ASPECTS OF THE PHYSIOLOGY AND ECOLOGY OF COROPHIUM VOLUTATOR (Pallas) IN RELATION TO SALINITY.

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by

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The work presented in this thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

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. . . . 19th June 1969 . . . Date

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ABSTRACT

The effects of salinity on the physiology and ecology of the mud-dwelling estuarine animal Corophium volutator (Pallas), a crustacean amphipod, have been investigated.

A study of the effects of salinity on the distribution and abundance of C. volutator on the estuary of the River Ythan, Aberdeenshire, indicates that 2°/oo is a critical minimum salinity controlling its distribution. In areas with salinities between 2 and 5°/oo C. volutator was present, but in reduced numbers. In areas with salinity greater than 5°/oo, the distribution and abundance of C. volutator were controlled by the nature of the substrate; however, in areas with suitable substrates, but where the salinity was below 5°/oo, the effects of salinity override the effects of the substrate.

Experimental studies indicated that, if supplied with mud, it will survive the salinity range of 2 - 50°/oo, and without mud, the range 7.5 - 47.5°/oo. Moulting occurred in salinities of 2.6 - 46°/oo, but most frequently in the range 5 - 20°/oo. Growth occurred at a maximum rate in 15.4°/oo, and only slightly slower at 4.4 and 30.6°/oo; but below 4.4°/oo the growth rate was progressively reduced. The effects of salinity on the various stages in the life cycle are discussed.

Freezing point studies show C. volutator to be a hyperosmotic regulator/...

regulator, having a tissue tolerance range of 13 – 50°/oo. The effect of size, sex, feeding and moulting on the freezing point have been investigated. *C. volutator* was found to produce urine hypo-osmotic to the blood when acclimated to low salinities, and isosmotic urine at salinities above 20°/oo. Over a range of salinities from 1 - 35°/oo, *C. volutator* was found to maintain Na^+, K^+, Ca^{++}, Cl^-, more concentrated than the medium, and Mg^{++} less concentrated.

The restricted permeable areas of the cuticle have been localised by silver staining. The oxygen consumption of animals of the same size, at the same level of activity, and at the same temperature, did not differ significantly between animals in different salinities. A salinity preference range of 10 - 30°/oo has been demonstrated.

Relevant literature on estuarine life, and osmoregulation of crustacea is reviewed. The adaptations of *C. volutator* to an environment with varying salinity are discussed.

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I INTRODUCTION

1.1 OBJECTIVES.

The mud-dwelling amphipod Corophium volutator (Pallas, 1766) is essentially a brackish water species, being found most commonly intertidally in estuaries, where its range extends from marine to almost fresh water conditions, although it may also be found in muds submerged to a depth of 10 m (Zenkevitch, 1963). In the extreme salinity conditions of estuaries, C. volutator is frequently one of the most abundant animals present. Previous work on C. volutator (see Sect. 1.3) has been primarily concerned with taxonomy, and its distribution as controlled by the nature of the substrate. Some authors have mentioned the importance of salinity, as a factor controlling the distribution of C. volutator, during the course of faunal surveys; however no previous work has been performed to quantify these effects of salinity. This study is concerned with the effects of salinity on C. volutator, and the adaptations, if any, that it has to a habitat with wide ranging salinity variations.

The approach that has been made to study the effects of salinity on C. volutator, has been one of "physiological ecology", following the recommendations of Kinne (1957, 1966, 1967), Carriker (1967), Jansson (1967) and Vernberg (1967). A study of the effects of salinity on the distribution and abundance of C. volutator on the Ythan estuary, Aberdeenshire, has been closely linked with a laboratory study of the effects of salinity on its physiology, and/...

and of its mechanism of osmoregulation. Problems revealed in the field, have been further examined in the laboratory, and conversely an attempt has been made to correlate laboratory findings with results from the field. The first year of this study was devoted to field work, with simultaneous laboratory experiments on salinity tolerance. Subsequent work has attempted to analyse and explain these findings, by reference to studies of the physiology of osmoregulation.

C. volutator is a very important food link in the Ythan and other estuaries. It feeds on organic detritus, and in turn forms a major food item for flounders, wading birds, and also perhaps, *Nereis diversicolor*. *C. volutator* is very widely distributed within N. Europe, and often present in vast numbers. Within estuaries the total number of animal species present may be low when compared with adjacent fresh waters and the sea (Remane, 1934; Alexander, *et.al.*, 1935), and in this situation *C. volutator* may reach its maximum abundance, and may be the most abundant animal, along with *Hydrobia jenkinsi* and *Nereis diversicolor*. It would appear that if an animal can adapt to the extreme environmental conditions of an estuary, then the rewards in terms of an abundant food supply are great. This study is an attempt to describe the adaptations of *C.volutator*, to its environment, and in particular salinity as an environmental factor.

1.2 REVIEW OF SYSTEMATICS OF COROPHIUM VOLUTATOR

The systematic position of *Corophium volutator* may be briefly listed:

Phylum Arthropoda
  Sub-Phylum Crustacea
    Class Malacostraca
      Order Amphipoda
        Family Corophiidae
          Genus Erichthonius
          Genus Unicola
          Genus Siphonectes
          Genus Corophium

Marine Biological Assn. (1957) records the presence of 8 species of *Corophium* in the Plymouth area, most of which are tube-dwelling species found amid weeds, stones, or on piers and buoys in shallow water. Crawford (1937a) reviews the genus *Corophium*, containing 32 species, of which 11 have been recorded from Britain, and divides the genus into 3 sections (A, B & C). Within section A, he enumerates 4 British species - *Corophium affine*, *C. curvispinum devium*, *C. volutator* and *C. arenarium*. To this group must be added *C. multisetosum* (Stock, 1952). Stock (1952) and Ingle (1963) have redefined the characters used for specific recognition of these species.

Sexual/...

Sexual differentiation may be performed using the characteristics of Watkin (1941) and Ingle (1963). In this study only one species, *C. volutator* (synonyms *C. longicorne*, *C. grossipes*) is involved. *C. volutator* has been recorded on European coasts from Western Norway to the Adriatic Sea, on the coasts of Nova Scotia, The Bay of Fundy and Main, and also in the Black and Azov Seas (Segerstrale, 1959a). Ingle (1963) has exhaustively listed references to the distribution of *C. volutator* all round the coasts of Britain. Distribution records particularly relevant to this study, on the East Coast of Scotland, are those of Bate & Westwood (1863), Outer Skerries Harbour, Shetland; Alexander (1932), Firth of Tay; Nicol (1935), Aberlady Bay, E. Lothian. Local, isolated races of *C. volutator* have been reported by Chevais (1937), based on differences in growth rate and in the appearance of the stages in the moulting cycle. The physical similarity of the various species of *Corophium*, and the variation within each species has led Muus (1967 a,b) to suggest that the group is in "a state of development".

The other species in the "A" group of Crawford (1937a) are not so widespread as the mud-dwelling *C. volutator*. *C. arenarium* is generally found on sand (Meadows, 1964c); *C. multisetosum* appears within Britain to be confined to the South of England (Ingle, 1963), burrowing in clay or mud (Stock, 1952); *C. affine*

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has been recorded northwards from the Firths of Forth and Clyde, and is found on the sea bottom, at depths of up to 60m, (Ingle, 1963); and C. curvispinum devium is known in Britain only from the River Avon at Tewkesbury (Crawford, 1937a).

1.3 REVIEW OF LITERATURE ON COROPHIUM VOLUTATOR

Many authors (Delage, 1881; Nicol, 1935; Thamdrup, 1935; Crawford, 1937b; Beanland, 1940; Spooner & Moore, 1940; Rees, 1940; Goodhart, 1941; Stopford, 1951; Rullier, 1959; Gee, 1961; Muus, 1967a) have described the substrate in which C. volutator has been found. They have agreed that C. volutator is found in mud or muddy sand, containing approximately 37% silt or clay. C. volutator is especially abundant in sheltered conditions. It is not found in conditions of heavy pollution, sand without a plentiful supply of detritus, or sulphide mud which is blackened by excessive organic detritus. Meadows (1964a-c, 1967) has experimentally studied substrate selection by C. volutator, and has shown how specific is its choice of the typical substrate in which it is found. Meadows & Reid (1966) have described the behaviour of C. volutator, in particular swimming, crawling, burrowing and feeding activities; the detritus feeding habits have also been described by Enequist (1949). Vader (1964) and Morgan (1965) have described a rhythmical swimming activity, which

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is entrained by tidal hydrostatic pressure. Data on C. volutator has been partially summarised by Segerstrale (1959a), who gives information on reproduction (Segerstrale, 1940), growth, abundance and economic importance.

The importance of C. volutator as a food source may be seen in Table 1.

Table 1

Predator		Reference
Calidris alpina Calidris minuta	Wading Birds	Bengtson & Svensson (1968)
Tringa totanus	Redshank	Goss-Custard (1966, unpublished thesis, University Aberdeen)
Platichthys flesus	Flounder	Emerson (Thesis in preparation, University Aberdeen)
Bembidion lateralle	Insect	Green (1954)

Preliminary notes on the bionomics of a C. volutator population in Whitby Harbour were made by Hart (1930) especially with regard to feeding habits, substrate selection and life cycle. Further data on its life cycle has been recorded by Segerstrale (1940, Baltic Sea), Movaghar (1961, Elbe estuary) and Muus (1967a, Denmark). The weights of C. volutator in Riga Bay have been given by Kostrichkina & Kostromina (1966). Data on its abundance (up to 65,000 ind./sq.m) have been reported by Linke (1939),

Movaghar/...

Movagher (1964) and Muus (1967a). Segerstrale (1937) reports on parasitisation of *C. volutator* by the yeast *Cryptococcus gammari*, Mackinnon and Hawks (1961) report parasitisation by *Gymnodiniodes inkystans*, and Muus (1967a) reports infection by unidentified cercaria.

The physiology of digestion, and the functional morphology of the stomach of *C. volutator* have been described by Agrawal (1963a,b). Upper temperature tolerance limits have been set at 36.5 - 37.5^{o}C by Huntsman & Sparks (1924). Muus (1967a) and Williams, *et. al.*, (1964) noted mass mortality following temperatures minus 1.5^{o}C. Oxygen consumption in relation to temperature has been measured by Thamdrup (1935). Fox (1952) has observed rectal pumping of water.

During the course of faunal surveys, many authors have reported a lower limit of salinity, below which *C. volutator* was not found (Table 2).

Table 2

Locality	Minimum Salinity (^{o}/oo)	Reference
Hampshire	6	Goodhart (1941)
Aberlady Bay	5	Nicol (1935)
Holland	4	Stock & Vos (1961)
Norfolk	3.6	Hart (1930)

Gulf of Finland/....

Table 2 contd.

Locality	Minimum Salinity (°/oo)	Reference
Gulf of Finland	2	Hellen (1919)
" " "	2	Gurjanova (1951)
Roscoff	1.5	Chevais (1937)
Tolerance range	3-34	Remane & Schlieper (1958)

These variations in the lower limit of salinity observed may reflect the varying distribution of suitable substrates in the areas studied. At the upper limit of salinity, it frequently occurs in sea water (33-35‰) and Schellenberg (in Fox, 1926-9) reported the presence of C. volutator f. orientalis in hypersaline (60‰) water in Lake Timsah in the Suez Canal. The latter was suspected by Crawford (1937a) and Segerstrale (1959) to be C. arenarium, although Stock (1960) claims it to be a new species, C. orientalis. C. volutator was present in the ZuiderZee (Ysselmer) prior to the construction of the North Sea Dyke which closed it from the sea. Following the construction of this dyke, and the introduction of fresh water conditions in the enclosed area, C. volutator disappeared from the ZuiderZee (Macan, 1963). The ability of C. volutator to withstand regular and irregular salt fluctuations is well known (Crawford, 1937 a,b: Movaghar, 1964). Mercier (1920) reported male and female C. volutator with aberrant armature

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of the first antenna, and also noted that they occurred more frequently in specimens from water with comparatively low salinity, than in marine specimens. Hart (1930) noted a similar aberration in males in summer. Stock (1952) suggests that these are not salinity or temperature effects on *C. volutator*, but that they are specimens of *C. multisetosum*, hitherto unrecognised; the recognition being supported by the finding that *C. multisetosum* may be more common in less saline areas than *C. volutator*. Hamond (1967) whilst contributing to the problem of specific identification in the genus *Corophium*, suggests a salinity distribution gradient, with *C. arenarium* in more saline conditions than *C. volutator* or *C. multisetosum*, and *C. lacustre* in the least saline conditions.

The ecology and distribution of *C. volutator* is clearly well documented, and it may conclusively be called a euryhaline species, although much of the older work has been rendered of reduced value due to reclassification and the creation of new or amended species. Recent autecological work has been centered on the effects of substrate on *C. volutator*. I have been unable to trace any published work on osmoregulation. This current study has been designed to study the multifold effects of salinity, and in conjunction with previous work to further explain the observed distribution of *C. volutator*.

2. FIELD STUDIES

2.1 THE PROPERTIES OF ESTUARIES.

The biological and physical features of estuaries and the estuarine environment have been exhaustively reviewed by Emery, Stevenson, and Hedgpeth (1957). Books on the biology of estuaries have been writted by Remane & Schlieper (1958) and Green (1968). More recent information has been provided by the Jekyll Island Conference on Estuaries (March 31 - April 3, 1964), the papers of which, with a supplementary bibliography, were published under the editorship of Lauff (1967).

Estuaries have been variously defined (for review see Caspers, 1967), the most widely accepted being that of Pritchard (1967) that "an estuary is a semi-enclosed coastal body of water, which has a free connection with the open sea, and within which sea water is measureably diluted with fresh water derived from land drainage". This definition whilst not completely exclusive, does exclude lagoons and brackish seas, which are usually more stable than the complex mixture of environments that constitute an estuary. Estuaries represent an intermediate between the sea, freshwaters and the land. Most of the substrate and food matter is derived from the land, transported to the estuary by fresh water, and there deposited, in a region subject to considerable influence from the sea.

Most of the animal species in estuaries are of marine origin (Emery et.al., 1957), although the number of species is usually considerably reduced compared to any adjacent biotope. The reasons for this paucity are not hard to find. Yonge (1953) points out that the substrate within an estuary is usually very different from adjacent marine coasts. Whereas marine coasts are usually rocky or sandy in nature, most estuarine areas are dominated by a large area of littoral mud. Mud although very rich nutritionally, presents a difficult environment to colonise, due to its softness and also frequently its instability. Locomotion is difficult, both over and through mud, and the fine particulate nature of mud may readily clog the respiratory and feeding mechanisms of many animals, for example, aspidobranch gastropods (Yonge, 1953). Although, as already stated mud may be one of the richest habitats from a nutritional point of view, it may also be readily oxidised by bacteria, leading to a deprivation of oxygen, or the accumulation of hydrogen sulphide. Thus mud is a very difficult environment, whilst mud-flats are a most characteristic feature of estuaries.

In addition to the problems of burrowing and surviving in mud, an estuarine animal must cope with a large range of salinities within the estuary. Whether it colonises the estuary from the sea or from freshwater, it has left an environment with stable

salinity/...

salinity, either 33-35‰, or 0-0.5‰, and entered an environment with salinity ranging from 0 - 35‰, and frequently above 35‰ due to evaporation. In addition the ratio of ions within estuarine water may be much more variable than the sea. The pattern of salt penetration in estuaries has been the subject of many hydrographic studies (Rochford, 1959; Lucht, 1964; Ippen, 1966; Bowden, 1967). Many studies have combined hydrography with biological surveys, and have compared salt penetration with the distribution of the fauna. The works of Remane (1934) in the Bay of Keil, and Alexander, et.al., (1935) on the Tees and Tay estuaries have laid the foundation for subsequent faunal surveys. In particular, studies have been made of the estuaries of the Tamar (Milne, 1938, 1940a; Crawford, 1937b; Spooner & Moore, 1940); Mersey (Bassindale, 1938); Aberdeenshire Dee (Milne, 1940b); Dovey (Beanland, 1940); Exe (Holme, 1949); Elbe (Caspers, 1959); Stour (Bull et.al., 1960); Ribble (Popham, 1966); Knyasa (Day, 1967); Danish estuaries (Muus, 1967a). These studies have all been primarily faunistic surveys, plotting the distribution of the animals of a particular estuary and also usually the salinity and substrates. Reports of C. volutator from these and other surveys, have already been mentioned (Section 1.3).

From this foundation of faunistic surveys, other workers have

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studied individual species and environments more intensively. For example: Amphipoda (Goodhart, 1941); Nereis diversicolor (Smith, 1956); Nematodes (Capstick, 1959); Harpacticoids (Barnett, 1959, unpublished thesis, University Southampton); Hydrobia ulvae (Newell, 1964); Cyathura spp. (Burbanck, 1967); Diatoms (Hopkins, 1966); Mytilus edulis (Baird, 1966); Balanus, Gryphae, Merciella, & Hydroides (Sandison & Hill, 1966); Pandalus montagui (Warren & Sheldon, 1967). Green (1968) extensively reviews estuarine animals, in general considering them species by species.

The influence of pollution on estuaries may be considerable, and may severely limit the abundance of animals (Newell, 1959), or may induce animals such as Corophiidae to migrate (Lubyanov, 1964). DeFalco (1967) has described estuaries as the "septic tank of the megalopolis", a fitting description for many estuaries. However, as will be seen later, the Ythan estuary is not affected by pollution.

In reviewing the biology of estuarine animals, and in particular the effects of salinity on the fauna, it is important to consider which parameters are measured. Early workers concentrated on the variation in the salinity of the estuarine water, however many animals burrow within the substrate, and are sealed

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off from the overlying water. Reid (1930, 1932) studied the interstitial water of sands and muds, and was the first to show how the range of salinity of the interstitial water was considerably reduced, in comparison to the overlying water. It has been generally recognised (Alexander, *et. al.*, 1932; Nicol, 1935; Smith, 1956; Capstick, 1957a) that the interstitial salinity reflects the salinity of the overlying water, but that such changes are considerably reduced in magnitude. In an intertidal estuarine mudflat, only covered by the high tide the top of the beach is only covered by waters of high salinity (i.e. sea water brought in by the tide), whereas the bottom of the beach is generally covered by waters of lower salinity. Hence the interstitial salinity is generally higher, further up a beach. This persistent pattern is regarded by Emery, *et.al.* (1957) as one of the most ecologically significant properties of the muddy bottoms of estuaries, and it permits animals living within the substrate, to penetrate a greater distance up an estuary along the upper region of intertidal mudflats, than animals which are subject to the salinity of the overlying water, such as plankton. A good example of this phenomenon was demonstrated by Newell (1964) with regard to the upstream distribution of *Hydrobia ulvae*, a mud-dwelling snail.

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From these studies of estuaries, various factors emerge which may limit the distribution and abundance of the fauna, viz: currents, sedimentation, oxygen, temperature, pH, inorganic ions, food, predation, exposure, substrate and salinity.

The currents within an estuary are of two principal varieties. The downstream current from the river, and tidal currents as the saline water ebbs and flows from the sea. The effect of these currents is considerably influenced by the volume of the estuary, a measure of which is the "flushing time" (Green, 1968). If the estuary is of a large volume, the currents entering the estuary will considerably slacken in velocity, and lead to a large area of mixing, with generally calm conditions prevailing. In these calm conditions, the sediment load of the river current will generally fall out of suspension, leading to extensive sedimentation (Yonge, 1953). Conversely if the volume of the estuary is relatively small, the momentum of the downstream current may be sufficiently strong to carry the suspended sediment load right through the estuary, and deposition will occur at sea. However the commonest situation is the former, and the Ythan estuary is of this type, with a large area of deposition. Within an estuary localised currents may lead to a variety of substrates. Areas subject to strong downstream currents, or tidal scour will usually have coarser, unsorted substrates; whereas areas which receive eddies, and are off the main channel, will usually contain finer, well sorted substrates since fine suspended matter being transported will be deposited out of suspension in such regions. The distribution of most animals which burrow within substrates is usually critically controlled by the physical nature of the substrate (for C. volutator, see Meadows (1964c)); and it may be seen how important are hydrographic features, such as currents and their corollary, sedimentation, in controlling the nature of the substratum.

Oxygen concentration has already been shown to be an important factor within estuaries, particularly with regard to mudflats, in that these areas may be subject to partial deoxygenation. Because of the regular influx of water from freshwaters and the sea, there is usually a good supply of oxygen in estuaries. However it may not be available to the macrofauna, due to the activity of bacteria, and other microorganisms, which may consume all the available oxygen and render deoxygenated conditions. The deleterious effects of deoxygenation may be seen most strikingly in severely polluted estuaries, where deoxygenation may lead to the exclusion of most animals, except specially adapted forms such as Tubifex (Alexander, et.al., 1935; Lubyanov, 1964; Palmer, 1968; Persoone & De Pauw, 1968). Romane & Schlieper (1958) report that C. volutator is particularly resistant to low

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oxygen concentrations, however it will not tolerate complete deoxygenation.

Temperature conditions may be very variable in estuaries, due in particular to their shallowness. They may be subject to warming by the sun, and may reach temperatures lethal to many animals, particularly those from the more temperature-stable sea. Estuaries may also be subject to frost, which despite the partially saline water, may cause the mud or water to freeze, resulting in catastrophic mortality, as in the winter of 1962-3 (Crisp, 1964a,b; Williams, et.al., 1964; Muus, 1967a, George, 1968). The activities of man, particularly with regard to power station effluents, may be an important temperature factor in estuaries, although Gammon (1968) was able to find little difference apart from improved oyster development, in the fauna of the Blackwater estuary, due to the effects of Bradwell Power Station. The temperature tolerance limits of C. volutator have already been reviewed. Kinne (1963b, 1964a, 1967) has emphasised the importance of temperature as a factor which may affect salinity tolerance.

The Hydrogen Ion Concentration (pH) of Sea Water is normally 8.1 (Nicol, 1967), although it may rise to 9.6 in tidal pools, or fall to 7.0 following deconposition of organic matter. The pH of river water is normally 6.5 - 7.3 (Dee-Don River Purification Board, 1967). The range of estuarine pH would thus be expected to be 7.0 - 8.0. Nicol (1967) suggests that some marine animals are very sensitive to small changes of pH, although Odum (1959) claims that most organisms seen to have a wide tolerance for the naturally occurring ranges of pH. I have been unable to trace any published work on the effects of pH on estuarine life.

Inorganic ions in estuaries (e.g. phosphate, nitrate, sulphate, ferric) have been studies with particular regard to plant production, and decomposition as occurs within muds (Green, 1968). I have found no records of the effects of individual inorganic ions on the macrofauna of estuaries.

Estuaries are usually considered to be more abundant in food than adjacent freshwater areas, and may be comparable to, or better than marine areas (Masse, 1968). This abundance of food is due to the nature of estuaries, as depositories for freshwater debris, the products of terrestrial erosion and agricultural drainage, which may be rich in fertilisers. These deposits are decomposed by bacteria leading to nutritionally rich sediments. This food is generally detritus, which has been defined by Darnell (1967a) as "all types of biogenic material in various stages of microbial decomposition, which represents potential energy sources for consumer species". Darnell (1967b) has surveyed the subject of organic detritus in relation to the estuarine ecosystem, and concludes that the estuarine ecosystem is really "vegetable soup". He also emphasises the prime importance of iliophagic animals (detritus feeders) within estuaries. Previous work has suggested that the detritus feeders are the primary consumers in an estuarine ecosystem, but Darnell (1967b) points out that the microbial decomposers are the real primary consumers. The importance of the microbial decomposers, as well as the complexity of the detritus food chain has been emphasised by Odum & de la Cruz (1967), who conclude that the bacteria-rich detritus is nutritionally a better food source for animals than is the plant tissue which forms the original base for most of the particulate matter. Identification of the microbial flora of detritus has been greatly assisted by the studies of Meadows & Anderson (1968), who have demonstrated the presence of colonies of micro-organisms (bacteria, blue-green algae, diatoms) on the surface of sand grains. The difficulties of defining the food of a detritus feeder (Newell, 1965, for *Hydrobia* & *Macoma*) are great, but the richness of detritus as a food source is clear.

I have no data to suggest that predation is any more, or less, important in estuaries than elsewhere - except to note the abundance

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of bird life usually found in estuaries, which feeds on the invertebrates which are accesible there. It would appear that the use of estuaries as feeding grounds for birds and fish, reflects the abundance of food, rather than a more intensive rate of predation.

Exposure to the weather and tides is a factor that intertidal estuarine areas share with intertidal marine areas. Tidal exposure may limit the time available for feeding, or may expose the animal to the extremes of the weather, with problems of osmotic dilution due to rainfall, or desiccation due to sunshine. The problems for an estuarine animal may thus be the same as those for a marine intertidal animal; with the addition in estuaries of factors such as a muddy substrate, or regular variation in salinity.

Estuaries have been shown to be typified by muddy substrates, and the problems of adapting to a muddy substrate have already been enumerated. Other substrates may occur; sand is frequently found at the seaward end of estuaries, and rocky outcrops may occur giving suitable substrates for barnacles or seaweeds, such as *Fucus ceranoides*. Carriker (1967) has extensively reviewed, and emphasised the importance of the nature of the substratum in the ecology of estuarine benthic invertebrates.

Salinity/...

Salinity was the principal environmental factor studied by many earlier estuarine workers (see above), and has remained a subject of intensive study, indeed the variability of the salinity within an estuary, compared to adjacent aquatic biotopes, is one of the most characteristic features of estuaries.

Salinity has been repeatedly claimed as the main limiting factor for the life of estuarine organisms. Petit & Schachter (1959) suggest that despite many other factors (oxygen conc., temperature, substrate, bacteria), salinity remains the primary basis for the classification of estuaries. Salinity has also been claimed to be the "ecological master factor", controlling the biology of estuarine organisms, by Gunter (1961), Wells (1961), Kinne (1964a,b, 1966) and Green (1968). Various authors (for historical review, see Segerstrale, 1959b) have proposed systems for classifying brackish and estuarine waters. The complexity of the existing classification systems led to a Symposium in Venice (8-14 April, 1958) which proposed a unified system, modified from Redeke-Valikangas, for classifying brackish waters (Venice System, 1959). This system which is now widely adopted is briefly as follows:-

<u>Zone</u>	<u>Salinity</u> $^{o}/oo$
Hyperhaline	>40

contd/...

contd:

Zone	Salinity °/oo
Euhaline	40 - 30
Mixohaline	(40)30 - 0.5
Mixoeuhaline	> 30 but < adjacent sea area
Polyhaline	30 - 18
Mesohaline	18 - 5
Oligohaline	5 - 0.5
Limnetic (Fresh Water)	< 0.5

Carriker (1967) has also classified estuarine organisms, into groups which correspond with the Venice System (1959):-

Zone	Salinity (°/oo)	Ecological Classification				
Euhaline	40-30	stenohaline		euryhaline	migrants	
Mixoeuhaline	> 30/25	marine	true			mixohaline
Polyhaline	30-18					
Mesohaline	18- 5					
Oligohaline	5- 0.5	oligohaline	estuarine	marine		
Limnetic	< 0.5	limnetic			↓	mixohaline

These ecological groups of Carriker have been based in particular on the extensive estuarine faunal surveys already referred to. These groupings also agree well with the classifications of Kinne

(1963a, 1967)/...

(1963a, 1967), which were based on osmoregulatory capability.

Kinne (1964c) has defined adaptation as the adjustment of organisms to alterations in the intensity pattern of variables in the environment, and further (Kinne, 1966) has suggested that for most estuarine animals, salinity is the principal variable factor. He suggests that the basic characteristics of estuaries are the increased gradients and fluctuations of the environmental factors relative to the more stable situation in the neighbouring sea and fresh-water areas. Given similar temperature conditions, the degree of dilution of sea water by river water and the physico-chemical properties of the resulting mixture, largely determines the performance and distribution of the estuarine organisms.

The estuarine environment may thus be seen as an area of mixture and change, but nevertheless a distinct biotope; An area of mixing sea water and fresh water, leading to deposition of frequently unstable substrates, subject to large temperature variations, and oxygen depletion; an environment which few animal species have managed to colonise, although these species may be very abundant; Estuarine animals are typically detritus feeding animals living in a muddy "vegetable soup".

Estuaries are geologically ephemeral (Kinne, 1966). Gorsline (1967) has suggested that all estuaries, as we know them today, are

less/...

less than 3000 years old. A suggestion which may explain the interesting observations of Muus (1967b) that estuarine species may be very plastic, and that most species within typically estuarine genera (e.g. Cardium, Hydrobia, Nereis, Gammarus, Corophium) are currently developing. A situation well seen in the changing taxonomy of C. volutator.

Estuaries thus present areas of significant biological interest; They are frequently areas which have been exploited and influenced greatly by mankind, and they present to the biologist a complex interaction of definable environmental and biotic factors. Kinne (1957) and others, have pointed to the need for a polyfactorial approach when studying estuarine life, for the efficiency of single environmental factors may depend on the whole environmental situation.

With regard to C. volutator, it has already been noted that many authers have referred to its ~~disturbing~~ distribution limits when conducting faunal surveys. From the published record it appears that the two principal environmental factors affecting its distribution and abundance are substrate and salinity. The substrate selection of C. volutator has been investigated by Meadows (1964 a-c, 1967) and it has been shown how specific is this selection. Hitherto salinity has not been critically investigated as a factor controlling C. volutator, and the present investigation is concerned to study the effects of salinity on the distribution and abundance of this crustacean.

2.2 THE YTHAN ESTUARY.

The Ythan estuary enters the North Sea, 21km north of the city of Aberdeen, and the estuary extends inland for 8.4 km, almost as far

far as the village of Ellon. Newburgh, with Aberdeen University Field Station (Culterty) is located on the western bank, and a National Nature Reserve (Sands of Forvie) is located on the eastern bank of the estuary. The catchment area of the River Ythan, which is mainly agricultural land, covers 690 km^2 of Aberdeenshire. At high tide the estuary forms a mass of water approximately $\frac{1}{2}$ - $\frac{2}{3}$ km wide, and 6 km long; at low tide it resembles a river approximately 50 m wide (Fig 1).

The Dee-Don River Purification Board (1967) report that slight pollution occurs due to field drainage (above Auchterless) and from small sewage works at Fyvie and Ellon. Except in the immediate vicinity of these locations, the pollution is not considered to be significant. The average data for the river at Ellon (just above the head of the estuary) is as Table 3.

Table 3. From Dee-Don River Purification Board (1967).

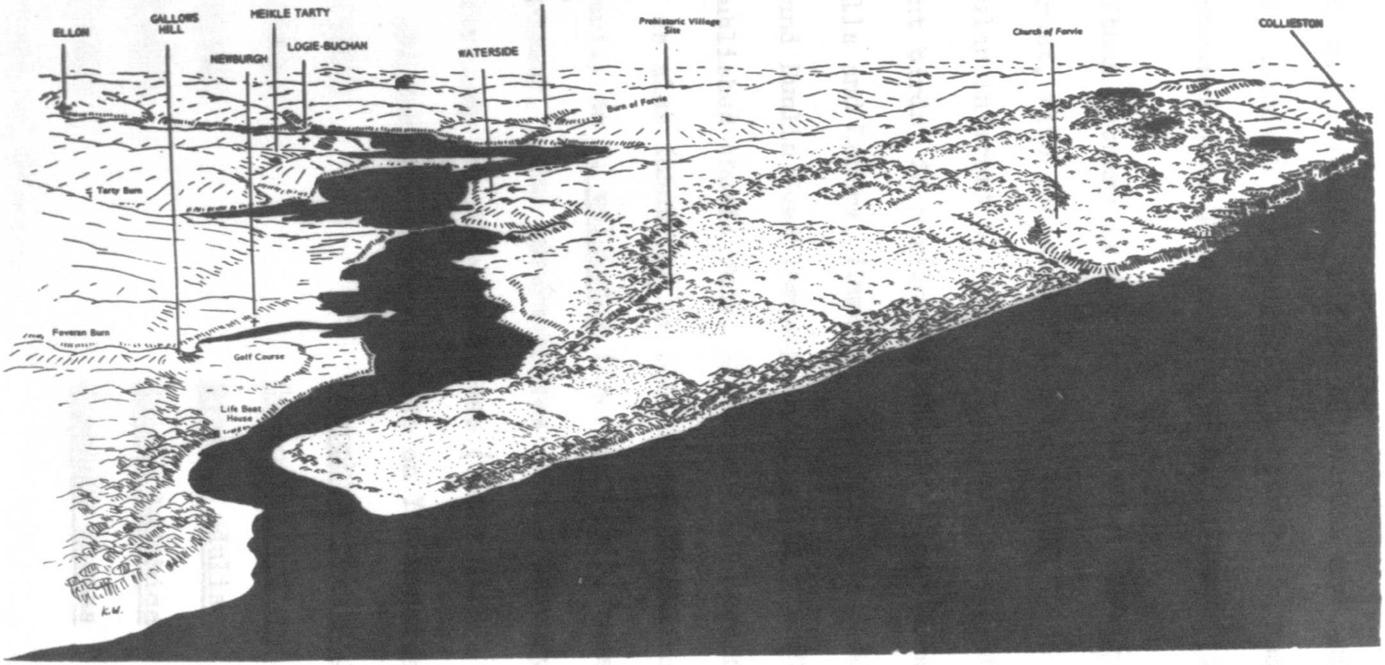
Biochemical Oxygen Demand	1.3 p.p.m.	Albuminoid Ammonia	0.10 p.p.m.
Suspended solids	7.0 p.p.m.	pH	6.8
Oxygen absorbed	2.0 p.p.m.	Free and Saline Ammonia, as N	0.05 p.p.m.

All these figures are regarded as acceptable from the standpoint of possible deliterious effects of pollution.

The/...

FIGURE 1.

General map of the Ythan estuary, Aberdeenshire, Scotland


ELLON
GALLOWS HILL
NEWBURGH
MEIKLE TARTY
LOGIE-BUCHAN
WATERSIDE
WESTFIELD OF AUCHMACOY
Prehistoric Village Site
Church of Forvie
COLLIESTON
Burn of Forvie
Tarty Burn
Foveran Burn
Golf Course
Life Boat House
K.W.

The estuary contains a wide variety of substrates, ranging from clean sand at the seaward end to sulphurous mud amid weed at the inland end, although the commonest substrate is mud or muddy sand. These substrates have been mapped by A. Anderson (in Dunnet, 1965).

The Ythan estuary is the location of a fishery, particularly for sea trout, with occasional salmon. The Ythan estuary is also of particular interest because of its large populations of resident and migrant birds, in particular, Terns, Eider Duck, Shellduck, Geese, Gulls and Waders, (Dunnet, 1965). The Waders, especially Redshank and Dunlin feed largely on C. volutator (Goss-Custard, 1966, unpublished thesis, Univ. Aberdeen). Apart from Sea Trout, the river is also inhabited by Gobies and Flounders. The Flounders feed primarily on C. volutator (Emerson, 1969, thesis in preparation, Univ. Aberdeen). The invertebrates of the estuary have been subject of study for many unpublished theses (Table 4).

Table 4. Aberdeen University theses on Ythan estuary.

Noble	(1962)	Corophium volutator
Keddie	(1966)	Scoloplos armiger
Marriott	(1966)	Mytilus edulis
Dunn	(1967)	Macoma balthica
Mackay	(1968)	Carcinus maenas

Research work in progress,

Leach/....

contd.

Table 4 contd.

Leach	Hydrography and plankton of estuary
Gidney	Effect of light on C. volutator
Gibson	Podocotyle atomon, the metacercaria of which are in C. volutator, other hosts being the Flounder & Littorina sp.
Wood	Production of C. volutator.

Under the auspices of the International Biological Programme, it is intended to construct a food web for the Ythan estuary, and to study certain food links to ascertain the productivity of the estuary. Since C. volutator is one of the most abundant food animals in the Ythan ecosystem, knowledge of the factors affecting its distribution and abundance are of particular value.

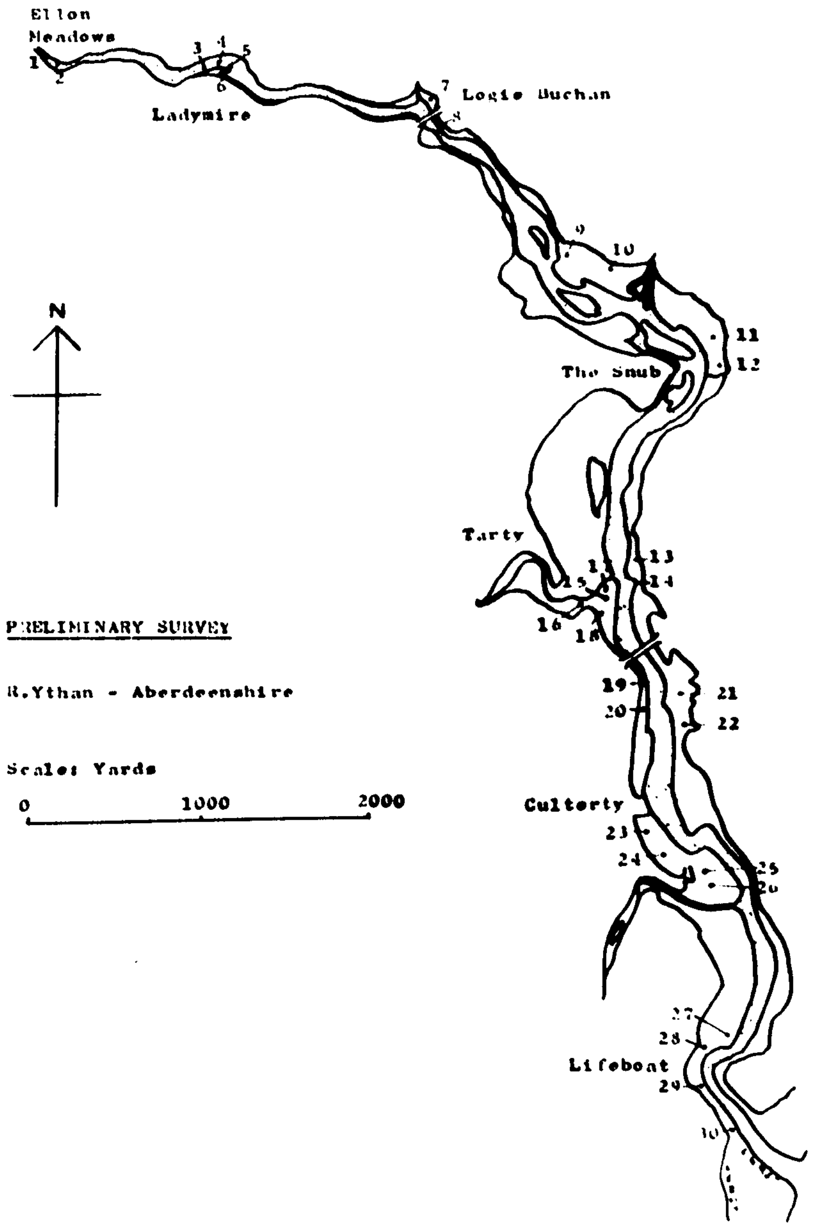
2.3 PRELIMINARY STUDY

A preliminary survey of the Ythan estuary was made and samples (each 10 x 10 x 10 cm) from 30 stations, along the length of the estuary were collected, the type of substrate noted, and any C. volutator counted (Fig. 2, Table 5). It was found that C. volutator was most abundant at stations with a high proportion of silt, stations which had mud or muddy sand. Stations with black sulphurous mud, mud overlain with Enteromorpha, or sand

lacking/...

FIGURE 2.

Preliminary survey of the Ythan estuary. Sampling stations.



PRELIMINARY SURVEY

R.Ythan - Aberdeenshire

Scale: Yards

		C. volutator	♀		♂		imm.	
Station No.	Description	Total No.	No.	%	No.	%	No.	%
1	Mud and Reed	0	0	0	0	0	0	0
2	Mud and Reed	0	0	0	0	0	0	0
3	Soft Mud	0	0	0	0	0	0	0
4	Firm Mud	0	0	0	0	0	0	0
5	Muddy Sand	0	0	0	0	0	0	0
6	Sand	0	0	0	0	0	0	0
7	Firm Black Mud	12	4	33	4	33	4	33
8	Soft Black Mud	3	2	66	1	33	0	0
9	Mud	35	26	74	9	26	0	0
10	Mud	17	12	70	5	30	0	0
11	Sandy Mud	92	19	20	12	13	61	66
12	Sandy Mud	66	20	30	8	12	38	57
13	Gravelly Mud	47	24	51	11	23	12	25
14	Gravelly Mud	86	41	47	24	27	21	24
15	Course Sandy Mud	46	28	60	11	23	7	15
16	Mud	44	26	59	12	27	6	13
17	Clean Sand	0	0	0	0	0	0	0
18	Sand	2	2	100	0	0	0	0
19	Gravel & Mud	33	12	36	6	18	15	45
20	Gravel & Mud	85	40	47	23	27	22	26
21	Mud	23	17	73	6	26	0	0
22	Mud	54	37	68	17	31	0	0
23	Sewage polluted Mud	83	23	27	16	19	44	53
24	Sewage polluted Mud	76	23	30	15	19	38	50
25	Sand and little Mud	32	7	21	5	15	20	37
26	Mud & *Enteromorpha*	0	0	0	0	0	0	0
27	Mud & Mussels	4	1	25	0	0	3	75
28	Muddy Sand	151	41	27	56	37	54	35
29	Muddy Sand	115	47	40	30	26	38	33
30	Sand	0	0	0	0	0	0	0
TOTALS:		1106	467	42	256	23	383	35

Of those sexed; Female 64.5%, Male 35.5%.

lacking in silt, all had few or no C. volutator. These findings are in agreement with the previous findings of Hart (1930), Spooner & Moore (1940), Goodhart (1941), Gee (1961) and Meadows (1964a-c).

Within the central part of the estuary, substrate was apparently the predominant factor limiting the distribution and abundance of C. volutator.

2.4 STUDY AREAS AND METHODS.

Having regard to this preliminary study, four areas were chosen in the upper half of the Ythan estuary for intensive study (Fig.3).

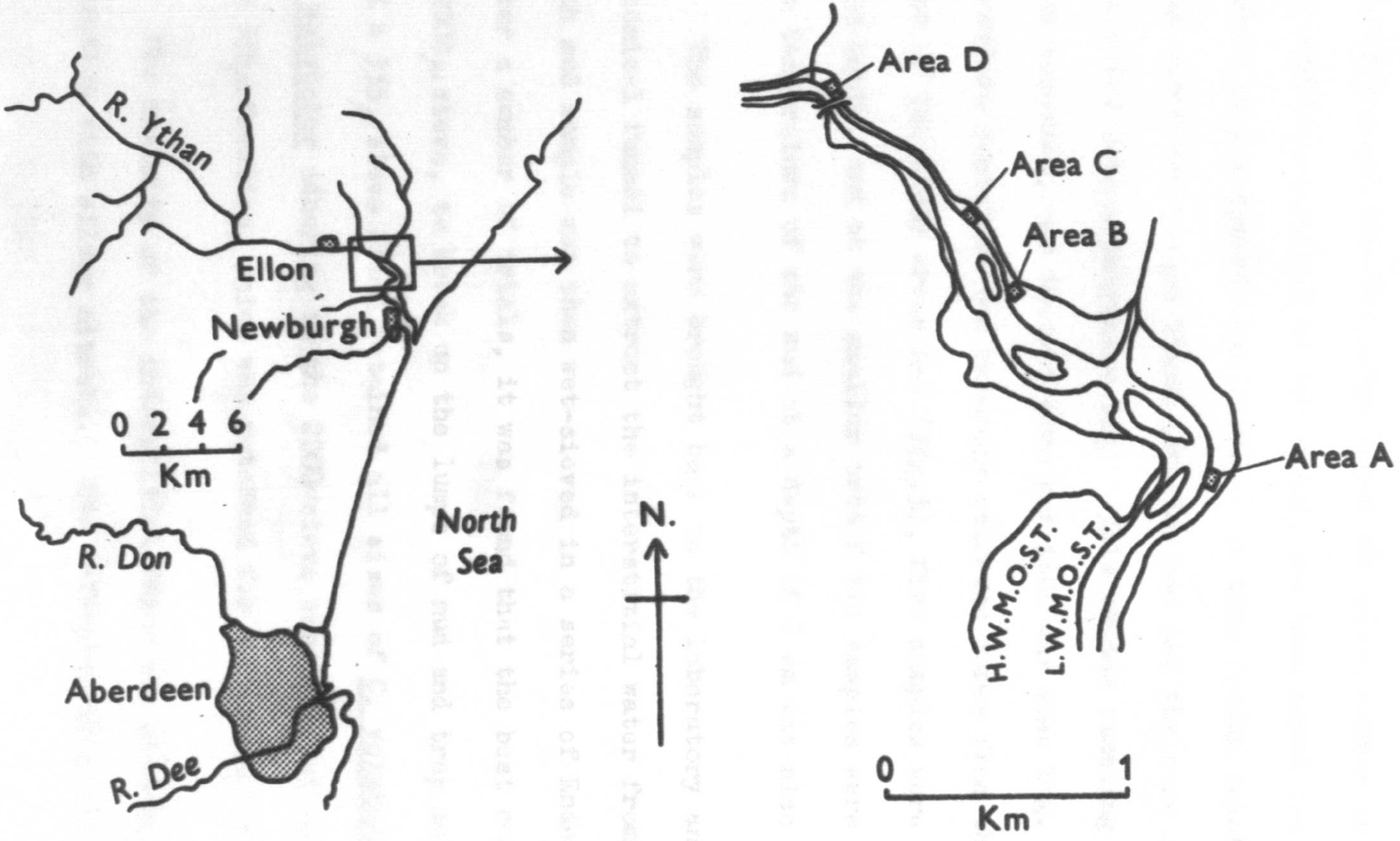
These areas were selected as having, as far as possible, uniform substrate conditions and representing a potentially wide range of salinity. Thus by ensuring uniform substrate conditions the effect of salinity could be analysed more easily.

At each of the four study areas, samples were collected at monthly intervals from October 1966 to November 1967. Samples were collected at the same state of the tide at 29 to 30-day intervals.

Each area was divided into a grid 50 m square; paired random numbers/...

FIGURE 3.

Map showing, on the left, the location of the estuary of the River Ythan, with inset on the right showing the position of the four study areas.


R. Ythan
Ellon
Newburgh
0 2 4 6
Km
R. Don
Aberdeen
R. Dee
North Sea
N.
Area D
Area C
Area B
Area A
H.W.M.O.S.T.
L.W.M.O.S.T.
0
1
Km

numbers were chosen (Fisher & Yates, 1963), then from the fixed starting point the collector moved the given number in $\frac{1}{2}$ m across the mudflat, parallel to the river, and then moved the next given number in $\frac{1}{2}$ m towards the river. At this random point, a 5 x 5 x 6cm high corer was pushed flush into the mud and the core of mud was collected into a polythene bag. This random sampling procedure was then repeated, and the next core put into the same bag. A sample therefore contained two randomly selected cores from the area. At each of the study areas A-C (Fig.3), five samples were collected each month, and at the smaller area D two samples were collected. The temperature of the mud at a depth of 2 cm was also noted.

The samples were brought back to the laboratory and filtered in a conical funnel to extract the interstitial water from the mud. Each mud sample was then wet-sieved in a series of Endecott sieves. After a number of trials, it was found that the best combination was a 2000μ sieve, to break up the lumps of mud and trap any pebbles, and a 335μ sieve which retained all sizes of C. volutator. Any C. volutator adhering to the 2000μ sieve were removed and added to the 335μ fraction, which was retained for subsequent examination.

The salinity of the interstitial water was determined by titration with silver nitrate. The interstitial water /...

water of the mud was sampled, rather than the estuarine water, as the interstitial water represents the immediate environment of C. volutator.

The 335μ sieve fraction was carefully examined to remove any individuals of C. volutator present. These were counted, measured from the tip of the rostrum to the end of the telson, and assigned to size categories (i.e. 1.0 - 1.9 mm, 2.0 - 2.9 mm, 3.0 - 3.9 mm, etc.). The percentage size composition and arithmetic mean size were calculated.

Rainfall data were obtained from Culterty Field Station.

2.5 RESULTS

The results are presented in Figs. 4(a-e) and 5 and Appendix I. At the start of the study (Oct. 1966) the population of C. volutator had just completed its reproductive season. Throughout the winter (Oct. - Mar.) the mean size of animals at areas A-C fluctuated between 3.0 and 3.6 mm (Fig.4e), whilst the salinity and temperature conditions remained fairly constant (Figs 4a,c). The temperature at all areas was between 2° and 7°C. At areas A and B the salinity averaged 10°/oo and 6°/oo respectively. At area C the salinity averaged 2.5°/oo and the number of animals was extremely low. At area D, with salinities of below 1°/oo no animals were present. From December onwards slow growth was observed/...

FIGURE 4.

(a) The interstitial salinity of the mud at each study area. 0, Area A; ● , area B; △ , area C; ▲ , area D. (b) Total rainfall recorded at Culterty Field Station, Newburgh, 30 days (■) and 10 days (□) prior to each monthly sample. (c) Mean temperature of the mud, at a depth of 2 cm, at the study areas. (d) The number of Corophium volutator, expressed as no./ m^2, at each study area (legend as before). (e) Mean size (mm) of C. volutator at each study area (legend as before).

