OSMOREGULATORY STUDIES ON SEVERAL INVERTEBRATES

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INTRODUCTION

To perform their specific function the internal cells of most multicellular organisms require to maintain an active exchange of material with the internal medium or body fluids. Obviously the internal fluids must carry in solution a sufficient quantity of the substances required by the cells and their total ~~percentage~~ content of solutes is a measure of their osmotic concentration. Animals have to control, within limits, not only the total amount of solutes in their internal medium, but also the nature of these solutes, and in particular, have to select a proper ratio of cations and anions, that is to exert some degree of ionic regulation. There has recently been some criticism of the physiological practice of expressing ion balance in biological systems as quantities of various single cations or anions (Nicol, 1962) in "the belief that effects of individual ion-species can be isolated in biology". It is pointed out that some ions are taken up, adsorbed or transported within particular colloids and that "biotic equilibrium is the resultant of metabolic and environmental situations".

A more appropriate application of classical inorganic chemistry would, Nicol thinks, be the recognition of the 'ecological concept' of the constancy of the ratio of anions to cations by weight denoted by G. Important biological values for G he indicates, are 1.7 for ocean water, for animal fluids 1.7 and not less than 1.54. What is meaningful in the relations of living organisms to their habitat is, therefore, G and osmotic pressure taken together.

The maintenance of the necessary minimum or threshold concentration of solutes in body fluids with the required balance of specific ions are interrelated but independent physiological mechanisms, and this also applies in respect of the relations of the cell to the extracellular fluids. Osmotic concentration and ion balance are physiological states common to all animals, terrestrial and aquatic, but the latter lend themselves more readily to the experimental study of the response of the internal medium to variations of the external medium, particularly those marine and fresh water invertebrates whose normal habitat confronts the animal with the need to cope with a range of natural variation.

An initial interest in the differences and changes in the composition of body fluids was the correlation of osmotic concentration with ecological distribution, and in recent times this has been combined with a quantitative analysis of ion ratios in internal and external media, together with investigations of the mechanism of selective uptake of particles and water transport from the immersion medium, and the differential excretion of substances and water from the internal medium. Whatever the experimental approach or the results of tests and observations, the problems centering on osmotic concentration and ionic regulation, as between internal and external media and intra- and extracellular components, finally converge on the properties of cell membranes. It is implicit in all theories of water permeability of living tissue that resistance to the passage of water is effectively confined to cell membranes, and cells which contain larger or smaller amounts of protein have to face the problem of water entry. There is now abundant evidence that cells expel sodium and take up potassium. It may be, as suggested by Glynn (1959) that they acquire the

ability to balance the osmotic pressure of their proteins in this way. The physico-chemical mechanisms involved in water permeability and active ion transport are not well understood and there is still a lack of knowledge concerning the structural basis of selective permeability.

It can be said, therefore, that all data obtained about the organisms' response in terms of the composition of its internal medium to natural or experimental variation in the external medium is a contribution toward our understanding of the lability of cell membranes in general.

This thesis is an account of experiments undertaken to test the effect of such conditions as temperature, season, sex and size, and any combination of these variables, on the osmotic concentration of certain marine and fresh water invertebrates when exposed to a range of immersion media from 100% sea water to fresh water.

Van't Hoff in 1886, using Pfeffer's results and arguing from thermodynamic principles, connected osmotic pressure with other colligative properties of

solutions. The freezing point depression (Δ) method of measuring osmotic concentration was probably first used by Bottazzi (1897) to test the body fluids of a number of marine invertebrates and vertebrates in sea water. This method has been criticised as inaccurate; for example, Blanchard (1940) suggested that measurement of the freezing point depression was never an accurate measure in colloid-containing solutions. Freezing point depression more recently determined as the melting point has been extensively used and is considered to give satisfactory results (Ramsay, 1949; Ramsay and Brown, 1955; Gross, 1954).

Other methods available for measuring osmotic concentration are described in Krogh (1939) and Prosser and Brown (1961). These include the thermoelectric vapour pressure method as originally described by Hill and Kupalov (1930), Barger's vapour pressure method and the chemical method of summing the total concentration of ions (Robertson, 1961).

The first laboratory experiments to test the response of aquatic animals to a change in salinity

were performed by Beudant in 1816 who claimed that over a period of five months he adapted a series of marine animals, including Mytilus edulis and Venus sp. to live in fresh water and, conversely, gradually adapted fresh water animals such as Lymnaea sp. and Ancylus fluviatilis to sea water over a period of five months. Anodonta sp. was not adapted to live in a higher salinity than 50% sea water. These experiments have never been successfully repeated. Bert (1871) recorded survival times of a number of fresh water fish and arthropods when plunged into sea water and found a longer survival time with a lower temperature. He was able to conclude from experiments and observation that death in sea water was due to the desiccation of the animals because of osmotic action. Among the first recorded comparisons of the concentration of blood and external medium were those by Fredericq (1882, 1885) who measured the concentration of the body fluids of a number of marine invertebrates which he found to be isosomotic with sea water. He was also the first to show that a euryhaline animal, Carcinus maenas, is hyperosmotic relative to the medium in brackish water. Bottazzi (1897) later reported an isosomotic internal concentration

in several marine invertebrates. Some of these results were based on measurements from pooled samples which Edmonds (1935) suggested masked significant small differences between internal and external concentration. His work includes the first reference to the differences in the osmotic concentrations of the elasmobranch and teleost blood. During the next ten years, both Fredericq (1898, 1901, 1904) and Bottazzi (1908) obtained further data concerning the osmotic balance in other marine and fresh water invertebrates, including values of $\Delta_i 0.80$ for crayfish, $\Delta_i 0.18$ to $\Delta_i 0.21$ for Anodonta anatina and $\Delta_i 0.22$ to $\Delta_i 0.23$ for Lymnaea stagnalis. Reporting the urine hypo-osmotic to the blood Bottazzi (1906) demonstrated the performance of osmotic work in the invertebrate Octopus vulgaris, and showed that a marine gastropod, Dolium galea, was isosomotic ($\Delta_i 2.24$, $\Delta_e 2.27$).

Quinton (1900b) demonstrated experimentally that the body surface of a marine invertebrate such as Aplysia punctata was permeable both internally and externally to salts and other experiments in the early part of the century by Quinton (1900a), Garrey (1905) and Monti (1914) added information about the osmotic

concentration in a number of echinoderms, molluscs, arthropods, elasmobranchs and teleosts, in marine, brackish and fresh water, while the range of salinity tolerance was explored by Colgan (1910) by survival experiments with molluscs from different intertidal levels. Fortunately, the great mass of information which has now accumulated about osmotic relations of aquatic invertebrates has been adequately collated at intervals in a series of reviews covering modern work (Schlieper, 1929, 1930; Krogh, 1939; Beadle, 1943, 1957; Prosser et al., 1950; Ramsay, 1954; Robertson, 1957, 1960; Shaw, 1960; Prosser and Brown, 1961).

Beadle, in his 1943 review, discusses at some length the evolution of what he terms 'osmotic independence' which enabled marine animals to penetrate into fresh or brackish water from their ancestral marine habitat. He points out that there are a large number of marine animals, for example, Mytilus edulis, which, although lacking any mechanism for osmotic regulation can live in much diluted sea water, and concludes that within the limits of salinity tolerance it is the rate of dilution which is the determining factor.

This capacity for a gradual adaptation must extend to the tissues of the animal. Other animals, such as the crabs, Eriocheir sinensis and Carcinus maenas, maintain a high level of osmotic concentration, presumably by some mechanism of osmoregulation. There are thus, he states, two possible methods of adaptation to dilute sea water, (1) by regulating the body fluid and thus insulating the tissues from the salinity changes of environment, or (2) by adapting the tissues to a diluted body fluid. An important component of the osmoregulatory mechanism in brackish water animals is, as Beadle showed, the active absorption of salts from the medium.

Some isopods and molluscs are found as inhabitants of fresh water where they maintain a hyperosmotic concentration and excrete a hypo-osmotic urine relative to the blood. The crab, Eriocheir sinensis, (Scholles, 1933), however, which maintains a high blood concentration in fresh water, does not secrete a hypo-osmotic urine, and Beadle suggests that as a first stage towards penetration of fresh water the animals maintain a high blood concentration not much below sea water by the absorption of ions, and thus protects the tissues

from dilution. The next step is the adaptation of the tissues to a lower blood concentration along with development of renal reabsorption and the secretion of a hypo-osmotic urine. Pott's (1954c) calculations on the amount of thermodynamic work required to maintain a high blood concentration makes it clear that animals such as Anodonta cygnea with a high value of PA (permeability per unit area of surface) could never have maintained a high blood concentration in fresh water. In the opposite direction, many fresh water animals can survive in salinities with an upper limit of tolerance which is related to the initial blood concentration when possibly there is an inability to obtain water from an isosomotic medium.

Ramsay (1954) in his general survey traces the development of ideas about the movements of water and electrolytes in invertebrates, considers the history of the penetration into fresh water by marine animals, and the consequent evolution of the ability to regulate the osmotic pressure of their blood above that of the dilute sea water and then in fresh water. He reviews the stages by which the various species succeed in leaving the sea water by active transport mechanisms

of the body surface and later secretion of hypo-osmotic urine. Complete adaptation to fresh water involves lowering of the osmotic pressure of the blood to a new general level to at least half that of sea water. He comments that in comparison very few fresh water invertebrates survive transference to sea water.

Reviews by Robertson (1957, 1960, 1962) of the osmotic and ionic regulation in aquatic invertebrates make clear the essential difference between ionic composition and osmotic concentration. As he points out, most marine invertebrates have an isosomotic concentration while practically all of them have some degree of ionic regulation, and this is brought out very clearly by reference to his ion analysis of an extensive series of invertebrates in practically all of which the ion ratio of the body fluids was different from that of the external medium. As would be expected, sodium and chloride, which make up most of the total ionic concentration, were the least variable ions. Robertson emphasises that in marine crustaceans with isosomotic blood concentration the central problem is ionic regulation as distinct from the crustaceans in brackish or fresh water who are confronted with the

task of maintaining an adequate osmotic concentration.

The aquatic invertebrates in respect of their osmotic relations to the external medium can thus be grouped broadly as follows:

A. Those animals in which the concentration of the blood is isosomotic and follows that of the medium. This includes most of the marine invertebrates.

B. Animals which generally have an internal concentration isosomotic in 100% sea water, but which regulate a hyperosmotic concentration in dilute sea water or fresh water. This includes many of the higher crustaceans and all fresh water animals.

C. Animals with an internal hypo-osmotic concentration in 100% sea water but which regulate hyperosmotically in dilute sea water. This includes the prawns and <u>Artemia salina</u>.

Group A, includes all the marine molluscs so far

investigated (Yazaki, 1929; Potts, 1954a; Beliaev, 1951; Robertson, 1962). The internal concentration follows the external concentration, that is, the animals tend to remain isosomotic. In the absence of osmoregulation, considered to be a primitive feature (Ramsay, 1954) there is still ion regulation. Some of the bivalves, however, maintain a hyperosmotic concentration in lower salinity solutions by closing the valves and so isolating themselves from the medium. "If this were an active process, i.e. if the adductor muscle of the valves did not possess a 'catch'-mechanism which enables the muscle to remain contracted without any increase in its metabolic rate, it could be dignified by the term osmoregulation." (Maloeuf, 1937.) The echinoderms are reported to be isosomotic in full strength and dilute sea water. Maloeuf (1937) has reported Asterias forbesii isosomotic down to 50% sea water.

Group B are animals with an internal hyperosmotic concentration in salinities below 100% sea water and this includes fresh water animals, all of which are necessarily hyperosmotic regulators. Fresh water animals such as Anodonta cygnea, Unio pictorum (Krogh, 1939) and Eriocheir sinensis (Krogh, 1938)

are able to absorb the necessary salts against a concentration gradient. Dakin (1935) records a value of Δ_i1.1 for Astacopsis sp. which indicates an ion concentration of a level considerably above that of the medium. Among the euryhaline animals, Carcinus maenas has been studied by Fredericq as early as 1885 followed by Duval (1925), Schlieper (1929), Margaria (1931), Schwabe (1933), Nagel (1934) and Picken (1936). This animal becomes increasingly hyperosmotic as the external salinity falls and it excretes a urine isosomotic to the blood which, as noted by Nagel and recently confirmed by Shaw (1961), becomes more abundant with decreasing salinity. This means an increased loss of salts and consequently a greater uptake of salts from the medium. Apart from the crustaceans few animals osmoregulate strongly in dilute water. Nereis diversicolor shows limited regulation in 25% sea water (Beadle, 1937).

In Group C, Palaemon serratus and Palaemonetes varians (Panikkar, 1941; Parry, 1954; 1955) are hypo-osmotic in 100% sea water and below 60% to 70% sea water are hyperosmotic. Over the salinity range in which they can regulate concentration of the blood, Metapenaeus monoceros (Panikkar and Viswanathan, 1948)

remains more or less constant throughout a manyfold change in the medium. Broekema (1941) demonstrated that Crangon vulgaris changed from hyper- to hypo-osmotic over the range 15°/oo to 45°/oo, and Williams (1960) showed that Panaeus duorarum and P. aztecus followed the same pattern of osmotic concentration as in the shrimps so far investigated. The animals were hypo-osmotic in 100% sea water and hyperosmotic in solutions below 25°/oo to 30°/oo. In addition, some crabs are known to have hypo-osmotic concentration in 100% sea water. Baumberger and Olmstead (1928) showed that Pachygrapsus crassipes had a value for blood concentration equal to Δ_i1.33 when the sea water was Δ_e1.97 but Gross (1955, 1957) and Prosser, Green and Chow (1955) found that animals may be isosomotic or hyperosmotic relative to 100% sea water and regulate strongly as the salinity decreases. Dakin and Edmonds (1931) found the Helocius cordiformis was hypo-osmotic (Δ_i1.89) in 100% sea water, (Δ_e1.98) but hyperosmotic in dilute sea water (Δ_i1.38, Δ_e0.72). These relationships were confirmed by Edmonds (1935) who also showed a similar response in Leptograpsus veriegatus and Sesarma erythrodactyla. Similarly, (Anderson and Prosser, 1953) Callinectes

sapidus is hypo-osmotic (≡0.46 N NaCl) to the medium in 100% sea water (≡0.51 N NaCl) but hyperosmotic (≡0.38 N NaCl) in dilute sea water (≡0.10 N NaCl). Artemia salina (Croghan, 1958a) maintains almost a constant ion and osmotic concentration. Over the external range 2% to 600% NaCl the animals showed only a 5% NaCl increase in blood concentration. They are hypo-osmotic above 25% sea water and hyperosmotic below that salinity.

Osmotic balance is primarily an adjustment between the concentrations of the internal and external media, but it can be influenced by other physical conditions of the environment. The temperature of the external medium is known to affect osmotic regulation. Commenting on this, Panikkar (1940) believes that temperature may be a factor of some ecological importance for the penetration of marine animals into brackish and fresh water. He found that raising the temperature lowers the blood osmotic concentration of the two prawns Palaemon serratus and Palaemonetes varians and speculates on the possibility of an 'optimum' blood concentration lowered, for example, by a raised temperature, which would in this way diminish the osmotic stress of changing salinity

conditions. There is the interesting observation of Beadle (1943) who records that the survival time of Nereis diversicolor in dilute sea water was much increased at low temperature, although the blood osmotic concentration was not affected. Robertson (1960) giving examples of higher blood concentration of certain Crustacea at lower temperatures, comments on the possible combined effects of temperature and salinity on ecological distribution. It has been suggested that the temperature influence on osmotic concentration is a by product of a disturbed metabolism, but such effects have been noted within normal ecological temperature range.

The combination of the salinity, and temperature and salinity, of the medium has been shown to affect the development of the eggs and larvae of Ostrea gigas (Amemiya, 1928) and of the eggs of Carcinus maenas (Broekhuysen, 1936). The range of salinity conditions in which Ostrea gigas eggs could develop was wider at 16°C than at 25°C and there was a lower optimum salinity for development at 16°C than at 25°C. In contrast, the eggs of Carcinus maenas developed more normally in a lower salinity at the higher

experimental temperature, 16°C, than at 9°C, and Broekhysen suggested that this could be explained as a different optimum temperature for egg development in the two species, or alternatively a possible specific difference in salinity response. Other relevant examples were demonstrated by Smith (1955a and b, 1957) in Nereis diversicolor and Neanthes lighti, both of which show alteration of regulation in low salinities as a temperature response.

Outside the scope of the present experiments but pertinent to their implications is the temporal influence of the moult cycle on osmotic balance in the Crustacea. Baumberger and Olmstead (1928) and Baumberger and Dill (1928) noted in both Pachygrapsus crassipes and Callinectes sapidus that the osmotic concentration was at its lowest in the intermoult stage and at its highest during actual moulting. Parry (1953) reported an increase in osmotic concentration of the blood of Ligia oceanica during moulting, and Robertson (1960) found a change in both total ionic and osmotic concentration in Carcinus maenas in relation to the moulting cycle. The increase in the total concentration in the premoult stage involved a rise in the concentration of sodium, calcium, magnesium

and chloride.

Other factors such as size and sex could influence osmotic balance and Gilbert (1959a,b and c) found that in Carcinus maenas blood conductivity, freezing point depression, chloride, sulphate and non-protein nitrogen all were significantly affected by the size and sex of the animals.

The seasonal effects on osmotic concentration response are naturally associated with temperature differences, but there are now available records from several species of fresh water fish (Hart, 1952), a planarian (Schlieper and Blüsing, 1953) and the rainbow trout (Keiz, 1953) showing a greater tolerance of high temperatures in summer than in winter after adaptation to the same temperature in both seasons. The seasonal effect where it exists must obviously reflect some cyclic adaptation.

There are therefore a sufficient number of random observations available on the influence of temperature and of temperature and salinity combined on osmotic concentration and tolerance of unfavourable media to

indicate the need for the systematic experimental tests which are the subject of this thesis. What is of immediate importance to the organism as an adaptive response to normal or emergency conditions is its internal osmotic concentration and not its ionic regulation and for that reason this study in the first instance is limited to measurements of osmotic concentration and survival tests.

The choice of experimental animals was dictated by a number of considerations, (1) that they should be indigenous and suitable for laboratory conditions, (2) that they should be representative of at least two of the phyla of the invertebrates, (3) that they should include animals adapted to a wide range of natural conditions, that is intertidal or estuarine, (4) a fresh water animal which also inhabited low salinity media such as salt marshes or brackish pools.

MATERIAL AND METHODS

The work reported here started in the winter of 1959-60 and extended over three years, finishing in 1962.

Experimental Animals

The experimental animals were two species of isopod crustaceans, Ligia oceanica (Linnaeus) and Idotea granulosa Rathke, and five species of gastropod molluscs, Littorina littorea (L.), Littorina littoralis (L.), Littorina saxatilis (Olivi), Hydrobia ulvae (Pennant) and Potamopyrgus jenkinsi (Smith, 1889, p.142, as Hydrobia). The nomenclature follows that of the Plymouth Marine Fauna, 1957, of the Marine Biological Association.

Animals collected during November to March were considered as belonging to the winter type and those collected from May to September as the summer type. A sample of water was obtained when possible, when the animals were collected. The isopods Ligia oceanica and Idotea granulosa, and the three species of Littorinidae, Littorina littorea, L. littoralis

and L. saxatilis were all collected on the shores of the Isle of Cumbrae in the Firth of Clyde. Ligia oceanica and Idotea granulosa were both collected from the rocky shores directly below and also to the east of the Marine Station on the island, the former from high tide levels and the latter from mid to low tide levels. The population density of Idotea granulosa fluctuated throughout the year. In the autumn and winter months, specimens were numerous and readily found on the shores, but the numbers declined until by the beginning of April only one individual was obtained after one-and-a-half-hours' searching (March 16, 1961). Thereafter, with a gradual increase through the summer, they became more numerous again with the approach of winter. Naylor (1955) found them to be most abundant on the Isle of Man from August to November.

Specimens from two populations of Littorina littorea were collected from two areas. Those from Population I, east of the Marine Station at the Wishing Well, had a normal operculum while about half of those from Population II, collected from the rocks just below the Station, either lacked an

operculum or else had a reduced one. Presumably animals with this defect cannot shut themselves off from the medium to the same extent as those with a normal operculum. A similar abnormality in Nucella from Ireland was recorded by Colgan (1910). The shells of a large proportion of both populations of L. littorea were bored by the polychaete, Polydora ciliata. Littorina littoralis collected from the same two localities differed in the colour pattern of the shell. The majority of those from the site below the Station had a brown and yellow checkered type of shell, while those from the east of the Station had a plain coloured shell. L. saxatilis was also collected from localities adjoining the Station and further east. The collected animals, put into polythene bags with damp seaweed and packed into an insulated container, were dispatched to the Glasgow laboratory where they arrived the same day and were immediately placed into the experimental media.

Of the Hydrobiidae, Hydrobia ulvae was collected in the Clyde river estuary, near Ardoch, and specimens of the giant type of the same species from

a salt marsh at Tynningham, East Lothian. Rothschild (1936) showed that H. ulvae attain a greater size when infected with larval trematodes which probably delay sexual maturity and thus prolong the growth period.

The second member of the Hydrobiidae investigated, Potamopyrgus jenkinsi, originally described by Smith (1889), has been separated by Warwick (1952) into three morphological types, A, B and C. The osmotic balance of these three types was tested to ascertain if any physiological differences occurred. Some specimens of all three types were provided by Mr T. Warwick of Edinburgh. Samples of the A type from fresh water were collected from Lochend Loch, just west of Glasgow and from brackish pools at Dunbar, East Lothian. These brackish pools had a salinity about 17% to 19% sea water, but were not in direct connection with the sea. Presumably the salinity was due to sea water percolating through the gravel substrate. Type B (corresponding to Smith's type specimen) had been collected from a brackish ditch at Aldeburgh, Suffolk, and the Pembrokeshire type C from fresh water ditches at

Bathesland, Newgale. Types B and C were kept at laboratory temperatures in 5% sea water before starting the experiments, but the A type was placed in the experimental media immediately.

Experimental Conditions

The osmotic relationships of each species of animal were tested in a series of temperature-salinity conditions. Whenever possible the experiments covered both summer and winter types.

Salinity

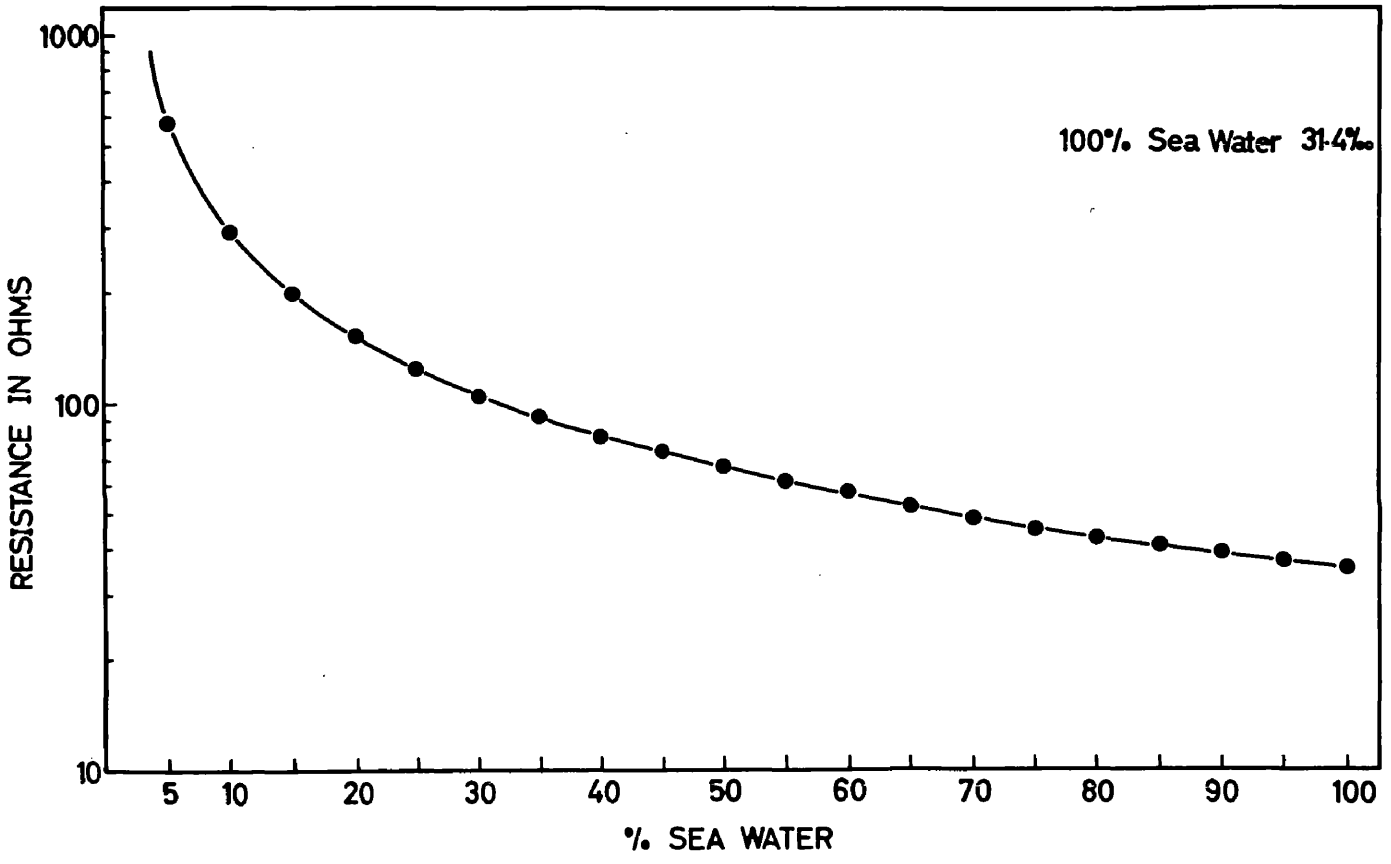
The experimental salinity solutions ranged from 150% sea water to fresh water. The different salinity concentrations were obtained by adding either Tidmann's Sea Salt or Glasgow tap water (total solids 21.3 mg/l, 1947 analysis, City of Glasgow) to fresh Millport sea water. An average value for surface salinity at Millport is 32.1‰ (Barnes, 1955) and this is taken as the salinity of 100% sea water. Sea water salinity changes seasonally, and this will be reflected in what constitutes a given per cent sea water at any one time, but such variations are insignificant in terms

of the total test range of experimental salinities.

Salinity determinations were made with a Phillips Conductivity-measuring Bridge, a method which is now being more generally used because of its simplicity and accuracy. The correlation of the properties of sea water with conductivity measurement has been investigated by Cox, Culkin, Greenhalgh and Riley (1962). Figure 1 represents a graph constructed from the conductivities of samples of known percentage salinity, from which, given the conductivity of the unknown sample, its percentage sea water (± 2%) could be read off. To avoid correction for temperature, conductivity of a sample was tested at 20 ± 0.2°C.

The fresh water used in the experiments came from either Lochend Loch (calcium content about 39.6 mg/l, Hunter, 1953), Loch Lomond (total solids about 45 mg/l, 1961 analysis, Clyde River Board) or Tynningham River (no analysis). No appreciable differences in osmotic concentration resulted from using fresh water from these different areas. In one set of experiments with Potamopyrgus jenkinsi, Cambridge tap water (total

Figure 1. Conductivity-measuring bridge calibration. Readings were taken at 20 $\pm$ 0.2°C.


1000
100
10
RESISTANCE IN OHMS
100% Sea Water 31·4‰
5
10
20
30
40
50
60
70
80
90
100
% SEA WATER

solids about 252.8 mg/l, Weil & Pantin, 1931) was the experimental medium.

Temperature

A high and a low experimental temperature were chosen in relation to the average seasonal sea and fresh water temperatures. For example, Macan & Worthington (1951, p.20) give a surface temperature of 15°C in summer (July) and 4°C in winter (February) for Lake Windermere. Barnes (1959) has investigated the surface fluctuations in temperature at Millport, and the range was from 13.4°C (August) to 6.8°C (March) for a ten-year average. The minimum temperature recorded was 4.8°C and the maximum was 15.5°C. From this temperature data, the low temperature chosen was 5 ± 1°C and the high temperature 15 ± 1°C. One group of Littorina littorea was tested at 20°C. The low temperature was maintained either by placing the animals in a refrigerated constant temperature room or by floating the containers in a water bath cooled by refrigerated antifreeze circulating in polythene tubing through the tanks. The high temperature was maintained by floating the animal

containers in water baths kept at a constant temperature by Techne Tempunits or by placing them in a constant temperature room.

Animal containers

During the period of the experiments, the animals were kept in plastic containers with a maximum of 35 animals per 3 litres of aerated water at a given salinity and temperature. The animals were starved and kept in constant darkness since photoperiod has been shown to affect metabolism (Dehnel, 1958). Difficulty in keeping the species alive when completely submerged has been reported by Barnes (1932) with Ligia baudiniana and by others with L. oceanica. Parry (1953), however, suggested this was due to lack of oxygen and certainly no difficulty was experienced in keeping L. oceanica alive in well aerated water. In preliminary experiments it was found that the two species of isopods could climb up the air hose and so out of the experimental media. This happened particularly if the salinity was not optimum, as for example 25% sea water. An animal which had thus been out of water for most

of the previous 24 hours would drop back into the medium when the lid of the container was lifted and so be sampled with the rest and in this way invalidate the results. This difficulty was counteracted by the simple expedient of placing a small glass funnel upside down on the air hose after the manner of rat shields on the ropes of ships.

Since under natural conditions the isopods cling to seaweed or rock surfaces, small washed, rough stones were placed in the container. The snails, however, crawled on the surface of the container as far as the edge of the water but the tested animals were always chosen from those completely submerged at the time, although they could have been out of the media some time previously. A series of control experiments with the animals enclosed in wide mesh muslin bags produced results no different from those in which the snails were not restrained in this way.

Dye experiments

In the lower salinities the snails may remain

retracted within the shell. To determine how much exchange there is between the body fluid and the external medium under these circumstances, vital dyes were added to the water. The amount of dye subsequently present in the tissues, estimated colorimetrically, was then taken as an index of the amount of exchange which had taken place. The dyes tested were: Edicol Pea Green, Phenol Red, Trypan Blue and Trypan Red. Of these, Phenol Red proved to be the most readily detectable. Two concentrations of the dye were tested, 0.01% and 0.1%, but with the more concentrated solution, accummulation of the dye was so rapid that intermediate concentrations in the tissues could not be detected. Phenol Red is an indicator which changes from yellow to red over the pH range 6.8 to 8.4. The pH of the tissues of the snail lies between 6.8 and 7.2 and over this range the colour of the indicator varies from yellow to pale orange and is difficult to distinguish from the normal body colour. If however the tissues are treated with an alkaline solution, the indicator turns red and the amount can then be readily estimated. Of the bases tried, potassium

hydroxide was found to penetrate cells sufficiently rapidly and to quickly demonstrate the presence of the dye in the tissues. After a test period in the Phenol Red solution, the snail was removed from its shell and by means of a glass micropipette, potassium hydroxide was added to the following: fluid or mucus in the mantle cavity; the gills; kidney tissue and urine in the renal cavity; to various regions of the alimentary canal from oesophagus to anus. In this way the different parts of the body were tested for Phenol Red and any dye present was recorded in terms of + to ++++ denoting a trace to strongly positive.

Sampling

Ramsay (1949) noted that even during short periods there was some diffusion out of hard glass capillaries such as Pyrex and therefore silica glass capillary tubes were used to hold the samples. The capillary tubes, drawn by hand using an oxy-acetylene flame, were chosen on the basis of a uniform diameter of about 0.2 mm external diameter. The capillary tube was broken into one-and-a-half-inch

lengths which were boiled in detergent followed by several changes of distilled water before drying. Gross's (1954) method of drawing the sample into the tube leaving air at each end and sealing the ends with vaseline, was unsatisfactory, the difference in the lengths of the samples in the tubes affecting the freezing point depression. Ramsay's (1949) technique was therefore adopted and the capillary tube joined with plasticene to a short pipette with a flexible plastic tube through which medicinal paraffin, the sample, then paraffin again were drawn up. In this way the sample was effectively sealed within the capillary tube. The loaded capillary tubes were then frozen immediately on solid carbon dioxide (dry ice) at -78°C.

