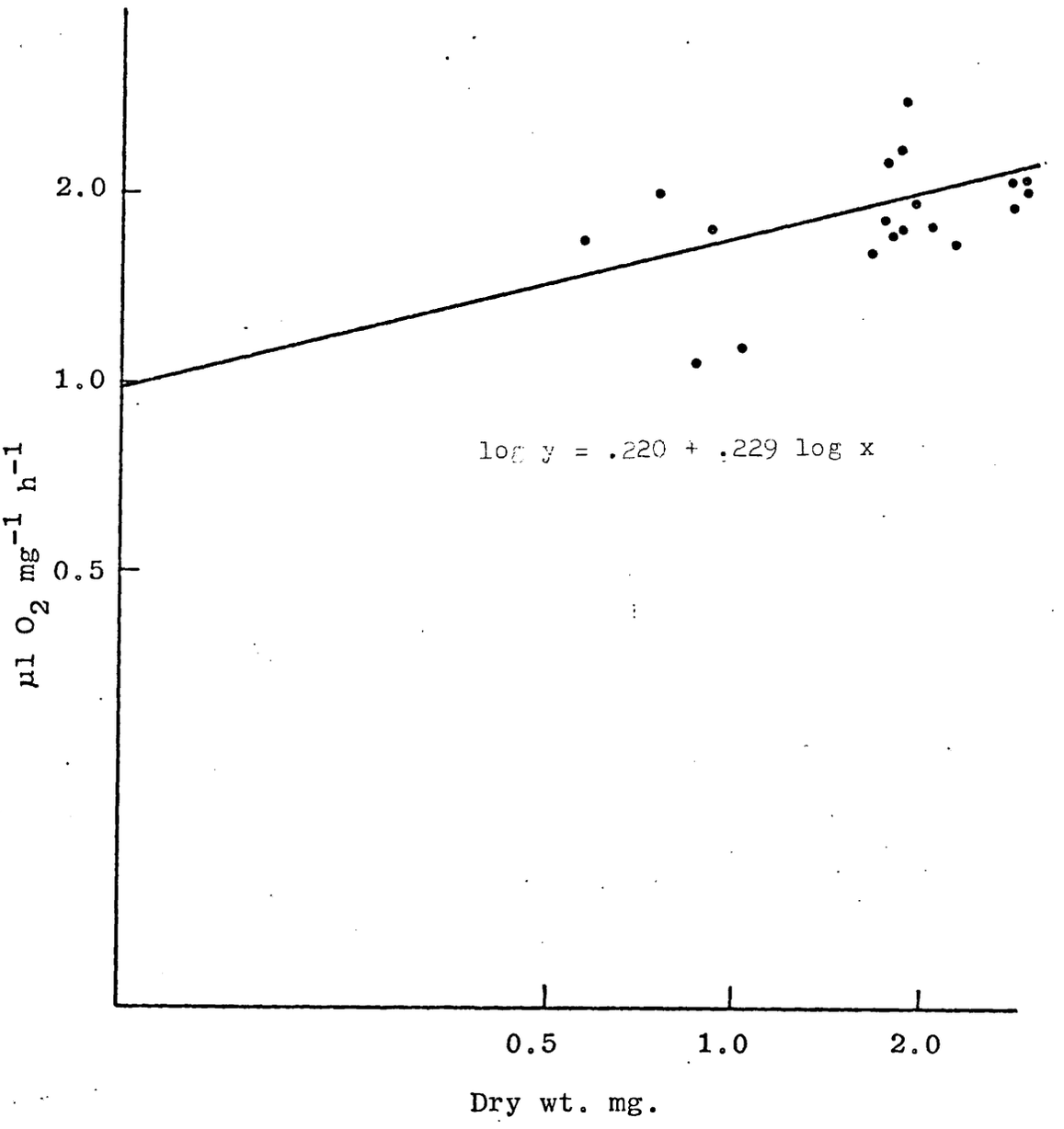


Fig. 11. The oxygen consumption of *Corophium volutator* induced to constant activity at 20 C.
(as fig. 10)

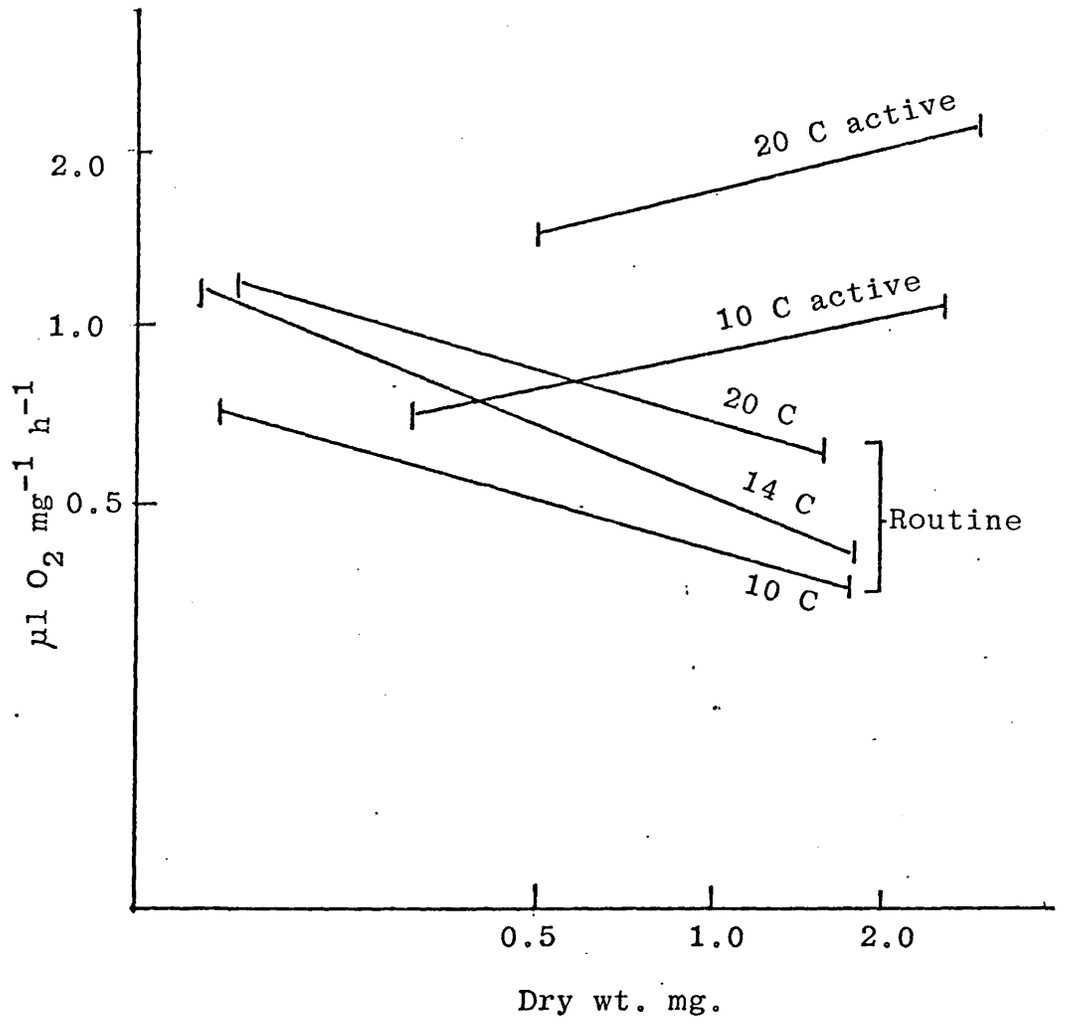


Fig. 12. "Routine" and "active" oxygen consumption of *Corophium volutator* at different temperatures

(Regression lines taken from figs. 7 - 11.)

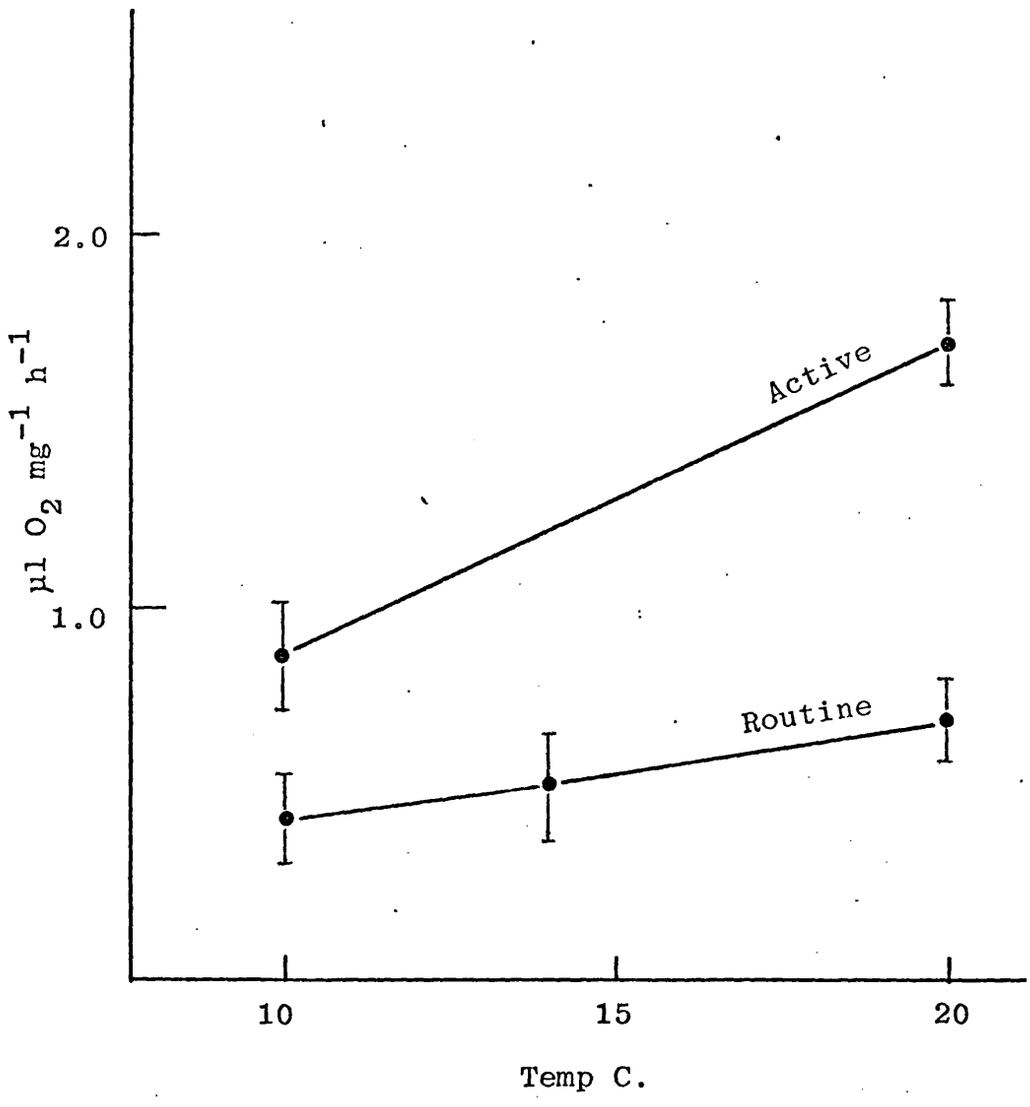


Fig. 13. The oxygen consumption of a 1 mg dry wt *Corophium volutator* - the effect of temperature

(with 95% confidence limits)

meters), the violent movements of the reaction flask imparts considerable stress upon them. While the larger individuals are able to respond and maintain swimming activity, small ones tend to be buffeted back and forth so that for much of the time they may not be exhibiting swimming activity at all. The effect can be seen in figs. 10 & 11.

Fortunately, this does not affect the calculation of R since data on active metabolism was only required for comparison and to test the assumption that temperature has a direct effect upon metabolic rate. It was noted that increased maintenance temperature caused an apparent rise in routine metabolic rates. Here too there is a temperature effect with the regression line for 20 C data lying markedly higher than that for 10 C. Regression lines for active and non-active animals have been plotted together in fig. 12. Fig. 13 shows the effect of temperature on the O_2 consumption of a hypothetical 1 mg dry wt. animal.

3.2.4. Discussion

The data presented here suggests a temperature effect upon the routine respiratory rates of *Corophium* in the laboratory. The question remains as to whether this is due to a direct effect of temperature on the rates of metabolic processes or is merely a function of temperature dependant activity levels. Newell & Northcroft (1967) proposed a reinterpretation of temperature effects in terms of increased activity. They found that, in a number of intertidal invertebrates, the active rate of O_2 uptake increased with temperature in approximate agreement with Arrhenius' law, while the quiescent rate (maintenance metabolism varied little over the normal environmental range. They suggested that only when minimal O_2 uptake corresponds with minimal activity can variation due to temperature be regarded as true variation in metabolic rate. Halcrow & Boyd (1967), simultaneously measuring activity and O_2 uptake in *Gammarus oceanicus* , agreed that O_2 uptake at comparable activity levels seemed to be less affected by temperature than had been previously assumed. Morgan (1965) found that *Corophium* was considerably more active at higher temperatures and this has certainly been confirmed in the present study.

It would seem likely, then, that the observed rises in O_2 uptake (routine) at higher temperatures is, at least in part, due to increased activity levels in experimental

animals, although a comparison of routine and active rates still seems to suggest a direct temperature effect.

A more detailed investigation is required to determine the precise nature of these effects but this is beyond the scope and indeed the requirments of the present study. The important point is that any increase in respiratory rate, due to temperature (direct or indirect) should be taken into account when assessing the energy requirments of an animal or population. Clearly, a rise in metabolic rate, even if entirely due to increased motor activity, represents a real increase in the value of R in an energy equation. The measurment of routine metabolic rate is of particular relevance to studies of energy flow since it embodies an estimate of the energy used by all the normal activities of the organism and not just the cost of maintenance.

O_2 uptake has been converted to energy required for metabolism by applying an oxycalorific coefficient. The energy equivalents for protein, fat and carbohydrate do differ slightlyand, strictly speaking, an appropriate figure should be calculated based upon the nature and composition of the food being respired. Energy equivalents given in the literature vary but it is generally accepted that a figure of 5.04 cals ml O_2^{-1} at N.T.P.(21.1 J) is applicable to carbohydrate, while the corresponding value for fat is somewhat lower at 4.69 cals (19.7 J). It has

been pointed out, however, that the oxidation of protein is incomplete and that it's energy equivalent is affected by the nature of the excretory end-products (Elliot & Davison, 1975). Both Elliot & Davison and Brafield & Solomon (1972) have proposed a value for protein respired by ammonotelic animals of 4.57 cals ml O_2^{-1} (19.2 J). The mean of these values is 4.77 cals or 19.99 ≡ 20 J. Crisp (1971) recommended the use of a similar value, 4.8 cals ml O_2^{-1}, while a slightly higher value , 4.83, was proposed by both Phillipson (1966) and Winberg (1971).

Detailed knowledge of the composition of ingested sediment and the excretory products to calculate an oxycalorific coefficient appropriate to *Corophium* feeding on a natural diet. Even then it would be necessary to assume that protein, fat and carbohydrate are respired in the proportions in which they are ingested. Moreover, the error involved in assuming a mean value is probably less than about 5% and when we consider the sort of errors inherent in the measurment of O_2 uptake it might be argued that time spent determining such a value would, perhaps, be mispent. Kay & Brafield (1973), for example, calculated an oxycalorific coefficient of 3.24 cals mg O_2^{-1}(the equivalent of 4.63 cals ml^{-1}) for *Neanthes virens* feeding on *Nephtys hombergii* but did not measure O_2 uptake under the same conditions, applying instead, data from a different study.

For most purposes the use of a mean value is likely

Table 7. Metabolic rates of adult *Corophium* (1 mg dry wt) as indicated by O_2 uptake

t C.	VO_2 $\mu l\ mg^{-1}\ h^{-1}$	Daily energy requirments J d^{-1}	cals d^{-1}
10	0.43	0.21	0.05
14	0.53	0.26	0.062
20	0.70	0.34	0.081

to be sufficiently accurate and so the figure 4.77 cals or 20 J ml O_2-1 has been used for calculations in this work (see section 3.4. The metabolic energy requirments of the Benfleet population.).

3.3. Excretion

3.3.1. Introduction

The energy content of excreted material, U, is a component of energy flow which has often been ignored in work on invertebrate energetics. In several recent studies the assumption has been made that it is so small in relation to the other components that it's measurment was unnecesary (Hughes, 1970, Nilsson, 1974). Hargrave (1971), on the other hand, suggested that U represents a more significant channel of energy flow than these workers had assumed. One of the reasons for this difference may lie in the interpretation of the term excreta and it is essential that the materials to be included are clearly defined.

In I.B.P. terminology (Holme & McIntyre, 1971), the excreta is taken to mean all material absorbed and later passed out of the body as urine and various secretions, such as mucus. The terms of their definition have been broadened to include such material as shed cuticle - regarded by others as part of the production component. In estimating U for *Neanthes virens* Kay & Brafield (1973) confined their attention to nitrogenous waste products. Their example has been followed in this study where the measurment of other secretions (like mucus) was deemed impracticable. The term excreta is used in the stricter

and narrower sense to describe the end-products of protein metabolism.