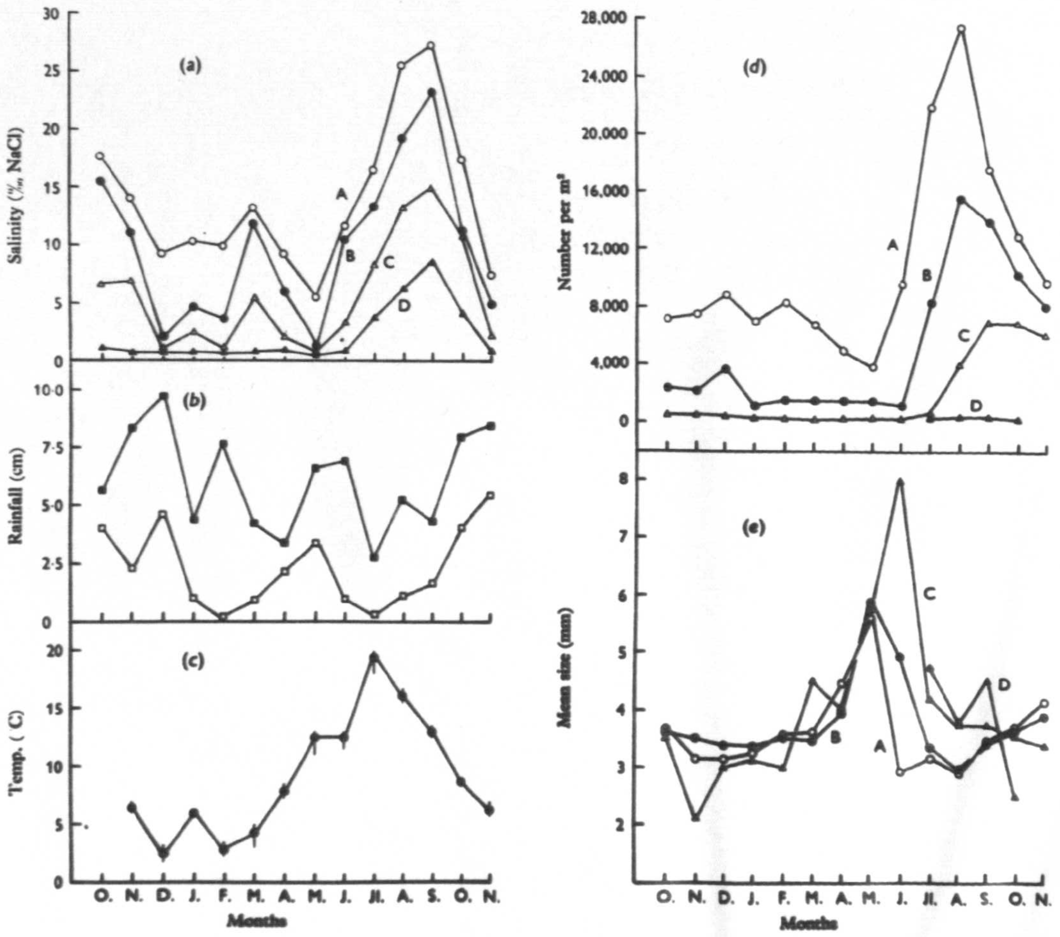
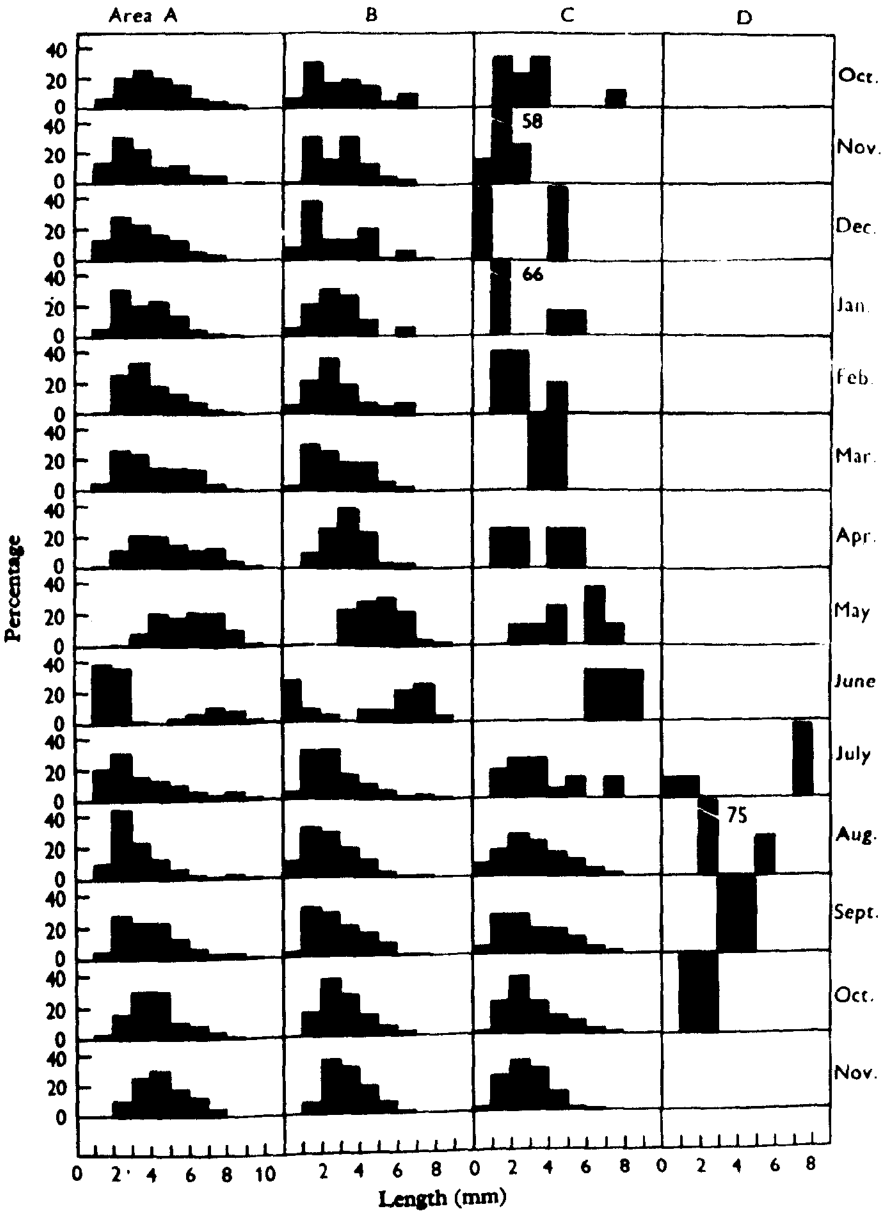

(a)
Salinity (‰ NaCl)
30
25
20
15
10
5
0
A
B
C
D
(b)
Rainfall (cm)
10·0
7·5
5·0
2·5
0
(c)
Temp. (°C)
20
15
10
5
0
O. N. D. J. F. M. A. M. J. Jl. A. S. O. N.
Months
(d)
Number per m²
28,000
24,000
20,000
16,000
12,000
8,000
4,000
0
A
B
C
D
(e)
Mean size (mm)
8
7
6
5
4
3
2
A
B
C
D
O. N. D. J. F. M. A. M. J. Jl. A. S. O. N.
Months

FIGURE 5.

The percentage size composition of populations of C. volutator at each study area, each month.


Area A
B
C
D
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
June
July
Aug.
Sept.
Oct.
Nov.
58
66
75
40
20
0
Percentage
0 2 4 6 8 10
2 4 6 8
0 2 4 6 8
0 2 4 6 8
Length (mm)

observed as the mean size of animals at area A increased from 3.17 mm in December to 3.62 mm in March (Fig. 4e).

In May, with warmer conditions (above 7°C), the mean size increased to 5.61 mm. The salinity fell sharply at all areas during this month, but this lowering was only temporary, following very heavy rainfall 4 days prior to sampling, and after this period the salinity rapidly returned to its former level. During May, females bearing eggs ('berried') were first noted, especially in area A. In June the number of animals in area A increased dramatically (Fig.4d) and the mean size fell, following the hatching of a large number of young animals, measuring 1.0 - 1.9 mm (Fig. 5). This increase in numbers at area A persisted into July, when a large increase in numbers due to young individuals occurred in area B (Figs. 4d, 5). Meanwhile salinity had increased to 16.5°/oo at area A, 13.6°/oo at area B, and 8.5°/oo at area C. With this persistent increase in salinity above 5°/oo in area C, the number of animals present also increased, partly because of hatching. At area D the salinity rose to 3.7°/oo and animals were recorded there in small numbers for the first time.

In August the populations at areas A and B were at a peak of abundance, having increased to a level of 6 - 10 times the over-wintering population. Animals in area C, at 13.5°/oo, were

breeding/...

breeding in small numbers. Animals persisted in area D at 6.4°/oo. In September the numbers of animals in areas A and B decreased, and their mean size was 3.45 mm. This decrease in numbers continued into October as, with all stock from the previous winter having died off, the new stock settled down to its winter level of abundance. In area C numbers continued to climb until September, following the later breeding season. In area D animals were present in September at 8.5°/oo and fewer were present in October at 4.1°/oo. Heavy rainfall during October caused the salinity to fall to 1.1°/oo at area D in November. With this lowering of salinity, animals were no longer found in this area. The number of animals at areas A - C fell, whilst the salinity fell to 7.5 - 2.2°/oo. As growth occurred in the animals born in the summer, the mean size increased from 2.90 mm (August) to 3.90 mm (November) (Fig. 4e).

The decrease of salinity in the autumn and in May was largely caused by changes in rainfall (Fig. 4b) affecting the downflow of river water and consequently modifying the inflow of salt water into the estuary. The summer increase in salinity was due partly to decreased rainfall and to increased temperature (Fig. 4c), causing increased evaporation.

From these results it can be seen that one single breeding season occurred per year in this population of <u>C. volutator</u>, extending from May to August. Animals born in the summer of one year grew to a maximum size, bred when over 5 mm, and subsequently died in the summer of the next year. The onset of the reproductive season was correlated with increased temperature and salinity conditions. Segerstråle (1940) reported that 7°C was the minimum temperature for reproduction in C. volutator, and the same temperature limit has been noted in this study. The breeding season finished whilst salinity and temperature conditions were still high, but these became reduced within a few weeks of the termination of breeding. Segerstråle (1940) found a similar annual cycle in a Baltic population of C. volutator, the only major difference being that in the Baltic the animals overwintered at 7.5 - 8.0 mm long, whilst in the Ythan they overwintered at 3.0 - 4.0 mm long and then grew to 7.5 - 8.0 mm in May prior to undergoing reproduction. It may be suggested that the animals grew in the autumn as long as conditions were favourable, then overwintered, and began growth again in the spring as conditions ameliorated. On the River Dovey, Watkin (1941) noted two generations per year, and in Denmark, Muus (1967a), suggested four generations per year, but in this study only one is indicated.

Comparing the four study areas, it can be seen that although they all experienced the same temperature conditions, breeding began first at area A, followed by areas B and C, in that order. It appears that breeding occurred only as the salinity increased above/...

above 7.5°/oo. The sequence of breeding reflected the successive increase of salinity up to and above this level. In June 73% of the animals in area A at 11.7°/oo measured less than 2.9 mm, whilst 34.5% of the animals in area B at 10.5°/oo were this size, having recently hatched. In area C newly hatched animals were first found in July, at 8.5°/oo (Figs. 4a, 5).

In area C the numbers of C. volutator remained extremely low all winter (December - June), with the interstitial salinity at 2 - 5°/oo. The numbers increased as the salinity increased above 5°/oo, and maintained the increase as long as salinities greater than 5°/oo were recorded. This increase was partly due to breeding, but also due to the immigration of individuals into the area as soon as conditions were favourable. The number of individuals larger than 5 mm in June, at 3.4°/oo was $120/m^2$, however, in August, at 13.5°/oo, the number had increased to $1120/m^2$. This increase is due entirely to immigration. The number of individuals smaller than 5 mm increased in the same period from 0 to $2640/m^2$, which it may be suggested is due to a combination of reproduction and immigration.

At area D there were no animals present during the winter and spring, when salinities were 0.5 - 1.0°/oo; however, in July, as the salinity increased above 2°/oo, C. volutator persisted in this area. In November the salinity in area D fell to 1.1°/oo and C. volutator was no longer found in this area.

Thus/...

Thus it can be seen that salinity appears to be critical in the control of the distribution and abundance of <u>C. volutator</u>. In an area with an interstitial salinity below $2^{\circ}/oo$ the species was absent. However, when the salinity of this area increased above $2^{\circ}/oo$, animals were found. In salinities 2 - $5^{\circ}/oo$ the numbers of animals present were always small; and the numbers only increased in salinities above $5^{\circ}/oo$. It may be suggested that <u>C. volutator</u> is ready to migrate into an area whenever conditions become favourable. Breeding (i.e. observation of "berried" females) appeared to be correlated with temperatures greater than 7°C and with interstitial salinity conditions above $7.5^{\circ}/oo$.

It has already been mentioned that the two principal environmental factors which may affect <u>C. volutator</u> are substrate and salinity. In the choice of study areas an attempt was made to eliminate the variability of the substrate, to assist in the study of the effect of salinity. An analysis was made of the nature of the substrate at each area in order to check on any variability which may have affected the distribution and abundance of <u>C. volutator</u>. Methods similar to those recommended by Morgans (1956) were adopted, and the mean results are given in Table 6.

Table 6/...

TABLE 6 RESULTS OF SUBSTRATE ANALYSIS AT THE FOUR STUDY AREAS

Area	Depth of aeration (cm)	Water composition (%)	Mean particle diameter (μ)	Skq ϕ (see Morgans, 1956)	Organic matter by ignition (%)
A	6.12	27.30	225	+0.250	2.46
B	1.37	29.65	235	-0.131	2.75
C	0.50	26.38	305	+0.081	2.08
D	0.35	49.43	220	-0.256	6.62

These results, which represent the means of random samples taken at each area, did not vary, except by very small amounts, throughout the year. Although the study areas were chosen for uniformity of substrate, some slight differences did occur. The depth of aeration showed a gradation A > B > C and D. The similarity in depths of aeration at areas C and D and the dis-similarity in records of C. volutator rule out this factor as being critical. Muds at areas A - C had 26 - 29% water content, whilst the mud at area D had 49% water content. The similarity of mud at areas A - C discounts this factor as a limiting factor controlling abundance, but the high value for area D may have partially accounted for the few animals ever recorded there. The differences between the mean particle diameters at the areas show no important difference. The Skq ϕ, which is a measure of the sorting of the particles of the substratum, indicates that in areas A and C, with positive results, particles larger than the

median/...

median are better sorted than the smaller particles. In areas B and D, with negative results, the smaller particles are better sorted than the larger ones. However, all the Skq ø results are extremely near zero, and the differences between the areas are very slight.

Thus it may be concluded that the textures of the substrates at the study areas are extremely similar, confirming the original choice of the study areas for their uniformity. The demonstrated uniformity, moreover, suggests that adverse currents, which usually affect the nature of the substrate, are not important in this study. The percentage organic matter showed no difference between areas A - C, averaging 2.43%. Area D was richer in organic matter (6.62%) than the other areas; however, Hart (1930) found C. volutator abundant in muds with 6 - 7% organic matter. This finding suggests that the amount of organic of matter is not limiting in this study. Muus (1967a) suggested that 15 - 20% organic matter may limit C. volutator.

2.6 DISCUSSION

The decrease in the number of animals (Fig. 4d) observed in area A between December and May and the decrease again from September to November may be due to either predation, natural mortality, or emigration. Especially between December and May, no great change in the engironmental factors occurred, whilst the population of C. volutator was more than halved. Goss-Custard (1966, unpublished thesis, Univ. Aberdeen/...

Aberdeen) noted that Redshank (Tringa totanus L.) ate 38% of C. volutator which were larger than 4 mm - a feature which would account for the spring decrease in numbers, due to predation.

The onset of breeding has been shown to be coincident with an increase in temperature above 7°C. In general, the temperature on a given day was uniform throughout the estuary; therefore, it is unlikely that temperature can cause the observed differences in the distribution of C. volutator between the study areas. Goss-Custard (1966, unpublished thesis, Univ. Aberdeen) has shown that temperature may affect the burrowing behaviour of C. volutator, which burrows deeper into the mud when the temperature is below 4°C. The results of the substrate analysis show that area D was slightly less suitable for C. volutator, but showed no factor which could adequately account for the variation in observed numbers between areas A - C. Also, the nature of the substrate at each area remained constant throughout the period of study, whereas the distribution and abundance of C. volutator varied considerably. In area D, animals were absent when the salinity was below $1^{\circ}/oo$, yet as soon as the salinity increased to above $2^{\circ}/oo$ animals were found there, albeit in small numbers. Similarly, the numbers in area C remained extremely low all winter, with salinities of $2 - 5^{\circ}/oo$, but when the salinity increased to above $5^{\circ}/oo$ the numbers of C. volutator increased, and when the salinity rose above $7.5^{\circ}/oo$ breeding was recorded in this area.

Thus the abundance and distribution of C. volutator at areas C and D were directly related to the prevailing salinity. The difference in abundance between areas A and B may be due to other factors, for example the difference in the depth of aeration.

Rainfall (Fig. 4b) - especially that of the 10 days prior to sampling - together with the tides is the chief factor affecting salinity. The salinity at any one point on the estuary represents a product of the interaction of the downflow of river water and inflow of salt water. The downflow of river water is directly related to the rainfall on the catchment area of the river. The drastic lowering of salinity in May and the autumnal decline in salinity were both due to periods of rainfall.

In this study the effect of maximum salinity was not studied.

The downstream limit of C. volutator on the Ythan estuary is delimited by the presence of clean sand at the mouth of the estuary.

The appearance of C. volutator at area D and its increase in number at area C, as salinity increased, suggest that it must undergo migration within the estuary. Morgan (1965) showed that C. volutator swim in the ebb tide, an action which will tend to carry them down-river; however, he also showed that a few animals swim in the flow tide, which would tend to carry them up-river, where they could colonize new substrates. Amanieu (1967) also suggested migration of Corophium insidiosum, in

response/...

response to salinity.

It has thus been found that within the central part of the estuary the nature of the substrate is the principal factor controlling the distribution and abundance of C. volutator. Within an area of suitable substrate, C. volutator became abundant when the salinity rose above $5^{\circ}/oo$ and bred when the salinity was greater than $7.5^{\circ}/oo$. Below $5^{\circ}/oo$ it was reduced in abundance and was not found at all below $2^{\circ}/oo$. Thus the effects of salinity can override substrate selection in the upper part of an estuary, and salinity becomes the 'ecological master factor' controlling the animal's distribution and abundance.

3. LABORATORY STUDIES

A series of experiments were undertaken to determine the effect of salinity on survival, moulting and growth of C. volutator. Studies were also made of the effect of salinity on the survival of animals of different size and sex; and on the effect of salinity on breeding.

3.1 EFFECT OF SALINITY ON SURVIVAL.

C. volutator were collected from the Ythan estuary, and transported to stock tanks in the laboratory, supplied with mud and maintained at 10 - 12°C and 15 - 20‰.

To each of a series of 5 cm Petri dishes was added 10 ml. of sea water of known salinity. Salinities below 35°/oo were prepared by diluting sea water with distilled water, and the salinity was then determined by titration. For hypersaline solutions, sea water was gently evaporated over a bunsen burner without boiling.

To each of these dishes at each salinity, an individual C. volutator was added; and then to half of the dishes a small quantity of fresh mud, which had been kept in the appropriate salinity, was added. The dishes were kept in a constant temperature room at 8°C, within a humid chamber, to prevent evaporation. The dishes were examined daily, any moults removed, and any deaths of C. volutator noted.

The/...

The results of this experiment (Fig. 6), indicate that the animals survived well under the restricted experimental conditions, some living for over 1500 h (2 months) in the Petri dishes. A clear difference between those animals supplied with mud, and those without mud can be seen. If survival of C. volutator is expressed as the ability for half of the population to live for 500 h (20 days), then it may be seen that animals supplied with mud can survive in water of salinities between 2 and 50°/oo. Using the same criterion, for the animals without mud, the range of salinities survived becomes 7.5 - 47.5°/oo. ~~Using the same criterion, for the animals without mud, the range of salinities survived becomes 7.5 - 47.5°/oo~~.

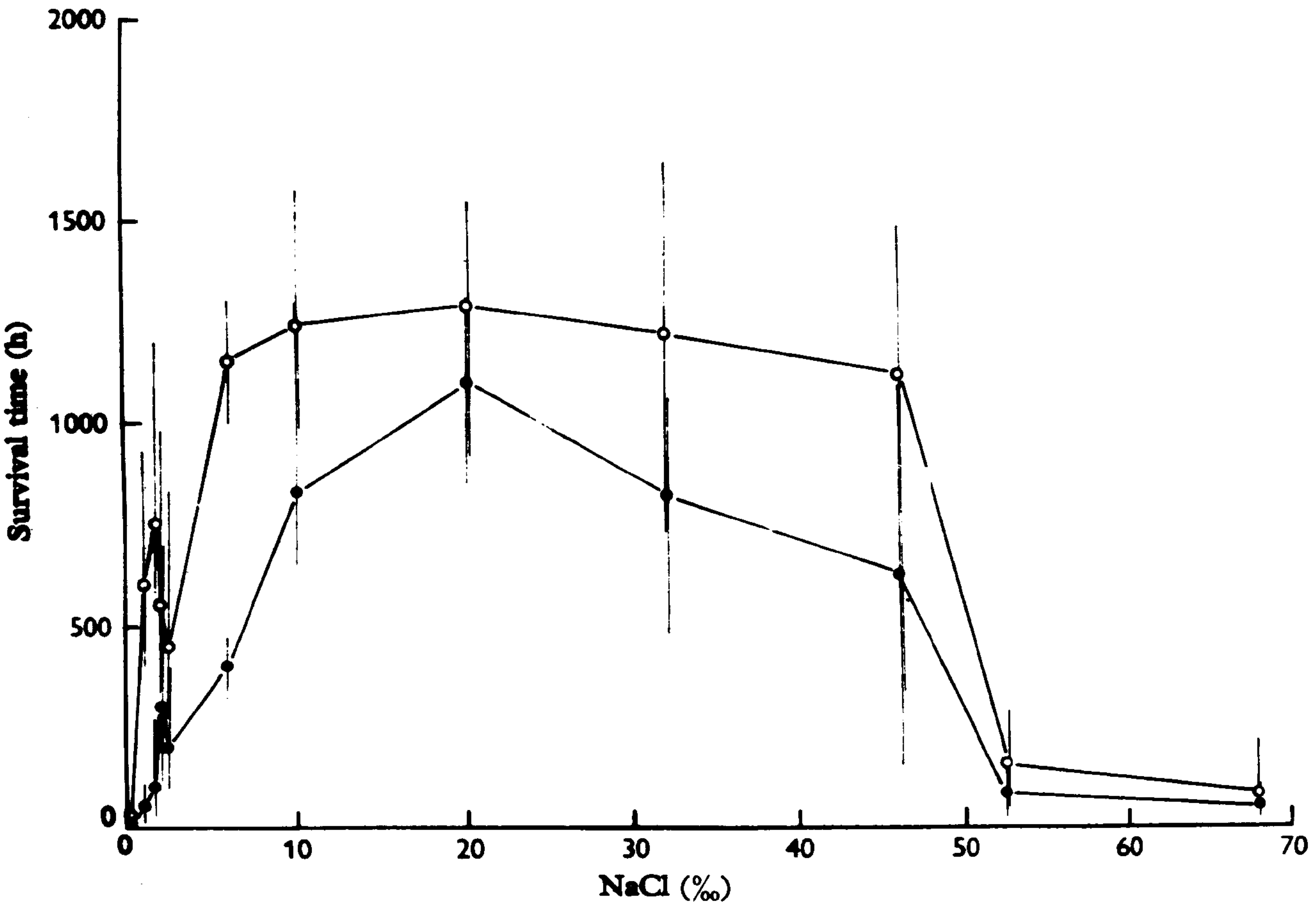
Individual variation in survival time occurred, but almost always those animals with mud survived longer than those without mud. In salinities 10 - 30°/oo the supply of mud increased survival time by an average of 34°/o. In salinities below 10°/oo, survival time was more markedly increased by the presence of mud (265% at 6°/oo, and 118% at 2.6°/oo).

An experiment was designed to test whether the dependence of C. volutator on the presence of mud was due to: (i) food value (including inorganic ion content), or (ii) physical presence. Animals were maintained at 5°/oo with acid-cleaned fine sand (B.D.H.), the sand being thoroughly washed, and the sea water sterilized. Others

were/...

FIGURE 6.

The effect of salinity on the survival time of animals with (open circles), and without (filled circles) mud.


2000
1500
1000
500
0
Survival time (h)
0
10
20
30
40
50
60
70
NaCl (‰)

were maintained in the sterilized sea water, some without any substrate and some with fresh mud. It was found that those with fine sand lived for an average of 164 h, and those without any substrate for an average of 178 h. This difference is not significant ($P = < 0.3$). Those animals supplied with mud lived for an average of 707 h, which is significantly different ($P = < 0.01$) from those with fine sand or without any substrate.

Thus it may be suggested that mud is necessary for the survival of C. volutator either for the supply of food as energy for osmoregulation, or for the direct supply of ions which are taken up via the gut.

3.2 EFFECT OF SALINITY ON MOULTING

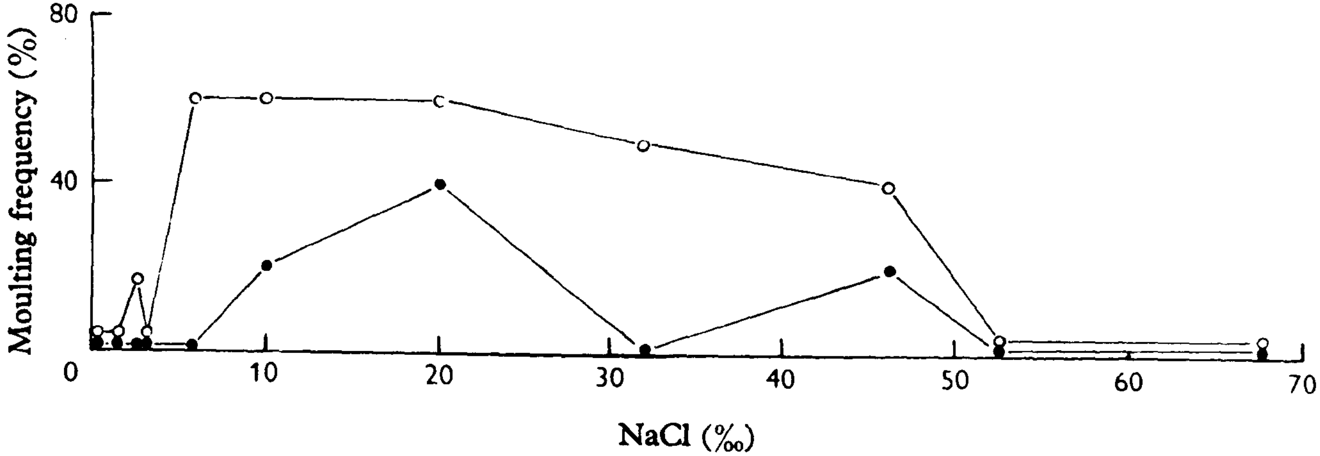
Any moults that occurred during the previous experiment were noted.

It was found that moulting occurred (Fig. 7), in animals supplied with mud, in sea water between 5 and 46^{o}/oo and in one individual at 2.6^{o}/oo. In animals without mud moulting occurred less frequently, and only in the salinity range 10 - 46^{o}/oo. By comparison with the data for survival time at different salinities, it may be seen that the range of salinities for successful moulting is smaller. This smaller range may be especially seen at low salinities - animals supplied with mud will survive down to 2^{o}/oo, but only moult regularly down to 5^{o}/oo. Moulting occurred most frequently in the range 5 - 20^{o}/oo and less

frequently/...

FIGURE 7.

The effect of salinity on moulting frequency in animals with (open circles), and without (filled circles) mud.


Moulting frequency (%)
80
40
0
10
20
30
40
50
60
70
NaCl (‰)

frequently up to 46°/oo.

3.3 EFFECT OF SALINITY ON GROWTH

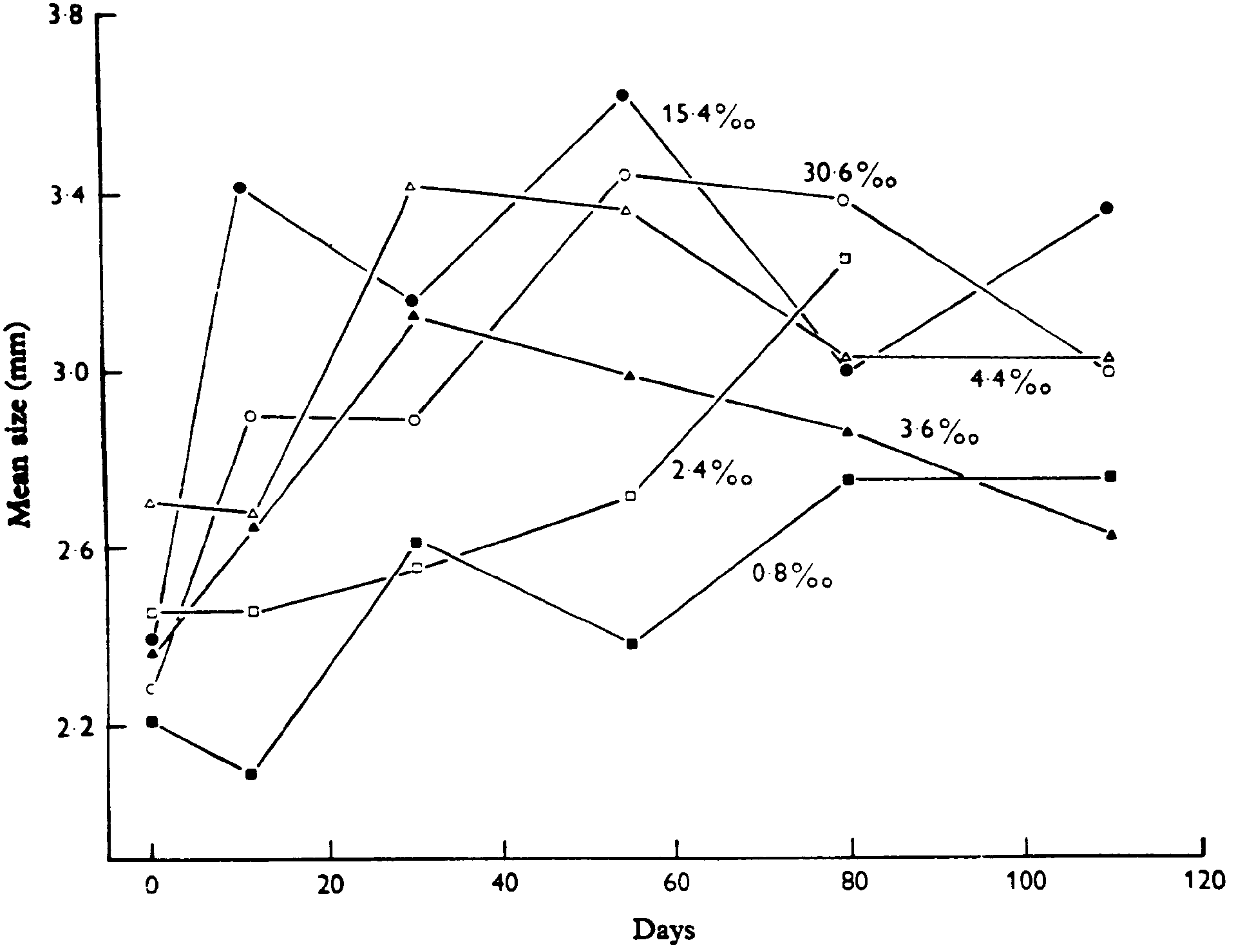
To determine the effect of salinity on growth, a large sample of mud was collected, and divided into six equal parts. Each part was placed in an aquarium tank, and covered to a depth of 15 cm with a sea-water solution of known salinity (0.8, 2.4, 3.6, 4.4, 15.4, 30.6°/oo). The mud and water were thoroughly mixed, and the mud allowed to settle. The salinity of the water was determined, and maintained at that salinity during the subsequent weeks. The tanks were aerated, and kept at 11°C. On the first day and after 11, 30, 55 and 80 days, a 40 ml. sample of the mud was collected, and sieved with a 335μ sieve. This sieving was performed in the water of the experimental tank, in order to conserve the mud. The animals so collected were counted, measured and assigned to millimetre size groups (i.e. 1.0 - 1.9 mm, 2.0 - 2.9 mm, 3.0 - 3.9 mm, etc), and then returned to their tanks.

The mean size of each population, at each salinity, during the experiment, can be seen in Fig. 8. The initial population, collected on 9 November 1966, had a mean size of 2.40 mm. During the first 55 days, the population growth rate was fastest at 15.4°/oo, increasing from a mean of 2.39 - 3.71 mm (+ 55%), 30.6 and 4.4°/oo were only slightly slower, and there was a serial decline in growth rate below 4.4°/oo, such that $3.6 > 2.4 > 0.8$°/oo.

An/...

FIGURE 8.

The mean size of populations maintained in tanks at 0.8, 2.4, 3.6, 4.4, 15.4 and 30.6‰.


15·4‰
30·6‰
4·4‰
3·6‰
2·4‰
0·8‰
Mean size (mm)
3·8
3·4
3·0
2·6
2·2
0
20
40
60
80
100
120
Days

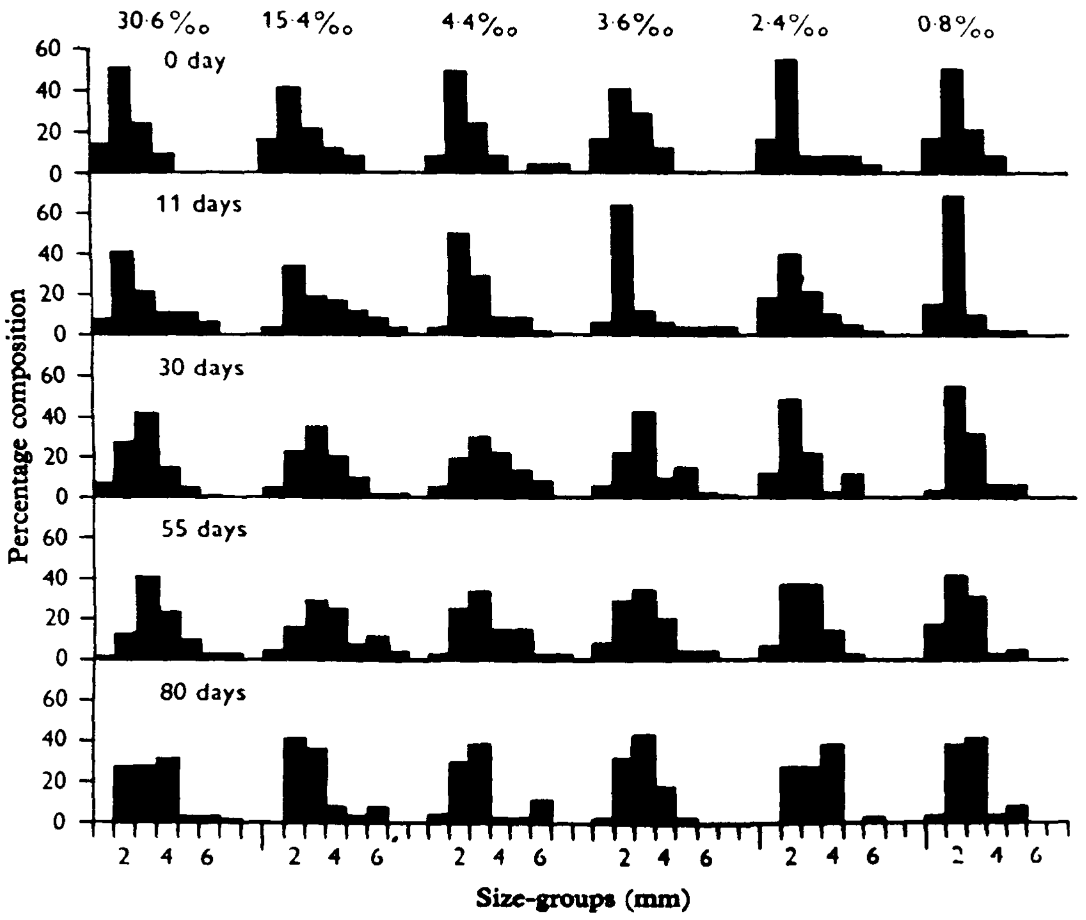
An analysis of the different sizes, by percentage, in each population (Fig. 9), shows more precisely the mode of growth in each population. Initially animals measuring 2.0 - 2.9 mm. formed 47% of the population. In the lowest salinity (0.8°/oo) little growth occurred, and after 55 days this category still formed 42% of the population, whilst the percentage of animals measuring 3.0 - 3.9 mm. had increased from 21 to 31%. In salinities 30.6 - 3.6°/oo, the percentage of animals in the 2 mm category became reduced from 47 to 23% within 30 days, by which time the 3 mm category had become more numerous than the 2 mm category. In 2.4°/oo, 55 days were needed for the 3 mm category to equal the 2 mm category (both 37%). These shifts in percentage composition represent growth of the individuals, as overall numbers remained constant, and reproduction did not occur.

Initially only 1.7% of the population was larger than 5 mm; however, growth of animals occurred rapidly in salinities 30.6 - 4.4°/oo, and within 55 days 25% at 15.4°/oo, 21% at 4.4°/oo, 17% at 30.6°/oo were larger than 5 mm. By comparison 8% at 3.6°/oo, 4% at 2.4°/oo and 5% at 0.8°/oo were larger than 5 mm. after 55 days.

As growth occurred, the number of animals in the 1.0 - 1.9 mm category fell. In 15.4°/oo there were initially 16% of the animals in this category, but by 55 days it had fallen to 4.2%. In all other salinities, except 0.8°/oo, a similar decrease in the percentage at 1mm occurred; however, in 0.8°/oo the percentage remained constant at 17%

FIGURE 9.

Per~~s~~centage size composition of population maintained in tanks at 0.8, 2.4, 3.6, 4.4, 15.4 and 30.6/o.


30·6‰
15·4‰
4·4‰
3·6‰
2·4‰
0·8‰
0 day
11 days
30 days
55 days
80 days
60
40
20
0
Percentage composition
2
4
6
Size-groups (mm)

After 55 days mortality occurred in the populations. In salinities of 30.6 - 3.6°/oo, this mortality affected the larger animals most severely, with the result that the percentages of smaller-sized animals increased. In 2.4 and 0.8°/oo mortality occurred, but appeared to be more uniformly spread throughout the size groups.

In conclusion, it can be seen that only very slow growth occurred at 0.8°/oo. Maximal growth was achieved at 15.4°/oo, and 20.6 and 4.4°/oo attained a slightly slower growth rate. Animals in 3.6°/oo grew well within the first 30 days, but failed to maintain that growth rate; and animals at 2.4°/oo had a slow but steady growth rate.

3.4 EFFECT OF SALINITY ON BREEDING.

<u>C. volutator</u> has been observed to breed successfully in laboratory stock tanks. This was noted in specimens which had been collected in November, and maintained in laboratory tanks supplied with mud and aerated water of 10 - 20‰ at 10°C, and when examined in the following April, "berried" females (i.e. bearing eggs on the ventral surface) were noted, and subsequently small individuals of <u>C. volutator</u> were found. It is unlikely that these females had been fertilised in the field prior to November, and thus it may be concluded that successful breeding had occurred in the laboratory.

Segerstrale/...

Segerstrale (1940) noted that there appears to be a minimum temperature of 7°C for breeding of C. volutator, and this has found confirmation on the Ythan (Sect. 2.5). During the field study it was noted that berried females were only observed after the salinity had risen above 7.5‰. However the salinity at this time was steadily increasing due to seasonal factors, and it is almost certain that fertilisation occurred in lower salinity conditions, and 7.5‰ may have been a coincidental limit. There was however a distinct pattern on the Ythan, whereby berried females were first noted at area A, and subsequently at areas B & C as the salinity increased. Experimental observations have been made to elucidate this problem of the effect of salinity on breeding.

I have been unable to observe the effect of salinity on fertilisation due to the problems of stimulating fertilisation in an isolated pair of animals in the laboratory. A study was therefore made of the effect of salinity on the hatching success of berried females. During the first series of experiments (May 1967) berried females were collected from the Ythan estuary, and 3 healthy individuals were placed into each of 250 ml. of various strengths of sea water (32, 24, 17, 9, 5, 3, 2, 1.3, 0.75‰).

These experimental dishes were observed regularly. The live adults, and any young which hatched out formed burrows, the openings of

which/...

which were clearly visible. Thamdrup (1935) points out that newly hatched C. volutator immediately form burrows, which may be side burrows off the mother's burrow. Moribund individuals generally left their burrows, and lay on the surface of the mud. Samples were also collected, in order to observe the growth rate of the newly hatched individuals, by removing and seiving 10 cm^3 of mud, and measuring any C. volutator present.

Within a week young animals were noted in all salinities. In the lowest salinity (0.75‰), after the young had hatched out, the 3 adults were observed to be dead within 170 hours, and within 234 hours the young were also found to be dead (56 individuals, mean size 1.0 mm). In order to avoid cannibalism, all adults were removed after 300 hours. At 583 hours the young were sampled, and mean size noted. (Table 7)

Table 7

Salinity	Mean size (mm)	
‰	483 h	795 h
0.75	DEAD.	
1.3	1.4	-
2	1.57	2.0
3	1.6	2.0
5	1.75	2.25
9	2.0	2.0
17	1.75	2.0
24	2.0	2.1
32	2.0	2.33

/...

These results indicate a slightly reduced growth rate below 5‰, up to 483 h. Samples were again collected after 795 hours (Table 7), when a slight increase in mean size was noted at 5, 24, 32‰, whilst those below 2°/oo had died. At this time the number of young was very low, due to mortality, or cannibalism within the dishes and the experiment was terminated.

The principal conclusion to be drawn from this experiment is that hatching occupied over a wide range of salinities (down to at least 0.75°/oo) but that below 1 - 2‰, the newly hatched individuals failed to grow and live. A tendency for reduced growth rate below 5‰ was also noted. These results indicating a wide salinity range for hatching, but immediate post-hatching mortality at low salinities, are in agreement with the observations of Hart (1930).

During a second series of experiments (May, 1968), berried females, from stock tanks, were kept in individual Petri dishes at salinities 30, 7.5, 3.7 & 1.0‰ - and comparison was made of the number of young individuals which were successfully hatched. At 1.0‰, half of the females died before hatching young, and the remainder averaged 3 offspring, which as in the first experiment failed to grow. At 3.7‰, the average number of offspring was 4.5; at 7.5‰, 8; at 30°/oo, 8. These females had been maintained in the laboratory for some time prior to the experiment and may have been less mature than

those/...

those in the preceding experiment, which may have accounted for their relatively low fecundity; nevertheless, the results do indicate a tendency for reduced fecundity at low salinities.

Kinne (1964a, 1966) has reviewed the effects of salinity on breeding and embryonic development, and suggests that sensitivity to salinity is at a maximum at these times. He further suggests that hatched young may tolerate a wider salinity than adults. I have no experimental data from C. volutator regarding the effect of salinity on fertilisation or embryonic development, but data on the survival of hatched young shows that they can tolerate a wide salinity range, down to perhaps 1 - 2‰, which may be lower than the adult tolerance level of 2‰. The observations from the Ythan estuary, of a lower limit of 7.5‰, may well reflect the adverse effect of low salinities on fertilisation or embryonic development.

These experiments have indicated a wide range of salinities for hatching, (down to at least 0.75°/oo), but have indicated post-hatching mortality below 1 - 2‰, in agreement with Hart (1930). Above 1 - 2‰ the hatched young survived, with lower growth rate below 5‰. The observation of 7.5‰ as a limit for breeding on the Ythan may well reflect the effect of salinity on fertilisation or embryonic development, this observation finding support in the reduction of fecundity noted in experimental animals at low salinities.

3.5/...

3.5 DISCUSSION ON THE EFFECTS OF SALINITY.

C. volutator has been found to survive for over 500 h, if supplied with mud, within the salinity range of 2 - 50°/oo. It is of interest to note that the lower salinity of 2°/oo coincides with the lowest salinity from which it has been recorded (Hellen, 1919; Gurjanova, 1951). If not supplied with suitable mud the range of salinities that it survived was reduced to 7.5 - 47.5°/oo.

Moulting occurred most frequently within the range of salinity 5 - 20°/oo. Growth occurred at a maximum rate at 15.4°/oo, and at a slightly slower rate at 4.4°/oo. It can be seen that although C. volutator can survive salinities within the range 2 - 50°/oo, frequent moulting occurred within a smaller range, and optimum growth was only achieved within an even smaller range. These results, which are similar to the pattern recorded by Kinne (1966), may be shown schematically, as in Fig. 10, 11, and compared with the results from the field study.

Hatching of young from berried females occurred in the laboratory in a wide range of salinities, at least 0.75 - 32‰, although those at 0.75‰ died rapidly and those at 1.4‰ died in under 795 hours. A tendency for reduced growth rate of young animals was found in salinities below 5‰, in agreement with the findings from adults. Reduced fecundity/...

FIGURE 10.

Summary of results on salinity tolerance.

SALINITY TOLERANCE

‰

5 - - - 30 Frequent moulting; Maximal growth

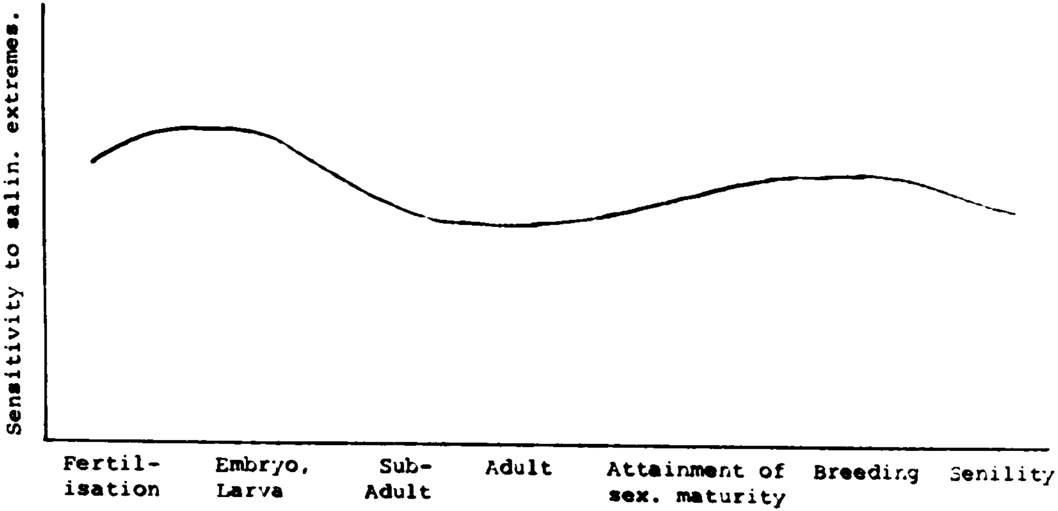
7.5 - - - 47.5 Survival - Without mud

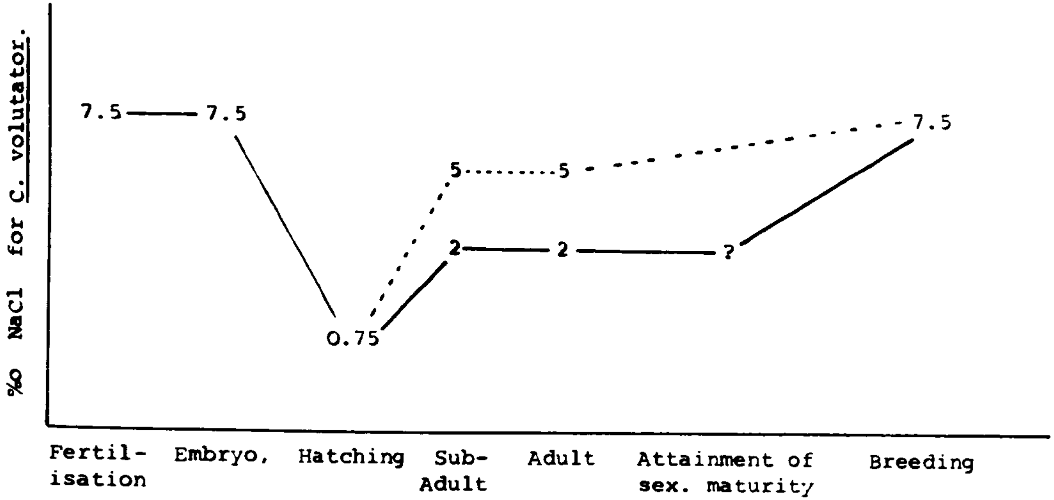
2 - - - - - - - - - - - 50 Survival - With mud

5 - - - - (36) Abundant on Ythan estuary
2 - - - - - - - - (36) Present on Ythan estuary

FIGURE 11.

Upper: Diagrammatic illustration of changes in sensitivity to limiting salinity intensities during ontogenetic development, after Kinne, (1966). Lower: Diagrammatic illustration of changes in sensitivity to salinity during the life cycle of *C. volutator*.