Pericardial fluid, blood or urine samples were drawn directly into the capillary tubes and there was no difficulty with blood clotting except in one experiment where tubes of 0.1 mm external diameter were used to obtain both blood and urine from the same specimen of Potamopyrgus jenkinsi. In the isopods, blood samples were taken from the heart, but attempts to get urine samples were

unsuccessful although a number of methods of vital staining of the kidney, Carmine (Cussans, 1904), Edicol Pea Green, Indigo-carmine (Hewitt, 1907), Neutral Red, Phenol Red, Trypan Blue and Trypan Red were tried. Different concentrations of dye in sea water were injected at the base of an appendage on the ventral surface of the animals and dyed food was given. Over periods ranging from 1 hour up to 5 days no staining of the saccule or labyrinth was detected by dissection under a binocular microscope. Repeated efforts were made to collect urine from the external apperture following Lockwood's (1961) procedure with Gammarus duebeni. The animal was enclosed round the middle with a tightly fitting wide rubber ring and the posterior segments with the pleopods were placed in the appropriate saline solution contained in a small glass vial; the vial was closed by means of the rubber ring. The whole unit was now placed in medicinal paraffin and the animal continued to respire by way of the pleopods. In Lockwood's experiments, a drop of urine appeared on the antennary cone in Gammarus duebeni, but in the present experiments with Ligia oceanica and Idotea granulosa, the labyrinth opens at the

base of the second maxilla and saline fluid would either seep up the animal from the vial or else an oily brown substance, possibly a stress response, was regurgitated before any urine could collect.

In the Littorinidae the shell was tapped gently until it cracked and could be picked off, exposing the heart in the pericardium and the kidney. Blood was taken from the ventricle and sometimes, in addition, fluid from the pericardial cavity was sampled. In the first experiments urine was sampled by pushing a capillary tube through the roof of the kidney into its lumen, but subsequently an opening was made for the tube with a clean needle. Every precaution was taken to prevent sample contamination and the paraffin was always kept under pressure at the end of the capillary until the desired area was reached.

Special techniques were necessary to sample both the blood and urine in Potamopyrgus jenkinsi because of the small size (maximum about 5 mm). Small calibre capillary tubes were used, although because of the greater surface area to volume there was the tendency for such a sample to stick

to the tube. The freezing point depression values for blood and for urine indicated that the results obtained from the urine samples could be regarded as representing osmotic balance (see also p.80). As samples of urine were readily available, and the test results consistent, this fluid alone was used throughout most of the experiments on the Hydrobiidae as the indicator of osmotic relationships. A screw clamp was used to crack the shell of Hydrobia ulvae but the fragile shell of Potamopyrgus jenkinsi was carefully picked off with a tungsten wire.

Various other regions of the body of the Hydrobiidae were tried as possible sources of body fluid. A mixture of fluid and cells could, for example, be obtained from the end of the spire close to the gonadal region and the sample prepared in the following way. Paraffin was drawn into the capillary tube, followed by the sample, followed by either mercury with sealing wax to close the end or melted paraffin wax which solidified at room temperature. A dense substance rather than the usual paraffin was required in order to centre the sample in the tube during centrifuging. The

tubes were now fitted upright into holes in a plastic rod, placed in the centrifuge and centrifuged for 30 minutes, 20,000 revolutions per minute, at 20°C. A clear fluid separated out at one end of the sample so that ice crystals could be easily observed after the sample was frozen. The disadvantage of this procedure was the inability to control the length of the clear sample which necessarily varied according to its original cellular content. This resulted in samples from different animals and also several samples from the same animal giving widely different results.

When both blood and urine from the same specimen of Potamopyrgus jenkinsi were tested, the sampling was carried out under liquid paraffin. Otherwise, no special precautions were taken in obtaining the samples since the procedure only took a few seconds. Freezing point depression determinations were made on volumes of about 0.04 microlitres (µl) using the 0.3 mm capillary tubes and 0.01 microlitres with the ultra fine 0.1 mm tubes. The volumes were estimated by weighing an equal amount of distilled water.

Preliminary experimental results with the isopods

indicated that 4 days adaptation to a temperature-salinity condition was required to reach a steady state. Blood concentration therefore was not tested until the fifth day. Results from an analysis of variance, (an example is given in Table I) showed that this length of time was sufficient, as further adaptation produced no change. In the case of the summer type Idotea granulosa, survival rate was low in 25% sea water. The tests on the blood were started on the second day rather than the alternative of improving the survival rate by using a higher percentage salinity, when the results would not have fitted into any comparative analysis. In the experimental media, the gastropods reached a steady state within a day and therefore the pericardial fluid, blood and urine could be sampled after 24 hours. Usually two or three animals from any one temperature-salinity condition were tested each day.

The isopods were weighed after sampling, the Littorinidae before sampling, and the sex of the animal was noted. Only isopods in the intermoult stage were used. The weights of Hydrobia ulvae and Potamopyrgus jenkinsi were not recorded since

Table I

LIGIA OCEANICA

Analysis of Variance

Summer animals in 100% sea water at 5°C

Days in medium	Δ_i°C a	b	c
5	2.20	2.27	2.22
6	1.98	2.28	2.26
11	2.02	2.26	2.13
12	2.24	2.26	2.24
13	2.33	2.30	2.15
14	2.21	2.20	2.09
15	2.14	2.45	2.36
17	2.17	2.13	(2.13)+

+Missing value

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Days in medium	7	0.0860	0.0123	1.1 N.S.
Residual	15	0.1674	0.0112	
Total	23	0.2534		

the tested animals fell within a uniform size range. Fluid samples could not be obtained from the smaller individuals with the 0.3 mm capillary tubes usually employed.

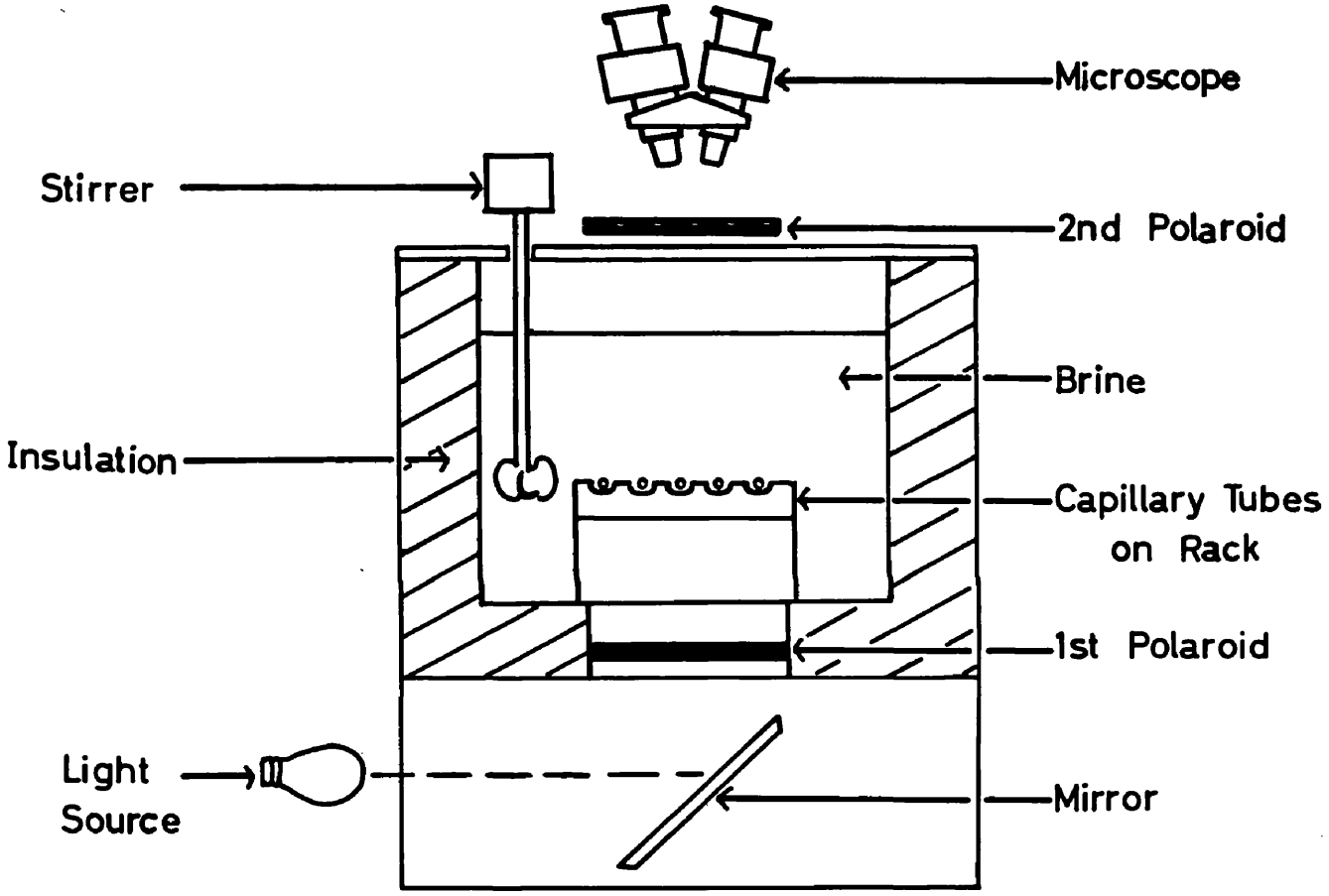
Freezing Point Depression Determination

The osmotic concentration of the sample was determined by the freezing point depression (Δ) method first described by Jones (1941) and later modified by Gross (1954). The freezing point depression of the animal's fluid is indicated by Δ_i and Δ_e refers to the external experimental medium which was also sampled. Repeated determinations of the freezing point depression of a standard solution showed the error to be within about 2%. In this technique the freezing point depression of small samples is deduced from comparison with that of known sodium chloride standards. What is determined is the melting point which has the same temperature as the freezing point but has the advantage of avoiding the difficulties arising from supercooling. The freezing point depression of the series of standards

was found using a Beckmann thermometer. For the present experiments known standards with freezing point depressions ranging approximately from 3.0 °C to 0.5 °C together with distilled water proved sufficient to cover the results. The frozen samples of both standard and unknown solutions were fixed on a notched stainless steel rack placed in a perspex box filled with -15°C brine and enclosed in a glass wool insulated perspex container. To prevent temperature gradients developing, the brine was stirred constantly and this was checked both with a thermometer and by changing the direction of the standard gradient on the rack from left to right or vice versa on alternate days. The same procedure was faithfully observed with all standard and unknown solutions.

Crossed polaroids were placed on either side of the capillary tubes. Light reflected from a mirror passed through the polaroids, the capillary tubes, and into a binocular microscope (Fig. 2). Observed in this way, the ice crystals of the frozen samples were birefrigent and the melting

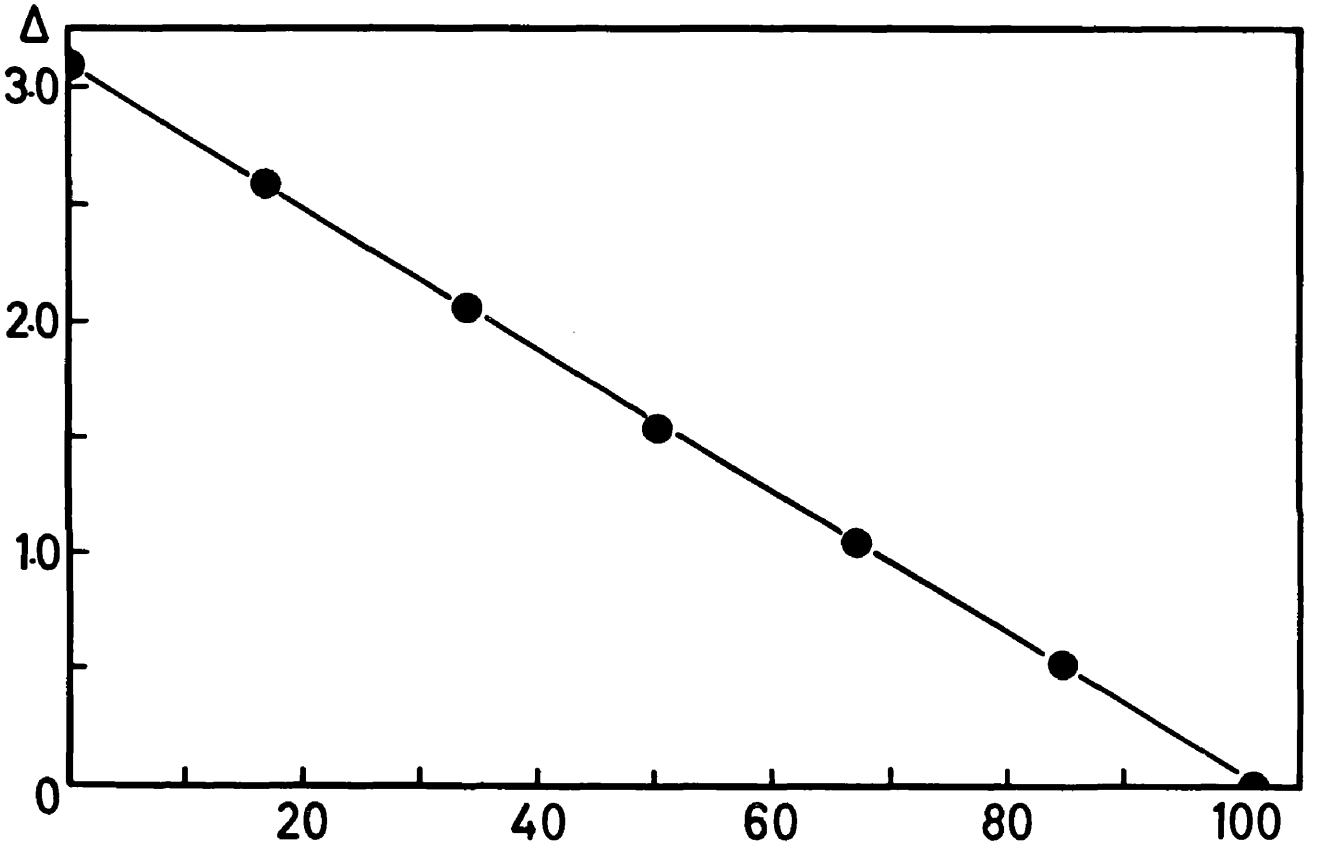
Figure 2. Diagram of the apparatus for measurement of the freezing point depression.


Microscope
Stirrer
2nd Polaroid
Brine
Insulation
Capillary Tubes
on Rack
1st Polaroid
Light
Source
Mirror

of the last crystal could be timed accurately by the abrupt disappearance of the light. Given zero as the melting time of the most concentrated standard solution, there is a straight line relationship between the melting times and the freezing point depressions of the standards (Fig. 3). When the melting time of a sample is known its freezing point depression can then be read off from the graph, in terms of sodium chloride freezing point depression. The time taken for the brine to warm 1°C was sufficiently slow, at least 32 minutes, to allow the samples and tubes to be in equilibrium with the brine temperature.

All the colligative properties of a solution are related but not necessarily directly. In the present range of freezing point depression, the sodium chloride concentration-freezing point depression is a straight line relationship and the formula $\frac{\Delta}{0.6}$ = % NaCl (Ramsay, 1949) gives a fairly accurate estimation of the sodium chloride concentration. The osmotic concentration can also be expressed in terms of molal of millimolal equivalents of sodium chloride or in osmoles.

Figure 3. An example of a graph showing freezing point depressions of the standard solutions. Given the time to melting of the last ice crystal of the unknown samples, the freezing point depression could then be read off.


Δ
3.0
2.0
1.0
0
20
40
60
80
100
Time to the Melting of last Ice Crystal
in Minutes

Statistical Treatment of Results

The results from the isopods were tested statistically by analysis of variance. Using this method which involves the calculation of a quantity F, it is possible to find whether the freezing point depression is affected by changes in salinity, temperature, season or by any combination of these factors. If the value of F for salinity, say, is greater than or equal to $F_{0.05}$ obtained from tables, then the probability of getting such an F value is $P < 0.05$ indicating that there are significant differences between the freezing point depression of animals in different salinities. Similarly for the other factors and interactions.

The results from any two groups of gastropods were analysed by "Student's" t-test for random samples for groups with unequal numbers (see for example Snedecor, 1956, p. 91).

$$t = (\bar{x}_1 - \bar{x}_2) \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{n_1 + n_2 \ (\Sigma x^2)}}$$

With blood and urine from the same specimen of Potamopyrgus jenkinsi, the test of significance for paired samples using the t-test was employed (see Snedecor,

1956, p 49) namely,

$$t = \frac{\bar{d} - \mu}{s_{\bar{d}}}$$

The original data are given in the appendix. Tables of the mean freezing point depression of each group of animals from a particular temperature-salinity condition also give the standard deviation and standard error of the mean.

There would seem to be no general agreement on the degree of difference between the freezing point depressions of body fluids and the medium which can be interpreted as hyperosmotic. Isosmotic is used in some reports where there is a consistent small difference between the internal and external concentrations, which in other reports would be described as hyperosmotic. In this investigation, mean values for body fluids $\pm$ 2% that of the medium are considered isosmotic. Within this adopted definition, in certain of the gastropods studied, particularly the Hydrobiidae, the blood or urine was consistently hyperosmotic to the medium. It might be objected that with marginal differences the term hyperosmotic did not

exclude the possibility of minor technical error, but if that were so, the expectation would be an equal number of values denoting hypo- as hyperosmotic. In fact this was not the case and the results were either isosomotic or hyperosmotic.

RESULTS

Isopoda

Any significant effect on the osmotic concentration of the blood due to salinity, temperature, season or interactions between these factors was determined by the analysis of variance. One complete analysis is given in the appendix (p.A9). Groups of equal numbers of animals from the different experimental media (either 15 or 18 measurements) were compared and comparison of the mean values from those groups with the mean values from the total numbers tested shows little difference. Several times "missing values" had to be inserted to make the groups equal in number and the method is given in the appendix (p. A 2).

Two other variables, size and sex of the animals, did not influence osmotic concentration of the blood and therefore were not included in the analysis of variance; they are discussed later.

Ligia oceanica

Osmotic balance

The osmotic balance was studied in Ligia oceanica

at 5°C and 15°C in both summer and winter animals over the range of salinities, 100% to 25% sea water (Fig. 4). Determinations of the freezing point depression were made on the blood of 190 summer animals and 167 winter animals. The number tested in each temperature-salinity condition is given in Table II with mean values, standard deviations and standard errors of the mean.

The blood of L. oceanica was hyperosmotic relative to the medium over the test range of salinities. The difference between the internal and external concentration increased as the salinity of the medium was lowered. This applied to summer and winter animals adapted to both 5°C and 15°C. The degree to which L. oceanica regulated concentration of its blood compared to the concentration of the medium is shown as a percentage in Table III.

The osmotic concentration of the blood was influenced by the salinity of the medium at both temperatures and in both seasons ($F = 13.68$ to 42.14, $P<0.01$; Table IV). For example, if mean values are compared over the range of

Figure 4. The relation of the osmotic concentration of the blood of Ligia oceanica to the concentration of the medium. Summer animals: 5°C —●—, 15°C --o--; winter animals: 5°C —■—, 15°C --□--

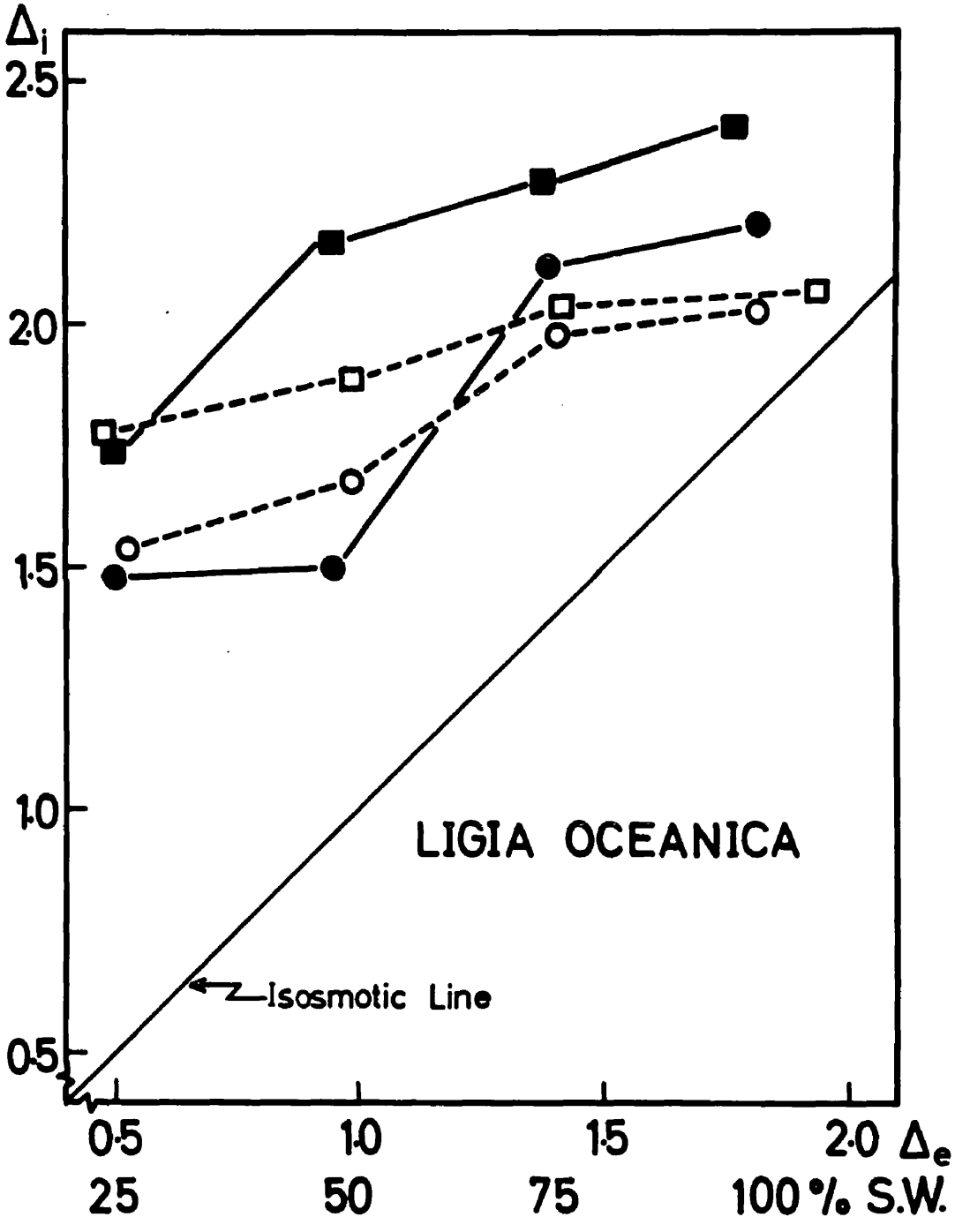

Δi
2·5
2·0
1·5
1·0
0·5
LIGIA OCEANICA
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100 % S.W.

Table II

LIGIA OCEANICA

The symbols and abbreviations used in the Tables are as follows: Temp. = Temperature, Sal. = Salinity in % sea water, Δ_e °C = Freezing point depression of the medium, N = Number of animals tested, Δ_i °C = Mean freezing point depression of the body fluid, S.D. = Standard deviation, S.E. = Standard error of the mean.

Summer

Temp.	Sal.	Δ_e °C	N	Δ_i °C	S.D.	S.E.
5°C	100%	1.82	23	2.21	0.105	0.022
	75	1.39	26	2.12	0.164	0.032
	50	0.95	24	1.50	0.408	0.083
	25	0.50	20	1.48	0.248	0.055
15°C	100%	1.82	25	2.03	0.075	0.015
	75	1.41	29	1.98	0.099	0.018
	50	0.99	17	1.68	0.200	0.049
	25	0.53	26	1.54	0.276	0.055

Winter

5°C	100%	1.77	19	2.41	0.115	0.026
	75	1.38	14	2.30	0.066	0.018
	50	0.95	20	2.17	0.134	0.030
	25	0.50	24	1.74	0.288	0.059
15°C	100%	1.94	27	2.07	0.098	0.019
	75	1.42	20	2.04	0.066	0.015
	50	0.99	21	1.79	0.216	0.047
	25	0.48	22	1.78	0.278	0.059

Table III

LIGIA OCEANICA

The hyperosmotic regulation is equivalent to:

percentage (%) = $\frac{\Delta_i - \Delta_e}{\Delta_e} \times 100$, Difference (Diff.) = $\Delta_i - \Delta_e$

Summer

Temp.	Sal.	Diff.	%
5°C	100%	0.39	21
	75	0.73	53
	50	0.55	58
	25	0.98	196
15°C	100%	0.21	12
	75	0.57	40
	50	0.69	70
	25	1.01	191

Winter

5°C	100%	0.64	36
	75	0.92	67
	50	1.22	128
	25	1.24	248
15°C	100%	0.13	7
	75	0.62	44
	50	0.80	81
	25	1.30	271

Table IV

LIGIA OCEANICA

Analysis of Variance

Temp.	Variation due to	Degrees of freedom	Sum of squares	Mean square	F.
		Summer			
5°C	Salinity	3	7.9694	2.6565	$F = \frac{2.6565}{0.0649} = 40.93^{**}$
	Residual	68	4.4134	0.0649	
	Total	71	12.3828		
15°C	Salinity	3	3.1085	1.0362	$F = \frac{1.0362}{0.0308} = 33.64^{**}$
	Residual	66+	2.0331	0.0308	
	Total	71	5.1416		
		+2 missing values			
		Winter			
5°C	Salinity	3	3.8055	1.2685	$F = \frac{1.2685}{0.0301} = 42.14^{**}$
	Residual	63+	1.8978	0.0301	
	Total	71	5.7033		
		+5 missing values			
15°C	Salinity	3	1.2559	0.4186	$F = \frac{0.4186}{0.0306} = 13.68^{**}$
	Residual	68	2.0839	0.0306	
	Total	71	3.3398		

salinities for summer animals at 5°C (Table II), it is seen that animals in 50% and 25% sea water had a significantly lower osmotic concentration than those in the two higher salinities, as indicated by the decrease in the mean freezing point depression values. There was little change in the mean values for animals in 100% and 75% sea water (Δ_i 2.21 and Δ_i2.12) or in those of animals in 50% and 25% (Δ_i1.50 and Δ_i 1.48). In other three groups, summer animals at 15°C and winter animals at 5°C and 15°C, the same relationship was found: there was without exception a significant drop in osmotic concentration of the blood of animals in 50% and 25% sea water from that of those in 100% and 75% sea water. The values of t from the t-test ranged from 2.26 to 9.84 ($P<0.05$ to $P<0.001$).

In the laboratory, L. oceanica lived indefinitely in 100% and 75% sea water, and even in 50% sea water there was a low mortality rate except in summer animals kept at 5°C. The threshold osmotic concentration of the blood compatible with survival of L. oceanica is

apparently within the range of $\Delta_i 0.97$ to $\Delta_i 1.16$ ($\Delta_e 0.50$ and $\Delta_e 0.95$); at these values intolerance was shown by oedema and loss of motility. Some animals, however, exhibited osmoregulation over prolonged periods even in 25% sea water. For example, after 13 days in the solution, three of the summer animals adapted to 15°C had values of $\Delta_i 1.81$, $\Delta_i 1.84$ and $\Delta_i 1.60$ - the mean was $\Delta_i 1.75$ - noticeably higher than the mean for the group, namely $\Delta_i 1.54$.

Temperature and salinity effects

Temperature and salinity each influence the osmotic concentration of the blood in L. oceanica, but are interdependent in their effect. Winter animals at 5°C had a mean osmotic concentration of the blood significantly higher than that of animals adapted to 15°C ($F = 67.58$, $P<0.01$; Table V). In summer, however, the mean osmotic concentration of the blood was not significantly affected by temperature. The variation in osmotic concentration due to salinity alone has been discussed previously.

The interdependence of temperature and salinity

Table V

LIGIA OCEANICA

Analysis of Variance

Summer

Variation due to	Degrees of freedom	Sum of squares	Mean square	F.
Salinity	3	10.4162	3.4721	72.19**
Temperature	1	0.0090	0.0090	< 1 N.S.
Sal. x Temp.	3	0.6617	0.2206	4.59**
Residual	134	6.4465	0.0481	
Total	143	17.5334		

Winter

Salinity	3	4.5960	1.5320	50.39**
Temperature	1	2.0545	2.0545	67.58**
Sal. x Temp.	3	0.4654	0.1551	5.10**
Residual	131	3.9817	0.0304	
Total	143	11.0976		

was shown by the significance of the temperature-salinity interaction in each season ($F = 4.59, 5.10$, $P<0.01$; Table V). From the examination of Table II, comparison of mean values for summer animals shows that the significant interaction is caused by the lower osmotic concentration at 15°C ($\Delta_i 2.03$ and $\Delta_i 1.98$) than at 5°C ($\Delta_i 2.21$ and $\Delta_i 2.12$) in 100% and 75% sea water, and the higher concentration at 15°C ($\Delta_i 1.68$ and $\Delta_i 1.54$) than at 5°C ($\Delta_i 1.50$ and $\Delta_i 1.48$) in 50% and 25% sea water. There is a similar effect in winter animals. In the salinity range 100% to 50% sea water, the animals adapted to 5°C had a higher osmotic concentration of the blood than those adapted to 15°C ($\underline{t} = 4.59, 5.29, 5.46$, $P<0.001$). At 25% sea water, however, there was no significant difference between osmotic concentration of the blood in animals adapted to 5°C and 15°C.

Seasonal effect

The physiological condition of the animals as measured by the osmotic concentration of the blood showed a seasonal change ($F = 100.68$, $P<0.01$; Table VI). The total mean values were $\Delta_i 1.82$ for summer animals and $\Delta_i 2.05$ for winter animals, over

Table VI

LIGIA OCEANICA

Analysis of Variance

Summer and Winter

Variation due to	Degrees of freedom	Sum of squares	Mean square	F.
Salinity	3	13.8508	4.6169	117.18**
Temperature	1	1.1680	1.1680	29.64**
Season	1	3.9668	3.9668	100.68**
Sal. x Temp.	3	0.7232	0.2411	6.12**
Sal. x Season	3	1.1614	0.3871	9.82**
Temp. x Season	1	0.8955	0.8955	22.73**
Sal. x Temp. x Season	3	0.4039	0.1346	3.42*
Residual	265	10.4282	0.0394	
Total	287	32.5978		

Coefficient of Variation = 10.3%

the range of salinities, calculated from the analysis of variance data. Seasonally, the osmoregulatory response differed with regard to both temperature ($F = 22.73$, $P<0.01$) and salinity ($F = 9.82$, $P<0.01$) and in addition differed in the temperature-salinity interaction in summer and winter ($F = 3.42$, $P<0.05$).

At a temperature of 5°C the mean freezing point depression of the blood differed significantly between summer and winter animals ($\underline{t} = 9.34$, $P<0.001$) and the winter animals maintained a higher osmotic concentration of the blood throughout the whole range of salinities. At a temperature of 15°C, the winter animals again maintained a higher osmotic concentration in all salinity solutions than summer animals ($\underline{t} = 3.61$, $P<0.001$), but the difference between summer and winter animals narrowed considerably.

In summer animals the freezing point depression levelled off at 50% sea water at 5°C and in the winter animals at 50% sea water at 15°C (Fig. 4). Thereafter it remained at more or less the same level in 25% sea water, indicating that at these particular temperature-salinity conditions the osmotic concentration

of the blood reached a threshold value determined by the viable range in the two seasons. Below a certain temperature-salinity parameter, the osmotic concentration of the blood would drop below this threshold value and the animals would not survive.

Idotea granulosa

Osmotic balance

The osmotic balance was studied in Idotea granulosa at 5°C and 15°C in both summer and winter animals over the range of salinities 100% to 25% sea water (Fig. 5). Determination of the freezing point depression of the blood was made on 137 summer animals and 147 winter animals. The number tested in each temperature-salinity combination is given in Table VII, with mean values, standard deviations and standard errors of the mean.

The blood of I. granulosa was hyperosmotic relative to the medium throughout the range of salinities. In 100% sea water the hyperosmotic condition was marginal, with an increase in 75% sea water and further increases at 50% and 25% sea water. The degree to which I. granulosa regulated

Figure 5. The relation of the osmotic concentration of the blood of Idotea granulosa to the concentration of the medium. Summer animals: 5°C —●—, 15°C --o--; winter animals: 5°C —■—, 15°C --□--.