It is generally accepted that ammonia forms the bulk of nitrogenous waste products in aquatic invertebrates. Parry (1960) has stated that, of the total nitrogen excreted by *Gammarus locusta* and *G. pulex*, 80 and 70% respectively was ammonia. In the littoral isopod *Ligia oceanica* the figure was 83%. It has been assumed that a similar situation occurs with *Corophium* and no account has been taken of other possible end-products. The production of ammonia by laboratory animals was measured and a comparison drawn with rates calculated from O_2 uptake.

3.3.2. Materials & methods

Measurments of excreted ammonia were made, in the laboratory, using a modified version (Liddicoat *et al*, 1975) of the phenol-hypochlorite method devised by Solorzano (1969). The method relies upon the spectrophotometric determination of the blue Indophenol colour complex which is formed when ammonia reacts with the oxidizing solution in the prescence of a phenol/alcohol mixture.

Determinations were made either on groups of small adults (5 - 6 mm, about 1 mg dry wt.) or on juveniles of about 3 mm. Groups of 10 adults or about 30 juveniles were placed in small dishes containing 50 mls of seawater which had been previously filtered through millipore membrane of pore size 0.2 um. Animals themselves were rinsed, twice, in filtered seawater to remove surface adhering sediment material in an attempt to minimize all sources of contamination. Antibiotics were not added to experimental water as they were found to interfere with colour formation. It was felt, nevertheless, that the combination of membrane filtration and the short duration of experiments (3 h) would mean that the influence of microorganisms would be insignificant (Butler *et al*, 1969).

All experiments were carried out on late autumn animals kept in seawater at 30‰ and 10 C - the conditions in stock tanks.from which the animals were collected.

After 3 h, experimental seawater was re-filtered and

treated with the oxidizing solution (for details see Liddicoat *et al*,1975). Animals, meanwhile, were removed to a drying oven so that dry wts. could be determined. Sample colour development was aided by placing flasks, containing the seawater oxidizing solution, phenol/alcohol and a potassium ferricyanide catalyst, in an aluminium box fitted with a long wave ultraviolet lamp, for 45 min at 25 C. Sample absorbance was measured in a Unicam Spectrophotometer. Wavelength was set at 640 nm and the instrument was zeroed against distilled water. Calibrations were made with seawater to which known ammounts of ammonium chloride had been added and seawater blanks were run with each set of samples to act as controls.

3.3.3. Results

The results of the calibration are illustrated in fig.14. Liddicoat *et al* claimed that Beer's law was obeyed over the concentration range 0 - 20 µg-atoms NH_3. A test for variation amongst replicate calibration samples was carried out using seawater to which the equivalent of 2 ug-atoms NH_3 had been added. After correction for seawater blanks, the mean absorbance was 0.156 $\pm$ 0.012 with a coefficient of variability of 7.6%(Table 8). Coefficients of variation for distilled water and seawater blanks were 3 and 3.8% respectively.

Some 30 experiments were carried out involving about 400 animals. Since groups of animals were used the figure obtained from each experiment is already a mean value for the animals in that group. Results have been expressed in µg mg-amphipod^{-1} d^{-1}. Mean excretion rates have been calculated for adults and juveniles as separate groups. Since the size range of animals was rather limited, no attempt was made to investigate possible effects of size on excretion rates. It is clear, however, that excretion rates were higher in juveniles than in adults (tables 9 & 10). For the adults, with a mean dry wt. of 1.08 mg, the mean rate of excretion was calculated as 1.92 µg mg^{-1} d^{-1} while in juveniles (mean dry wt. 0.41 mg) the mean rate rose to 4.47 µg mg^{-1} d^{-1}(equivalent to 4 and 6.4% of body N daily). Butler *et al* (1969) reported total N excreted

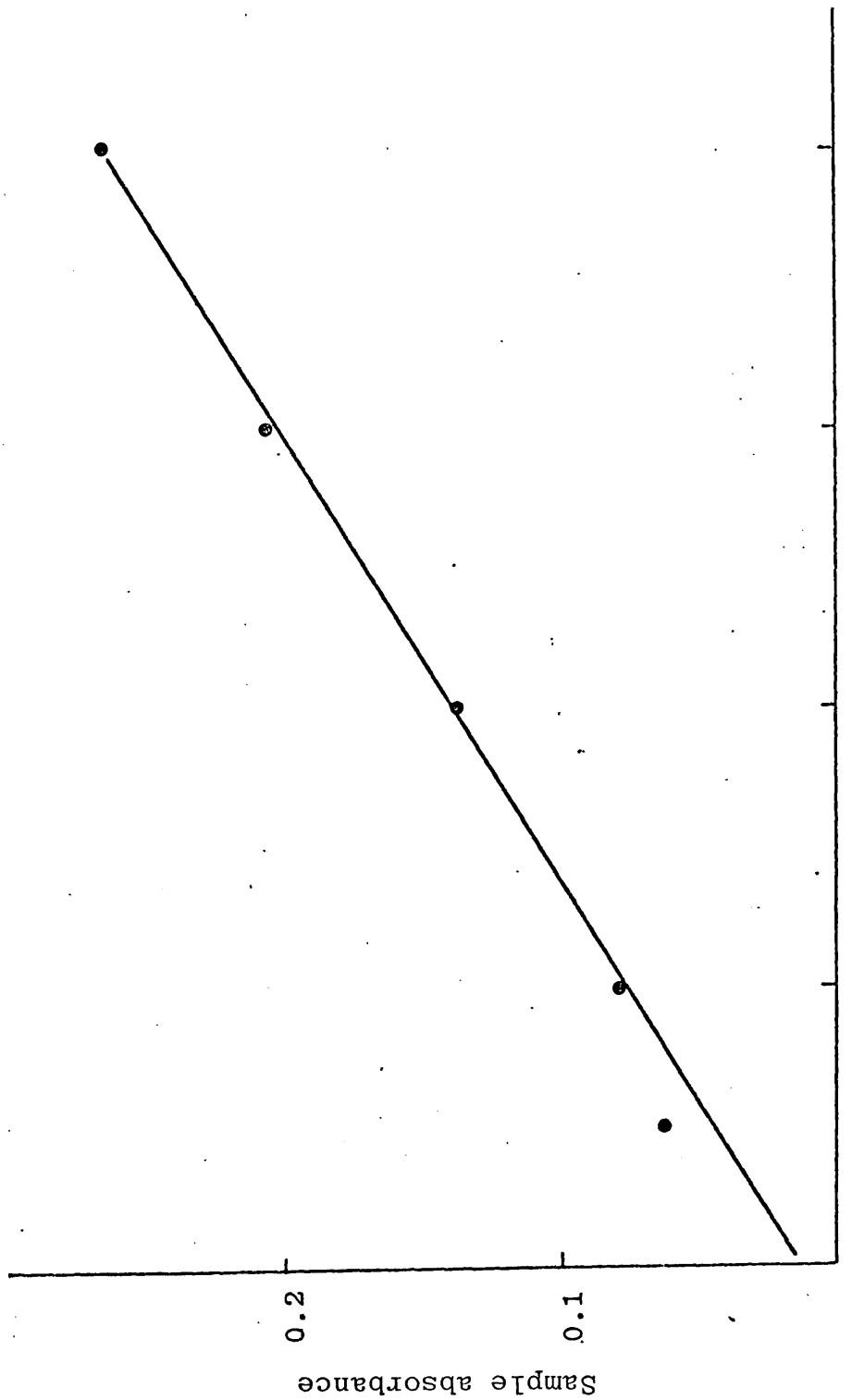


Fig. 14. Relationship between NH_3 concentration and absorbance.

Table 8. Absorbance of replicate calibration Samples *

Sample no.	Absorbance (corrected for seawater blanks)
1	0.136
2	0.149
3	0.151
4	0.159
5	0.181
6	0.153
7	0.154
8	0.171
9	0.145
10	0.157
Mean	0.156
S.D.	0.012 C.V.7.6%

* seawater containing the equivalent of 2 µg-atom NH_3 l^{-1}.

Table 9. Mean excretion rates for groups* of *Corophium* (5 - 6 mm adults)

Mean individual dry wt.(mg)	Excretion Rate ($\mu g\ NH_3\ mg^{-1}\ d^{-1}$)
1.51	0.97
1.43	1.32
1.46	1.86
1.20	1.05
1.20	1.07
1.14	1.58
1.12	1.18
1.09	4.59
1.08	0.93
0.95	1.72
0.95	2.58
0.98	1.06
0.98	1.15
0.88	1.55
0.88	3.26
0.82	3.64
0.75	3.12
Mean 1.08	Mean & S.D. 1.92 ± 1.07 (c.v.56%)

* 10 animals in a group

Table 10. Mean excretion rates for groups* of juveniles (about 3 mm)

0.58	3.77
0.58	4.02
0.49	3.90
0.44	4.47
0.38	3.12
0.23	4.33
0.20	7.69
Mean 0.41	Mean & S.D. 4.47 ± 1.22 (c.v.27%)

* 30 animals in a group

by two species of *Calanus* to be between 4.97 and 14.94 µg mg^{-1} d^{-1} (7.6 - 13.4% of body N),depending upon size, sex and season. Unfortunately comparable data for other amphipods is sparce.

To convert the mean weights of NH_3 excreted into units of energy passed out of the system, an energy equivalent for excreted NH_3 can be applied. The figure adopted here is that of Elliot & Davison (1975) - 4.89 cals mg NH_3^{-1} (83.2 kcals $mole^{-1}$- from heats of formation). When this value is applied to the mean rates from tables 9 & 10 it follows that an adult excretes 0.039 J mg^{-1} d^{-1}(0.0094 cals) while a juvenile excretes 0.092 J mg^{-1} d^{-1} (0.022 cals).

3.3.4. Discussion

The ammount of energy lost as the end products of nitrogen metabolism must always be small in relation to the major components of an energy budget. Kay & Brafield (1973), for example, gave figures that suggest that nitrogenous excretion represents only 2.5% of the total budget of *Neanthes virens*. Using the results of the experiments described above it would appear that for an adult 1 mg dry wt. *Corophium* excretion accounts for about 3% of the energy intake at 10 C. Closer examination of these results suggests, however, that on a molar basis this estimate is rather high.

When protein is oxidized the rate of production of excretory end products should be proportional to O_2 uptake. Brafield & Solomon (1972) and Elliot & Davison (1975) have made use of this relationship and have suggested that energy lost as excreted nitrogen can be calculated from O_2 uptake rates. They have calculated energy equivalents for converting O_2 uptake to energy lost through excretion. The appropriate value depends upon the nature of excretory end-products so that Elliot & Davison have proposed a figure equivalent to 3.71 J ml O_2^{-1}(0.89 cals). Applying this figure to rates of O_2 consumption for the animal (section 3.2.), and assuming protein to be respired exclusively, there is good agreement between observed and calculated U values for 1 mg adults thus:

	Daily U value J mg^{-1} d^{-1} at 10 C.
Observed from excretion experiments	0.039
Calculated from O_2 uptake (fig. 7)	0.038

It is unlikely, however, that protein is the sole respiratory substrate so that the lower figure (0.038) is the maximum possible U value for the given O_2 uptake - the true value probably falling some way below this. When the same comparison is made for juveniles the gulf between observed and calculated U widens considerably (observed - 0.092 J mg^{-1} d^{-1}, calculated - 0.047).

What these experiments do give is an illustration of the relatively minor role of excretion in the overall energy budget . Indeed, the magnitude of U is probably less than the margins of error involved in the measurment of any one of the major components. In a sense, those authors who have ignored it were perhaps justified. Where excretion does become important, however, is in the calculation of the energy requirments of a population, such as that in Benfleet Creek. Where such large numbers of animals are present U must represent a significant loss of energy from the population although it will still be small in relation to overall energy flow.

3.4. The metabolic energy requirements of the Benfleet population

Using data from laboratory metabolism studies it is now possible to estimate the energy requirments of the *Corophium* population in Benfleet Creek. Since it is not possible to measure respiration and excretion in the field it becomes necessary to assume that laboratory measurements reflect the natural situation. Given adequate precautions and reasonable judgement this may well be true but the researcher can seldom be entirely satisfied with such an assumption while the purist will always criticize the practice

Calculations

For each month of the year, the mean dry wt. of the animals making up the population has been determined (sect. 2.3.). The rate of O_2 uptake of animals at these dry wts. has been determined from regressions of dry wt. on O_2 consumption (sect. 3.2.3.-Figs. 7, 8 & 9). Mean monthly air temperatures (Southend-on-Sea - coutesy of the Meteorological office) have been rounded to the nearest 5 C and the appropriate regression line used. The O_2 consumption for the population has been calculated by multiplying VO_2 for the mean dry wt. by monthly biomass m^{-2}. Finally an oxycalorific coefficient (20 J ml O_2^{-1}) has been app-

lied to express population metabolism in energy units. These calculations are summarized in table 11.

Excreted energy has been calculated from O_2 uptake rates using the method of Kay & Brafield (1973). An energy equivalent has been used to convert the rate of O_2 uptake into the rate of energy lost as excretory products. The figure 3.71 J ml O_2^{-1} has been applied to the population O_2 consumption, and protein has been assumed to make up 60% of respired food (Blazka, 1972, Parsons *et al*, 1961).This is essentially a simple continuation of the calculations described above. Computations are summarized in table 12.

The monthly figures from tables 11 and 12 have been plotted on a graph (fig. 15) which illustrates a very clear trend, closely following the changes in environmental temperatures. Estimates of population production (sect. 2.3.3) follow a similar pattern.

Energy requirments reach a maximum in the summer months from June to September when the population is in it's most productive stage. The high figures reflect the large numbers of small animals which make up the population at this time as well as an increase in metabolic rate due to temperature. During the summer months the animals in the creek are often subjected to temperatures well in excess of 20 C. In July and August Temperatures as high as 28 C were recorded in the upper layers mud where *Corophium* was actively foraging.

Month	Temp. C.	(1) Biomass $g\ m^{-2}$	Mean ind. (mg.)	(2) VO_2 $\mu l\ O_2\ mg^{-1}\ h^{-1}$	R - Respiration $kJ\ m^{-2}$
November	5*	14.74	0.5	0.42	89.1
December	5*	13.0	0.5	0.42	78.6
January	5*	6.6	0.54	0.41	38.9
February	5*	3.8	0.29	0.48	26.2
March	5*	6.96	0.34	0.47	47.1
April	10	11.5	0.58	0.49	81.1
May	15	16.1	1.73	0.44	101.9
June	20	20.3	0.63	0.79	230.8
July	20	17.8	0.22	1.03	271.4
August	20	12.9	0.23	1.02	189.1
September	15	16.0	0.22	0.92	211.8
October	15	10.0	0.22	0.92	132.4
				Total	1499 $kJ\ m^{-2}\ yr^{-1}$

Table 11. Calculation of monthly population respiration for Benfleet animals

5 C.VO_2 s were extrapolated. R = (1) x (2) x oxy-cal. coeff.(20 $J\ mg^{-1}$)

Table 12. Population excretion of the Benfleet animals

Month	Temp. t C.	Biomass (g m^{-2})	Excretion - U kJ m^{-2} *
November	5	14.74	9.97
December	5	13.0	8.78
January	5	6.6	4.36
February	5	3.8	2.93
March	5	6.96	5.28
April	10	11.5	9.09
May	15	16.1	10.89
June	20	20.3	25.85
July	20	17.8	29.46
August	20	12.9	21.20
September	15	16.0	23.72
October	15	10.0	14.83
		Total	166.3 kJ m^{-2} yr^{-1}

* Calculated from population O_2 consumption

Biomass x VO_2 x 3.71 J (see text for explanation)

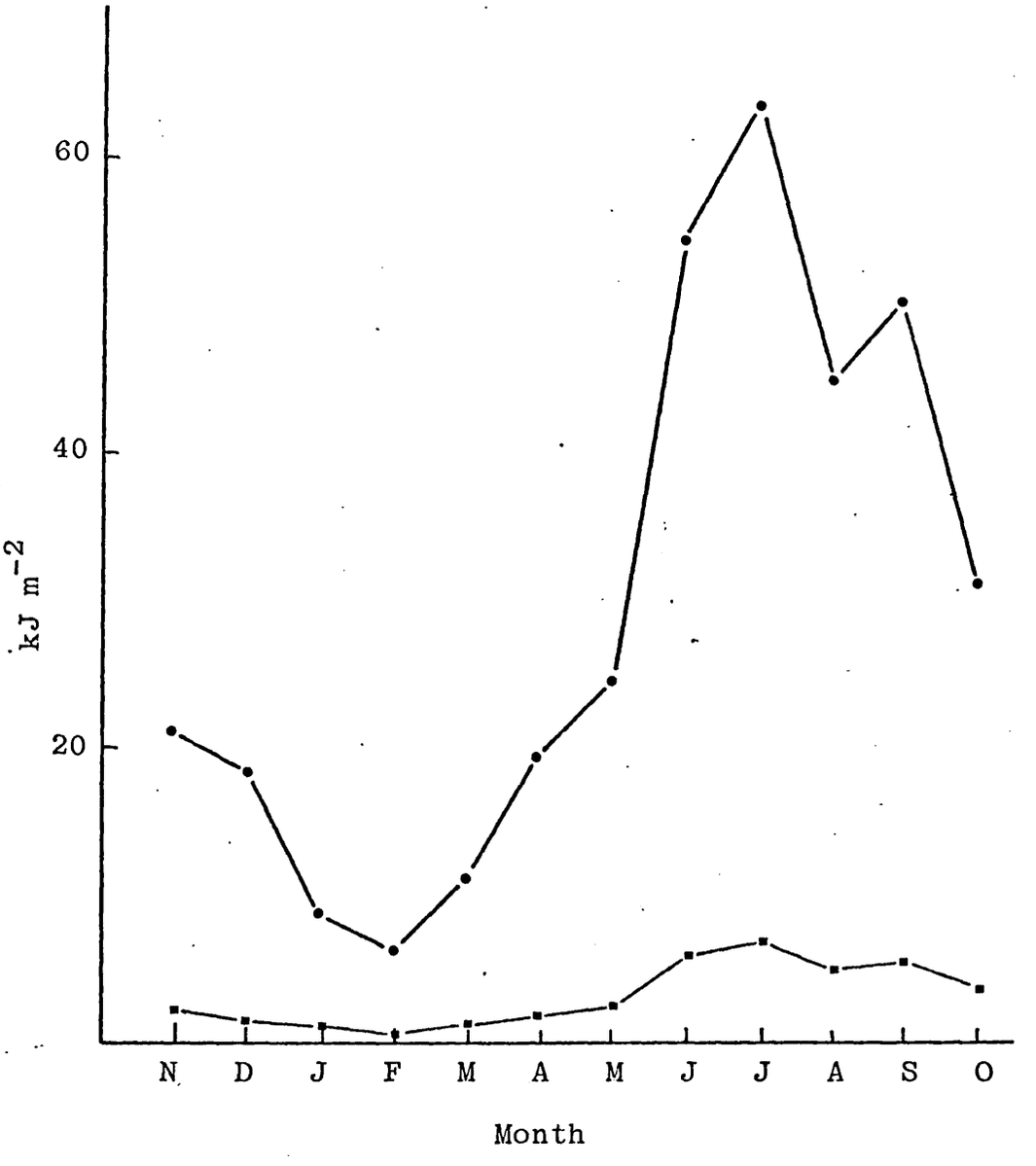


Fig. 15. Metabolic energy requirements of *Corophium* in Benfleet Creek (kJ m^{-2})

Circles - respiration, squares - excretion.