Sensitivity to salin. extremes.
Fertil-isation
Embryo, Larva
Sub-Adult
Adult
Attainment of sex. maturity
Breeding
Senility


%o NaCl for C. volutator.
7.5
7.5
0.75
5
5
7.5
2
2
?
Fertil-isation
Embryo,
Hatching
Sub-Adult
Adult
Attainment of sex. maturity
Breeding

fecundity was noted at salinities 1 & 3.7‰, compared with animals at 7.5 & 30‰. On the Ythan estuary, a lower limit of 7.5‰ for breeding was suggested, which may be due to the limiting effects of salinity on fertilisation or embryonic development.

Kinne (1964a, 1966) has discussed the effects of salinity on different stages of the life cycle of an animal. He noted in Cyprinodon macularius that the salinity range for optimal growth and reproduction was smaller than the range of salinities in which it could survive (Kinne, 1960). In estuarine animals, Kinne (1966) noted that older larval stages and hatched young may tolerate a wider salinity range than older individuals, and that sensitivity to salinity extremes reaches a maximum value during embryonic development and in the adult during reproduction. Considering the results from this study on Corophium volutator it may be possible to draw similar conclusions i.e. that maximum sensitivity to salinity extremes occurs during fertilisation and embryonic development, as seen in the field limit of 7.5‰ and the experimental reduction in fecundity at low salinities; and that hatching may occur in a wider range of salinities than adults will tolerate. These findings may be shown diagramatically, as in Fig. 11.

4. THE BLOOD AND BODY FLUIDS OF COROPHIUM VOLUTATOR

In order to follow in detail the physiological changes and adaptations that take place in C. volutator subjected to water of various salinities, a study was made of the changes occurring in the osmotic and ionic concentration of the blood (haemolymph) of C. volutator, in relation to the salinity of the external medium; and also the effect of various factors on the osmotic concentration of the blood. A study was also made of the osmotic concentration of urine produced by C. volutator.

4.1 FREEZING POINT STUDIES - EFFECT OF SALINITY ON THE OSMOTIC CONCENTRATION OF THE BLOOD.

Studies of the osmotic concentration of the blood were made by the freezing-point method. This method was adopted since the volume of blood in an individual C. volutator is very small. The technique of Ramsay and Brown (1955), was adopted to determine accurately the osmotic concentration of the micro-sample of blood obtainable from an individual C. volutator.

To obtain a sample of the blood of C. volutator, the live animal was quickly dried with a tissue, to remove any surface moisture, and placed on a watch-glass coated with a hydrofuge varnish (Bakelite damarda), and covered with medicinal paraffin. The animal was cut

transversely/...

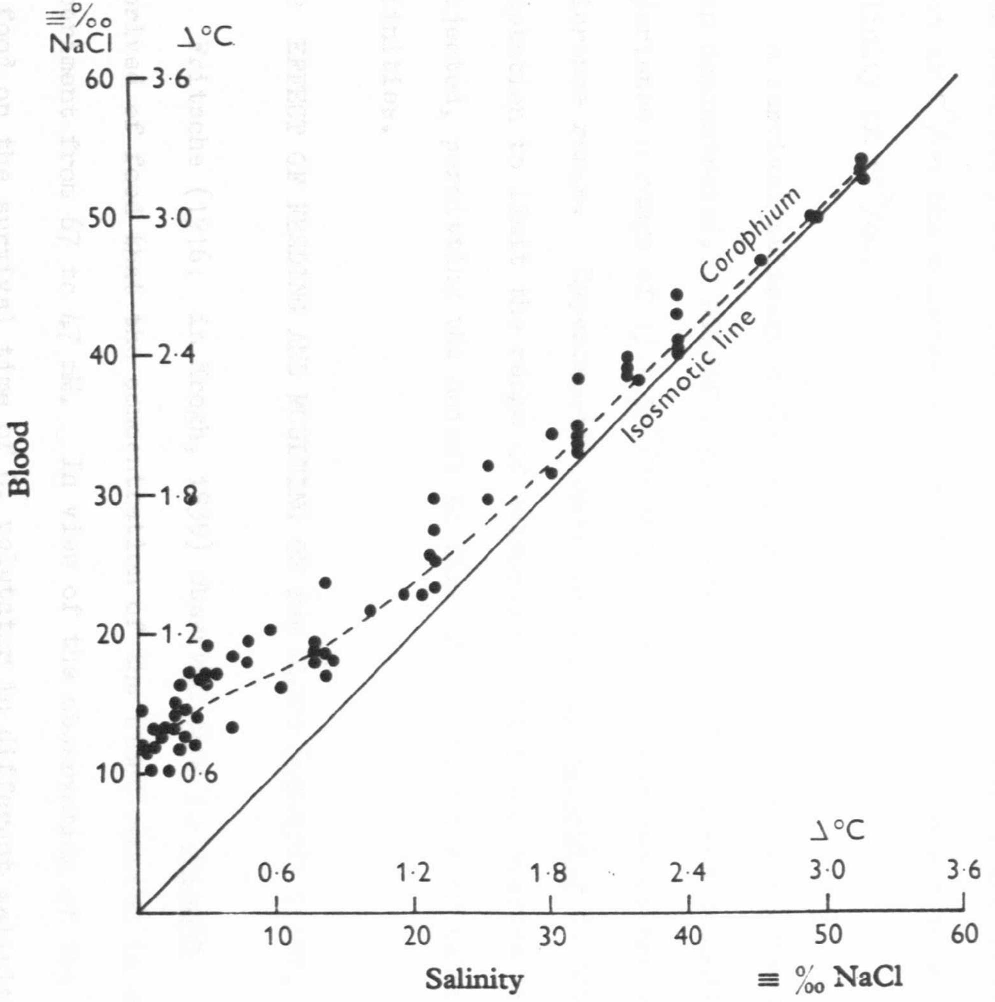
transversely in half, whilst under the paraffin. A small amount of paraffin was sucked into a fused silica microtube, and then the tube inserted into the haemocoel, at this point the animal was gently squeezed by a pair of forceps, and a small amount ($10^{-3}mm^3$) of blood sucked into the microtube, followed by more paraffin, so that four or five samples from one animal were collected into each microtube. The blood sample was then quickly frozen, and the melting point determined. Prior to sampling, animals were kept supplied with fresh mud, in various strengths of sea water, for at least 2 days. The freezing point of the animal's blood and of the water in which they had been living was determined.

The results indicate a clear pattern of regulation (Fig. 12). At 50°/oo, the blood is isosmotic with sea water. Between 20 and 50°/oo, the blood concentration follows closely that of the external medium, just maintaining a hyperosmotic level. Below 20°/oo the hyperosmocity increases progressively, such that the concentration of the blood never falls below a salinity equivalent to 11°/oo ($\Delta ^{o}C = 0.66$).

It appears that between 20 and 50°/oo the blood concentration of <u>C. volutator</u> passively drifts with the concentration of the external medium, but just maintaining a slight hyperosmocity. Below 20°/oo, the animal begins actively to regulate the concentration of the blood, and/...

FIGURE 12.

The effect of salinity on the osmotic concentration of the blood of C. volutator


≡‰ NaCl
Δ°C
60
3·6
50
3·0
40
2·4
30
1·8
20
1·2
10
0·6
Blood
Corophium
Isosmotic line
Δ°C
0·6
1·2
1·8
2·4
3·0
3·6
10
20
30
40
50
60
Salinity
≡ ‰ NaCl

and maintains itself hyperosmotic to the outside medium; such that at 20°/oo, the blbod is equivalent to a salinity of 23°/oo, at 10°/oo it is equivalent to 17°/oo, and at 2°/oo it is equivalent to 13°/oo - a concentration difference of 650%. Below 2°/oo the blood concentration parallels, but remains consistently above, that of the water, so that at 0°/oo the concentration of the blood is equivalent to a salinity of 11°/oo.

A survival salinity tolerance range of 2 - 50°/oo has already been demonstrated. Within these salinities, the tissues actually experience a range of 13 - 50°/oo which may be delimited as the tissue tolerance range. Hyperosmotic regulation in C. volutator is thus an adaptation to limit the range of salinity to which the tissues are subjected, permitting the animal to live in a wide range of external salinities.

4.2 EFFECT OF FEEDING AND MOULTING ON THE BLOOD FREEZING POINT.

Fritsche (1916; in Krogh, 1939) observed that in Daphnia deprived of food that the concentration of the blood reduced in one experiment from 67 to 47 mM. In view of the observation of the effect of food on the survival time of C. volutator in different salinities (Section 3.1), experiments were undertaken to determine the effect of food on the blood freezing point.

A/...

A group of animals, all fed, and acclimated to a given salinity was divided into two; one group was then maintained with food (mud) and the other maintained without food. The freezing point of the blood of individuals was determined at regular intervals. The results (Figs 13 & 14) indicate no change in the freezing point of the blood of the control animals over the experimental periods, in excess of 300 hours. The freezing point of the blood of animals without food generally fell during the period. Statistical treatment (Student's t-test) indicates the following results. (Table 8)

Table 8

Fig.	Medium Δ °C	Date	t	p	comment
13a	0.3	11/66	1.775	<0.2	not signif. small sample
13b	0.4	11/66	0.722	<0.5	not signif.
14a	0.6	1/68	4.268	<0.01	Significant
14b	0.7	2/67	1.582	<0.2	not signif. small sample
All expts.	-	-	4.933	<0.001	Significant

Only the individual experiment of 1/68 indicated a significant drop in the freezing point of unfed animals, however when the results were combined, a significant decline in the freezing point of the unfed animals was noted.

These/...

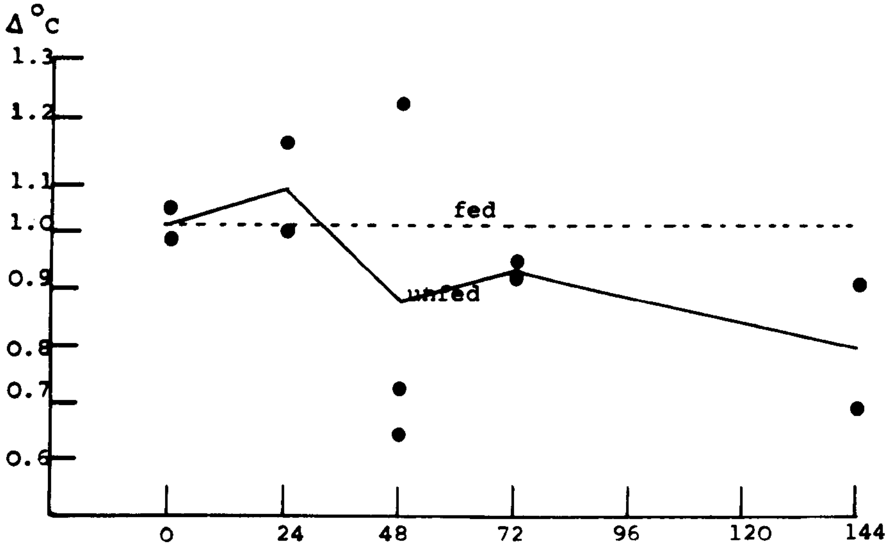
FIGURE 13.

a) The effect of feeding on the freezing point of the blood, (November 1966) b) The effect of feeding on the freezing point of the blood (November 1966).

● unfed animals

(A)

Medium = 0,3 Δ°C



(B)

Medium = 0.4 Δ°C

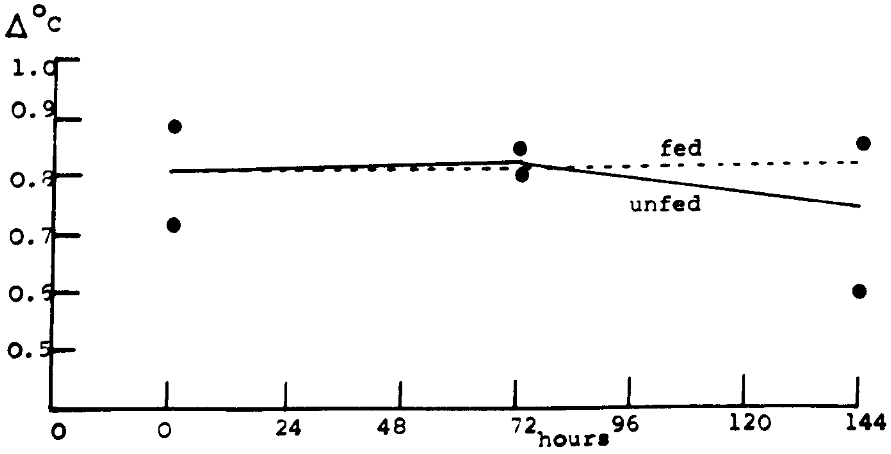
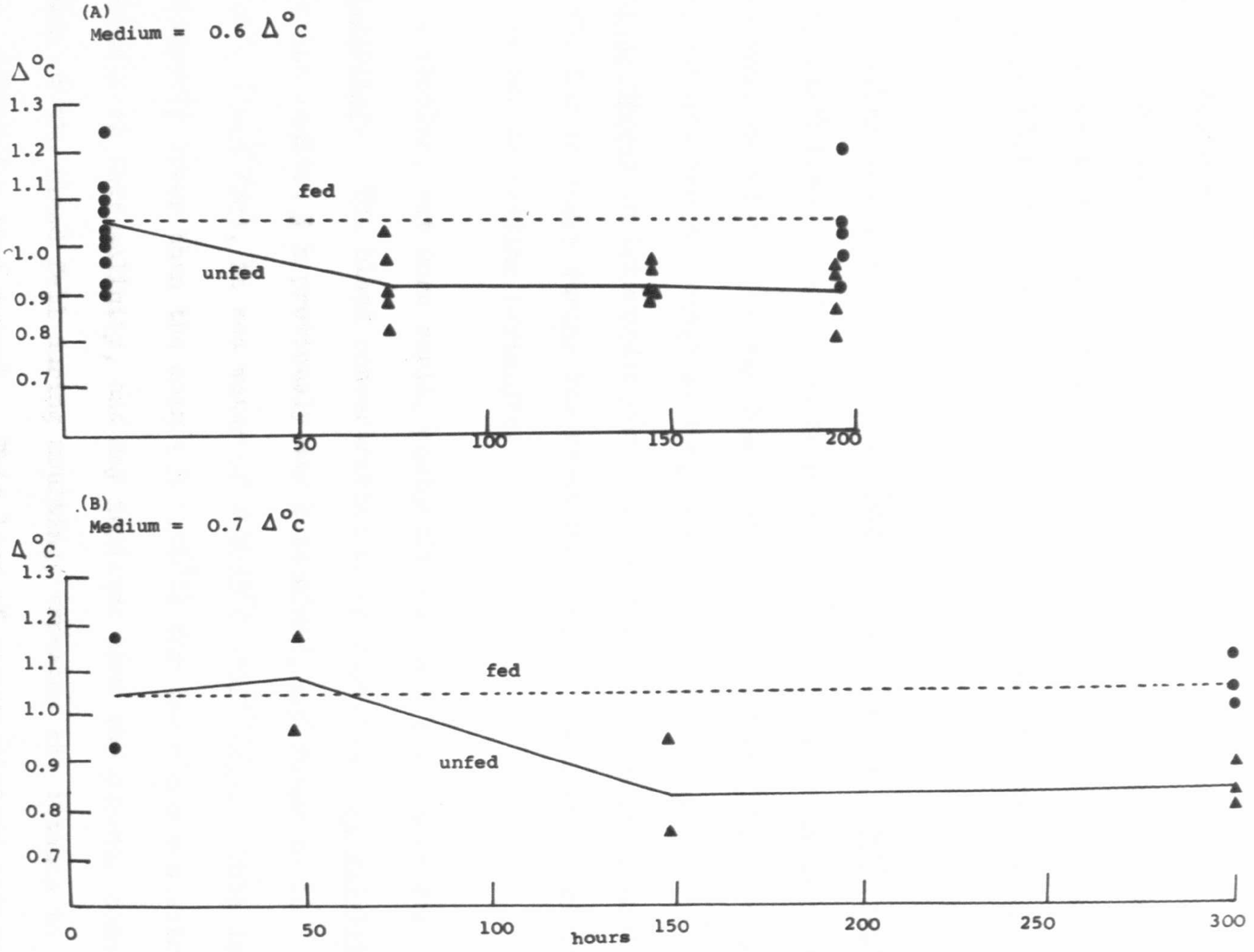


FIGURE 14.

a) The effect of feeding on the freezing point of the blood (January 1968). b) The effect of feeding on the freezing point of the blood (February 1967).

● fed animals
▲ unfed animals


(A)
Medium = 0.6 Δ°C
Δ°C
1.3
1.2
1.1
1.0
0.9
0.8
0.7
fed
unfed
50
100
150
200
(B)
Medium = 0.7 Δ°C
Δ°C
1.3
1.2
1.1
1.0
0.9
0.8
0.7
fed
unfed
0
50
100
150
200
250
300
hours

These results, indicating a drop in the freezing point of the blood of starved animals, suggests that food is necessary for the maintenance of the osmotic pressure of the blood. Whether this is due to the role of food as a direct source of ions, or as an energy source for other osmoregulatory processes cannot as yet be decided. Lockwood (1962, p. 274) suggests this fall is due to a fall in overall metabolic rate.

Studies on the moult cycle of Crustacea (Robertson, 1937, 1960a; Glynn, 1968) have revealed considerable physiochemical changes in the haemolymph in relation to the moult cycle. Robertson (1960a) showed that, considering sea water as 100, the blood concentration of *Carcinus maenas* in intermoult was 100.7, rising to 107.5 in premoult, and falling to 102.9 during the first 24 hours of postmoult, and to 98.2 in the succeeding fortnight.

A similar, but more rapid, postmoult drop has been shown for *C. volutator*. The blood concentration of an individual *C. volutator* which had moulted 3 h previously was determined, and found to be Δ 0.86°C (14.5°/oo), in sea water of Δ 0.63°C (10.7°/oo). This is considerably lower than the mean (Δ 1.05°C) for the blood concentration of animals at this salinity, and may indicate that the osmotic concentration of the blood fell during moulting, exposing the tissues to lower salinities than normal. This loss of osmoregulatory powers,

indicated/...

indicated during moulting, may be the explanation for the observed difference between the range of salinities survived and the range of salinities in which moulting occurred (Section 3.2) because moulting at low salinities would cause the concentration of the internal medium to fall below the tissue tolerance range, which is 13 - 50°/oo. Hart (1930) noted that mortality in C. volutator at low salinities generally occurred during moulting; and in the course of these experiments animals in low salinities undergoing a moult were observed to die.

4.3 EFFECT OF SIZE AND SEX ON THE BLOOD FREEZING POINT

Previous experiments on the freezing point of the blood of C. volutator (Sections 4.1, 4.2) have been performed on animals of randomly assorted sex and size. A check has been made to determine if there was any effect of sex or size on the blood f.pt. Determinations were made of the blood f.pt. of fed individuals acclimatized to 10‰ ($\Delta\,^{\circ}C = 0.60$). Their length (rostrum-telson), and sex (characters of Watkin (1941)) were noted.

The results (Fig.15) indicate that there was no significant difference in the freezing point of the blood, in relation to length or sex. The correlation coefficient (R) was 0.17 (N=28; $y = 2.326x + 2.737$), a result which signifies no correlation of freezing point with length. The mean freezing point was 1.027 ± 0.12°C. Comparison by Student's t-test between length and sex groups indicated no significant differences. (Table 9)/...

FIGURE 15.

The effect of length and sex on the freezing point of the blood.

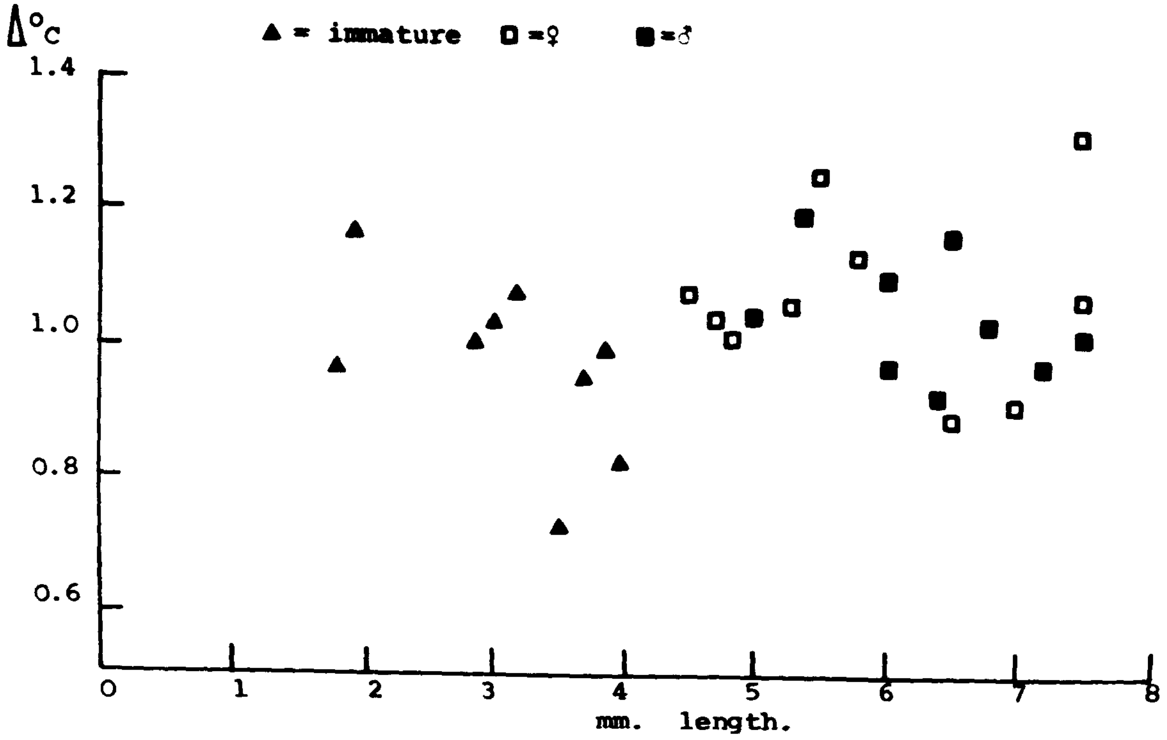

▲ = immature
□ = ♀
■ = ♂
Δ°C
1.4
1.2
1.0
0.8
0.6
0
1
2
3
4
5
6
7
8
mm. length.

Table 9

Comparison	t	N	P
Immature* v. Male	1.31	18	<0.2
Immature* v. Female	1.70	19	<0.1
Male v. Female	0.62	19	<0.5

* Below 5mm length.

Thus no significant difference was found between the freezing point of the blood of animals of different sex or size, maintained at 10°/oo.

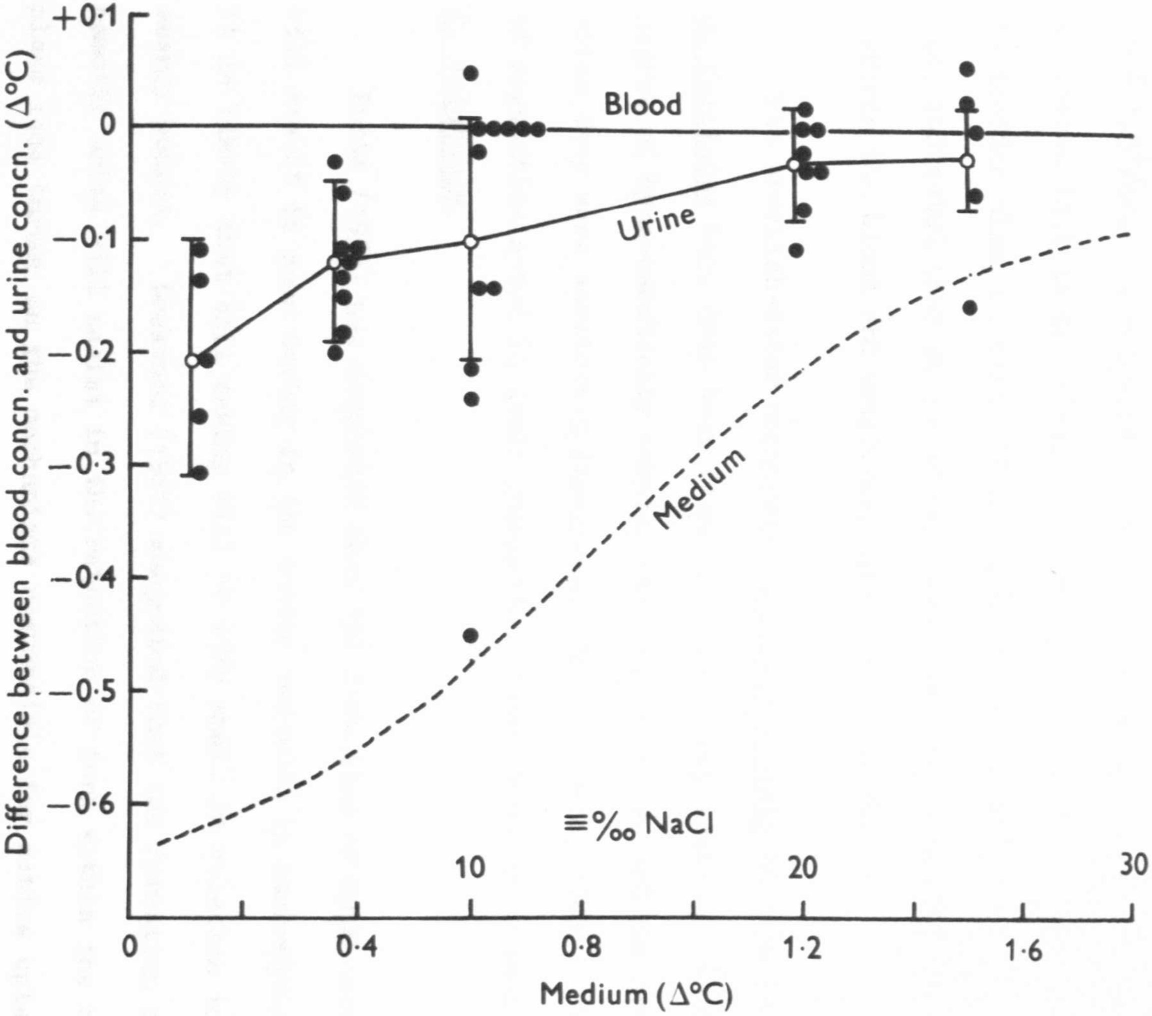
4.4 URINE CONCENTRATION

Samples of urine were collected by carefully drying off the individual animal on an absorbent tissue, and then immersing the animal in paraffin, in a watch-glass. A droplet of urine was observed to form at a small papilla at the base of the 2nd antenna. This droplet was carefully collected into a fine silica capillary. The freezing-point of the urine sample, and of a blood sample collected from the same animal, were determined.

At 25 and 20°/oo. the urine produced was almost isosmotic with the blood (Fig. 16), the differences were not significant ($P = < 0.4$ in both salinities). At salinities, 10, 6, 1.8°/oo, the urine was found to be hypo-osmotic to the blood, the differences were all significant ($P = < 0.02$/...

FIGURE 16.

Relationship of urine concentration to blood concentration in different salinities; showing the differences between the urine concentration and blood concentration in individual animals (●), and mean differences (○) (vertical lines indicate standard error). The difference between blood concentration and the medium is shown by the dotted line.


+0·1
0
−0·1
−0·2
−0·3
−0·4
−0·5
−0·6
Difference between blood concn. and urine concn. (Δ°C)
Blood
Urine
Medium
≡‰ NaCl
10
20
30
0
0·4
0·8
1·2
1·6
Medium (Δ°C)

(P = < 0.02 in all salinities). The degree of hypo-osmoticity was greater at 1.8°/oo than at 6°/oo, and at 10°/oo. Whilst the medium was 1.8°/oo, the blood concentration was 14.5 ± 1.6°/oo, and the urine concentration was 11 ± 1.6°/oo. Similarly, when the medium was 6°/oo, the blood concentration was 15 ± 1°/oo, and the urine concentration was 12 ± 1.5°/oo. A calculation of the coefficient of linear correlation indicated that as the blood concentration decreased, so the urine concentration also decreased (R = 0.9190, P = < 0.001). This calculation also indicated that at low blood concentrations, a greater difference between the blood and urine concentration occurred.

The brackish-water amphipods, _Gammarus duebeni_ Lillejeborg and _G. fasciatus_ Say, have been found to produce hypo-osmotic urine, the degree of hypo-osmoticity varying with the species, and the medium to which they were acclimated (Lockwood, 1961; Werntz, 1963). The pattern of regulation noted in these gammarids is very similar to that noted in _C. volutator_.

Potts (1954) has suggested that the formation of hypo-osmotic urine will result in some saving in the energy expended in osmoregulation, but it is likely that this saving will be very small in relation to the total energy budget. Lockwood (1961) suggested that the formation of hypo-osmotic urine will assist in the retention of ions within the body, and place less burden on the mechanisms responsible for active uptake at the body surface. C. _volutator_ ranges in size from 1 to 8 mm, and like all small/...

small animals has a large surface/volume ratio - hence any mechanism which helped to retain ions within the body, and maintain hyperosmotic conditions in the internal environment, would be of value to the animal. It should be emphasized that some ions are likely to be lost via the urine, for example when the blood was hyperosmotic by 9°/oo to a medium of 6°/oo, the urine was hypo-osmotic to the blood by 3°/oo, and hyper-osmotic to the medium by 6°/oo. Lockwood (1965) stated that: 'the conservation of ions within the body by the production of hypotonic urine is likely to be found to be a common feature of the smaller brackish water crustacea', and the present findings lend support to this hypothesis.

4.5 IONIC ANALYSIS OF THE BLOOD - CATIONS AND CHLORIDE IONS

A study has been made of the regulation of sodium potassium, calcium, magnesium, and chloride ions in animals acclimatized to salinities 1 - 35°/oo. Robertson (1960b), Potts & Parry (1963) and Nicol (1967) have reviewed the subject of ionic regulation in the crustacea, however most of the data is available from animals maintained in sea water only. Dehnel & Carefoot (1965) and Dehnel (1966) have studied ionic regulation in the estuarine crabs, Hemigrapsus spp., which, like C. volutator, maintain their blood hyperosmotic to the medium, and found that sodium, potassium, calcium and chloride ions in animals in 0 - 100% sea water, were more concentrated than the medium, whilst magnesium ion was less concentrated.

Similar/....

Similar results have been found in studies by Parry (1953), Ligia oceanica; Prosser, Green & Chow (1955), Pachygrapsus crassipes; Gross (1959), Pachygrapsus crassipes; De Leersnyder (1967 a, b), Eriocheir sinensis.

Blood samples were collected by a method similar to that for the freezing point, except that the animal was not under paraffin. Up to 0.25 μl of blood could be collected into a silica micropipette from an individual animal. This was sufficient for the determination of one ion only. The volume of the blood sample was determined by weighing an equal volume of de-ionized water.

Cations were analysed using a Unicam SP 900 flame spectrophotemeter. For sodium analysis, the machine was used conventionally, with the blood sample made up to 10 ml. with de-ionized water. For analysis of potassium, calcium and magnesium, for which the machine was less sensitive, and where the concentration of ions in the blood was considerably lower, the conventional atomizer and vaporizer were replaced by a hot chamber vaporizer devised by Dr C.M.M. Begg of Aberdeen University. This vaporizer consisted of a hypodermic needle set into a carburettor jet, which fitted into a brass cylinder, around which was wound a heating element maintained at a constant temperature. In the conventional atomizer and vaporizer, 92.5% of the sample ran away to waste; in the hot chamber vaporiser all of the sample was maintained as fine droplets, all/...

all of which passed to the burner, thus increasing the sensitivity of the spectrophotometer by X 12.5. For potassium analysis the blood sample was made up to 10 ml. with de-ionized water, and for calcium and magnesium to 1 ml. with de-ionized water.

For chloride ion analysis, the first method of Ramsay, Brown & Croghan (1955) was used. A Beckman Expandomatic pH meter was used as a millivolt meter for the electrometric titration, performed on a plate of PTFE, in a drop of acetic acid/acetone buffer solution.

In addition to the determination of the ionic content of the blood of C. volutator, determinations were also made of the ionic content of the sea-water solutions that they had been living in, and of standard solutions.

The results of this analysis are presented in Figs. 17 - 21 and Table 10. In all figures the concentration of the ion (in milli-equivalents/litre) is plotted against the salinity of the medium in which C. volutator had been living.

From Fig. 17 it can be seen that the concentration of the sodium ion in the blood was consistently maintained above that of the sea water, maintaining a difference of 33 m-equiv./l. at 35°/oo, and 105 m-equiv./l. at 5°/oo. The concentration of potassium (Fig. 18) was maintained well above that of sea water. At 30°/oo, C. volutator blood contained 17.7 m-equiv./l., whilst sea water had 8.6 m-equiv./l., and at 5°/oo the blood/...

FIGURE 17.

The concentration of sodium ions in the blood, of individuals maintained in various salinities. Filled circles indicate the concentration of ions in individual animals, and the open circles, the mean concentrations. The straight line indicates the concentration of ions in the medium.

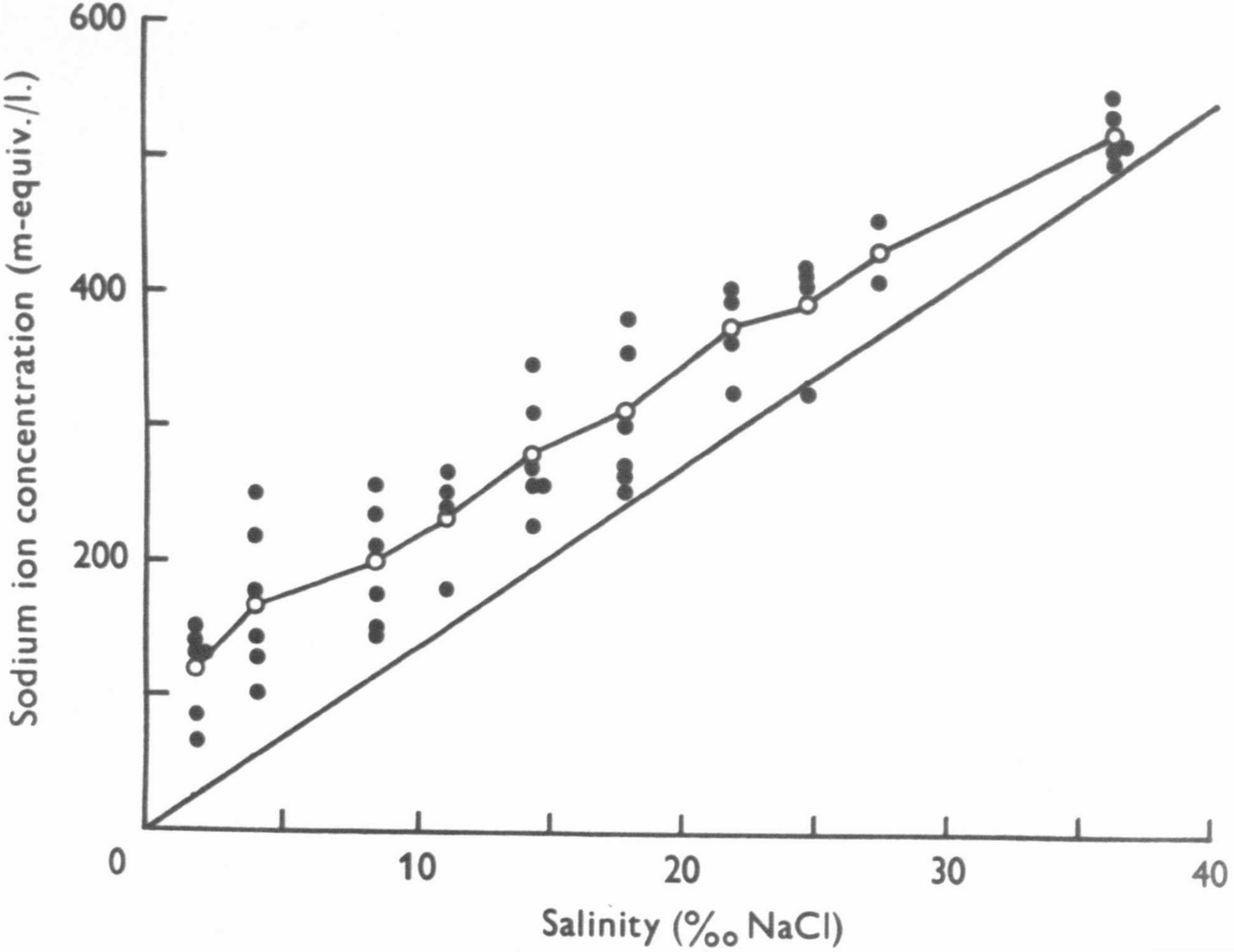

Sodium ion concentration (m-equiv./l.)
600
400
200
0
10
20
30
40
Salinity (‰ NaCl)

FIGURE 18.

The concentration of potassium ions in the blood, of individuals maintained in various salinities. Explanation as Fig. 17.

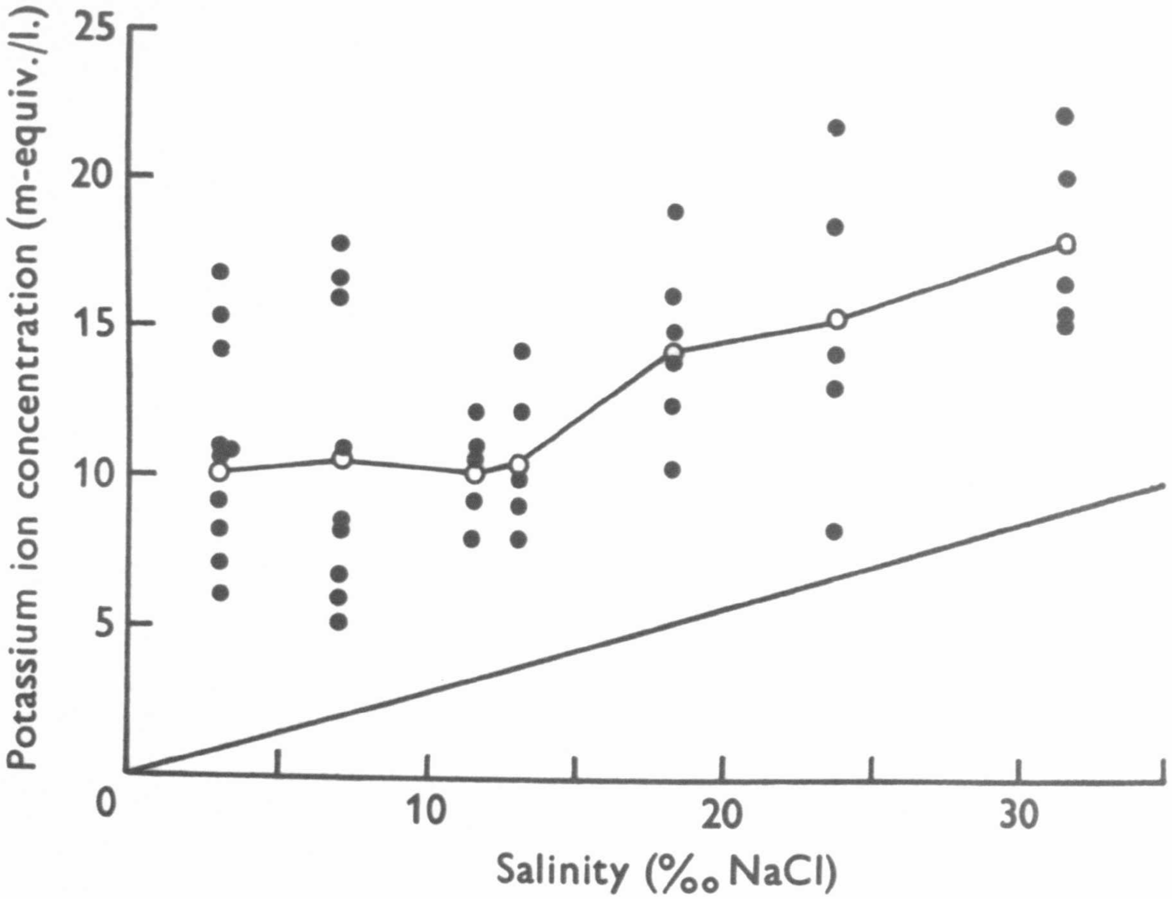

Potassium ion concentration (m-equiv./l.)
25
20
15
10
5
0
10
20
30
Salinity (‰ NaCl)

FIGURE 19.

The concentration of calcium ions in the blood, of individuals maintained in various salinities. Explanation as Fig. 17.

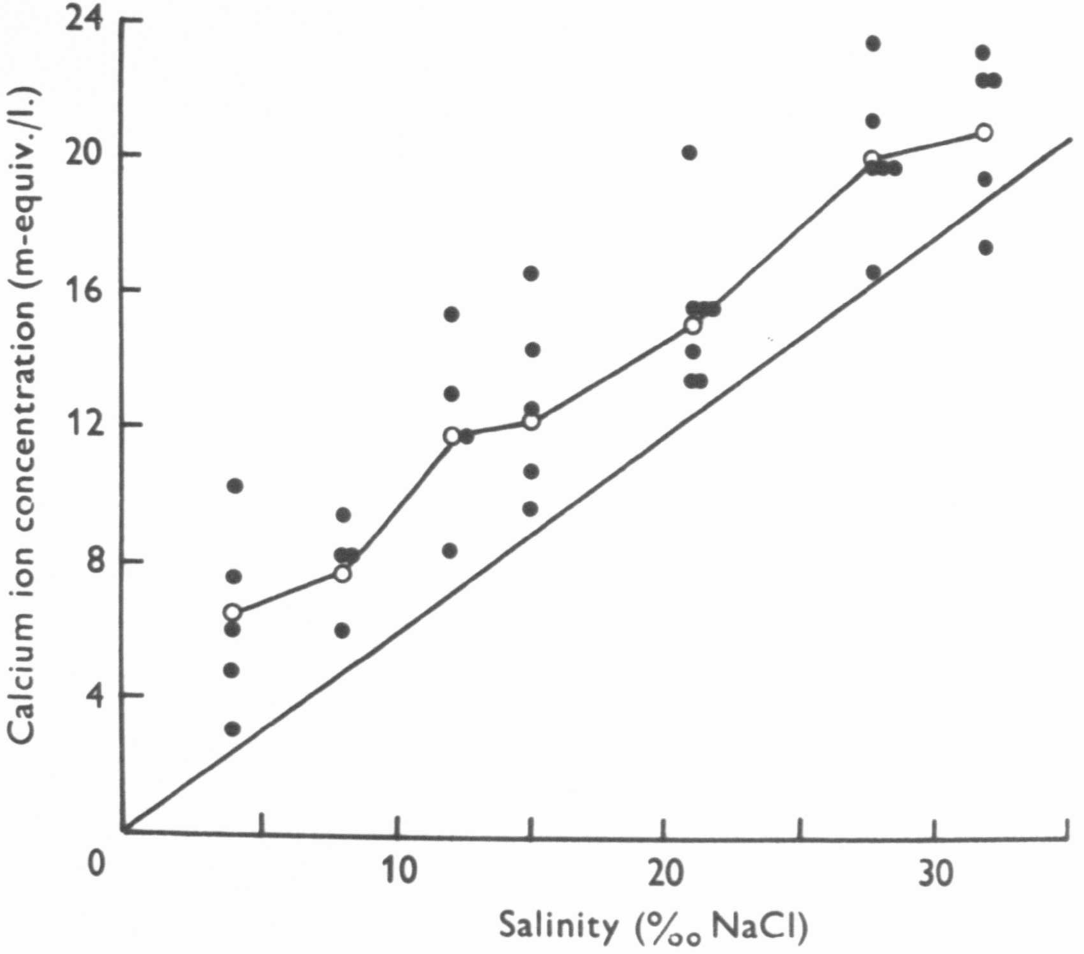

Calcium ion concentration (m-equiv./l.)
24
20
16
12
8
4
0
10
20
30
Salinity (‰ NaCl)

FIGURE 20.

The concentration of magnesium ions in the blood, of individuals maintained in various salinities. Explanation as Fig. 17.

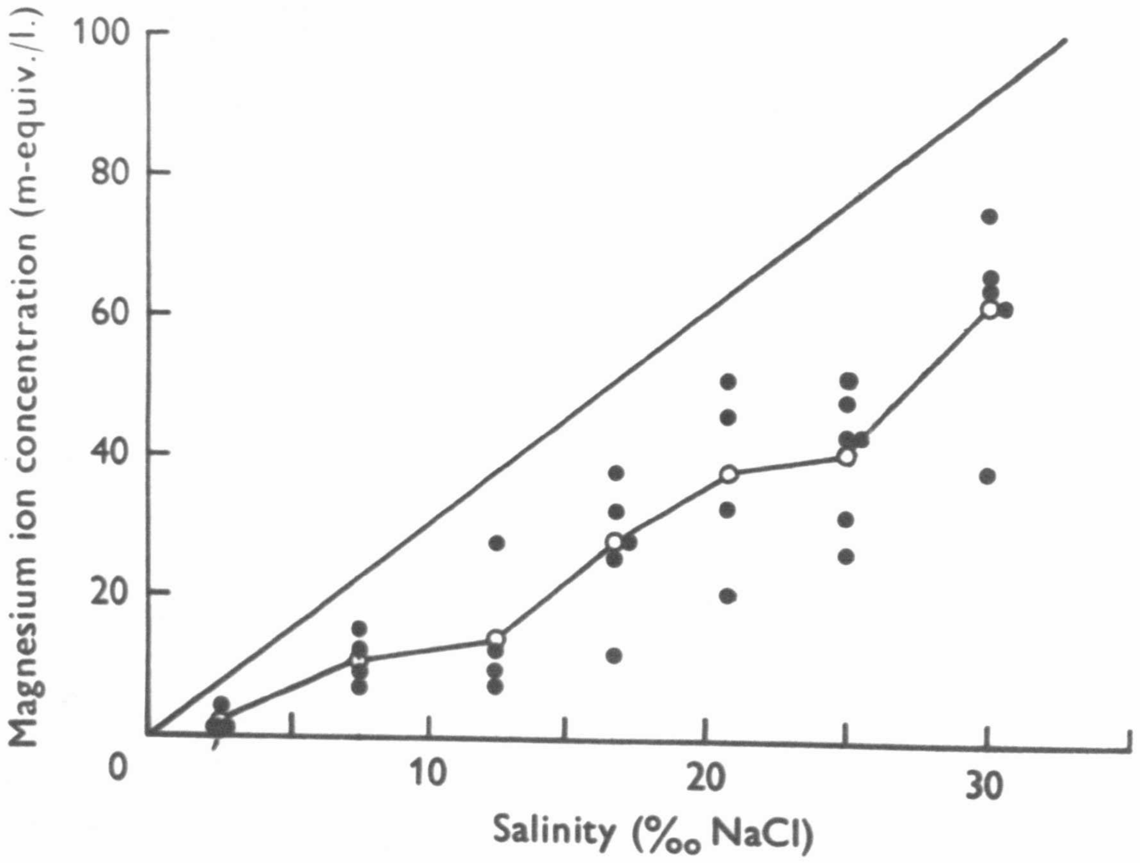
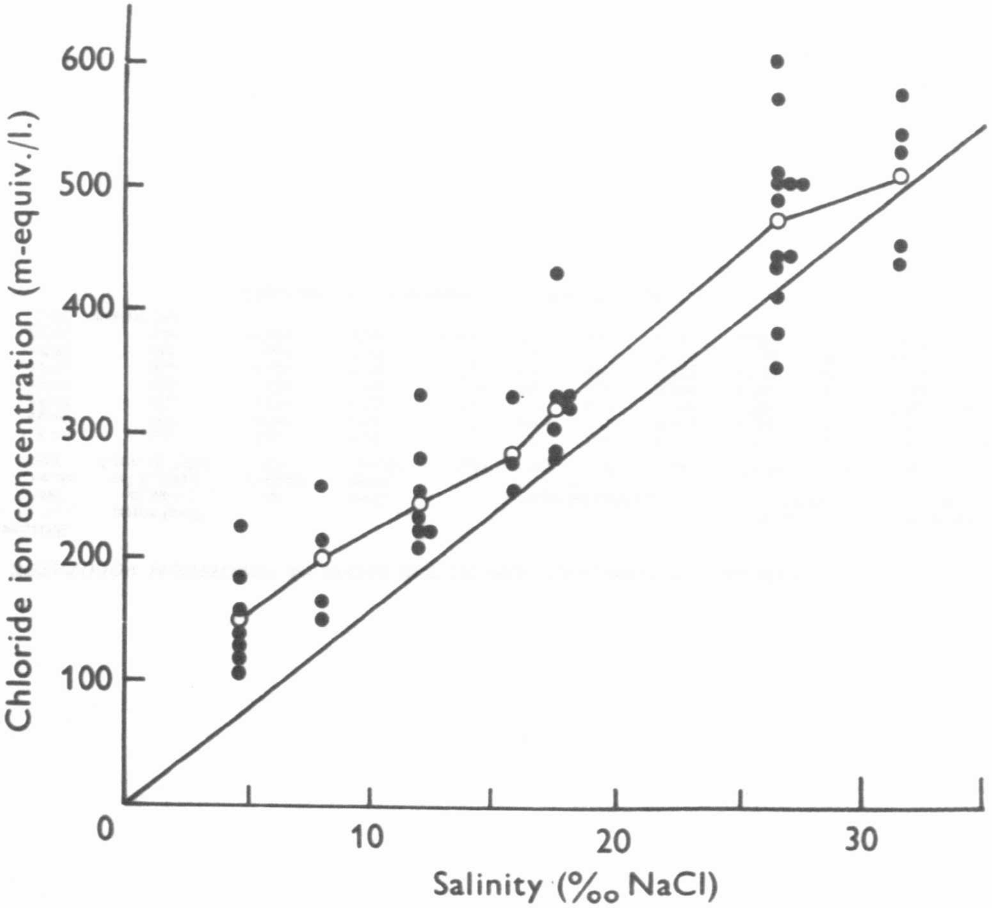

Magnesium ion concentration (m-equiv./l.)
100
80
60
40
20
0
10
20
30
Salinity (‰ NaCl)

FIGURE 21.