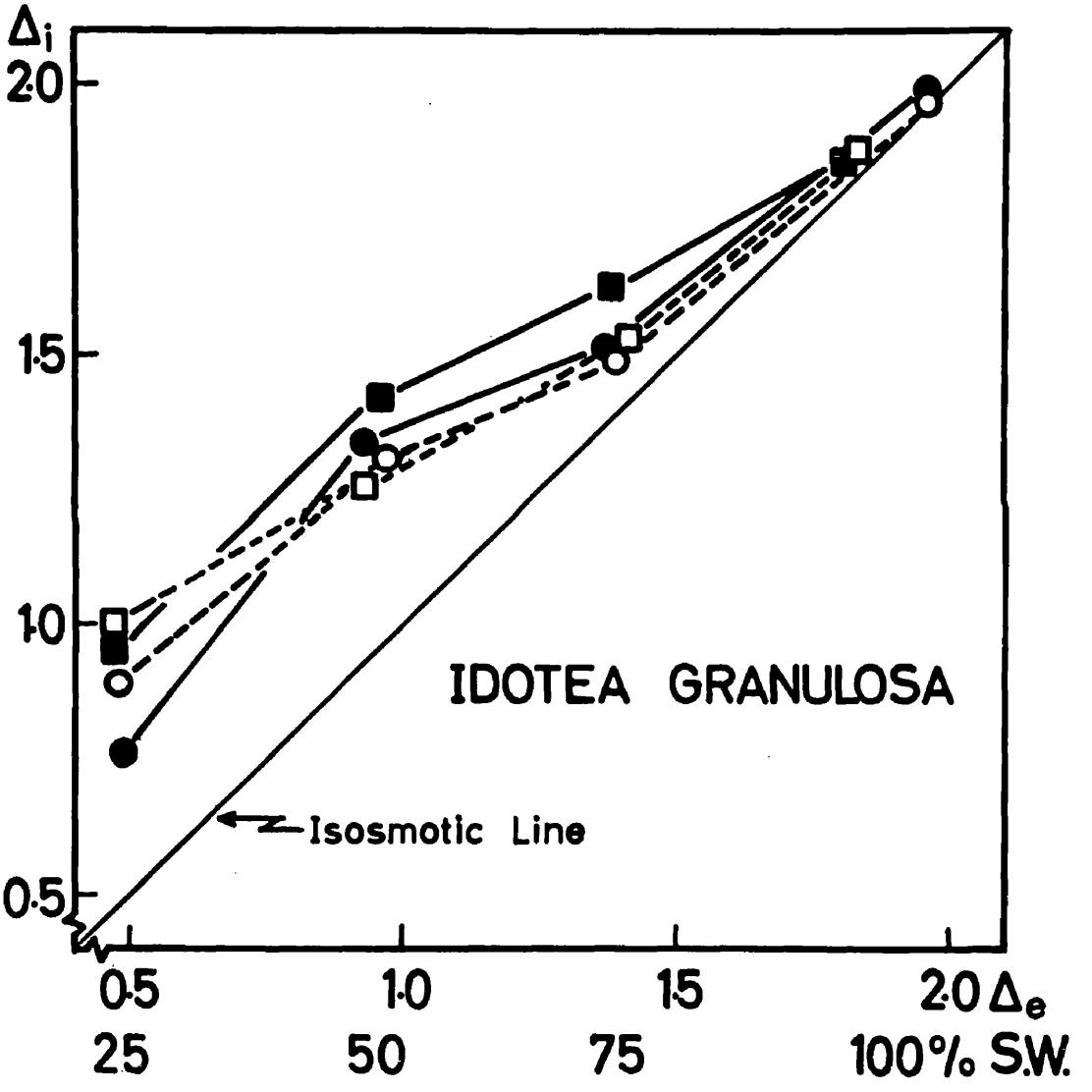

Δi
2·0
1·5
1·0
0·5
IDOTEA GRANULOSA
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100% S.W.

Table VII

IDOTEA GRANULOSA

Summer

Temp.	Sal.	Δ_e °C	N	Δ_i °C	S.D.	S.E.
5°C	100%	1.96	19	1.99	0.092	0.021
	75	1.37	25	1.52	0.062	0.012
	50	0.93	13	1.34	0.124	0.034
	25	0.49	10	0.76	0.142	0.045
15°C	100%	1.96	18	1.97	0.066	0.015
	75	1.39	19	1.49	0.050	0.011
	50	0.97	23	1.31	0.170	0.035
	25	0.48	10	0.89	0.117	0.037

Winter

5°C	100%	1.81	19	1.86	0.075	0.017
	75	1.38	27	1.63	0.089	0.017
	50	0.96	12	1.42	0.070	0.020
	25	0.47	15	0.95	0.175	0.045
15°C	100%	1.83	19	1.87	0.039	0.009
	75	1.41	22	1.53	0.058	0.012
	50	0.93	15	1.26	0.097	0.025
	25	0.47	18	1.00	0.167	0.039

the concentration of the blood compared to the concentration of the medium is given as a percentage in Table VIII.

The salinity of the medium influenced the freezing point depression of the blood and there was a close relationship between the two with a descent from the 100% value in summer and winter animals and at both temperatures ($F = 189.36$ to 375.18, $P<0.01$; Table IX). The mean values for freezing point depression of the blood in summer animals at 5°C are $\Delta_i 1.96$, $\Delta_i 1.52$, $\Delta_i 1.34$ and $\Delta_i 0.76$ for the salinity range 100% to 25% sea water. There is a significant drop in osmotic concentration of animals in 75% from those at 100%, of animals in 50% from those at 75% and of animals in 25% from those in 50% sea water. When mean values for animals in any two salinities are compared (Table VII), whether at 5°C or 15°C, the difference is always significant in both summer and winter animals. Results from the t-tests ranged from 4.88 to 33.06 ($P<0.001$).

Temperature and salinity effects

I. granulosa survived in the laboratory in the

Table VIII

IDOTEA GRANULOSA

Hyperosmotic regulation. (Symbols as in Table III)

Summer

Temp.	Sal.	Diff.	%
5°C	100%	0.03	2
	75	0.15	11
	50	0.41	44
	25	0.27	55
15°C	100%	0.01	1
	75	0.10	7
	50	0.34	35
	25	0.41	85

Winter

5°C	100%	0.05	3
	75	0.25	18
	50	0.46	48
	25	0.48	102
15°C	100%	0.04	2
	75	0.12	9
	50	0.33	35
	25	0.53	113

Table IX

IDOTEA GRANULOSA

Analysis of Variance

Summer

Temp.	Variation due to	Degrees of freedom	Sum of squares	Mean square	F.
5°C	Salinity	3	11.4803	3.8268	375.18**
	Residual	49+	0.4981	0.0102	
	Total	59			

+7 missing values

15°C	Salinity	3	9.0578	3.0193	282.18**
	Residual	51+	0.5437	0.0107	
	Total	59			

+5 missing values

Winter

5°C	Salinity	3	6.9441	2.3147	189.73**
	Residual	53+	0.6485	0.0122	
	Total	59			

+3 missing values

15°C	Salinity	3	6.2491	2.0830	189.36**
	Residual	56	0.6164	0.0110	
	Total	59	6.8655		

salinity range 100% to 50% sea water, but the threshold osmotic concentration of the blood was reached around 25% sea water. It was possible to obtain blood samples from living winter animals after 4 days adaptation, but the summer animals which regulated less efficiently at this low salinity had to be tested after 2 days adaptation to obtain sufficient results for comparison. The longest survival time at this salinity was 9 days (winter animals, 15°C). The higher temperature prolonged survival, possibly by contributing to the physical or physiological conditions which prevent dilution of the blood rather than by increasing tolerance for a lower blood osmotic concentration; the mean value for the freezing point depression was actually greater at 15°C.

The effect of the temperature-salinity interaction was significant in both summer and winter animals ($F = 4.49, 6.04$; $P<0.01$; Table X), indicating that temperature and salinity are interdependent in the influence they exert on osmotic concentration of the blood of I. granulosa. The freezing point depression of the blood was affected in winter by the

Table X

IDOTEA GRANULOSA

Analysis of Variance

Summer

Variation due to	Degrees of freedom	Sum of squares	Mean square	F.
Salinity	3	20.3980	6.7993	653.78**
Temperature	1	0.0033	0.0033	<1 N.S.
Sal. x Temp.	3	0.1400	0.0467	4.49**
Residual	100	1.0419	0.0104	
Total	119	21.5832		

Winter

Salinity	3	12.9829	4.3276	373.07**
Temperature	1	0.0940	0.0940	8.10**
Sal. x Temp.	3	0.2103	0.0701	6.04**
Residual	109	1.2649	0.0116	
Total	119	14.5521		

temperature to which the animals were adapted ($F = 8.10$, $P<0.01$), and the level of the osmotic concentration of the blood was higher in animals adapted to 5°C in the salinity range 100% to 50% sea water. Below 50% sea water the position was reversed and animals adapted to 15°C had the higher osmotic concentration of the blood. This difference in the concentration at the two temperatures becomes significant in salinities of 75% and 50% sea water ($t = 3.08$, 4.10, $P<0.005$ and $P<0.001$). In summer animals the mean osmotic concentrations were also higher in the animals adapted to 5°C than to 15°C in 100% to 50% sea water, and in 25% the position was again reversed. Comparison of mean values given in Figure 5 and Table VII indicates this interdependent relationship of temperature and salinity on osmoregulation.

Seasonal effect

The physiological condition of I. granulosa as measured by the osmotic concentration of the blood showed a seasonal change ($F = 6.09$, $P<0.05$; Table XI). The total mean values were $\Delta_i 1.41$ for summer animals and $\Delta_i 1.44$ for winter animals over the range

Table XI

IDOTEA GRANULOSA

Analysis of Variance

Summer and Winter

Variation due to	Degrees of freedom	Sum of squares	Mean square	F.
Salinity	3	32.7917	10.9306	999.37**
Temperature	1	0.0311	0.0311	2.83 N.S.
Season	1	0.0670	0.0670	6.09*
Sal. x Temp.	3	0.3129	0.1043	9.48**
Sal. x Season	3	0.5892	0.1964	17.85**
Temp. x Season	1	0.0662	0.0662	6.02*
Sal. x Temp. x Season	3	0.0375	0.0125	1.14 N.S.
Residual	209	2.3067	0.0110	
Total	239	36.2023		

Coefficient of Variation = 7.3%

of salinites, calculated from the analysis of variance data. The temperature-season interaction is significant ($F = 6.02$, $P<0.05$) and also the salinity-season interaction ($F = 17.85$, $P<0.01$). When all the animals tested were grouped together, the temperature effect was almost significant at the 5% level, and values of t from the t-test showed that winter animals adapted to 5°C had a significantly higher mean osmotic concentration of the blood than summer animals adapted to the same temperature over the salinity range ($t = 3.68$, $P<0.001$). In animals adapted to 15°C, there was no significant difference when mean values for summer and winter animals over the whole range of salinities were compared.

Size and Sex

There was no evidence that size had any effect on the freezing point depression of the blood in either *Ligia oceanica* or *Idotea granulosa*. This is apparent in the graphs of weight plotted against freezing point depression for winter *Ligia oceanica* adapted to 5°C and 15°C in 100% sea water (Fig. 6) and summer *Idotea granulosa* adapted to 100%, 75% and 50% sea water at 5°C (Fig. 7).

Figure 6. Scatter diagram plotting the freezing point depression values of summer male and female Ligia oceanica of different weights. The animals were in 100% sea water (Δ_e1.82) at either 5°C or 15°C.

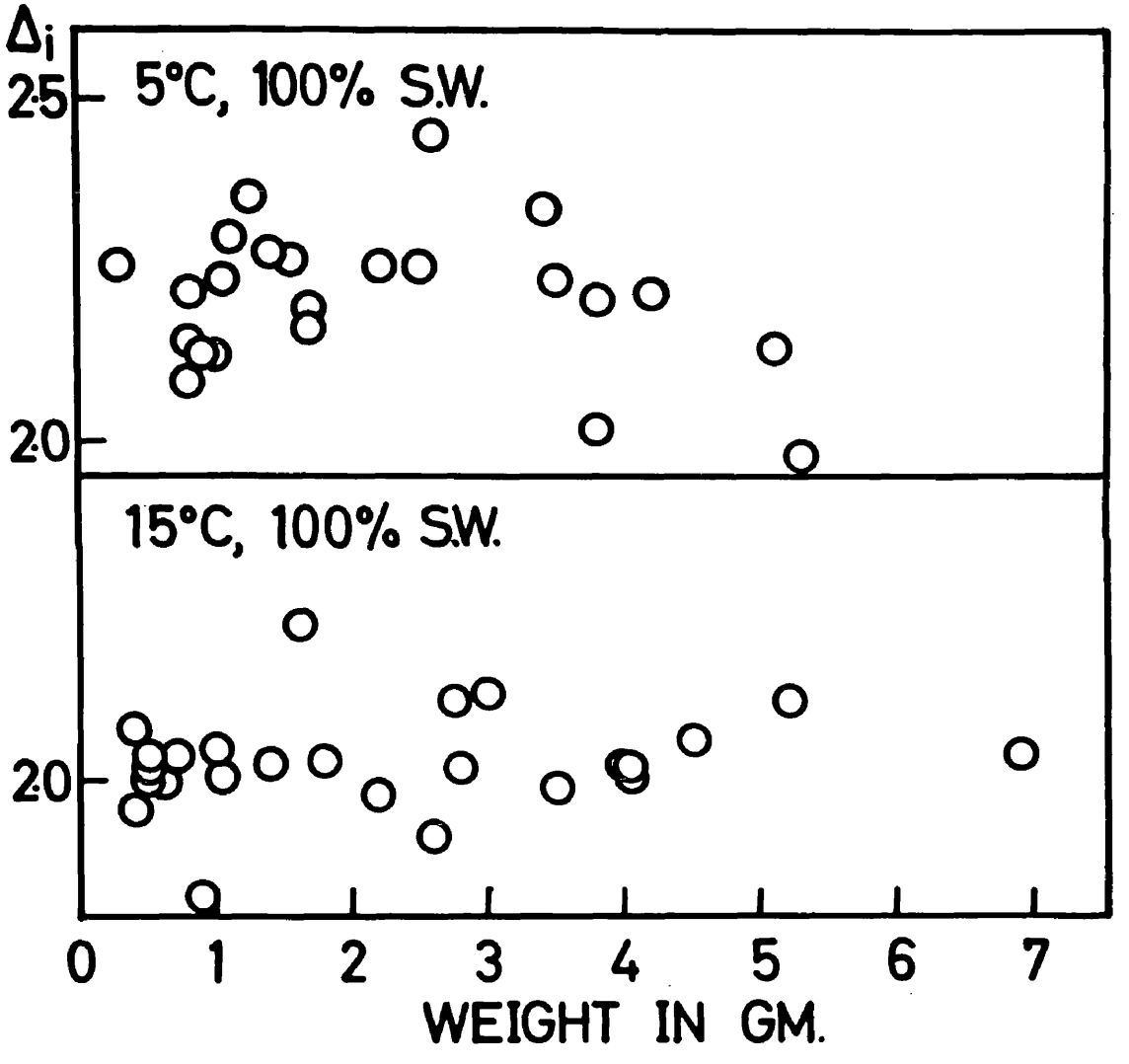
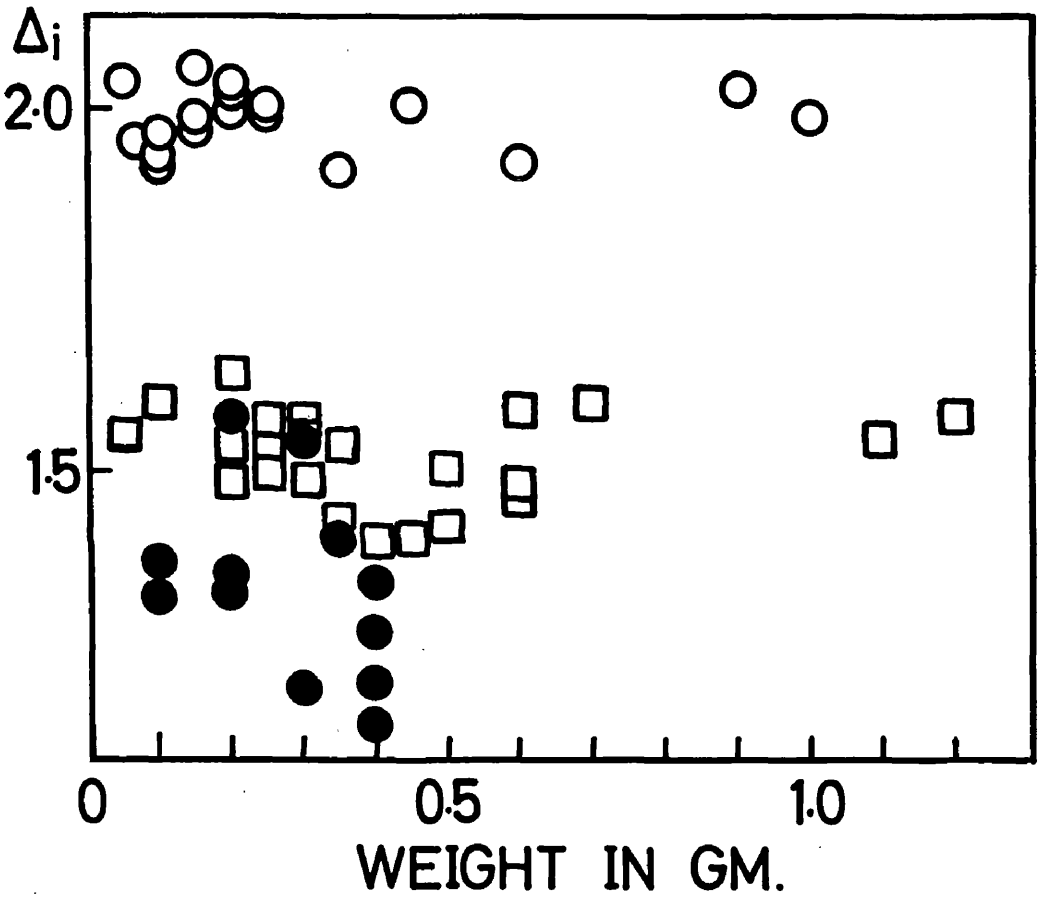

Δi
25
5°C, 100% S.W.
20
15°C, 100% S.W.
20
0
1
2
3
4
5
6
7
WEIGHT IN GM.

Figure 7. Scatter diagram plotting the freezing point depression values of summer male and female Idotea granulosa of different weights. The animals were in 100% (Δ_e1.96), 75% (Δ_e1.37) and 50% sea water (Δ_e0.93) at 5°C.


Δi
2·0
1·5
0
0·5
1·0
WEIGHT IN GM.

The sex ratio of Ligia oceanica was not the same in summer and winter (Table XII). In summer males outnumbered females in the proportion of 2.6:1, but in winter the ratio approached 1:1 which could be expected in a balanced population. In Idotea granulosa the ratio of males to females was 0.7:1 in summer and 2.2:1 in winter, more females in summer and more males in winter (Table XIII).

Sex was not a factor influencing the osmotic concentration of the blood in these experimental conditions (Tables XII and XIII). There was no significant difference between the freezing point depression of the blood of males and females in any experiment. The largest value of t obtained from the t-test comparing mean values for males and females was 1.77 ($P>0.05$). The osmotic concentration of the blood in female Ligia oceanica was slightly higher than that of the males in 10 out of 15 groups and equal in 1 group. In Idotea granulosa the variation of the osmotic concentration of the blood due to sex was even less. Out of the 14 groups listed, males had a slightly higher concentration in 7, but again the differences were

Table XII

LIGIA OCEANICA

Summer

Temp.	Sal.	N	Mean Δ_i°C ♂	N	Mean Δ_i°C ♀
5°C	100%	16	2.19	7	2.27
	75	17	2.15	6	2.07
	50	17	1.49	7	1.55
	25	14	1.51	6	1.41
15°C	100%	19	2.03	6	2.03
	75	24	1.97	5	2.02
	50	16	1.74	10	1.76
	25	18	1.60	8	1.42
	Total	141		55	
	Sex ratio		2.6:1		

Winter

5°C	100%	9	2.40	10	2.43
	75	11	2.29	3	2.35
	50	9	2.14	11	2.19
	25	12	1.72	12	1.76
15°C	100%		not recorded		
	75	9	2.02	11	2.05
	50	8	1.81	13	1.78
	25	13	1.83	7	1.67
	Total	71		67	
	Sex ratio		1:1		

Table XIII

IDOTEA GRANULOSA

Summer

Temp.	Sal.	N	Mean Δ_i°C ♂	N	Mean Δ_i°C ♀
5°C	100%	7	2.01	11	1.98
	75	4	1.52	21	1.52
	50	10	1.34	10	1.34
	25	7	0.76	3	0.77
15°C	100%	7	1.99	10	1.95
	75	4	1.52	15	1.48
	50	13	1.36	7	1.22
	25	6	0.88	4	0.91
	Total	58		81	
	Sex ratio		0.7:1		

Winter

5°C	100%		not recorded		
	75	8	1.65	4	1.72
	50	10	1.43	2	1.39
	25	3	0.95	3	1.00
15°C	100%		not recorded		
	75	6	1.53	2	1.51
	50	9	1.29	3	1.30
	25	6	0.95	5	0.92
	Total	42		19	
	Sex ratio		2.2:1		

not significant, while 2 groups had the same mean freezing point depression in males and females.

Osmoregulation in Ligia oceanica and Idotea granulosa

1. The osmoregulatory response of Ligia oceanica and Idotea granulosa to the range of the experimental variables was similar. They were both hyperosmotic relative to the medium and the difference between internal and external concentration increased as the salinity of the medium decreased.

2. In 100% sea water the osmotic concentration of the blood of Ligia oceanica was markedly above that of the medium whereas in Idotea granulosa the blood was only marginally hypersomotic.

3. In Ligia oceanica the blood concentration changed little in 100% and 75% sea water, but dropped significantly between 75% and 50% sea water whereas blood concentration in Idotea granulosa dropped significantly throughout the test range of salinities.

4. The more efficient osmoregulation of Ligia oceanica in 25% sea water is reflected in the mean

freezing point depression of the blood $\Delta_i 1.65$, compared with $\Delta_i 0.90$ in Idotea granulosa.

5. This is also evident in a comparison of survival times in 25% sea water, namely more than 20 days for Ligia oceanica and a maximum of 9 days for Idotea granulosa.

6. In both species the osmotic concentration of the blood was influenced by season, by temperature and by a temperature-salinity interaction.

Gastropoda: Littorinidae

The Littorinidae were placed directly into the experimental media from the environmental salinity, about 32°/oo. Thus, the animals were in effect transferred from 100% sea water to either 75%, 50% or 25% sea water. Any one group of animals, therefore, was only tested in a single temperature-salinity condition. The samples of body fluid were taken after the animals had been in the experimental media for at least 24 hours, and the mean freezing point depression value for a particular group was derived from all the animals tested, over a period varying from 1 to 40 days. The freezing point depression of the blood of each individual, along with the number of days in the medium are given in the appendix.

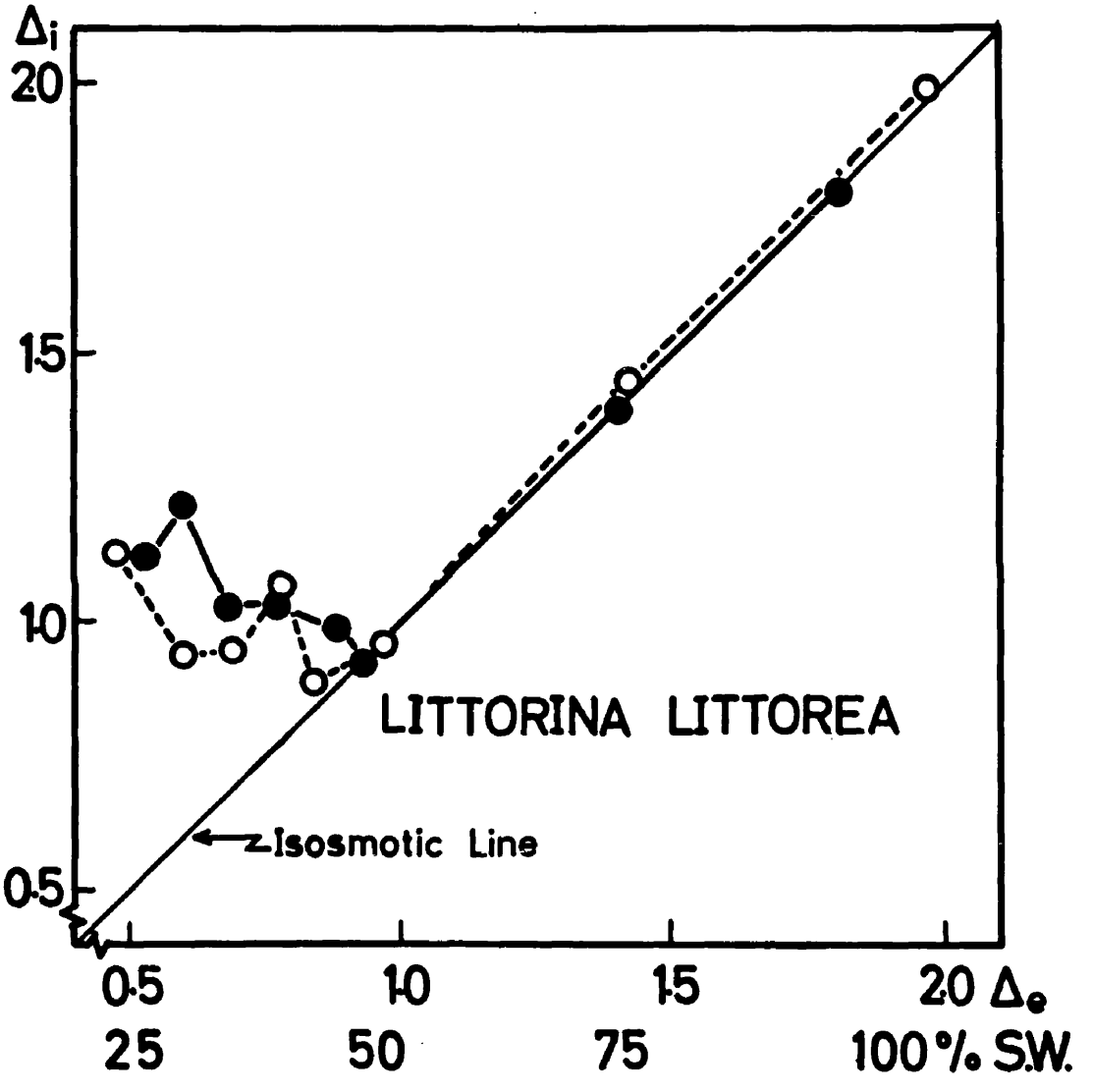
Littorina littorea

Osmotic balance

The osmotic balance of Littorina littorea was studied in the following conditions:

a. Summer animals in 100% to 25% sea water at 5°C and 15°C (Fig. 8).

Figure 8. The relation of the osmotic concentration of the blood of Littorina littorea to the concentration of the medium. Summer animals: 5°C —●—, 15°C --o--.


Δi
2·0
1·5
1·0
0·5
LITTORINA LITTOREA
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100% S.W.

b. Winter animals in 150% to 25% sea water at 5°C and 125% to 25% sea water at 15°C (Fig. 9).

c. One winter group only in 125% sea water at 20°C (Fig. 9).

The freezing point depression of the blood was determined in 355 summer animals and 173 winter animals. Table XIV and Table XV give mean values, standard deviations and standard errors of the mean for the various groups in the experimental conditions.

In summer and winter animals in salinities of 100% to 50% sea water the blood was usually isosomotic at 5°C and 15°C, and in such laboratory conditions the animals were active and survived for a prolonged period. In salinities below 50% sea water, they tended to withdraw into the shell, and the concentration of the blood was significantly hyperosomotic in 25% sea water (t = 6.46 to 10.27, $P<0.001$). A favourable environment is indicated when a snail attaches itself to the substrate and crawls. When conditions are marginal, crawling may not occur and L. littorea did not attempt to crawl out of the lowest salinity

Figure 9. The relation of the osmotic concentration of the blood of Littorina littorea to the concentration of the medium. Winter animals: 5°C -■-, 15°C -□--, 20°C -x-.

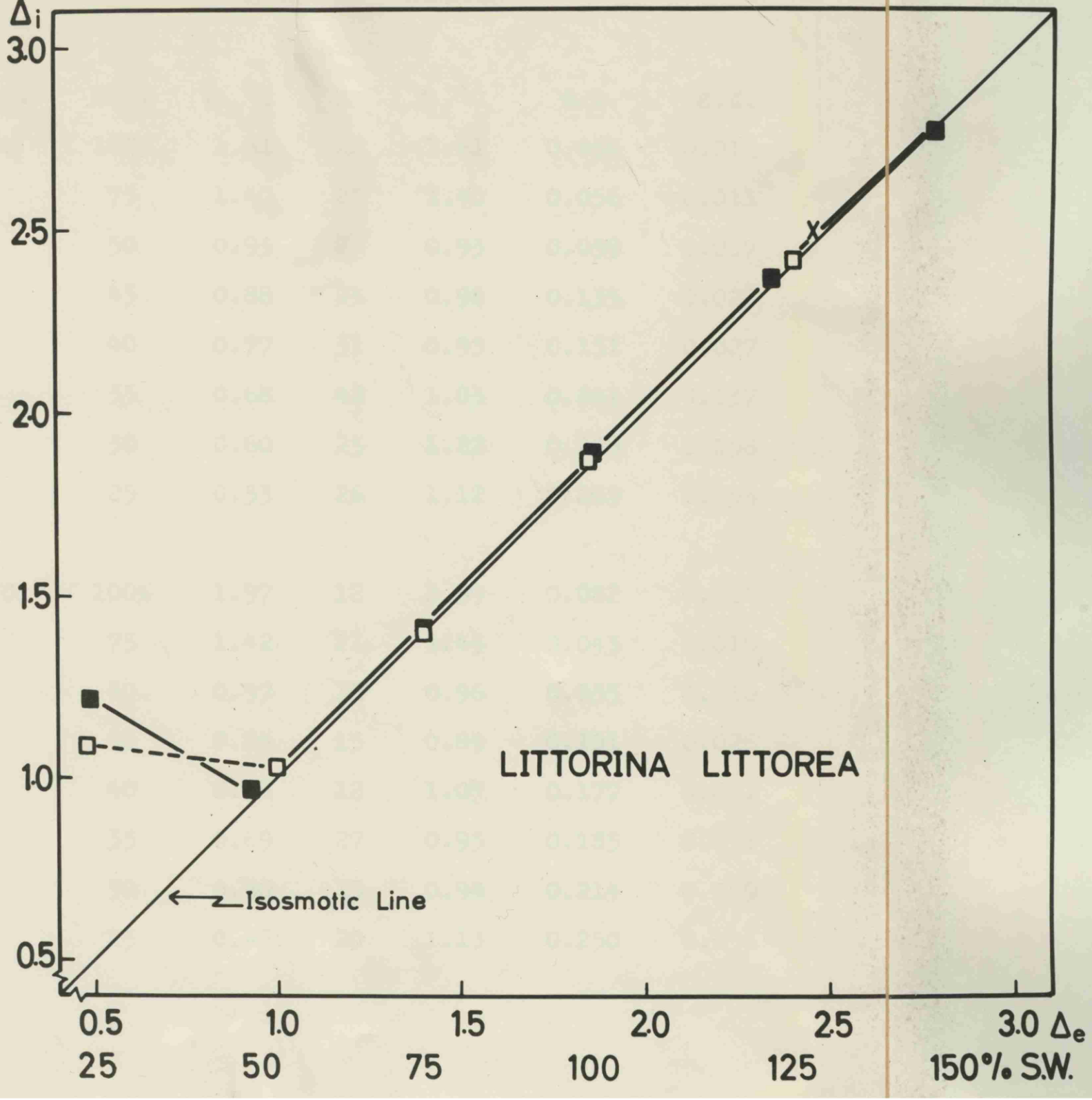

Δi
3.0
2.5
2.0
1.5
1.0
0.5
LITTORINA LITTOREA
Isosmotic Line
0.5
1.0
1.5
2.0
2.5
3.0 Δe
25
50
75
100
125
150% S.W.