Conversely the energy requirments of the winter population fall to a minimum in February. At this time of the year biomass is at it's lowest and metabolic rates are, doubtless, lowered by reduced temperatures. The mean for the month of February 1976 was 4.3 C.

4. CONSUMPTION

4.1. Introduction

The measurment of intake of food or energy (consumption) by *Corophium volutator* is complicated by the feeding habits of the animal. *Corophium* is a selective deposit feeder (Hart, 1930, Meadows & Reid, 1966) utilizing organic material which is present in it s native sediment. Deposit feeding is still poorly understood, but it has often been suggested that it is microorganisms, bacteria and diatoms, associated with detritus and sand grains which are important in the nutrition of deposit feeding animals (Newell, 1965, Meadows, 1964 b, Hargrave, 1970, Fenchel *et al*, 1975).

Attempts to measure feeding rates under natural conditions are fraught with difficulties and, consequently, there have been few serious studies of the energetics of deposit feeding. Those authors who have undertaken such work have tended to estimate ingestion by indirect means. Hughes (1970), for example, derived an equation for calculating ingestion by *Scrobicularia plana* based upon the calorific values and rates of production of faeces and pseudofaeces. Direct measurment of sediment ingestion is not always possible, although radiotracers may offer a solution in some cases. Hargrave (1970) estimated rates

of ingestion for the amphipod *Hyalella azteca* by mixing ^{14}C labelled microorganisms with lake sediment.

The selective feeding habits of *Corophium* and the complexity of the material on which it feeds have meant that the methods used by previous investigators could not (for reasons which, it is hoped, will become apparent) be applied to estimate ingestion in the present study. The greatest difficulties arose from the collection and analysis of faeces. *Corophium* produces faecal pellets bound in a thin chitinous membrane. Pellets are of variable size and are easily ruptured. In appearance they are indistinguishable from sediment and cannot, therefore, be separated from it. This meant that even the elegant ^{14}C radiotracer approach had limited applications and, while experiments using ^{14}C labelled diatoms were informative in terms of the feeding biology of the animal, reliable estimates of sediment ingestion were not possible.

Instead, measurements were made of the rate at which sediment passed through the gut of laboratory animals and the average weight of the gut contents. From this data average ingestion rates for adult (1 mg dry wt.) animals were calculated. The mean calorific content of the food assumed to be ingested was then applied to convert these proposed rates into energy units.

The aim, throughout, was to obtain estimates of ingestion which would, as far as possible, reflect the normal feeding activity of animals in stock tanks.

4.2. Materials & methods

4.2.1. Experiments with ^{14}C labelled diatoms

A pennate diatom, *Nitzschia closterium (≡ Phaeodactylum tricornatum)*, seeded from stock cultures, was grown up in 75% seawater enriched with Miquel-Allen solutions. ^{14}C was supplied as Na $H^{14}CO_3$ (specific activity - 40 mci $mmol^{-1}$) at a radioactive concentration of 1 uci per 10 ml of seawater and the whole was maintained at 20 C under fluorescent lighting. Cells were allowed to grow in labelled culture for 10 days before use, by which time it was assumed that labelling was uniform. When required for experiments cells were spun down from culture medium and resuspended, twice, in fresh seawater to remove extracellular label.

Adult *Corophium*, between 1 and 1.5 mg dry wt., were supplied with the labelled food organism either as a pure suspension or mixed with natural sediment. Killed animals were used as controls in each experiment. Radioactivity accumulated by the animals, and that of controls and food, was determined in a Panax Liquid Scintillation Counter. Samples to be counted were rinsed, three times, in fresh seawater, to remove surface adhering label, and then oven dried at 45 C. Dry material was then weighed, ground up in a small glass tissue homogenizer and suspended in Bray's solution. To prevent homogenate particles from sinking

to the bottom of the counting vial, Cab-O-Sil, a finely divided silica powder (Cabot Carbon of Canada Ltd.), was added to the cocktail to form a gel. Counting efficiency was measured by adding small volumes of a radiochemical standard, n-hexadecane (The Radiochemical Centre, Amersham) to precounted samples (Dyer, 1974).

Counting efficiency was between 65 and 75%, the degree of quenching being dependent upon the nature and quantity of the sample material. Efficiency was not significantly affected by the addition of Cab-O-Sil. Count rates were corrected for counting efficiency, background radiation and the radioactivity of controls and are expressed in d.p.s. mg-amphipod^{-1}.

4.2.2. Rate of food passage through the gut

The rate at which sediment passed through the guts of adult *Corophium* was estimated by visual examination of feeding animals under a binocular microscope. Groups of animals were presented with pellets of diatoms (*Nitzschia closterium*) and green algae (*Brachiomonas submarina* and *Chlorella* sp.) spun down from stock cultures. Animals had been previously feeding on natural sediment, under similar experimental conditions so that the interface between normal gut contents and coloured algal food could be traced through the gut. A number of animals were removed at intervals and examined under low power magni-

fication. The rate of food passage could, thus, be estimated. Observations were made at 10 and 20 C and reciprocal experiments were carried out with animals transferred back to sediment after feeding on coloured algae.

4.2.3. Weight of gut contents

The average weight of gut contents of 5- 6 mm animals was determined both for actively feeding animals and ran-om samplesof the laboratory population. For active feeders, gut contents were simply teased out in a watch glass and somatic tissue and carapace sparated from it. Gut contents are expressed as a percentage of animal dry wt.

For samples of the laboratory population, large numbers of adults with variable ammounts of sediment in their guts, were collected from stock tanks. Separation of gut material from individuals was, in this case, not possible and the method of Fenchel *et al* (1975) was used. Animals were killed in 70% alcohol immediately after collection and oven dried at 60 C. A weighed ammount of dry *Corophium* tissue was then digested for 2 h in conc. nitric acid held at 90 C. After this time, only the mineral particles derived from the gut contents, remained at the bottom of the vessel while a certain ammount of carapace material floated on the top of the acid. Acid and carapace were discarded and the mineral residue was rinsed,

several times, with distilled water and dried overnight. This material was then expressed, as above, as a percentage of animal dry wt.

4.2.4. Energy content of sediment

Surface sediment, collected from *Corophium* stock tanks, was oven dried at 60 C. Sub-samples (30 - 50 mg) of the dry material were transferred to conical flasks and calorific contents determined by the wet-oxidation method described in section 2.2.3. The technique yields results in either % Carbon (organic) or energy units (J g^{-1}). Fenchel *et al* (1975) found that the mineral particles ingested by *Corophium* were, in the main, less than 60 um in diameter. Further analyses were carried out on particles of this size fraction (material passing through a sieve of mesh size 63 μm). % total organic material was determined by burning pre-weighed samples in a muffle furnace at 550 C for 4 h.

4.3. Results

4.3.1. Experiments with ^{14}C labelled diatoms

The initial aims of tracer experiments were, firstly, to ascertain whether *Corophium* could utilize a diatom (*Nitzschia*) and then to obtain estimates of ingestion rates and assimilation efficiency when the diatom was ingested along with natural sediment.

The results showed that *Corophium* was indeed able to ingest and assimilate *Nitzschia* whether it was presented with sediment or as a pure culture (figs. 16). Fig. 17 shows the effect of temperature on feeding activity. Here animals were allowed to feed on a diatom/sediment mixture at 10 and 20 C but in otherwise identical conditions, and it is clear from the graph that they exhibited a markedly increased level of feeding activity at the higher temperature. This is consistent with the observation that, in general, activity seems to be temperature dependent (sect. 3.2).

Ingestion rates.

From this data it is possible, knowing the specific activity of the labelled food cells, to calculate short term ingestion rates. The expression "short term" must be emphasized here since one of the difficulties encountered inall feeding experiments lay in the collection of faeces.

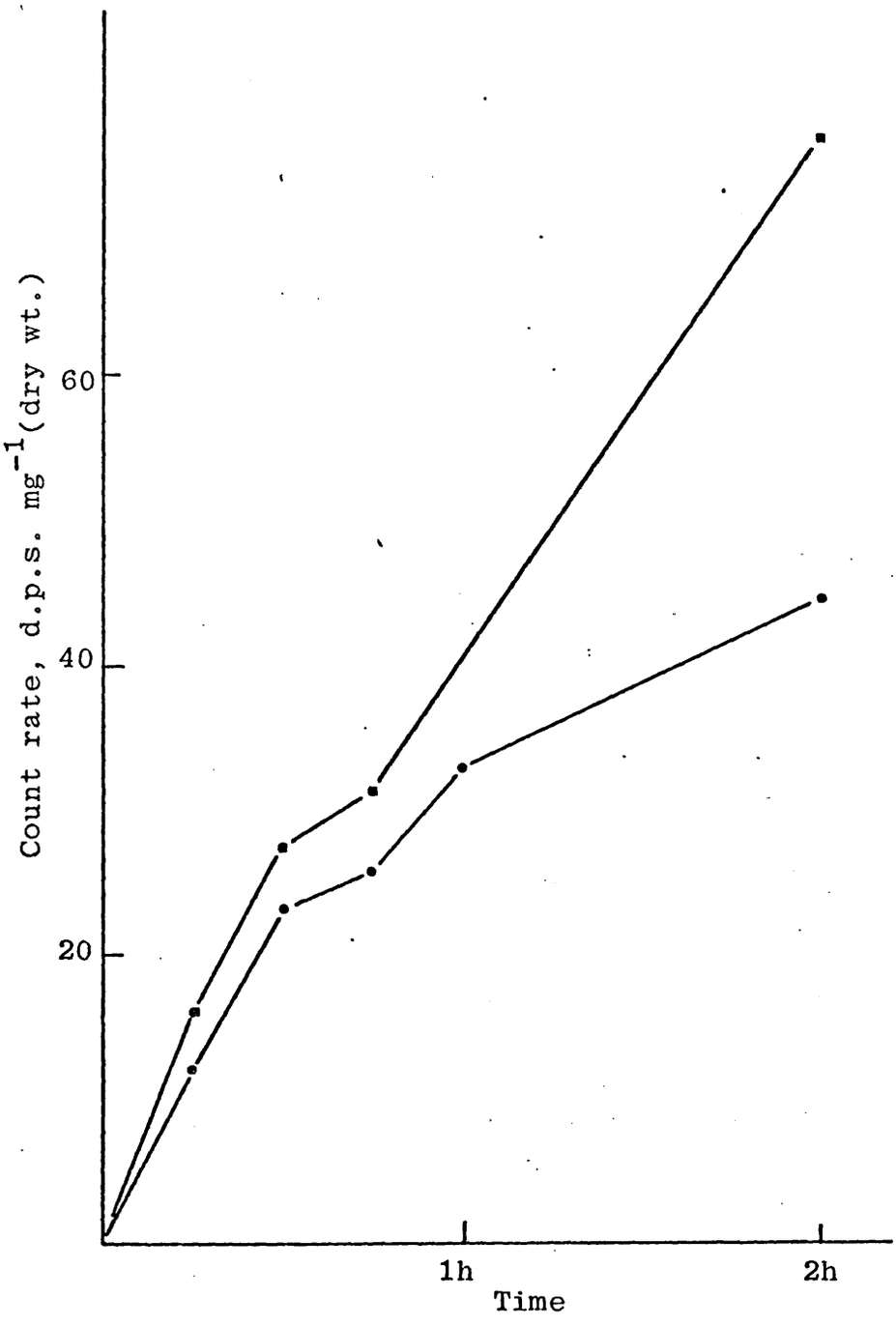


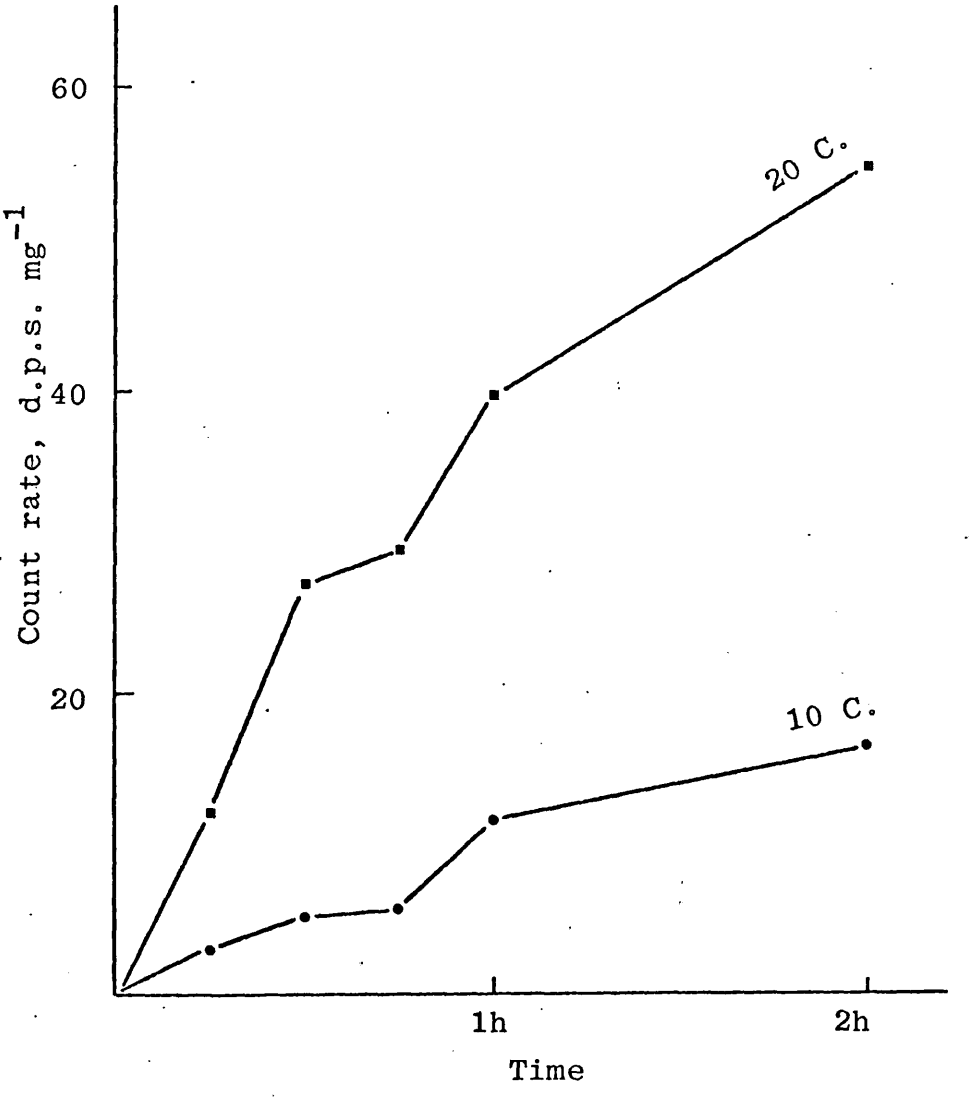
Fig. 16. Uptake of ^{14}C labelled diatoms by *Corophium*

Squares - from suspension

Circles - from a sediment/diatom mixture

(Each point represents a mean for a group of 10 adult animals)

Fig. 17. Uptake of ^{14}C labelled diatoms by *Corophium* at two temperatures, 10 & 20 C.



Each point represents the mean count rate of 10 experimental animals

Table 13. Ingestion of ^{14}C labelled diatoms by *Corophium volutator*

Experimental conditions	Count rates (d.p.s. mg^{-1})		Wt. of diatoms ingested (µg)	
	15 min	30 min	15 min	30 min
1) 10 C. (Diatom/sed)	2.93	5.23	9.3	16.7
2) 20 C. (diatom/sed	11.8	27.0	37.3	85.4
3) 20 C. (diatom/sed)	12.3	23.7	39.0	75.0
4) 20 C. (pure culture)	16.2	27.4	51.0	86.0

Spec. activity of diatom cells 316 d.p.s. mg^{-1} (dry wt.)