The concentration of chloride ions in the blood, of individuals maintained in various salinities. Explanation as Fig. 17.



Chloride ion concentration (m-equiv./l.)
600
500
400
300
200
100
0
10
20
30
Salinity (‰ NaCl)

TABLE 10

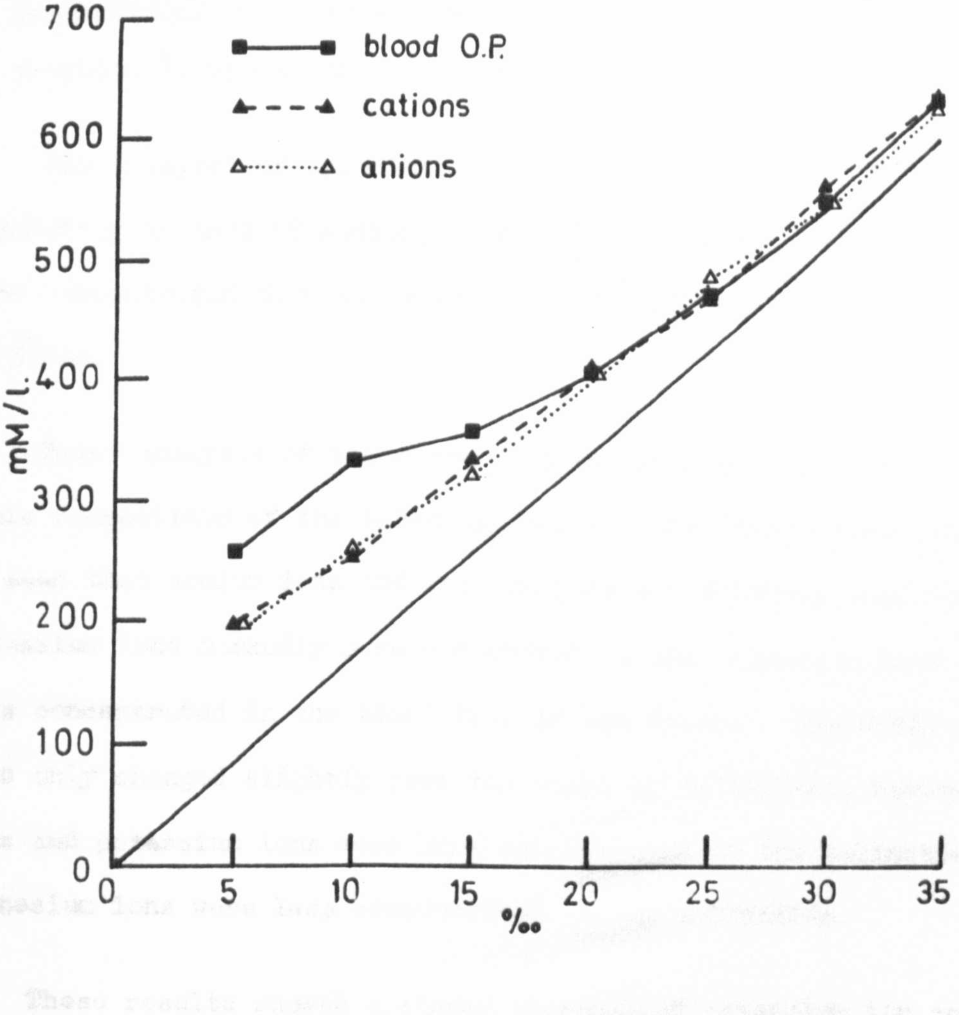
ANALYSIS OF INORGANIC IONS IN THE BLOOD OF *COROPHIUM VOLUTATOR*

Medium NaCl (‰)	Sea water (mM/l.*)		*C.v.* blood (mM/l.*)		Cations (m-equiv./l.)				Total cation (mM/l.)	m-equiv./l Cl^-	Total anion (+40 m-equiv./l for SO_4^{2-}‡) mM/l.	Relative ionic comp. Cl^- = 100			
	Δ °C.	NaCl	Δ °C.	NaCl	Na^+	K^+	Ca^{2+}	Mg^{2+}				Na^+ at. wt.	K^+ at. wt.	Ca^{2+} at. wt.	Mg^{2+} at. wt.
												23·0	39·1	40·1	24·5
5	0·3	85·5	0·9	256·5	173·0	10·3	6·6	6·0	195·9	155·0	195	72·3	7·32	2·41	1·33
10	0·6	171·0	1·17	333·4	219·0	10·3	9·4	12·0	250·7	220·0	260	64·49	5·16	2·41	1·88
15	0·9	256·5	1·25	356·2	290·0	11·9	12·6	21·0	335·5	277·0	317	67·83	4·73	2·56	2·62
20	1·2	342·0	1·43	407·5	349·0	14·6	14·6	36·0	414·2	365·0	405	61·50	4·40	2·25	3·40
25	1·5	427·5	1·66	473·1	396·0	15·9	18·0	42·0	470·9	450·0	490	57·01	3·89	2·25	3·15
30	1·8	513·0	1·92	547·2	462·0	17·7	20·6	61·4	561·7	509·0	549	58·81	3·83	2·28	4·16
35	2·1	598·3	2·21	629·8	515·0	19·2†	22·6†	78·0†	634·8	583·0†	623	57·23	3·62	2·19	4·61
											Sea water‡	55·5	2·01	2·12	6·69

* mM = Δ × 286. † Extrapolated. ‡ Nicol (1967).

FIGURE 22.

The sum of anions, and cations in the blood, compared with the results from freezing point studies.


blood O.P.
cations
anions
mM/L.
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
‰

blood contained 10.3 m-equiv./l., whilst sea water had 1.4 m-equiv./l. Like sodium and potassium, calcium (Fig. 19) was maintained consistently more concentrated than sea water - a difference of 2.6 m-equiv./l. at 30°/oo and 3.6 m-equiv./l. at 5°/oo. Magnesium (Fig. 20) was maintained at a consistently lower concentration in the blood than sea water; in 30°/oo, whilst sea water contained 92 m-equiv./l., the blood of <u>C. volutator</u> had 61.4 m-equiv./l., and at 5°/oo, sea water had 15 m-equiv./l. whilst the blood had 6.0 m-equiv./l.

The analysis of chloride ions (Fig. 21) showed a similar pattern of regulation to that of sodium. The blood at 30°/oo was 28 m-equiv./l. more concentrated than sea water, and 76 m-equiv./l. more concentrated at 5°/oo.

These analyses of blood are collated in Table 10. When the relative ionic composition of the blood is compared with that of sea water, it may be seen that sodium ions and calcium ions are slightly more concentrated, potassium ions markedly more concentrated, and magnesium ions considerably less concentrated in the blood than in sea water. The ratio of calcium ions only changed slightly over the range of salinities, however sodium ions and potassium ions were more concentrated at low salinities, and magnesium ions were less concentrated at low salinities.

These results showed a strong increase of potassium ion concentration in the blood of <u>C. volutator</u> relative to the medium, a slight increase of/...

of sodium ions, calcium ions, and chloride ions, and a strong decrease in magnesium ion concentration. This pattern of regulation, and the relative ionic content is similar to that noted for other crustaceans (Robertson, 1960 b; Nicol, 1967). The concentration of potassium ions was rather high, which may well be due to the fact that whole blood was used for the analyses, and breakdown of any cells contained in it may elevate the potassium ion concentration (Potts & Parry, 1963).

It has already been noted (Sect. 4.1) that C.volutator is a hyperosmotic regulator, maintaining its blood markedly more concentrated than the medium at low salinities, and tending towards isosmoticity at high salinities. The same pattern was noted for sodium, potassium, calcium, and chloride ions, which were all more hypertonic at low salinities, than at high salinities. Magnesium ion was always less concentrated in the blood than in sea water. Similar patterns of hypertonic regulation of magnesium ion have been found in estuarine Hemigrapsid crabs (Dehnel & Carefoot, 1965; Dehnel 1966).

When summation is made of the ions found in this analysis (Table 10 & Fig. 22), and compared with the osmotic concentration of the blood as determined by freezing-point studies a discrepancy is found at 6 and 10°/oo, and to a lesser extent at 15°/oo, whilst at 20 - 35°/oo, the sum of the ionic concentrations adequately accounts for the observed osmotic concentrations. An estimate of the sulphate ion concentration has been included in the anion total, using the value 40 m-equiv./l. (Nicol, 1967),

Dehnel/...

Dehnel (1966) noted a discrepancy between osmotic pressure and sodium ion concentration of the blood of Hemigrapsus nudus, and suggested that the protein content of the blood may change with changes of salinity, and account for the discrepancy noted. Sutcliffe (1961), in the caddis fly larva, Limnephilus affinis Curtis, which is a hypotonic regulator, found a marked discrepancy between osmotic pressure and the ionic content of the blood as determined by conductivity measurements, in animals maintained in certain salinities only. He suggested that the increase in the concentration of the non-electrolyte fraction may be brought about by the liberation of osmotically active substances into the haemolymph, perhaps due to the mobilization of amino acids from a protein reservoir. A significant rise in blood protein in crayfish in 50% sea water, compared to those in fresh water, has been found by Sharma (1968), who has suggested that this rise may contribute to a rise in the osmotic pressure of the blood. Gilbert (1959a,b) found in shore crabs of different sizes which were maintained in the same salinity, a discrepancy between the ionic composition and the total osmotic pressure of the blood. He postulated that this discrepancy was due to non-electrolytes, and subsequently found (Gilbert 1959c) that the concentration of non-protein nitrogen in the blood was inversely proportional to the concentration of ions. As one fraction increased in concentration, so the other decreased, in such a manner that the total osmotic pressure remained constant. Drilhon-Courtois (1934) also/...

also found in individual crabs that blood protein increased as the ionic concentration decreased. Horn & Kerr (1963) have analysed the haemolymph protein concentration of adult blue crabs, and summarized the data available from other Decapoda. Pampapathi Rao & Ramachandra (1961) and Padmanabhanaidu & Ramamurthi (1961) have studied the free amino acids in the blood of various species of crabs, and point to concentrations which may cause marked differences in the overall osmotic concentration of the blood. Robertson (1960a) and Bricteux-Gregoire, Duchateau-Bosson, Jeauniaux & Florkin (1966) however found only very small concentrations of amino acids in the blood of *Carcinus maenas* and *Limulus polyphenus*.

In insects, osmotic regulation of the blood is frequently controlled by adjustment of the aminoacidaemia (Florkin, 1966).

In polychaetes, Clarke (1968) showed that osmotic dilution causes a rise in coelomic fluid amino acid levels, suggesting a direct transfer from tissues to body fluid.

Camien, Sarlet, Duchateau & Florkin (1951) showed that in Crustacean muscle, amino acids were important for osmoregulation but that in the blood, amino acids were relatively unimportant. ~~Muscle~~ Blood was usually in osmotic and ionic equilibrium with sea water, whilst muscles were low in ionic concentration. Muscles had a high concentration of organic/...

organic molecules (amino acids) which exerted an osmotic pressure and hence created osmotic equilibrium with the blood. The adjustment of the intracellular osmotic pressure by **amino** acids has now been shown to occur widely in crustacea (Jeauniaux, Bricteux-Gregoire & Florkin, 1961). The importance of **amino** acids for cellular osmotic pressure has also been emphasised by Dick (1966) & Lockwood (1968). Raymont, Austin & Linford (1968) have recently studied the biochemical composition of whole Neomysis integer, and have shown that the concentration of non-protein nitrogen (NPN) increased with increasing environmental salinity. NPN was composed mainly (80%) of the amino acids, glycine, alanine and glutamate. They also demonstrated an increase in ammonia excretion rate following a sudden drop in salinity. This effect of a reduction of environmental salinity causing an increase in nitrogen excretion, and a decrease in free amino acid concentration has also been reported by Jeuniaux & Florkin (1961) for Eriochier sinensis. Raymont, Austin & Linford (1968) suggest that protein irreversibly provides free amino acids, which are broken down and excreted when no longer required. These interesting observations however do not mention the role, if any, of amino acids in osmoregulation of the blood.

From the published record it might be postulated that the discrepancy noted in C. volutator between the total ionic composition and the osmotic pressure of the blood, of animals maintained in salinities below/...

below 15°/oo, is due to non-electrolytes. A thin-layer chromatogram performed on the combined blood of ten individual *C. volutator*, has shown that amino acids are present in the blood, however quantitative results are not available, due to the extremely small volume of blood in an individual animal.

4.6 DISCUSSION.

A study of the osmotic concentration of the blood, as determined by the freezing point method, has shown that *C. volutator* is a hyperosmotic regulator, like many other brackish water crustaceans (Lockwood, 1962; Kinne, 1964b). Below 20‰ it was noted that the degree of hyperosmoticity increased with decreasing salinity of the medium. Above 20‰, the blood concentration tended towards ismoticity with the medium. Below 20°/oo, and especially below 10°/oo, *C. volutator* is living under conditions of potential osmotic imbalance which entails maintaining a difference in concentration between the internal and external medium of up to 650%. This difference between internal and external medium would appear to be too great below 2°/oo, causing rapid mortality.

No significant difference has been found in the blood concentration of animals of different size or sex. Evidence has been found that the osmotic concentration of the blood falls after a moult.

Food/...

Food has been shown to be necessary for the long term maintenance of hyperosmotic conditions in the blood, by a demonstration of a significant fall in osmotic concentration following starvation. The difference between the survival time of animals, especially at low salinities, with and without mud, has already been noted. It may be suggested that when the animal is under the osmotic stress of hyperosmotic regulation, the detritus in the mud is necessary as a source of energy for osmoregulation, or as a direct supply of ions taken up via the gut. Potts and Parry (1963) have suggested how small is the amount of energy needed for hyperosmotic regulation; and this finding suggests that the idea that detritus is necessary to C. volutator as a source of energy for osmoregulation is unlikely. Shaw (1955) has found that Sialis lutaria larvae can take up ions from food in its gut, and has no mechanism for the active uptake of ions from dilute media, which supports the hypothesis that detritus may be necessary to C. volutator because of the ions that it contains. The absorption of ions from food, for body-fluid regulation in marine invertebrates, has also been suggested by Robertson (1939). Fox (1952), whilst studying rectal pumping in C. volutator, suggested that in animals, such as C. volutator, with a relatively impermeable exoskeleton, salt entry must take place, at least partly, through the gut wall. This problem will be discussed further in the concluding discussion, (Section 6).

It has thus been shown that C. volutator is able to achieve success in an environment with wide fluctuations in salinity, due to its ability to survive low salinities, by preventing excessive changes in the blood concentration and the internal medium, by the process of hyperosmotic regulation.

Various approaches have been made to the problem of the maintenance of hyperosmotic conditions in the blood of C. volutator and have indicated that in salinities of 20°/oo and upwards, as the osmotic pressure of the blood tended towards isosmoticity with the medium, C. volutator produced urine isosmotic to the blood, and the osmotic pressure of the blood was adequately accounted for by the concentrations of inorganic ions found. In salinities below 20°/oo, as the blood became hyperosmotic to the medium, so urine hypo-osmotic to the blood was produced, and the urine appeared to be produced in larger volumes than at higher salinities. In lower salinities, the concentration of ions increased, and accounted for the majority of the hyperosmoticity of the blood, but a discrepancy was noted, especially below 10°/oo, which it may be postulated was due to amino acids or other non-electrolytes. Sutcliffe's (1961) suggestion that an increase in the non-electrolyte fraction could be due to the mobilization of amino acids from a protein reservoir is of particular interest in this respect, as are the findings of Sharma (1968) that a significant rise in blood protein and a shift towards/...

towards ureotelism occurred in crayfish under osmotic stress. However Robertson (1960a) in the crab Carcinus maenas points to the small osmotic concentration of non-protein amino N, lactic acid, reducing sugar, ammonium and inorganic phosphate in the blood (2 - 3 mOsm or mg. ions compared to the total osmotic concentration of 1080 mg/kg.), and suggests that amino acids etc. are of limited importance in osmoregulation. The present findings from C. volutator, therefore remain an unexplained discrepancy.

The maintenance of hyperosmotic conditions in the blood, in animals in low salinities is assisted by the production of hypo-osmotic urine, which will in part relieve the mechanism for the uptake of ions, whether it be at the body surface, or via the intestine. The ions taken up have been shown to be generally regulated in a manner comparable to other brackish water crustacea.

5. OTHER ASPECTS OF OSMOREGULATION.

In addition to determinations of the effects of salinity on the biology and the body fluids of C. volutator, other aspects of osmoregulation have been studied: 1) the permeability of the body surface, 2) the distribution of vital stains, with respect to drinking etc., 3) the effect of changing C. volutator from one salinity to another, 4) oxygen consumption in relation to salinity, 5) salinity preference.

5.1 PERMEABILITY

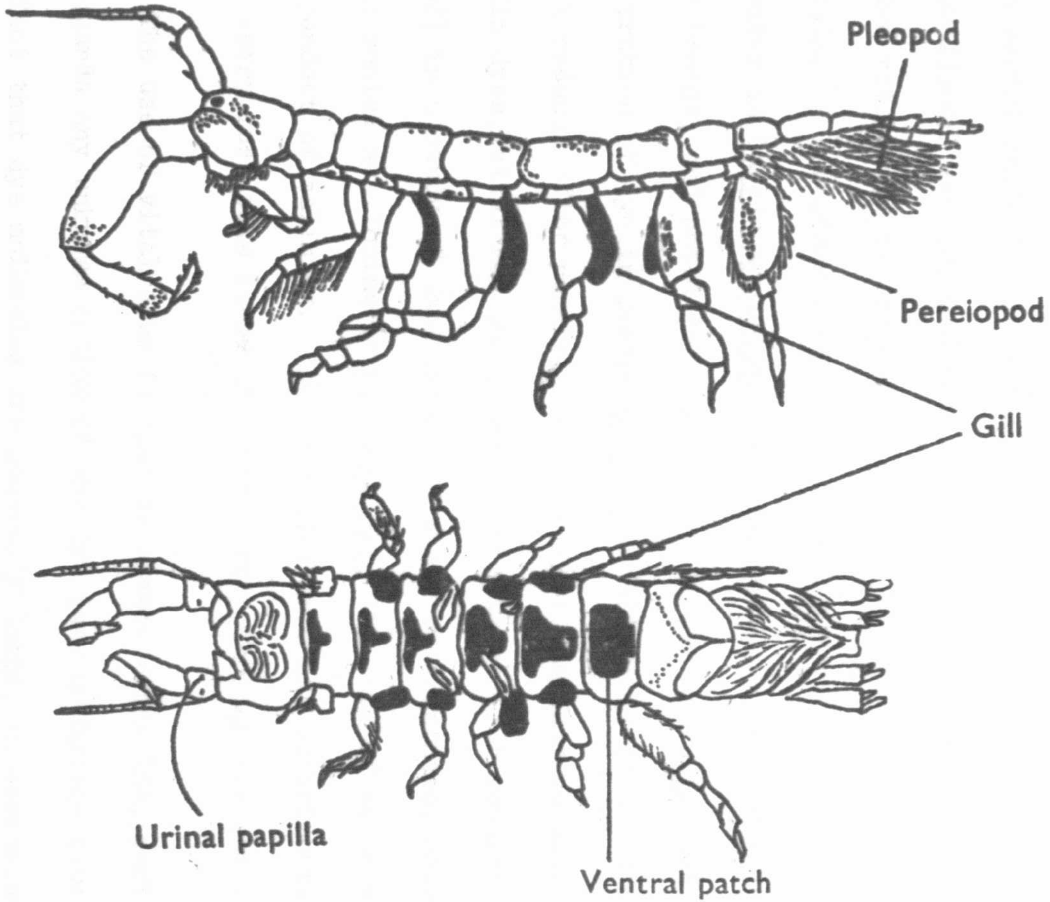
In a small brackish-water crustacean such as C. volutator, any reduction in the permeability of the surface will assist in the maintenance of hyperosmotic conditions in the blood. Permeable areas of the cuticle were localized by means of a silver-staining technique, essentially the same as that used by Croghan (1958) and Ralph (1967). The living animals were washed in changes of distilled water to remove any adherent chloride, and then placed in dilute silver nitrate solution for 5 min. They were then again carefully washed in distilled water, and put into photographic developer (Suprol). This reduced any silver ions that had been taken up to black metallic silver. The principle of this technique is that silver ions will diffuse into the permeable areas of the cuticle and there be reduced by meeting halide ions (e.g. chloride) from the blood. The precipitate is then developed to metallic silver.

This technique stained four pairs of gills attached to the bases of pereiopods 1-4. T-shaped patches were also revealed on the sterna of abdominal segments 2-7 (Fig. 23). Animals were examined from different locations on the Ythan estuary, after maintenance in different salinity conditions, and of different sizes. No consistent differences were found between animals of different sizes or from different conditions, but it was found that certain individuals lacked the stained T-patches. Hart (1930) commented on a current of water produced by the pleopods of C. volutator. It may be noted that the silver-stained areas were all located on the ventral side of the animal, where this current passes underneath the animal. It may be suggested that these areas of the cuticle and the gills are permeable, to allow interchange of water, gases and ions. The dorsal and lateral surfaces of the animal were never stained by this technique, which suggests that they are areas of reduced permeability. the restriction of the permeable areas of the cuticle to certain confined areas on the ventral surface is likely to assist in the maintenance of hyperosmotic internal conditions. The importance of restricted permeability to brackish-water crustaceans for the maintenance of internal conditions has been emphasized by Shaw (1959), Smith (1967), and Rudy (1967). Although silver staining may indicate the presence of permeable areas of the cuticle, it does not necessarily define the actual site of active transport of ions. (Lockwood, 1962).

5.2/...

FIGURE 23.

C. volutator, showing the gills and ventral patches, revealed by silver staining, lateral view (above) and ventral view (below)


Pleopod
Pereiopod
Gill
Urinal papilla
Ventral patch

5.2 VITAL STAINING

Krogh (1939) reports the use of phenol red by Homer Smith (1930) as a useful indicator in cases of hypotonicity as to whether water is swallowed and absorbed from the intestine. Krogh also recommended vital dyes as indicators of drinking in iso - and hypertonic animals. Robertson (1957) used phenol red to indicate oral uptake of water in *Carcinus maenas*. Pannikar (1941a) used methylene blue with Prawns, and noted low penetration in estuarine *Leander* and fast penetration of dye in marine *Crangon*. Methylene blue was criticised as it reduces after penetration, therefore Pannikar changed to acidic dyes, vital red and neutral red with similar results. Fox (1952) in a study of anal intake of water by Crustacea, noted rhythmical rectal swallowing in *C. volutator*, the rate of which was independent of salinity. He concluded from his experiments that salt entry must take place at least partly through the gut wall.

The use of vital dyes is open to severe criticism, particularly as regards any unknown action of the dye (see methylene blue, above), the fact that dye molecules are generally large, in comparison to the normal ions in the medium of *C. volutator*, and also accumulation of dye in the gut of an animal may cause stress which will severely alter the animal's physiology. Despite these drawbacks vital dyes are useful indicators of drinking, either orally or anally. Individual *C. volutator* were placed in solutions of methylene blue, methyl green/...

green, phenol red, evans blue, toluidine blue, methyl violet, neutral red, indigo carmine and congo red, made with ½ strength sea water, and observed.

Methyl violet and methyl green caused rapid death. In the remaining dyes the animals lived for at least 48 hours. In these dyes anal pumping of water and oral swallowing of water was observed - particularly in phenol red. After periods of 36 - 48 hours, the gut and caecae were found to be stained. Otherwise no staining was observed, except for a tinging of the gills, especially in methylene blue and toluidine blue. In a dead animal the dye permeated the body tissues in about 6 hours.

These observations, suggesting possible salt entry via the gut wall, confirm those of Fox (1952) of anal pumping and also indicated oral entry of water, as suggested by Krogh (1939) and Robertson (1957), leading to aggregation of dye in the mid-gut and paired dorsal and ventral caecae (Agrawal, 1963a).

The importance of drinking for hypo-osmotes has been emphasized by Dall (1967). With the aid of inulin C^{14}, Thuet, Motais and Maetz (1968), have shown in Artemia salina, that one third of sodium influx occurs via the intestine, following oral drinking, and Bryan (1967) showed that crayfish take up zinc ions via the stomach.

The experiments with silver nitrate (Section 5.1) indicated the areas of the cuticle (gills and sterna) permeable to halide ions, and the present observations with vital dyes indicate that oral swallowing and anal pumping occur. These experiments have indicated possible entry routes of salts to the internal medium of an animal with a relatively impermeable exoskeleton, namely the 4 pairs of gills, 6 sternal patches, anal pumping, and oral swallowing leading to the midgut and associated caeacae.

5.3 EFFECT OF A CHANGE OF MEDIUM.

The ability to endure changes of medium and to regulate body volume appears to be an important characteristic of euryhaline organisms (Gross, 1957; Kinne, 1964a). The regulation of body water volume by excretion, or by loss or gain of salts or water, leads to a new steady state of balance, between an animal and its environment. Volume regulation is well known in annelids and molluscs as well as crustacea (Prosser & Brown, 1961; Kinne, 1964a). Osmotic conformers (usually stenohaline marine organisms) generally have poor volume regulation, whilst osmotic regulators can maintain physiological balance, and avoid any large changes of weight or volume. Annelids such as Nereis diversicolor or Eudistylia vancouveri, which volume regulate, may gain up to 40% of body volume or weight when changed from sea water to dilute media, before adjusting/...

adjusting back to their original weight, in a new state of osmotic balance; whilst crustacea which volume regulate, e.g. Carcinus maenas, hardly swell at all, following a similar change, although weight increase following such a change was found to be 10X greater when the excretory pores were blocked, due, almost certainly, to the prevention of urine production (Prosser & Brown, 1961; Hoar, 1967).

From the published record it may be seen that the ability to withstand changes of medium, and protect the most sensitive tissues from severe salinity changes in changes of ~~hydustalic~~ hydrostatic pressure would be of great value to the survival of a relatively highly organised animal such as C. volutator. To investigate the effect of a change of medium on C. volutator, which in a steady state has been shown to minimise the effects of salinity by the process of hyperosmotic regulation, a study has been made of the effects of a sudden change of salinity, on the freezing point of the blood, and the wet weight of the body.

Animals, both fed and unfed, were acclimated fully to one salinity, and then changed into another salinity. Blood samples were taken from individual animals at regular intervals, and the freezing point determined. Experimental details as Table 11:

Table 11/...

Expt.	Initial salinity °/oo	Final salinity °/oo	Food	Results Fig. No.
1.	32.8	4.6	fed	24
2.	5.8	30.3	fed	25
3.	2.8	27.2	unfed	26

The results (Figs. 24 - 26) show that accommodation of the freezing point of the blood to a new medium was largely accomplished within 3 hours, and then stabilised gradually over a period of up to 24 hours. This limitation of rapid changes in the blood concentration, allows time for adjustment of intracellula~~tion~~r concentrations (Lockwood, 1962). No significant effect of feeding was noted.

The effect of a change of medium on the body weight was also determined. In each experiment 15 animals were acclimated to one salinity, and then 10 were changed into another salinity, and 5 were changed into medium of the same salinity as the initial medium, as a control. The wet weight of animals, which had been quickly dried on a tissue, was determined at regular intervals. The results are presented in Fig. 27A (33 to 3, & 33 to 33‰) and Fig 27B (3 to 33, & 3 to 3‰).

It can be seen that large fluctuations occurred in the weight of the control animals, reflecting large experimental errors involved/...

FIGURE 24.

The effect of a change from 32.8 to 4.6‰ on the **freesing point** of the blood.

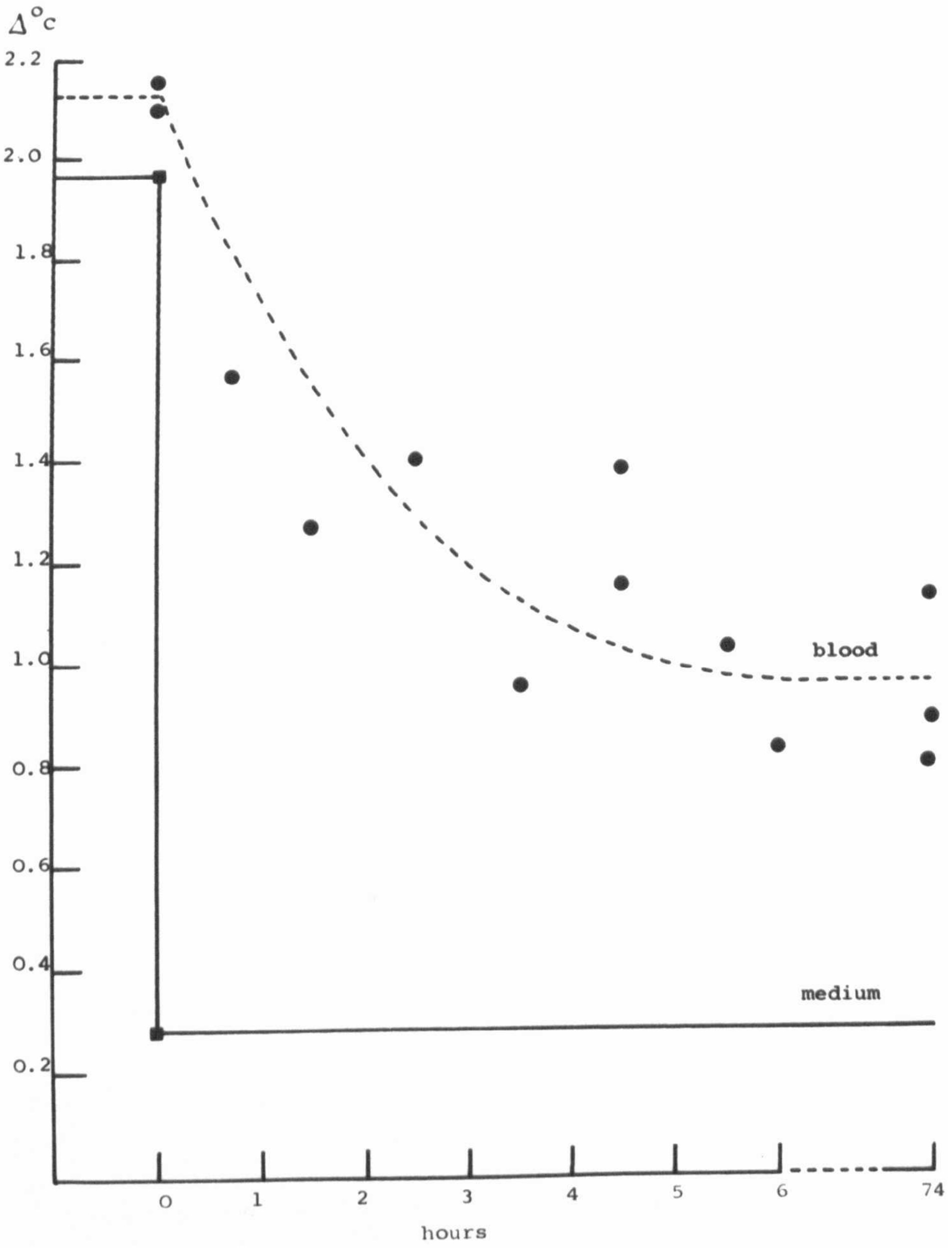

Δ°c
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
blood
medium
0
1
2
3
4
5
6
74
hours

FIGURE 25.

The effect of a change from 5.8 to 30.3%o on the freezing point of the blood.

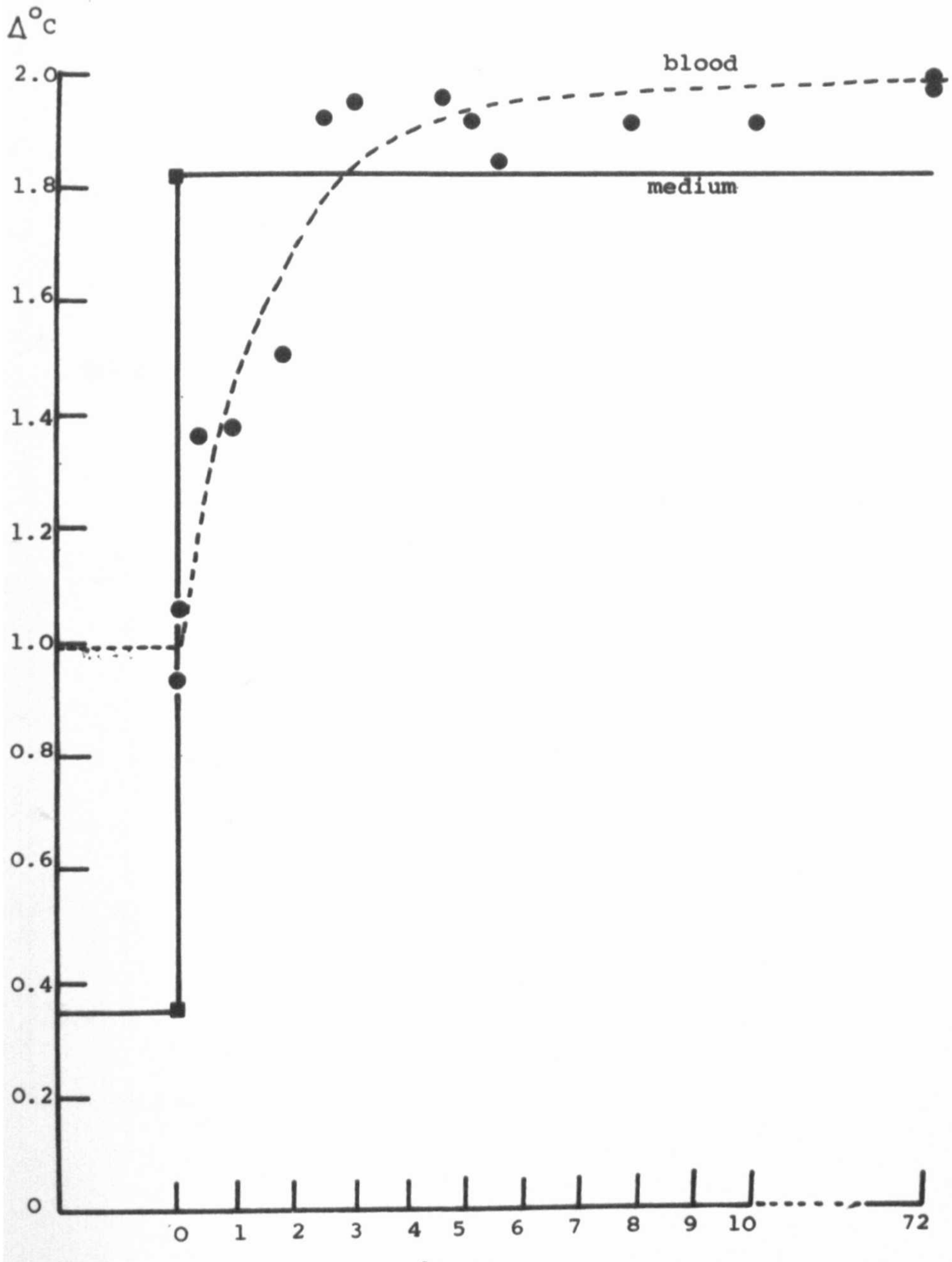

Δ°C
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
blood
medium
0
1
2
3
4
5
6
7
8
9
10
72
hours

FIGURE 26.

The effect of a change from 2.8 to 27.2‰ on the freezing point of the blood of unfed animals.

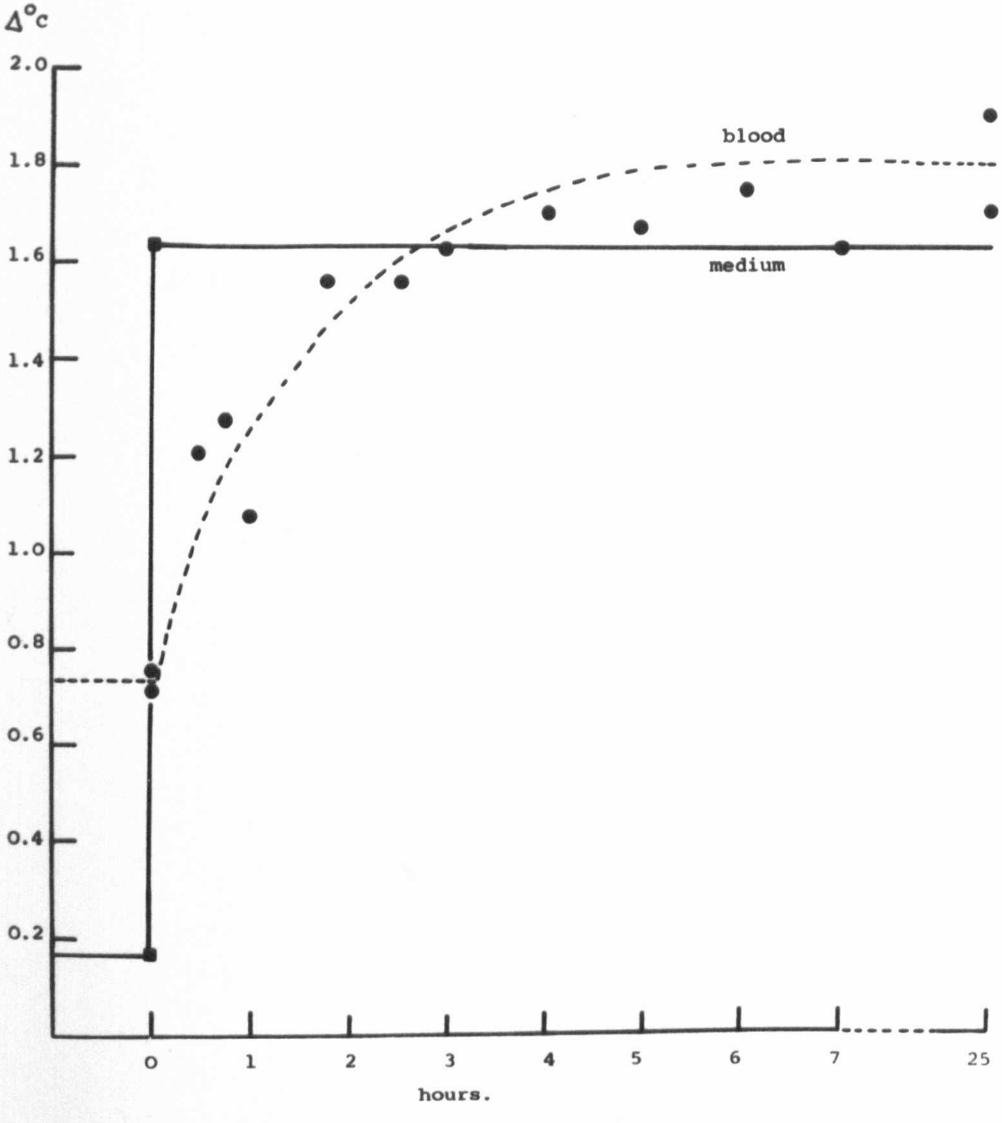
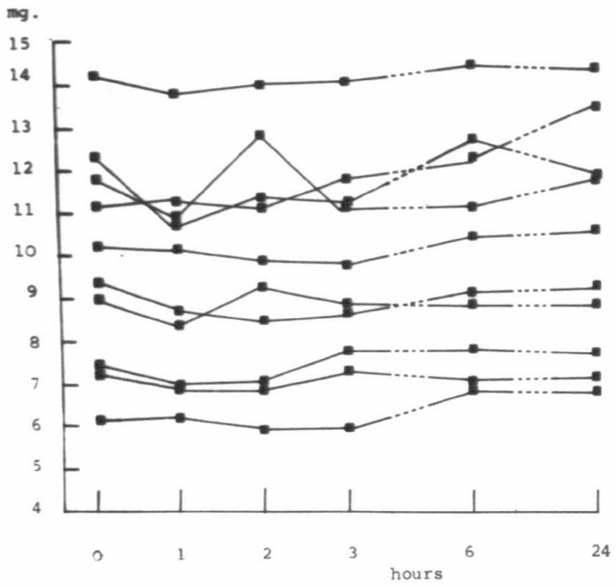

Δ°c
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
blood
medium
0
1
2
3
4
5
6
7
25
hours.

FIGURE 27a.

The effect of a change of medium from 3 to 33‰ on the weight of animals.


3‰ to 33‰

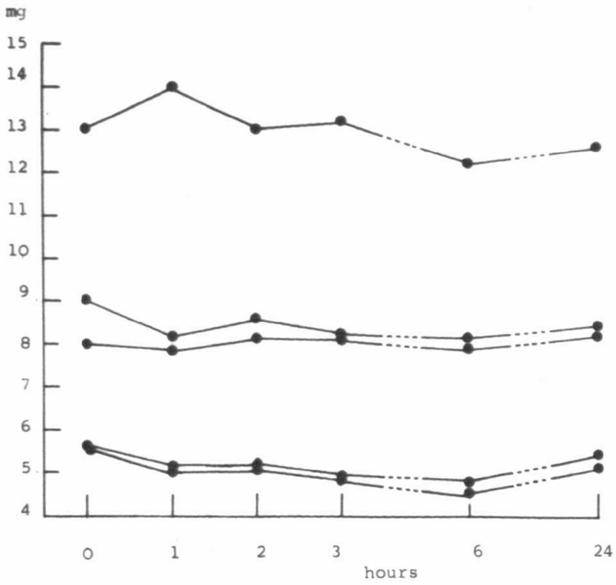
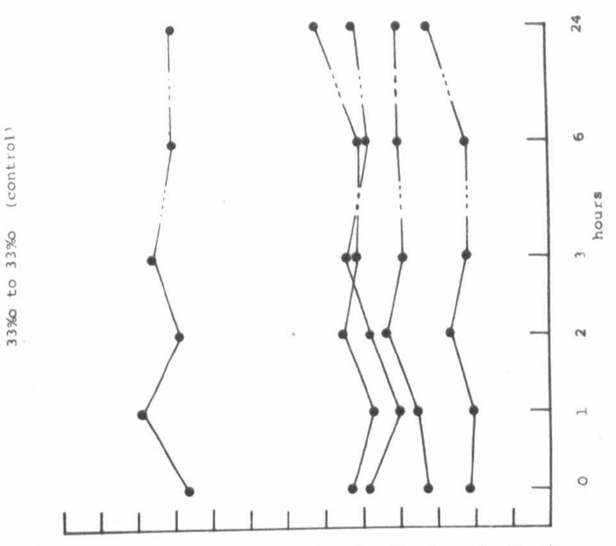
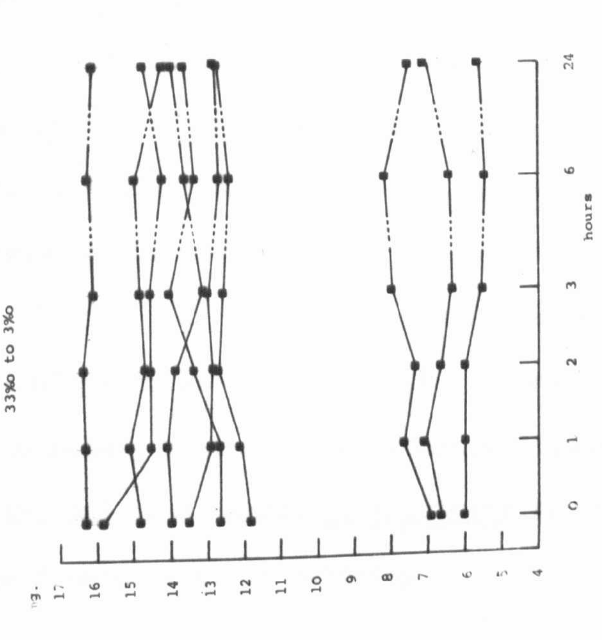

3‰ to 3‰ (control)

FIGURE 27b.

The effect of a change of medium from 33 to 3% on the weight of animals.


33‰ to 33‰ (control)
mg.
17
16
15
14
13
12
11
10
9
8
7
6
5
4
0
1
2
3
6
24
hours


33‰ to 3‰
mg.
17
16
15
14
13
12
11
10
9
8
7
6
5
4
0
1
2
3
6
24
hours

involved in the wet weight determination of a small animal with a large surface area, such as C. volutator.

In an attempt to eliminate the weighing errors of the previous experiment, C. volutator were weighed whilst swimming in a known weight of water. The previous experiment was repeated, except that the animals were removed from the experimental dish, quickly dried on a tissue to remove adherent water, and then weighed in a known weight of water of the same salinity as the experimental medium. The results (Fig. 28) indicate similar fluctuations in the weight of the control animals, as in the previous experiment, being due perhaps to **variations** in weight caused by the need to remove adherent water. In both of these experiments no consistent changes were found in the weights of the experimental animals subjected to a change of salinity. Like the control animals, considerable variability occurred in the weight of the experimental animals. These experiments, by the lack of any consistent change in weight, indicate volume regulation, which in addition to the adjustment of the blood concentration noted, protects the internal tissues from rapid changes in salinity, and thus adapts C. volutator to an estuarine habitat with large fluctuations in salinity.

Lockwood (1968) has pointed to the vital importance of volume regulation for controlling hydrostatic pressure in any Crustacean with a relatively impermeable exoskeleton, and suggests that volume regulation/...

regulation is largely brought about by urine production (and drinking). A similar suggestion may be made for C. volutator.

Nagel (1934) demonstrated active uptake of ions at the body surface by changing a hyperosmotic animal from a dilute medium to a more concentrated medium, which was still less concentrated than the original blood concentration, and found an increase in the osmotic pressure of the blood, suggesting active uptake of ions. I have repeated this experiment on C. volutator, by changing them from a medium of 3‰ to one of 10‰. These experiments were performed on animals fed and unfed, prior to and during the experiments. It was found (Fig. 29) in all animals that the mean blood concentration increased from 12‰ to 16‰ within 3 hours, and no significant differences in the rate of change were found, between fed and unfed animals. This finding leads to the conclusion that active uptake of ions may be accomplished without food.

Attempts were made to determine the density of C. volutator, maintained in different media. The animals were weighed in air, and then suspended in water of known salinity (Denton & Gilpin-Brown, 1961). The findings ~~(Fig.29)~~ indicated densities of 1.02 - 1.23, but showed considerable variation, again due to the necessity of removing excess

water/...

FIGURE 28.

The effect of a change of medium from 32 to $3^{\circ}/oo$, and vice versa, on the weight of animals, weighed under water.