Table XIV

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.81	22	1.81	0.056	0.012
	75	1.40	27	1.40	0.056	0.011
	50	0.93	27	0.93	0.039	0.007
	45	0.88	23	0.98	0.135	0.028
	40	0.77	31	0.93	0.151	0.027
	35	0.68	42	1.03	0.241	0.037
	30	0.60	25	1.22	0.289	0.058
	25	0.53	26	1.12	0.269	0.053
15°C	100%	1.97	12	1.99	0.082	0.024
	75	1.42	21	1.45	0.045	0.010
	50	0.97	12	0.96	0.035	0.010
	45	0.84	15	0.89	0.101	0.026
	40	0.78	12	1.07	0.177	0.051
	35	0.69	27	0.95	0.185	0.036
	30	0.60	13	0.94	0.214	0.059
	25	0.45	20	1.13	0.250	0.056

Table XV

LITTORINA LITTOREA

Winter

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.86	13	1.88	0.098	0.027
	75	1.40	13	1.41	0.056	0.016
	50	0.93	13	0.97	0.030	0.008
	25	0.49	11	1.22	0.119	0.040
15°C	100%	1.85	10	1.87	0.069	0.022
	75	1.40	14	1.40	0.087	0.023
	50	1.00	17	1.03	0.103	0.025
	25	0.48	11	1.09	0.108	0.033
5°C	150%	2.78	3	2.77	0.046	0.026
	125	2.35	34	2.37	0.059	0.010
15°C	125%	2.41	21	2.42	0.076	0.017
20°C	125%	2.46	13	2.50	0.130	0.036

nor did it attach to the substrate.

Within the range 50% to 25% sea water, the summer animals were tested at 5% salinity intervals, and Figure 8 shows how the difference between internal and external concentration increased as the salinity decreased. When the snails were immersed in 50% sea water, the freezing point depression and therefore the osmotic concentration of the blood was reduced to half its value in 100% sea water with no apparent behavioural response to the decreased salinity. However, withdrawal occurred in solutions below 50% sea water before there was any appreciable dilution of the blood, perhaps triggered off by some peripheral salinity detector. The mean values of the osmotic concentration of the blood were higher at 25% than at 50% sea water when the species is isosomotic. The difference is significant except in winter animals at 15°C ($\underline{t}$ = 2.33, 3.63 and 7.33, $P<0.05$ and $P<0.001$).

Temperature and salinity effects

The threshold salinity for survival was influenced by temperature. After 31 days in 35% sea water summer animals at 5°C seemed healthy, and several of them were attached to the substrate, indicating a

suitable environment. In the attached animals, or in those with the foot out but not attached, the osmotic concentration of the blood $\Delta_i 0.75$, was nevertheless significantly higher than that of the medium $\Delta_e 0.68$ ($t = 3.15$, $P<0.01$). On the other hand, L. littorea at 15°C in 40% sea water, that is 5% stronger, were all dead by the ninth day; none survived after 12 days in 35% sea water at 15°C. In 30% sea water the animals were alive after 17 days when the temperature was 5°C, but were dead by 14 days when the temperature was 15°C. In salinities less than 45% sea water, survival times of animals at 15°C increased as the salinity decreased but were always shorter than that of animals at 5°C in the same solution. The maximum survival time under the various conditions is summarised in Table XVI.

The average values for osmotic concentration of the blood decreased according to the length of time the animals were in the very low salinities but as there was considerable variation between individual values, the decrease in osmotic concentration is not

Table XVI

LITTORINA LITTOREA

		Maximum time survived	
	Salinity	5°C	15°C
Summer	45% S.W.	21+*	5+
	40	23+	9
	35	31+	12
	30	17+	14
	25	13+	7+
Winter	25	13+	13+

+* = 100% survival when experiment discontinued

directly proportional to the time in the solution.

There is no fixed threshold value for osmotic concentration of the blood below which it can be stated L. littorea will not survive. For example, the mean osmotic concentration of the blood of animals which subsequently failed to survive in low salinity solutions at 15°C was actually higher (for example in 40%, Δ_i1.07) than that well tolerated by animals at the lower temperature or in solutions of higher salinity at 15°C. When the mean values of the freezing point depression of summer animals in the salinity range 50% to 25% sea water are compared, significant differences due to temperature are revealed only in 30% (t = 3.07, $P<0.005$) and 40% (t = 2.59, $P<0.025$). In winter animals in 25% sea water, the mean osmotic concentration of the blood was significantly higher in animals at 5°C than in those adapted to 15°C (t = 2.69, $P<0.025$). The particular properties of the medium favouring longer survival within the critical range of conditions is not wholly a level of salinity or of temperature but is a function of a temperature-salinity combination.

Above 50% sea water, the temperature had no significant effect on osmotic concentration of the blood in either season.

Seasonal effect

There was no detectable seasonal influence on osmotic concentration of the blood of animals in 100% to 50% sea water or those in 25% sea water when summer animals with or without a normal operculum were compared with the winter counterparts all of which had a normal operculum.

The operculum

Animals from two populations of L. littorea were tested in the experiments, Population I in which all the animals had a normal operculum and Population II in which a number of the animals had no operculum or a functionally deficient one. The condition of the operculum in animals of Population II was recorded; the cause of the abnormality is unknown. Of 156 animals from Population II tested in the salinity range 100% to 50% sea water, 53% had an abnormal operculum (24% with no operculum, 29% with a small operculum) occurring equally in both sexes. As

there was minimal individual variation of osmotic concentration at these salinities, an abnormal operculum would have a negligible effect on osmotic concentration. Below 50% sea water, however, when the animals retract into the shell, the lack of a complete operculum could influence osmotic concentration. All animals with an abnormal operculum were grouped together and the mean freezing point depressions are plotted for the two groups in Figure 10. Table XVII shows the means, standard deviations and standard errors of the mean for both groups at the different salinities. Animals in 40% and 35% sea water at 5°C all had a normal operculum as they came from Population I and so were not included.

Snails with an abnormal operculum had a lower osmotic concentration of the blood in salinities below 50% sea water than those with a normal operculum. The conclusion is that the operculum normally acts as a partial physical barrier to osmotic equilibrium in low salinity solutions, but nevertheless even with an abnormal operculum the osmotic concentration remained markedly hyperosmotic relative to the medium.

Figure 10. The relation of the osmotic concentration of the blood of summer Littorina littorea with a complete operculum (—●—) and an abnormal one (--o--). The animals were tested at 5°C and 15°C over the salinity range 50% to 25% sea water.

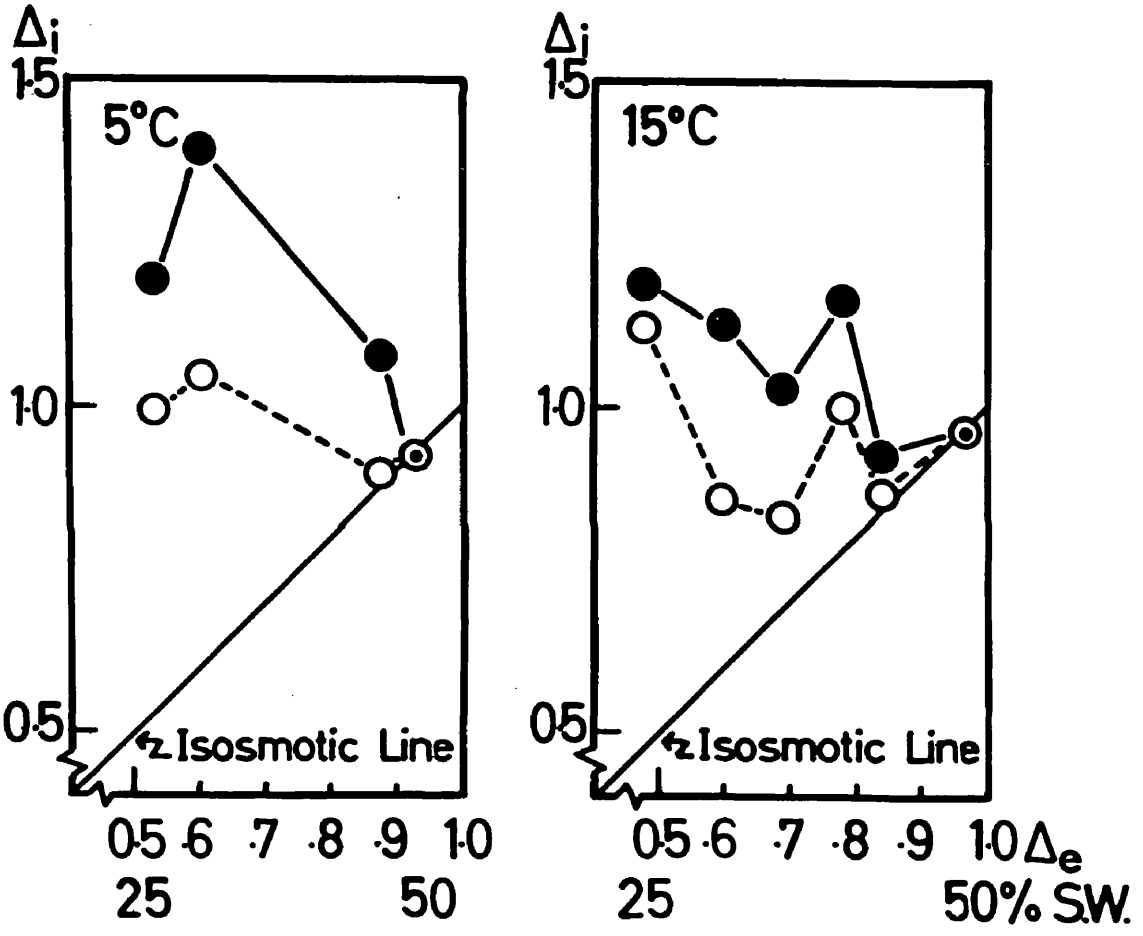

Δi
1·5
5°C
1·0
0·5
Isosmotic Line
0·5 ·6 ·7 ·8 ·9 1·0
25
50
Δj
1·5
15°C
1·0
0·5
Isosmotic Line
0·5 ·6 ·7 ·8 ·9 1·0 Δe
25
50% S.W.

Table XVII

LITTORINA LITTOREA

Normal Operculum

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	45%	0.88	9	1.08	0.150	0.030
	40	0.77	31	0.93	0.151	0.027
	35	0.68	42	1.03	0.241	0.037
	30	0.60	12	1.40	0.089	0.026
	25	0.53	14	1.20	0.217	0.058
15°C	45	0.84	4	0.93	0.145	0.072
	40	0.78	5	1.17	0.235	0.105
	35	0.69	17	1.03	0.158	0.038
	30	0.60	4	1.13	0.168	0.084
	25	0.48	6	1.19	0.115	0.047

Abnormal Operculum

5°C	45	0.88	14	0.90	0.057	0.015
	30	0.60	13	1.05	0.308	0.085
	25	0.53	11	1.00	0.303	0.091
15°C	45	0.84	11	0.87	0.085	0.026
	40	0.78	7	1.00	0.082	0.031
	35	0.69	10	0.83	0.167	0.053
	30	0.60	9	0.86	0.179	0.059
	25	0.48	14	1.12	0.259	0.069

Breaking of the shell

A ready explanation of the prolonged hyperosmotic state of the blood of the Littorinidae maintained in 25% sea water is the possible prevention of osmotic equilibrium by the simple process of achieving isolation from the medium by the withdrawal response invariably elicited in low salinities. Otherwise, it must be assumed that there is active regulation of the osmotic concentration of the blood. A rough test of the efficacy of the operculum mechanism in this respect was carried out by breaking the shells of a number of summer L. littorea and returning them to the 25% sea water solution at 5°C. It is recognised, however, that such a procedure is open to criticism as an uncontrolled experiment leaving the extent of body surface exposed to the medium as a variable for each animal. Moreover, it is impossible to eliminate sublethal injury to the animal. The results from such experiments are given in Table XVIII and not surprisingly are inconclusive. Both blood and urine were tested, and the mean value of the osmotic concentration of the urine was slightly higher than that of the blood, but with no significant difference.

Table XVIII

LITTORINA LITTOREA

Temp.	Sal.	Δ_e °C	Days in medium	Δ_i °C Blood	Δ_i °C Urine	Operc.	Hours shell broken
5°C	25%	0.53	4	0.66	0.76	x	20
				0.82	0.90	x	20
			5	0.73	0.77	S	18*
				0.60	—	P	18*
			7	0.60	0.59	O	48
			8	0.65	0.71	P	20
			10	0.51	0.54	S	68
			11	0.56	0.52	P	24
			12	0.54	0.56	S	48‡
			13	0.62	0.63	P	72
			Mean	0.63	0.65		

Operc. = Operculum x = Condition not recorded

P = Present

S = Small

O = Absent

* = Shell almost removed

‡ = Shell removed

The mean osmotic concentration of the blood, $\Delta_i 0.63$, however, is significantly lower than that of undamaged animals under the same conditions, $\Delta_i 1.12$ ($t = 5.59$, $P<0.001$; see Table XIV). After 72 hours one animal with a broken shell had blood $\Delta_i 0.62$ ($\Delta_e 0.53$).

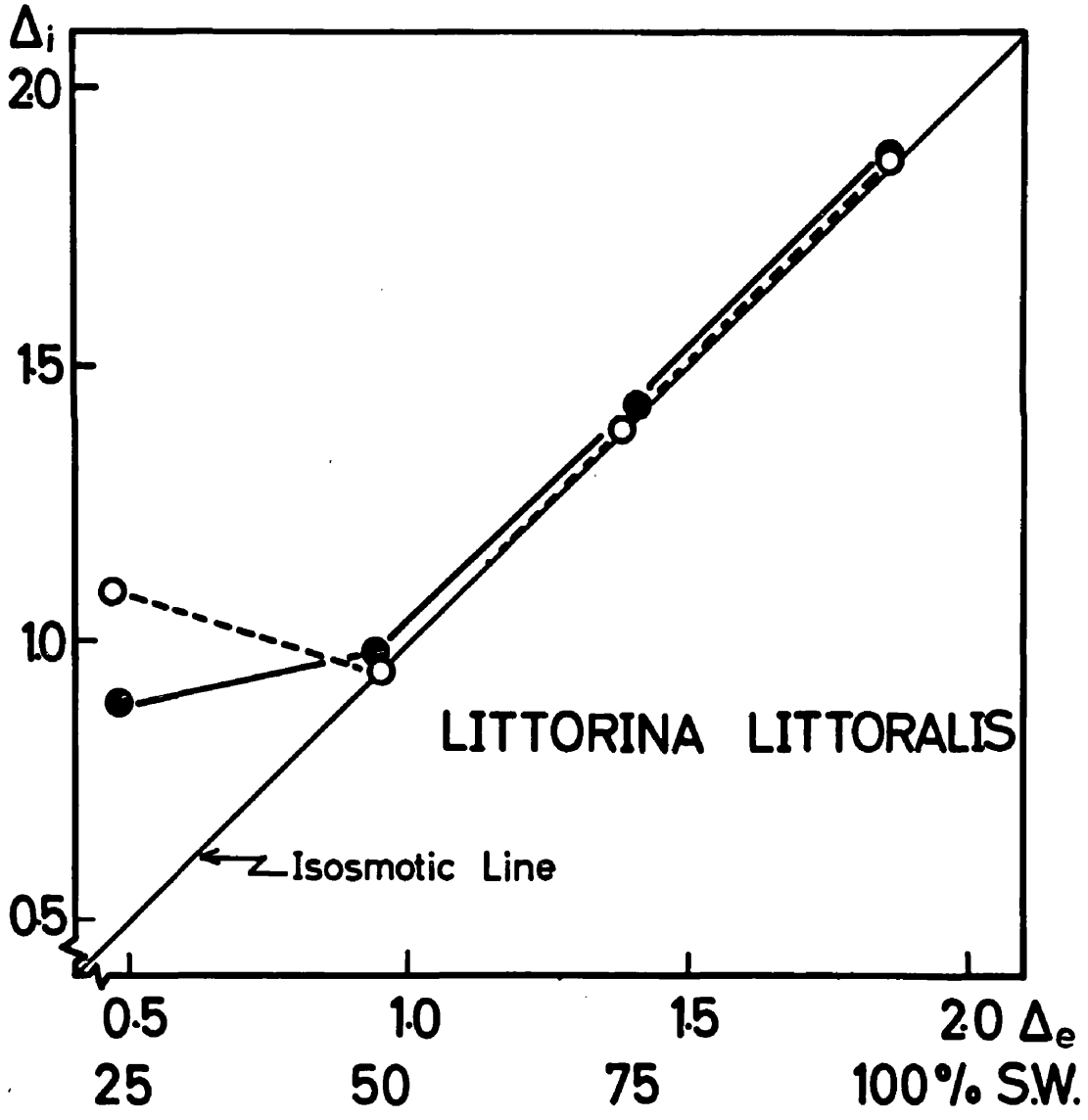
The passive dilution of the blood either proceeded very slowly or else an active maintenance of the hyperosmotic state was effective for a limited period after which the animals died. Certainly in L. littorea from Population II with an absent or functionally defective operculum there was throughout the tests in the low salinities, a lower mean osmotic concentration of the blood than in animals with a normally functioning operculum, although the blood still remained markedly hyperosmotic relative to the medium (Fig. 10).

Littorina littoralis

Osmotic balance

The osmotic balance of L. littoralis was studied in the range of salinities 100% to 25% sea water at 5°C and 15°C, and the freezing point depression of the blood was tested in 146 summer animals (Fig. 11)

Figure 11. The relation of the osmotic concentration of the blood of Littorina littoralis to the concentration of the medium. Summer animals: 5°C —●—, 15°C --o--.


Δi
2·0
1·5
1·0
0·5
LITTORINA LITTORALIS
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100% S.W.

and 120 winter animals (Fig. 12). The number of L. littoralis tested in each group with the mean values, standard deviations and standard errors of the mean are given in Table XIX. In 125% sea water, the freezing point depression of the blood $\Delta_i 2.37$ (average of two) approximated to that of the medium $\Delta_e 2.34$. Animals with a checkered shell and those with a plain coloured shell were tested in the experiments. Since there were no differences in the mean osmotic concentrations of the blood of the two shell types in any of the experiments, the results have been grouped together. On two occasions, however, animals with the checkered shell were attached in 25% sea water at 15°C and were active for some time in that solution, evidence of tolerance of the medium, while the others were never active at this salinity.

The blood of L. littoralis was isosomotic or slightly hyperosmotic in salinities down to 50% sea water and the animals were active, while in solutions below 50% sea water they were usually retracted into the shell and the blood was significantly hyperosmotic (t = 4.25 to 9.34, $P<0.001$).

Figure 12. The relation of the osmotic concentration of the blood of Littorina littoralis to the concentration of the medium. Winter animals: 5°C —■—, 15°C --□--.

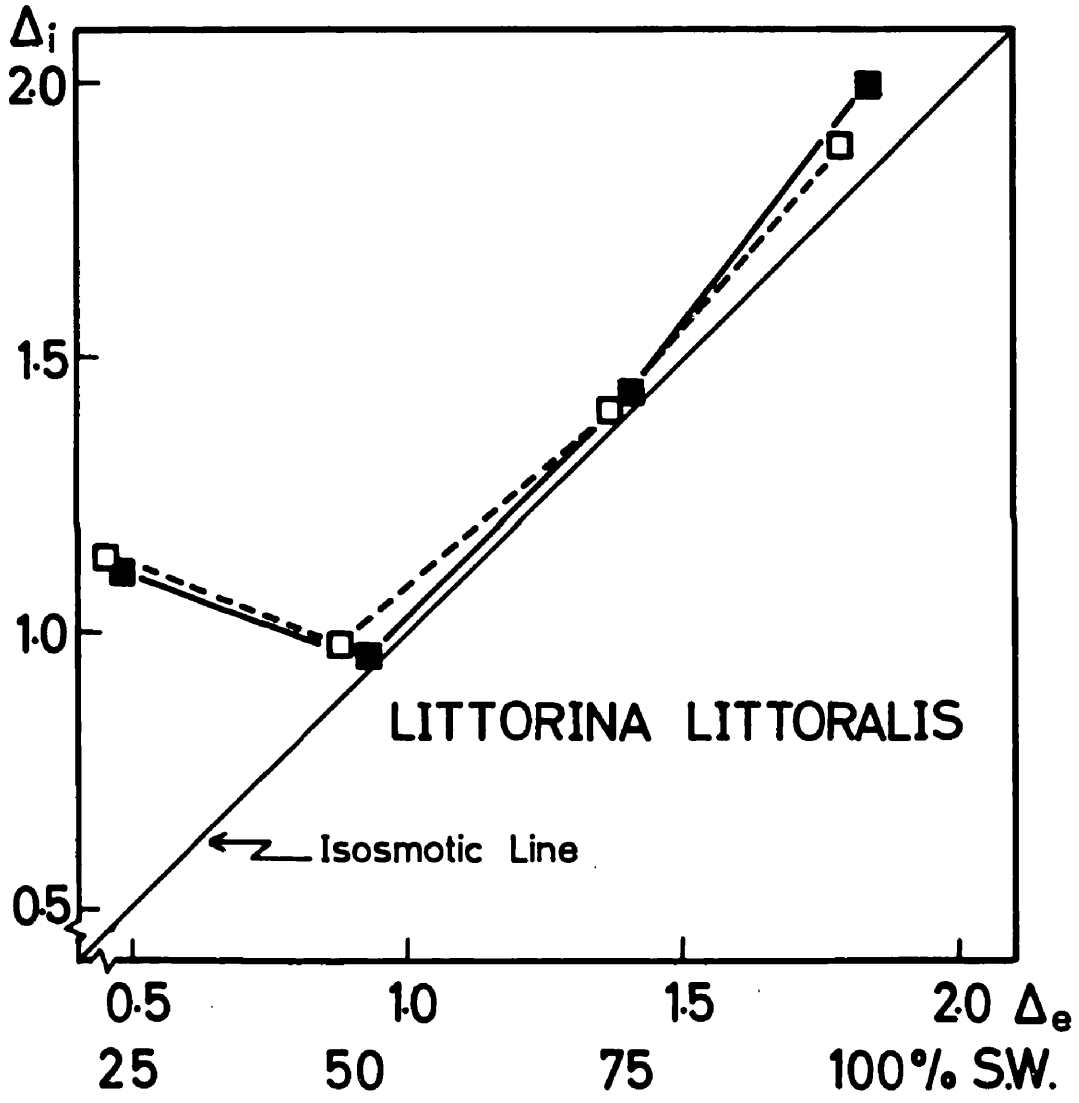

Δi
2·0
1·5
1·0
0·5
LITTORINA LITTORALIS
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100% S.W.

Table XIX

LITTORINA LITTORALIS

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.86	16	1.88	0.056	0.014
	75	1.43	11	1.41	0.136	0.040
	50	0.94	23	0.98	0.099	0.021
	25	0.48	29	0.89	0.265	0.049
15°C	100%	1.86	15	1.87	0.077	0.020
	75	1.38	14	1.39	0.036	0.010
	50	0.95	12	0.95	0.028	0.008
	25	0.47	26	1.09	0.253	0.050

Winter

5°C	100%	1.84	14	2.00	0.113	0.030
	75	1.41	11	1.44	0.061	0.018
	50	0.93	11	0.96	0.044	0.013
	25	0.48	25	1.11	0.387	0.077
15°C	100%	1.79	18	1.89	0.070	0.016
	75	1.37	17	1.41	0.066	0.016
	50	0.88	11	0.98	0.085	0.026
	25	0.45	13	1.14	0.159	0.044

Temperature and salinity effects

In summer animals the mean osmotic concentration of the blood in 25% sea water was significantly higher (t = 2.84, $P<0.01$) in animals held at 15°C than in those at 5°C. Nevertheless, the survival rate was better at the lower temperature. In three separate groups of animals held at 15°C in 25% sea water, there were no survivors on the seventh day in one group and none on the eighth day in the other two groups. In contrast there was a 100% survival rate in two groups of animals after 9 days in 25% sea water at 5°C. Of the winter animals tested in 25% sea water, the rather higher mean osmotic concentration of the blood in the group held at 15°C was not significantly different from that of the group at 5°C. Here also low temperature favoured survival - more than 17 days whereas none survived after 8 days at the higher temperature. In contrast to L. littorea, L. littoralis survived for a limited time in 50% sea water at 15°C. There were no living summer animals after 9 days or winter animals after 10 days. Maximum survival times are shown in Table XX. There is no simple correlation between the level of osmotic

Table XX

LITTORINA LITTORALIS

	Maximum time survived			
	Summer		Winter	
Salinity	5°C	15°C	5°C	15°C
50%	22^{+}	9	12^{+}	10
25%	9^{+}	7	17^{+}	8
	8^{+}	8		
		8		

LITTORINA SAXATILIS

50%	22^{+}	8^{+}	13^{+}	19
25%	13^{+}	9	17^{+}	15
				13

concentration of the blood and ability to tolerate low salinity solutions. For example, the mean freezing point depression of a winter group which died in 25% sea water at 15°C was Δ_i1.14, whereas Δ_i0.96 was tolerated in 50% sea water at 5°C. The results of the experiments with L. littoralis are similar to those of L. littorea, indicating that tolerance of low salinities is a function of the temperature-salinity interaction.

In 100% and 75% sea water the experimental temperature did not affect blood concentration or survival.

Seasonal effect

There was no difference in the osmotic concentration of winter and summer animals at the two temperatures in solutions over 50% sea water. There are significant differences between the mean values of the osmotic concentration of the blood of winter animals in 25% sea water at 5°C and 15°C and those of summer animals in 25% sea water at 5°C (summer and winter at 5°C, t = 2.46, $P<0.025$; summer 5°C, winter 15°C, t = 3.15, $P<0.005$), but survival at 25% is similar in both seasons.

Littorina saxatilis

Osmotic balance

The osmotic balance of L. saxatilis was studied in the salinity range 100% to 25% sea water at 5°C and 15°C. The mean freezing point depressions of the blood of 98 summer animals and 147 winter animals are shown in Figure 13 and Figure 14. The mean values, standard deviations and standard errors of the mean of animals from the different temperature-salinity conditions are given in Table XXI. Two animals tested in 125% sea water gave the expected result Δ_i 2.31, medium Δ_e 2.25. In 100% to 50% sea water, the blood was slightly hyperosmotic relative to the medium and at these salinities the animals were active. In 25% sea water the snails retracted into the shell and the blood was markedly hypersomotic (t = 8.30 to 20.11, $P<0.001$).

Temperature and salinity effects

The observed effect of the experimental conditions on the survival time of L. saxatilis in 50% and 25% sea water is given in Table XX. Visible evidence of cardiac activity in the form of frequent and vigorous

Figure 13. The relation of the osmotic concentration of the blood of Littorina saxatilis to the concentration of the medium. Summer animals: 5°C —●—, 15°C --o--.

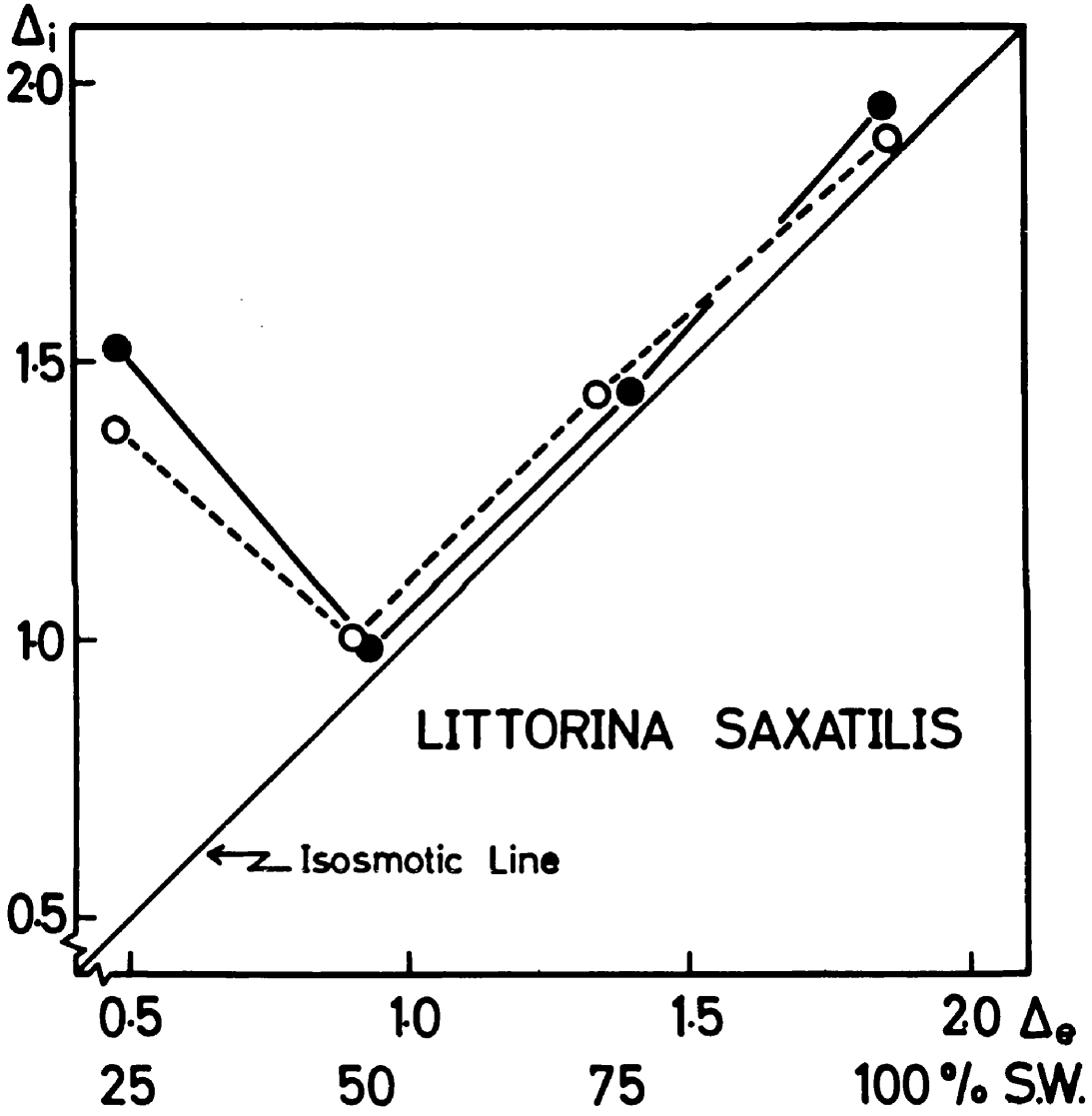

Δi
2·0
1·5
1·0
0·5
LITTORINA SAXATILIS
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100 % S.W.

Figure 14. The relation of the osmotic concentration of the blood of Littorina saxatilis to the concentration of the medium. Winter animals: 5°C -■-, 15°C --□--.

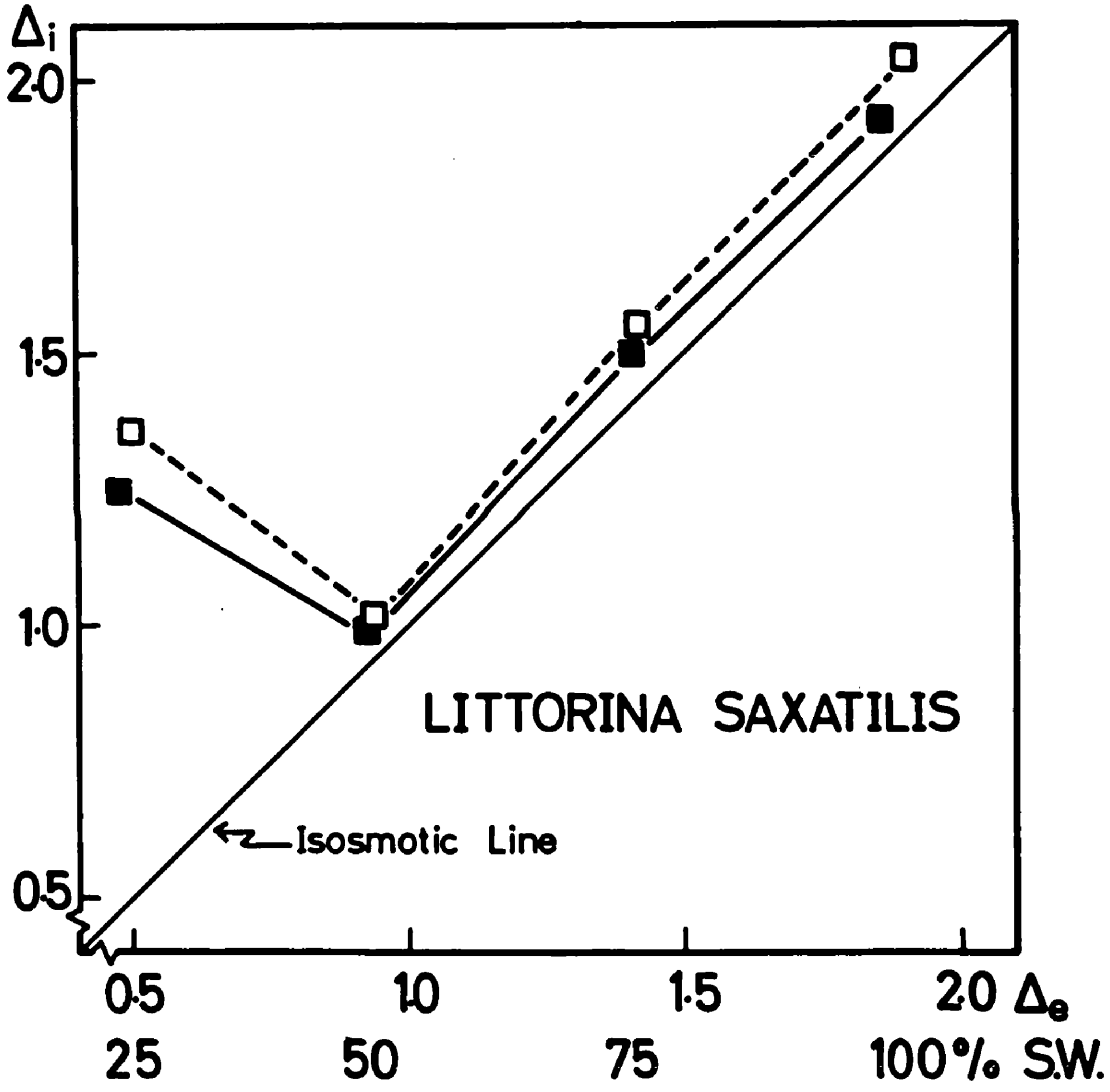

Δi
2·0
1·5
1·0
0·5
LITTORINA SAXATILIS
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
25
50
75
100% S.W.