Much of the faecal material produced by *Corophium* is in the form of distinct pellets bound in a thin chitinous membrane but even in this form it is almost impossible to separate it from the sediment. This makes the direct measurment of long term ingestion rates impracticable. As an animal continues to feed it accumulates radioactivity in it's body and assuming a constant rate of ingestion, a graph of radioactive uptake against time will continue as a straight line. At some point, X, however, the animal's gut will be full and it will begin to produce radioactive faeces. When this point is reached, the slope of the line will change (Rigler, 1961) so as to represent net accumulation of label due to assimilation (ingestion - faeces). Clearly, then, ingestion rate can only be directly measured up to the point when drop in the rate of ^{14}C uptake denotes the production of labelled faeces. In these experiments such a drop appeared after about 30 min at 20 C (figs. 16 & 17) so that short term in the present context means 30 min or less. Table 13 gives estimates of ingestion rates calculated from these figures.

After 15 min feeding at 10 C animals had ingested 9.3 µg dry wt. mg-amphipod^{-1} of diatom cells while at 20 C the figure rose to 37.3 µg mg-amphipod^{-1}.(animals at 10 C took about 1 h to ingest the same ammount of cells. The 10 C rise in temperature produced a 4-fold increase in the feeding rates of laboratory animals. These figures refer only to the weight of diatom cells and do not represent

the total weight of ingested material. Since sediment was also taken in, the true ingestion rates (overall) in these experiments may have been considerably higher.

Assimilation efficiency

The measurment of assimilation efficiency was beset by the same problems described above, namely, the collection of faeces. Normally an estimate of total ingestion is required (including faeces produced). Since faeces could not be collected an indirect, graphical method has been used to calculate assimilation efficiency as follows:

Assimilation rate (the net uptake of radioactivity during the experiment) was plotted just as in figs. 16 & 17, and the initial observed ingestion rate (table 13) was extrapolated beyond the point X, where ^{14}C labelled faeces were produced and a change in slope was seen (figs. 18 & 19). Thus, we have two lines, one (line A),which is the observed assimilation rate and the other (line B),which represents the expected ingestion rate. The area between the two lines corresponds to the ^{14}C label rejected, mainly as faeces, over the course of the experiment. Before the assimilation efficiency can be calculated, a third line must be added. This is a new baseline, C, which corrects for the radioactivity of the gut contents and is taken, again, from the point X (where the guts of the experimental animals are assumed to be full). Gut contents must be excluded from calculations since by no means all of the labelled material present is likely to be assimilated.

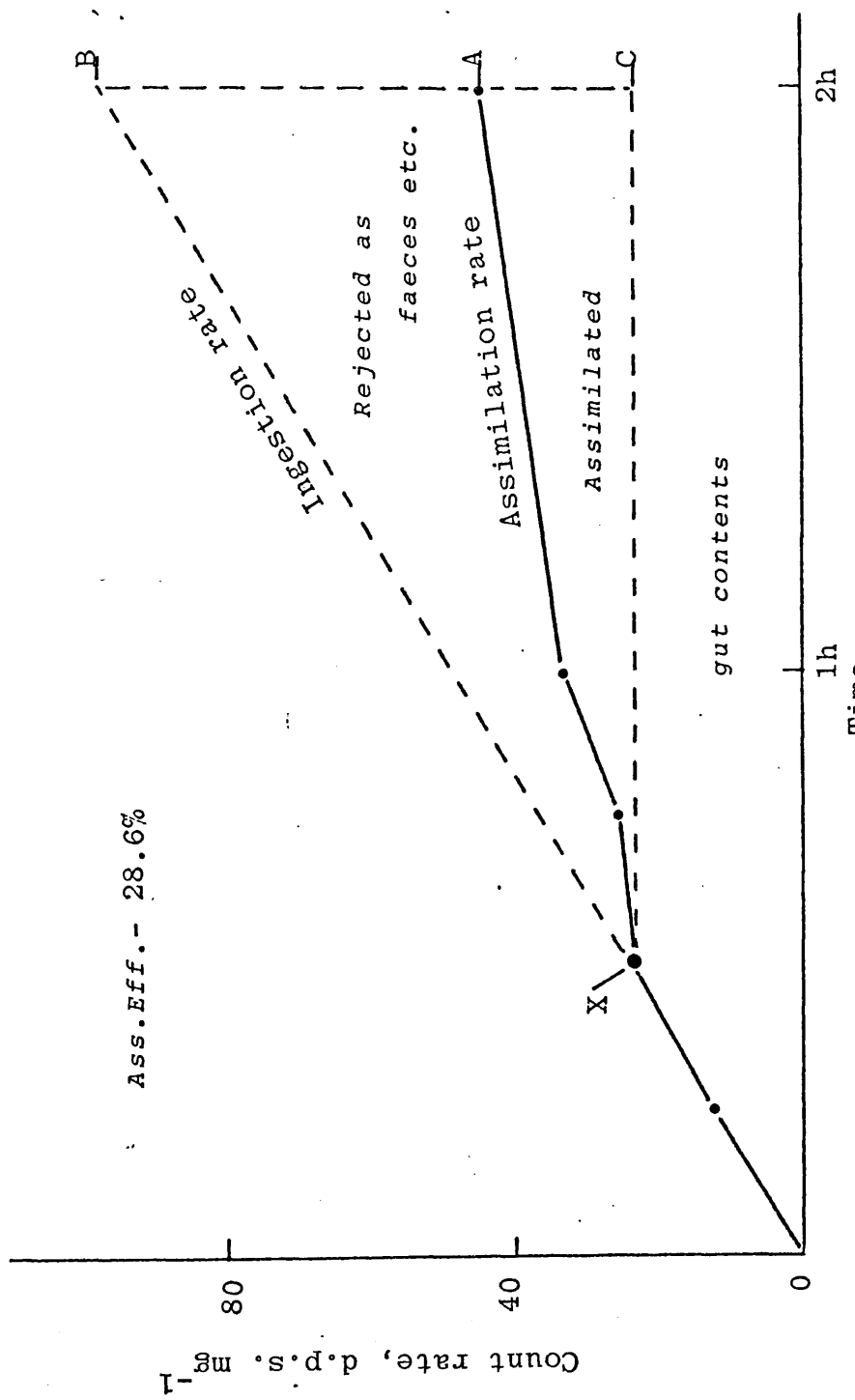


Fig. 18. Graphical estimation of assimilation efficiency for *Corophium* feeding on labelled diatoms (see text for explanation).

(Each point represents a mean for a group of 10 adults)

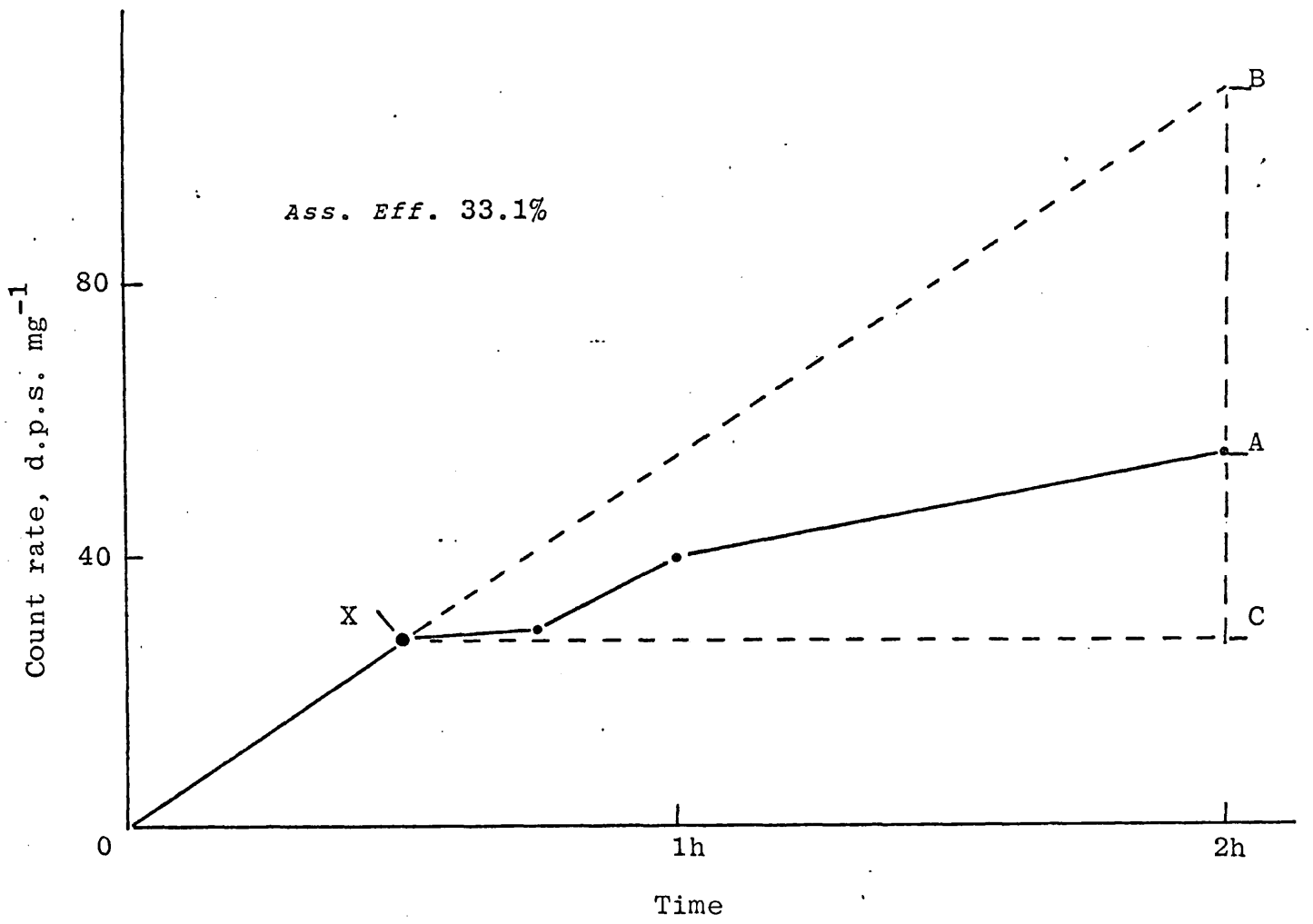


Fig. 19. Graphical estimation of assimilation efficiency for *Corophium* feeding on labelled diatoms (see text)

(Each point represents a mean for a group of 10 adults)

Without this correction assimilation efficiency may be overestimated.

The total area of the graph between lines B & C now represents the theoretical ingestion while that between A & C denotes the actual assimilation. Assimilation efficiency is now given by the expression:

$$* \% \text{ Ass. Eff.} = \frac{\text{Area of sector between A \& C}}{\text{Area of sector between B \& C}} \times 100$$

* The relationship holds true until the specific activity of the feeding animals equals that of the food, after which the net accumulation of ^{14}C label would denote growth. In practise this situation would only arise after many hours of feeding.

This calculation has been done for the examples drawn in figs. 18 & 19 and assimilation efficiencies were 28.6 and 33.1% respectively. Much higher efficiencies than these were reported for the amphipod *Hyalella azteca* by Hargrave (1970).. He noted 75% efficiency with the diatom *Navicula* sp. and a mean of 50% with green algae. It is felt, however, that the figures calculated here probably offer a realistic estimate since the large am ounts of inorganic material present must reduce the efficiency of digestion.

4.3.2. Rate of food passage through the gut

Visual observations of food passage times showed certain striking features. Firstly, in all experiments a percentage of animals could be classed as non-feeders, and while others had filled their guts and begun to produce colour labelled faecal pellets, these animals had no algal food in their guts at all. At first it was thought that the non-feeders had, in some way, been damaged when they were transferred to the experimental vessels, but analysis of animals from stock tanks and from Benfleet Creek revealed a similar situation with up to 35% of the animals in a random sample without food in their guts. No explanation can be given for this phenomenon, except to suggest that individuals may undergo periods when they do not feed, perhaps during moulting. At any rate, the nutritional state of animals in the field and in stock tanks was by no means constant and at a given time the amount of food in the guts of individuals varied considerably. Table 14 shows the percentages of non-feeders in short term laboratory experiments and in a random sample from a stock tank.

It can be seen from this that the proportion of animals without food in their guts is remarkably constant throughout. The percentage of animals exhibiting maximal feeding activity (those with full guts) is not so constant.

Table 14. Feeding activity of *C. volutator* in laboratory experiments and in stock tanks

	% active feeders	% non-feeders	% with full guts
10 C lab expt.	69	31	6 (n = 80)
20 C lab expt.	72	28	72 (n = 70)
Random sample Stock tank	65	35	45 (n = 120)

Lab data from 25 min feeding experiments

At 10 C it was only 6% while at 20 C all feeding animals (72%) had full guts. At the higher temperature animals fed either at maximal rates or not at all while at 10 C feeding activity was substantially reduced. This is consistent with the results of radiotracer experiments where feeding activity was seen to be affected by temperature.

The rate of food passage is rapid and reflects these activity differences. At 20 C the time taken for animals to fill their guts was 19.4 $\pm$ 4.3 min (n=20). At 10 C, the meantime was 56.5 $\pm$ 10.9 min (n=19). It would seem therefore, that the rate of food passage is a function of feeding activity rather than a direct physiological effect. The rate of food passage seemed unaffected by the nature of the food presented to the animals but they would not ingest sediment from which organic material had been removed. (see also, Meadows, 1964 b).

Fenchel et al (1975) estimated that the time taken for *Corophium* to fill its gut was between 30 and 40 min, although they did not state the conditions under which this was determined.

4.3.3. Weight of gut contents

The gut contents of adult *Corophium* (with guts full of sediment) have been estimated to represent about 20% of total body dry wt. (table 15). This means that an animal of 1 mg dry wt. (about 5 mg live wet wt.) could ingest around 200 µg dry wt. of sediment in 1 h at 10 C and around 600 µg h^{-1} at 20 C. In other words, the animal could ingest it s own weight of sediment in 5 h at 10 C or in less than 2 h at 20 C. Heywood & Edwards (1962) reported comparable sediment turnover rates for *Potamopyrgus jenkinsi*. They calculated that the snail produced a mean of 114 µg dry wt. h^{-1} of faeces, some 25% of body dry wt. (exclusive of shell).

Sustained sediment turnover at these sort of rates is difficult to envisage , if we assume that deposit feeders like *Corophium* feed continuously. Indeed, from observations described in the preceeding section it is clear that this is not the case. It has been pointed out that many of the animals collected from stock tanks and from the field had not been feeding while others had varying ammounts of food in their guts. What is really needed, then, is an estimate of the average weight of gut contents of random samples of the population. Such an estimate would take into account the observed variations in feeding activity. For this, gut contents were separated

Table 15. Weight of gut contents in actively feeding *C. volutator* (animals with full guts

	Total dry wt. (mg)	Dry wt of gut contents(mg)	Gut contents as % of total dry wt.
1)	6.61	1.63	24.7
2)	6.00	1.32	22.0
3)	8.04	1.18	14.7
		mean	20.5

6 adult animals in each sample.

Table 16. Weight of gut contents of random samples of *C. volutator*

	Total dry wt. (mg)	Dry wt. of gut contents(mg)	gut contents as % of total dry wt.
1)	379.08	8.31	2.19
2)	312.01	11.88	3.81
3)	201.10	12.88	6.41
4)	200.00	5.33	2.67
5)	83.21	2.36	2.84
		Mean	3.58%
		S.D.	1.5

Gut material separated by HNO_3 digestion

out by the acid digestion described in the materials & methods section. This technique, inevitably, leads to a loss of the organic material from the gut contents and, therefore, only estimates the weight of mineral particles. The organic content of the sediment is low, however, and the error involved will be small. Table 16 gives the results of five digestions from which a mean of 3.6 $\pm$ 1.5% of body dry wt. has been calculated. Thus, on average, the gut of a 1 mg animal would contain 36 µg of sediment. This new figure falls substantially short of the estimate based on actively feeding animals but is thought to be a realistic average taking into account periods when an animal is not feeding.

4.3.4. Energy content of sediment

The energy content of the sediment of laboratory stock tanks varied little. Tanks were replenished at approximately 8 week intervals and calorific and organic carbon contents were measured at a number of occasions throughout the year. Table 17 gives the range of values experienced. The mean, 1.35% organic C, compares well with previously reported values for natural sediments (George, 1964, Buchanan & Longbottom, 1970, Newell, 1965), and corresponds to a mean calorific content of 511 J g^{-1} dry wt.(122 cals). As expected the calorific content of the material is low (equivalent to 1800 cals g^{-1} AFDW), reflecting the small ammount of organic material present- less than 7% of the total dry wt. Hughes (1970) reported the mean calorific content of sediment ingested by *Scrobicularia plana* to be 152 cals g^{-1} (637 J).

Newell (1965) showed that organic carbon content was highest in the smaller size fractions of the sediment at Whitstable in Kent while Meadows (1964 a) suggested that by selecting small particle sizes *Corophium* might be making use of a richer microflora. Fenchel *et al* (1975) have demonstrated that *Corophium* ingests particles of a limited size range, mostly less than 60 µm diameter. In view of this evidence it is quite probable that the energy content of the material ingested by the animal is not the

Table 17. Energy content of laboratory sediment

Mean values for random samples from stock tanks - n = 3 in each case.