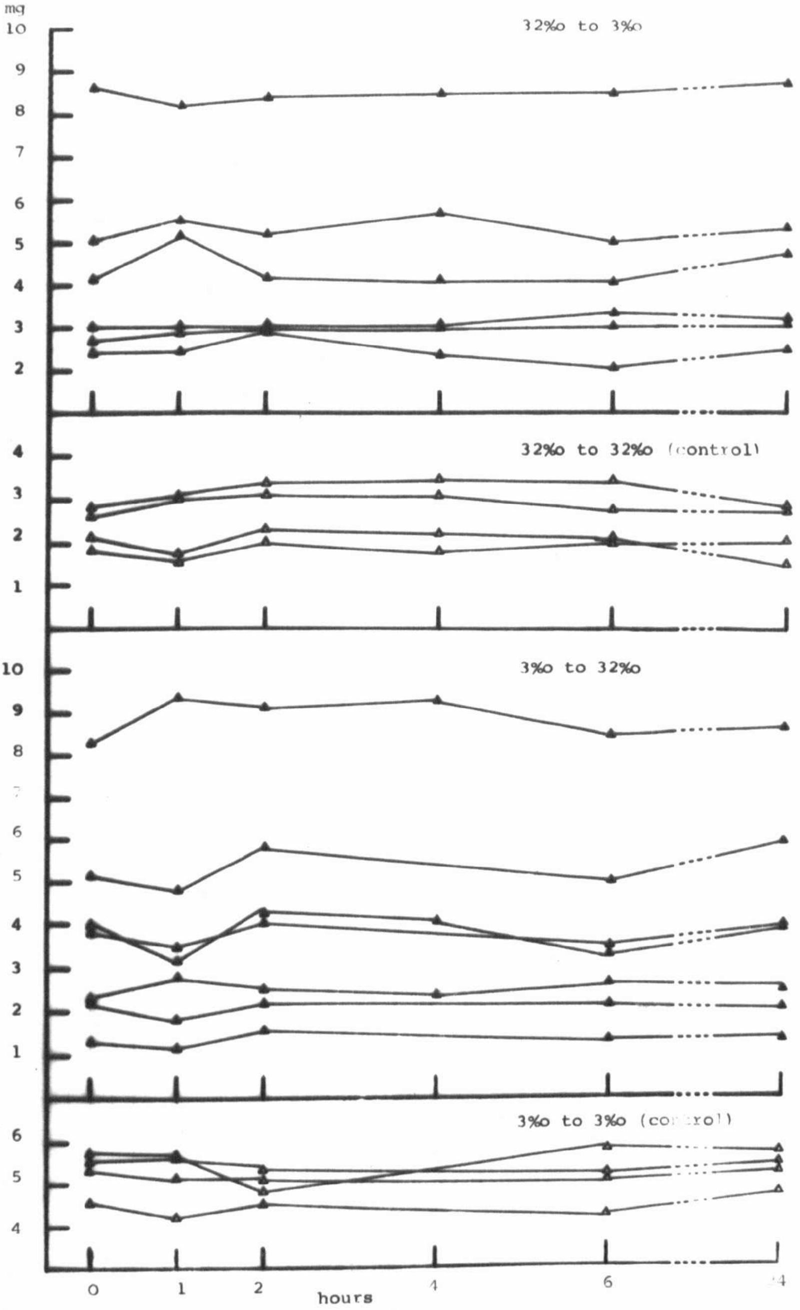
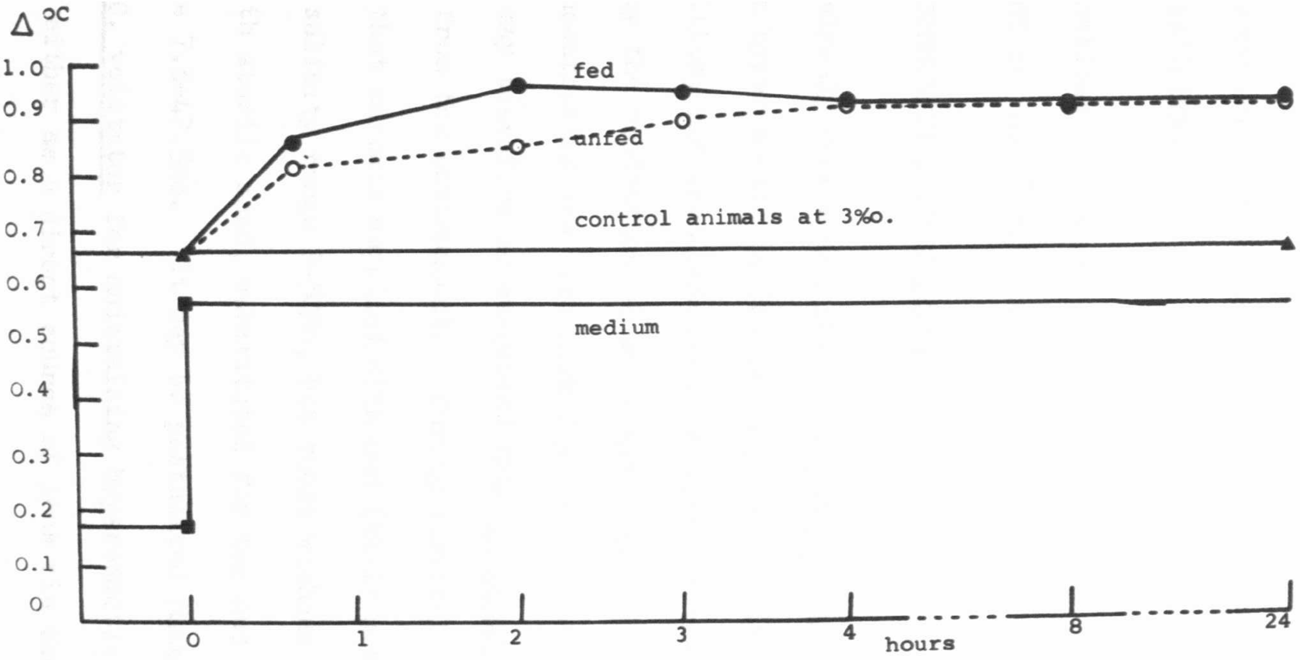

mg
10
9
8
7
6
5
4
3
2
32‰ to 3‰
4
3
2
1
32‰ to 32‰ (control)
10
9
8
7
6
5
4
3
2
1
3‰ to 32‰
6
5
4
3‰ to 3‰ (control)
0
1
2
4
6
24
hours

FIGURE 29.

The effect of changing individuals (fed and unfed) from a medium of 3‰ to one of 10‰, on the mean freezing point of the blood.


Δ °C
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
fed
unfed
control animals at 3‰.
medium
0
1
2
3
4
hours
8
24

water and then weighing in air, and also to surface tension effects when suspended in water. Because of this variability it is impossible to conclude if the density of <u>C. volutator</u> changed in relation to salinity.

Determinations of the dry weight of <u>C. volutator</u>, show that the dry weight is 14.7 ± 3.2% of the wet weight.

5.4 OXYGEN CONSUMPTION IN RELATION TO SALINITY.

It has already been noted that <u>C. volutator</u> maintains its blood concentration hyperosmotic to the medium, especially when living in low salinities, and that retention of salts in the blood may be assisted by the production of urine hypo-osmotic to it, however the urine concentration was also markedly hyperosmotic to the medium. It may therefore be suggested that <u>C. volutator</u> must also take up ions from the environment. During survival experiments, it was noted that animals supplied with mud (their normal food) survived the salinity range 2-50‰, but those without any substrate at all, or with sterile sand, substituted for the mud survived the salinity range 7.5-47.5‰. It may be postulated that food is necessary in <u>C. volutator</u> for maintaining hyperosmotic conditions in the blood, either as a direct source of ions via the gut, or as an energy source for the active uptake of ions at the body surface. If food is necessary as an energy source, it is likely that the uptake of ions for osmoregulation will be reflected in an increase in the metabolism of C. volutator under osmotic stress, and that this increase in metabolism will be expressed by a change in oxygen consumption in relation to salinity, provided that there is no shift in the oxygen requirements between different metabolic processes of the animal. For example it might be that under osmotic stress, energy could be diverted from gonad maturation into osmoregulation.

The extensive literature on the effects of salinity on respiration presents a conflicting picture. Many authors (Schwabe , 1933, for Carcinus; Peters, 1935; Flemister & Flemister, 1951; Lofts 1956; Dehnel, 1960; Lance, 1965; Ramamurthi, 1967) have found that oxygen consumption was increased under conditions of osmotic stress. Madanmohanrao & Pampapathirao (1962) noted lowest oxygen consumption in medium of adaptation, in spite of osmotic stress. Krogh (1939) and Potts & Parry (1963) have postulated that any change that would occur due to osmotic stress would be small, and many authors (Schwabe, 1933, for Eriocheir; Gilchrist, 1956; Frankenberg & Burbanck, 1963; Holliday, Blaxter & Lasker, 1964; Eltringham, 1965; McFarland & Pickens, 1965; Palmer, 1968) have found no significant changes in respiration rate which may be correlated with salinity. Gross (1957) and Duncan & Klekowski (1967) have pointed to the effects of salinity on the locomotory activity of an animal, and suggest that certain observed changes in respiration rate in relation to salinity

are/...

are due to changes in activity. The importance of activity in relation to oxygen consumption has also been emphasised by McFarland & Picken, (1965); Newell & Northcroft (1965) and Halcrow & Boyd (1967).

In the present study an attempt has been made to induce a continual locomotory activity, and to measure the oxygen consumption of animals at a constant level of activity, acclimatized to different salinities. Anaesthetics were not used, since these may induce sub-basal oxygen consumption levels, or may selectively depress only certain metabolic processes of the animal.

Animals were collected from the Ythan estuary and transported to stock tanks, supplied with mud and water (15 - 20‰) from their natural habitat, and maintained at 10°C. The animals were kept in the tanks for at least a fortnight, to extinguish any tidal rhythm (Morgan, 1965) which may have affected oxygen consumption (Weiser, 1962). They were maintained prior to oxygen consumption measurements in experimental salinities (1, 10, 20, 30°/oo; at 10°C), supplied with mud for 10 days.

Oxygen consumption was measured using Microvolumetric respirometers (supplied by Mark Co.), to the design of Scholander & Iversen (1958), following the technique of Scholander, et.al. (1952). Standard reaction vials were used, and CO_2 was absorbed

with/...

with 20% KOH on Whatman No. 40 filter paper. All measurements were made with the respirometers immersed in a constant temperature water bath at 10±0.02°C. Two thermobarometric blanks, with 1 ml of medium in each were maintained. The animals in the respirometers were in 1 ml of medium.

An advantage of this design of respirometer was that it permitted continual observation of the experimental animals. Whilst the respirometers were shaken concentrically at a rate of 30 shakes per minute, to maintain equilibrium of the liquid and gas phases (Scholander, et.al., 1952; Scholander & Iversen, 1958), the animals were observed to swim continually. At a faster speed of shaking the animals were severely stressed, however at 30 shakes per minute they were able to swim constantly. These active animals appeared to settle down to a constant rate of oxygen consumption after a maximum period of one hour; all measurements have therefore been made after one hour's acclimatisation, the constant level of swimming activity attained by all animals, permitting comparison of animals of different sizes, or in different salinities.

"Resting" or "Routine" (Fry, 1957) metabolism was measured by leaving the respirometers still, except for periods of equilibration. During these still periods, the animals swam or crawled sporadically, or rested. The larger animals were observed to swim more

frequently/...

frequently than the smaller individuals. Spontaneous activity may change with the salinity of the medium but no objective measure was made in this study, however observations suggested greater activity in lower salinities.

The results showed that in all salinities (1, 10, 20, 30‰) that the oxygen consumption of active animals was logarithmically related to the weight of the animal (Figs. 30, 31, 32, 33). The statistical treatment of the data, the equations of the regression lines, and the correlation coefficients are presented in Table 12.

TABLE 12

OXYGEN CONSUMPTION OF <u>C. VOLUTATOR</u> IN RELATION TO WEIGHT AND SALINITY

‰ NaCl.	$y = a + bx$	N.	Corr. Coeff.
1	$y = 1.933-0.818x$	22	-0.581
10	$y = 1.981-0.758x$	40	-0.603
20	$y = 1.989-0.961x$	32	-0.532
30	$y = 1.976-0.782x$	37	-0.723

This exponential relationship was first demonstrated by Zeuthen (1947), and has since been frequently confirmed (Nicol, 1967; Newell & Northcroft, 1965; McFarland & Pickens, 1965).

When the oxygen consumption of all the active animals was compared with the salinity of the medium (Fig. 34), it was found that/...

FIGURE 30.

The oxygen consumption in relation to dry weight of animals at 10°C and 1‰.

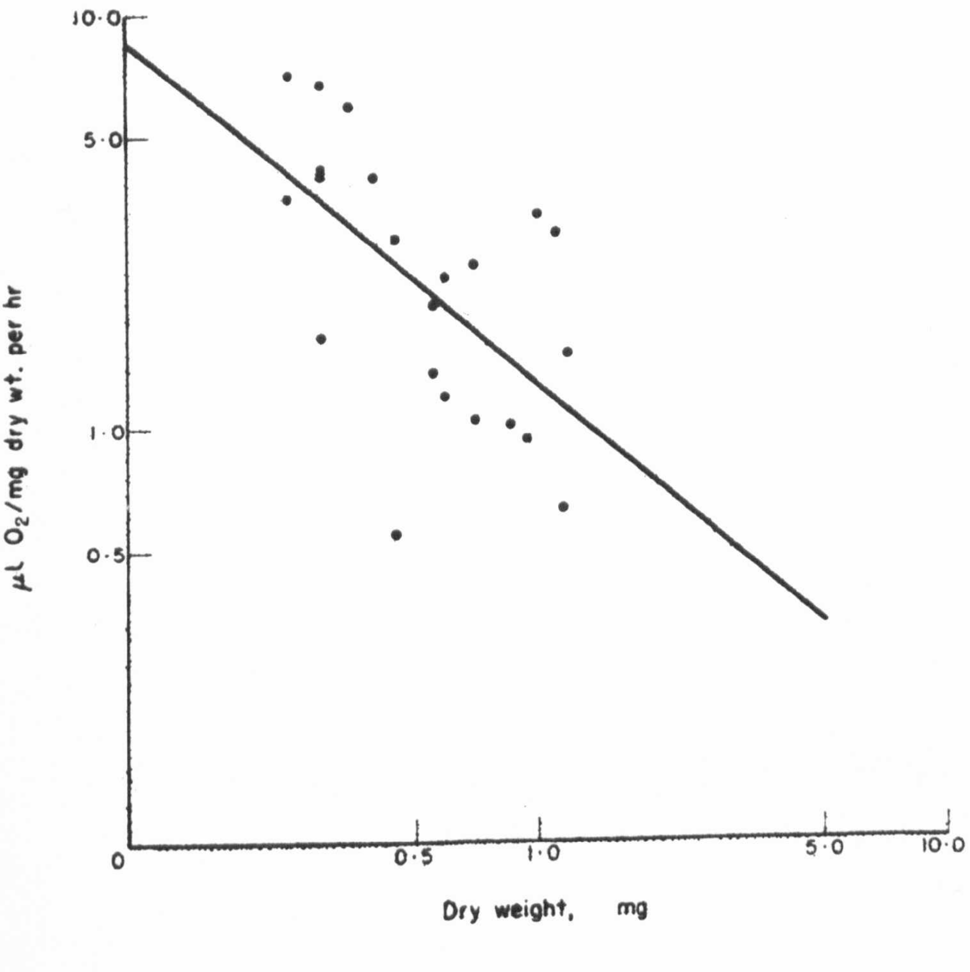

10·0
5·0
1·0
0·5
0
0·5
1·0
5·0
10·0
μl O_2/mg dry wt. per hr
Dry weight, mg

FIGURE 31.

The oxygen consumption in relation to dry weight of animals at 10°C and 10‰.

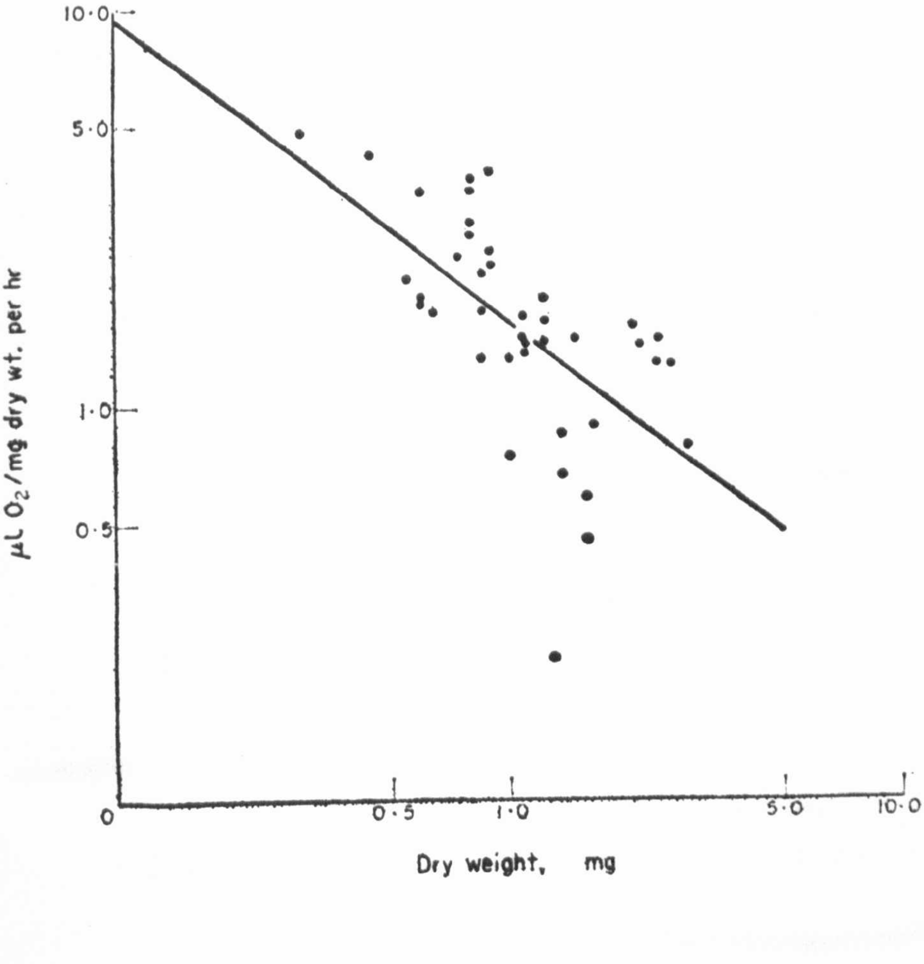

10·0
5·0
1·0
0·5
μl O$_2$/mg dry wt. per hr
0
0·5
1·0
5·0
10·0
Dry weight, mg

FIGURE 32

The oxygen consumption in relation to dry weight of animals at 10°C and 20‰.

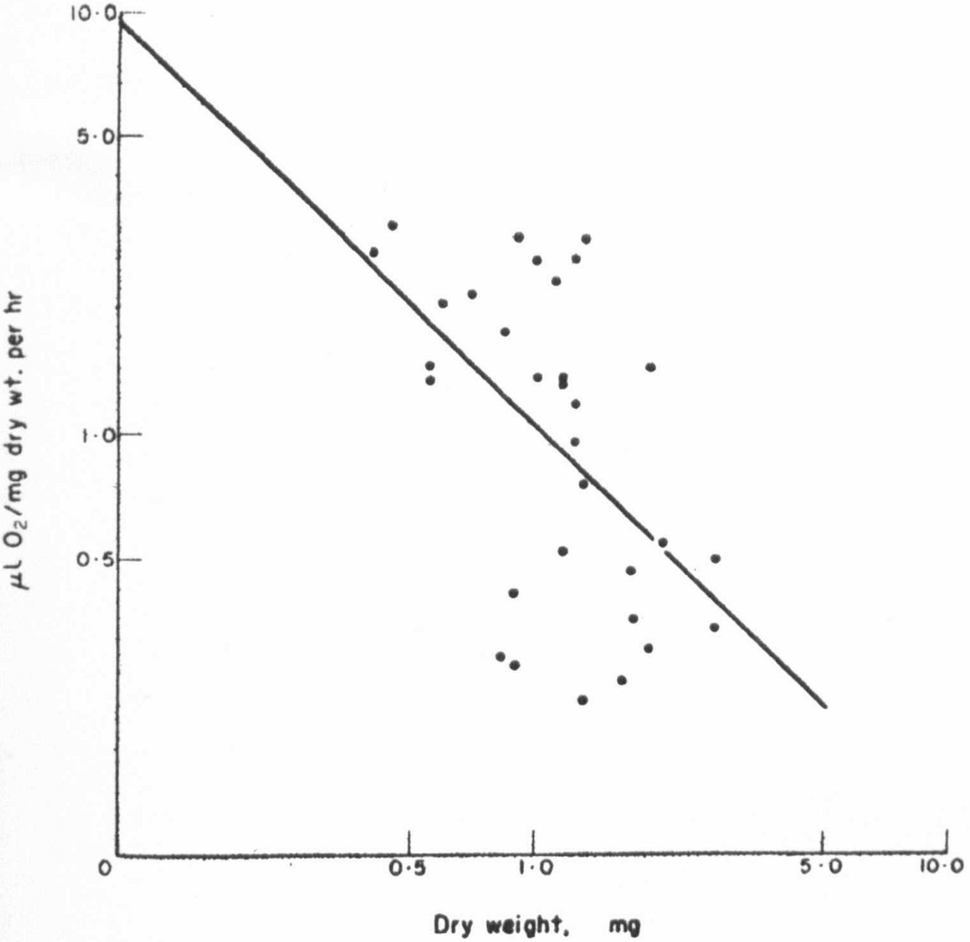

10·0
5·0
1·0
0·5
0
0·5
1·0
5·0
10·0
μl O₂/mg dry wt. per hr
Dry weight, mg

FIGURE 33.

The oxygen consumption in relation to dry weight of animals at 10°C and 30‰.

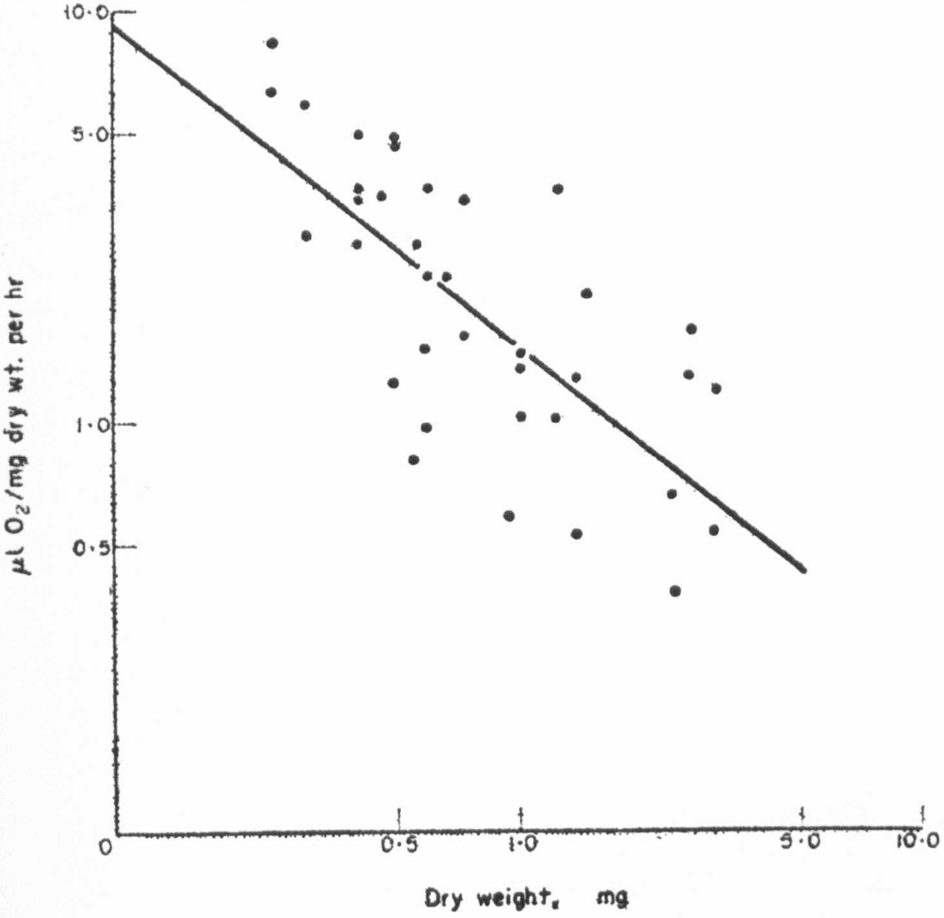
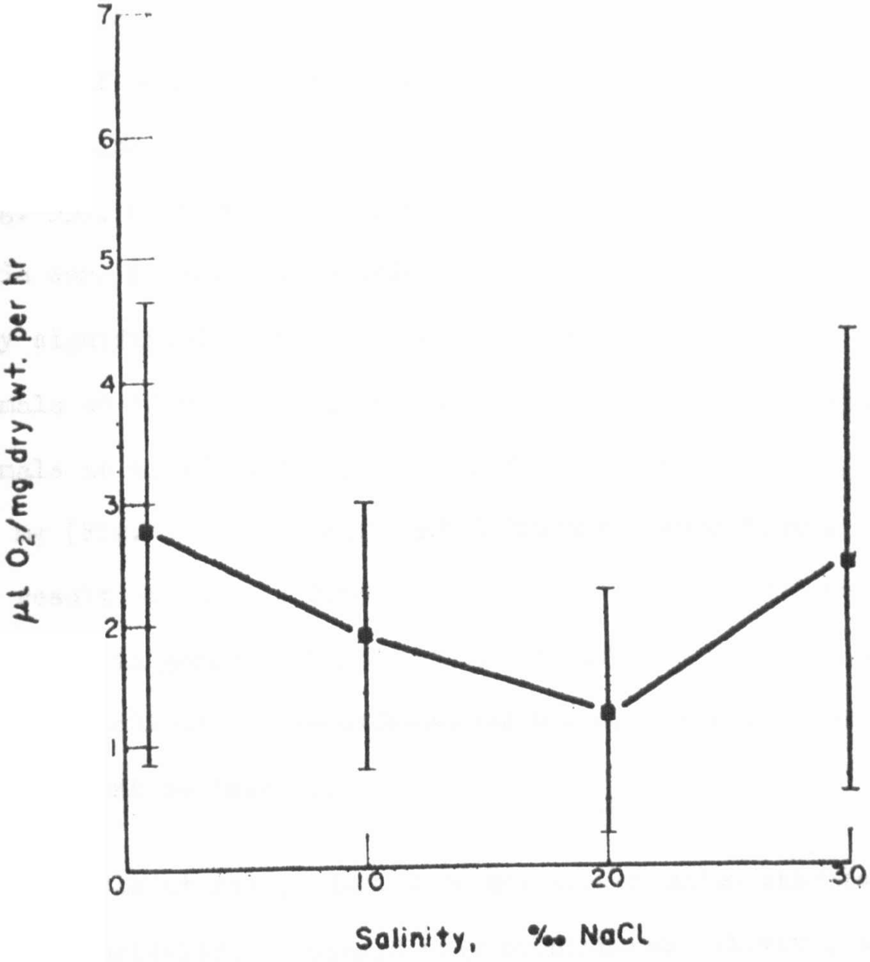

10·0
5·0
1·0
0·5
0
0·5
1·0
5·0
10·0
μl O_2/mg dry wt. per hr
Dry weight, mg

FIGURE 34.

The oxygen consumption of active C. volutator in relation to salinity.

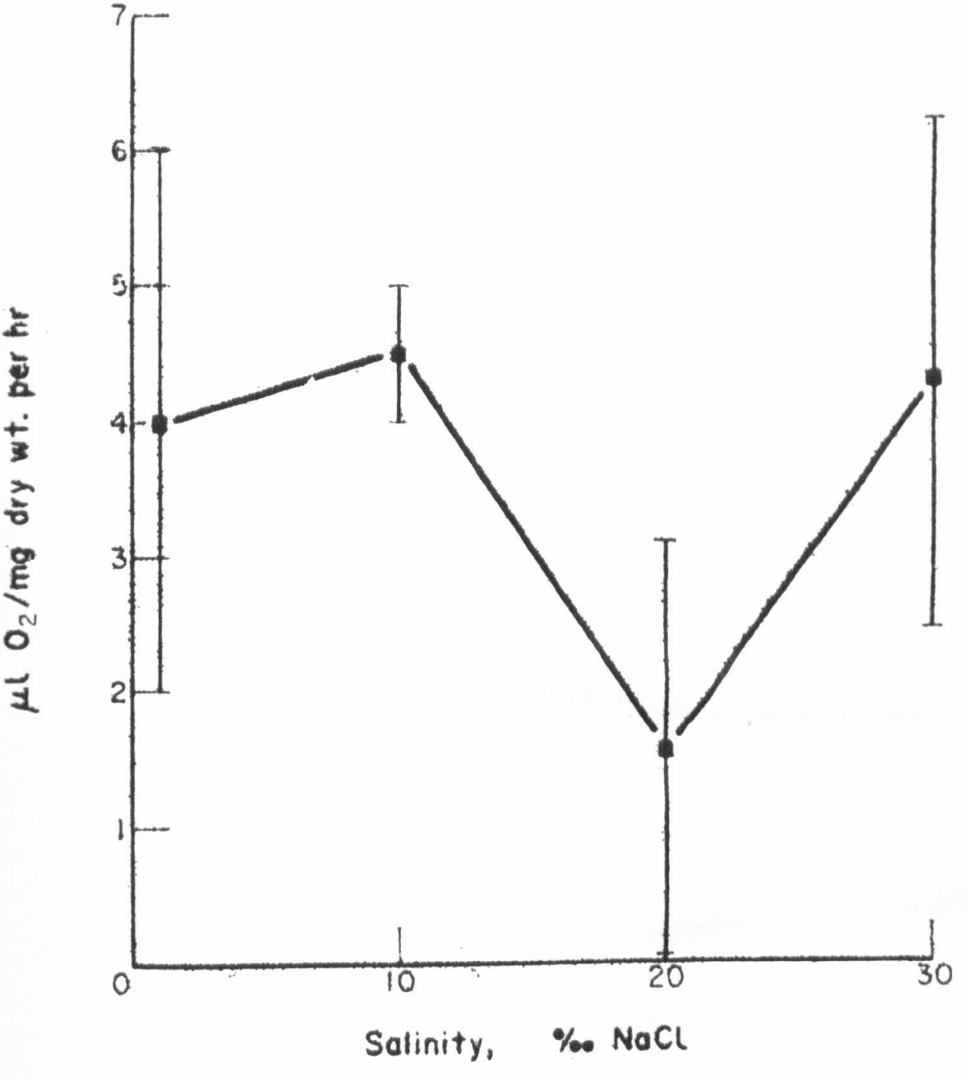

7
6
5
4
3
2
1
0
10
20
30
µl O$_2$/mg dry wt. per hr
Salinity, ‰ NaCl

that the oxygen consumption of animals at 20‰ was significantly different (at the 98% level) from that of animals at 1, 10 or 30‰. However oxygen consumption per unit of weight is not a valid basis for comparison (Gilchrist, 1956), and if comparison is made of animals of similar dry weights, these differences largely disappeared. With animals all weighing less than 0.50 mg (Fig. 35), no significant differences between animals in different media were found. With animals 0.55 - 0.95 mg (Fig. 36) the only significant difference (at the 98% level) noted was between animals at 10 & 20‰; there were no significant differences between animals at 1, 10, 1 & 20, or 20 & 30‰. With animals greater than 1.0 mg (Fig. 37) no significant differences were found. Despite the result of animals 0.55 - 0.95 mg at 10‰ compared to 20‰, it is seen that in general there were no significant differences between animals acclimatised ~~to differences between animals acclimatised~~ to different salinities.

Animals at rest, which were not shaken and so stimulated to constant activity, displayed only spontaneous activity, and showed a lower level of oxygen consumption, in relation to the lower level of activity (Fig. 38). The oxygen consumption of animals of all sizes at 20‰ was significantly lower (at the 99% level) than that of animals at 1 or 10‰, but not significantly different from those at 30‰. However, as with the continuously active animals/...

FIGURE 35.

The oxygen consumption of active animals less than 0.50 mg dry wt., in relation to salinity.



μl O_2/mg dry wt. per hr
7
6
5
4
3
2
1
0
10
20
30
Salinity, ‰ NaCl

FIGURE 36.

The oxygen consumption of active animals 0.55 - 0.95 mg dry wt., in relation to salinity.

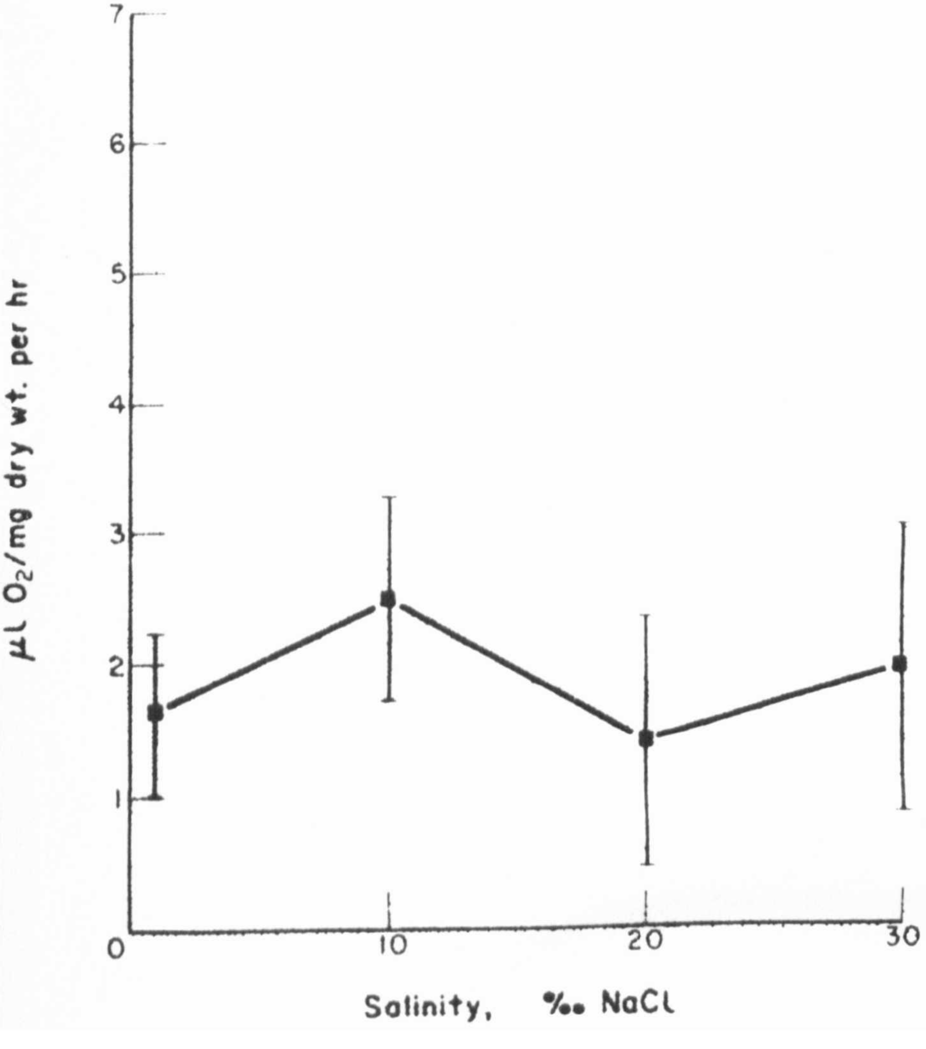

7
6
5
4
3
2
1
0
10
20
30
µl O₂/mg dry wt. per hr
Salinity, ‰ NaCl

FIGURE 37.

The oxygen consumption of active animals greater than 1.0 mg dry wt., in relation to salinity.

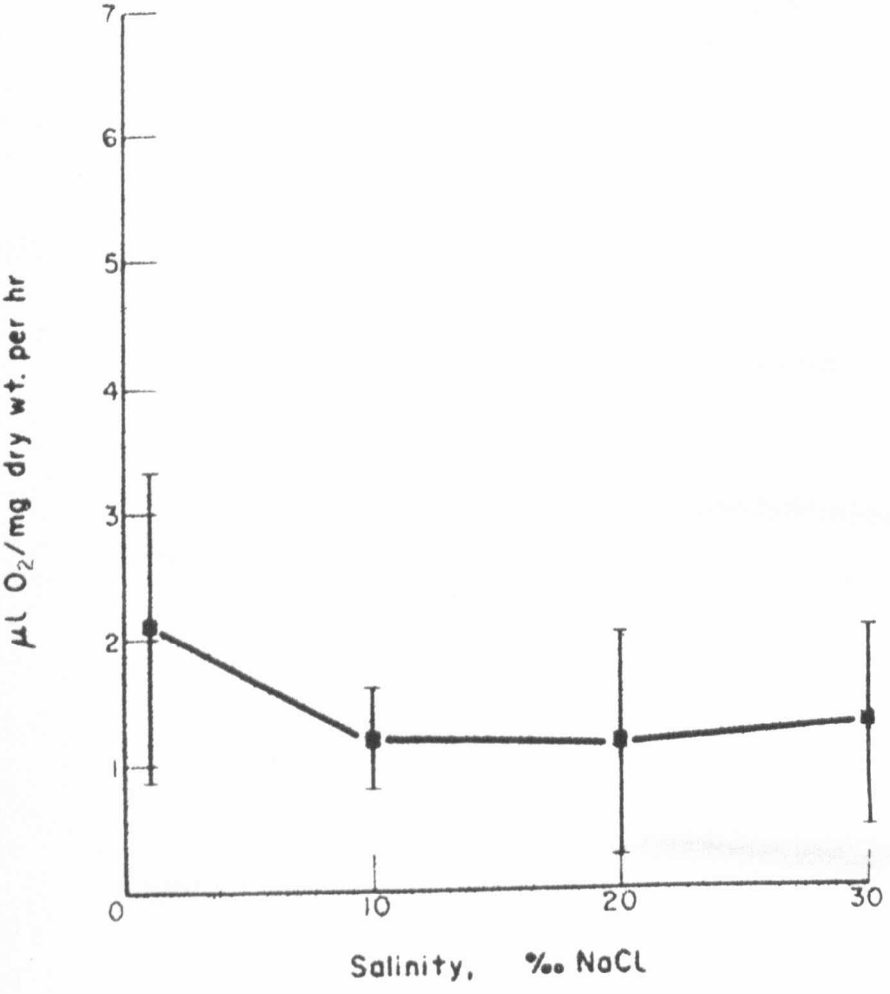
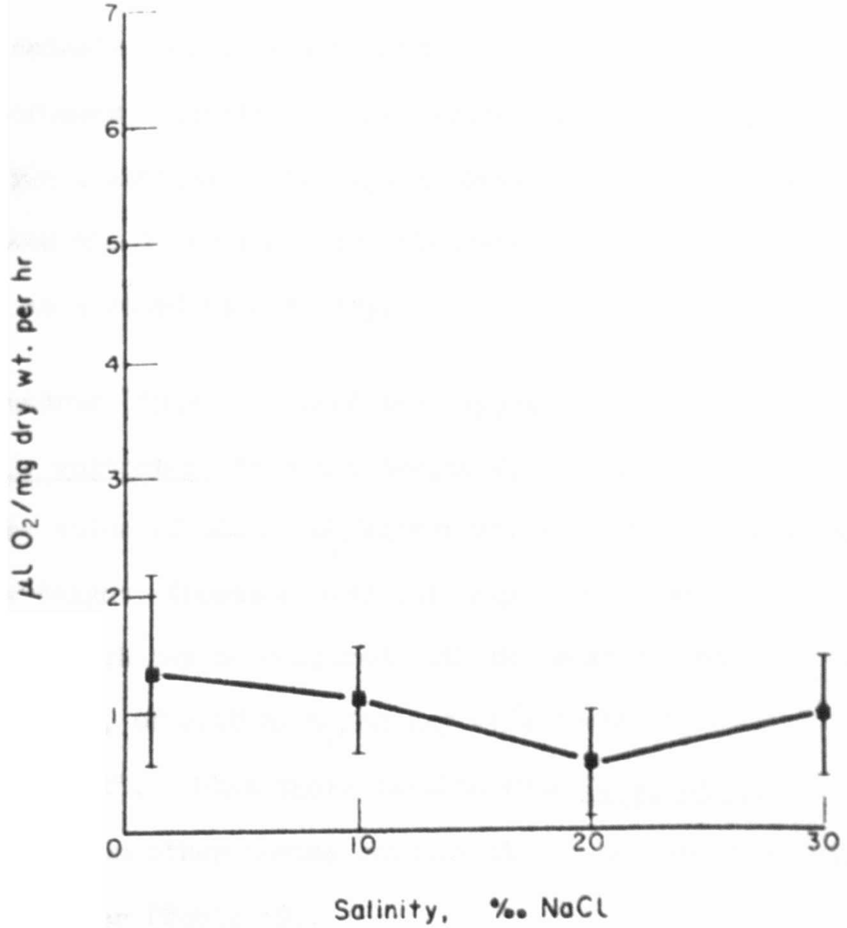

µl O₂/mg dry wt. per hr
7
6
5
4
3
2
1
0
10
20
30
Salinity, ‰ NaCl

FIGURE 38.

The oxygen consumption of animals not induced to continual activity in relation to salinity.


μl O2/mg dry wt. per hr
7
6
5
4
3
2
1
0
10
20
30
Salinity, ‰ NaCl

animals, this difference was not found when comparison was made of animals of similar sizes. It was also found with the spontaneously active animals that there was no linear or logarithmic relationship between weight and oxygen consumption. It had been noted that larger animals swam more frequently, and it may be suggested that this increased activity pattern, which was also noted by Meadows & Reid (1966), increased the oxygen consumption of the large animals, and masked the logarithmic relationship of Zeuthen (1947), noted in animals at a standard activity.

Thamdrup (1935) measured the oxygen consumption of spontaneously active C. volutator, in sea water,at 2, 10, 20 & 28°C. At 10°C he noted the value of 600 cc O_2/kg/wet wt. h. Considering wet weight as 6.6x dry weight, (Section 5.3) this may be converted to 0.91 µl/O_2/mg dry wt/h, which may be compared with the results from the present study at 30‰, of 2.48 µl O_2/mg dry wt/h "active", or 0.93 µl O_2/mg dry wt/h "rest". When these results from C. volutator are compared with those from other marine animals it may be seen that they are broadly similar (Table 13).

Table 13:

Clupea harengus (larva)	2.3-2.5	µl O_2/mg dry wt/h.	Holliday, et.al., 1964
Limnoria	1.253-1.853	"	Eltringham, 1965
Euphausia pacifica	1.47	"	Lasker, 1966

It/...

It has been found ~~that~~ when comparison was made of the effects of salinity on respiration of *C. volutator* of similar sizes, at the same level of activity, that no significant differences were noted between animals acclimated to different salinities, and these results will be discussed in the conclusion (Section 6.2).

5.5 SALINITY PREFERENCE.

The earlier sections have described the various ecological and physiological responses of *C. volutator* to salinity. It was suggested (Section 2.5) on the Ythan estuary that migration of animals in response to salinity occurred; this suggestion pre-supposes that the animals are able to detect the salinity, and exhibit a behavioral response to it. The presence and nature of such a response was investigated in the laboratory by means of simple choice experiments. Animals were presented with an alternative choice of salinity, in an apparatus (Fig. 39) based on that of Jansson (1962); a similar design has also been used by McGrorty (1968). Each compartment of the apparatus contained 500 ml. of a sea water solution of known salinity, and the animals were confined by means of bolting silk to two compartments each of 125 ml; the remaining 375 ml acting as a reservoir to ensure stable salinities. Salinities over a range of 0-50‰ were prepared by diluting sea water (collected at Aberdour, Fife) with copper-free tap water as appropriate, or for hypersaline media by evaporation of sea water. Into each compartment of the apparatus were/...

were carefully placed 10 C. volutator from the Ythan population, which had been acclimated to the salinity range of the experiment. The experiment was arranged so that the animals had a 5‰ choice (i.e. 5 v 10, 10 v 15‰), except in the case of 0 v 2.5 and 2.5 v 5‰. Uniform light and temperature conditions were maintained.

The animals were free to swim or crawl within each compartment, and to change compartments they were free to crawl across a ramp 2.5 mm wide. The animals were regularly observed, and counts made at 20 minute intervals. The final number in each compartment after 2 hours was noted. 2 hours was chosen because most C. volutator appeared to choose a compartment within 1 hour, (e.g. Table 14), and over a period of 2 h, the salinity in each compartment remained virtually constant. Minor changes in salinity could be detected after 5 - 6 h.

TABLE 14:

Time (min.)		0	20	40	60	80	100	120
No. in compartment.								
0‰	A	11	6	5	4	4	3	3
2.5‰	B	11	16	17	18	18	19	19

The individual results are presented in Fig. 40, and the cumulative results in Fig. 41. The significance of the numbers of animals in each compartment was compared by means of the binomial test.

From/...

FIGURE 39.

Apparatus for preference experiments.

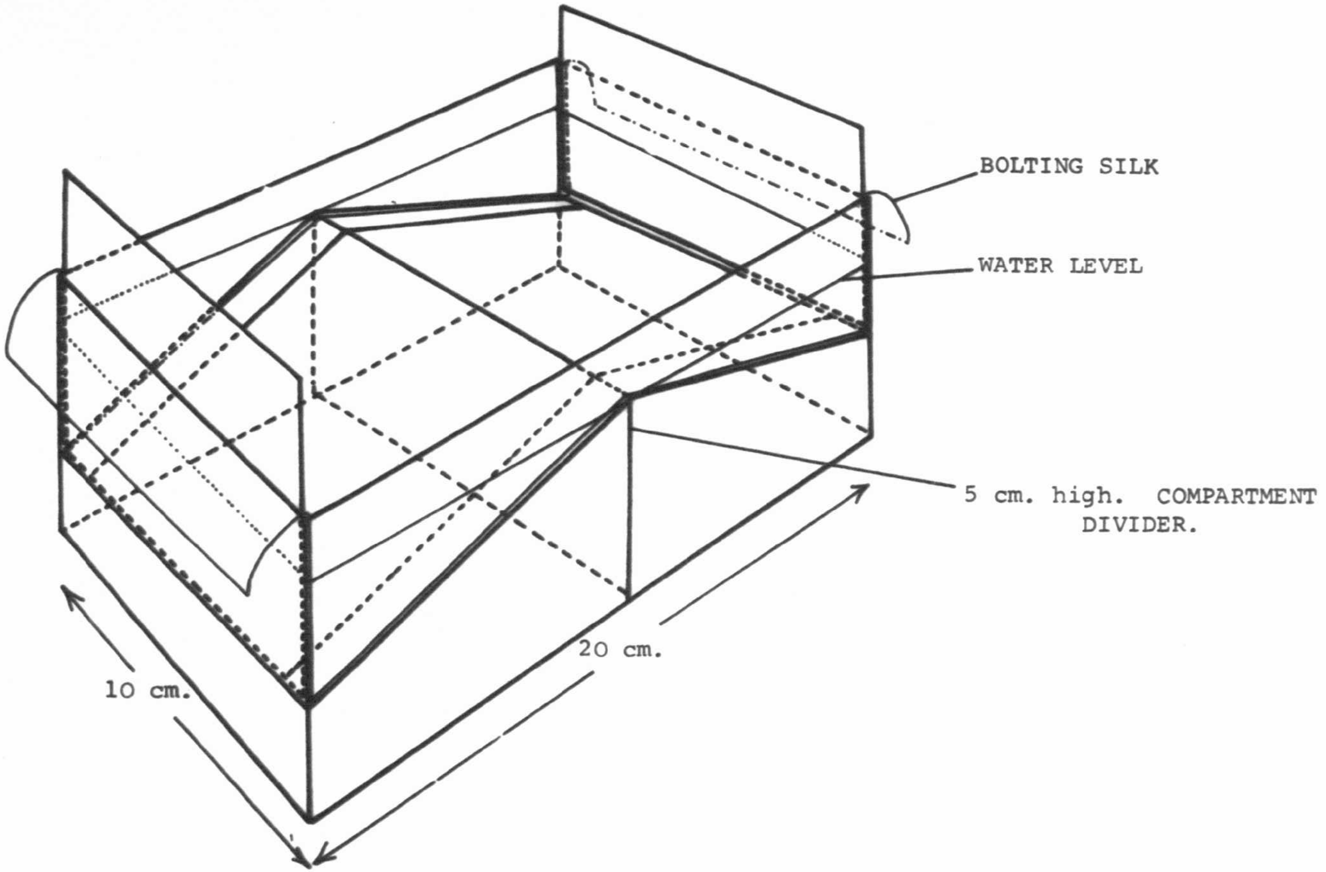

BOLTING SILK
WATER LEVEL
5 cm. high. COMPARTMENT DIVIDER.
20 cm.
10 cm.

FIGURE 40.

Salinity preference experiment. Percentage choices made in each experiment.