Table XXI

LITTORINA SAXATILIS

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.85	10	1.96	0.097	0.031
	75	1.40	13	1.45	0.062	0.017
	50	0.93	12	0.99	0.077	0.022
	25	0.48	15	1.53	0.246	0.064
15°C	100%	1.86	10	1.90	0.079	0.025
	75	1.34	12	1.44	0.079	0.023
	50	0.90	12	1.01	0.089	0.026
	25	0.48	14	1.38	0.238	0.064

Winter

5°C	100%	1.86	14	1.93	0.035	0.009
	75	1.41	14	1.50	0.104	0.028
	50	0.93	20	0.99	0.072	0.016
	25	0.48	20	1.25	0.159	0.036
15°C	100%	1.90	22	2.04	0.148	0.031
	75	1.42	23	1.56	0.107	0.022
	50	0.94	15	1.02	0.074	0.019
	25	0.50	19	1.36	0.156	0.036

contractions could always be observed in the surviving L. saxatilis in 25% sea water although this was seldom shown in L. littorea or L. littoralis in this salinity. The survival time for winter L. saxatilis was over 17 days in 25% sea water at 5°C compared with a maximum of 13 to 15 days in 25% sea water at 15°C. No summer animals survived 9 days in 25% sea water at 15°C but they were all alive after 13 days in the solution at 5°C. Survival time at the higher temperature in 25% sea water therefore is longer in the winter animals than in summer animals. A group of winter animals at 15°C in 50% sea water did not survive beyond 19 days, but a group of summer animals at 5°C were still active after 22 days. As in the other two species of Littorinidae, the critical survival factor at the low salinities was a combination of temperature and salinity rather than a threshold osmotic concentration.

In salinities above 50% sea water, there was no appreciable temperature effect on osmotic concentration of the blood in either winter or summer animals.

Seasonal effect

The winter animals tested in 25% sea water had a

higher mean value for the osmotic concentration of the blood at 15°C than at 5°C ($\underline{t}$ = 2.15, $P<0.05$), but the summer animals in the two temperatures in 25% sea water showed no such significant difference. When the mean osmotic concentrations of the blood of summer and winter animals at 25% are compared, the summer group at 5°C had a significantly higher mean osmotic concentration than winter animals at 5°C ($\underline{t}$ = 4.08, $P<0.001$) and at 15°C ($\underline{t}$ = 2.43, $P<0.025$) but there were no significant differences between summer and winter animals tested at 15°C.

Experiments with Phenol Red

The experiments with 0.01% Phenol Red were carried out on the Littorinidae to obtain a relative measure of the exchange between body fluids and external medium in retracted animals in 25% sea water, at both 5°C and 15°C. Controls were placed in the same concentration of Phenol Red in 100% sea water. An arbitrary measure of dye concentration as indicated by colour intensity after addition of potassium hydroxide was assessed as + to ++++, and since any absorbed dye rapidly accumulated in the kidney, each value mainly represented the dye concentration in

that organ. Controls in 100% sea water were always ++++ within a few hours.

In the first experiments, groups of winter L. littorea were placed in the dye solution at 15°C after 30 to 40 days in 100% or 75% sea water. The detailed results for the groups are set out in Table XXII and Tables XXIII. The dye reached the mantle cavity in the experimental animals in less than 5 hours in 25% sea water, and although after 2 days the dye concentration in the kidney was perceptibly less than that in the controls in 100% sea water, it is evident that the tissues of the animal are in contact with the dye solution. A random sample of freezing point depressions made it clear that there is no obvious connection between the dye concentration in the tissues and the diminished osmotic concentration of the blood.

In further experiments, groups of winter L. littorea were examined after 1 and 4 days in 25% sea water at 5°C and at 15°C (Table XXIV). The subjective assessment showed a bias favouring greater dye concentration at a temperature of 15°C.

Table XXII

LITTORINA LITTOREA

Dye Experiments

Winter

Animals previously in 75% sea water

Temp.	Sal.	Δ_i°C	Time in dye sol.	Operc.	Dye value	Remarks
15°C	25%	—	4.45 hours	P	+	Mantle cavity and rectum pink
		1.20	27	P	++	Kidney, Mantle cavity, rectum, pink
		1.10	27	P	+++	Kidney dark pink, mantle cavity, rectum pale pink
		1.21	2 days	P	++	Kidney, mantle cavity pink
		—	3	P	++++	Kidney red, rectum pink

Animals previously in 100% sea water

15°C	25%	1.12	23 hours	S	++	Kidney pink, rectum, mantle cavity pale pink
		1.28	2 days	S	+++	Kidney dark pink, oesophagus, rectum pale pink
		—	3	P	++++	Kidney red, rectum pink

Sol. = Solution
Results are for individual animals representative of the group
Operc. = Operculum P = Present S = Small

Table XXIII

LITTORINA LITTOREA

Dye Experiments

Winter

Animals previously in 75% sea water

Temp.	Sal.	Time in dye sol.	Operc.	Dye value	Remarks
15°C	100%	1.5 hours	P	++++	Kidney red, muscle of foot pink
		2.75	P	++++	Dye in mantle cavity, dark red kidney, rectum pink
		27	P	++++	Kidney dark red, mantle cavity, rectum pink
		30.5	P	++++	Kidney dark red, mantle cavity, oesophagus, rectum pink

Animals previously in 100% sea water

15°C	100%	23	S	++++	Kidney dark red, rectum pale pink

Results are for individual animals representative of the group

Table XXIV

LITTORINA LITTOREA

Dye Experiments

Summer

Temp.	Sal.	Time in dye sol.	Operc.	Dye values
5°C	25%	1 day	P	+++
			P	+++
		2	P	++++
			P	++++

Winter

5°C	25%	1	P	++
			S	+
15°C		1	S	++
			S	++
5°C		4	S	+
			S	+
			P	-*
15°C		4	P	++
			P	+
			S	++

Results are for individual animals representative of the group

* No dye detected.

In summer L. littorea all with a complete operculum, tested in the dye solution at 5°C, concentration was +++ after 24 hours, which could be interpreted as indicating a more rapid accumulation of the dye in summer animals although the osmotic concentration of the blood was the same in summer and winter animals at 5°C.

The results of the dye experiments with summer L. littoralis at 5°C and 15°C (Table XXV) suggested that to begin with penetration of the dye was faster with greater accumulation at the higher temperature, but by the third day the dye concentration was equally strong at both temperatures.

Tested in 25% sea water at 5°C (Table XXV), summer L. saxatilis had initially a slower penetration and accumulation of the dye than that in L. littorea and L. littoralis which could be expected with the higher mean osmotic concentration of the blood in L. saxatilis in 25% sea water.

Comparison of Blood, Pericardial Fluid and Urine

The possibility of the animals maintaining an active osmoregulation by excreting a hypo-osmotic

Table XXV

Summer

LITTORINA LITTORALIS

Temp.	Sal.	Time in dye sol.	Dye values a	b
5°C	25%	1 day	−	−
15°C		1	−	++
5°C		2	−	+++
15°C		2	+	++
5°C		3	++++	++++
15°C		3	++++	++++
5°C		6	++++	++++

LITTORINA SAXATILIS

5°C	25%	1	+?	+
		2	−	+?
		3	+	+?
		6	++++	++++

a and b refer to two different animals tested which were representative of the group

urine in low salinities was tested by examining blood, pericardial fluid and urine, and compared with similar tests in the higher salinities where the animals are approximately isosomotic.

According to Goodrich (1945) the pericardial and renal cavities communicate in gastropods through a renal-pericardial pore: pericardial fluid is regarded as an ultra-filtrate of the blood, and the urine as modified pericardial fluid. Figure 15 is a diagram giving a general plan of the relationship of heart, pericardial cavity and kidney, common to the three species of Littorinidae and the two species of Hydrobiidae to be described later.

The freezing point depression of blood and urine of 10 summer L. littorea in 25% sea water at 5°C is given in Table XXVI, and it is apparent that in any one animal the freezing point depression of the blood and urine approximate within narrow limits; the means of $\Delta_i 1.26$ and $\Delta_i 1.24$ are not significantly different.

In another group at 15°C, in 30% sea water (Table XXVII), blood, pericardial fluid and urine

Figure 15. Diagram showing the relationships of the heart, pericardial cavity and kidney in the gastropods studied here.

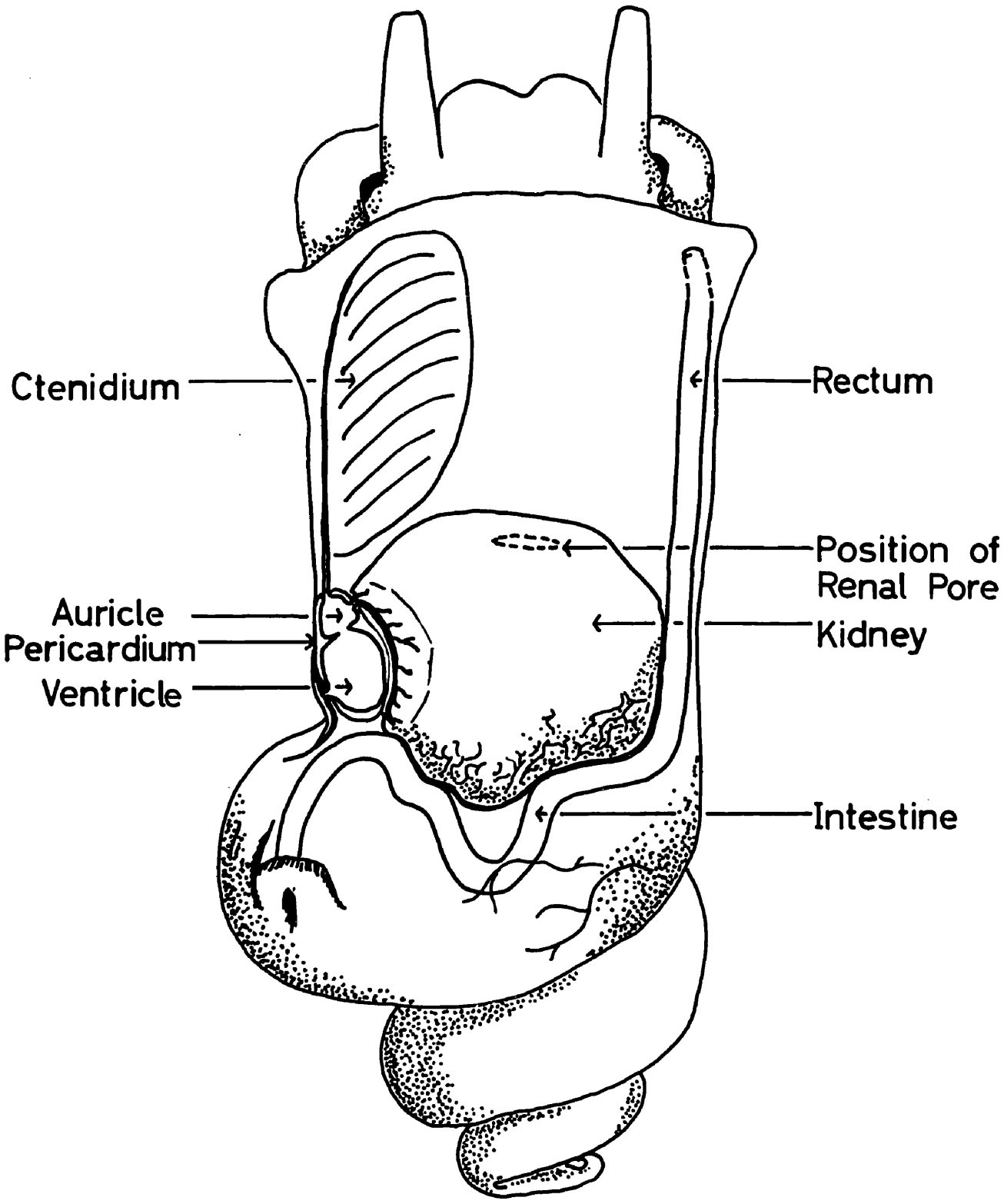

Ctenidium
Rectum
Position of
Renal Pore
Auricle
Pericardium
Ventricle
Kidney
Intestine

Table XXVI

LITTORINA LITTOREA

Temp.	Sal.	Δ_e°C	Days in medium	Δ_i°C blood	Δ_i°C urine
5°C	25%	0.53	3	1.32	1.31
				1.23	1.31
				1.31	1.26
			4	1.27	1.24
			5	1.17	1.19
				1.32	1.15
				1.44	1.42
			7	0.72	0.75
				1.36	1.34
				1.43	1.41
			Mean	1.26	1.24

Table XXVII

LITTORINA LITTOREA

Temp.	Sal.	Δ_e °C	Days in medium	Δ_i °C blood	Δ_i °C p.c. fluid*	Δ_i °C urine
15°C	30%	0.60	1	1.27	1.26	1.29
			2	1.05	1.20	1.21
			3	0.76	0.76	0.81
			4	0.83	0.81	0.92
			5	0.75	0.76	0.78
			2	0.83	0.88	0.92
			3	1.17	1.14	1.10
				0.97	0.92	1.01
			5	0.73	0.72	0.76
			6	0.90	0.88	0.81
			7	0.96	0.93	0.93
				0.68	0.68	0.70
			Mean	0.91	0.91	0.94

* p.c. fluid = fluid from the pericardial cavity

were tested in 12 animals. The freezing point depressions of the three fluids were similar in each animal and the means show no significant differences. This was confirmed by the results of similar tests on other animals in different salinities.

Tests from a further series with L. littoralis and L. saxatilis are listed in Table XXVIII and Table XXIX and the values in respect of any one animal with the absence of any significant difference between the mean values for the groups again confirm that the fluid from the three areas in these species can be regarded as more or less isosomotic. Therefore, the hyperosmotic condition in low salinities is not due to the excretion of a urine hypo-osmotic relative to the blood.

Size and Sex

There was no obvious connection between size, sex and freezing point depression of the blood in the Littorinidae. Since the values for the freezing point depression of the blood in individual animals in salinities above 50% sea water fell within a narrow range, size and sex are not likely to be significant

Table XXVIII

LITTORINA LITTORALIS

Winter

Temp.	Sal.	Δ_e °C	Days in medium	Δ_i °C blood	Δ_i °C p.c. fluid	Δ_i °C urine
5°C	75%	1.41	2	1.42		1.46
				1.43		1.46
				1.51		1.57
			7	1.46		1.48
				1.43		1.47
			Mean	1.45		1.49
5°C	50%	0.93	2	1.00		1.02
				0.97		0.98
				1.03		1.07
				0.97		0.96
			7	0.95		0.97
				0.97		0.97
			Mean	0.98		0.99

Summer

15°C	25%	0.47	2	0.70	—	0.69
			3	0.93	0.86	1.00
				0.64	0.65	0.63
			6	1.11	1.03	—
				1.04	1.04	—
			7	1.06	1.09	1.03
			Mean (N=4)	0.83		0.84
			(N=5)	0.96	0.93	

Table XXIX

LITTORINA SAXATILIS

Temp.	Sal.	Δ_e °C	Days in medium	Δ_i °C blood	Δ_i °C p.c. fluid	Δ_i °C urine
15°C	25%	0.48	3	1.23		1.24
				1.32		1.37
				1.38		1.55
			4	1.56		1.68
				1.08		1.12
				1.57		1.60
			7	1.37		1.17
				1.27	1.26	1.43
			8	1.19		1.20
			Mean	1.33		1.37

factors in these experimental conditions. Below 50% sea water, however, size may affect osmotic concentration of the blood and Figure 16 is an example of the freezing point depressions of the blood plotted against weight for summer L. littorea males at 5°C, 35% sea water, with a peak suggesting an optimum size range apparently more capable of maintaining hyperosmotic blood. An example of the comparison of mean freezing point depressions of the blood for males and females is shown for summer L. littorea and winter L. littoralis over the range of salinities from 100% to 25% sea water (Table XXX). The values are similar in males and females and the highest value of t from the t-test was 1.04 ($P > 0.05$). The sex ratio in the three species is close to 1:1.

Osmotic Balance in the Littorinidae

1. The three species of Littorinidae investigated, L. littorea, L. littoralis and L. saxatilis had an isosomotic or slightly hyperosmotic concentration of the blood relative to the medium in the range of salinities 100% to 50% sea water.

2. The animals responded to the low salinity solutions (less than 50% sea water) by retracting into

Figure 16. Scatter diagram plotting the freezing point depression values of summer male Littorina littorea of different weights. The animals were in 35% sea water (Δ_e0.68) at 5°C.

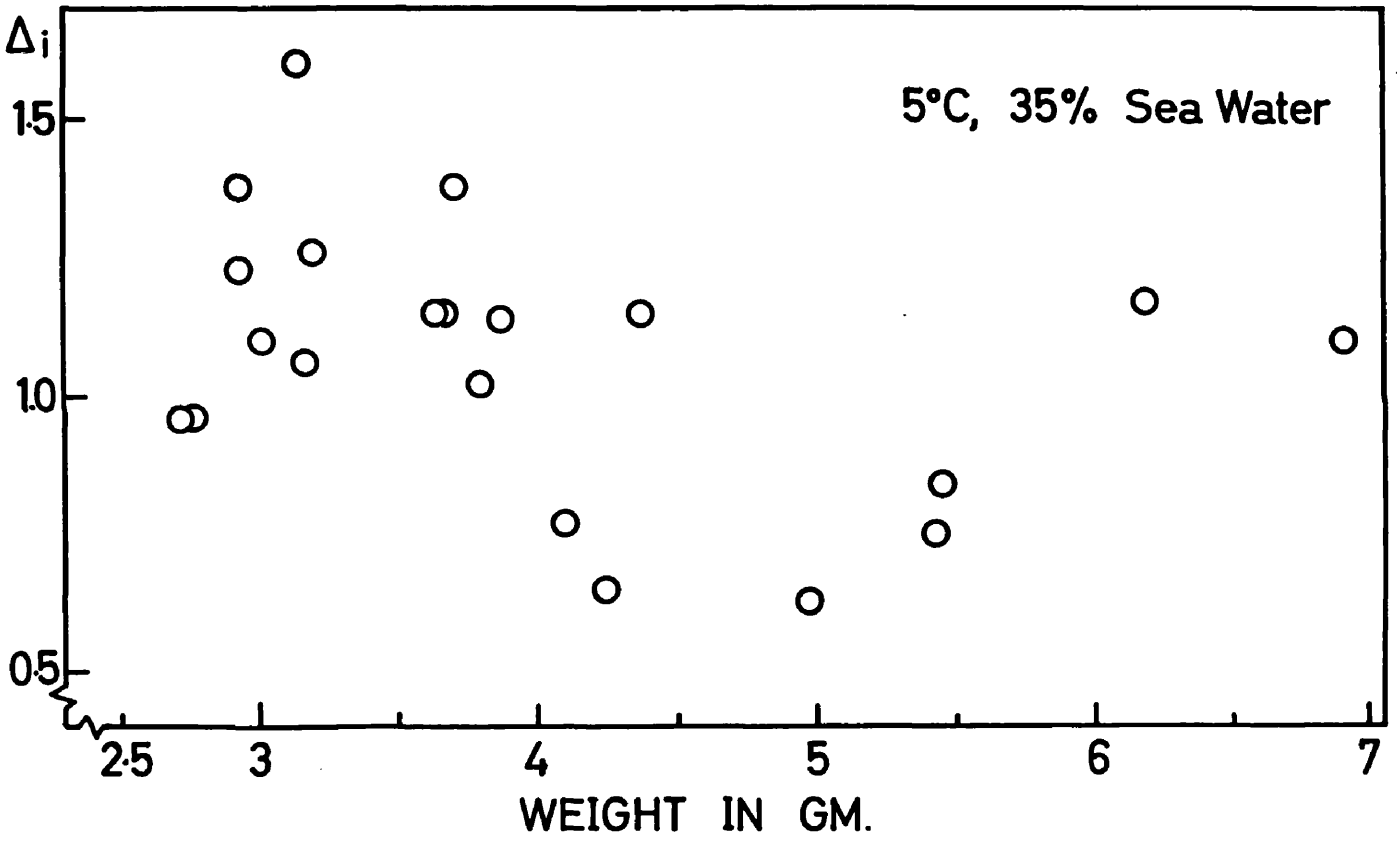

Δi
1.5
1.0
0.5
5°C, 35% Sea Water
2.5
3
4
5
6
7
WEIGHT IN GM.

Table XXX

LITTORINA LITTOREA

Summer

Temp.	Sal.	N	Δ_i °C ♂	N	Δ_i °C ♀
5°C	100%	12	1.81	9	1.83
	75	14	1.39	13	1.41
	50	10	0.94	17	0.93
	25	15	1.15	9	1.03
15°C	100%	4	1.95	8	2.02
	75	8	1.44	13	1.45
	50	6	0.98	6	0.94
	25	7	1.12	12	1.08
	Total	76		87	
	Sex ratio		0.9:1		

LITTORINA LITTORALIS

Winter

5°C	100%	6	2.03	4	2.10
	75	5	1.41	5	1.46
	50	4	1.03	5	0.96
	25	4	1.21	7	1.18
	Total	19		21	
	Sex ratio		0.9:1		

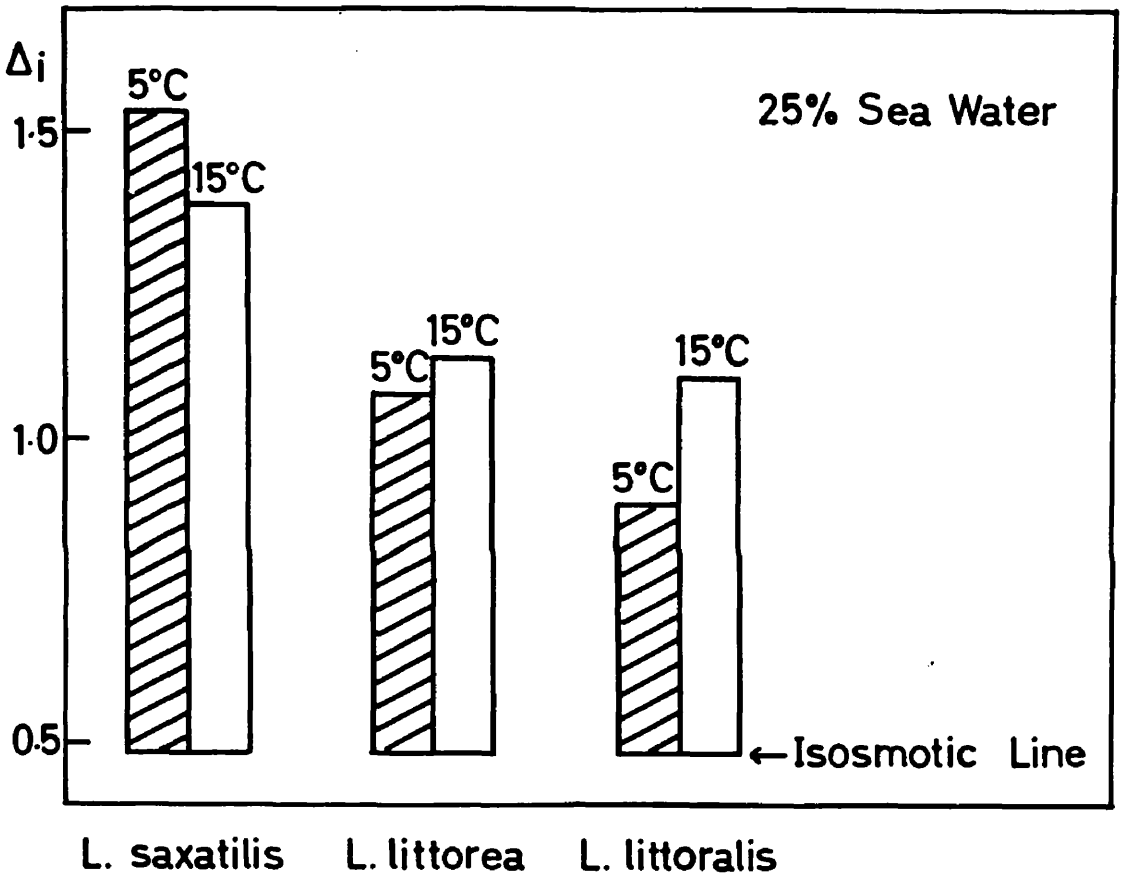
the shell before the blood was appreciably diluted. In 25% sea water the blood was significantly hyper-osmotic, but the animals' tissues were in contact with the medium, as shown by the intake of Phenol Red.

3. The values for the freezing point depression of the blood, pericardial fluid and urine in the same animal were similar. Moreover, even at the lowest salinity, 25% sea water, the urine tended to be hyperosmotic rather than hypo-osmotic relative to the blood.

4. The threshold osmotic concentration of the blood necessary for survival is within the experimental parameters a function of the temperature-salinity interaction.

5. The osmotic concentration of the blood in 25% sea water was highest in L. saxatilis and lowest in L. littoralis, and this is shown in Figure 17 by the extent to which the blood is hyperosmotic relative to 25% sea water in the three species, collected in summer and kept at 5°C and 15°C for periods varying from 1 to 13 days.

Figure 17. Comparison of freezing point depression values of summer Littorina saxatilis, L. littorea and L. littoralis in 25% sea water at 5°C and 15°C. Isosomotic base line was taken as $\Delta_e 0.48$.


Δi
1·5
1·0
0·5
5°C
15°C
25% Sea Water
5°C
15°C
5°C
15°C
←Isosmotic Line
L. saxatilis
L. littorea
L. littoralis

6. In 50% and 25% sea water, the greatest tolerance as assessed by survival time was shown by L. littorea and the least by L. littoralis.

7. Season, size and sex had no pronounced effect on the osmotic balance in the Littorinidae.

Gastropoda: Hydrobiidae

Except in one group of animals, the osmotic balance was studied in *Hydrobia ulvae* and *Potamopyrgus jenkinsi* by determining the freezing point depression of the urine since it was difficult to sample the blood. The results from the Littorinidae showed that the urine was approximately isosomotic relative to the blood in saline solutions and it is assumed that this will apply to the Hydrobiidae. In experiments with *P. jenkinsi* in fresh water, the osmotic concentration of the urine was shown to be 83% that of the blood.

Hydrobia ulvae: Estuarine Animals

Osmotic balance

The osmotic balance of *Hydrobia ulvae* was studied over the range of solutions 100% sea water to fresh water at 5°C and 15°C except for winter animals tested in fresh water at one temperature, 5°C. The freezing point depression of the urine was tested in 162 summer animals and 133 winter animals, and the results in the different experimental salinities are given in Figure 18 and Figure 19. The number of

Figure 18. The relation of the osmotic concentration of the urine of Hydrobia ulvae, estuarine animals, to the concentration of the medium. Summer animals: 5°C -●-, 15°C --o--.

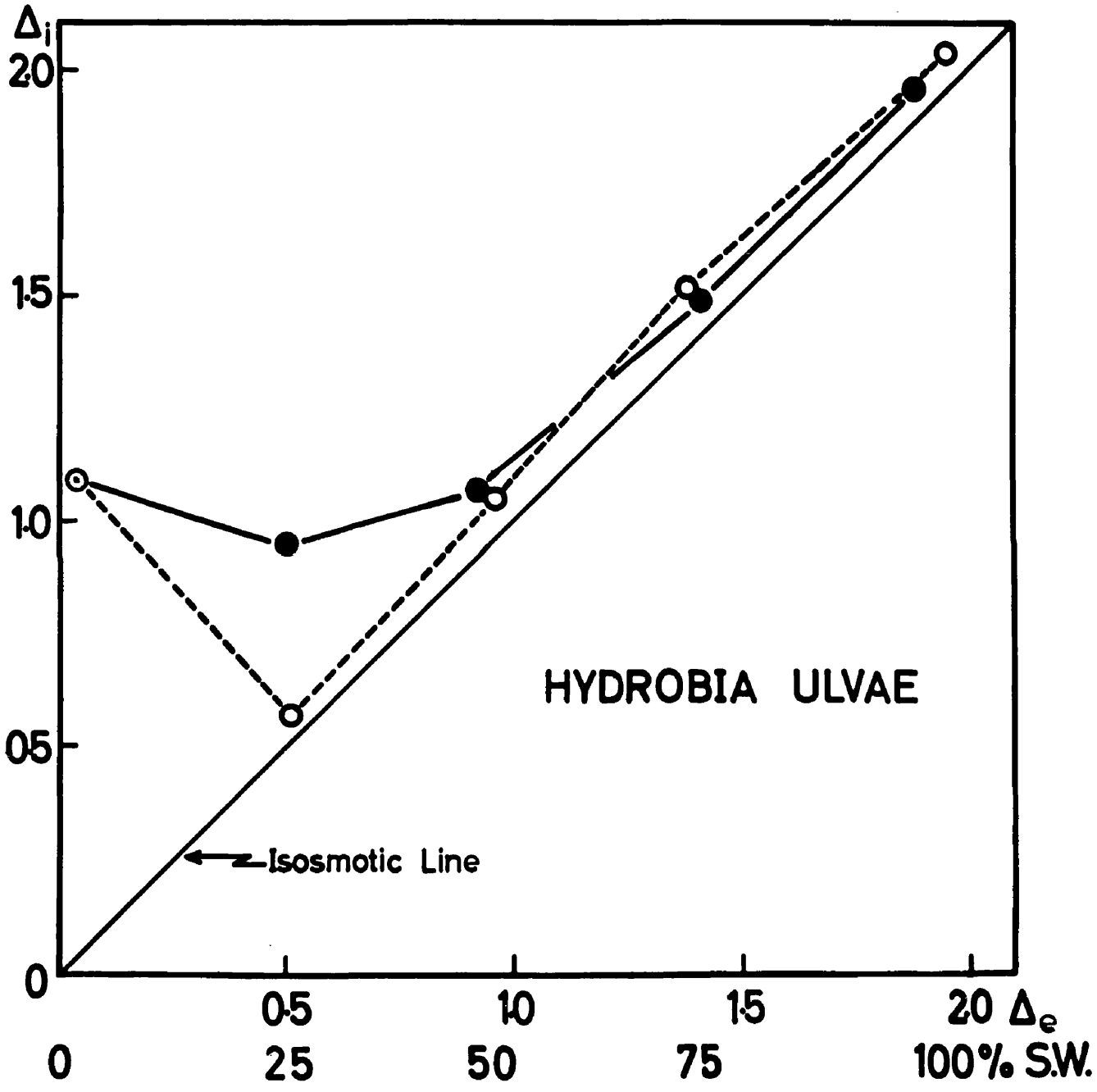
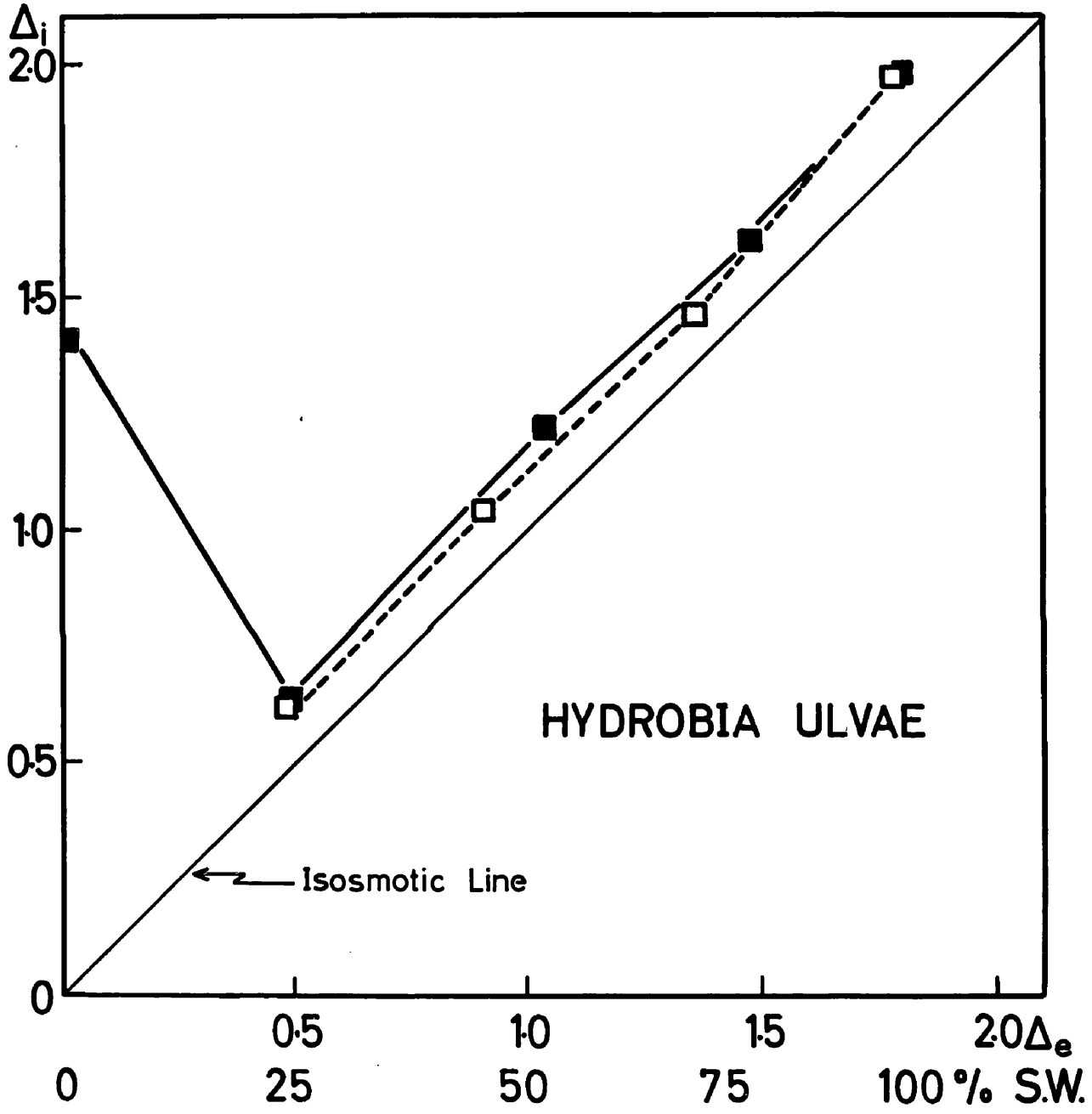

Δi
2.0
1.5
1.0
0.5
0
HYDROBIA ULVAE
Isosmotic Line
0.5
1.0
1.5
2.0 Δe
0
25
50
75
100% S.W.