% organic C		$J\ g^{-1}$ dry wt.	
2.10		796.1	
1.55		585.6	
1.33		502.8	
1.44		544.7	
1.22		460.9	
1.33		502.8	
1.22		460.9	
1.10		419.0	
0.88		331.0	
Mean	1.35 ± 0.32	Mean	511.2 ± 121.5

Mean total organic content = 6.7%

Table 18. Energy content of laboratory sediment

Particle diameter 63 um

$J\ g^{-1}$ dry wt.
1341
1425
1341
1215
1425
Mean & S.D. 1349 ± 75.4 (322 cals g^{-1})

Mean total organic content = 8.8%

All values determined by wet-oxidation

same as that of uningested sediment. The calorific content of the material which can be ingested by *Corophium* was, therefore, estimated. The mean value for this material (less than 63 μm particle diameter)was found to be 1349 J g^{-1} dry wt. (322 cals g^{-1} or 3650 cals g^{-1}AFDW). By selecting this fraction of the sediment, the am ount of energy available to *Corophium* from a given weight of material is doubled and even this may be an underestimate if selection goes beyond the particle size level. (table 18).

It should now be possible to propose average ingestion rates.

4.3.5. Calculation of ingested energy

Data from the previous sections can now be combined to estimate average ingestion rates for adult (1 mg dry wt.) *Corophium*. The calculation relies upon data on

a) Rate of food passage

b) Mean weight of gut contents

c) Calorific content of ingested food

The rate of food passage has been estimated as 20 min at 20 C and 1 h at 10 C (4.3.2). The average weight of gut contents of a 1 mg adult was found to be 36 µg dry wt. Taking into account an organic content of 8.8% this figure round up to approximately 40 µg so that a 1 mg animal would ingest 40 µg h^{-1} at 10 C and 120 µg h^{-1} at 20 C. Hargrave (1971) estimated that a 700 µg dry wt. *Hyalella azteca* ingested 25 µg h^{-1} of lake sediment at 14 C.

The calorific content of the material ingested by *Corophium* was estimated at 1349 J g^{-1} dry wt.(4.3.4). The energy intake of the 1 mg animal is now determined by multiplying the calorific value of the food by the weight of food ingested in unit time. The animal would, therefore, ingest 0.054 J h^{-1} at 10 C and 0.162 J h^{-1} at 20 C. (table 19).

These calculations were only possible for 1 mg adults. Evaluation of equivalent rates for all size classes would have been prohibitively complex and time consuming.

Table 19. Ingestion rates of 1 mg dry wt *Corophium*

	10 C.	20 C.
µg mg-amphipod^{-1} h^{-1}	40	120
J mg-amphipod^{-1} h^{-1}	0.054	0.163
cals mg-amphipod^{-1} h^{-1}	0.013	0.039

Sediment calorific value taken as 1349 J g^{-1} (dry wt.)

4.3.6. Energy intake of the Benfleet population

As with R and U, energy intake or consumption (C) has been calculated from the results of laboratory studies. Certain assumptions have had to be made. Firstly it has been assumed that the feeding rates of animals in the laboratory are comparable to those of animals in the salt marsh. The extent to which this assertion is true is impossible to gauge (so often the case in work of this nature) except, perhaps,in the final analysis when results can be evaluated against the findings of previous studies. Secondly, because of the complexity of the size structure of the *Corophium* population, ingestion rates could only be calculated for a "standard" adult of 1 mg dry wt. (at about 6 mm length). This corresponds approximately to the median dry wt. for Thames animals and was, therefore, considered a convenient size for feeding experiments. It has been necessary to assume that rates of ingestion calculated for these animals provides a reasonable basis for calculation of population energy intake.

Average ingestion rates for 1 mg animals have been calculated in the previous section (4.3.5)Rates were seen to be affected by temperature and so the appropriate rate for each month of the year has been chosen on the basis of mean monthly temperature rounded to the nearest 5 C.

Month	Temp. t° C.	Biomass g m^{-2}	Ingestion Rate J mg^{-1} h^{-1}*	Monthly intake kJ m^{-2}
November	5	14.74	0.037 J	386.9
December	5	13.0	"	341.3
January	5	6.6	"	173.3
February	5	3.8	"	99.8
March	5	6.96	"	182.7
April	10	11.5	0.054	451.0
May	15	16.1	0.084	947.2
June	20	20.3	0.163	2388.5
July	20	17.8	"	2088.5
August	20	12.9	"	1515.5
September	15	16.0	0.084	941.3
October	15	10.0	"	588.3
			Total -	10104.3

Table 20. Calculation of monthly energy intake of the Benfleet population

* 5 & 15 C. rates extrapolated from 10 & 20 C.

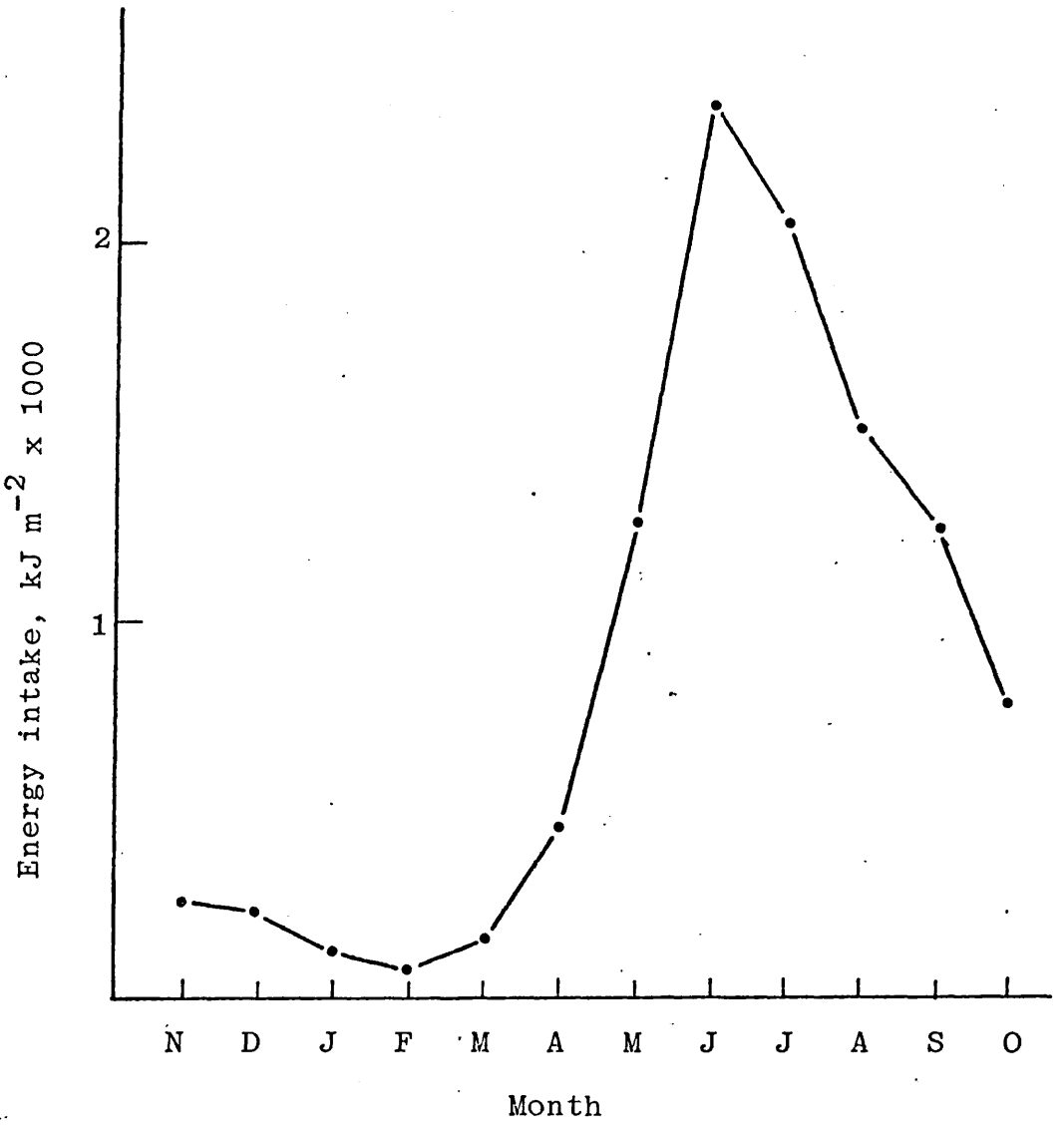


Fig. 20. Energy intake of the Benfleet population
Calculated from lab feeding rates using a model of the *Corophium* population

Rates for 5 and 15 C. were extrapolated from 10 and 20 C. data. Ingestion rates were multiplied by standing stock biomass (g m^{-2}) for each month, giving the monthly energy intake of the population. The sum of these estimates is, of course, the total annual consumption (C). A summary of these calculations is given in table 20 and monthly estimates have been plotted in fig. 20.

4.4. Discussion

It soon became clear, when studying the feeding biology of *Corophium volutator*, that the direct measurement of ingestion rates and assimilation efficiencies would be impracticable. The specialized deposit feeding habits of the animal (Hart, 1930, Meadows & Reid, 1966) meant that the well tried methods of previous studies could not, strictly, be applied. Most of the problems arose out of the need to maintain *Corophium* in it s native sediment.

To ensure it s survival,growth and normal feeding behaviour, the animal needs a supply of mud in which to burrow. Most of it s time is spent in the burrow and it is here that normal feeding takes place - the animal scraping detritus and sand grains towards the opening by means of it s enlarged antennules (Meadows & Reid, 1966). Most of it s nutritional requirments are derived from organic material in the surface sediment along with it's associated microflora so the need to maintain *Corophium* in it s native mud is obvious. Strictly speaking,then, for an energy budget for the animal under these conditions, consumption should be estimated in terms of the natural food.

Much of the work on invertebrate feeding biology has been carried out under artificial feeding regimes with perhaps a single food organism in pure culture. This

approach has been used frequently with filter feeders although it has also been adopted for *Neanthes virens* (Kay & Brafield, 1973). The calculation of ingestion rates and assimilation efficiency is fairly straightforward under such conditions and could easily have been applied to *Corophium* but the real interest and, unfortunately, the real difficulty lies in measuring feeding rates under natural conditions. It was felt that, despite the difficulties of working with natural sediment, maintaining *Corophium* without it would be too far removed from the natural situation to produce ecologically meaningful results.

A number of authors have studied the energetics of deposit feeding. Hargrave (1970) measured ingestion rate and assimilation efficiencies in *Hyalella*. He used a number of different ^{14}C labelled microorganisms mixed with lake sediment and estimated ingestion rate from the specific activity of the sediment and the amount of ^{14}C label taken up by the animals. His method relied upon two basic assumptions, firstly, that the labelled cells were evenly distributed throughout the sediment and, secondly, that feeding was non-selective. Neither assumption could be made in this work. Even when sediment with particle size diameters less than 63 um was used the ingestion rates which were calculated were, to say the least, improbable. It was not clear whether this meant that the animals were selecting diatoms or whether the diatom/sediment mixture was simply not homogenous. Hargrave, it seems, did not

encounter these problems.

Some authors have adopted indirect methods based upon the rates at which faeces are produced and differences in biochemical composition of faeces and uningested sediment. (Heywood & Edwards, 1962, Hughes, 1970).Assimilation efficiencies have also been calculated from information of this sort. Heywood & Edwards (1962) measured the organic carbon content of the food and faeces of *Potamopyrgus jenkinsi* and estimated an assimilation efficiency of 4%. Hargrave (1970) measured both total organic and protein contents of lake sediment and calculated efficiencies for *Hyalella* of 6.5 - 14.9% of total organic material and 13.7 - 23.2% of protein. Once again the assumption has been made that feeding is non- selective and once again the feeding habits of *Corophium* make calculations of this type innappropriate in the present study (Fenchel *et al*, 1975). Since the composition of the material ingested by *Corophium* is likely to be different from that of the uningested sediment, the strict application of these indirect methods could result in serious errors.

The situation is further complicated by the observation that faecal pellets produced by *Corophium* are very much richer in organic material than the sediment. Microscopic examination of freshly voided faeces showed that, in addition to the thin, transparent, chitinous membrane that binds it into pellet form (Gauld, 1957, Reeve, 1963), the material is densely packed with micro-

organisms. Johannes & Satomi (1966) noted this same feature of the faecal pellets of *Palaemonetes pugio* and concluded that this microflora was of intestinal origin, since few bacteria were present in the food supplied to the animals. Since we are considering a food whose total organic content is less than 10%, it follows that even small additions of this type, to the faeces, will result in a significant increase in calorific value. This possibility seems to have been ignored in the earlier studies cited above. Indeed, Hargrave assumed the calorific content of faeces to be the same as that of sediment.

The method finally adopted here was, of necessity, indirect so that average ingestion rates for adult *Corophium* have been proposed from a combination of observations and experimental data (4.3.5). The method, while rather unconventional, has provided data which is consistent with observed feeding activity and the results of radiotracer experiments. Moreover the rates are broadly comparable to those estimated by other workers. A number of previous studies have demonstrated the rapid turnover of sediment by deposit feeders. Heywood & Edwards (1962) reported that *Potamopyrgus* produced faeces at a rate of 25% of body dry wt. h^{-1}. Hargrave (1972) estimated that daily faeces production by *Hyalella* was between 32 and 252% of body weight. The daily turnover of sediment by a 1 mg dry wt. *Corophium* can be calculated to be 96% of body dry wt. at 10 C. and 288% at 20 C.

Such rapid turnover of sediment must mean that little is digested. It has already been noted that the deposits on which *Corophium* feeds are made up, in the main, of inorganic mineral material while included in the small percentage of organic matter there is probably a good deal of indigestible material. This will almost certainly include plant remains made up of lignin and cellulose, chitinous material of animal origin and shell debris, none of which can really be regarded as available food. This prompted George (1964) to estimate the proportion of this organic matter which could be utilized by the deposit feeding worm *Cirriformia tentaculata*. From enzyme digestion experiments he concluded that only 14% of the organic matter was digestible, while the worm itself only made use of a little over half of this. Rapid turnover is clearly a prerequisite if useful ammounts of energy are to be derived from sediment.

Corophium has, to some extent, reduced this problem by feeding selectively. It has been seen earlier that by selecting small particle sizes the animal makes use of a richer microflora and hence a richer fraction of the mud. It is not clear whether selectivity has evolved as a nutritional strategy or is merely a constraint dependent upon the size of the mouthparts and oesophagus. Indeed, it is possible that the degree to which *Corophium* can select material has been underestimated.

Assimilation efficiencies reported for deposit

are variable. Efficiencies calculated in terms of total organic material or organic carbon tend to be low, e.g. 4% for *Potamopyrgus* (Heywood & Edwards, 1962), 7.9% for *Cirriformia* (George, 1964), 6 - 15% for *Hyalella* (Hargrave, 1970). On the other hand, Hargrave showed that algae and bacteria present in deposits were assimilated with very high efficiencies. He calculated figures between 45 and 90% when *Hyalella* was fed on a range of microorganisms. Kofoed found similar high efficiencies for *Hydrobia ventrosa* (Kofoed, 1975), lending further weight to the idea that deposit feeders derive much of their assimilated energy from microorganisms associated with organic detritus rather than from the detritus itself, which, after all has been largely broken down already. *Corophium* assimilated the diatom *Nitzschia closterium* with an efficiency of about 30% - low in comparison to *Hyalella* but, perhaps, realistic considering the large ammount of inorganic material present and the rapid rates of food turnover. In terms of the total calorific content of ingested sediment assimilation efficiency was in the order of 25 - 30%. This figure and it's derivation will be discussed further in the final section.

4.5. The effect of *Corophium volutator* on algal production in salt marsh sediments

4.5.1. Introduction

Radiotracer experiments and visual observations of the feeding behaviour of laboratory animals have indicated that *Corophium* utilizes diatoms and green algae as food. Field observations in Benfleet Creek have suggested that the *Corophium* population may have a significant effect on algal productivity. It was noted that areas inhabited by *Corophium* were largely devoid of the greenish/brown algal crust that forms in other parts of the creek where the animal is less prevelant. These observations alone, while they are based on a fair knowledge of the creek, prove little as there are many factors affecting the ecology of a salt marsh, any combination of which might offer an explanation.

The same situation was seen, however, in salt marsh pools. These pools lie well above mean high water levels and are only inundated on spring tides so that the effects of current and sediment movements are negligable. The substrate and general conditions in the pools are apparently similar in all respects but one. If *Corophium* is present in a pool there is no diatom crust, except where submerged objects have raised the level of the bottom.

Conversely, neighbouring pools may have abundant algal growth over the entire bottom area but no amphipod population.

It was decided, therefore, to attempt to use an index of algal productivity, in this case, the photosynthetic uptake of ^{14}C, to measure possible effects of *Corophium* on primary production in two areas of the creek.