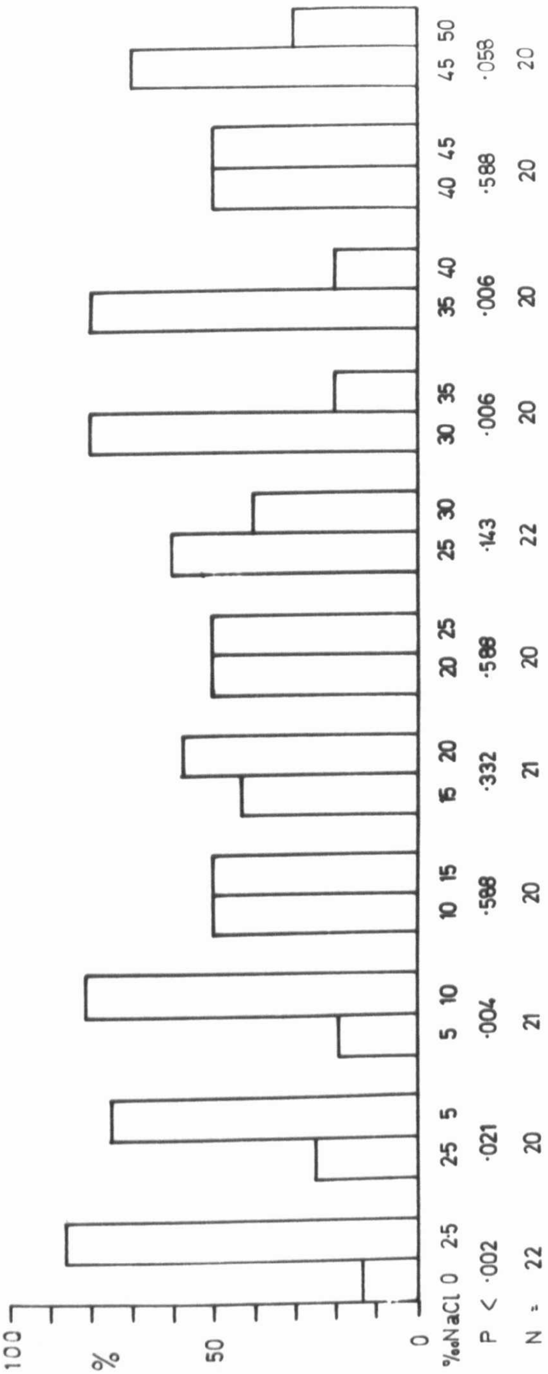
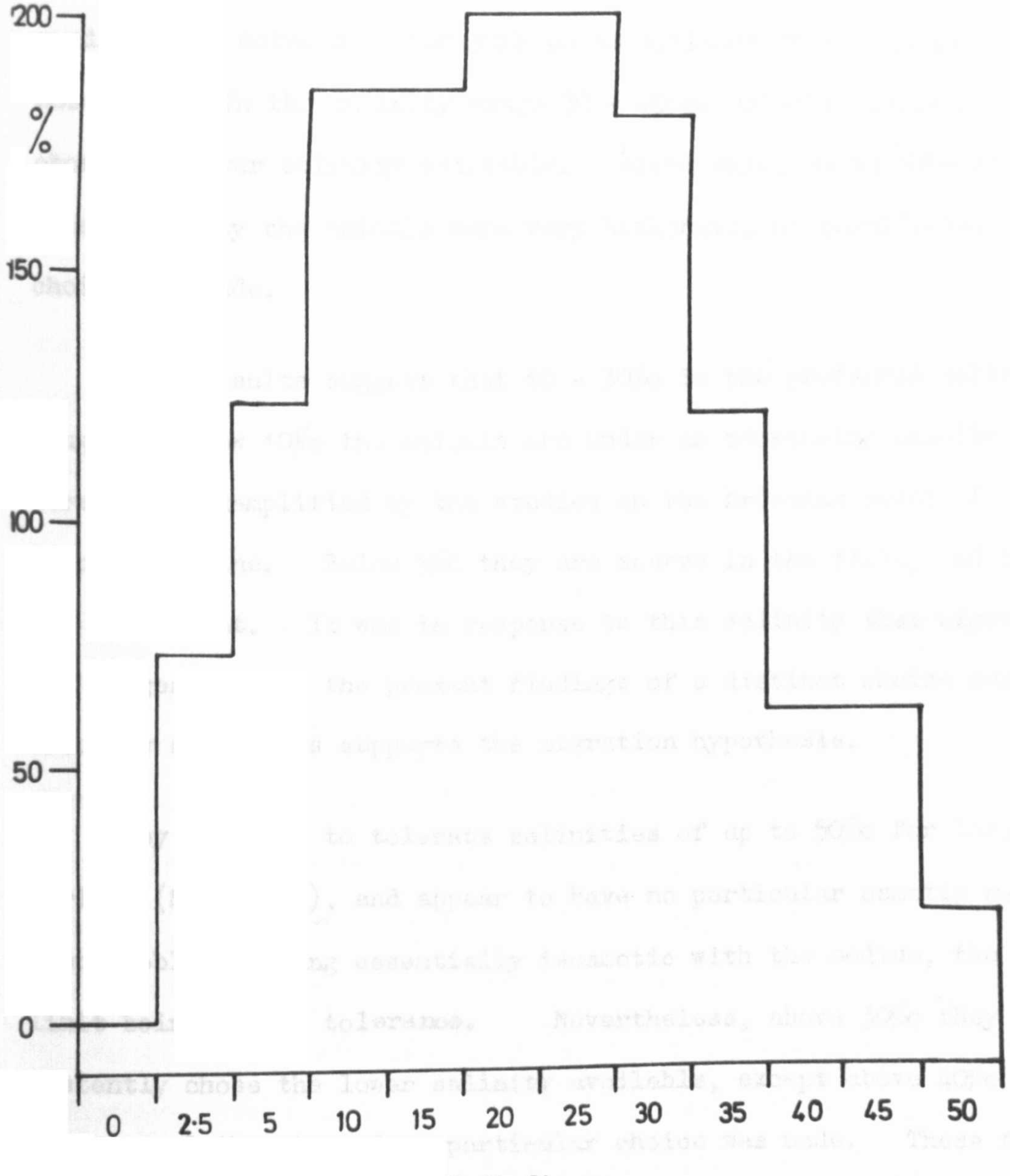

100
%
50
0
‰NaCl 0 2·5 2·5 5 5 10 10 15 15 20 20 25 25 30 30 35 35 40 40 45 45 50
P < ·002 ·021 ·004 ·588 ·332 ·588 ·143 ·006 ·006 ·588 ·058
N = 22 20 21 20 21 20 22 20 20 20 20

FIGURE 41.

Salinity preference experiment. Cumulative percentage choice.


200
%
150
100
50
0
0
2·5
5
10
15
20
25
30
35
40
45
50
‰ NaCl

From these results it may be seen that animals in salinities below 10‰ significantly and regularly chose the higher salinity available. Between 10 and 30‰ no significant choice pattern was observed. In the salinity range 30 - 40‰. animals significantly chose the lower salinity available. Above 40‰, up to 50‰ at which salinity the animals were very lethargic, no significant choice was made.

These results suggest that 10 - 30‰ is the preferred salinity range. Below 10‰ the animals are under an increasing osmotic stress, as exemplified by the studies on the freezing point of blood and urine. Below 5‰ they are scarce in the field, and below 2‰ are absent. It was in response to this salinity that migration was suggested, and the present findings of a distinct choice away from low salinities supports the migration hypothesis.

They are able to tolerate salinities of up to 50‰ for long periods (Sect. 3.1), and appear to have no particular osmotic regulation problems, being essentially isosmotic with the medium, the only limit being tissue tolerance. Nevertheless, above 30‰ they consistently chose the lower salinity available, except above 40‰ when they were lethargic and no particular choice was made. These findings suggest long-term acclimatisation to the salinity range 10 - 30‰, indeed this was also the optimal range for growth and moulting in this population.

It is thus seen that above 20‰, C. volutator is almost isosmotic to the medium, and the preference limit is set at 30‰, probably due to long-term acclimatisation. Below 20‰, the blood is maintained progressively hyperosmotic to the medium, and the animal is under "osmotic stress", however only below 10‰ will they significantly choose a higher salinity if available. C. volutator thus exhibits a distinct preference reaction to maintain it within the salinity range 10 - 30‰, which is also the normal salinity range of its estuarine habitat, and is thus behaviorally as well as physiologically adapted to the estuarine habitat.

Lindroth (1949; in Jansson, 1962) introduced the concept of stable and unstable preferences. Species with stable preferences showed a more or less constant prefered temperature, probably not influenced by previous treatment or changes in environmental factors. Animals with unstable temperature preferences showed a preferendum which varied with the time of day or year, the previous treatment etc. Lindroth stated that especially in eurytopic species the unstable preference type may be the normal one. Despite prior acclimatisation to the salinity ranges of the choice experiments, C. volutator showed a distinct stable preference for the range 10 - 30‰, which is the normal salinity range of its Ythan habitat. Perhaps this Ythan population of C. volutator has acclimatised over a prolonged period to this salinity range, and this preferance would indeed be proved to be "unstable" if compared with populations from, for example, hypersaline salt marshes. Jansson (1962) for Marionina preclitellochaeta found different preferenda for different populations suggesting prolonged salinity adaptation, and a similar suggestion might be made for C. volutator.

6. DISCUSSION

6.1 OSMOREGULATION OF CRUSTACEA

The subject of osmoregulation in crustacea has been extensively reviewed*. Reviews during the period up to 1962 were primarily concerned with descriptions of osmotic and ionic regulation, in particular blood concentration, in relation to environmental salinity. Since that time a dichotomy has occurred, with workers such as Kinne tending to concentrate on physiological ecology aspects of osmoregulation in crustacea, in particular the effects of salinity on whole crustacea, whether ecological, behavioral or physiological; whilst other workers (Lockwood, 1968; Scheer, 1965; Shaw, 1964) have concentrated on analyses of the dynamics of salt balance, primarily using results obtained with radio-active techniques. The work of Florkin and his associates (Sect. 4.5) on the analysis of intracellular regulation has also been given prominence.

Kinne (especially 1964a) has reviewed the effects of salinity on marine and brackish-water animals, including crustacea. He has pointed out that the factors, including salinity, which affect crustacea may be of a multi-variate nature, and it may frequently be difficult/...

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* Beadle, 1943, 1957, 1959; Dehnel, 1967; Green, 1968; Kinne, 1958, 1963a, 1963b, 1964a, 1964b, 1964c, 1966, 1967; Krogh, 1939; Lockwood, 1962, 1966, 1968; Magnin, 1964; Nicol, 1967; Pearse & Gunter, 1957; Potts, 1968; Potts & Parry, 1963, Prosser & Brown, 1961; Robertson, 1957, 1960b; Scheer, 1965; Schlieper, 1964; Shaw, 1964.

/...

difficult to assess the specific causes of the observed effects. Nevertheless, he suggests that salinity may affect an animal through changes in 1) total osmo-concentration, 2) relative proportion of solutes, 3) coefficient of absorption and the saturation of dissolved gases, 4) density and viscosity. As already noted (Section 3.6) the salinity tolerance of an animal may vary markedly according to the stage of its life cycle. In general salinity tolerance is more restricted during a sexual phase, than an asexual phase. Death at extremes of salinity may be usually attributed to osmotic phenomena, or changes in the concentration of gasses. Changes in salinity, in particular the total osmo-concentration may considerably affect metabolism, growth and food conversion of an animal.

Salinity stress may invoke various responses in a crustacean, including, 1) reduction of contact, 2) escape, 3) passive tolerance, 4) active regulation leading to homeostasis, 5) genetic and non-genetic functional and structural adaptation.

Reduction of contact may involve a reduction of surface permeability, the formation of burrows to avoid salinity stress, or the secretion of a protective film of mucus. Behavioral responses to salinity, including escape, have particularly been demonstrated by Gross (1957). Passive tolerance, as will be seen later, is only of limited value in wide-ranging salinity conditions. Active regulation will be dealt with extensively, later in this section. Functional and structural adaptations to salinity have been described by Kinne (1964 a, c).

Salinity stress may cause various non-genetic adaptations, (or acclimatisation) involving changes of metabolic rate, activity, body volume, or the concentration of the internal medium. Various genetic adaptations to salinity stress have also been noted, viz: 1) increased absorption and excretion of water and salt, 2) increased capacity for regulation of ionic ratios and osmoconcentration, 3) ability to store water and salts in tissues, 4) decreased permeability of surface, 5) behavioral regulation, 6) increased tissue tolerance, 7) structural modifications.

These various adaptations and mechanisms for regulation, will now be discussed in relation to osmoregulation in crustacea.

Marine species are generally unspecialised with regard to their osmotic physiology. They are usually in a state of osmotic equilibrium (isosmoticity) with sea water, although the ionic composition of the blood may differ significantly from that of sea water. In particular the concentration of magnesium is generally lower in the blood than in sea water, a feature related to the suggestion that magnesium inhibits activity, or nerve transmission (Robertson, 1953).

Following/...

Following dialysis experiments, Robertson (1957) has shown that the dialysate differed from blood in all species studied, a feature which is interpreted as showing that active transport of ions is involved in the maintenance of the blood concentration.

The evolution of crustacea from the sea to brackish water or fresh water has occurred independently many times. For life to proceed in media markedly different from sea water, physiological devices are required to protect the most sensitive tissues from extremes of salinity. This process, the evolution of osmoregulation, is generally considered as an extension of ionic regulation, as seen in marine forms.

More than one physiological solution has occurred to solve the problems of life in media markedly different from sea water, and bring about the evolution of euryhalinity from stenohaline animals.

When considering the demands of a euryhaline life, it is seen that the paramount requirement is to enable the tissues and cells to function normally and adequately, in spite of gross changes in the external environment. Claude Bernard's famous statement in 1857 that "la fixite du milieu interieur, est la condition de la vie libre" (see, Holmes, 1965) is both the basis and the main criterion of success in a euryhaline habitat. The cells must be protected from gross changes in volume or osmotic pressure, to enable the functioning/...

functioning of the cell to proceed. A stability must be preserved within the cell, and the cell must also be adapted to the osmotic pressure of its environment. Similarly the tissues may need osmotic control to enable them to function efficiently.

In crustacea, with their relatively hard exoskeleton, it can be seen that volume changes must be controlled, as far as possible, otherwise extreme hydrostatic pressures would occur. The tissues and cells of crustacea are bathed in an open blood system, the haemocoel, which permeates all parts of the body, and occupies a large part of the volume of the body. Since the blood of crustacea is the main fluid component of the body (le milieu interieur), it may be seen that the blood is vitally involved in any osmoregulatory process, since the blood is virtually the sole intermediary between the external environment and the cells and tissues. The regulation of blood concentration and volume by various mechanisms is therefore the principal feature of crustacean osmoregulation.

Various patterns of osmoregulation of blood concentration may be seen in euryhaline crustacea. Lockwood (1962) has classified these patterns as 1) isosmotic conformers, 2) hyper/isosmotic regulators, 3) hyper/hypo-osmotic regulators. Examples of these patterns are given in Fig. 42.

The/...

FIGURE 42.

Various examples of the patterns of crustacean osmoregulation. Based on Kinne (1963a).

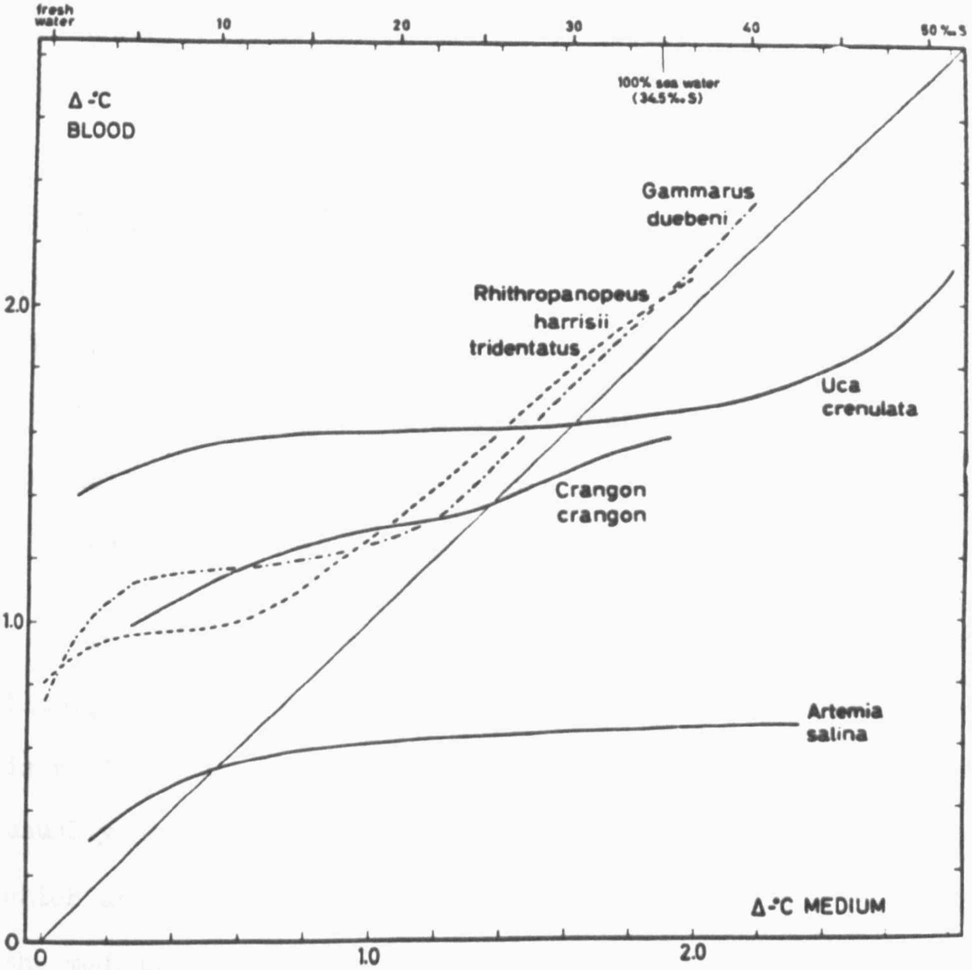


FIGURE 1. Blood-osmoconcentration as function of salinity in euryhaline Crustacea. Hyperosmotic regulators: amphipod *Gammarus* (from Kinne, 1952) and decapod crab *Rhithropanopeus* (Kinne and Rotthauwe, 1952). Hyper-hypo-osmotic regulators: decapod land crab *Uca* (Jones, 1941), shore shrimp *Crangon* (Flügel, 1959), and anostracan brine shrimp *Artemia* (Croghan, 1958b). (From Kinne, 1962).

The mechanisms bringing about these patterns of osmotic regulation may be briefly listed. (Lockwood, 1962, 1968):

1) Restriction of the permeability of the body surface.
2) Active uptake or extrusion of ions or water, via surface, gills or gut.
3) Regulation of body water volume.
4) Conservation of water or ions by excretory organ.
5) Regulation of cellular osmotic pressure.
6) Toleration at the cellular level of variations in blood concentration.
7) Ability to transport inorganic ions against a concentration gradient, and evolution of ion uptake mechanisms with high affinities.

In order to maintain an osmotic gradient between the medium and the blood, it is of great value if the permeability of the body surface is restricted. Marine crustacea which are isosmotic to the medium, usually have a freely permeable cuticle, whereas in euryhaline crustacea, which are often actively maintaining a blood concentration different from the medium, a freely permeable cuticle would allow a loss of ions, or make it otherwise difficult to maintain an osmotic pressure difference. The permeability of the cuticle is therefore strictly controlled. In most cases the only permeable areas are the gills, with in addition certain discrete patches on the cuticle. The remainder of the cuticle is regarded as non-permeable to ions. The permeability is controlled especially in small animals since the larger the turnover of ions, by gain or loss across permeable areas of the cuticle, the greater will be the energy that must be expended, per unit mass of tissue, in order to maintain a given gradient between blood and medium (Lockwood, 1962).

Active uptake of ions was first conclusively demonstrated by Nagel (1934), and was later confirmed, in particular by Krogh (1939). It is generally agreed that the majority of the osmotic pressure of crustacean blood is due to inorganic ions, especially sodium and chloride. In order to maintain hyperosmotic conditions in the blood, ions must betaken up from the environment. This uptake of ions must be almost continuous, since loss of ions may occur continually through urine, or across those parts of the body surface which are permeable. Branchipus apus (Krogh, 1939) and Chirocephalus diaphanus (Pannikar, 1941b) are unable to maintain their blood concentrations unless fed, a finding which has led to the suggestion that ions are taken up from the food. Ion uptake from food has also been suggested in Gammarus duebeni, Coenobita perlatus, Triops cancriformis. (Kinne, 1964a). Although Lockwood (1962) points out that the lack of food may cause a decline in metabolic rate, causing the observed decline in blood concentrations. It is also well known that terrestrial crustacea obtain ions from food.

Robertson (1957) suggested water and salt exchange via the gut, and the gut has been shown to be important for drinking by Dall (1967). Otherwise active uptake of ions has been suggested to occur at the body surface, or through organs such as gills. Krogh (1939) demonstrated that ions may be related to 1) concentration of the blood, 2) concentration of the medium, 3) temperature. Ion uptake mechanisms against a concentration gradient, and the evolution of transport systems with a high affinity for particular ions have been particularly studied by Shaw (1964), and the results of these experiments have been summarised by Lockwood (1968) and Potts & Parry (1963). The latter have diagrammatically represented the sodium fluxes (the balance of ion uptake and loss) for <u>Carcinus maenas</u> (Potts & Parry, 1963; Fig. IV.10). From this diagram, reproduced in Fig. 43, it may be seen that the active uptake of ions is related to the consentration of the blood and the medium, and how adjustments are made to maintain hyperosmotic conditions.

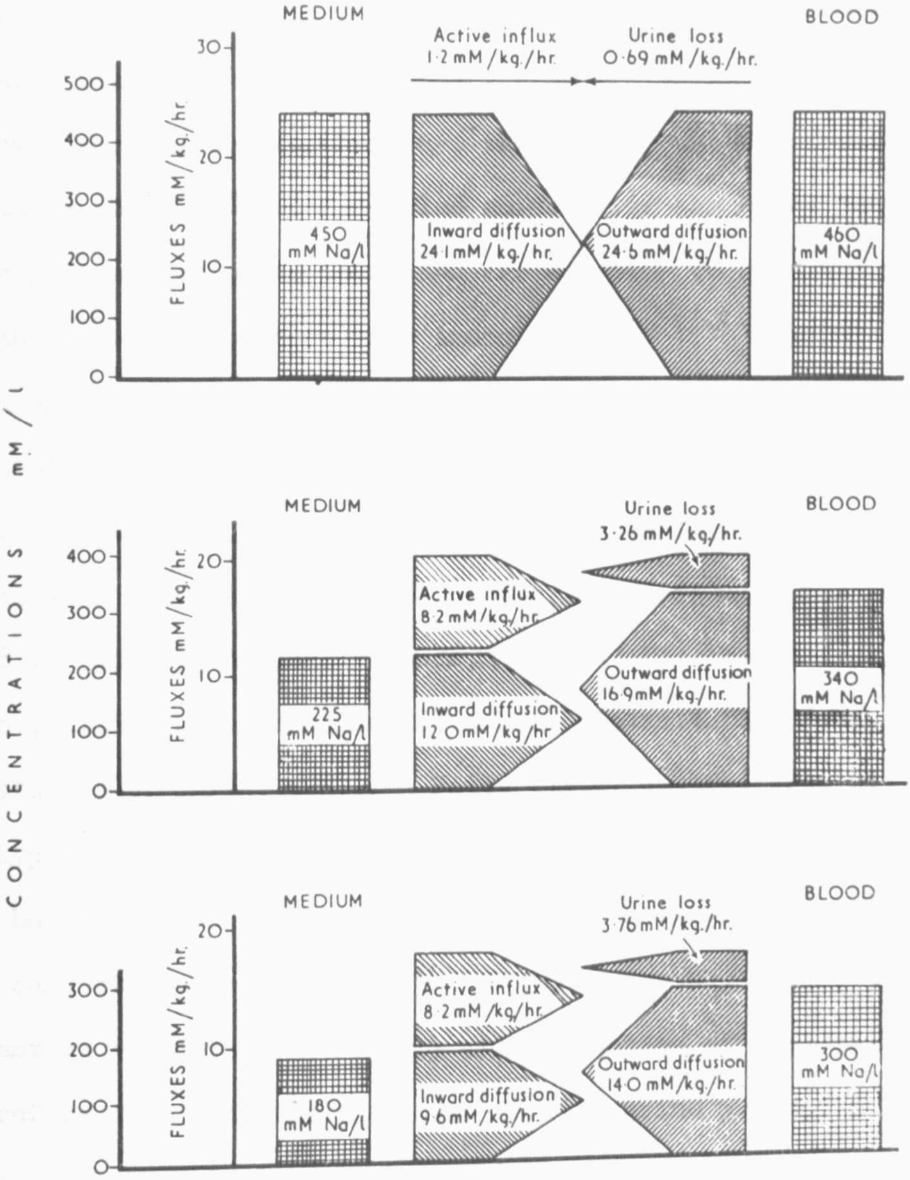
Active uptake, inward and outward diffusion, and active extrusion of salts and water, serve to maintain a relatively constant body volume which is a feature of osmoregulatory processes in crustacea, bound as they are by an exoskeleton.

Active extrusion of ions and water, as distinct from outward diffusion of ions or water, can occur via urine, usually from an antennary gland, via the gut, or via the body surface. In hypo-osmotic /...

FIGURE 43.

Diagrammatical representation of the sodium fluxes through *Carcinus maenas*. Reproduced from Potts & Parry (1963, Fig. IV.10).

DIAGRAMMATIC REPRESENTATION OF THE
SODIUM FLUXES THROUGH CARCINUS MAENAS



hypo-osmotic forms, the principal mechanisms for maintenance of the blood concentration has been shown to be drinking of water (Dall, 1967), rather than active extrusion of ions. In hyperosmotes passive extrusion of ions is believed to occur via the gut or body surface, with active extrusion occuring via the antennary gland. Urine formation serves to remove from the crustacean body, the waste products of metabolism (usually 60 - 85%ammonia; plus amino acids and other compounds) excess magnesium and sulphate ions, and excess water to regulate body volume. Metabolic waste, particularly ammonia, needs to be excreted in a liquid form and a certain amount of water must necessarily be excreted with it. Magnesium is excreted, since it is believed that an excess inhibits nervous activity, (Robertson, 1953). Urine formation is a distinct process of ultrafiltration, which may be modified by resorption (Potts, 1968). On hypothetical grounds, hyperosmotes should produce hypo-osmotic urine, although few examples have been produced (Lockwood, 1961, 1965; Werntz, 1963; Smith, 1967). Potts (1954) claimed that the formation of hypo-osmotic urine would conserve energy, however Croghan (1961) and Lockwood (1962) point out that if the mechanism of ion transport is the same at the body surface and in the excretory organ, then no energetic advantage would be conferred by the production of hypo-osmotic urine. Therefore they conclude that hypo-osmotic urine production may be related more to fine control of ionic regulation, than to energy conservation. Nevertheless the production of hypo-osmotic urine remains a distinct feature of small brackish water crustacea (Lockwood, 1965; Smith, 1967).

Cell osmotic pressure has been shown to be well regulated by Florkin and his co-workers (e.g. Camien, et.al., 1951; Jeuniaux, et.al., 1961; also Lange, 1968). Osmotic regulation of crustacean cells is largely accomplished by changes in the amino acid composition of the cells. Intracellular fluid is composed of approximately $\frac{1}{3}$ inorganic ions and $\frac{2}{3}$ small organic molecules. Gross shifts of water from cell to blood or vice versa, must be avoided in a homeostatic animal, and therefore cellular osmotic adjustment occurs, largely by an adjustment in the concentration of amino acids. Amino acids could be supplied from the blood, but this is doubted; or they could be formed <u>de novo</u>, but this is also unlikely. They are probably released within a cell by protein degradation. The amino acids enable a cell to maintain osmotic equilibrium with the blood, although clearly not ionic equilibrium. The external medium and the blood are usually in ionic balance, but may be osmotically different. Thus the blood acts as an osmotic barrier between the tissue cells and the external environment, preventing excess changes in the osmotic pressure of the cell. However it is energetically impracticable, especially in small crustacea, to maintain the blood concentration completely independent from the environment, thus the blood concentration in a euryhaline crustacean, is adapted to change in relation to the environment. But the changes in the blood concentration are generally smaller

than/...

than changes in the medium - thus the blood serves to "dampen" the osmotic extremes of the environment. Since the cells are in osmotic equilibrium with the blood, they must tolerate these dampened changes in osmotic pressure. The evolution of the cellular ability to withstand limited changes in osmotic pressure, is therefore vital to euryhaline crustacea. This dampening effect of the blood is well seen in the slow adaptation of blood concentration following a sudden change of medium, as the blood buffers the cells from the sudden change in osmotic pressure.

It has thus been seen that a variety of patterns and mechanisms have been evolved for osmoregulation in euryhaline crustacea. Considering hyperosmotic regulation of crustacea in low salinities, (which is or particular relevance to this study of *C. volutator*) it may be seen that the maintenance of hyperosmotic conditions may be brought about by active uptake and extrusion of ions, via the body surface, gut and antennary gland. The amount of work required to combat diffusion may be lessened by a reduction in the permeability of the body surface, and a restriction in ion turnover rate. Hyperosmotes tend to gain water and lo~~o~~se ions, therefore to maintain equilibrium they particularly take up ions (via body surface or gut) and remove water (via hypo-osmotic urine). Cellular control of osmotic pressure has also been shown to be an

important/...

important means of maintaining an internal homeostasis. These mechanisms combine to give a hyperosmotic crustacean some measure of a constant internal environment, in the face of a wide ranging external environment.

The energy expended in osmoregulation has been seen to be conserved by permitting some decrease in the internal environment, in response to a decrease in the concentration of the external medium. This conservation of energy being particularly vital to a small animal, with a large surface area.

Osmoregulation in crustacea is therefore a well developed physiological system, which maintains a balance, rather than a perfect homeostasis, within the crustacean body. Osmoregulation maintains a constancy of the internal environment, which permits life in a fluctuating euryhaline or dilute environment, in a group of animals which first evolved in the sea.

6.2 CONCLUSION

Laboratory and field studies of the effects of salinity on C. volutator have shown that fed adult animals may tolerate the salinity range 2 - 50‰, and newly hatched young may tolerate down to 0.75‰, although fertilisation, embryonic development and fecundity appear to be limited below 7.5‰. Highest growth rate, moulting and maximum field abundance occurred between 5 and 30‰. *C. volutator* was found experimentally to choose the salinity range 10 - 30‰. The effects of salinity on the life cycle may be observed, in particular the changes between young, adults and fertilisation stages (Fig. 11), following a general crustacean pattern noted by Kinne (1964a, 1966). The adverse effects of low salinity on the physiological processes of *C. volutator*, such as growth and moulting, and the survival of unfed animals have also been seen.

A study of the osmotic regulation of *C. volutator* has shown that it is a hyperosmotic regulator, maintaining its blood concentration almost isosmotic to the medium above 20‰, and progressively more hyperosmotic to the medium below 20‰, reaching a maximum hyperosmoticity at 2‰. Below 2‰ it would appear that a concentration gradient in excess of 650% is too great for *C. volutator* to maintain. It was suggested (Sect. 4.3) that in low salinities that the reduction in blood concentration concomitant with moulting may be fatal. This may explain the inhibition of moulting below 5‰.

The ions within the blood, which contribute to the majority of the hyperosmoticity have been found to be regulated in a manner comparable with other brackish water crustacea.

Osmoregulation/...

Osmoregulation has been noted to be assisted by a low surface permeability, and by the production of hypo-osmotic urine. The generalisation of Smith (1967), based on that of Lockwood (1965), that: "the conservation of ions within the body by the production of hypotonic urine is likely to be found to be a common feature in those brackish and freshwater crustacea which combine a high rate of water turnover with a significant reduction in the salt-permeability of the body-surface" has found support in this study.

Measurements of the oxygen consumption of C. volutator have shown no significant changes in relation to salinity.

The various findings of this study lead to three alternative hypotheses:

1) food is more important as a direct supply of inorganic ions than as an energy supply.
2) the metabolic requirements of osmoregulation are negligible.
3) a shift in the energy requirements occurs from one metabolic process to another.

It has been shown that unfed animals by comparison with fed animals, cannot survive low salinities or maintain their blood concentration (Sect. 4.2) and it was suggested that this may be due to their use of food (mud) as a direct supply of ions, or as an energy source for osmoregulation, but Nagel's (1934) experiments, repeated on C. volutator which were both fed and unfed, demonstrated that active uptake of ions may be accomplished without food. When comparing the results of these experiments, it is necessary to consider the time scale, the adaptation to a salinity increase was completed in 3 hours, whereas the survival experiment was made over a period in excess of 500 hours. It is seen that food is necessary for long-term survival at low salinities, but that short-term uptake of ions and maintenance of hyperosmotic conditions may be accomplished without direct feeding and that the energy reserves of the animal are adequate to cope with the uptake of inorganic ions. Lockwood (1962) points out that the lack of food may cause a decline in metabolic rate, causing the observed decline in blood concentration.

It has been noted that C. volutator produces urine hypo-osmotic to the blood under conditions of osmotic stress, and the demonstration of Potts (1954) that the production of urine hypo-osmotic to the blood, even if more concentrated than the medium, can greatly reduce the osmotic work of an animal, may lead to a partial explanation for the lack of any change in oxygen consumption in different salinities, although Croghan (1961) and Lockwood (1962) suggest that the production of hypo-osmotic urine may be related more to the fine control of ionic regulation than to energy conservation.

It/...

It has been noted that the optimum salinity range for maximal growth was smaller than the range of salinities tolerated. It may be that in low salinities, under osmotic stress, the animal is able to survive, and no changes in oxygen consumption are noted, because a metabolic shift occurs within the animal, and energy is diverted from growth to assist in osmoregulation. Thus the energy requirements for osmoregulatory processes would be increased under osmotic stress, but no overt change in oxygen consumption could be detected.

Lockwood (1962) rejects the importance of food for the ions contained in it, and also dismisses any energetic advantantage conferred by the production of hypo-osmotic urine. However, Oglesby (1965) suggested for <u>Nereis limnicola</u> that the production of hypo-osmotic urine and a supply of ions via the food were important factors in osmoregulation.

It is most likely that the various mechanisms discussed each contribute in some part to hyperosmotic regulation and permit <u>C. volutator</u> to live successfully and efficiently in the nutritionally rich estuarine habitat. These mechanisms are briefly:

1) Restricted surface permeability
2) Hypo-osmotic urine
3) Food as a supply of ions and/or energy
4) Active uptake and regulation of ions
5) The ability to divert energy from one physiological process (probably growth or reproduction) into osmoregulation.

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APPENDIX I

Data from each Field Study Area~~s~~, for each month.

ANIMALS PRESENT AT AREA A.

MEAN NUMBER PER SAMPLE PER MONTH

MONTH	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JLY	AUG	SEPT	OCT	NOV
INTERSTITIAL SALINITY ‰	17.8	14.0	9.2	10.3	9.9	13.2	9.3	5.5	11.7	16.5	25.8	27.2	17.6	7.5
1.0 - 1.9 m.m.	2.0	4.8	5.2	2.0	0.2	1.4	0.4	0	17.0	22.2	12.8	3.8	1.6	0
2.0 - 2.9	6.0	11.2	11.6	8.8	10.0	8.4	2.8	0.2	15.2	31.2	60.2	24.8	10.6	4.8
3.0 - 3.9	7.4	8.0	9.4	6.0	13.2	7.6	5.4	1.4	0.4	16.8	30.8	20.0	19.0	12.2
4.0 - 4.9	5.6	3.6	7.0	6.4	7.4	4.6	5.2	3.4	0.2	13.2	17.6	19.8	19.0	15.0
5.0 - 5.9	4.2	4.0	5.0	4.0	5.0	4.4	4.2	3.0	1.0	10.4	8.6	11.4	7.2	8.8
6.0 - 6.9	2.0	2.0	2.2	1.2	3.0	4.0	2.8	3.8	2.4	5.2	3.6	6.0	5.4	6.0
7.0 - 7.9	1.4	2.0	1.4	0.4	1.2	1.2	3.2	3.8	4.0	2.8	1.6	2.4	2.4	2.8
8.0 - 8.9	0.6	0	0	0.2	0.6	0.2	0.8	1.8	3.2	5.2	2.8	0.6	0.2	0
9.0 - 9.9	0	0.2	0	0	0	0	0.2	0.2	0.8	0.8	0.4	0	0	0
TOTAL	29.2	35.8	41.8	29.0	40.6	31.8	25.0	17.6	44.2	107.8	138.4	88.8	65.4	49.6

ANIMALS PRESENT AT AREA B.

MEAN NUMBER PER SAMPLE PER MONTH.

MONTH	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JLY	AUG	SEPT	OCT	NOV
INTERSTITIAL SALINITY %o	15.6	11.4	2.25	4.8	3.5	12.0	6.2	1.15	10.5	13.6	19.3	23.6	11.8	5.0
1.0 - 1.9 m.m.	0.6	0.2	1.3	0.2	0.4	0.2	0	0	1.4	1.6	8.4	1.8	0.4	0
2.0 - 2.9	2.8	3.4	6.0	0.8	1.6	2.0	0.6	0	0.4	12.4	24.4	21.4	7.4	3.0
3.0 - 3.9	1.6	1.8	2.0	1.2	2.6	1.8	1.8	0	0.2	12.8	21.2	17.2	18.4	14.4
4.0 - 4.9	1.8	3.2	2.0	1.0	1.4	1.2	2.8	1.2	0	6.2	13.6	14.2	14.0	12.4
5.0 - 5.9	1.4	1.4	3.3	0.4	0.6	1.2	1.6	1.6	0.4	3.6	8.2	9.4	7.0	7.2
6.0 - 6.9	0.4	0.6	0.3	0	0.4	0.4	0.2	2.0	0.4	2.4	2.0	5.6	3.4	3.2
7.0 - 7.9	1	0.4	1.0	0.2	0.6	0.2	0.2	1.8	1.0	0.2	0.4	1.2	1.4	0.6
8.0 - 8.9	0	0	0.3	0	0	0	0	0.4	1.2	1.0	0.2	0	0	0
9.0 - 9.9	0	0	0	0	0	0	0	0.2	0.2	0.6	0	0	0	0
TOTAL	9.6	11.0	16.2	3.8	7.6	7.0	7.2	7.2	5.2	40.8	78.4	70.8	52.0	40.8

ANIMALS PRESENT AT AREA C.

MEAN NUMBER PER SAMPLE PER MONTH

MONTH	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JLY	AUG	SEPT	OCT	NOV
INTERSTITIAL SALINITY ‰	6.7	6.9	1.05	2.4	1.05	5.4	2.25	0.9	3.4	8.5	13.5	15.0	11.8	2.2
1.0 - 1.9 m.m.	0	0.4	1.0	0	0	0	0	0	0	0	1.4	1.8	0.4	1.0
2.0 - 2.9	0.6	1.4	0	0.8	0.4	0	0.2	0	0	0.6	2.8	8.4	7.0	7.0
3.0 - 3.9	0.4	0.6	0	0	0.4	0	0.2	0.2	0	0.8	5.0	7.0	12.8	10.6
4.0 - 4.9	0.6	0	0	0	0	0.2	0	0.2	0	0.8	4.0	5.8	7.0	8.6
5.0 - 5.9	0	0	1.0	0.2	0.2	0.2	0.2	0.4	0	0.2	2.6	5.8	4.0	3.8
6.0 - 6.9	0	0	0	0.2	0	0	0.2	0	0	0.4	2.0	3.4	2.8	0.6
7.0 - 7.9	0	0	0	0	0	0	0	0.6	0.2	0	0.8	1.8	1.0	0.2
8.0 - 8.9	0.2	0	0	0	0	0	0	0.2	0.2	0.4	0.2	0.4	0.2	0
9.0 - 9.9	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0
TOTAL	1.8	2.4	2.0	1.2	1.0	0.4	0.8	1.6	0.6	3.2	18.8	34.4	35.2	31.8

ANIMALS PRESENT AT AREA D.

MEAN NUMBER PER SAMPLE PER MONTH

MONTH	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JLY	AUG	SEPT	OCT	NOV
INTERSTITIAL SALINITY ‰	1.3	0.75	0.8	1.05	0.95	1.1	1.3	0.8	0.9	3.7	6.4	8.5	4.1	1.1
1.0 - 1.9 m.m.	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0
2.0 - 2.9	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0
3.0 - 3.9	0	0	0	0	0	0	0	0	0	0	1.5	0	0.5	0
4.0 - 4.9	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0
5.0 - 5.9	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0
6.0 - 6.9	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0
7.0 - 7.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8.0 - 8.9	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0
9.0 - 9.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	0	0	0	0	0	0	0	0	0	2.0	2.0	2.0	1.0	0

APPENDIX 2

Published Papers.

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SOME EFFECTS OF SALINITY ON THE SURVIVAL, MOULTING, AND GROWTH OF *COROPHIUM VOLUTATOR* [AMPHIPODA]

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(Text-figs. 1–5)

An experimental study of the effects of salinity on the mud-dwelling amphipod, *Corophium volutator* (Pallas), indicates that, if supplied with mud, it will survive the salinity range of 2–50 ‰, and without mud the range 7·5–47·5 ‰. Moulting occurred in salinities of 2·6–46 ‰, but most frequently in the range 5–20 ‰. Growth occurred at a maximum rate in 15·4 ‰, and only slightly slower at 4·4 and 30·6 ‰; but below 4·4 ‰ the growth rate was progressively reduced. Freezing-point studies show *C. volutator* to be a hyperosmotic regulator, having a tissue tolerance range of 13–50 ‰. The importance of a supply of mud, and the significance of hyperosmotic regulation are briefly discussed.

INTRODUCTION

The amphipod *Corophium volutator* (Pallas) is an inhabitant of littoral muds, the populations often reaching high densities. It has been most commonly reported from shores of estuaries, although Zenkevitch (1963) has stated that it may also occur in muds submerged to a depth of 10 m. Segerstråle (1959) has summarized the data available on the occurrence and distribution of *C. volutator*. Since that date, work has been done on substrate selection (Meadows 1964*a–c*), on burrowing behaviour (Meadows & Reid, 1966) and on rhythmical swimming activity under tidal control (Morgan, 1965). Many authors (Nicol, 1935; Thamdrup, 1935; Beanland, 1940; Spooner & Moore, 1940; Rees, 1940; Goodhart, 1941; Stopford, 1951; Rullier, 1959; Gee, 1961) have described the substrate in which *C. volutator* occurs. They have agreed that *C. volutator* is found in mud or muddy sand, containing approximately 37 % silt or clay. *C. volutator* is especially abundant in sheltered conditions, and is not found in conditions of heavy pollution, sand without a plentiful supply of detritus, or sulphide mud which is blackened by excessive organic detritus. From the published record it is clear that the nature of the substrate is a most important factor in controlling distribution.

C. volutator has been most frequently reported in estuarine waters, or seas such as the Baltic, that have a reduced salinity. During the course of faunal surveys, many authors have reported a lower limit of salinity below which

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C. volutator was not found. Goodhart (1941) reported 6‰ from Hampshire; Nicol (1935), Aberlady Bay, 5‰; Stock & Vos (1961), Holland, 4‰; Hart (1930), Norfolk, 3·6‰; Zenkevitch (1963), Sea of Azov, 3·6‰; Beanland (1940), River Dovey, 3‰; Hellén (1919), and Gurjanova (1951), Gulf of Finland, 2‰. These variations in the lower limits observed may reflect the varying distribution of suitable substrates in the areas studied. At the upper limit of salinity it frequently occurs in sea water (33–35‰), and Fox *et al.* (1926–29) reported the presence of *C. volutator* f. *orientalis* in hyper-saline water in Lake Timsah in the Suez Canal. However the latter was probably *C. arenarium* Crawford (see Segerstråle, 1959).

C. volutator is therefore clearly a euryhaline species, being found in a wide range of salinities, and its ecology and distribution is well documented. However, little of its physiology appears to have been studied. Agrawal (1963) studied the physiology of digestion. *C. volutator* is a selective deposit feeder, which feeds by the ingestion of mud (Meadows & Reid, 1966). I have been unable to trace any published work on osmo-regulation.

The muddy shores of estuaries have been colonized by relatively few animal species (Yonge, 1953), and the ability to live in waters liable to a wide range of salinity fluctuations is seldom achieved. Clearly, adaptation to such conditions is necessary before an animal can thrive in the estuarine habitat. Kinne (1964*a*) has defined adaptation as the adjustment of organisms to alterations in the intensity pattern of variables in the environment. Two of the principal variables in the environment inhabited by *C. volutator* are substrate, studied by Meadows (1964*a–c*), and salinity.

On the estuary of the River Ythan, Aberdeenshire, Scotland, a study has been made of the distribution of *C. volutator*. It was clear that within the central part of the estuary, in the salinity range 5–35‰, *C. volutator* occurred most abundantly wherever there was a suitable substrate, which is in agreement with the findings of Hart (1930). At the seaward end of the estuary, *C. volutator* did not occur, because of the presence of clean sand containing little detritus. At the inland part of the estuary, despite the occurrence of apparently suitable substrate, it was never found where the interstitial salinity of the mud was below 2‰. Within the range 2–5‰ *C. volutator* was present, but in lesser numbers than in muds with an interstitial salinity greater than 5‰.

An experimental study has been made in order to ascertain more precisely the effect of salinity on *C. volutator* and, to determine the adaptation, if any, of the organism to salinity.

SURVIVAL

C. volutator were collected from the River Ythan, and transported to stock tanks (maintained at 18‰, and at 11° C) in Aberdeen.

To each of a series of 5 cm Petri dishes was added 10 ml. of sea water of known salinity. Salinities below 35‰ were prepared by diluting sea water with distilled water, and the salinity was then determined by titration. For hypersaline solutions, sea water was gently evaporated over a bunsen burner without boiling.

To each of these dishes at each salinity, an individual *C. volutator* was added; and then to half of the dishes a small quantity of fresh mud, which had been kept in the appropriate salinity, was added. The dishes were kept in a constant temperature room at 8 °C, within a humid chamber, to prevent evaporation. The dishes were examined daily, any moults removed, and any deaths of *C. volutator* noted.

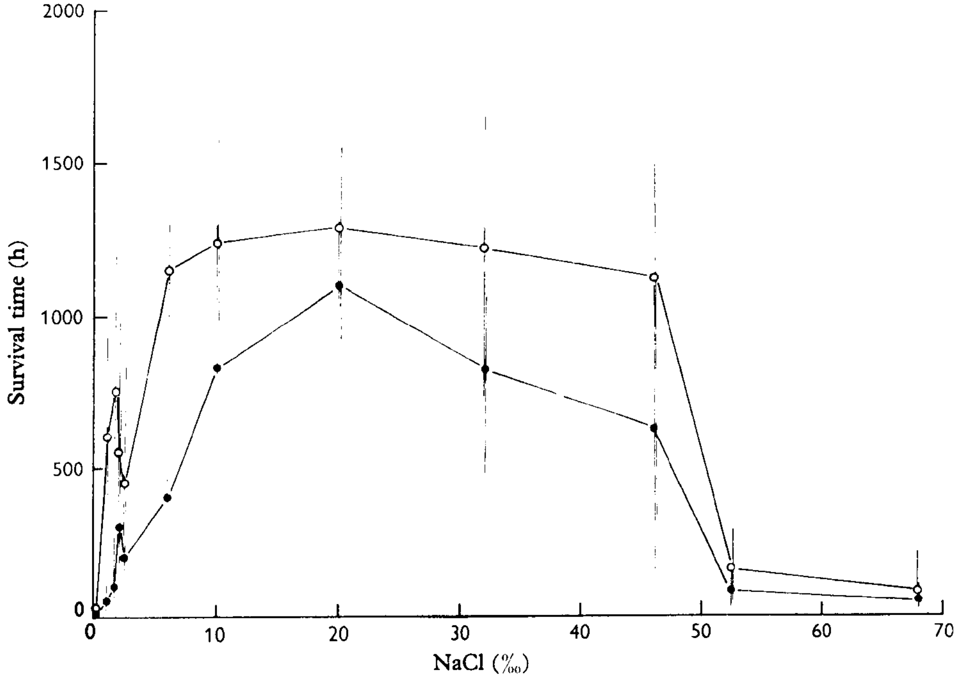

Fig. 1. The effect of salinity on the survival time of animals with (○), and without (●) mud.

The results of this experiment (Fig. 1), indicate that the animals survived well under the restricted experimental conditions, some living for over 1500 h (2 months) in the Petri dishes. A clear difference between those animals supplied with mud, and those without mud can be seen. If survival of *C. volutator* is expressed as the ability for half of the population to live for 500 h (20 days), then it may be seen that animals supplied with mud can survive in water of salinities between 2 and 50‰. Using the same criterion, for the animals without mud, the range of salinities survived becomes 7·5–47·5‰.

Individual variation in survival time occurred, but almost always those animals with mud survived longer than those without mud. In salinities 10–35‰ the supply of mud increased survival time by an average of 34%. In salinities below 10‰, survival time was more markedly increased by the presence of mud (265% at 6‰, and 118% at 2·6‰).

An experiment was designed to test whether the dependence of *C. volutator* on the presence of mud was due to: (i) food value (including inorganic ion content), or (ii) physical presence. Animals were maintained at 5‰ with acid-cleaned fine sand (B.D.H.), the sand being thoroughly washed, and the sea water sterilized. Others were maintained in the sterilized sea water, some without any substrate and some with fresh mud. It was found that those with fine sand lived for an average of 164 h, and those without any substrate for an average of 178 h. This difference is not significant ($P = < 0.3$). Those animals supplied with mud lived for an average of 707 h, which is significantly different ($P = < 0.01$) from those with fine sand or without any substrate.

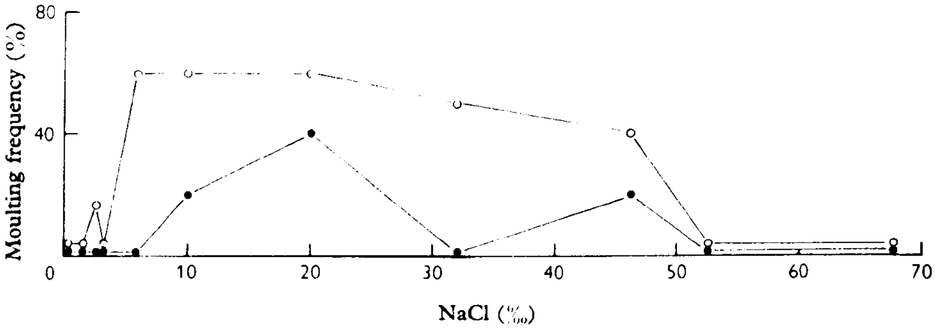

Fig. 2. The effect of salinity on moulting frequency in animals with (○), and without (●) mud.

Thus it may be suggested that mud is necessary for the survival of *C. volutator* either for the supply of food as energy for osmo-regulation, or for the direct supply of ions which are taken up via the gut.

MOULTING

Moulting occurred (Fig. 2), in animals supplied with mud, in sea water between 5 and 46‰, and in one individual at 2·6‰. In animals without mud moulting occurred less frequently, and only in the salinity range 10–46‰. By comparison with the data for survival time at different salinities, it may be seen that the range of salinities for successful moulting is smaller. This smaller range may be especially seen at low salinities—animals supplied with mud will survive down to 2‰, but only moult regularly down to 5‰. Moulting occurred most frequently in the range 5–20‰, and less frequently up to 46‰.