Figure 19. The relation of the osmotic concentration of the urine of Hydrobia ulvae, estuarine animals, to the concentration of the medium. Winter animals: 5°C -■-, 15°C --□--.


Δ_i
2·0
1·5
1·0
0·5
0
HYDROBIA ULVAE
Isosmotic Line
0·5
1·0
1·5
2·0 Δ_e
0
25
50
75
100 % S.W.

animals tested in each group with mean values, standard deviations and standard errors of the mean are given in Table XXXI.

The concentration of the urine was slightly hyperosmotic in 100% to 25% sea water solutions, but in fresh water when the animals withdrew into the shell, the urine was markedly hyperosmotic relative to the medium ($\underline{t}$ = 8.83 to 17.70, $P<0.001$). This species was active except in fresh water.

Temperature, salinity and seasonal effects

Summer animals in 25% sea water at 15°C were active very quickly, but those at 5°C remained withdrawn into the shell for 8 days. Thereafter, the foot was protruded in some animals but crawling was not evident until the fourteenth day. During the withdrawn period, the freezing point depression of the animals at 5°C was Δ_i1.19 but from 9 days onward was Δ_i0.67, that is, approaching the freezing point depression of animals at 15°C. (In Figure 18, the freezing point depression mean is calculated from all animals in 25% at 5°C for 1 to 27 days.) The temperature of the pools from which the summer animals

Table XXXI

HYDROBIA ULVAE

Estuarine Animals

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.88	10	1.96	0.082	0.026
	75	1.41	18	1.49	0.105	0.025
	50	0.92	19	1.07	0.099	0.023
	25	0.50	19	0.95	0.286	0.066
	F.W.*	0.04	17	1.09	0.155	0.037
15°C	100%	1.95	17	2.04	0.145	0.035
	75	1.38	16	1.52	0.122	0.029
	50	0.96	18	1.05	0.116	0.027
	25	0.51	18	0.57	0.075	0.018
	F.W.	0.04	10	1.09	0.261	0.082

Winter

5°C	100%	1.80	11	1.98	0.088	0.027
	75	1.48	13	1.62	0.151	0.042
	50	1.04	12	1.22	0.169	0.049
	25	0.49	12	0.63	0.069	0.020
	F.W.	0.01	12	1.41	0.196	0.057
15°C	100%	1.78	16	1.97	0.066	0.016
	75	1.36	16	1.46	0.094	0.023
	50	0.91	18	1.04	0.077	0.018
	25	0.48	23	0.62	0.062	0.013

*Fresh water

were collected was often over 20°C and it is probable that the initial withdrawal and inactivity at 5°C in these poikilotherms was a temperature response pending adaptation to the low temperature. This same response did not occur in winter animals at 5°C in 25% sea water.

H. ulvae in fresh water showed no difference in the mean freezing point depression values for summer animals at 5°C and 15°C, although survival was noticeably better at 5°C, the count being 19/23 alive after 14 days compared to 1/26 at 15°C. The osmotic concentration of the urine of winter animals in fresh water at 5°C was significantly different from that of summer animals in fresh water at the same temperature (t = 4.91, $P<0.001$) and from those at 15°C (t = 3.30, $P<0.005$).

In 50% and above sea water solutions, neither temperature nor season affected the osmotic concentration of the urine of tested animals.

Hydrobia ulvae: Salt Marsh Animals

Osmotic balance

Osmotic balance was studied in summer animals

from the salt marsh population in the range of solutions 100% sea water to fresh water at 5°C and 15°C (Fig. 20). Freezing point depression of the urine was tested in 164 animals and the number in each group along with mean values, standard deviations and standard errors of the mean are shown in Table XXXII.

The urine of the animals tested at 5°C and 15°C was slightly hyperosmotic relative to the medium down to 25% sea water (except animals at 5°C, 25%) and thereafter was markedly hyperosmotic ($\underline{t}$ = 11.88 and 16.16, $P<0.001$).

Temperature and salinity effects.

The urine of animals tested at 5°C was more hyperosmotic than that of those at 15°C during the initial adaptation period; as with estuarine H. ulvae, the animals in 25% sea water at 5°C were withdrawn but for a more prolonged period - up to 28 days, and even after 43 days no animal had become attached to the substrate. The freezing point depression of two animals tested on the forty-third day was $\Delta_i 0.70$, approaching the value for animals at 15°C. (The

Figure 20. The relation of the osmotic concentration of the urine of Hydrobia ulvae, salt marsh animals, to the concentration of the medium. Summer animals: 5°C -●-, 15°C --o--.

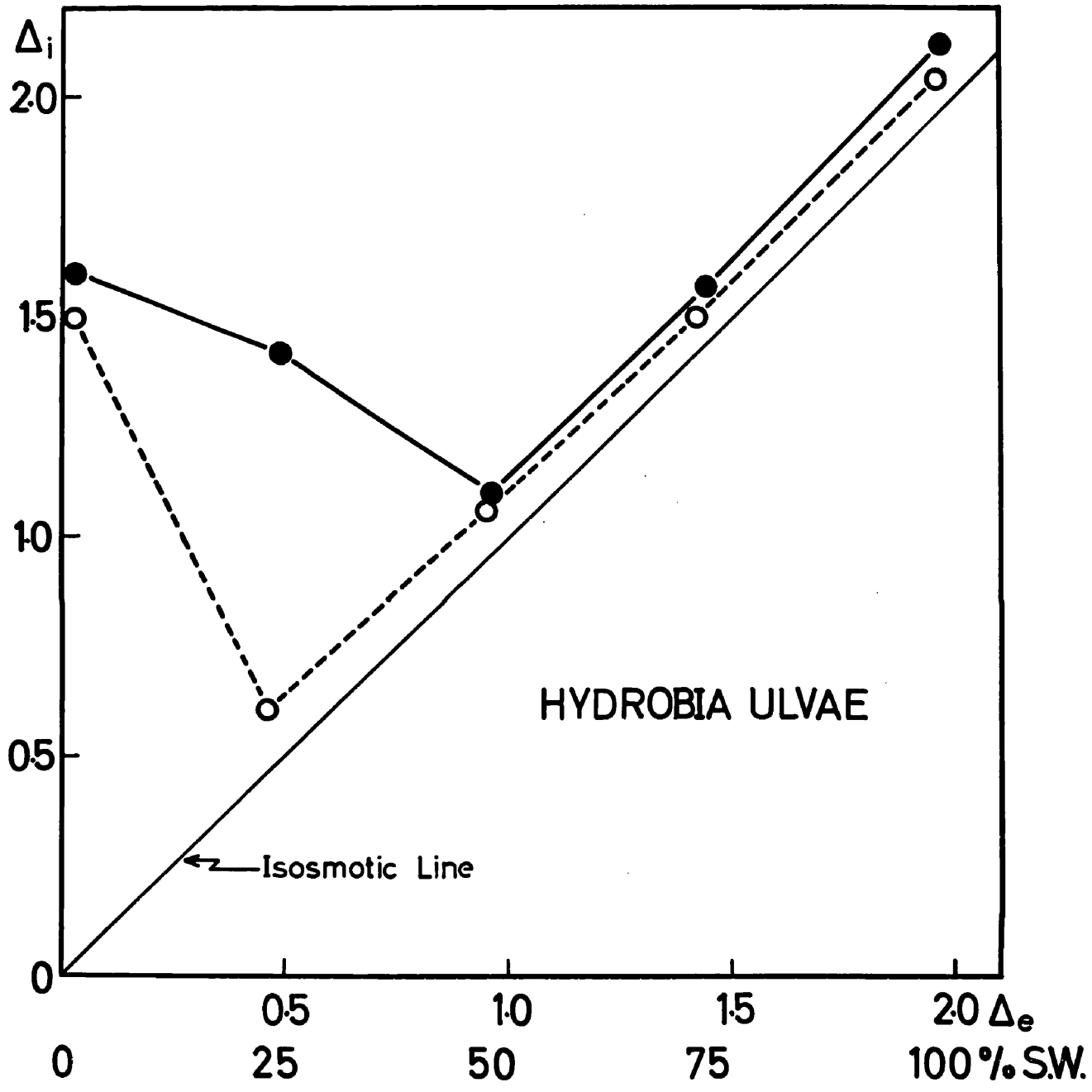

Δi
2·0
1·5
1·0
0·5
0
HYDROBIA ULVAE
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
0
25
50
75
100 % S.W.

Table XXXII

HYDROBIA ULVAE

Salt Marsh Animals

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.97	16	2.12	0.112	0.028
	75	1.44	13	1.57	0.155	0.043
	50	0.96	18	1.10	0.173	0.041
	25	0.49	19	1.42	0.319	0.073
	F.W.	0.03	18	1.50	0.346	0.082
15°C	100%	1.96	16	2.04	0.058	0.015
	75	1.42	16	1.50	0.096	0.024
	50	0.95	18	1.06	0.108	0.025
	25	0.46	14	0.61	0.174	0.047
	F.W.	0.03	16	1.60	0.278	0.069

value in Figure 20 is the mean for all animals tested from 1 to 43 days.) The salt marsh animals remained withdrawn in the 50% sea water solution at 5°C until after 8 days and none were crawling until the twelfth day. The salt marsh animals were less active, indicating less tolerance, than the estuarine animals at the low temperature and in low salinities.

In fresh water, half of the animals were dead after 12 days at 15°C and all of them after 20 days, whereas 11/11 at 5°C were alive after 20 days. There was no significant difference between the mean osmotic concentration of the urine of animals in fresh water at 5°C and 15°C although they live longer at 5°C.

In fresh water the mean osmotic concentration of the urine of the salt marsh animals exceeded that of the summer estuarine animals both at 5°C ($\underline{t}$ = 4.48, $P<0.001$) and at 15°C ($\underline{t}$ = 4.66, $P<0.001$). As in the estuarine population, there are no significant differences due to temperature within the salinity range 100% to 50% sea water.

Potamopyrgus jenkinsi

It was found that all the types of Potamopyrgus

jenkinsi, including those from fresh water, will live indefinitely in salinities up to 100% sea water if conditioned first in lower salinities, so that a comparison between the different ecological groups and types was only possible by submitting the animals directly into the test temperature-salinity medium. In contrast to survival, conditioning had no effect on osmotic concentration of the urine, which reached a steady state within 24 hours when the animals were transferred from a lower to a higher salinity.

Potamopyrgus jenkinsi: Type A, Fresh Water

Osmotic balance

The osmotic balance was studied in P. jenkinsi, type A, from fresh water over the range fresh water to 100% sea water. Freezing point depressions of the urine were tested in 144 summer animals at 5°C and 15°C and in 52 winter animals at 5°C (Fig. 21). Mean values, standard deviations and standard errors of the mean are given in Table XXXIII with the number of animals tested in each group.

The urine was hyperosmotic relative to the

Figure 21. The relation of the osmotic concentration of the urine of Potamopyrgus jenkinsi, Type A, from fresh water, to the concentration of the medium. Summer animals: 5°C -●-, 15°C -o-. Winter animals: 5°C -■-.

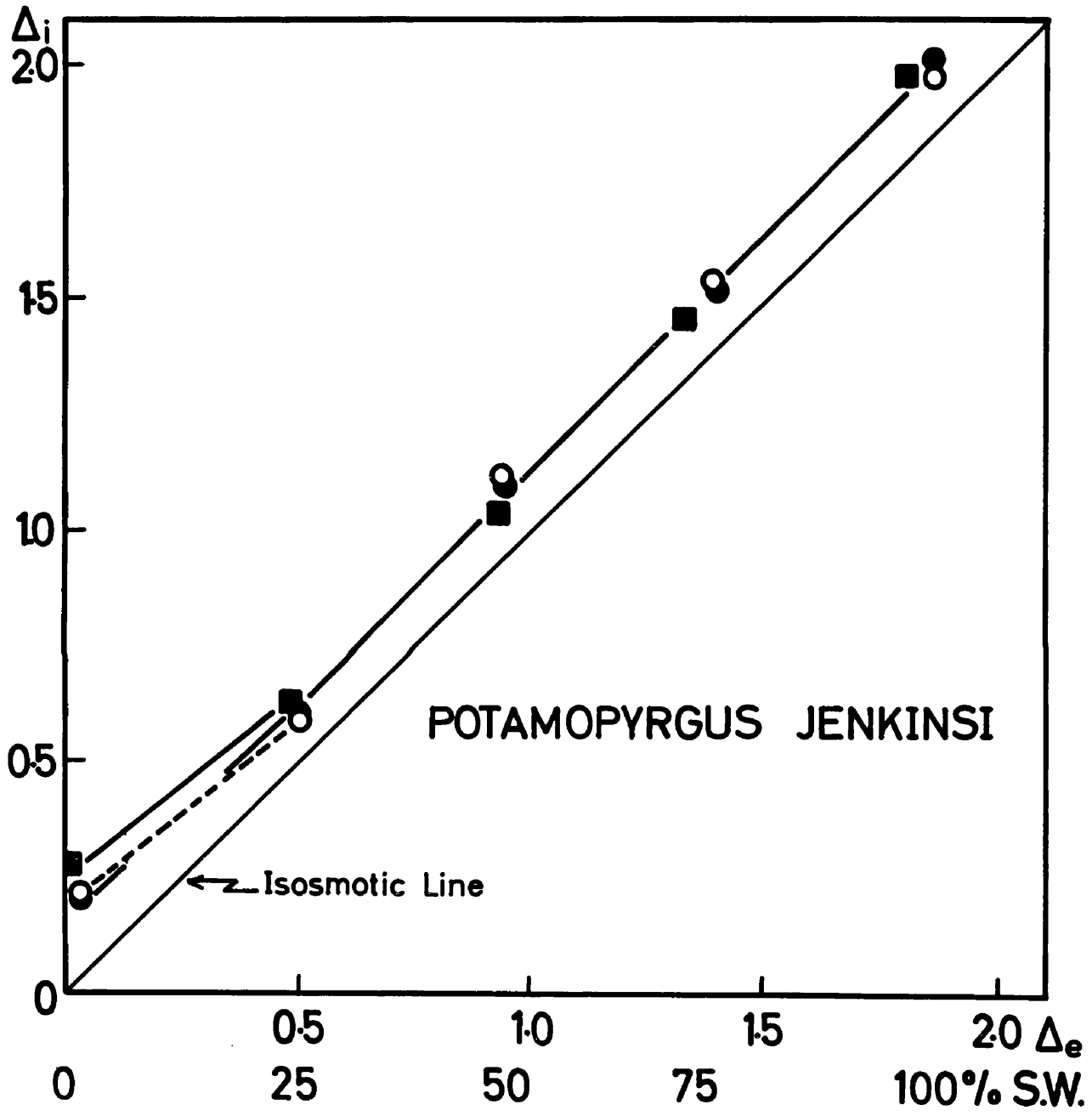

Δi
2·0
1·5
1·0
0·5
0
POTAMOPYRGUS JENKINSI
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
0
25
50
75
100% S.W.

Table XXXIII

POTAMOPYRGUS JENKINSI

Type A, Fresh Water

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.86	7	2.02	0.181	0.068
	75	1.40	9	1.52	0.196	0.065
	50	0.95	18	1.10	0.103	0.024
	25	0.50	22	0.61	0.071	0.015
	F.W.	0.03	20	0.21	0.045	0.010
15°C	100%	1.86	5	1.98	0.088	0.039
	75	1.39	8	1.54	0.137	0.049
	50	0.94	17	1.12	0.102	0.025
	25	0.50	22	0.59	0.084	0.018
	F.W.	0.03	16	0.22	0.030	0.007

Winter

5°C	100%	1.80	6	1.98	0.136	0.055
	75	1.33	5	1.46	0.073	0.033
	50	0.93	13	1.04	0.100	0.028
	25	0.48	14	0.63	0.084	0.022
	F.W.	0.01	14	0.28	0.063	0.017

medium over the whole range of salinities and even more so in fresh water ($\underline{t}$ = 9.76 to 32.30, $P<0.001$).

Temperature, salinity and seasonal effects

Summer snails in both 75% and 100% sea water (direct from fresh water) were dead after 8 days at 5°C and after 7 days at 15°C. In 50% sea water the snails were active within 24 hours at 15°C and were still normal after 120 days, while at 5°C they all were withdrawn until the seventh day and none (15/15) survived beyond the nineteenth day. In 25% sea water and fresh water all the animals were active immediately and lived indefinitely.

Winter animals also died in 75% and 100% sea water, but in contrast to the summer animals lived indefinitely in 50% sea water at 5°C. About half were crawling (11/24) by the eighteenth day and all of them by the twentieth day. In 25% sea water and in fresh the animals were active immediately and survived.

In the range fresh water to 100% sea water, temperature did not influence the mean osmotic

concentration of the urine of the summer animals. The only difference in the osmotic concentration of the urine in winter and summer animals was in the groups tested in fresh water where there was a significantly higher osmotic concentration of the urine in winter than summer animals at 5°C (t = 3.69, $P<0.001$) and also at 15°C (t = 3.37, $P<0.005$).

Potamopyrgus jenkinsi: Type A, Brackish Water

Osmotic balance

P. jenkinsi from brackish water were studied in solutions of fresh water to 100% sea water at 5°C and 15°C and also in Cambridge tap water at 15°C. The freezing point depression of the urine was determined in 133 summer animals (Fig. 22). Table XXXIV gives mean freezing point depressions, standard deviations and standard errors of the mean for the animals in the different conditions.

The concentration of the urine was hyperosmotic relative to the medium over the whole range of salinities, in fresh water (t = 8.83 and 12.51, $P<0.001$) and in Cambridge tap water (t = 27.37, $P<0.001$).

Figure 22. The relation of the osmotic concentration of the urine of Potamopyrgus jenkinsi, type A, from brackish water, to the concentration of the medium. Summer animals: 5°C —●—, 15°C --o--, Cambridge tap water at 15°C -x- .

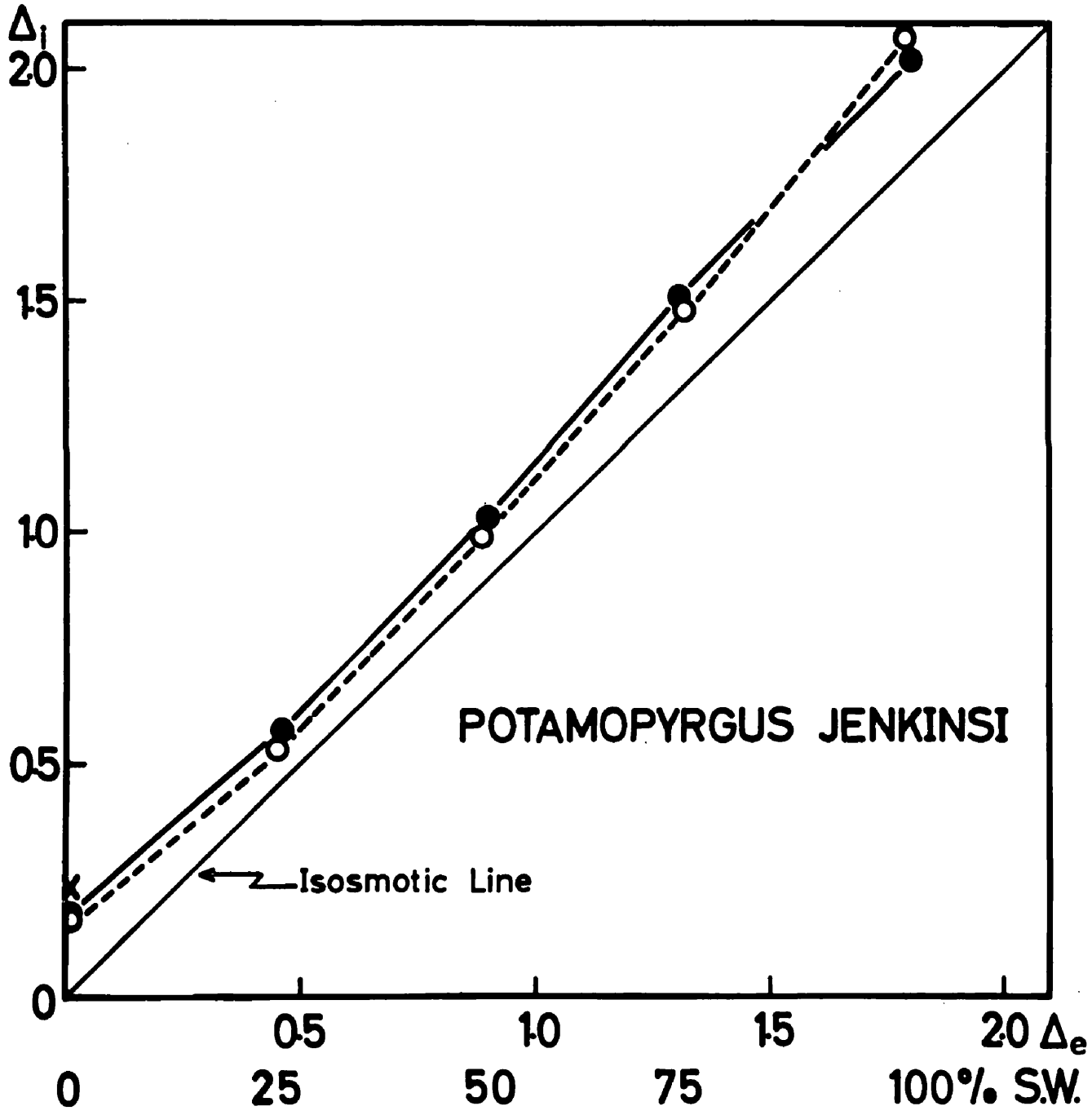

Δi
2.0
1.5
1.0
0.5
0
0.5
1.0
1.5
2.0 Δe
0
25
50
75
100% S.W.
POTAMOPYRGUS JENKINSI
Isosmotic Line

Table XXXIV

POTAMOPYRGUS JENKINSI

Type A, Brackish Water

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
5°C	100%	1.81	13	2.02	0.102	0.028
	75	1.31	12	1.51	0.114	0.033
	50	0.90	11	1.03	0.049	0.015
	25	0.46	12	0.57	0.051	0.015
	F.W.	0.01	9	0.18	0.041	0.014
15°C	100%	1.80	12	2.07	0.247	0.071
	75	1.32	13	1.48	0.095	0.026
	50	0.89	13	0.99	0.054	0.015
	25	0.45	13	0.53	0.026	0.007
	F.W.	0.01	11	0.17	0.028	0.008
	C.T.W.*	0.01	14	0.24	0.017	0.005

*Cambridge tap water

Temperature and salinity effects

In contrast to the fresh water group, the brackish water animals showed much greater tolerance in 75% and 100% sea water. None of the animals were active when placed directly into 100% sea water. At a temperature of 5°C the snails were all alive on the tenth day whereas at 15°C they had all died after 7 days (16/16). In 75% sea water at 15°C, the animals all lived and were immediately active and at 5°C were showing signs of activity by 7 days. In salinities below 75% sea water the animals were active immediately at both temperatures and lived indefinitely.

Three groups of animals introduced directly into 100% sea water either from 25%, 50% or 75% sea water had mean urine Δ_i 2.11 (average of eight), medium Δ_e 1.79. There were no significant differences in osmotic concentration of the urine due to temperature in any salinity, but the mean osmotic concentration of the urine of animals in Cambridge tap water at 15°C was significantly higher than in those at 5°C ($\underline{t}$ = 4.87, $P<0.001$) and those at 15°C ($\underline{t}$ = 7.86, $P<0.001$) in Lochend Loch water.

In fresh water at 15°C, summer <u>P. jenkinsi</u>

type A, brackish water, had a significantly lower mean osmotic concentration of the urine than summer P. jenkinsi, type A, from fresh water at 15°C (t = 4.33, $P < 0.001$). The difference, however, was not significant when both groups were at 5°C. In Cambridge tap water, the mean osmotic concentration of the urine of the summer brackish water P. jenkinsi was significantly higher than that of the summer fresh water animals at 5°C (t = 2.26, $P<0.05$, and at 15°C (t = 2.17, $P<0.05$).

Potamopyrgus jenkinsi: Type B

Osmotic balance

The osmotic balance of 24 summer P. jenkinsi, type B, were studied in solutions of fresh water to 100% sea water at 15°C (Fig. 23). These animals previously had been kept in 5% sea water at laboratory temperatures. Mean freezing point depressions, standard deviations and standard errors of the mean are given in Table XXXV. The animals died in 100% sea water in 5 days, but were immediately active and survived in 75% sea water and lower salinities. Values given are from animals transferred from lower salinities.

The concentration of the urine was hyperosmotic

Figure 23. The relation of the osmotic concentration of the urine of Potamopyrgus jenkinsi, type B, to the concentration of the medium. Summer animals: 15°C --o--.

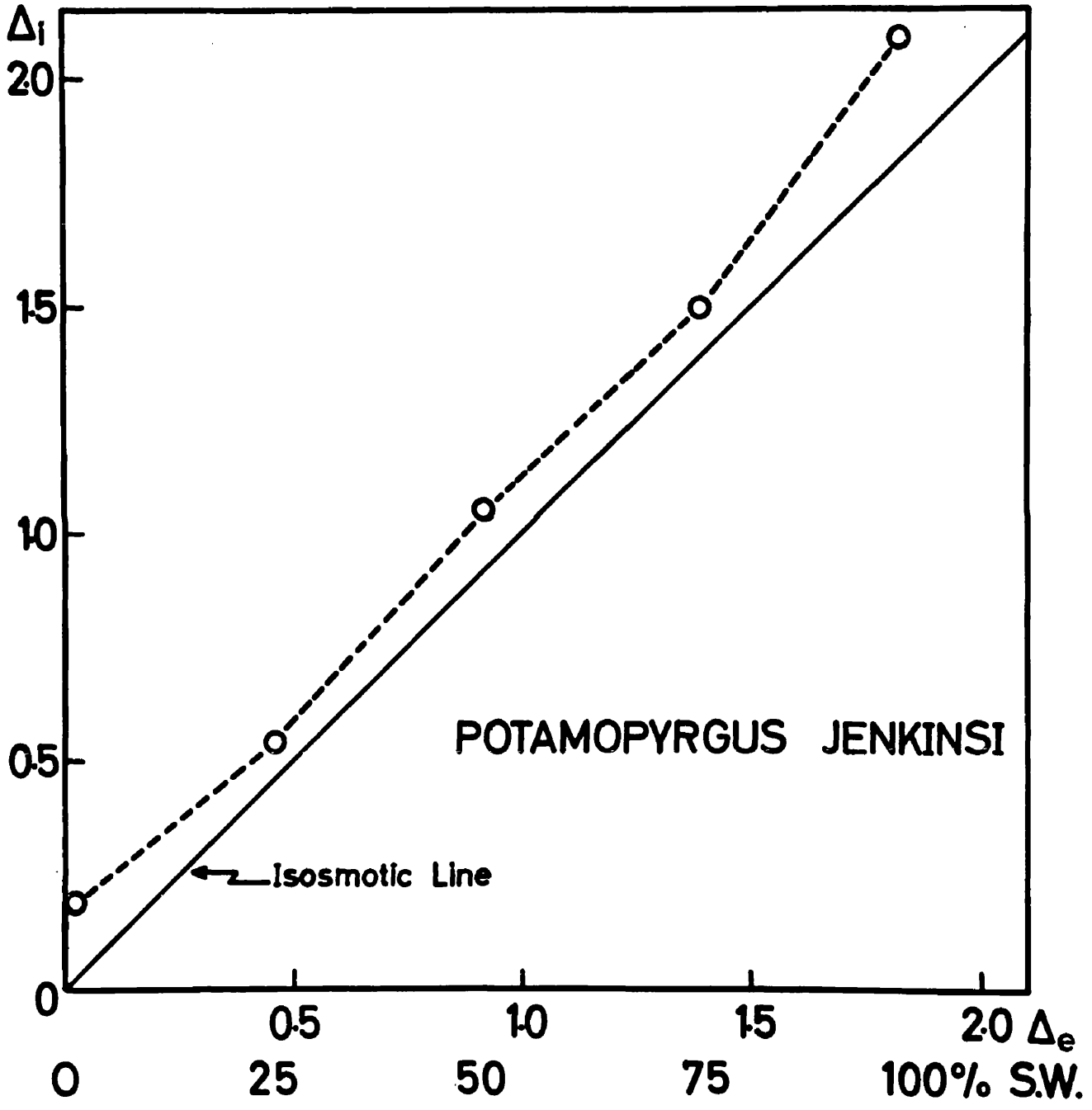

Δi
2·0
1·5
1·0
0·5
0
POTAMOPYRGUS JENKINSI
Isosmotic Line
0·5
1·0
1·5
2·0 Δe
0
25
50
75
100% S.W.

Table XXXV

POTAMOPYRGUS JENKINSI

Type B

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
15°C	100%	1.82	4	2.09	0.241	0.121
	75	1.39	5	1.50	0.078	0.035
	50	0.92	6	1.05	0.120	0.049
	25	0.46	6	0.54	0.036	0.015
	F.W.	0.02	3	0.19	0.032	0.018

in fresh water (t = 4.54, $P<0.05$) and over the salinity range. The mean osmotic concentration of the urine was not significantly different from that of fresh water animals or brackish water animals of type A, when all were tested in fresh water at 15°C.