4.5.2. Materials & methods

The method used to estimate algal productivity was, in essence, that described by Van Raalte *et al* (1974). Cores of natural sediment from two areas, A, a *Corophium* bed and B, a diatom rich area with no amphipod population, were incubated, *in situ*, in plastic cylinders. The cores were 1 cm deep with a surface area of 13.6 cm^{-2} and were provided with 10 mls of creek water containing 2.8 uci of Na $H^{14}CO_3$ (Specific act.- 58.5 mci $mmol^{-1}$, from The Radiochemical centre, Amersham). Cores from area A were also incubated at B and *vice versa*, while three cores at each site contained animals at a density of 10 per core (the equivalent of 7300 m^{-2}). After 4 h incubation cores were "killed" by the addition of buffered formalin and brought to the laboratory for analysis. Here each core was digested in 10 mls of conc. nitric acid for several hours, centrifuged, and the supernatent (containing dissolved ^{14}C label fixed by the algae in the core) diluted in 0.75N tris-basic buffer. Aliquots were then counted in Bray's solution (Bray, 1960) in a Panax Liquid Scintillation Counter. Colour and acid quenching was determined, as before (4.2.1), using the internal standard technique. Net uptake of ^{14}C was determined as the difference between light and dark incubated cylinders. A killed core was also included as a control for absorption of label by sediment.

A similar experiment was carried out in the laboratory using cores of sediment incubated in a fume cupboard under constant illumination.

Count rates of experimental cores were converted to mg C fixed m^{-2} h^{-1} by the following expression:

$$\text{Mg C } m^{-2} h^{-1} = \frac{I \times Co \times V}{Ca \times A}$$

where I = the concentration of inorganic C in creek water (59.9 mg C l^{-1} - calculated from pH and chlorinity by the method of Strickland & Parsons, 1968).

Co = the observed activity of the core (d.p.s. sec^{-1}).

V = volume of water present (l)

Ca = total activity added to the water (d.p.s. sed^{-1})

A = area of the core (m^{-2})

4.5.3. Results

Table 21 summarizes the results of the field investigations.

At area A the mean algal productivity was 0.38 mg C m^{-2} h^{-1}, less than 4% of the figure at the diatom rich site. The result of adding *Corophium* to diatom rich cores was a highly significant reduction in the photosynthetic uptake from a mean of 10.83 to 4.89 mg C m^{-2} h^{-1}, a drop of 55%. The animal effect was less clear at A, the *Corophium* zone, where productivity was already very low. When the experiment was repeated under laboratory conditions the mean production figure from three cores was 4.98 mg C m^{-2} h^{-2}. Cores to which *Corophium* had been added showed no net production at all - uptake of ^{14}C label by light cores was of the same order as dark incubated controls.

There is a clear indication that algal production is considerably higher where *Corophium* is absent. This is most clearly demonstrated by a histogram (fig. 20) showing the results of the field experiment.

Table21. Accumulation of ^{14}C label in sediment cores due to algal photosynthesis

Site	Count rate of core (d.p.s.)		Mg C fixed m^{-2} h^{-1}
A. *Corophium* bed	440		0.44
	567		0.65
	66		0.06
	330r		0.33
		Mean	0.38 ± 0.19
. animals added	440		0.44
	no net uptake		-
	"		-
B. Diatom rich	9950		10.5
	9450		10.0
	10720r		11.3
	10890r		11.5
		Mean	10.8 ± 0.51
animals added	5064		5.38
	5674		6.05
	3026		3.23
		Mean	4.89 ± 1.18

r - reciprocal incubation

Cores were incubated *in situ* for 4 h.

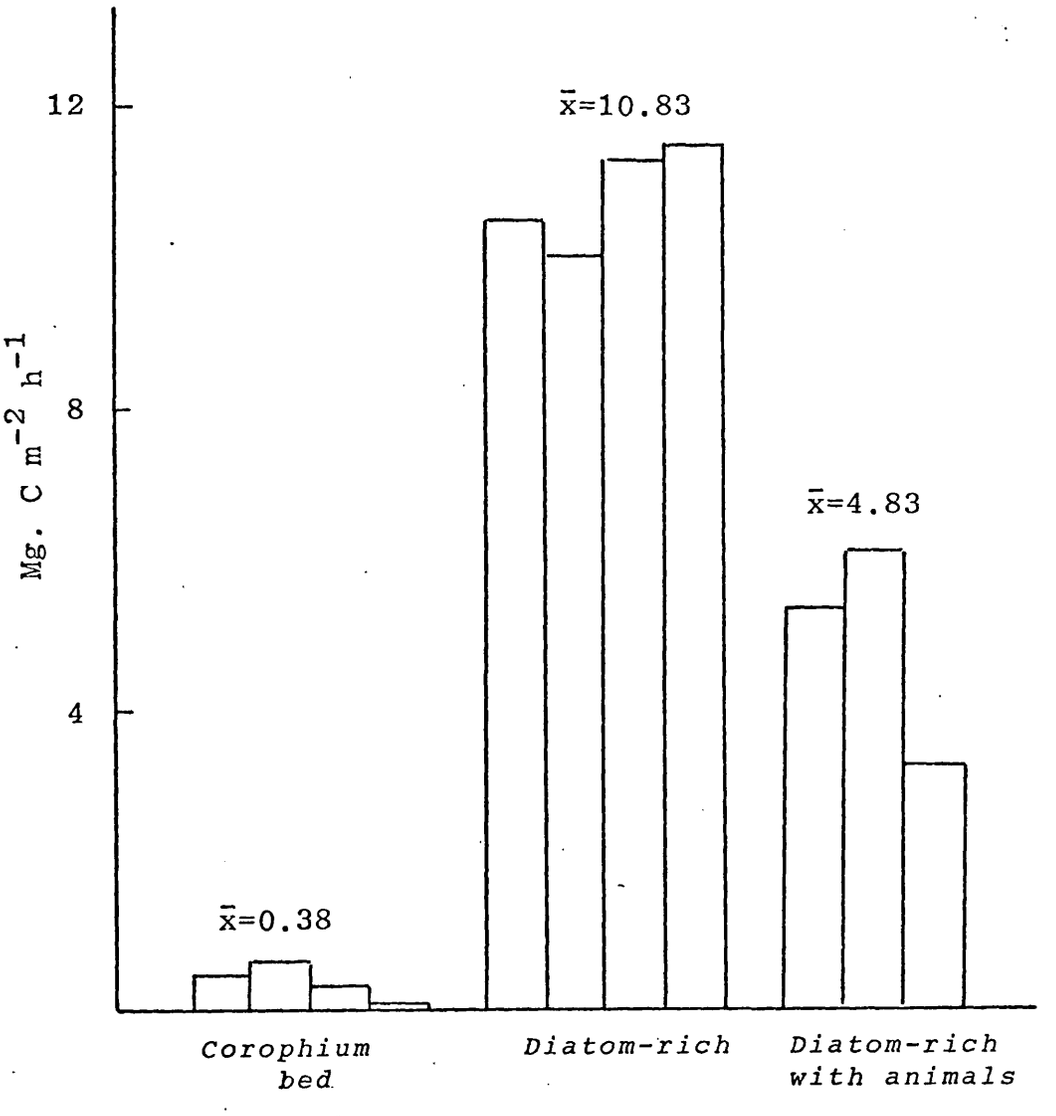


Fig. 21. Algal productivity in sediment cores showing the effect of the presence of *Corophium*

Results of a field expt. using ^{14}C to measure algal productivity

4.5.4. Discussion

The observations described here suggest that algal productivity is low in areas populated by *Corophium* and very much higher in areas where the amphipod is absent. Now it has already been pointed out that such observations, alone, are not sufficient evidence to suggest that the presence of the animal is a factor regulating the primary productivity of the salt marsh. It is unlikely that the explanation would be so simple. *Corophium* is notoriously patchy in it's distribution. Meadows (1964 a & b) suggested that the animal can select areas with a pre ferred particle size distribution and that it can detect the nature of the organic film around sand grains. More recently gregariousness (Campbell & Meadows, 1974) and the mechanical sheer strength of the sediment (Meadows, personal communication) have been added to the list of influences. It could be suggested that what we are seeing is, in fact, an exclusion effect whereby *Corophium* avoids areas rich in micro-algae, although this is by no means supported by laboratory feeding studies where *Corophium* has been seen to feed actively on such material.

More weight is added to the observations by the finding that the addition of animals to experimental cores resulted in a drop in algal photosynthesis. The reasons for this drop could be twofold. Either the animals are

feeding on the algae present or the disturbance created as they burrow and forage over the surface of the mud is sufficient to reduce the photosynthetic potential of the core. Measurements of ^{14}C label taken up by the animals were not carried out but there were indications from the laboratory experiment that individuals certainly accumulated label far in excess of the levels seen in controls. In other words, feeding was, at least in part, responsible for the observed reductions in algal productivity. The true impact of grazing by the *Corophium* population will only be determined by a more detailed and objective investigation.

The results of these preliminary experiments do tend to support the initial observations and, as such, offer the beginnings of an explanation of the observed differences in primary productivity in different regions of Benfleet Creek.

4.6.Uptake of a ^{14}C labelled amino acid by *Corophium volutator*

4.6.1. Introduction

Since the beginning of the century uptake of dissolved material has been proposed as a source of energy available to aquatic animals. Jorgensen (1976) has reviewed the history and development of the major theories on the importance of dissolved organic matter (DOM) in animal nutrition. The recent revival of interest in these theories has produced a wealth of new data and since the mid-1950s the possibilty of dissolved uptake has been shown in a wide range of aquatic invertabrates from corals (Stephens, 1960) to echinoderms (Ferguson, 1971).

Stephens & Schinske (1961) investigated some 35 representatives of 11 phyla and found that the capacity to remove glycine from solution was widespread, with the notable exception of the 6 arthropod species studied.

Inevitably, attempts have been made to estimate the importance of DOM in energy budgets. Southward & Southward (1972) suggested that pogonophores and some polychaetes were capable of obtaining energetically useful ammounts of amino acids and glucose from seawater. Since then, Jorgensen (1976) has compared the rates at which a number of organisms take up amino acids with their energy

requirements, suggesting that infaunal polychaetes like *Capitella capitata* and *Nereis diversicolor* appear to be capable of covering the major part of their maintenance energy requirements from dissolved uptake. Such theories are not without their critics, however, and some would argue that the importance of DOM in the nutrition of higher metazoa has been overplayed (Johannes, Coward & Webb, 1969). There is little evidence of uptake in the crustacea, at any rate, and Stephens & Anderson (1969) re-examined earlier findings that arthropods did not accumulate dissolved nutrients. In experiments on four small crustacean species (including *Corophium acherusicum)* they found that when adequate precautions were taken to inactivate microorganisms, uptake of ^{14}C glycine was insignificant. It might follow, then, that dissolved uptake is unlikely to represent a significant source of energy to *Corophium volutator*. It was felt, nevertheless, that a brief, preliminary study should be carried out so that this assumption could be either confirmed or rejected.

4.6.2. Materials & methods

Three experiments were carried out, the first with no added antibiotics and the second with Streptomycin and Penicillin in seawater at the respective concentrations of 50 and 30 mg l^{-1}. In a third experiment the concentrations of these drugs was doubled since it was found that a few bacterial colonies formed when water containing the lower concentrations was plated out on Zobell's medium.

Animals were incubated for 18 h in 100 mls of membrane filtered seawater (30%) containing 15 uci of ^{14}C-glycine (spec.act.- 114 mci $mmol^{-1}$, from The Radiochemical Centre). This was equivalent to a molar concentration of 1.3×10^{-6} M. (Southward & Southward, 1968, used concentrations of 10^{-6} and 10^{-7} M of glycine). Several killed controls were included in each experiment. After incubation, animals were removed from labelled seawater, rinsed thoroughly and dried, overnight, at 45 C. Dried samples were mounted on special wire sample holders attached to the screw tops of standard counting vials. Vials were evacuated and filled with pure O_2 and mounted on the turntable of a Micromat B.5010 Semi-automatic Combustion apparatus (Camlab, Cambridge). Here, light focused from a projector lamp ignited each sample in turn. Sample radioactivity in the form of $^{14}CO_2$ was absorbed in 1 ml of phenylethylamine to which 10 mls of a toluene based scintillant was added. Loss of label was assumed to be negligible (Gupta, 1966) and count rates and efficiencies were determined as before.

4.6.3. Results

The results of uptake experiments are detailed in tables 22a & b and summarized in table 23.

In all three experiments there was an apparent uptake of ^{14}C-glycine by the animals. That is to say, the count rates of experimental animals were significantly higher than the count rates of killed controls. The effect of the addition of antibiotics was to reduce uptake by 46% in experiment 2 and by 89% in experiment 3.

Thus, the high count rates seen in the first experiment (with no antibiotics) were probably almost entirely due to uptake of glycine by microorganisms adhering to *Corophium*'s body surfaces. In the second experiment, where count rates were still high, there was considerable doubt as to the effectivness of the antibiotic treatment. In the final experiment, net uptake was considerbly less significant when compared to the count rates of controls. Indeed, the apparent uptake of ^{14}C-glycine by live animals was well below that of the dead controls used in the first experiment. Clearly, the accumulation of radioactivity from solution must be regarded with some suspicion. Equally clear is the overiding influence of microorganisms upon the uptake of ^{14}C-glycine by *Corophium volutator*.

Table 22a. Uptake of ^{14}C-glycine by *Corophium*

Animal dry wt. (mg)	D.p.s	D.p.s. mg^{-1}(dry wt)
1)Controls		
0.78	158.8	203.6
0.72	138.7	192.6
0.69	194.0	142.5
1.52	98.3	127.6
Mean 0.93		166.6 ± 32
Live		
0.91	1300.0	1428.6
0.99	1087.0	1128.3
0.82	488.0	595.2
0.78	749.0	1295.8
1.19	1542.5	961.2
Mean 1.10		1082.0 ± 289
2)Controls		
1.70	45.6	26.8
0.75	35.6	47.5
0.86	31.0	36.1
1.06	22,6	21.3
Mean 1.10		32.9 ± 9.9
Live		
2.83	1249.0	441.0
0.51	330.0	647.0
0.41	144.0	351.0
0.54	370.0	685.0
1.25	656.0	525.0
Mean 1.11		530.0 ± 124

1) No antibiotics

2) Strep. 50 mg l^{-1}/Pen. 30 mg l^{-1}

Table 22 b. Uptake of ^{14}C-glycine by *Corophium*

	Animal dry wt. (mg)	D.p.s.	D.p.s. mg^{-1}(dry wt.)
3) Controls			
	0.86	36.5	42.5
	1.05	27.5	26.2
	0.79	27.8	34.3
	0.81	21.8	29.9
	1.21	49.3	40.7
	1.51	45.8	96.6
	0.57	22.6	39.6
	1.25	55.6	44.5
Mean	1.06		44.3 ± 21
	2.83	333.7	117.9
	0.70	168.8	241.1
	2.90	234.4	80.8
	1.16	81.2	70.0
	0.72	136.1	189.0
	1.46	240.2	164.5
	0.77	140.0	181.8
	1.80	247.0	137.2
	0.46	79.0	171.7
	0.61	52.0	85.2
Mean	1.34		143.9 ± 53

3) Strep. 100 mg l^{-1}/Pen. 60 mg l^{-1}.

Table 23. Summary of data from ^{14}C-glycine Expts.

	Mean count rates (d.p.s.) with S.D.s.		Net d.p.s.	Equivalent rate* of glycine uptake (μmoles $g^{-1}d^{-1}$)
	Controls	live		
1)	166.6 ± 32	1082 ± 289	916	0.29
2)	32.9 ± 9.9	530 ± 124	497	0.16
3)	44.3 ± 20.6	143.9 ± 52.7	99.6	0.03

* given by the expression

$$\mu\text{moles } g^{-1}\ d^{-1} = \frac{Co \times k \times 1000}{Ca} \times \frac{4}{3}$$

where Co = observed count rate(d.p.s.mg^{-1})

k = concentration of glycine

Ca = total activity added to water(d.p.s.)

4.6.4. Discussion

It appears from the results of these preliminary studies that, when suitable precautions are taken against micro-organisms, uptake of dissolved glycine by *Corophium* is negligible. The removal of ^{14}C-glycine was almost certainly due to these associated microorganisms and, as such, did not represent true uptake by the animal itself. These findings confirm those of Anderson & Stephens (1969) who reported that several crustaceans, including the closely related form, *Corophium acherusicum*, appeared to take up ^{14}C-glycine from concentrations similar to those used in these experiments. Uptake was variable, however, and was reduced to insignificant levels by the addition of antibiotics. Moreover, the measurment of uptake of ^{14}C-amino acids takes no account of possible eflux, measuring only unidirectional movement of material. Jorgensen (1976) pointed out that in order to obtain ecologically meaningful estimates, measurments should be of net influx from concentrations which reflect the natural levels of the dissolved compound. While the concentration used in this study is comparable to levels in natural seawater, they may represent an underestimate in terms of sediment and interstitial waters. Southward & Southward (1972) have suggested a range of 2.5 - 25 µmoles of amino acids in sediments while Stephens (1975) measured concentrations of

up to 50 µmoles (glycine equivalents) in interstitial water.

If it is assumed that the rates calculated in table 23 were to represent a real influx of useful material it is possible to estimate thevalue of this uptake in terms of *Corophium*'s energy requirments. Assuming that the oxidation of 1 µg of amino acid requires 0.93 µl O_2 (from Jorgensen, 1976) and taking 0.53 µl mg^{-1} h^{-1} as the mean O_2 consumption of a 1 mg *Corophium*, a simple calculation shows that even the highest rate of uptake (0.29 µmoles g^{-1} d^{-1}) would account for less than 0.2% of the animal's O_2 requirments. When proper controls were applied to inactivate microorganisms the apparent uptake rate dropped to 0.03 µmoles g^{-1} dry wt. d^{-1}- insignificant as an energy supply. Stephens (1975) measured net influx rates of 0.75 µmoles g^{-1}(wet wt.) h^{-1} in *Capitella capitata* and 0.84 in *Nereis diversicolor*.