GROWTH

To determine the effect of salinity on growth, a large sample of mud was collected, and divided into six equal parts. Each part was placed in an aquarium tank, and covered to a depth of 15 cm with a sea-water solution of known salinity (0·8, 2·4, 3·6, 4·4, 15·4, 30·6‰). The mud and water were thoroughly mixed, and the mud allowed to settle. The salinity of the water was determined, and maintained at that salinity during the subsequent weeks.

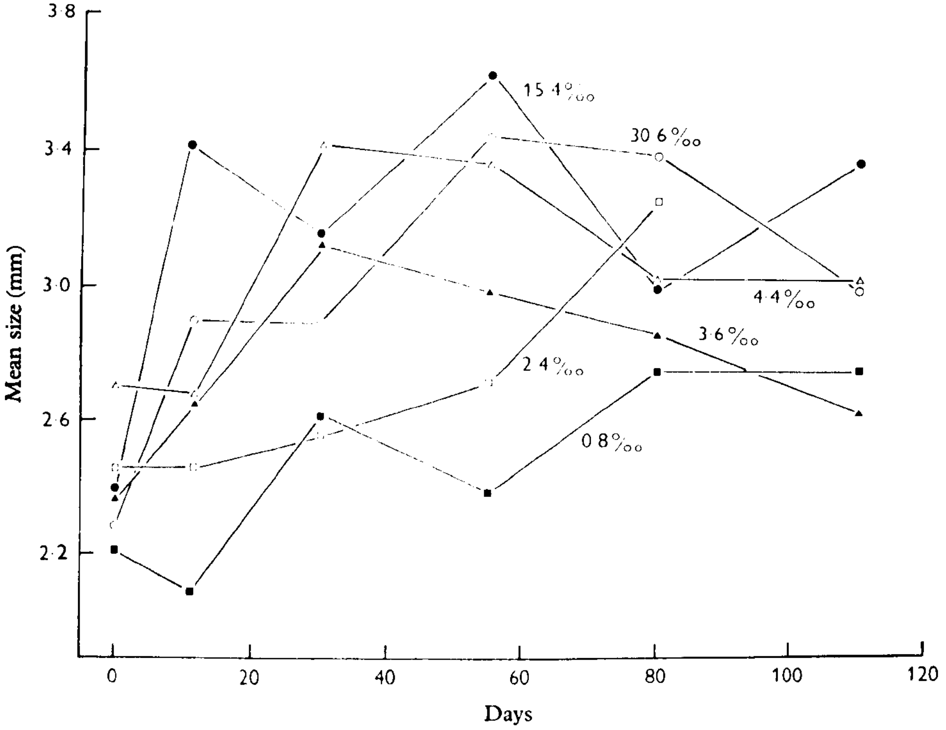

Fig. 3. The mean size of populations maintained in tanks at 0·8, 2·4, 3·6, 4·4, 15·4 and 30·6‰.

The tanks were aerated, and kept at 11 °C. On the first day and after 11, 30, 55 and 80 days, a 40 ml. sample of the mud was collected, and sieved with a 335 μ sieve. This sieving was performed in the water of the experimental tank, in order to conserve the mud. The animals so collected were counted, measured and assigned to millimetre size groups (i.e. 1·0–1·9 mm., 2·0–2·9 mm., 3·0–3·9 mm., etc.), and then returned to their tanks.

The mean size of each population, at each salinity, during the experiment, can be seen in Fig. 3. The initial population, collected on 9 November 1966, had a mean size of 2·40 mm. During the first 55 days, the population growth rate was fastest at 15·4‰, increasing from a mean of 2·39–3·71 mm (+55%), 30·6 and 4·4‰ were only slightly slower, and there was a serial decline in growth rate below 4·4‰, such that 3·6 > 2·4 > 0·8‰.

An analysis of the different sizes, by percentage, in each population (Fig. 4), shows more precisely the mode of growth in each population. Initially animals

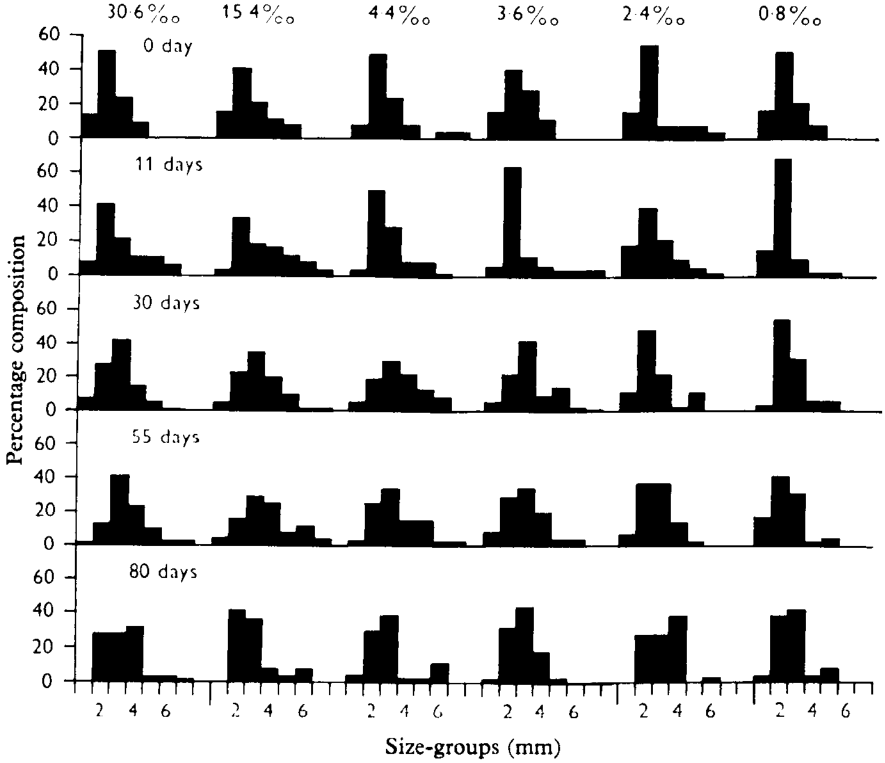

Fig. 4. Percentage size composition of populations maintained in tanks at 0·8, 2·4, 3·6, 4·4, 15·4 and 30·6‰, sampled initially and at 11, 30, 55 and 80 days.

measuring 2·0–2·9 mm. formed 47% of the population. In the lowest salinity (0·8‰) little growth occurred, and after 55 days this category still formed 42% of the population, whilst the percentage of animals measuring 3·0–3·9 mm. had increased from 21 to 31%. In salinities 30·6–3·6‰, the percentage of animals in the 2 mm category became reduced from 47 to 23% within 30 days, by which time the 3 mm category had become more numerous than the 2 mm category. In 2·4‰, 55 days were needed for the 3 mm category to equal the 2 mm category (both 37%). These shifts in percentage composition represent growth of the individuals, as over-all numbers remained constant, and reproduction did not occur.

Initially only 1·7% of the population was larger than 5 mm; however, growth of animals occurred rapidly in salinities 30·6–4·4‰, and within 55 days 25% at 15·4‰, 21% at 4·4‰, 17% at 30·6‰ were larger than 5 mm. By comparison 8% at 3·6‰, 4% at 2·4‰ and 5% at 0·8‰ were larger than 5 mm. after 55 days.

As growth occurred, the number of animals in the 1·0–1·9 mm category fell. In 15·4‰ there were initially 16% of the animals in this category, but by 55 days it had fallen to 4·2%. In all other salinities, except 0·8‰, a similar decrease in the percentage at 1 mm occurred; however, in 0·8‰ the percentage remained constant at 17%.

After 55 days mortality occurred in the populations. In salinities of 30·6–3·6‰, this mortality affected the larger animals most severely, with the result that the percentages of smaller-sized animals increased. In 2·4 and 0·8‰ mortality occurred, but appeared to be more uniformly spread throughout the size groups.

In conclusion, it can be seen that only very slow growth occurred at 0·8‰. Maximal growth was achieved at 15·4‰, and 30·6 and 4·4‰ attained a slightly slower growth rate. Animals in 3·6‰ grew well within the first 30 days, but failed to maintain that growth rate; and animals at 2·4‰ had a slow but steady growth rate.

BLOOD CONCENTRATION

In order to follow in detail the physiological changes and adaptations that take place in *C. volutator* subjected to water of various salinities, a study was made of the changes occurring in the blood concentration of *C. volutator*, in relation to the salinity of the external medium.

Prior to sampling, animals were kept supplied with fresh mud, in various strengths of sea water, for at least 2 days. The freezing point of the animal's blood and of the water in which they had been living was determined. Since the volume of blood in an individual *C. volutator* is very small, the depression of the freezing-point method, using equipment based on that of Ramsay & Brown (1955) was adopted, to determine accurately the osmotic concentration of the blood.

To obtain a sample of the blood of *C. volutator*, the live animal was quickly dried with a tissue, to remove any surface moisture, and placed on a watch-glass coated with a hydrofuge varnish (Bakelite damarda), and covered with medicinal paraffin. The animal was cut transversely in half, whilst under the paraffin. A small amount of paraffin was sucked into a fused silica microtube, and then the tube inserted into the haemocoel, at this point the animal was gently squeezed by a pair of forceps, and a small amount (10^{-3}mm^3) of blood sucked into the microtube, followed by more paraffin, so that four or five samples from one animal were collected into each microtube. The blood sample was then quickly frozen, and the melting point determined.

Results indicate a clear pattern of regulation (Fig. 5). At 50‰, the blood is isosmotic with sea water. Between 20 and 50‰, the blood concentration follows closely that of the external medium, just maintaining a hyperosmotic level. Below 20‰ the hyperosmoticity increases progressively, such that the concentration of the blood never falls below a salinity equivalent to 11‰ (Δ °C = 0·66).

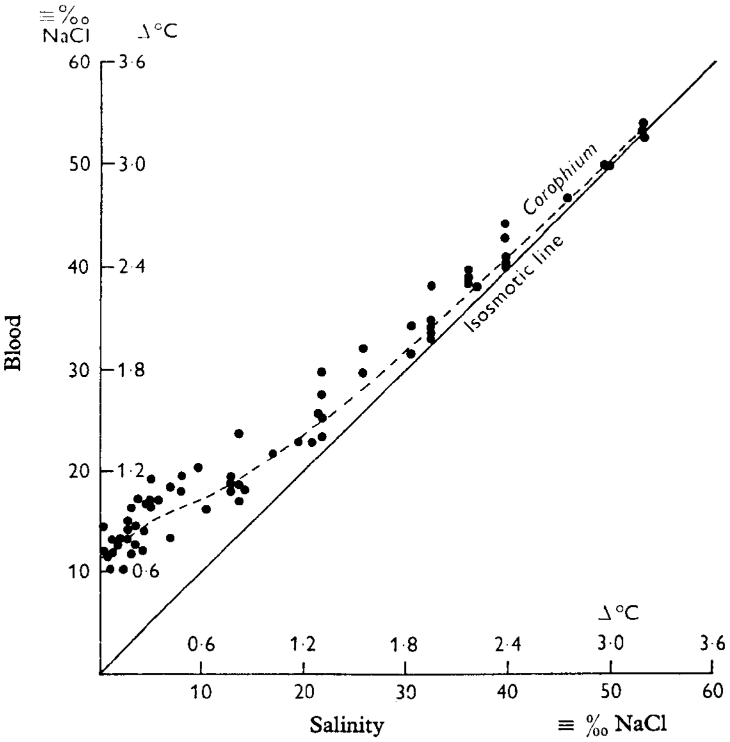

Fig. 5. The effect of salinity on the osmotic concentration of the blood of *Corophium volutator*.

It appears that between 20 and 50‰ the blood concentration of *C. volutator* passively drifts with the concentration of the external medium, but just maintaining a slight hyperosmoticity. Below 20‰, the animal begins actively to regulate the concentration of the blood, and maintains itself hyperosmotic to the outside medium; such that at 20‰, the blood is equivalent to a salinity of 23‰, at 10‰ it is equivalent to 17‰, and at 2‰ it is equivalent to 13‰—a concentration difference of 650%. Below 2‰ the blood concentration parallels, but remains consistently above, that of the water, so that at 0‰ the concentration of the blood is equivalent to a salinity of 11‰.

A survival salinity tolerance range of 2–50‰ has already been demonstrated. Within these salinities, the tissues actually experience a range of 13–50‰, which may be delimited as the tissue tolerance range. Hyperosmotic regulation in *C. volutator* is thus an adaptation to limit the range of salinity to which the tissues are subjected, permitting the animal to live in a wide range of external salinities.

DISCUSSION

C. volutator has been found to survive for over 500 h, if supplied with mud, within the salinity range of 2-50‰. It is of interest to note that the lower salinity of 2‰ coincides with the lowest salinity from which it has been recorded (Hellén, 1919; Gurjanova, 1951). If not supplied with suitable mud the range of salinities that it survived was reduced to 7·5–47·5‰.

Moulting occurred most frequently within the range of salinity 5–20‰. Growth occurred at a maximum rate at 15·4‰, and at a slightly slower rate at 4·4‰. It can be seen that although *C. volutator* can survive salinities within the range 2–50‰, frequent moulting occurred within a smaller range, and optimum growth was only achieved within an even smaller range. Kinne (1960), in a similar study of *Cyprinodon macularius*, found that the salinity range for optimal growth and reproduction was smaller than the range of salinities in which it could survive.

Studies of the osmotic concentration of the blood of *C. volutator* have shown it to be a hyperosmotic regulator, like many other brackish water crustaceans (Lockwood, 1962; Kinne, 1964*b*). The blood concentration of an individual *C. volutator* which had moulted 3 h previously was determined, and found to be Δ 0·86 °C (14·5‰), in sea water of Δ 0·63 °C (10·7‰). This is considerably lower than the mean (Δ 1·05 °C) for the blood concentration of animals at this salinity, and may indicate that the osmotic concentration of the blood fell during moulting, exposing the tissues to lower salinities than normal. This loss of osmoregulatory powers, indicated during moulting, may be the explanation for the observed difference between the range of salinities survived and the range of salinities in which moulting occurred; because moulting at low salinities would cause the concentration of the internal medium to fall below the tissue tolerance range, which is 13–50‰. Hart (1930) noted that mortality in *C. volutator* at low salinities generally occurred during moulting; and in the course of these experiments animals in low salinities undergoing a moult were observed to die.

Below 20‰, and especially below 10‰, *C. volutator* is living under conditions of potential osmotic imbalance which entails maintaining a difference in concentration between the internal and external medium of up to 650 %. This difference between internal and external medium would appear to be too great below 2‰, causing rapid mortality.

The difference between the survival time of animals, especially at low salinities, with and without mud, has already been noted. It may be suggested that when the animal is under the osmotic stress of hyperosmotic regulation, the detritus in the mud is necessary as a source of energy for osmoregulation, or as a direct supply of ions taken up via the gut. Potts (1954) has shown how small is the amount of energy needed for hyperosmotic regulation; and this finding suggests that the idea that detritus is necessary to *C. volutator* as a source of energy for osmoregulation is unlikely. Shaw (1955) has found that *Sialis lutaria* larvae can take up ions from food in its gut, and has no mechanism for the active uptake of ions from dilute media, which supports the hypothesis that detritus may be necessary to *C. volutator* because of the ions that it contains. The absorption of ions from food, for body-fluid regulation in marine invertebrates, has also been suggested by Robertson (1939). Fox (1952), whilst studying rectal pumping in *C. volutator*, suggested that in animals, such as *C. volutator*, with a relatively impermeable exoskeleton, salt entry must take place, at least partly, through the gut wall. This problem of whether *C. volutator* needs detrital food for energy for osmoregulation, or for the ions that are contained in it, is currently under investigation.

Corophium volutator is thus able to achieve success in an environment with wide fluctuations in salinity, due to its ability to survive low salinities, by preventing excessive changes in the blood concentration and the internal medium, by the process of hyperosmotic regulation.

I wish to thank Prof. F. G. T. Holliday for his advice and encouragement. The work was carried out during the tenure of a Kilgour Senior Scholarship.

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SOME EFFECTS OF SALINITY ON THE DISTRIBUTION AND ABUNDANCE OF *COROPHIUM VOLUTATOR* IN THE YTHAN ESTUARY

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(Text-figs. 1–3)

A study of the effects of salinity on the distribution and abundance of the amphipod *Corophium volutator* (Pallas) on the estuary of the River Ythan, Aberdeenshire, indicates that 2 ‰ is a critical minimum salinity controlling its distribution. In areas with salinities between 2 and 5 ‰ *C. volutator* was present, but in reduced numbers. Breeding occurred only in salinities greater than 7·5 ‰. In areas with salinity greater than 5 ‰, the distribution and abundance of *C. volutator* were controlled by the nature of the substrate; however, in areas with suitable substrates, but where the salinity was below 5 ‰, the effects of salinity override the effects of the substrate. The annual life-cycle is discussed. The results of this study are compared with those of a recent experimental study.

INTRODUCTION

The amphipod *Corophium volutator* (Pallas) is a frequent inhabitant of mudflats in North European estuaries, where it lives in U-shaped burrows, which penetrate up to 6 cm into the mud. In the extreme salinity conditions of estuaries, *C. volutator* is frequently found in conditions from marine to almost fresh water. As a component in the food web of an estuary, *C. volutator* is an important link, feeding on mud and organic detritus, and in turn forming a major constituent in the diet of, for example, flounders, *Platichthys flesus* (L.), and wading birds (Dunnet 1965).

Segerstråle (1959) has summarized the data available on the occurrence and distribution of *C. volutator*, and McLusky (1967) has reviewed the more recent literature as part of a study of the effect of salinity on survival, moulting and growth of *C. volutator*. The latter found experimentally that *C. volutator*, if fed, can survive a salinity range of 2–50‰, and undergoes most frequent moulting and attains a maximal growth rate in salinities 5–30‰.

Ingle (1963) and Hamond (1967) have pointed to certain confusion in specific records of the family Corophiidae, especially in the South of England. However, in this study only one species, *C. volutator*, is involved.

The distribution of an estuarine mud-dwelling animal, such as *C. volutator*, may be subject to control by many environmental factors. Two principal

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factors are likely to be substrate and salinity. Meadows (1964) has shown experimentally that *C. volutator* selects and forms burrows only in suitable substrates. These substrates may be delimited as mud or muddy sand, with a plentiful supply of detritus, but without excessive organic matter, and not severely affected by pollution or adverse currents. This type of substrate may occur over a wide range of salinities. Kinne (1966) has emphasized the frequently reported view that salinity is the 'ecological master factor' controlling the life of estuarine animals. This study has been made to ascertain the effect of salinity on the distribution and abundance of *C. volutator* on the estuary of the River Ythan, Aberdeenshire, Scotland.

Study area

The River Ythan enters the North Sea 21 km north of Aberdeen, and the estuary extends inland for 8·4 km (Fig. 1). The catchment area of the river covers 690 km^2 of Aberdeenshire. During the course of its 8·4 km, the estuary contains a wide variety of substrates, ranging from clean sand at the seaward end to sulphurous mud amidst reeds at its inland end (Dunnet, 1965).

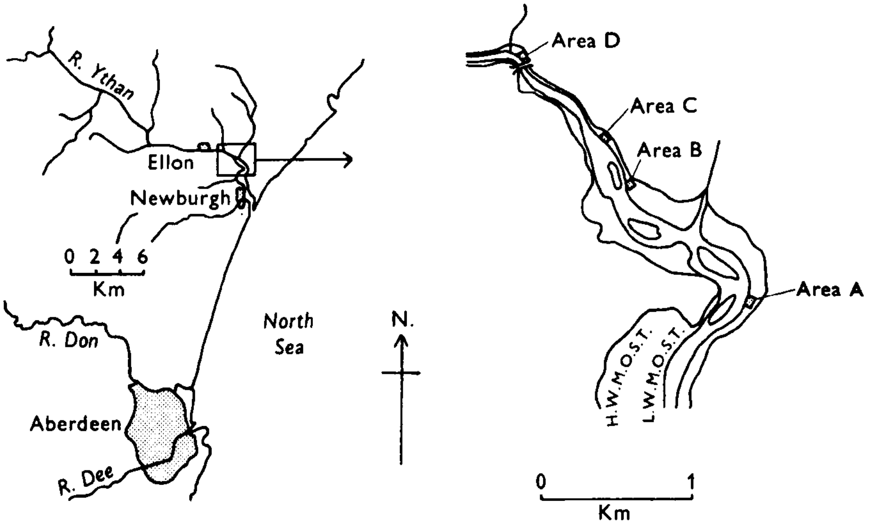

Fig. 1. Map showing, on the left, the location of the estuary of the River Ythan, with inset on the right showing the position of the four study areas.

In a preliminary study, samples of substrate were collected along the length of the estuary, and sieved to ascertain the presence and abundance of *C. volutator*. It was confirmed that in the lower and middle parts of the estuary *C. volutator* was abundant in mud or muddy sand, but absent from clean sand lacking any detrital food and in conditions of heavy pollution or in mud severely blackened by organic detritus. After this preliminary study, four areas in the upper half of the estuary were chosen for intensive study (Fig. 1). These areas were selected as having, as far as possible, uniform substrate conditions and representing a potentially wide range of salinity. Thus by ensuring uniform substrate conditions the effect of salinity could be analysed more easily.

METHODS

At each of the four study areas samples were collected at monthly intervals from October 1966 to November 1967. Samples were collected at the same state of the tide at 29- to 30-day intervals.

Each area was divided into a grid 50 m square; paired random numbers were chosen (Fisher & Yates, 1963), then from the fixed starting point the collector moved the given number in ½ m across the mudflat, parallel to the river, and then moved the next given number in ½ m towards the river. At this random point, a 5 × 5 × 6 cm high corer was pushed flush into the mud and the core of mud was collected into a polythene bag. This random sampling procedure was then repeated, and the next core put into the same bag. A sample therefore contained two randomly selected cores from the area. At each of the study areas A–C (Fig. 1), five samples were collected each month, and at the smaller area D two samples were collected. The temperature of the mud at a depth of 2 cm was also noted.

The samples were brought back to the laboratory and filtered in a conical funnel to extract the interstitial water from the mud. Each mud sample was then wet-sieved in a series of Endecott sieves. After a number of trials, it was found that the best combination was a 2000 μ sieve, to break up the lumps of mud and trap any pebbles, and a 335 μ sieve which retained all sizes of *C. volutator*. Any *C. volutator* adhering to the 2000 μ sieve were removed and added to the 335 μ fraction, which was retained for subsequent examination.

The salinity of the interstitial water was determined by titration with silver nitrate. The interstitial water of the mud was sampled, rather than the estuarine water, as the interstitial water represents the immediate environment of *C. volutator*. The interstitial salinity shows little fluctuation from hour to hour, unlike the river water (Reid, 1930; Capstick, 1957). The interstitial salinity is controlled by the salinity of the high-tide water overlying the mud, but interchange of water is slow; therefore only persistent changes in the salinity conditions of the estuarine water will be reflected in the interstitial salinity.

The 335 μ sieve fraction was carefully examined to remove any individuals of *C. volutator* present. These were counted, measured from the tip of the rostrum to the end of the telson, and assigned to size categories (i.e. 1·0–1·9 mm, 2·0–2·9 mm, 3·0–3·9 mm, etc.). The percentage size composition and arithmetic mean size were calculated.

Rainfall data were obtained from Culterty Field Station, Newburgh.

RESULTS

The results are presented in Figs. 2 (*a–e*) and 3. At the start of the study (Oct. 1966) the population of *C. volutator* had just completed its reproductive season. Throughout the winter (Oct.–Mar.) the mean size of animals at areas A–C fluctuated between 3·0 and 3·6 mm (Fig. 2*e*), whilst the salinity and temperature conditions remained fairly constant (Figs. 2*a*, *c*). The temperature at all areas was between 2° and 7 °C. At areas A and B the salinity averaged 10‰ and 6‰ respectively. At area C the salinity averaged 2·5‰, and the number of animals was extremely low. At area D, with salinities of below 1‰, no animals were present. From December onwards slow growth was observed, as the mean size of animals at area A increased from 3·17 mm in December to 3·62 mm in March (Fig. 2*e*).

In May, with warmer conditions (above 7 °C), the mean size increased to 5·61 mm. The salinity fell sharply at all areas during this month, but this lowering was only temporary, following very heavy rainfall 4 days prior to sampling, and after this period the salinity rapidly returned to its former level. During May, females bearing eggs ('berried') were first noted, especially in area A. In June the number of animals in area A increased dramatically (Fig. 2*d*) and the mean size fell, following the hatching of a large number of young animals, measuring 1·0–1·9 mm (Fig. 3). This increase in numbers at area A persisted into July, when a large increase in numbers due to young individuals occurred in area B (Figs. 2*d*, 3). Meanwhile salinity had increased to 16·5‰ at area A, 13·6‰ at area B, and 8·5‰ at area C. With this persistent increase in salinity above 5‰ in area C, the number of animals present also increased, partly because of hatching. At area D the salinity rose to 3·7‰, and animals were recorded there in small numbers for the first time.

In August the populations at areas A and B were at a peak of abundance, having increased to a level of 6–10 times the overwintering population. Animals in area C, at 13·5‰, were breeding in small numbers. Animals persisted in area D at 6·4‰. In September the numbers of animals in areas A and B decreased, and their mean size was 3·45 mm. This decrease in numbers continued into October as, with all stock from the previous winter having died off, the new stock settled down to its winter level of abundance. In area C numbers continued to climb until September, following the later breeding season. In area D animals were present in September at 8·5‰, and fewer were present in October at 4·1‰. Heavy rainfall during October caused the salinity to fall to 1·1‰ at area D in November. With this lowering of salinity, animals were no longer found in this area. The number of animals at areas A–C fell, whilst the salinity fell to 7·5–2·2‰. As growth occurred in the animals born in the summer, the mean size increased from 2·90 mm (August) to 3·90 mm (November) (Fig. 2*e*).

The decrease of salinity in the autumn and in May was largely caused by changes in rainfall (Fig. 2*b*) affecting the downflow of river water and consequently modifying the inflow of salt water into the estuary. The summer increase in salinity was due partly to decreased rainfall and to increased temperature (Fig. 2*c*), causing increased evaporation.

From these results it can be seen that one single breeding season occurred per year in this population of *C. volutator*, extending from May to August. Animals born in the summer of one year grew to a maximum size, bred when over 5 mm, and subsequently died in the summer of the next year. The onset of the reproductive season was correlated with increased temperature and salinity conditions. Segerstråle (1940) reported that 7 °C was the minimum temperature for reproduction in *C. volutator*, and the same temperature limit has been noted in this study. The breeding season finished whilst salinity and temperature conditions were still high, but these became reduced within a few weeks of the termination of breeding. Segerstråle (1940) found a similar annual cycle in a Baltic population of *C. volutator*, the only major difference being that in the Baltic the animals overwintered at 7·5–8·0 mm. long, whilst in the Ythan they overwintered at 3·0–4·0 mm long and then grew to 7·5–8·0 mm in May prior to undergoing reproduction. It may be suggested that the animals grew in the autumn as long as conditions were favourable, then overwintered, and began growth again in the spring as conditions ameliorated. On the River Dovey, Watkin (1941) noted two generations per year, but in this study only one is indicated.

Comparing the four study areas, it can be seen that although they all experienced the same temperature conditions, breeding began first at area A, followed by areas B and C, in that order. It appears that breeding occurred only as the salinity increased above 7·5‰. The sequence of breeding reflected the successive increase of salinity up to and above this level. In June 73 % of the animals in area A at 11·7‰ measured less than 2·9 mm, whilst 34·5 % of the animals in area B at 10·5‰ were this size, having recently hatched. In area C newly hatched animals were first found in July, at 8·5‰ (Figs. 2*a*, 3).

In area C the numbers of *C. volutator* remained extremely low all winter (December–June), with the interstitial salinity at 2–5‰. The numbers increased as the salinity increased above 5‰, and maintained the increase as long as salinities greater than 5‰ were recorded. This increase was partly due to breeding, but also due to the immigration of individuals into the area as soon as conditions were favourable. The number of individuals larger than 5 mm in June, at 3·4‰, was 120/m^2; however, in August, at 13·5‰, the number had increased to 1120/m^2. This increase is due entirely to immigration. The number of individuals smaller than 5 mm increased in the same period from 0 to 2640/m^2, which it may be suggested is due to a combination of reproduction and immigration.

At area D there were no animals present during the winter and spring, when

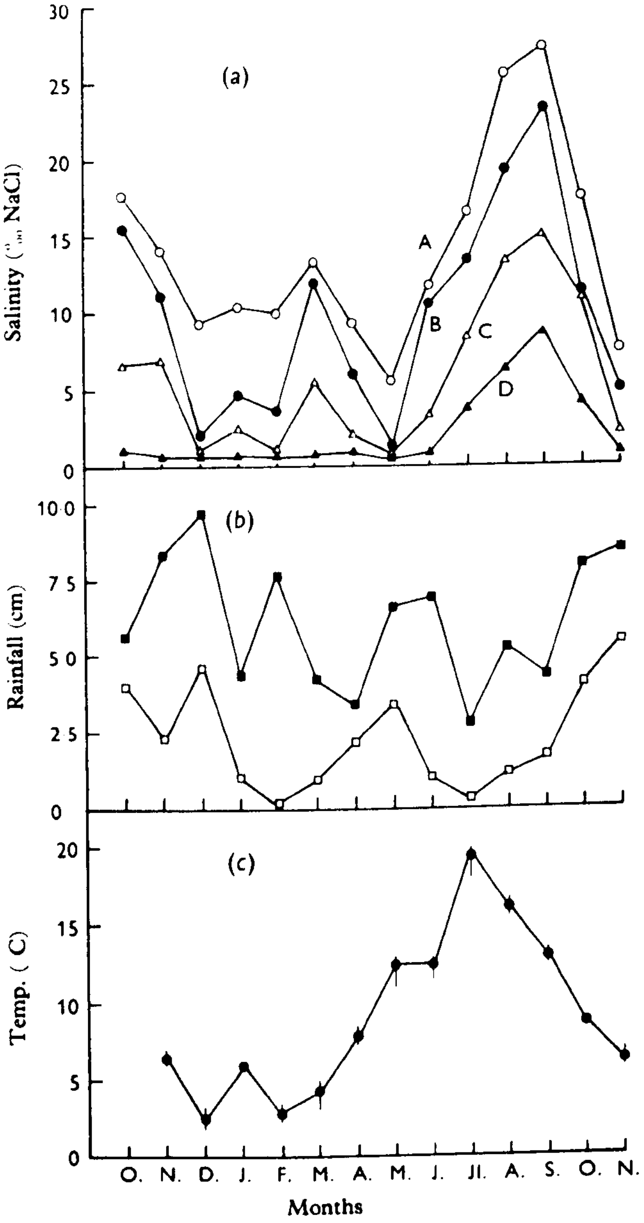

Fig. 2. For legend see opposite page.

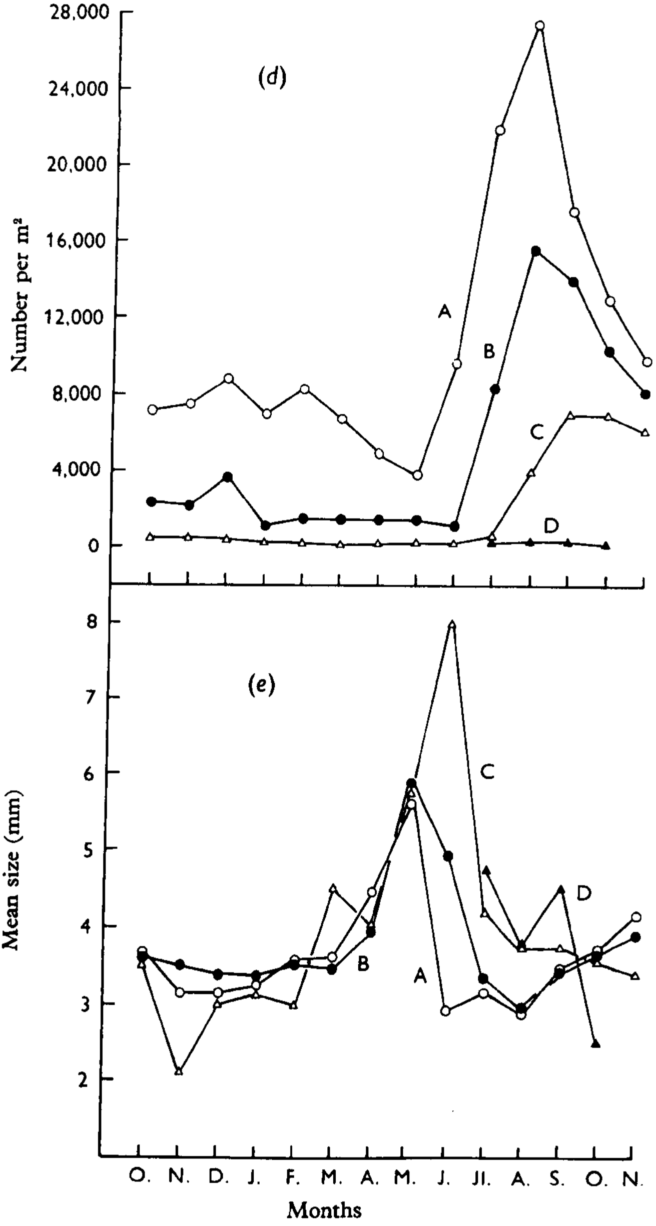

Fig. 2. (*a*) The interstitial salinity of the mud at each study area. ○, Area A; ●, area B; △, area C; ▲, area D. (*b*) Total rainfall recorded at Culterty Field Station, Newburgh, 30 days (■) and 10 days (□) prior to each monthly sample. (*c*) Mean temperature of the mud, at a depth of 2 cm, at the study areas. (*d*) The number of *Corophium volutator*, expressed as no./m², at each study area (legend as before). (*e*) Mean size (mm) of *C. volutator* at each study area (legend as before).

salinities were 0·5–1·0‰; however, in July, as the salinity increased above 2‰, *C. volutator* appeared in the area. From August to October, at salinities 4·1–8·5‰, *C. volutator* persisted in this area. In November the salinity in area D fell to 1·1‰, and *C. volutator* was no longer found in this area.

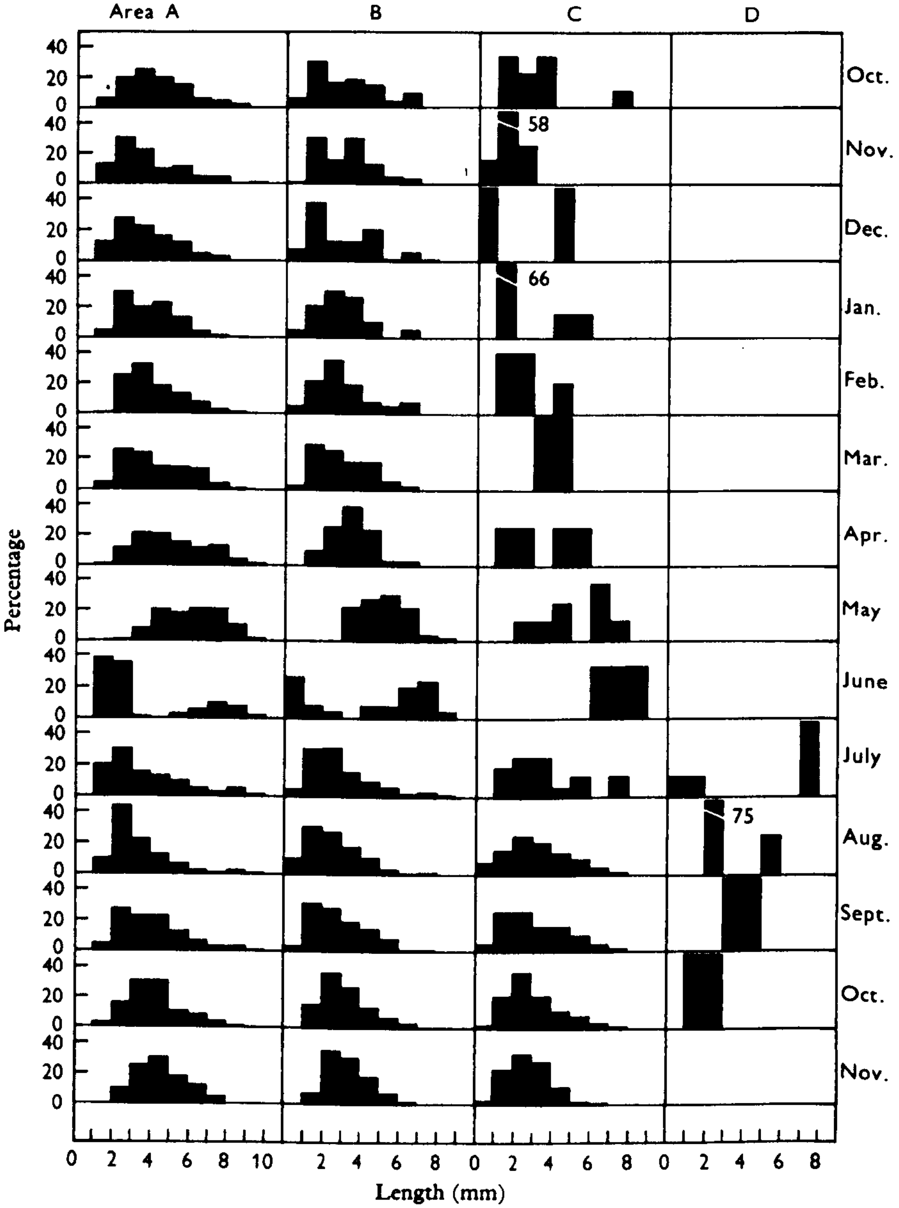

Fig. 3. The percentage size composition of populations of *Corophium volutator*, at each study area, each month.

Thus it can be seen that salinity appears to be critical in the control of the distribution and abundance of *C. volutator*. In an area with an interstitial salinity below 2‰ the species was absent. However, when the salinity of this area increased above 2‰, animals were found. In salinities 2–5‰ the numbers of animals present were always small; and the numbers only increased in salinities above 5‰. It may be suggested that *C. volutator* is ready to migrate into an area whenever conditions become favourable. Breeding appeared to be correlated with temperatures greater than 7 °C and with interstitial salinity conditions above 7·5‰.

Substrate analysis

It has already been mentioned that the two principal environmental factors which may affect *C. volutator* are substrate and salinity. In the choice of study areas an attempt was made to eliminate the variability of the substrate, to assist in the study of the effect of salinity. An analysis was made of the nature of the substrate at each area in order to check on any variability which may have affected the distribution and abundance of *C. volutator*. Methods similar to those recommended by Morgans (1956) were adopted, and the mean results are given in Table 1.

TABLE 1. RESULTS OF SUBSTRATE ANALYSIS AT THE FOUR STUDY AREAS

Area	Depth of aeration (cm)	Water composition (%)	Mean particle diameter (μ)	Skq ϕ (see Morgans, 1956)	Organic matter by ignition (%)
A	6·12	27·30	225	+0·250	2·46
B	1·37	29·65	235	−0·131	2·75
C	0·50	26·38	305	+0·081	2·08
D	0·35	49·43	220	−0·256	6·62

These results, which represent the means of random samples taken at each area, did not vary, except by very small amounts, throughout the year. Although the study areas were chosen for uniformity of substrate, some slight differences did occur. The depth of aeration showed a gradation A > B > C and D. The similarity in depths of aeration at areas C and D and the dissimilarity in records of *C. volutator* rule out this factor as being critical. Muds at areas A–C had 26–29% water content, whilst the mud at area D had 49% water content. The similarity of mud at areas A–C discounts this factor as a limiting factor controlling abundance, but the high value for area D may have partially accounted for the few animals ever recorded there. The differences between the mean particle diameters at the areas show no important difference. The Skq ϕ, which is a measure of the sorting of the particles of the substratum, indicates that in areas A and C, with positive results, particles larger than the median are better sorted than the smaller particles.

In areas B and D, with negative results, the smaller particles are better sorted than the larger ones. However, all the Skq ϕ results are extremely near zero, and the differences between the areas are very slight.

Thus it may be concluded that the textures of the substrates at the study areas are extremely similar, confirming the original choice of the study areas for their uniformity. The demonstrated uniformity, moreover, suggests that adverse currents, which usually affect the nature of the substrate, are not important in this study. The percentage organic matter showed no difference between areas A–C, averaging 2·43 %. Area D was richer in organic matter (6·62 %) than the other areas; however, Hart (1930) found *C. volutator* abundant in muds with 6–7 % organic matter. This finding suggests that the amount of organic matter is not limiting in this study.

DISCUSSION

Various factors are liable to affect the distribution and abundance of a mud-dwelling estuarine animal such as *C. volutator*. Some of these are tides, temperature, substrate, currents, rainfall, salinity, oxygen concentration and predation.

No study of the effect of oxygen concentration has been undertaken during this study. Remane & Schlieper (1958) report that *C. volutator* is particularly resistant to low oxygen concentrations. The effect of tidal pressures has already been studied by Morgan (1965), who has shown that *C. volutator* maintains a 12·4 h activity in the laboratory rhythm. A swimming response is entrained by a decrease in pressure as the tide ebbs. This swimming behaviour may be of importance when considering the possibility of migration by *C. volutator*.

The decrease in the number of animals (Fig. 2*d*) observed in area A between December and May and the decrease again from September to November may be due to either predation, natural mortality, or emigration. Especially between December and May, no great change in the environmental factors occurred, whilst the population of *C. volutator* was more than halved. Goss-Custard[1] noted that Redshank (*Tringa totanus* L.) ate 38 % of *C. volutator* which were larger than 4 mm—a feature which would account for the spring decrease in numbers, due to predation.

The onset of breeding has been shown to be coincident with an increase in temperature above 7 °C. In general, the temperature on a given day was uniform throughout the estuary; therefore, it is unlikely that temperature can cause the observed differences in the distribution of *C. volutator* between the study areas. Goss-Custard[1] has shown that temperature may affect the burrowing behaviour of *C. volutator*, which burrows deeper into the mud when the temperature is below 4 °C. The results of the substrate analysis

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[1] Ph.D. Thesis, University of Aberdeen, 1966.

show that area D was slightly less suitable for *C. volutator*, but showed no factor which could adequately account for the variation in observed numbers between areas A–C. Also, the nature of the substrate at each area remained constant throughout the period of study, whereas the distribution and abundance of *C. volutator* varied considerably. In area D, animals were absent when the salinity was below 1‰, yet as soon as the salinity increased to above 2‰ animals were found there, albeit in small numbers. Similarly, the numbers at area C remained extremely low all winter, with salinities of 2–5‰, but when the salinity increased to above 5‰, the numbers of *C. volutator* increased, and when the salinity rose above 7·5‰ breeding was recorded in this area.

Thus the abundance and distribution of *C. volutator* at areas C and D were directly related to the prevailing salinity. The difference in abundance between areas A and B may be due to other factors, for example the difference in the depth of aeration.

Rainfall (Fig. 2*b*)—especially that of the 10 days prior to sampling, rather than of the whole month prior to sampling—together with the tides is the chief factor affecting salinity. The salinity at any one point on the estuary represents a product of the interaction of the downflow of river water and inflow of salt water. The downflow of river water is directly related to the rainfall on the catchment area of the river. The drastic lowering of salinity in May and the autumnal decline in salinity were both due to periods of rainfall.

In this study the effect of maximum salinity was not studied. It has already been shown (McLusky, 1967) that *C. volutator* can tolerate salinities up to 50‰, and on the Ythan estuary the maximum salinity that has been recorded is 36‰. The downstream limit of *C. volutator* on the Ythan estuary is delimited by the presence of clean sand at the mouth of the estuary.

The appearance of *C. volutator* at area D and its increase in number at area C, as salinity increased, suggest that it must undergo migration within the estuary. Morgan (1965) showed that *C. volutator* swim in the ebb tide, an action which will tend to carry them down-river; however, he also showed that a few animals swim in the flow tide, which would tend to carry them up-river, where they could colonize new substrates.

In an experimental study (McLusky, 1967) 2‰ was found to be the minimum salinity for the survival of fed individuals for more than 500 h. The same salinity, 2‰, has been shown to be critical for the distribution of *C. volutator* on the Ythan estuary. *C. volutator* can tolerate brief periods of salinity below this level, as seen on the estuary in May. It was also found experimentally that for moulting, growth and the survival of unfed animals, salinities above 5‰ were necessary. This finding correlates with the few animals at area C during the winter, and at area D in the summer, when the salinity was 2–5‰.

It has thus been found that within the central part of the estuary the nature of the substrate is the principal factor controlling the distribution and abundance of *C. volutator*. Within an area of suitable substrate, *C. volutator* became abundant when the salinity rose above 5‰ and bred when the salinity was greater than 7·5‰. Below 5‰ it was reduced in abundance and was not found at all below 2‰. Thus the effects of salinity can override substrate selection in the upper part of an estuary, and salinity becomes the 'ecological master factor' controlling the animal's distribution and abundance.

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ASPECTS OF OSMOTIC AND IONIC REGULATION IN *COROPHIUM VOLUTATOR* (PALLAS)

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(Text-figs. 1–7)

Experiments have been made to elucidate the problem of hyperosmotic regulation in the mud-dwelling euryhaline amphipod, *Corophium volutator*. The animal produced urine hypo-osmotic to the blood when acclimatized to low salinities, and isosmotic urine at salinities above 20 ‰. The restricted permeable areas of the cuticle have been localized by silver staining. Over a range of salinities from 1 to 35 ‰ *C. volutator* was found to maintain Na^+, K^+, Ca^{2+}, Cl^-, more concentrated than the medium, and Mg^{2+} less concentrated. The importance of these mechanisms in assisting hyperosmotic regulation is discussed.

INTRODUCTION

The mud-dwelling amphipod, *Corophium volutator* (Pallas), is essentially a brackish-water species, being found most commonly in estuaries, where its range extends from marine to almost freshwater conditions. Segerstråle (1959) and Ingle (1963) have summarized the data available on its life-cycle and distribution. *C. volutator* occurs abundantly on the Ythan estuary, Aberdeenshire, where a study has been made of its distribution (McLusky, 1968). Animals were found in salinities 2–36‰, but were reduced in abundance below 5‰, and breeding was noted only in salinities greater than 7·5‰. A complementary experimental study (McLusky, 1967) showed that *C. volutator* survived the salinity range 2–50‰, but moulted, and attained maximal growth in 5–30‰. It was also found that *C. volutator* was a hyperosmotic regulator, which in low salinities maintained its blood concentration hyperosmotic to the medium, and in salinities above 20‰ tended towards isosmoticity. The tissue tolerance range was delimited as 13–50‰.

Further experiments have been made to elucidate the mechanism of hyperosmotic regulation in *C. volutator*, in particular, studies of urine concentration, permeability of the body surface, and ionic regulation.

MATERIALS AND METHODS

Animals were collected from the River Ythan, and maintained in tanks at 15‰ and at 10 °C, supplied with mud from their natural habitat. Prior to sampling, the animals were maintained in various dilutions of sea water, supplied with mud, for at least 48 h. The freezing-point of urine and blood samples was determined using equipment based on that of Ramsay & Brown (1955). Blood samples were collected as described by McLusky (1967). Other methods are described in the relevant sections.

URINE CONCENTRATION

Samples of urine were collected by carefully drying off the individual animal on an absorbent tissue, and then immersing the animal in paraffin, in a watch-glass. A droplet of urine was observed to form at a small papilla at the base of the 2nd antenna. This droplet was carefully collected into a fine silica capillary. The freezing-point of the urine sample, and of a blood sample collected from the same animal, were determined.

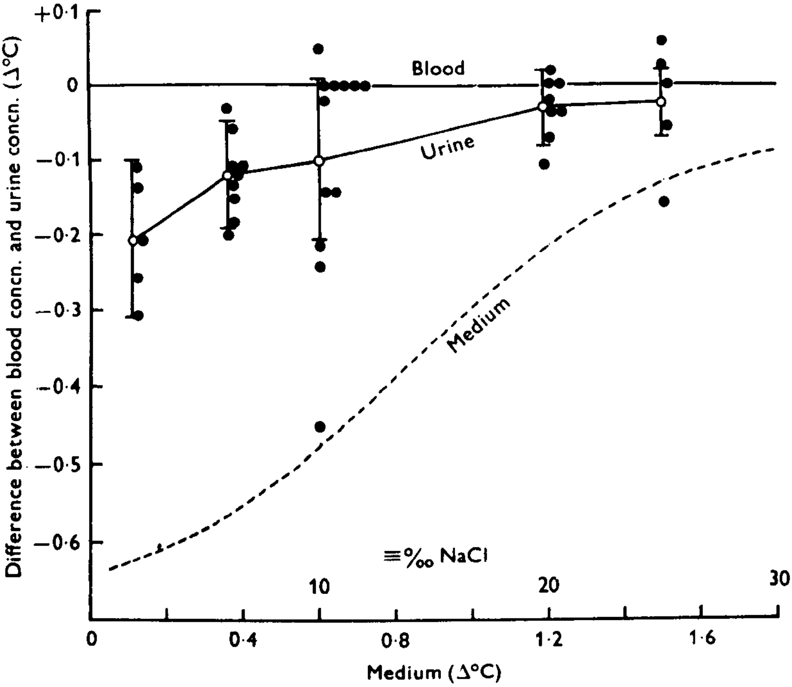

Fig. 1. Relationship of urine concentration to blood concentration in *C. volutator*, in different salinities; showing the differences between the urine concentration and blood concentration in individual animals (●), and mean differences (○) (vertical lines indicate standard error). The difference between blood concentration and the medium is shown by the dotted line.