Potamopyrgus jenkinsi: Type C

Osmotic balance

The osmotic balance of P. jenkinsi, type C, was studied over the range of solutions, fresh water to 100% sea water at 15°C (Fig. 24). The freezing point depression of the urine was determined in 37 animals and standard deviations and standard errors of the mean are given in Table XXXVI. These animals were kept in 5% sea water, at laboratory temperatures, before the experiments were started.

In salinities of 75% sea water and lower, the animals were immediately active and lived indefinitely. Put directly in 100% from 5% sea water, they died in 5 days and the urine for testing was obtained from animals which survived in 100% after transfer from 75% sea water. The concentration of the urine was hyperosmotic over the test range, and in fresh water

Figure 24. The relation of the osmotic concentration of the urine of Potamopyrgus jenkinsi, type C, to the concentration of the medium. Summer animals: 15°C --o--.

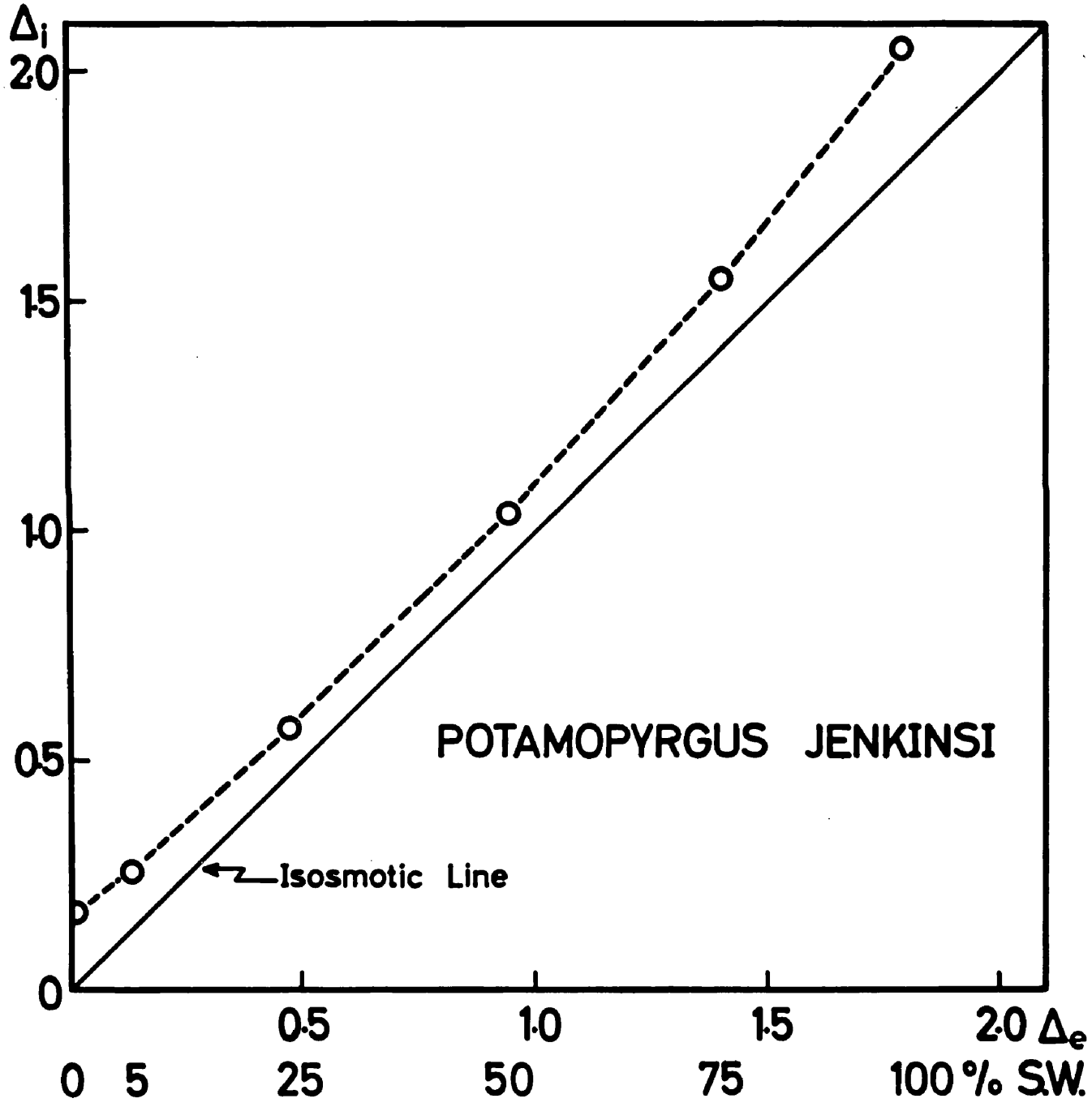

Δi
2.0
1.5
1.0
0.5
0
POTAMOPYRGUS JENKINSI
Isosmotic Line
0.5
1.0
1.5
2.0 Δe
0 5
25
50
75
100 % S.W.

Table XXXVI

POTAMOPYRGUS JENKINSI

Type C

Summer

Temp.	Sal.	Δ_e°C	N	Δ_i°C	S.D.	S.E.
15°C	100%	1.79	2	2.05	0.149	0.105
	75	1.40	8	1.55	0.113	0.040
	50	0.94	8	1.04	0.126	0.044
	25	0.47	8	0.57	0.068	0.024
	5	0.13	2	0.26	0.036	0.026
	F.W.	0.01	9	0.17	0.030	0.010

(t = 10.20, $P<0.01$). In fresh water, the mean freezing point depression of the urine was significantly lower than that of fresh water type A at 15°C (t = 3.96, $P<0.001$).

Blood and Urine

The freezing point depression of both blood and urine of eight brackish water *P. jenkinsi* of type A and three type B *P. jenkinsi*, all in fresh water at 15°C, were tested using the ultra fine capillary tubes. Since the mean values for the urine were not significantly different in these two types, the results have been grouped together for analysis (Table XXXVII). The urine was hypo-osmotic relative to the blood (t = 3.35, $P<0.01$), indicating that in fresh water, *P. jenkinsi* maintains the osmotic balance in part by the excretion of a hypo-osmotic urine.

Osmotic Balance in the Hydrobiidae

1. The urine of *Hydrobia ulvae* was slightly hyperosmotic relative to the medium over the salinity range 100% to 25% sea water, and may be initially markedly hyperosmotic in low salinity solutions at 5°C.

Table XXXVII

POTAMOPYRGUS JENKINSI

		Δ_i°C blood	Δ_i°C urine
Type A	1	0.14	0.10
	2	0.19	0.15
	3	0.18	0.14
	4	0.26	0.17
	5	0.21	0.14
	6	0.19	0.16
	7	0.16	0.19
	8	0.12	0.10
Type B	9	0.17	0.18
	10	0.16	0.13
	11	0.19	0.14
Mean		0.18	0.15

The urine was markedly hyperosmotic in fresh water. There were differences between the estuarine and salt marsh animals in respect of osmotic concentration and tolerance to low salinities.

2. The capacity for survival of Hydrobia ulvae in fresh water was influenced by temperature.

3. Potamopyrgus jenkinsi had slightly hyper-osmotic urine in the range 25% to 100% sea water.

4. Excretion of a urine hypo-osmotic relative to the blood contributes to the osmotic regulation in fresh water, in P. jenkinsi.

5. The combination of temperature and salinity determined survival capacity of animals transferred directly from salinities below 25% sea water into 50% to 100% sea water, but with gradual changes of increasing salinity, P. jenkinsi survived throughout the entire range of solutions.

6. P. jenkinsi, type A, from different habitats, showed differences in survival at the higher salinities and in the osmotic concentration of the urine in fresh

water.

7. In Cambridge tap water, type A, brackish water, had a significantly higher mean osmotic concentration of the urine than type A, fresh water, and types B and C.

8. In both Hydrobia ulvae and Potamopyrgus jenkinsi the previous environmental, seasonal and laboratory adaptation influenced survival and osmotic concentration in the experimental media.

DISCUSSION

Ecology

The results of the experimental investigation of osmotic balance in the representatives of two phyla, the Arthropoda and Mollusca, have to be considered along with their ecology and the range of salinities and temperatures which they normally tolerate.

The three species, Ligia oceanica, Littorina saxatilis and L. littorea, are found in the upper intertidal regions, exposed to the vagaries of the environment, on the one hand possible desiccation, on the other dilution from rain, streams and rivulets. In addition, they penetrate into estuaries and populate pools of brackish water (Bassindale and Barrett, 1957; Naylor and Slinn, 1958).

Ligia oceanica is commonly found at or above high tide level and has been reported up to 450 feet above the high water mark (Darling, 1947). The gills are, however, kept moist by dipping the uropods into water, a procedure also observed by Barnes (1932) in L. baudiniana. L. oceanica (Naylor and Slinn, 1958) occurs in pools where the surface salinity may

become as low as 1.6°/oo (5% sea water). Littorina saxatilis, also found in the upper intertidal zone (Chumley, 1918; Bakker, 1959), and up to seven feet above high water (Evans, 1947), has been known to start feeding during heavy rain (Barkman, 1955). L. littorea, although sometimes inhabiting the subtidal zone, occurs on exposed rocks in the upper intertidal regions (Moore, 1940; Evans, 1947; Smith and Newell, 1955; Newell, 1958) and has been recorded as far as 400 feet above sea level (Waterston and Taylor, 1906), thereby indicating a wide range of adapability. It lives in estuaries with a low tide salinity of $\Delta_e 0.40$, less than 25% sea water (Topping and Fuller, 1942).

Idotea granulosa and Littorina littoralis are among the intertidal fauna of the seaweed zone, characteristic of the mid or lower tidal stretches. Idotea granulosa is usually found clinging to Ascophyllum sp. or Fucus sp. (Naylor, 1955) and Littorina littoralis lives mainly on Fucus (Barkman, 1955). While Idotea granulosa does not penetrate into estuaries or brackish water to any extent in Britain, it is recorded from the Gulf of Finland in salinities about 6°/oo, about 19% sea water (Segerstråle,

1947). In their intertidal habitat, both species are thus insulated from evaporation and dilution by the large salt and water reserves in the seaweed.

In the two isopods, the blood was more hyperosmotic in 50% sea water than in 100% sea water whereas the osmotic concentration of the Littorinidae dropped linearly with that of the medium in 100% to 50% sea water. In salinities below 50% sea water there was a further increase in the hyperosmotic state in the isopods while the Littorinidae retracted into their shell and the blood was also markedly hyperosmotic.

Of the Hydrobiidae, Hydrobia ulvae is a common inhabitant of mud flats in estuaries (Rees, 1940; Eales, 1961; Hunter and Hunter, 1962) and salt marsh pools (Nicol, 1935). These are habitats where, because of rain and tidal fluctuations, there are wide ranges of temperature and salinity; the temperature of salt marsh pools can on occasion be more than 25°C. H. ulvae was still active in 25% sea water with a urine slightly hyperosmotic relative to the medium but the animals withdrew into their shells in fresh water when the urine was markedly hyperosmotic.

Potamopyrgus jenkinsi is also found in estuaries or brackish water pools (Hunter and Warwick, 1957), and is one of the relatively few pectinibranch snails which have successfully penetrated fresh water. There are what appear to be early records of this shell under the name Rissoa castanea Jeffreys (Kennard and Woodward, 1899) and shells have been found in Suffolk deposits (Warwick, 1954) so it is probable that it did occur in Britain before being officially recorded from the Plumstead Marshes by Smith (1889). As it was first reported living inland by Daniel (1894) it was suggested that up to that time it had been confined to brackish water. P. jenkinsi is unique among the molluscs in being parthenogenetic (Robson, 1923), a factor favouring an initial rapid increase in numbers and the first fresh water report was followed within a short time by many others (Morris, 1894; Overton, 1894).

P. jenkinsi was the one experimental animal which survived in solutions from 100% sea water to fresh water. In fresh water the hyperosmotic condition of the urine relative to the medium did not greatly exceed that in saline solutions but there was

evidence of active osmoregulation as the urine was hypo-osmotic relative to the blood.

The experimental animals, therefore, fall into several ecological groups and this accords with their response in terms of osmotic balance to the test range of salinities and temperatures. The osmoregulation of Ligia oceanica, for example, is superior to that of Idotea granulosa, and similarly the hardier Littorinidae, Littorina littorea and L. saxatilis, are more tolerant of low salinities than the ecologically less adaptable L. littoralis. L. littorea, more ubiquitous than L. saxatilis, has the greater tolerance of variations of salinity. Hydrobia ulvae is active at even lower salinities than the Littorinidae and the related species Potamopyrgus jenkinsi can live in fresh water.

Isopoda

Osmoregulation in Ligia oceanica was investigated by Bateman (1933) when the animals were living on moist seaweed and he records for the blood a mean value of≡0.63 M NaCl (Δ_i2.15), that is an osmotic concentration higher than the concentration of normal

sea water. In concentrations above 100% sea water he found that the blood was isosomotic with the medium, and he reported erratic survival in dilutions down to 50% sea water, and in 100% sea water, only the smallest animals survived for the maximum period of eight days. Barnes (1940) found that the average survival time for the youngest (newly released) L. baudiniana was longer than that of adults, but never more than thirteen days in 100% sea water (Barnes, 1932). This relationship between size and survival could, however, be attributed to the higher ratio of surface area to volume in the smaller animals giving the optimum utilisation of the oxygen available in an insufficiently aerated medium. Parry (1953) found for example, that L. oceanica seldom survived for longer than seven days without aeration.

There was no evidence from the present work that size affected osmoregulation within the range of experimental salinities. In two size groups of Crangon vulgaris (Broekema, 1941) similarly showing no difference in the osmotic concentration of the blood, the smaller animals were more tolerant of the very low salinities as were the smaller Palaemon serratus

(Pannikkar, 1941), but again without a higher osmotic concentration than larger animals to account for the results.

Widmann (1935) gives the average values of the freezing point depression of the blood of L. oceanica as $\Delta_i 2.25$ in summer and $\Delta_i 2.36$ in winter. This agrees with the seasonal effect on osmotic concentration of the blood in 100% sea water in the present experiments for animals adapted to 5°C, $\Delta_i 2.21$ in summer and $\Delta_i 2.41$ in winter. The seasonal effect is also evident in Parry's (1953) figures of $\Delta_i 2.15$ for L. oceanica tested in April to June and of $\Delta_i 2.28$ for animals tested in October to December and kept at 18-20°C living on wet sand. Parry's results with summer animals adapted to 8°C over the salinity range 100% to 25% sea water have comparable freezing point depressions to those reported here for animals at 5°C. Parry's results obtained with temperatures of 18-20°C when the animals were living on wet sand gave a higher blood osmotic concentration than the present summer series from totally immersed animals at 15°C. The different experimental conditions probably account for

the discrepancy.

Widmann (1935) found a sex bias in the freezing point depressions of the blood of L. oceanica on the basis of differences of 0.10°C and 0.17°C between males and females; the females had the higher osmotic concentration. The present data show a maximum difference of 0.18°C between males and females in 25% sea water at 15°C but this difference was not statistically significant.

Considering the ecological range, the response of Idotea granulosa in respect of osmotic concentration of the blood and survival in the test range of salinities was to be expected, compared with the response of Ligia oceanica. Naylor (1955) reported a satisfactory survival of 80% of Idotea granulosa in solutions of 50% sea water but over 20% mortality after 48 hours in 25% sea water at temperatures of 10-14°C. This confirms the present findings of good tolerance in solutions of 50% sea water, and poor survival in 25% sea water. I. granulosa thriving in the Gulf of Finland (Segerstråle, 1947) in salinities about 19% sea water may be a physiological race, resembling in this respect Mesidotea entomon investigated by Lockwood and Croghan

(1957) who reported for this isopod a blood chloride concentration approximately that of 100% sea water, but the animals became increasingly hyperosmotic in solutions of lower salinity. The brackish water animals could not live in fresh water, but the fresh water animals could be adapted to 100% sea water. In 20% sea water, the natural habitat for the brackish water animals, the osmotic concentration of the blood was equivalent to 330 mM/l Cl (Δ_i1.12) compared to a mean of Δ_i0.90 for Idotea granulosa in 25% sea water, although in 50% sea water, the blood of I. granulosa was rather more hyperosmotic than that of Mesidotea entomon. In an early study of osmoregulation in M. entomon by Bogucki (1932), hyperosmotic regulation (Δ_i1.07) in a salinity of Δ_e0.41 was a similar finding. Thus the body fluids of both M. entomon and Idotea granulosa are approximately isosomotic in 100% sea water but show hyperosmotic regulation in lower salinities.

While other crustaceans regulate the osmotic concentration of the blood in the same way as I. granulosa, only Ligia oceanica has been shown to have marked hyperosmotic regulation in 100% sea water, about 119%

that of the medium. Carcinus maenas (Duval, 1925; Schlieper, 1929; Margaria, 1931) and Gammarus duebeni (Beadle and Cragg, 1940; Lockwood, 1961) have approximately isosomotic blood in 100% sea water but show hyperosmotic regulation in lower salinities similarly to Idotea granulosa. Callinectes sapidus which is hyperosmotic in an estuarine habitat, has a hypo-osmotic blood concentration in 100% sea water (Anderson and Prosser, 1953). Homarus americanus (Cole, 1940; Burger, 1957), Maia squinado and Portunus depurator (Margaria, 1931) have an osmotic concentration near the concentration of 100% sea water. Homarus americanus is able to maintain some hyperosmotic regulation, and Cole found it living in $\Delta_e 0.65$ (about 35% sea water) with blood $\Delta_i 0.94$. Garrey (1905), however, found that in the animals he used, death occurred within six hours in 50% sea water ($\Delta_e 1.02$) with blood $\Delta_i 1.32$. Maia squinado and Portunus depurator have no regulating ability, the osmotic concentration drops with the decreasing salinity, and the animals die in a few hours in 50% sea water.

In 25% sea water, Ligia oceanica and Idotea granulosa maintained mean blood concentrations equivalent

to Δ_i1.63 and Δ_i0.90, compared with Δ_i2.18 and Δ_i1.92 in 100% sea water respectively. To drop to those levels merely by passive dilution would require a water intake that would show as an increase in volume particularly in I. granulosa. In Ligia oceanica this did not happen in the animals kept in 25% sea water except after a week or more when oedema and loss of motility preceded death, indicating a breakdown in the regulation mechanism. In Idotea granulosa, similarly, there was no marked increase in volume until the animals became moribund. Similar results have been reported for other euryhaline animals. Bethe (1930) concluded that there must be an exchange of salt in Carcinus maenas in dilute sea water since there was no weight change, and Croghan (1958a) suggested the main changes in blood concentration of Artemia salina were due more to movements of sodium chloride than water.

Since the present experimental animals were not fed, hyperosmotic regulation presumes an active absorption of salts against the gradient of osmotic pressure, representing some form of metabolic work by the organism. The most likely site for active uptake is the pleopods and Parry (1953) suggested that

regulation may be possible in Ligia oceanica when only the uropods are in contact with the solution. Croghan (1958b) demonstrated localised staining of cells of the branchiae by silver salts in Artemia salina which may be interpreted as the area of ion exchange, and Krogh (1939) cites a number of experiments where similar cells have been found in insects.

Littorinidae

Although the osmotic balance over a range of salinities has been investigated in a number of bivalves, there are few similar data for the marine prosobranch gastropods, to compare with those given here for the Littorinidae. Littorina littorea, L. littoralis and L. saxatilis, had blood concentrations isosomotic or slightly hyperosmotic in immersion media of 100% to 50% sea water and considerably more hyperosmotic in 25% sea water.

In any mollusc with an external shell, it is always difficult to know to what extent the animal is in contact with the medium. For instance, in Mytilus edulis in distilled water (Maloeuf, 1937) and Venus mercenaria in fresh water (Garrey, 1905) there

is a large osmotic concentration differential between blood and medium when the valves are kept closed. In dilute sea water or fresh water, a steady osmotic state is attained very quickly if the valves are forceably kept open or if the animal normally feeds and respires in the test solution.

Conklin and Krogh (1938) found the blood of Mytilus edulis isosomotic when the animals were in 30°/oo to 10°/oo (100% to 30% sea water). In the low salinities, there was some delay in reaching an isosomotic blood concentration since the mussels kept the valves closed for several days. Potts (1952) gave the mean freezing point depression of M. edulis blood as Δ_i2.079 in sea water Δ_e2.077, and reported (1954a) the blood to be isosomotic to sea water over the range Δ_e2.09 to Δ_e0.58. Other molluscs with isosomotic concentration of the blood in sea water of Δ_e1.82 are Busycon canaliculatum, Venus mercenaria and Mya arenaria. Busycon canaliculatum tested in 50% sea water (Δ_e1.02) showed a decrease in osmotic concentration of the blood (Δ_i1.07) within 30 hours (Garrey, 1905). The Littorinidae, therefore, have the same kind of osmotic balance as other

euryhaline molluscs in immersion media of 100% to 50% sea water, and within 24 hours reach a steady state of blood concentration similar to the concentration of the medium. Other bivalves tested and giving comparable results were Venus mercenaria from a naturally brackish water habitat Δ_e1.336 with slightly hyperosmotic blood Δ_i1.386 (Cole, 1940), and Ostrea edulis, Cassis sulcosa and Mytilus edulis in Δ_e2.11 to Δ_e2.22 also slightly hyperosmotic, recorded by Monti (1914).

Scrobicularia plana (Freeman and Rigler, 1957) tested at 15°C, gave results similar to those obtained in the Littorinidae; after 48 hours in 100% to 50% sea water the osmotic concentration of the blood corresponded closely to the concentration of the media, but was significantly hyperosmotic after 60 hours in 30% sea water. The animals were not tested to confirm the maintenance of hyperosmotic condition after more prolonged immersion in this salinity.

Littorina littorea and L. saxatilis have a greater tolerance to 25% sea water than L. littoralis, and L. littorea has a better survival rate than L. saxatilis

despite the higher mean osmotic concentration of the blood of the latter in 25% sea water. There are a number of records of salinity survival experiments with similar results. In tolerance experiments in fresh water, L. littorea and L. saxatilis lived for seven days compared with two days for L. littoralis (Colgan, 1910). Gowanloch and Hayes (1926) recorded the survival rate of L. littorea and L. saxatilis in low salinities. Both species survived for over thirteen days in 15.0°/oo (less than 50% sea water), presumably at room temperature. In 11.25°/oo (35% sea water) all the specimens of L. saxatilis were dead by four and half days compared with twelve days for L. littorea, corresponding to the present observations on L. littorea in 35% sea water at 15°C. Manigault (1932) reported indefinite survival of L. littoralis at 17°C in salinities above a density of 1.015 (65% sea water). In a density of 1.007 (about 32% sea water) maximum survival period for L. littoralis was 28 hours compared with seven to eight days in the present experiments. The higher test temperature may be responsible in part for the poorer tolerance of low salinities, since in the present experiments there was a shorter survival

period in the low salinity with a higher temperature. MacMillan (1950) found that L. saxatilis died in 33% sea water at 14.5°C when gradually brought to that salinity over a period of 28 days, but the gradual change in salinity would influence the results. Nicol (1936) found L. saxatilis living in 10°/oo (32% sea water) at temperatures sometimes higher than 20°C, a greater tolerance of low salinities than in the present experiments where maximum survival time at 15°C in 50% sea water was nineteen days.

In Newell's (1958) experiments L. littorea ceased crawling at 8°C and he showed that this was due to a combination of low light intensity with low temperature. That light intensity may be more important than temperature seems possible from present observations on L. littorea kept in constant darkness at 5°C and 15°C when there was no obvious decrease in crawling activity of the group at the lower temperature.

Hydrobiidae

Within the viable salinity range, the Hydrobiidae adapt better in media at the higher temperature. For

example, summer Hydrobia ulvae in 25% sea water were active more quickly at 15°C than at 5°C, and similarly, Potamopyrgus jenkinsi in 50% and 75% sea water, depending on the previous environmental history, crawled at 15°C before movement started at 5°C. Outwith this viable range, which is determined by both temperature and salinity adaptation, survival is prolonged at the low temperature whether animals are transferred from a low to a high salinity or vice versa, although in media within the animal's adaptive capacity, a higher temperature is more conducive to immediate activity.

Hydrobia ulvae adapted better to low salinities at 15°C than to those at 5°C, and this was also observed by Ellis (1925) in H. ulvae from a salt marsh. Within the temperature range 12-18°C all were inactive in 25% sea water, but at temperatures 18-25°C, all were active for more than ten days in 20% sea water. Ellis's experimental animals showed less tolerance than the present salt marsh group which were active in 25% sea water at 15°C. In the same salinities the present estuarine animals, however, were not only active at a temperature of 15°C, but at 5°C

were displaying some movement on the eighth day, whereas the salt marsh animals at the low temperature did not crawl even after forty-three days in the solution. The distinctive reactions of the two groups imply basic physiological differences and MacMillan (1948) suggested that H. ulvae could, on the basis of variation in tolerance to low salinities, be regarded as consisting of different physiological races. MacMillan found that animals from a brackish ditch and an estuary were not active below about 25% sea water, but the salt marsh animals were active in a salinity as low as 7.5% sea water which indicates greater tolerance than either Ellis's (1925) or the present results, and substantiates the suggestion that physiological differences do occur. Johansen (1918) reported H. ulvae living in 3% sea water ($1^{o}/oo$) representing toleration of a salinity lower than that reported under laboratory conditions. Littorina saxatilis also lives in the Randers Fjord in 19% sea water ($5\text{-}6^{o}/oo$), a salinity lower than that tolerated in Britain, and in both species this may be connected with the lower temperature in the more northern region which could favour survival in a

lower salinity.

Other reports assign a much higher salinity threshold to Hydrobia ulvae. For example, Ellis (1932) found H. ulvae common in salt marsh pools in the Adur Estuary in salinities of 1.37% to 3.47% NaCl (43% to over 100% sea water), but when the channel changed and salinity dropped to 1.16% NaCl (35% sea water), H. ulvae did not survive (Ellis, 1931). Robson (1920) connected the presence of H. ulvae to Ulva sp. in tidal ditches rather than to salinity, and the lowest salinity in which Nicol (1936, 1938) found H. ulvae living in the Hebrides and Orkneys was 10°/oo (about 32% sea water).

The maximum survival time reported for H. ulvae in fresh water at 15°C, about fifteen days, is less than the eighteen days recorded by Colgan (1910) who does not however give the experimental temperature and states without explanation that his observation may be an underestimate.

Robson (1920) and Ellis (1925) have commented on the tendency of H. ulvae to crawl out of a container, a feature which Ellis attributed to the

influence of reflected light as it did not happen to the same extent in earthenware containers. It was noted in the present work that whereas the salt marsh animals sometimes crawled out of the solution in darkness and practically always with normal lighting, the estuarine animals never left the solution in darkness and seldom with normal lighting. The different behaviour patterns, pronounced phototaxis in one group, feeble response in the other, support the claim for the existence of distinct physiological races of H. ulvae.

The wide range of experimental tests, fresh water to 100% sea water, tolerated by Potamopyrgus jenkinsi reflects an ability to adapt to a broad spectrum of natural conditions and is apparent in the results from the present experiments showing how the change in the osmotic concentration of the urine closely followed that of the medium.

P. jenkinsi in the Randjers Fjord (Johansen, 1918) lives in 1°/oo to 20°/oo (3% to 65% sea water), it is abundant in the estuarines and salt marshes of the Moray Firth area (Richter, 1959), and is recorded in

the Solway Firth (Nicol, 1936; Boycott, 1938) although when collecting in the latter locality in 1961 for the present experiments only Hydrobia ulvae was identified. Potamopyrgus jenkinsi inhabits stretches of the River Leven where salinity fluctuations of from 0.05°/oo to 25°/oo (1.5% to 78% sea water) over 24 hours have been recorded (Yeoh, unpublished). Boycott (1936) reported that no difficulty was experienced in keeping P. jenkinsi alive in solutions ranging from fresh water to 100% sea water, and transference from 17°/oo (over 50% sea water) directly into fresh water was possible without ill effects (Robson, 1923), both of these results corresponding to the present findings.

Gresens (1928) reported that fresh water animals (Dendrocoelum lacteum, Glossiphonia complanata and Herpobdella atomaria) when transferred from fresh water to 25°/oo (78% sea water) survived two to three times longer at 5°C than at 15°C. This was also the case with Potamopyrgus jenkinsi transferred from fresh water into 75% and 100% sea water when survival was prolonged at the low temperature. The suggestion of Wikgren (1953) that the longer survival time at the lower temperature could be attributed to a decrease in tissue permeability was not supported by

the values obtained in the present work for the internal osmotic concentration which in P. jenkinsi were the same for animals at the two temperatures.

The pattern of osmoregulation in P. jenkinsi in fresh water resembles that which has been reported for wholly fresh water molluscs and for those that in addition inhabit brackish water.

In Randjers Fjord, Johansen (1918) found the prosobranch, Theodoxus fluviatilis, in salinities up to 14°/oo (43% sea water) although it also lives in fresh water. Tested in the range of solutions from fresh water to 75% sea water (Neumann, 1960), the results were similar to those for Potamopyrgus jenkinsi, a hyperosmotic concentration of the blood increasing in fresh water. Tested in saline solutions up to about 75% sea water, Theodoxus fluviatilis again showed results similar to these obtained for Potamopyrgus jenkinsi. Animals from brackish water were more tolerant than those from fresh water. Moreover, in agreement with the experiments with P. jenkinsi, there was better survival in solutions at 8°C than in solutions at 20°C. The freezing point depression

of the blood of Theodoxus fluviatilis in fresh water at 20°C was given as $\Delta_i 0.15$, of the same order as the mean value obtained for the blood of Potamopyrgus jenkinsi, $\Delta_i 0.18$, at 15°C.

The freezing point depression of the blood of fresh water pulmonate gastropods has been reported as $\Delta_i 0.17$ for Viviparus viviparus (Monti, 1914), $\Delta_i 0.21$ for Viviparus fasciatus (Obuchowicz, 1958) and 0.43% NaCl ($\Delta_i 0.26$) for Lymnaea peregra (Picken, 1937). Of these, only L. peregra has been shown to have a urine hypo-osmotic relative to the blood (Picken, 1937).

The osmotic concentration of the blood of the gastropods in fresh water is generally higher than that of the lamellibranchs, which may in part be connected with greater activity in the gastropods. Only Hydridella australis with blood $\Delta_i 0.16$ (46mM NaCl in Hiscock, 1953a) comes within the gastropod range of values. Rotthauwe (1958) reported for Dreissensia polymorpha blood $\Delta_i 0.09$. The latest determination of the osmotic concentration of the blood of Anodonta cygnea by Potts (1954b) gives a value of $\Delta_i 0.078$ for the blood which is slightly higher than Picken's value (1937) of 0.10°/o NaCl

(Δ_i0.06) but corresponds to Koch's (1917) and Florkin's (1938) estimation of Δ_i0.09. Picken demonstrated that A. cygnea excretes a urine hypo-osmotic relative to the blood. Potts (1954c) points out that with the penetration into fresh water there is an initial selective advantage in the reduction of the osmotic concentration of the body fluids and from calculations of the thermodynamic work involved in the maintenance of a water and salt balance has shown that this together with the excretion of a hypo-osmotic urine greatly reduces the load of osmotic work, These physiological conditions have been fulfilled by Potamopyrgus jenkinsi in fresh water.

P. jenkinsi shares the almost unique ability to adapt to a range of solutions from fresh water to 100% sea water with but a few other animals such as the crab, Eriocheir sinensis (Scholles, 1933) which excretes an isosomotic urine but reduces its osmotic work by tolerance of a lower blood concentration (Potts, 1954c) and Gammarus duebeni (Lockwood, 1961) which excretes a hypo-osmotic urine in fresh water.

Both Potamopyrgus jenkinsi and Hydrobia ulvae tolerated wide variations of internal osmotic

concentration and maintained a hyperosmotic concentration in brackish water. Hyperosmotic regulation even at higher salinities is a useful adaptation for survival at very low salinities and it is possible that organic as well as inorganic constituents contribute to the hyperosmotic condition. Since Potamopyrgus jenkinsi has a concentration above that of the medium when placed in saline solutions from fresh water, this suggests that it is not a straightforward establishment of ion equilibrium with the medium.

If we assume that P. jenkinsi at one time inhabited only brackish water, a further decline in the salinity of the medium down to fresh water would require only a minor adjustment of existing adaptations, since the need for an increased tolerance of diminished total osmotic concentration of body fluids is kept to a minimum by the relative increase in the hyperosmotic concentration. P. jenkinsi only inhabits hard waters, that is those with a fairly high calcium content (Nicol, 1936; Hunter, 1953) which decreases permeability and so possibly the amount of work necessary to maintain an ion concentration against the gradient. Certainly in the present experiments, when P. jenkinsi was tested in water with a very high calcium content

(Cambridge tap water) the osmotic concentration of the urine was significantly higher than that of animals tested in normal soft water, although no significant difference could be detected in the actual freezing point depressions of both hard and soft water.