The implication of these results is clear. It is unlikely that dissolved compounds such as amino acids, represent a significant supply of energy to *Corophium* so that consumption can be regarded, exclusively, as that energy derived from ingestion of particulate food material.

5. ENERGY FLOW MODELS FOR *COROPHIUM VOLUTATOR* BASED ON THE BENFLEET POPULATION

It has been the aim of this work to measure, where possible, the major components of *Corophium*'s energy budget. In some cases it became clear that laboratory studies would not yield reliable estimates. Production, for example, could only be estimated from field data while energy rejected in the faeces (F), had to be estimated as the difference between ingested and absorbed energy (F = C - P + R + U). Direct measurment of faeces production was not tried. Faecal pellets could only be collected from animals which were isolated without food and, under these conditions, rates of defaccation fall off rapidly. Difficulties were also encountered in determining the calorific content of faecal material (4.4).

In order to calculate production (Ch. 2) a fairly detailed knowledge of the structure of the Benfleet population was required. This same knowledge has been used to estimate energy used for metabolism (sect. 3.4) and the energy intake of the population (4.3.6). It is now possible to combine all of this information (table 24 & fig. 21) and to construct a model of energy flow)fig. 22).

Such a model gives a valuable illustration of the fate of ingested energy and the efficiencies at which the various transformations operate. It is now possible, for

Table 24. Energy flow through the *Corophium volutator* population in Benfleet Creek

Month	C	= P	+ R	+ U	+ F
		(kJ m^{-2})			
Nov.	386.9	-0.9	89.1	9.8	
Dec.	341.3	7.4	78.6	8.8	
Jan.	173.3	-22.1	38.9	4.4	
Feb.	99.8	79.4	26.2	2.9	
Mar.	182.7	50.7	47.1	5.3	
Apr.	451.0	178.2	81.1	9.2	
May.	947.2	186.4	101.9	10.9	
Jun.	2388.5	217.2	230.8	25.9	
Jul.	2088.5	36.0	271.4	29.5	
Aug.	1515,5	148.2	189.1	21.2	
Sep.	941.3	0.2	211.8	23.7	
Oct.	588.3	109.2	132.4	14.8	
Total	10104.3	1007.0	1499.0	166.3	7432.0*

* Egesta found by difference.

Assimilation efficiency = 26.5% $\left(\frac{C - F}{C}\right)$.

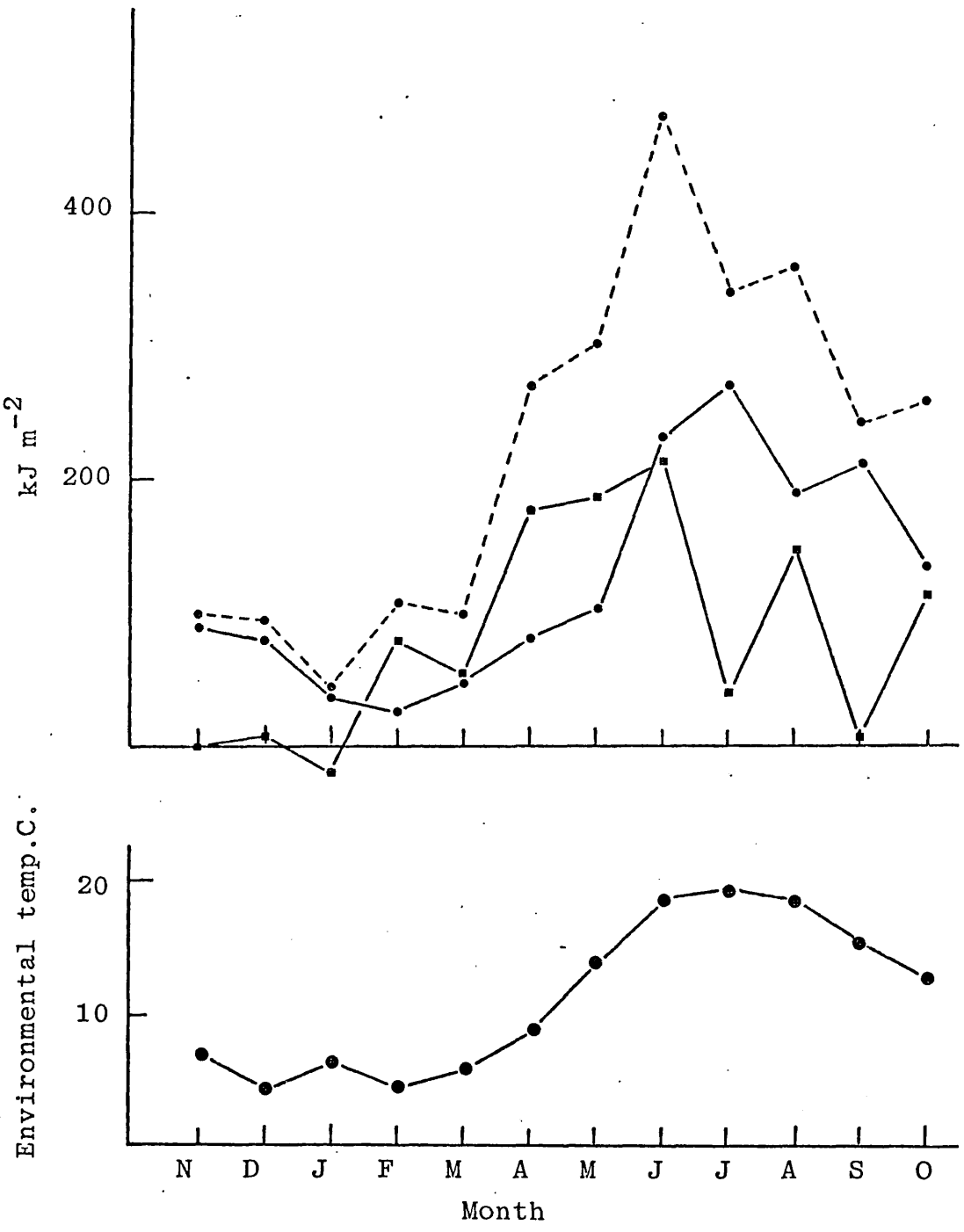


Fig. 22. Energy required for growth and maintenance by the *C. volutator* population at Benfleet

Squares - Production, circles (solid line), Respiration, circles (hatched line)- Assimilation.

(P+R+U)

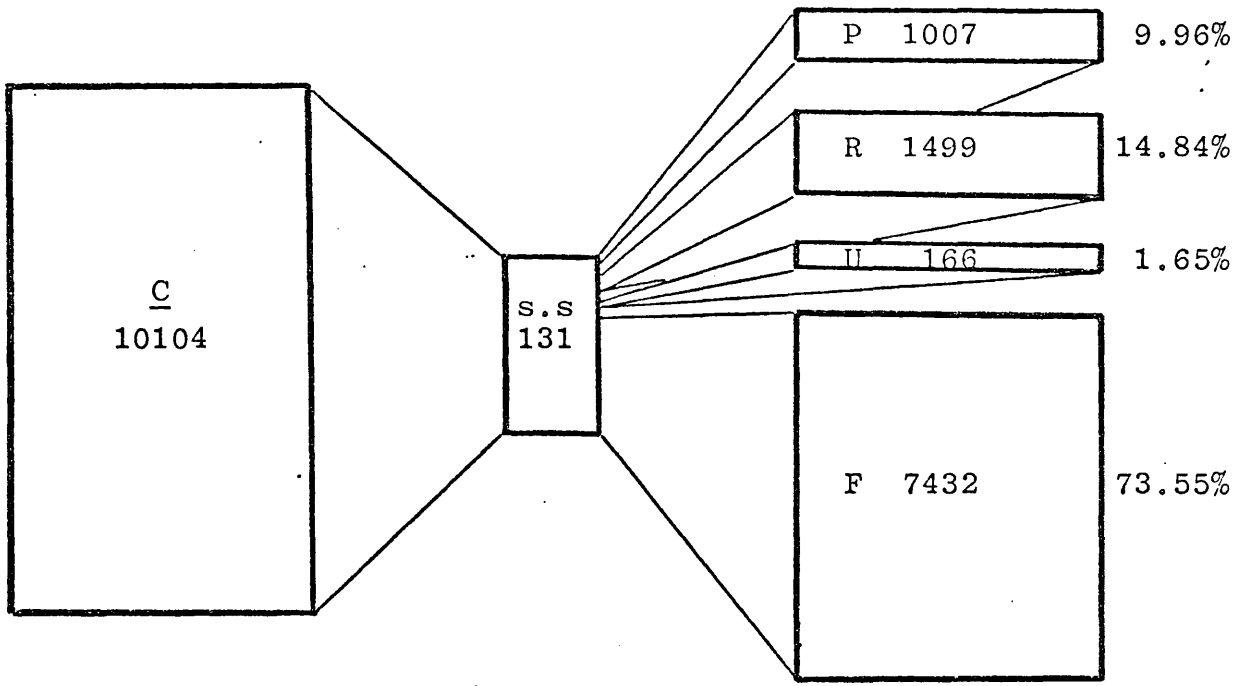


Fig. 23. Energy flow through the *C. volutator* population in Benfleet Creek, Essex. (all figures in kJ m^{-2} yr^{-1})

(Egg and moult production not included in calculations)

example, to estimate assimilation and growth efficiencies.

The outstanding feature of this particular model is the scale of energy flow in relation to standing stock. Over 75% of the total ingested energy is returned to the sediment - 73.6% as faeces and 1.6% as nitrogenous waste. Only 24.8% was converted into physiologically useful energy and used for growth and metabolism. Assimilation (Absorption) efficiency was 26.5%. Much higher efficiencies have been reported for animals feeding on high energy diets (e.g. Hargrave, 1970, Kay & Brafield, 1973) but it must be borne in mind that *Corophium* utilizes sediment and associated organic detritus and it has already been pointed out that much of the energy content of this material is bound up in forms which *Corophium* is probably unable to digest. Assimilation efficiencies for deposit feeders tend to be rather low and in the light of previous studies the present estimate seems fairly realistic. An efficiency of about 30% was calculated for *Corophium* feeding on a ^{14}C labelled diatom mixed with natural sediment.

9.96% of ingested energy went into production (gross growth efficiency, $^{P}/_{C}$). Estimates of growth efficiency from the literature vary. At one end of the scale, Kay & Brafield (1973) gave a figure of 60% for *Neanthes virens* fed on a diet of *Nephtys hombergii* tissue. The data of Hargrave (1971), on the other hand, suggests a growth efficiency of only 2.3% for the deposit feeder *Hyalella*. The figure of Kay & Brafield must be regarded with caution

since it is based upon laboratory maintenance experiments, the authors taking care to point out that the animal, in the field, was unlikely to feed on such a rich diet.

Production accounted for 40% of the total energy assimilated by the population. This is the net growth efficiency (the net population production efficiency of McNeil & Lawton, 1970) and is the equivalent of Ivlev's second order coefficient of food utilization, k_2. (Winberg, 1971). Winberg reviewed published data on k_2 values and found that they fell within the range 0.2 - 0.6 (20 - 60% efficiency) with an average value for crustaceans of 0.3 or 30%. *Artemia salina, Daphnia magna, D. pulex* and the copepod *Calanus heligolandicus*, however, all gave a k_2 value equivalent to that calculated for *Corophium* in the present study.

The net growth efficiency of the Benfleet population is clearly high as is the ratio of production to biomass (P/B), which at 7.7 is higher than in other amphipod populations. The sheer size of the population in the creek, where *Corophium* is without doubt the dominant species, is proof of the animal's success. It is an interesting feature of tidal mudflats that the fauna tends to be made up of a small number of very successful species. The habitat, at first sight, rather inhospitable, supports large numbers of these successful colonists - animals like *Corophium*, the polychaete *Nereis diversicolor* and the bivalves *Scrobicularia plana* and *Cerastoderma edule*.

The annual production of *Corophium* in Benfleet Creek has been estimated as 1007 kJ m^{-2} yr^{-1}. This figure can be brought into perspective by a comparison with some of the earlier estimates of production for salt marsh animals. Odum & Smalley (1959) quoted a figure equivalent to 170kJ m^{-2} yr^{-1} for *Littorina irrorata*, Hughes (1970) estimated a mean of 297 kJ (70.8 kcals) m^{-2} yr^{-1} for *Scrobicularia plana*, and Kay & Brafield (1973), 189 kJ (45.2 kcals) m^{-2} yr^{-1} for *Neanthes virens*. All of these figures fall some way short of the present estimate though Milne & Dunnet (1972) found rather higher production for *Mytilus edulis* in the Ythan estuary at 5450 kJ (1300 kcals) m^{-2} yr^{-1}.

An alternative to the ecological approach is the construction of a laboratory budget like those calculated for the amphipods *Hyalella* (Hargrave, 1971) and *Gammarus pulex*(Nilsson, 1974). Fig. 24 is a diagramatic representation of the daily energy budget for a 1 mg dry wt. adult *Corophium*. Ingestion, respiration and excretion were calculated directly from the results of laboratory studies at 10 C. (close to the annual mean environmental temperature in the lower Thames estuary). Production was calculated on the basis of the ratio of P:R in the Benfleet population (0.67). Egestion was calculated by difference.

From the daily intake of 1.31 J mg amphipod^{-1}, 16% was converted into heat of metabolism, 10.7% was used for production, 3% was excreted and the remaining 70.3% was passed out in the faeces. Assimilation efficiency, at

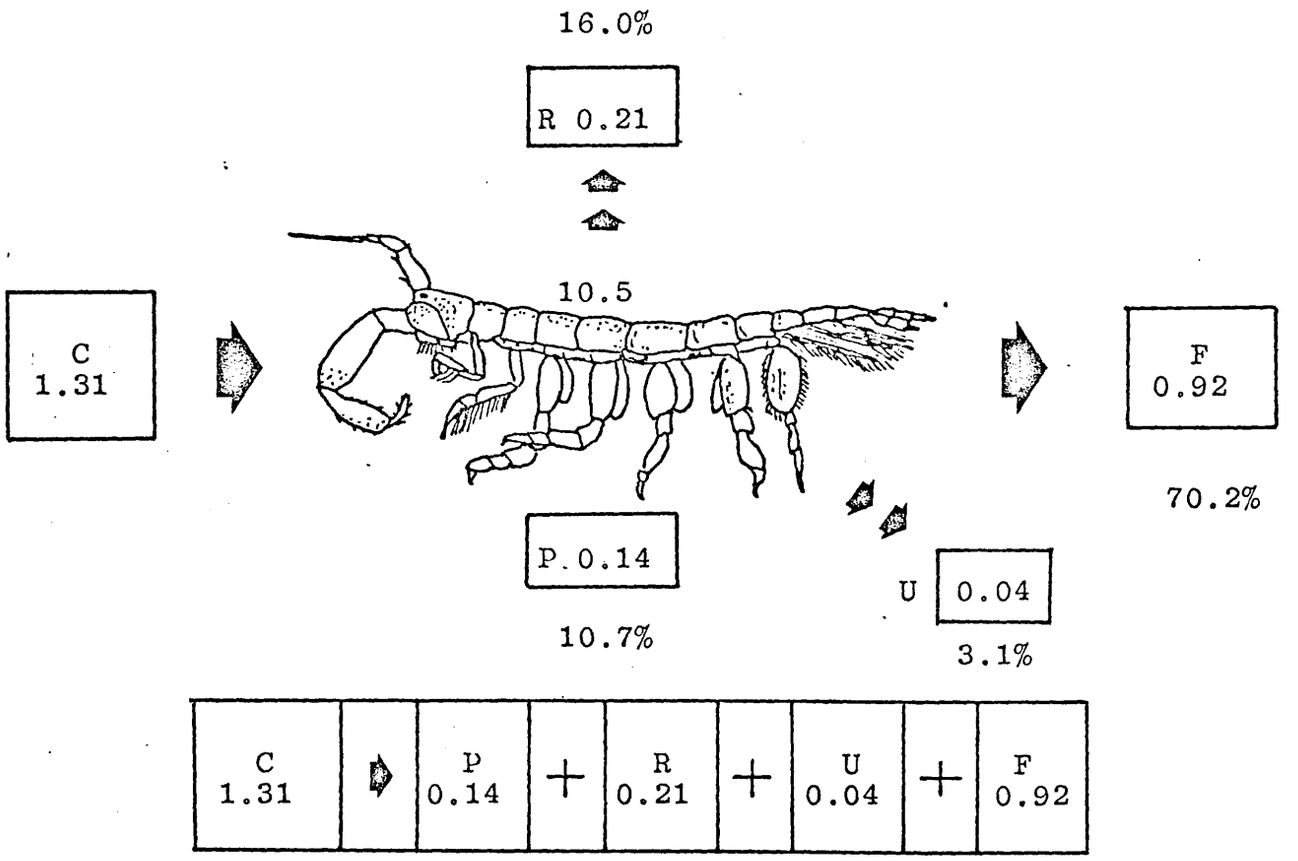


Fig. 24. The daily energy budget for a 1 mg dry wt. *Corophium volutator* (all figures are J d^{-1})

(Egg and moult production not included in calculations)

29.7% is slightly higher than was calculated for the natural population. U is also higher since it was calculated, here, from the observed excretion rates of the animal in the laboratory and not from O_2 uptake. The discrepancy is small in relation to the size of the budget and is, doubtless, well within the limits of accuracy with which consumption was measured.