At 25 and 20‰, the urine produced was almost isosmotic with the blood (Fig. 1), the differences were not significant ($P = < 0{\cdot}4$ in both salinities). At salinities, 10, 6, 1·8‰, the urine was found to be hypo-osmotic to the blood, the differences were all significant ($P = < 0{\cdot}02$ in all salinities). The degree of hypo-osmoticity was greater at 1·8‰ than at 6‰, and at 10‰. Whilst the medium was 1·8‰, the blood concentration was 14·5 ± 1·6‰, and the urine concentration was 11 ± 1·6‰. Similarly, when the medium was 6‰, the blood concentration was 15 ± 1‰, and the urine concentration was 12 ± 1·5‰. A calculation of the coefficient of linear correlation indicated that as the blood concentration decreased, so the urine concentration also decreased ($R = 0{\cdot}9190$, $P = < 0{\cdot}001$). This calculation also indicated that at low blood concentrations, a greater difference between the blood and urine concentrations occurred.

The brackish-water amphipods, *Gammarus duebeni* Lillejeborg and *G. fasciatus* Say, have been found to produce hypo-osmotic urine, the degree of hypo-osmoticity varying with the species, and the medium to which they were acclimated (Lockwood, 1961; Werntz, 1963). The pattern of regulation noted in these gammarids is very similar to that noted in *C. volutator.*

Potts (1954) has suggested that the formation of hypo-osmotic urine will result in some saving in the energy expended in osmoregulation, but it is likely that this saving will be very small in relation to the total energy budget. Lockwood (1961) suggested that the formation of hypo-osmotic urine will assist in the retention of ions within the body, and place less burden on the mechanisms responsible for active uptake at the body surface. *C. volutator* ranges in size from 1 to 8 mm, and like all small animals has a large surface/volume ratio—hence any mechanism which helped to retain ions within the body, and maintain hyperosmotic conditions in the internal environment, would be of value to the animal. It should be emphasized that some ions are likely to be lost via the urine, for example when the blood was hyperosmotic by 9‰ to a medium of 6‰, the urine was hypo-osmotic to the blood by 3‰, and hyperosmotic to the medium by 6‰. Lockwood (1965) stated that: 'the conservation of ions within the body by the production of hypotonic urine is likely to be found to be a common feature of the smaller brackish water crustacea', and the present findings lend support to this hypothesis.

PERMEABILITY

In a small brackish-water crustacean such as *C. volutator*, any reduction in the permeability of the surface will assist in the maintenance of hyperosmotic conditions in the blood. Permeable areas of the cuticle of *C. volutator* were localized by means of a silver-staining technique, essentially the same as that used by Croghan (1958) and Ralph (1967). The living animals were washed in changes of distilled water to remove any adherent chloride, and then placed in dilute silver nitrate solution for 5 min. They were then again carefully washed in distilled water, and put into photographic developer (Suprol). This reduced any silver that had been taken up to black metallic silver. The principle of this technique is that silver ions will diffuse into the permeable areas of the cuticle and there be reduced by meeting halide ions (e.g. chloride) from the blood. The precipitate is then developed to metallic silver.

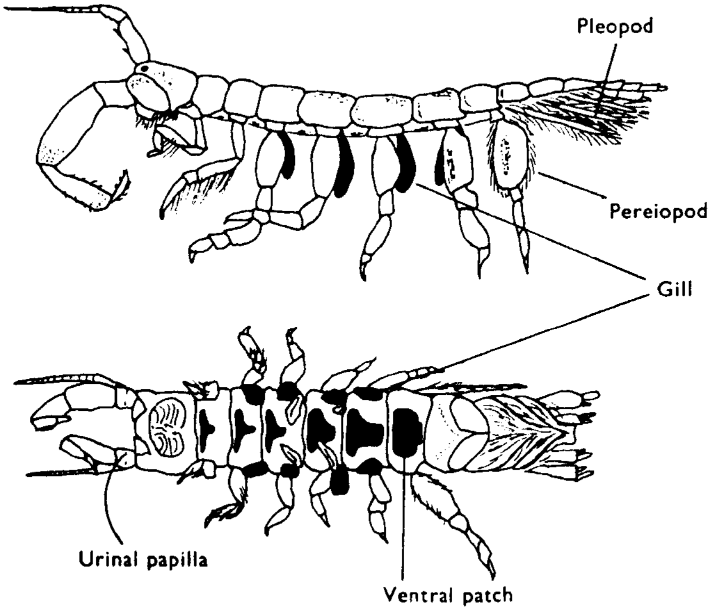

Fig. 2. *C. volutator*, showing the gills and ventral patches, revealed by silver-staining, lateral view (above) and ventral view (below).

This technique stained four pairs of gills attached to the bases of pereiopods 1–4. T-shaped patches were also revealed on the sterna of abdominal segments 2–7 (Fig. 2). Animals were examined from different locations on the Ythan estuary, after maintenance in different salinity conditions, and of different sizes. No consistent differences were found between animals of different sizes or from different conditions, but it was found that certain individuals lacked the stained T-patches. Hart (1930) commented on a current of water produced by the pleopods of *C. volutator*. It may be noted that the silver-stained areas were all located on the ventral side of the animal, where this current passes underneath the animal. It may be suggested that these areas of the cuticle and the gills are permeable, to allow interchange of water, gases and ions. The dorsal and lateral surfaces of the animal were never stained by this technique, which suggests that they are areas of reduced permeability. The restriction of the permeable areas of the cuticle to certain confined areas on the ventral surface is likely to assist in the maintenance of hyperosmotic internal conditions. The importance of restricted permeability to brackish-water crustaceans for the maintenance of internal conditions has been emphasized by Shaw (1959), Smith (1967), and Rudy (1967).

IONIC REGULATION

A study has been made of the regulation of sodium, potassium, calcium, magnesium, and chloride ions in animals acclimatized to salinities 1–35‰. Robertson (1959), Potts & Parry (1964) and Nicol (1967) have reviewed the subject of ionic regulation in the crustacea, however most of the data is available from animals maintained in sea water only. Dehnel & Carefoot (1965) and Dehnel (1966) have studied ionic regulation in the estuarine crabs, *Hemigrapsus* spp., which, like *C. volutator*, maintain their blood hyperosmotic to the medium, and found that sodium, potassium, calcium and chloride ions in animals in 0–100% sea water, were more concentrated than the medium, whilst magnesium ion was less concentrated. De Leersnyder (1967*a*, *b*) has made a comprehensive study of the hyper/hypo-osmotic regulator, *Eriocheir sinensis* M-Ed., with similar results.

Blood samples were collected by a method similar to McLusky (1967), except that the animal was not under paraffin. Up to 0·25 μl. of blood could be collected into a silica micropipette from an individual animal. This was sufficient for the determination of one ion only. The volume of the blood sample was determined by weighing an equal volume of de-ionized water.

Cations were analysed using a Unicam SP 900 flame spectrophotometer. For sodium analysis, the machine was used conventionally, with the blood sample made up to 10 ml. with de-ionized water. For analysis of potassium, calcium and magnesium, for which the machine was less sensitive, and where the concentration of ions in the blood was considerably lower, the conventional atomizer and vaporizer were replaced by a hot chamber vaporizer devised by Dr C. M. M. Begg of Aberdeen University. This vaporizer consisted of a hypodermic needle set into a carburettor jet, which fitted into a brass cylinder, around which was wound a heating element maintained at a constant temperature. In the conventional atomizer and vaporizer, 92·5% of the sample ran away to waste; in the hot chamber vaporizer all of the sample was maintained as fine droplets, all of which passed to the burner, thus increasing the sensitivity of the spectrophotometer by × 12·5. For potassium analysis the blood sample was made up to 10 ml. with de-ionized water, and for calcium and magnesium to 1 ml. with de-ionized water.

For chloride ion analysis, the first method of Ramsay, Brown & Croghan (1955) was used. A Beckman Expandomatic pH meter was used as a millivolt meter for the electrometric titration, performed on a plate of PTFE, in a drop of acetic acid/acetone buffer solution.

In addition to the determination of the ionic content of the blood of *C. volutator* determinations were also made of the ionic content of the sea-water solutions that they had been living in, and of standard solutions.

The results of this analysis are presented in Figs. 3–7 and Table 1. In all figures the concentration of the ion (in milli-equivalents/litre) is plotted against the salinity of the medium in which *C. volutator* had been living.

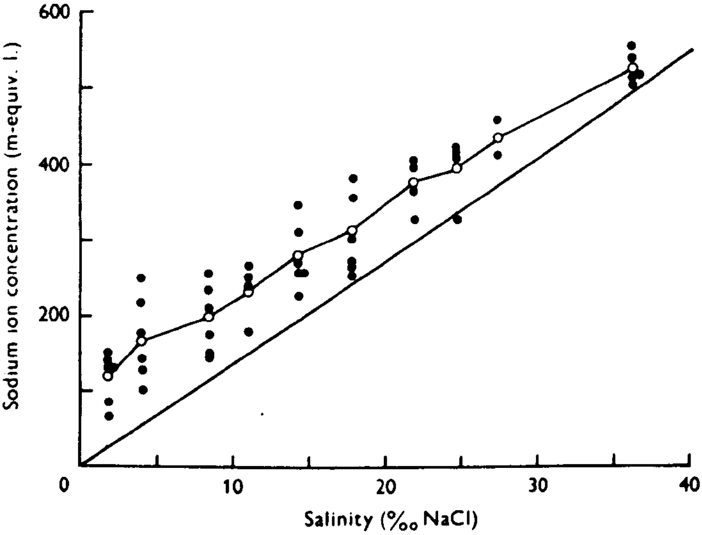

Fig. 3. The concentration of sodium ions in the blood of *C. volutator*, maintained in various salinities. Filled circles indicate the concentration of ions in individual animals, and the open circles, the mean concentrations. The straight line indicates the concentration of ions in the medium.

From Fig. 3 it can be seen that the concentration of the sodium ion in the blood was consistently maintained above that of the sea water, maintaining a difference of 33 m-equiv./l. at 35‰, and 105 m-equiv./l. at 5‰. The concentration of potassium (Fig. 4) was maintained well above that of sea water. At 30‰, *C. volutator* blood contained 17·7 m-equiv./l., whilst sea water had 8·6 m-equiv./l., and at 5‰ the blood contained 10·3 m-equiv./l., whilst sea water had 1·4 m-equiv./l. Like sodium and potassium, calcium (Fig. 5) was maintained consistently more concentrated than sea water—a difference of 2·6 m-equiv./l. at 30‰, and 3·6 m-equiv./l. at 5‰. Magnesium (Fig. 6) was maintained at a consistently lower concentration in the blood than sea water; in 30‰, whilst sea water contained 92 m-equiv./l., the blood of

TABLE 1. ANALYSIS OF INORGANIC IONS IN THE BLOOD OF *COROPHIUM VOLUTATOR*

Medium NaCl (‰)	Sea water (mM/l.*)		*C.v.* blood (mM/l.*)		Cations (m-equiv./l.)				Total cation (mM/l.)	m-equiv./l. Cl^-	Total anion (+40 m-equiv./l for SO_4^{2-}‡) mM/l.	Relative ionic comp. Cl^- = 100			
	Δ °C.	NaCl	Δ °C.	NaCl	Na^+	K^+	Ca^{2+}	Mg^{2+}				Na^+ at. wt. 23·0	K^+ at. wt. 39·1	Ca^{2+} at. wt. 40·1	Mg^{2+} at. wt. 24·5
5	0·3	85·5	0·9	256·5	173·0	10·3	6·6	6·0	195·9	155·0	195	72·3	7·32	2·41	1·33
10	0·6	171·0	1·17	333·4	219·0	10·3	9·4	12·0	250·7	220·0	260	64·49	5·16	2·41	1·88
15	0·9	256·5	1·25	356·2	290·0	11·9	12·6	21·0	335·5	277·0	317	67·83	4·73	2·56	2·62
20	1·2	342·0	1·43	407·5	349·0	14·6	14·6	36·0	414·2	365·0	405	61·50	4·40	2·25	3·40
25	1·5	427·5	1·66	473·1	396·0	15·9	18·0	42·0	470·9	450·0	490	57·01	3·89	2·25	3·15
30	1·8	513·0	1·92	547·2	462·0	17·7	20·6	61·4	561·7	509·0	549	58·81	3·83	2·28	4·16
35	2·1	598·3	2·21	629·8	515·0	19·2†	22·6†	78·0†	634·8	583·0†	623	57·23	3·62	2·19	4·61
											Sea water‡	55·5	2·01	2·12	6·69

* mM = Δ × 286. † Extrapolated. ‡ Nicol (1967).

C. volutator had 61·4 m-equiv. l., and at 5‰, sea water had 15 m-equiv./l. whilst the blood had 6·0 m-equiv. l.

The analysis of chloride ions (Fig. 7) showed a similar pattern of regulation to that of sodium. The blood at 30‰ was 28 m-equiv./l. more concentrated than sea water, and 76 m-equiv. l. more concentrated at 5‰.

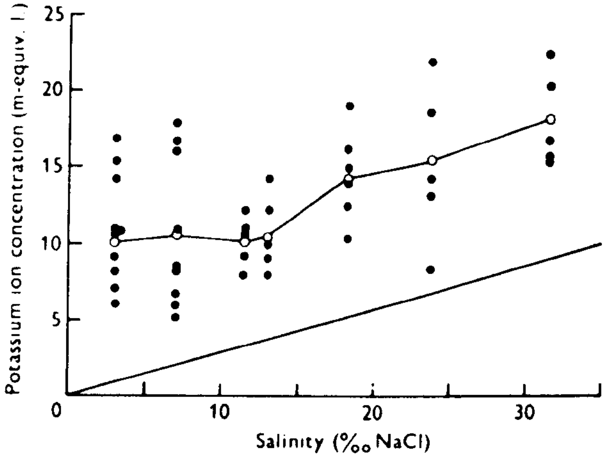

Fig. 4. The concentration of potassium ions in the blood of *C. volutator*, maintained in various salinities. Explanation as Fig. 3.

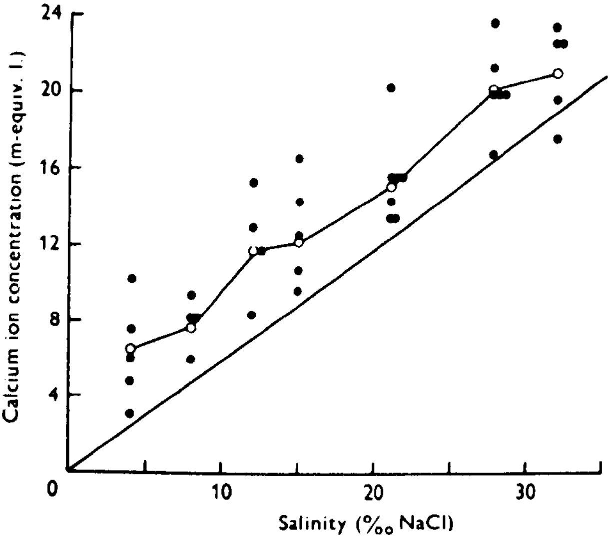

Fig. 5. The concentration of calcium ions in the blood of *C. volutator*, maintained in various salinities. Explanation as Fig. 3.

These analyses of blood are collated in Table 1. When the relative ionic composition of the blood is compared with that of sea water, it may be seen that sodium ions and calcium ions are slightly more concentrated, potassium ions markedly more concentrated, and magnesium ions considerably less concentrated in the blood than in sea water. The ratio of calcium ions only changed slightly over the range of salinities, however sodium ions and potassium ions were more concentrated at low salinities, and magnesium ions were less concentrated at low salinities.

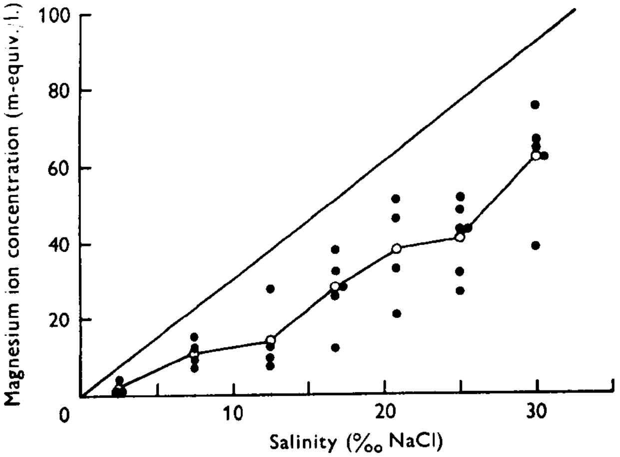

Fig. 6. The concentration of magnesium ions in the blood of *C. volutator*, maintained in various salinities. Explanation as Fig. 3.

These results showed a strong increase of potassium ion concentration in the blood of *C. volutator* relative to the medium, a slight increase of sodium ions, calcium ions, and chloride ions, and a strong decrease in magnesium ion concentration. This pattern of regulation, and the relative ionic content is similar to that noted for other crustaceans (Robertson, 1959; Nicol, 1967). The concentration of potassium ions was rather high, which may well be due to the fact that whole blood was used for the analyses, and breakdown of any cells contained in it may elevate the potassium ion concentration (Potts & Parry, 1964).

It has already been noted that *C. volutator* is a hyperosmotic regulator, maintaining its blood markedly more concentrated than the medium at low salinities, and tending towards isosmoticity at high salinities. The same pattern was noted for sodium, potassium, calcium, and chloride ions, which were all more hypertonic at low salinities, than at high salinities. Magnesium ion was always less concentrated in the blood than in sea water. Similar patterns of hypertonic regulation of sodium, potassium, calcium and chloride ions, and hypotonic regulation of magnesium ion have been found in estuarine Hemigrapsid crabs (Dehnel & Carefoot, 1965; Dehnel, 1966).

When summation is made of the ions found in this analysis (Table 1), and compared with the osmotic concentration of the blood as determined by freezing-point studies (McLusky, 1967), a discrepancy is found at 5 and 10‰, and to a lesser extent at 15‰, whilst at 20–35‰ the sum of the ionic concentrations adequately accounts for the observed osmotic concentrations. An estimate of the sulphate ion concentration has been included in the anion total, using the value 40 m-equiv./l. (Nicol, 1967).

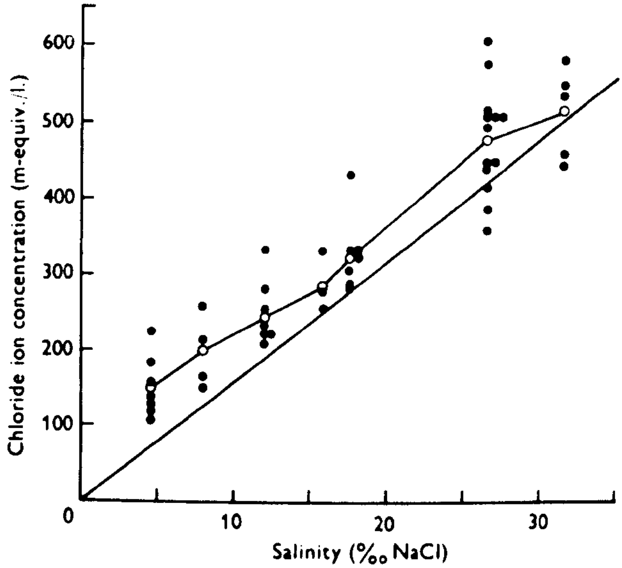

Fig. 7. The concentration of chloride ions in the blood of *C. volutator*, maintained in various salinities. Explanation as Fig. 3.

Dehnel (1966) noted a discrepancy between osmotic pressure and sodium ion concentration of the blood of *Hemigrapsus nudus*, and suggested that the protein content of the blood may change with changes of salinity, and account for the discrepancy noted. Sutcliffe (1961), in the caddis fly larva, *Limnephilus affinis* Curtis, which is a hypotonic regulator, found a marked discrepancy between osmotic pressure and the ionic content of the blood as determined by conductivity measurements, in animals maintained in certain salinities only. He suggested that the increase in the concentration of the non-electrolyte fraction may be brought about by the liberation of osmotically active substances into the haemolymph, perhaps due to the mobilization of amino acids from a protein reservoir. A significant rise in blood protein in crayfish in 50% sea water, compared to those in fresh water, has been found by Sharma (1968), who has suggested that this rise may contribute to a rise in the osmotic pressure of the blood. Gilbert (1959*a*, *b*) found in shore crabs of different sizes which were maintained in the same salinity, a discrepancy between the ionic composition and the total osmotic pressure of the blood. He postulated that this discrepancy was due to non-electrolytes, and subsequently found (Gilbert, 1959*c*) that the concentration of non-protein nitrogen in the blood was inversely proportional to the concentration of ions. As one fraction increased in concentration, so the other decreased, in such a manner that the total osmotic pressure remained constant. Drilhon-Courtois (1934) also found in individual crabs that blood protein increased as the ionic concentration decreased. Horn & Kerr (1963) have analysed the haemolymph protein concentration of adult blue crabs, and summarized the data available from other Decapoda.

From the published record it is reasonable to postulate that the discrepancy noted in *C. volutator* between the total ionic composition and the osmotic pressure of the blood, of animals maintained in salinities below 15‰, is due to non-electrolytes. A thin-layer chromatogram performed on the combined blood of ten individual *C. volutator*, has shown that amino acids are present in the blood, however quantitative results are not yet available, due to the extremely small volume of blood in an individual animal.

DISCUSSION

These three approaches to the problem of the maintenance of hyperosmotic conditions in the blood of *C. volutator* have indicated that in salinities of 20‰ and upwards, as the osmotic pressure of the blood tended towards isosmoticity with the medium, *C. volutator* produced urine isosmotic to the blood, and the osmotic pressure of the blood was adequately accounted for by the concentrations of inorganic ions found. In salinities below 20‰, as the blood became hyperosmotic to the medium, so urine hypo-osmotic to the blood was produced, and the urine appeared to be produced in larger volumes than at higher salinities. In lower salinities, the concentration of ions increased, and accounted for the majority of the hyperosmoticity of the blood, but a discrepancy was noted, especially below 10‰, which it may be postulated was due to amino acids or other non-electrolytes. Sutcliffe's (1961) suggestion that an increase in the non-electrolyte fraction could be due to the mobilization of amino acids from a protein reservoir is of particular interest in this respect, as are the findings of Sharma (1968) that a significant rise in blood protein and a shift towards ureotelism occurred in crayfish under osmotic stress.

The permeable areas of the cuticle were found to be confined to certain ventral patches and the gills. This restriction of the permeable areas of the cuticle may assist in the maintenance of internal hyperosmotic conditions. The generalization of Smith (1967), based on that of Lockwood (1965), that:

'the conservation of ions within the body by the production of hypotonic urine is likely to be found in those brackish and freshwater crustaceans which combine a high rate of water turnover with a significant reduction in the salt-permeability of the body surface', has found support in this study.

It has already been suggested (McLusky, 1967) that hyperosmotic regulation allowed an animal to live in a wide range of external salinity conditions, and permitted it to fully exploit the nutritionally rich estuarine habitat. To assist in maintaining hyperosmotic conditions, *C. volutator* has only limited areas of surface permeability, and in low salinities it produces urine hypo-osmotic to the blood. The formation of hypo-osmotic urine will in part relieve the mechanism for the uptake of ions, whether it be at the body surface, or via the intestine. The ions taken up have been shown to be regulated in a manner comparable with other brackish-water crustaceans.

This study was begun at the University of Aberdeen, and completed at the University of Stirling. I should like to thank Professor F. G. T. Holliday, Dr C. M. M. Begg and Dr R. Ralph for their advice and criticism. The work was carried out during the tenure of a Kilgour Senior Scholarship.

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THE OXYGEN CONSUMPTION OF *COROPHIUM VOLUTATOR* IN RELATION TO SALINITY

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Abstract—1. The oxygen consumption of *Corophium volutator*, a mud-dwelling euryhaline amphipod, has been measured using microvolumetric respirometers.

2. Comparison was made of animals of the same size, at the same level of activity, at 10°C, acclimatized to salinities 1, 10, 20 and 30%.

3. No significant differences were found in oxygen consumption, between animals in different salinities.

4. In all salinities a logarithmic relationship was noted between oxygen consumption and dry weight.

5. These findings, in conjunction with previous findings, are discussed in relation to the energy requirements of osmoregulation, with particular regard to the conservation of energy by the production of hypo-osmotic urine, the suggested uptake of ions from food, and the possibility of a shift in the energy requirements of different metabolic processes under osmotic stress, perhaps from growth into osmoregulation.

INTRODUCTION

PREVIOUS studies of the effects of salinity on the mud-dwelling euryhaline amphipod, *Corophium volutator* (Pallas), have shown that, if fed, it survived salinities in the range 2–50‰ and underwent frequent moulting and attained maximal growth in salinities 5–30‰. It has also been found that the blood concentration was almost isosmotic to the medium in salinities above 20‰ and maintained increasingly hyperosmotic to the medium below 20‰ (McLusky, 1967). On the Ythan estuary (Aberdeenshire) it has been found that the critical minimum salinity limiting its distribution was 2‰(McLusky, 1968a). A study of ionic regulation has shown that regulation of inorganic ions largely accounted for hyperosmotic regulation of the blood, the retention of ions being assisted by a reduction in the permeable areas of the cuticle. It was also noted that *C. volutator* produced urine hypo-osmotic to the blood when acclimatized to media below 20‰ (McLusky, 1968b). The retention of inorganic ions in the blood must be assisted by the production of urine hypo-osmotic to it, however the urine concentration was also markedly hyperosmotic to the medium. It may therefore be suggested that *C. volutator* must take up ions from the environment. During survival experiments, it was noted that animals supplied with mud (their normal food) survived the salinity range 2–50‰, but those without any substrate at all or with sterile sand substituted for the mud survived the salinity range 7·5–47·5‰ (McLusky, 1967). It may be postulated that food is necessary in *C. volutator* for maintaining hyperosmotic conditions in the blood, either as a direct source of ions via the gut or as an energy source for the active uptake of ions at the body surface. If food is necessary as an energy source, it is likely that the uptake of ions for osmoregulation will be reflected in an increase in the metabolism of *C. volutator* under osmotic stress, and that this increase in metabolism will be expressed by a change in oxygen consumption in relation to salinity, provided that there is no shift in the oxygen requirements between different metabolic processes of the animal. For example, it might be that under osmotic stress, energy could be diverted from gonad maturation into osmoregulation.

The extensive literature on the effects of salinity on respiration presents a conflicting picture. Many authors (Schwabe, 1933, for *Carcinus*; Peters, 1935; Flemister & Flemister, 1951; Lofts, 1956; Rao, 1958; Dehnel, 1960; Lance, 1965; Ramamurthi, 1967) have found that oxygen consumption was increased under conditions of osmotic stress. Madanmohanrao & Pampapathirao (1962) noted lowest oxygen consumption in medium of adaptation, in spite of osmotic stress. Krogh (1939) and Potts & Parry (1964) have postulated that any change that would occur due to osmotic stress would be small, and many authors (Schwabe, 1933, for *Eriocheir*; Gilchrist, 1956; Suomalainen, 1956; Frankenberg & Burbanck, 1963; Holliday *et al.*, 1964; Eltringham, 1965; McFarland & Pickens, 1965; Palmer, 1968) have found no significant changes in respiration rate which may be correlated with salinity. Gross (1957) and Duncan & Klekowski (1967) have pointed to the effects of salinity on the locomotory activity of an animal and suggest that certain observed changes in respiration rate in relation to salinity are due to changes in activity. The importance of activity in relation to oxygen consumption has been emphasized by McFarland & Pickens (1965), Newell & Northcroft (1967) and Halcrow & Boyd (1967).

In the present study an attempt has been made to induce a continual locomotory activity, and to measure the oxygen consumption of animals at a constant level of activity, acclimatized to different salinities. Anaesthetics were not used, since these may induce sub-basal oxygen consumption levels, or may selectively depress only certain metabolic processes of the animal.

MATERIALS AND METHODS

Animals were collected from the Ythan estuary, Aberdeenshire, and transported to stock tanks, supplied with mud and water (15–20‰) from their natural habitat, and maintained at 10°C. The animals were kept in the tanks for at least a fortnight, to extinguish any tidal rhythm (Morgan, 1965) which may have affected oxygen consumption (Wieser, 1962). They were maintained prior to oxygen consumption measurements in experimental salinities (1, 10, 20, 30‰; at 10°C), supplied with mud for 10 days.

Oxygen consumption was measured using microvolumetric respirometers (supplied by Mark Co.), to the design of Scholander & Iversen (1958), following the technique of Scholander *et al.* (1952). Standard reaction vials were used, and CO_2 was absorbed with 20% KOH on Whatman No. 40 filter paper. All measurements were made with the respirometers immersed in a constant-temperature water-bath at 10 ± 0·02 °C. Two thermobarometric blanks, with 1 ml of medium in each, were maintained. The animals in the respirometers were in 1 ml of medium. The respirometers were shaken concentrically at a rate of 30 shakes/min, to maintain equilibrium of the liquid and gas phases (Scholander *et al.*, 1952; Scholander & Iversen, 1958), and to induce a continual swimming activity in *C. volutator*. "Resting" or "routine" (Fry, 1957) metabolism was measured by leaving the respirometers still, except for periods of equilibration.

Animals were dried at 105°C for 24 hr, allowed to cool and weighed. Results are expressed at S.T.P. in units of μl O_2/mg dry wt. per hr.

RESULTS

It was found in all salinities (1, 10, 20, 30‰) that the oxygen consumption of active animals was logarithmically related to the weight of the animal (Figs. 1, 2, 3, 4). The statistical treatment of the data, the equations of the regression lines and the correlation coefficients are presented in Table 1.

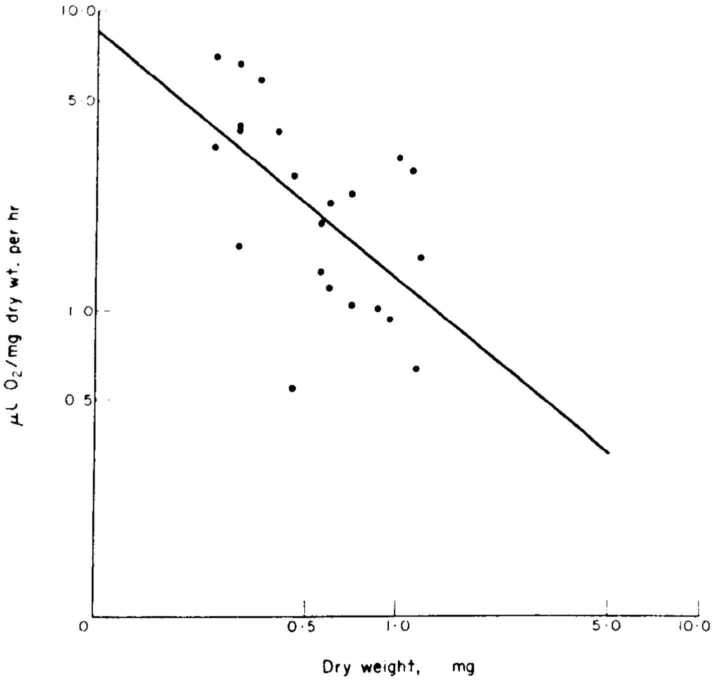

FIG. 1. The oxygen consumption in relation to dry wt. of *C. volutator* at 10°C and 1‰.

This exponential relationship was first demonstrated by Zeuthen (1947) and has since been frequently confirmed (Nicol, 1967; McFarland & Pickens, 1965; Newell & Northcroft, 1967).

When the oxygen consumption of all the active animals was compared with the salinity of the medium (Fig. 5), it was found that the oxygen consumption of animals at 20‰ was significantly different (at the 98 per cent level) from that of animals at 1, 10 or 30‰. However oxygen consumption per unit of weight is not a

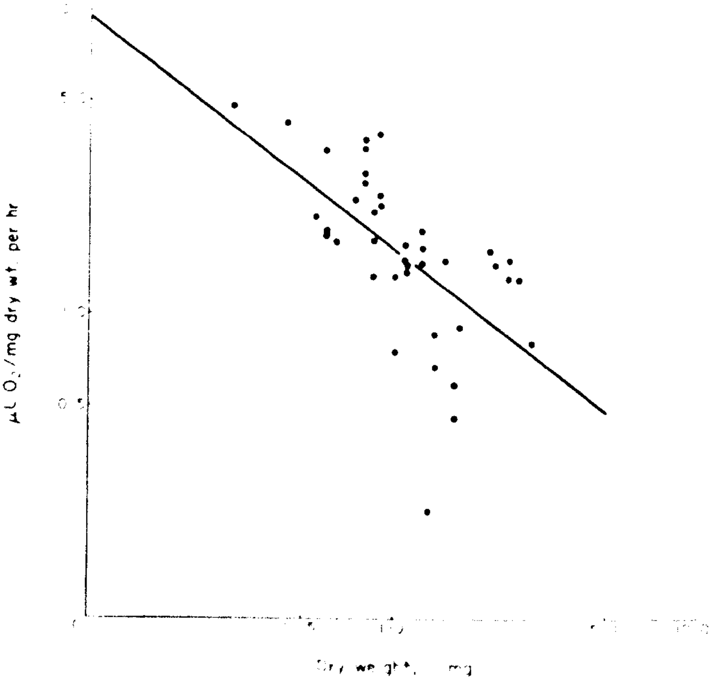

Fig. 2. The oxygen consumption in relation to dry wt. of *C. volutator* at 10°C and 10‰.

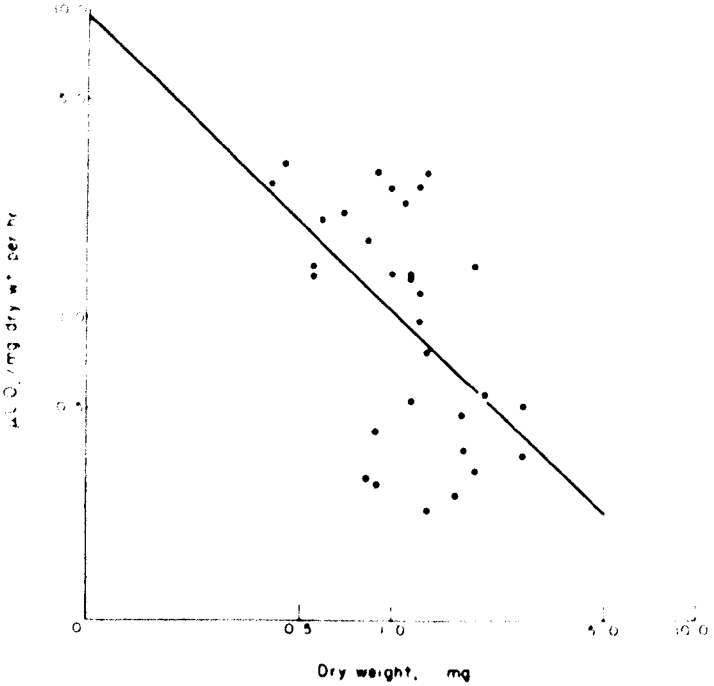

Fig. 3. The oxygen consumption in relation to dry wt. of *C. volutator* at 10°C and 20‰.

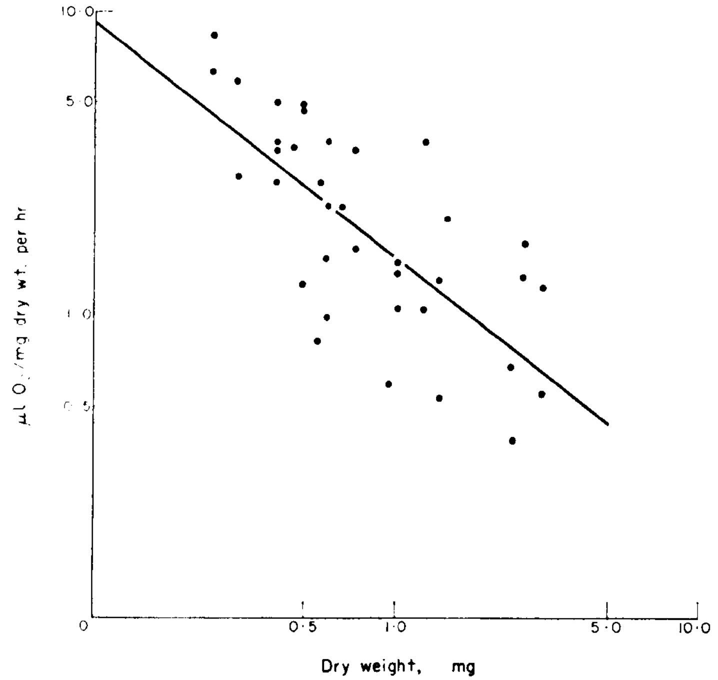

FIG. 4. The oxygen consumption in relation to dry wt. of *C. volutator* at 10°C and 30‰.

TABLE 1—OXYGEN CONSUMPTION OF *C. volutator* IN RELATION TO WEIGHT AND SALINITY

NaCl ‰	$y = a+bx$	n	Correlation coefficient
1	$y = 1{\cdot}933{-}0{\cdot}818x$	22	−0·581
10	$y = 1{\cdot}981{-}0{\cdot}758x$	40	−0·603
20	$y = 1{\cdot}989{-}0{\cdot}961x$	32	−0·532
30	$y = 1{\cdot}976{-}0{\cdot}782x$	37	−0·723

valid basis for comparison (Gilchrist, 1956), and if comparison is made of animals of similar dry weights, these differences largely disappeared. With animals all weighing less than 0·50 mg (Fig. 6), no significant differences between animals in different media were found. With animals 0·55–0·95 mg (Fig. 7) the only significant difference (at the 98 per cent level) noted was between animals at 10 and 20‰; there were no significant differences between animals at 1 and 10, 1 and 20 or 20 and 30‰. With animals greater than 1·0 mg (Fig. 8) no significant differences were found. Despite the result of animals 0·55–0·95 mg at 10‰ compared to 20‰, it is seen that in general there were no significant differences between animals acclimatized to different salinities.

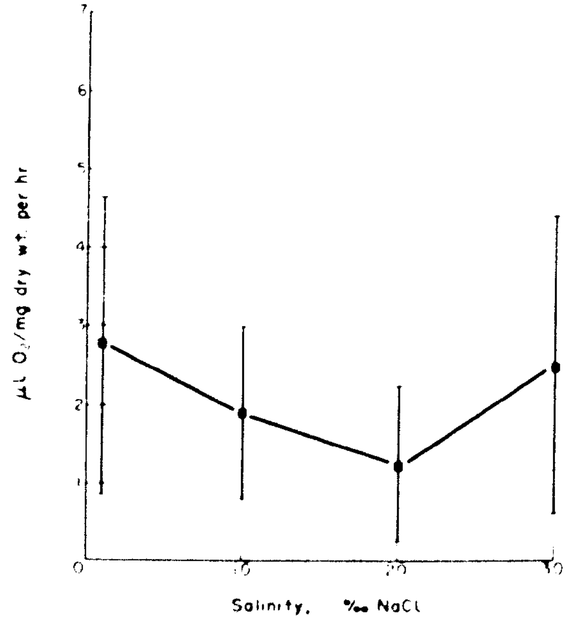

FIG. 5. The oxygen consumption of active *C. volutator* in relation to salinity. Square indicates mean; vertical lines indicate standard error.

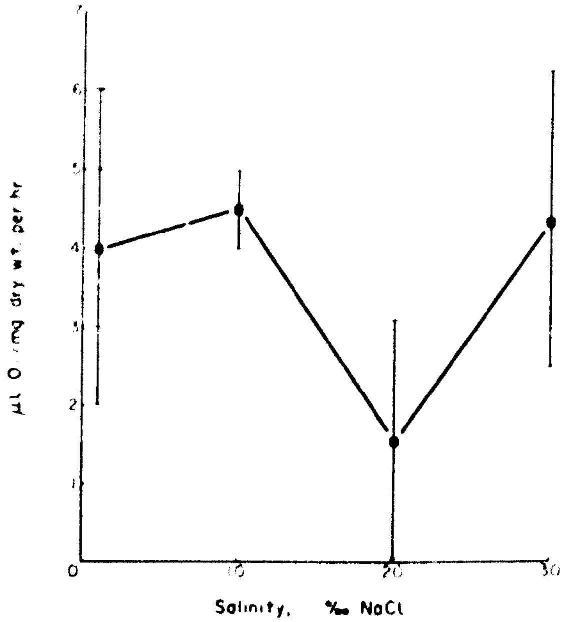

FIG. 6. The oxygen consumption of active *C. volutator* less than 0·50 mg dry wt., in relation to salinity. Square indicates mean; vertical lines indicate standard error.

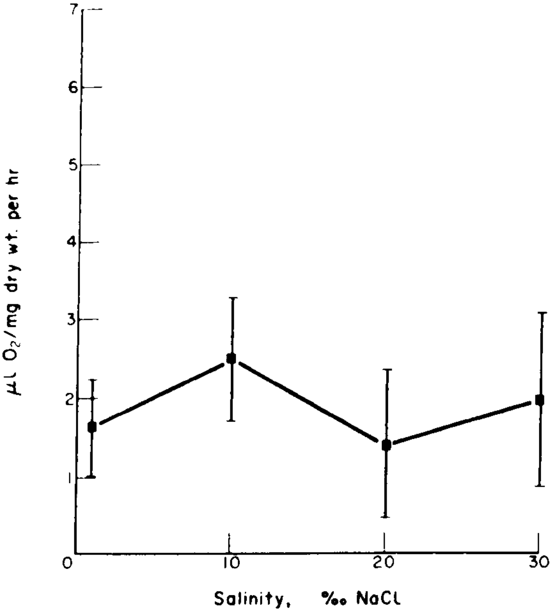

FIG. 7. The oxygen consumption of active *C. volutator* 0·55–0·95 mg dry wt., in relation to salinity. Square indicates mean; vertical lines indicate standard error.

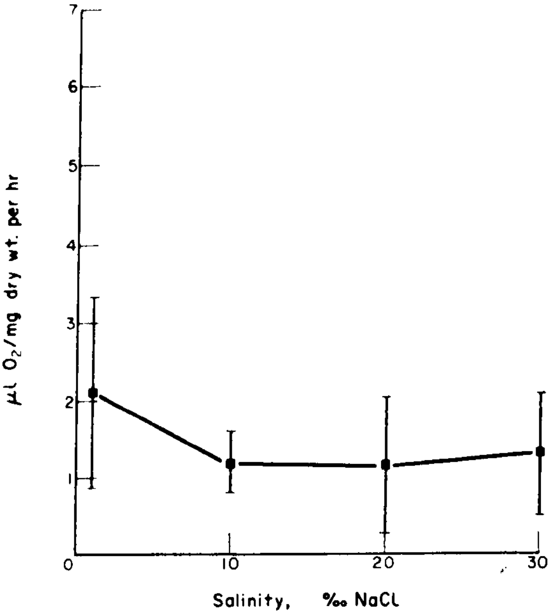

FIG. 8. The oxygen consumption of active *C. volutator* greater than 1·0 mg dry wt., in relation to salinity. Square indicates mean; vertical lines indicate standard error.

Animals at rest, which were not shaken and so stimulated to constant activity, displayed only spontaneous activity and showed a lower level of oxygen consumption, in relation to the lower level of activity (Fig. 9). The oxygen consumption of animals of all sizes at 20‰ was significantly lower (at the 99 per cent level) than that of animals at 1 or 10‰, but not significantly different from those at 30‰. However, as with the continuously active animals, this difference was not found when comparison was made of animals of similar sizes. It was also found with the spontaneously active animals that there was no linear or logarithmic relationship between weight and oxygen consumption. It has been noted (Meadows & Reid, 1966) that larger animals swim more frequently, and it may be suggested that this increased activity pattern, which was also noted in the experimental animals, increased the oxygen consumption of the large animals and masked the logarithmic relationship of Zeuthen (1947), noted in animals at a standard activity.

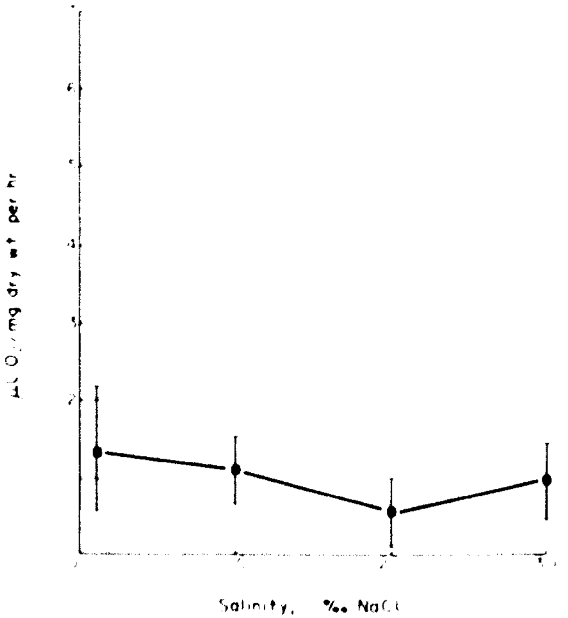

FIG. 9. The oxygen consumption of *C. volutator* not induced to continual activity (i.e. at rest) in relation to salinity. Square indicates mean; vertical lines indicate standard error.

Thamdrup (1935) measured the oxygen consumption of *C. volutator*, in sea water, at 2, 10, 20 and 29° C. At 10° C he noted the value of 600 ml O_2/kg wet wt. per hr. Considering wet weight as five times dry weight (Butterworth, 1968), this may be converted to 1·2 μl O_2/mg dry wt. per hr, which may be compared with the results from the present study at 30‰ of 2·48 μl O_2/mg dry wt. per hr "active" or 0·93 μl O_2/mg dry wt. per hr "rest". When these results from *C. volutator* are compared with those from other marine animals, it may be seen that they are broadly similar:

Clupea harengus (larva)	2·5–2·3 µl O_2/mg dry wt. per hr (Holliday *et al.*, 1964);
Limnoria	1·853–1·253 µl O_2/mg dry wt. per hr (Eltringham, 1965);
Euphausia pacifica	1·47 µl O_2/mg dry wt. per hr (Lasker, 1966).

DISCUSSION

It has been found that when comparison was made of the effects of salinity on respiration of *C. volutator* of similar sizes, at the same level of activity, no significant differences were noted between animals acclimated to different salinities. Three hypotheses may be made from these findings: either food is necessary as a direct supply of inorganic ions, or the metabolic cost of osmoregulation is nil, or a shift of energy requirements occurs from one metabolic process to another.

Nagel (1934) demonstrated active uptake of ions at the body surface by changing a hyperosmotic animal from a dilute medium to a more concentrated medium, which was still less concentrated than the original blood concentration, and found an increase in the osmotic pressure of the blood, suggesting active uptake of ions. I have repeated this experiment on *C. volutator*, by changing them from a medium of 3‰ to one of 10‰. These experiments were performed on animals fed and unfed, prior to and during the experiments. It was found in all animals that the mean blood concentration increased from 12‰ to 16‰ within 3 hr, and no significant differences in the rate of change were found between fed and unfed animals. This finding leads to the conclusion that active uptake of ions may be accomplished without food.

There remains, however, the finding (McLusky, 1967) that the range of salinities survived was increased, especially at low salinities, when the animals were supplied with food. When comparing the results of these experiments, it is necessary to consider the time scale, the adaptation to a salinity increase was completed in 3 hr, whereas the survival experiment was made over a period in excess of 500 hr. It is seen that food is necessary for long-term survival at low salinities, but that short-term uptake of ions and maintenance of hyperosmotic conditions may be accomplished without direct feeding, and that the energy reserves of the animal are adequate to cope with the uptake of inorganic ions.

It has already been noted that the optimal salinity range for maximal growth was smaller than the range of salinities tolerated (McLusky, 1967). It may be that in low salinities, under osmotic stress, that the animal is able to survive, and no changes in oxygen consumption are noted, because a metabolic shift occurs within the animal, and energy is diverted from growth to assist in osmoregulation. Thus the energy requirements for osmoregulatory processes would be increased under osmotic stress, but no overt change in oxygen consumption could be detected.

It has been noted that *C. volutator* produces urine hypo-osmotic to the blood under conditions of osmotic stress, and the demonstration of Potts (1954) that the production of urine hypo-osmotic to the blood, even if more concentrated than the medium, can greatly reduce the osmotic work of an animal leads to a partial explanation for the lack of any change in oxygen consumption in different salinities. Food, as a direct supply of ions for long-term survival at low salinities, would also minimize the metabolic cost of osmoregulation, by minimizing the need for active uptake of ions at the body surface.

Oglesby (1965) suggested that in *Nereis limnicola* the production of hypo-osmotic urine and a supply of ions via the food were important factors in osmoregulation, and a similar suggestion may be made for *C. volutator*, explaining the lack of any change in respiration rate in relation to salinity. The lack of any overt change in respiration rate may, however, conceal a shift of energy requirements within the animal.

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Key Word Index—Oxygen consumption; salinity; osmoregulation; euryhaline; *Corophium volutator*; amphipoda; Crustacea.