The partition of energy is similar in both models. The agreement is reassuring although, in a sense, it is to be expected since the same investigations form the basis of each. The population model, however, takes into account environmental temperature and the size structure of the population as well as the biomass for each month.

The laboratory budget for *Corophium*, is in some ways comparable to Hargrave's (1971) budget for *Hyalella*. Bearing in mind that conditions of study were rather different (Hargrave used data for 700 µg animals kept at 15 C and feeding on a somewhat richer diet) both models describe a similar situation. Like *Corophium*, *Hyalella* assimilated a relatively small fraction of ingested energy with 82% being rejected as faeces. P and R were of the same order as in the present study. Production accounted for the equivalent of 0.12 J d^{-1} (0.14 in the present study) and respiration for 0.39 J d^{-1} (0.21 for *Corophium*).

The impact of *Corophium* on the ecology of the creek is difficult to assess without a detailed study of energy

flow at all levels of the community. A look at some estimates of salt marsh primary production might give some idea, though. Jefferies (1972) estimated the production of the salt marsh grass *Spartina anglica* in Norfolk and in Bridgewater Bay to be 980 and 960 g m^{-2} yr^{-1}. If an average calorific value of 4.07 kcals g^{-1} is assumed (after Golley, 1961) this would mean that 16500 kJ m^{-2} yr^{-1} enters the detritus system from this source. Algal productivity, on the other hand, is ,by comparison probably rather less important. Leach (1970) estimated algal productivity on the Tarty mudflat in the Ythan estuary to be 31 g C m^{-2} yr^{-1}. Applying a conversion factor of 41.9 kJ g C^{-1} (Winberg, 1971 b) this would represent an energy supply of only 1300 kJ m^{-2} yr^{-1}. Assuming similar levels of primary production in Benfleet Creek it is clear that *Corophium* with an annual population assimilation of 2500 kJ m^{-2} yr^{-1}, must be an important component of the salt marsh community.

Having made this hypothetical comparison it is, perhaps, fair to point out that most of the energy consumed by *Corophium* probably remains within the sediment/detritus system. It has already been shown that 75% of this energy is returned directly to the sediment as rejecta. Most of the energy used in production will also be returned through the normal processes of mortality and decomposition while a certain ammount of material must be lost through predation. Predation losses are not thought

to be heavy in Benfleet Creek - there is no resident wildfowl population and there are few visitors other than gulls (and starlings from nearby woods). In general the gulls leave the *Corophium* beds undisturbed, preferring to feed lower down the shore. *Corophium*, itself, is not an active wanderer so there is probably little export of material.

Like other deposit feeders, *Corophium* undoubtedly reingests it's own faecal material along with surface sediment. Freshly voided faecal pellets are packed with microorganisms (Bacteria and ciliates) and are soon broken down to become incorporated into the sediment again. In view of the density of the *Corophium* population and the close proximity of neighbouring burrows it seems inevitable that there is considerable recycling of material. It has been suggested (Stephens, personal communication) that irrigation of the sediment either artificially or by animal activity, increases the levels of primary amines, and that it is probably facultative aerobes which release this material into the medium. Hylleberg (1975) presented evidence that *Abarenicola* grows up a crop of bacteria in a feeding pocket just in front of the mouth. The idea of "gardening" might well be applied to *Corophium* too. By irrigating the top few centimetres of mud the amphipod population is, perhaps, promoting microbial activity and, at the same time, increasing it's own energy supply.

If this is true then it raises interesting questions

about the relationship between deposit feeders and the sediment in which they live and suggests that *Corophium* is instrumental at both ends of the decomposer food chain - perhaps, as important a contributor as it is a consumer.

6.REFERENCES

Anderson, J. W. & Stephens, G. C. (1969) Uptake of organic material by aquatic invertebrates. VI. The role of epiflora in apparent uptake of glycine by marine crustaceans. *Mar Biol.* 4, pp 243-249.

Bate, C. S. & Westwood, J. O. (1863) *A History of the British sessile-eyed crustacea.* I. London (Ray Society)

Blazka, P. (1972) The ratio of crude protein, carbohydrate and lipid in particulate matter. In *Detritus and it's role in aquatic ecosystems* - proceedings of an I.B.P/ UNESCO symposium. (Eds. Melchiorri, Santolini & Hapton) *Mem. Inst.Ital. Idrobiol.* Suppl. 29, pp 529-531.

Brafield, A. E. & Solomon, D. J. (1972) Oxy-calorific coefficients for animals respiring nitrogenous substrates. *Comp. Biochem. Physiol.* 43, pp 837-841.

Bray, G. A. (1960) A simplified liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt. Biochem.* 1, pp279-285.

Buchanan, J. B. & Longbottom, M. R. (1970) The determination of organic matter in marine muds: The effect of the presence of coal and the routine determination of protein. *J. exp. mar. Biol. Ecol.* 5, pp 158-169.

Butler, E.I., Corner, E. D. S. & Marshall, S. M. (1969) On the nutrition and metabolism of zooplankton: VI. Feeding efficiency of *Calanus* in terms of nitrogen and phosphorus. *J.mar. biol. Ass. U.K.* 49, 977-1001.

Campbell, J. I. & Meadows, P. S. (1974) Gregarious behaviour in a benthic marine amphipod (*Corophium volutator*). *Experientia*. 30, p 1396.

Clay, E. (1965) Literature survey of the common fauna of estuaries. 15. *Corophium volutator*. Imperial chemical Ind., Paints division. 17 pp.

Crawford,G. I. (1937) A review of the amphipod genus *Corophium* with notes on the British species. *J. mar. biol. Ass. U.K.* 21, pp 589-630.

Crisp, D. J. (1971) Energy flow measurments. In *Methods for the study of marine benthos*.(Eds. Holme, N. A. & McIntyre, A. D.) IBP handbook no. 16, pp 197-280. Oxford & Edinburgh (Blackwell).

Dyer, A. (1974) *An introduction to Liquid Scintillation Counting*. London (Heyden). 111pp

Elliot, J.M. & Davison, W. (1975) Energy equivalents of oxygen consumption in animal energetics. *Oecologia*. 19, pp 195-201.

Fenchel, T.,Kofoed, L. H. & Lappalainen, A. (1975) Particle size selection in two deposit feeders: th amphipod *Corophium volutator*, and the prosobranch *Hydrobia ulvae*. *Mar. Biol*. 30, pp 119-128.

Ferguson, J. C. (1971) Uptake and release of free amino acids by starfishes. *Biol.Bull. Woods Hole*. 141, 122-129.

Fry, F. E. J. (1957) The aquatic respiration of fish. In *Physiology of Fishes*. (Ed. Brown, M. E.), vol I, pp 1-64. New York (Academic Press)

Gauld, D. T. (1957) A peritrophic membrane in calanoid copepods. *Nature. Lond*. 179, pp 325-326.

Gee, J. M. (1961) Ecological studies of South Benfleet Creek, with special reference to the amphipod genus *Corophium*. *Essex nat.* ,pp 291-309.

George, J. D. (1961) Organic matter available to the polychaete *Cirriformia tentaculata*, (Montagu), living in an intertidal mudflat. *Limnol & Oceanogr.* 9, pp 453-454.

Golley, F. (1961) Energy values of ecological materials. *Ecology*. 42, pp 581-583.

Goss-Custard, J. D. (1977) The energetics of prey selection by redshank, *Tringa totanus* (L) in relation to prey density. *J. Anim. Ecol.* 46, pp1-19.

Goss-Custard, J. D. (1969) The winter feeding ecology of the redshank, *Tringa totanus. Ibis.* 111, pp338-356.

Gupta, G. N. (1966) A simple in-vial combustion method for assay of Hydrogen-3, Carbon-14 and Sulphur-35 in biological, biochemical and organic materials. *Analyt. Chem.* 38 pp 1356-1359.

Halcrow, K. & Boyd, C. M. (1967) The oxygen consumption and swimming activity of the amphipod *Gammarus oceanicus* at three different temperatures. *Comp. Biochem. Physiol.* 23, pp 233-242.

Hargrave, B. T. (1970) The utilization of benthic microorganisms by *Hyalella azteca*(Amphipoda). *J. Anim. Ecol.* 39, pp427-437.

Hargrave, B. T. (1971) An energy budget for a deposit feeding amphipod. *Limnol. Oceanogr,* 16, pp 99-103.

Hargrave, B. T. (1972) Prediction of egestion by the deposit feeding amphipod *Hyalella azteca*. *Oikos*. 23, pp 116-124.

Hart, T. J. (1930) Preliminary notes on the bionomics of the amphipod *Corophium volutator* (Pallas).*J. mar. biol. Ass. U.K.* 16, 761-789.

Heywood, J. & Edwards, R. W. (1962) Some aspects of the ecology of *Potamopyrgus jenkinsi* (Smith). *J. Anim. Ecol.* 31, pp 239-250.

Holme, N. A. & McIntyre, A. D. (1971) *Methods for the study of Marine Benthos*. IBP handbook no. 16. Oxford & Edinburgh (Blackwell) 334pp

Hughes, R. N. (1970) An energy budget for a tidal flat population of the bivalve *Scrobicularia plana* (Da Costa) *J. Anim. Ecol*. 39, pp357-379.

Hylleberg, J. (1975) Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and the concept of gardening in lugworms. *Ophelia*. 14, 113-138.

Jefferies, R. L. (1972) Annual mean net productivity of salt marsh plants. In *The Estuarine Environment*. (Eds. Barnes, R. S. K. & Green, J.) pp London (Applied SCience publishers).

Johannes, R. E., Coward, S. J. & Webb, K. L. (1969) Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.* 29, pp283-288.

Johannes, R. E. & Satomi, M. (1966) Composition and nutritive value of faecal pellets of a marine crustacean. *Limnol. Oceanogr.* 11, pp191-197.

Johnson, W. S. (1976) Biology and population dynamics of the intertidal isopod *Cirolana harfordi*. *Mar. Biol.* 36, pp 343-350.

Jorgensen, C. B. (1976) August Putter, August Krogh and modern ideas on the use of dissolved organic matter in aquatic environments. *Biol. Rev.* 51 (3), pp 291-328.

Kay, D. J. & Brafield, A. E. (1973) The energy relations of the polychaete *Neanthes (Nereis) virens* (Sars). *J. Anim. Ecol.* 42, 673-692.

Kofoed, L. H. (1975) The feeding biology of *Hydrobia ventrosa* (Montagu): I. The assimilation of different components of the food. *J. exp. mar. Biol. Ecol.* 19, pp 233-241.

Leach, J. H. (1970) Epibenthic algal production in an intertidal mudflat. *Limnol. Oceanogr.* 15, pp 514-521.

Liddicoat, M. I., Tibbets, S. & Butler, E.I. (1969) The determination of ammonia in seawater. *Limnol. Oceanogr.* 20, pp 131-132.

McLusky, D. S. (1967) Some effects of salinity on the Survival, Growth and Moulting of *Corophium volutator*. *J. mar. biol. Ass. U.K.* 47, pp 607-617.

McLusky, D. S. (1968) Some effects of salinity on the distribution of *Corophium volutator*. *J. mar. biol. Ass. U.K.* 48, pp 443-454.

McLusky, D. S. (1969) The oxygen consumption of *Corophium volutator* in relation to salinity. *Comp. Biochem. Physiol.* 29, pp743-753.

McLusky, D. S. (1970) Salinity preference in *Corophium volutator*. *J. mar. biol. Ass. U.K.* 50, pp 747-752.

McNeil, S. & Lawton, J. H. (1970) Annual production and respiration in animal populations. *Nature. Lond.* 225, pp 472-474.

Maciolek, J. A. (1962) Limnological organic analyses by quantitative dichromate oxidation. *Res. Rep. U.S. Fish Wildl. Serv.* 60, 1-61.

Meadows, P. S. (1964 a) Substrate selection by *Corophium* species: The particle size of substrates. *J. Anim. Ecol.* 33, 387-394.

MEadows, P. S. (1964 b) Substrate selection by *Corophium* Species: Films and bacteria on sand particles. *J. exp. Biol.* 41, pp 499-511.

Meadows, P. S. (1964 c) Experiments on substrate selection by *Corophium* species: depth selection and population density. *J. exp. Biol.* 41, pp677-687.

Meadows, P. S. (1967) Discrimination, previous experince and substrate selection by the amphipod *Corophium. J. exp. Biol.* 47, pp 553-559.

Meadows, P. S. & Reid, A. (1966) The behaviour of *Corophium volutator* (crustacea: amphipoda). *J. Zool. Lond.* 150, pp 387- 399.

Milne, H. & Dunnet, G. M. (1972) Standing crop, productivity and trophic relationships of the fauna of the Ythan estuary. In *The Estuarine Environment*. (Eds. Barnes, R. S. K. & Green, J.). pp86-106. London (Applied Science Publishers).

Morgan, E. (1965) The activity rythm of the amphipod *Corophium volutator* (Pallas) and it's possible relationship to change in hydrostatic pressure associated with the tides. *J. Anim. Ecol.* 34, 731-746.

Newell, R. C. (1965) The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*. *Proc. Zool. Soc. Lond.* 144, pp25-45.

Newell, R. C. & Northcroft, H. R. (1967) A reinterpretation of the effect of temperature on the metabolism of certain marine invertebrates. *J. Zool. Lond.* 151, pp 277-298.

Nilsson, L. (1974) Energy budget for a laboratory population of *Gammarus pulex* (Amphipoda). *Oikos*. 25, pp 35-42.

Odum, H. T. (1957) Trophic structure and productivity of Silver Springs, Florida. *Ecol. Monogr.* 27, pp55-112.

Odum, E. P. & Smalley, A.E. (1959) Comparison of population energy flow of an herbivorous and a deposit feeding invertebrate in a salt marsh ecosystem. *Proc. natn. Acad. Sci. U.S.A.* 45, pp 617-622.

Parry, G. (1960) Excretion. Ch 10 in *The Physiology of the Crustacea*. I. (Ed. Watermann, T. H.) pp 341-363. New York & London (Academic Press).

Parsons, T. R., Stephens, K. & Stickland, J. D. H. (1961) On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Canada*. 18, 1001-1016.

Phillipson, J. (1964) A miniature bomb calorimeter for small biological samples. *Oikos*. 15, pp 130-139.

Phillipson, J. (1966) *Ecological energetics*. London (Arnold) 57 pp.

Reeve, M. R. (1963) The filter feeding of *Artemia*: III. Faecal pellets and their associated membranes. *J. exp. Biol*. 40, 215-221.

Ricker, W. E. (1968) (Ed.) *Methods for assessment of fish production in fresh waters*. IBP handbook no. 3. Oxford & Edinburgh.(Blackwell) 313 pp.

Rigler, F. H. (1971) Methods for the measurment of Assimilation of food by zooplankton. In *A manual on methods for the assessment of secondary productivity in freshwaters*. (Eds. Edmondson, W. T. & Winberg, G. G.) IBP handbook no. 17, pp 264-269. Oxford & Edinburgh (Blackwell).

Royal Society, The. (1972) Metric units, conversion factors and nomenclature in nutritional and food sciences. London (The Royal Society).

Solorzano, L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* 14, pp 799-801.

Southward, A. J. (1952) Organic matter in littoral sediments. *Nature. Lond.* 169, p 888.

Southward, A. J. & Southward, E. C. (1968) Uptake and incorporation of labelled glycine by pogonophores. *Nature. Lond.* 218, pp875-876.

Southward, A. J. & Southward, E. C. (1972) Observations on the role of organic compounds in the nutrition of benthic invertebrates: III. Uptake in relation to the organic content of the habitat. *Sarsia*. 50, pp 29-45.

Stephens, G. C. (1960) Uptake of glucose from solution by the solitary coral *Fungia*. *Science*. 131, pp 1352.

Stephens, G. C. (1975) Uptake of naturally occuring primary amines by marine annelids. *Biol. Bull. Woods Hole*. 149 , pp 397-407.

Stephens, G. C. & Schinske, R. A. (1961) Uptake of amino acids by marine invertebrates. *Limnol. Oceanogr.* 6, pp 175-181.

Strickland, J. D. H. & Parsons, T. R. (1968) *A practical hanbook of seawater analysis. Bull. Fish. Res. Bd. Canada.* 167, 311pp.

Swiss, J. J. & Johnson, M. G. (1976) Energy dynamics of two benthic crustaceans in relation to diet. *J. Fish. Res. Bd. Canada*. 33, pp 2544-2550.

Teal, J. M. (1957) Community metabolism in a temperate cold spring. *Ecol. Monogr.* 27, pp 283-302.

Van Raalte, C., Stewart, W. C., Valiela, I. & Carpenter, E. J. (1974) A ^{14}C technique for measuring algal productivity in salt marsh muds. *Bot. Marina*. 17, pp 186-188.

Watkin, E. E. (1941) The yearly life-cycle of the amphipod *Corophium volutator*. *J. Anim. Ecol.* 10, 77-93.

Winberg, G. G. (1971 a) (Ed)*Methods for the estimation of production of aquatic animals*. London (Academic Press) 175 pp.

Winberg, G. G. (1971 b) (Ed) *Symbols, units and conversion factors in studies of freshwater productivity*. London. IBP Central office. 23pp.

Zeuthen, E. (1947) Body size and metabolic rate in the animal kingdom with special regard to the marine microfauna. *C. r. Trav. Carlsberg. Ser. Chim.* 26, pp 17-161.