It should be noted that the two operculate snails normally found in both fresh and brackish water, Theodoxus fluviatilis (Neumann, 1960) and Potamopyrgus jenkinsi, maintain a hyperosmotic blood concentration over the tolerated range of salinities. Fresh water molluscs, such as Viviparus fasciatus (Obuchowicz, 1958), Dreissensia polymorpha (Rotthauwe, 1958) and Anodonta cygnea (Florkin, 1938; Potts, 1954b) tested in brackish water are isosomotic.

Both Hydrobia ulvae and Potamopyrgus jenkinsi appear to have within the species, physiological races with distinctive qualities. In Hydrobia ulvae these are differentiated by variation in the tolerance to low salinities whereas the fresh and brackish water Potamopyrgus jenkinsi, type A, showed significant differences in the osmotic concentration of the urine tested in fresh water. Types B and C in fresh water

excrete a urine of the same osmotic concentration, but type C has a lower urine concentration than that of type A from fresh water.

In the salinity range in which they are active, the gastropods reached a new steady state of osmotic concentration within 24 hours after transfer from a higher to a lower salinity without accompanying marked change in volume. This suggests that in the Littorinidae and Hydrobia ulvae the lowered osmotic concentration involves a salt loss. Bethe (1930) showed that in sea water down to 50% there was a ready exchange of water and salts through the body wall of Aplysia limacina and although Weel (1957) at first questioned the validity of this work on the grounds of damage to the animals in the test solution, he (Weel) obtained similar results with a test solution of 95% sea water and so confirmed Bethe's conclusions. Since the body surface of the gastropods is readily permeable and appears to provide little barrier to rapid exchange with the external medium, the maintenance of a hyperosmotic condition in 25% sea water by the Littorinidae and in fresh water by Hydrobia ulvae implies work against an osmotic pressure gradient.

Similarly, Potamopyrgus jenkinsi transferred from fresh water to 100% sea water increases the internal osmotic concentration without any perceptable shrinkage which could be expected if this change were due to water loss only. Presumably there is in addition uptake of salts, and animals in the new steady state are slightly hyperosmotic.

Withdrawal Response

Littorina littoralis and L. saxatilis survived in 50% sea water at 15°C for only a limited period (up to nineteen days), yet if they had reacted by withdrawing as they did in 25% sea water, they would have lived longer. A survival period of a week or more, however, provides an ample safety margin for salinities of 50% sea water which in normal intertidal conditions are likely to endure for a matter of hours only. On the other hand, in the absence of the withdrawal response to 25% sea water and the accompanying rapid drop in osmotic concentration of body fluids, the animals would die very quickly. The increasingly longer survival period of L. littorea at 15°C in decreasing salinities is evidence of the greater protective response evoked by the lower

salinities.

The withdrawal response occurred in the gastropods before the body fluids were diluted and could well be triggered off as in Jasus lalandii (Krijgsman and Krijgsman, 1954) by a peripheral salinity detector. Freeman and Rigler (1957) have suggested that the free edges of the mantle lobes in Scrobicularia plana may have a 'osmoreceptive function'. Another physiological response is associated with spontaneous activity of the pedal ganglia of two land gastropods, Agriolimax reticulus and Arion ater, which have been shown to increase when bathed in solutions less concentrated than the animals' blood and to be sensitive to change in concentration of body fluids at the rate of 1% in four minutes (Hughes and Kerkut, 1956; Kerkut and Taylor, 1956). In the gastropods studied here, a peripheral receptor could on the same principle be stimulated to increased activity in low salinities, and trigger the response of foot retraction. Arnold (1957) found that only the cephalic tentacles and mantle fringe in Patella vulgata contracted quickly when tested with fresh water which suggested that the salinity receptors were in those areas.

Osmotic Adaptations

Prosser and Brown (1961) group animals as osmoregulators and osmoconformers and in general the isopods are osmoregulators while the gastropods are osmoconformers. These are not necessarily, however, rigid categories and although for instance the osmotic concentration of the body fluids of the gastropods within their active range followed that of the medium, the blood or urine was often consistently hyperosmotic relative to the medium particularly in the Hydrobiidae. Potamopyrgus jenkinsi maintained a hyperosmotic concentration in fresh water by active osmoregulation by an amount slightly more hyperosmotic than when in saline solutions. The Hydrobiidae along with the Littorinidae therefore may be considered as osmoregulators in certain environmental and experimental conditions.

Animals survive changes in the salinity of their natural environment either because they are able to regulate their osmotic concentration or able to tolerate variations in their internal osmotic concentration which means in essence that the cells can continue an adequate exchange of ions and other material with the body fluids. In most euryhaline animals the

mechanism is some combination of the two since even strict osmoconformers have ion regulation (Robertson, 1939, 1949, 1953). Osmotic regulation is generally considered to favour successful ecological adaptation, but Potamopyrgus jenkinsi was outstanding in its tolerance of changes in internal osmotic concentration in response to a range of external media from fresh water (Δ_i 0.18) to 100% sea water (Δ_i 2.03). It could be argued that tolerance of a low osmotic concentration of body fluids along with some active regulation is a more useful adaptation than the relatively high internal concentration, with greater osmotic work, as found in the fresh water crustaceans such as Astacus fluviatilus (Herrmann, 1931) and Potamon niloticus (Shaw, 1959). Certainly Littorina littorea classified as an osmoconformer although it may in part regulate in low salinities, survives longer in 25% sea water solutions than the osmoregulators Idotea granulosa and Ligia oceanica,

In bivalves such as Mytilus edulis there is no penetration of dye when the shells are closed to form apparently a functional barrier between the animals and the unfavourable medium (Maloeuf, 1937) and the

accompanying decrease in respiration (Hiscock, 1953b) and slowing of the heartbeat (Koch, 1917) are further adaptations that aid survival. As shown, however, in the dye experiments, the Littorinidae, withdrawn in low salinities were not isolated from the medium but nevertheless maintained a hyperosmotic concentration.

Temperature

In the consideration of the properties of the experimental media of the present work, the relevant parameters are the ion composition and the temperature. Although either the temperature or the salinity of the medium singly can influence survival and osmotic concentration of the blood and urine, the organism's response to the immersion medium in certain of the experiments is to the combined effect of temperature and salinity which cannot however be expressed as a simple relation.

Osmotic pressure is defined as the excess pressure which must be applied to a solution to bring it into equilibrium with the pure solvent when they are separated by a perfectly semipermeable membrane (Dick, 1959), and in non-living systems is proportional to

the absolute temperature. A difference of 10°C as in the present experiments, would produce a 0.3% difference in the osmotic pressure of a solution measured in atmospheres. With the order of osmotic pressures in the present results, a 0.3% difference in terms of atmospheres would fall within limits of variation between individual animals and would therefore not be significant. Biological systems, however, depart from the ideal behaviour of non-living systems and the response to temperature reported here varied under the conditions of the experiment, with the type of animal, salinity of the medium, season, etc.

Temperature response in the form of a change in concentration of body fluids is now known to occur in a number of animals in a variety of conditions and accounts for differences in the estimations of ion and osmotic concentrations. Dakin (1908) for example, reported a higher osmotic concentration of the blood of Pleuronectes platessa (difference of Δ_i0.08) when measured in the field (Heligoland) and in the laboratory, probably due to the different temperatures in the natural and laboratory media.

Lockwood (1960) studied the effect of temperature on the fresh water isopod, Asellus aquaticus, and found an increase in sodium ion concentration of the blood with both a rise and a fall of temperature. The minimum sodium ion concentration occurred in the temperature range 8°C to 12°C, depending on the concentration of the external medium, from 57μM/l to 2.4mM/l NaCl. Lockwood also cites Heut's (1943) work on the same species and Gammarus duebeni which demonstrated an increase in blood osmotic concentration both above and below 4°C. Brooks and Brooks (1941) quote the experiments of Fauré-Fremiet (1924) on the effect of temperature on the eggs of the polychaete Sabellaria sp. There was a gradual decrease in egg volume starting from a low temperature up to the normal, 18-25°C, for the egg and over that range the volume remained constant to decrease again as the temperature was further raised. No explanation of this effect was given.

Bateman (1933) found no significant differences in Carcinus maenas blood concentration in 50% sea water after four days' adaptation to 2.3°C or 15.7°C, but the adaptation time may not have been long enough since Atwood (unpublished) found that the blood potassium,

after the crabs were transferred from 5°C to 17°C, required about nine days to reach a steady level. Rao and Ramachandra (1961) investigated the change in chloride, free amino acids and total osmotic concentration in the fresh water mussel, Lamellidens marginalis, at 26°C and 33°C. All three had increased values at the higher temperature, but the relatively smaller change in the total osmotic concentration they concluded was not due to chloride and amino acids. The fresh water crab, Paratelphusa sp., on the other hand, showed a decrease in the chloride, amino acids and total osmotic concentration at the higher temperature. Otto (1937) demonstrated a higher osmotic concentration of the blood in Eriocheir sinensis in fresh water at temperatures of 0-6°C compared with 24-25°C.

There are a number of explanations of the effect of temperature on osmoregulation. Lockwood (1960) suggested that the increase in blood sodium ion concentration in Asellus aquaticus with a fall in temperature might be due to the accumulation of metabolites in the cells followed by a shift of water from the blood. This would imply that the animals responded to a temperature of about 8°C by

some departure from normal metabolic processes and this is difficult to accept. Reigel (1959) compared the weights of sphaeromid isopods at temperatures of 5°C and 16°C in both low and high salinity solutions and reported a weight gain in the low salinities at the lower temperature, but a weight loss in the high salinities at the lower temperature. Along with the change in weight which he explained as an upset in metabolism there was also a shorter survival period at the low temperature.

Verwey (1957, 1958) interpreted the temperature effect as the animals' attempt to maintain a constant osmotic pressure measured in atmospheres by increasing osmotic concentration of body fluids at the lower temperature. His calculations certainly showed a reasonably constant osmotic pressure of the blood of Crangon vulgaris, based on Broekema's (1941) work, but could not explain the lower osmotic concentration of the blood at the low temperature in low salinity solutions. The reversal of the temperature effect on Crangon vulgaris and in Panaeus aztecus and P. duorarum (Williams, 1960) occurred within the natural range of temperature conditions

and suggests that any question of a breakdown of osmoregulation at the low temperature is unlikely.

Osmotic concentration of body fluids in the final analysis depends on the special biological property of cell membranes which is selective permeability, or the regulation of the kind of particle, ion and molecule, which can pass through the same membranes at any given time. Moreover, organisms are capable of osmotic work in the form of a differential uptake or secretion of ions and water against a pressure gradient. In general low temperature decreases cell membrane permeability (Heilbrunn, 1952). In Asellus aquaticus, however, Lockwood (1960) found that sodium loss was unaffected by temperature, but he omitted the critical temperature range where the blood sodium ion concentration was at the lowest. In addition, his results are contrary to those of Wikgren (1953) who found an increasing relative loss of chloride as the temperature decreased in another fresh water crustacean, Astacus fluviatilis, and also in Lampetra fluviatilis and Cyprinus carassius.

It is recognised that there is a quite unexpectedly

large effect of small temperature differences in the active transport of water (Spanner, 1954) - it is said that a difference of 0.01°C can cause movement of water at the same rate as a pressure difference of almost one atmosphere and, this being so, it is probable the alteration in the temperature gradient between external medium and poikilothermic organisms will affect its membrane permeability.

The influence of temperature on osmotic concentration has been shown both by quantitative ion analysis and by determining total osmotic concentration which includes all solutes. When osmotic and ion concentration have been estimated at different seasons and temperatures, it has become apparent that the increase in the ion concentration is not sufficient to account for the increase in total osmotic concentration at, for example, the lower temperature. Scholander, van Dam, Kanwisher, Hammel and Gordon (1957) and Pearcy (1961) demonstrated that the greater osmotic concentration of the blood of teleosts in winter as compared to summer could not be explained as an increase in ion concentration represented by sodium chloride. Of the total osmotic concentration in summer about 80% was due to sodium chloride but only about 50-60% in winter,

and the remaining osmolabile substance or substances has not yet been identified.

Pearcy (1961) showed that the relative proportions of Na^+ and Cl^- differed in Pseudopleuronectes americanus seasonally. A similar fluctuation in ion ratios in teleosts in cold and warm waters was found by Eliassen and Leivestad (1961) and in Pachygrapsus crassipes (Atwood, 1960) adapted to 7°C and 22°C.

It is an obvious selective advantage for Arctic fish to increase their osmotic concentration of body fluids in winter and thereby diminish the possibility of freezing in surface water; deep summer fish at the same temperature as the winter fish but in no danger of freezing did not increase their osmotic concentration to the same extent (Scholander et al., 1957).

Temperature and Salinity

Only the interdependence of temperature and salinity can explain the reversal effect noted in Ligia oceanica and Idotea granulosa, namely a higher mean value for the osmotic concentration of the blood

in 100% sea water at 5°C, but a lower mean value at 5°C in 25% sea water. It can be assumed that the maintenance of a hyperosmotic state involves metabolic work, and the association of a higher osmotic concentration with the higher temperature in the medium which demands more osmotic work could be related to the initial increase in the metabolic rate (a factor of Q_{10} of 2 to 3 is generally given for a 10°C difference in temperature), and to an increase in cell permeability.

Crangon vulgaris (Broekema, 1941) tested at 4°C and 21°C also had the higher osmotic concentration of the blood at 4°C in 100% sea water and at 21°C in less than 65% sea water (21°/oo). Williams (1960) showed a like reversal effect of temperature in Panaeus aztecus and P. duorarum when results from the highest and lowest experimental temperatures (28.5°C and 8.8°C) are compared. Although the prawns in contrast to the isopods are hypo-osmotic regulators in 100% sea water, they show the same temperature effect on osmoregulation as the isopods studied here. On the other hand, Gammarus duebeni (Kinne, 1952) and Rithropanopaeus harrisi (Kinne and Rotthauwe, 1952)

had the higher value for the osmotic concentration of the blood in fresh water at the lower temperature and the reverse effect in 100% sea water. Otto (1934) previously had thought that the decrease in osmotic concentration of the blood of Rithropanopaeus harrisi in the laboratory was probably connected with a rise in room temperature.

Brooks and Brooks (1941) describe the experiments of Lucké and McCutcheon (1930) where there was an increase in the permeability of Arbacia sp. eggs to water in 50% sea water at 18°C, and it was suggested that the large value for the coefficient of permeability was the product of the combined action of temperature and 'hypotonicity'.

The data obtained so far in the Crustacea with regard to the nature of the temperature effect on osmotic concentration of body fluids can be connected with their range of ecological adaptation. For example, Gammarus duebeni, Eriocheir sinensis and Rithropanopaeus harrisi are physiologically adapted to fresh water, even though they also live in brackish water or 100% sea water, and have in common the higher osmotic concentration of the blood in fresh

water with a low temperature. Marine and brackish water Ligia oceanica, Idotea granulosa, Crangon vulgaris, Panaeus aztecus and P. duorarum, on the other hand, have a lower osmotic concentration of the blood in low salinities at the lower temperature and the reverse temperature effect in 100% sea water. This could be interpreted to mean that in the 'optimum' medium, the lower temperature is associated with the higher osmotic concentration of the blood. For example, a higher salinity associated with a lower temperature was optimum for survival of Crangon vulgaris (Broekema, 1941). Schlieper (1958) supports the idea of an optimum temperature as an adaptive parameter of osmotic concentration of the body fluids, and Kinne (1956) considers the same relationship to be important for adaptive radiation in tropical areas.

In the gastropods there is no ready explanation of the better survival of the Littorinidae in 25% sea water, of Hydrobia ulvae in fresh water and of Potamopyrgus jenkinsi in 50% to 100% sea water at the lower temperature (5°C). Temperature alone is not the significant factor as 5°C to 15°C is well within the seasonal range for the species and further, these

temperatures do not affect survival in more favourable salinities. An analysis of the composition of the blood at the high and low temperatures might provide useful information.

Although no satisfactory explanation can be offered here to account for the variety of response as between the different species to temperature and salinity conditions, the results justify the inference that there is a physiologically effective temperature-salinity interaction. The two important properties of the medium are therefore the salinity alone and the temperature-salinity combined.

Season

Temperature affects most metabolic processes and seasonal adaptations are generally associated with temperature differences. It is probable that there is a common cause for the temperature effect on osmotic concentration in either summer or winter animals. There are no other data available for comparison with the results obtained in these experiments relating season to osmoregulation over a range of salinities in both summer and winter animals after adaptation to the same temperature.

Kinne (1952) tested winter Rithropanopaeus harrisi in fresh water after adaptation to 20°C, the summer temperature, and found no difference in osmotic concentration between the summer and winter animals. This result, however, could be expected since adaptation to a higher temperature generally proceeds more rapidly than adaptation to a lower temperature (Broekema, 1941; Brett, 1944, 1946), and, for example, summer and winter Idotea granulosa tested at 15°C showed no significant difference in mean blood freezing point depressions. Again, the seasonal differences in Ligia oceanica were less with the experimental temperature at 15°C, that at 5°C. Widmann (1935) reported a seasonal change in the osmotic concentration of several isopods, amphipods and decapods. Blood osmotic concentration was reported higher in winter Palaemon serratus and Palaemonetes varians (Panikkar, 1940). According to Lockwood (1960), the concentration of the blood of Asellus aquaticus increased at the beginning of winter and later decreased to the summer value, which suggests that the animals regulate concentration within very narrow limits.

Seasonal differences in the physiological processes

of animals cannot always be causally related to temperature change. In the present experiments with Ligia oceanica and Idotea granulosa, the mean value for the osmotic concentration of the blood over the whole salinity range is higher in winter animals than summer animals after comparable laboratory adaptation. In addition, summer and winter Ligia oceanica had a different response to the interdependent effect of temperature and salinity: summer animals had a higher osmotic concentration of the blood at 15°C in 50% and 25% sea water, while the winter animals had the same osmotic concentration at 5°C and 15°C in 25% sea water. Complete seasonal adaptation might involve photoperiod as Hoar (1956) found that seasonal variations in temperature tolerance were 'photoperiodically controlled' in goldfish. Again, experiments on tolerance to high temperatures in Hemigrapsus nudus and H. oregonensis after adaptation to various temperature-salinity combinations (Todd and Dehnel, 1960) showed that summer and winter animals were physiologically different. Another seasonal effect independent of temperature is recorded by Woodhead and Woodhead (1959) who found that summer

Gadus morhua lived in water below 0°C without alteration in osmotic relationships, but that in winter there was an increase in the osmotic concentration at temperatures below 2°C.

SUMMARY

1. Osmotic balance was studied in Ligia oceanica, Idotea granulosa, Littorina littorea and L. saxatilis over the range of salinities from 100% to 25% sea water, and in Hydrobia ulvae and Potamopyrgus jenkinsi from 100% sea water to fresh water.

2. Ligia oceanica and Idotea granulosa showed hyper-osmotic regulation over the test range of salinites. In both animals, the mean osmotic concentration of the blood was higher in winter animals than in summer animals.

3. The osmotic concentration of the blood of the two isopods showed a response to the adaptation temperature; temperature and salinity had an interdependent effect.

In both isopods, there was a reversal of the temperature-salinity response, with the higher mean osmotic concentration of the blood in 100% to 50% sea water at 5°C, and in 25% sea water at 15°C.

4. Sex and size has no apparent effect on osmotic concentration of the blood.

5. The osmotic concentration of the blood of the gastropods, in the range in which they were active, followed the concentration of the medium, although both Hydrobia ulvae and Potamopyrgus jenkinsi were consistently slightly hyperosmotic relative to the medium.

6. Littorina littorea, L. littoralis and L. saxatilis were active in the salinity range 100% to 50% sea water, but withdrew into the shell in 25% sea water before the blood was appreciably diluted. L. littorea was tested from 50% to 25% sea water at 5% salinity intervals and the withdrawal response was triggered off in some animals in 45% sea water. The reaction of Hydrobia ulvae in the salinity range 100% to 25% sea water was conditioned by natural habitat, season and adaptation temperature, but they withdrew into the shell in fresh water.

7. The retracted gastropods had an osmotic concentration of the blood or urine which was significantly

hyperosmotic relative to the medium, including Littorina littorea with an abnormal operculum.

8. Dye experiments with Phenol Red indicated that the tissues of the Littorinidae were not shut off from the external medium when withdrawn at the low salinities, suggesting that the hyperosmotic condition in low salinities was not the result of isolation of the tissues. This was confirmed by the results of the experiments with L. littorea with the shell broken.

9. In the Littorinidae, the blood, fluid from the pericardial cavity and urine had the same osmotic concentration.

10. The influence of temperature on the osmotic concentration of the blood or urine of the gastropods was not consistent.

11. The survival time of marine or brackish water gastropods in low salinities was found to be function of a temperature-salinity interaction, with longer survival at the lower experimental temperature, 5°C.

12. There was no fixed threshold osmotic concentration of the blood or urine below which the gastropods did not survive.

13. Season, size and sex had no consistent effect on the osmotic concentration of the blood in the Littorinidae.

14. Potamopyrgus jenkinsi was the one experimental animal which could be adapted to solutions from fresh water to 100% sea water, and maintained osmotic balance in fresh water in part by the excretion of a urine hypo-osmotic relative to the blood.

15. In the Hydrobiidae, transferred directly from a low to a high salinity or vice versa, activity occurred more rapidly at 15°C than at 5°C, but the range of solutions in which they became active was determined by previous environmental adaptation.

16. Survival of Potamopyrgus jenkinsi in higher salinities, from either fresh or brackish water, was a function of a temperature-salinity interaction.

17. Estuarine and salt marsh Hydrobia ulvae differed in respect of tolerance of low salinities and this was more marked when adapted to a temperature of 5°C.

18. The three morphological types of Potamopyrgus jenkinsi, A, B and C, showed some physiological differences with respect to osmotic concentration of the urine. Type A from fresh and brackish water, taken directly from the environmental conditions, had different survival times in high salinities and different osmotic concentrations of the urine in fresh water.

19. Ligia oceanica showed stronger hyperosmotic regulation in 100% to 25% sea water than Idotea granulosa.

20. Of the Littorinidae, Littorina saxatilis had the highest mean osmotic concentration of the blood in 25% sea water, and L. littoralis the lowest. L. littorea, however, survived longer in 50% and 25% sea water than L. saxatilis or L. littoralis.

21. The results are discussed in relation to the information available about osmotic balance.

The present observations give information so far unrecorded about osmotic balance in the experimental animals.

There is no simple formula to express the relation of temperature or temperature and salinity to osmotic concentration or survival.

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Data for Analysis of Variance

LIGIA OCEANICA

Summer

Temp.	Sal.			
5°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	2.20	2.19	1.25	1.67
	1.98	2.33	1.14	1.79
	2.02	2.17	1.16	1.18
	2.24	2.06	1.36	1.48
	2.33	2.18	2.14	1.70
	2.21	2.32	1.86	1.62
	2.27	2.10	1.29	1.17
	2.28	2.11	1.37	1.49
	2.26	2.07	1.20	1.62
	2.26	2.18	1.37	1.08
	2.30	1.68	2.07	1.68
	2.20	2.22	1.54	1.08
	2.22	2.25	1.24	1.37
	2.26	1.90	1.01	1.73
	2.13	2.14	1.48	1.57
	2.24	1.96	1.13	1.26
	2.15	2.22	1.87	1.82
	2.09	1.91	2.42	1.58
Mean	2.20	2.11	1.49	1.49

LIGIA OCEANICA

Summer

Temp.	Sal.			
15°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	2.01	2.07	1.91	1.66
	2.02	2.02	1.79	0.97
	2.04	1.94	1.26	1.39
	2.02	1.97	1.83	1.77
	1.99	1.87	(1.78)*	1.40
	2.06	2.01	1.46	1.81
	2.05	2.13	1.61	1.65
	2.12	1.99	1.82	2.06
	1.92	1.97	1.56	1.42
	2.13	1.98	1.72	1.41
	2.02	1.93	1.79	1.85
	2.03	2.09	1.68	1.84
	2.04	2.08	1.87	1.16
	2.01	1.89	1.52	1.37
	1.96	2.00	1.81	1.28
	2.03	1.96	1.27	1.66
	2.02	1.99	1.75	1.57
	2.08	2.02	(1.52)	1.60
Mean	2.03	1.99	1.66	1.55

*Bracketed numbers were inserted by the 'missing value' technique. Snedecor (1956, p. 310) gives

$$= \frac{tT + bB - S}{(t-1)(b-1)}$$

where t = number of rows
b = number of columns
T = sum of observed measures in row containing missing value
B = sum of observed measures in column containing missing value
S = grand total of observed values

Data for Analysis of Variance

LIGIA OCEANICA

Winter

Temp.	Sal.			
5°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	2.59	2.35	2.10	1.38
	2.28	2.32	2.31	1.74
	2.25	2.27	2.03	1.86
	2.52	2.31	2.23	1.74
	2.28	2.30	2.29	2.16
	2.50	2.23	1.97	1.99
	2.53	2.28	2.13	1.76
	2.43	(2.34)	2.15	1.72
	2.42	2.35	2.34	2.07
	2.44	(2.33)	2.07	1.83
	2.38	2.33	2.41	1.38
	2.32	2.33	1.99	2.15
	(2.55)	2.31	2.12	2.01
	2.47	(2.30)	2.18	1.83
	2.26	2.33	2.27	1.25
	2.33	(2.30)	2.26	1.76
	2.52	2.42	2.26	2.21
	2.30	2.13	1.90	1.60
Mean	2.41	2.31	2.17	1.80

Data for Analysis of Variance

LIGIA OCEANICA

Winter

Temp.	Sal.			
15°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	2.21	2.07	2.03	1.83
	2.12	2.04	1.92	1.85
	2.25	1.99	1.93	1.45
	2.19	2.03	1.86	1.82
	2.02	2.04	1.96	1.88
	2.08	2.05	1.92	1.88
	2.03	2.02	1.82	1.72
	2.15	1.97	2.02	2.20
	2.06	2.05	1.88	1.60
	2.01	1.92	1.85	1.92
	1.96	2.02	1.79	2.03
	2.12	2.16	1.80	1.94
	2.13	1.98	1.98	1.38
	2.03	2.06	2.03	1.96
	2.08	1.92	1.67	1.11
	2.12	1.97	1.83	1.86
	2.08	2.08	1.58	1.16
	2.00	2.08	1.53	2.07
Mean	2.09	2.02	1.86	1.76

Data for Analysis of Variance

IDOTEA GRANULOSA

Summer

Temp.	Sal.			
5°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	1.99	1.60	1.28	0.83
	2.03	1.55	1.35	0.73
	2.01	1.43	1.15	0.63
	1.99	1.51	1.21	0.68
	2.01	1.59	1.20	(0.68)
	1.93	1.48	1.55	1.05
	1.92	1.54	1.33	0.97
	1.93	1.41	1.41	0.73
	1.94	1.54	(1.40)	0.67
	2.05	1.41	1.58	(0.83)
	2.03	1.50	1.35	(0.88)
	2.04	1.56	1.36	(0.82)
	1.97	1.54	1.38	0.67
	1.96	1.57	(1.25)	0.67
	2.04	1.52	1.33	(0.66)
Mean	1.99	1.52	1.34	0.77

Data for Analysis of Variance

IDOTEA GRANULOSA

Summer

Temp.	Sal.			
15°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	1.93	1.50	1.03	0.87
	1.91	1.59	1.29	0.82
	2.06	1.48	1.44	0.95
	2.01	1.43	1.42	0.78
	1.80	1.49	1.50	(0.83)
	1.93	1.52	1.01	0.87
	1.99	1.49	1.32	0.97
	1.96	1.47	1.23	0.77
	2.00	1.48	1.22	0.78
	1.98	1.44	1.39	(0.80)
	1.99	1.51	1.50	1.14
	2.10	1.43	1.26	(1.00)
	2.02	1.49	1.25	0.97
	1.92	1.51	1.33	(0.92)
	2.01	1.55	1.26	(0.94)
Mean	1.97	1.49	1.30	0.89

Data for Analysis of Variance

IDOTEA GRANULOSA

Winter

Temp.	Sal.			
5°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	1.95	1.55	1.41	0.94
	1.87	1.60	1.50	0.83
	1.85	1.60	1.37	1.02
	1.87	1.69	1.33	1.12
	1.87	1.70	(1.38)	1.14
	1.79	1.63	1.45	1.19
	1.86	1.65	1.54	1.03
	1.83	1.76	1.46	1.16
	1.96	1.81	1.42	0.84
	1.81	1.67	(1.44)	0.90
	1.84	1.64	1.36	0.87
	1.80	1.64	1.31	0.98
	1.82	1.75	1.46	0.52
	1.91	1.74	1.47	0.82
	1.86	1.42	(1.38)	0.84
Mean	1.86	1.66	1.42	0.95

Data for Analysis of Variance

IDOTEA GRANULOSA

Winter

Temp.	Sal.			
15°C	100%	75%	50%	25%
	Δ_i°C	Δ_i°C	Δ_i°C	Δ_i°C
	1.84	1.58	1.37	0.81
	1.90	1.47	1.34	1.11
	1.87	1.45	1.25	0.82
	1.81	1.60	1.25	0.92
	1.88	1.48	1.12	1.01
	1.87	1.54	1.29	0.98
	1.88	1.50	1.18	0.77
	1.86	1.57	1.36	1.26
	1.84	1.60	1.29	0.90
	1.87	1.65	1.11	1.27
	1.83	1.53	1.35	0.88
	1.86	1.56	1.12	1.16
	1.93	1.48	1.25	0.76
	1.81	1.59	1.41	1.10
	1.87	1.53	1.21	1.16
Mean	1.86	1.54	1.26	0.99

Calculations for Analysis of Variance

LIGIA OCEANICA, summer animals at 5°C:

Total sum of squares

$$= 2.20^2 + 1.98^2 + 2.02^2 + \text{to 72 terms} - \frac{(131.42)^2}{72}$$

$$= 252.2608 - 239.8780 = 12.3828$$

No. 'days in medium' sum of squares will be required if it is assumed that no difference is caused by varying the days of adaptation.

Salinity sum of squares

$$= \frac{(39.64)^2}{18} + \frac{(37.99)^2}{18} + \frac{(26.90)^2}{18} + \frac{(26.89)^2}{18} - \frac{(131.42)^2}{72}$$

$$= 247.8474 - 239.8780 = 7.9694$$

See Table IV for analysis of variance.

LIGIA OCEANICA, summer animals at 15°C:

Total sum of squares

$$= 2.01^2 + 2.02^2 + 2.04^2 + \text{to 72 terms} - \frac{(130.28)^2}{72}$$

$$= 240.8760 - 235.7344 = 5.1416$$

Salinity sum of squares

$$= \frac{(36.55)^2}{18} + \frac{(35.91)^2}{18} + \frac{(29.95)^2}{18} + \frac{(27.87)^2}{18} - \frac{(130.28)^2}{72}$$

$$= 238.8429 - 235.7344 = 3.1085$$

See Table IV for analysis of variance

Summer and winter animals combined:

Total sum of squares

$$= (240.8760 + 252.2608) - \frac{(261.70)^2}{144}$$

$$= 493.1368 - 475.6034 = 17.5334$$

Salinity sum of squares

$$= \frac{(36.55 + 39.64)^2}{36} + \frac{(35.91 + 37.99)^2}{36} + \frac{(29.95 + 26.90)^2}{36}$$

$$+ \frac{(27.87 + 26.89)^2}{36} - \frac{(261.70)^2}{144}$$

$$= 486.0196 - 475.6034 = 10.4162$$

Temperature sum of squares

$$= \frac{(131.42)^2}{72} + \frac{(130.28)^2}{72} - \frac{(271.60)^2}{144}$$

$$= 475.6124 - 475.6034 = 0.0090$$

First order of interaction table

Sal.	Temp. 5°C	Temp. 15°C	Totals
100%	39.64	36.55	76.19
75	37.99	35.91	73.90
50	26.90	29.95	56.85
25	26.89	27.87	54.76
Totals	131.42	130.28	26.170

Sal. x temp. + temp. x sal. sum of squares

$$= \frac{(39.64)^2}{18} + \text{to 8 terms} - \frac{(261.70)^2}{144}$$

$$= 486.6903 - 475.6034 = 11.0869$$

Hence temp. x sal. sum of squares

$$= 11.0869 - 0.0090 - 10.4162 = 0.6617$$

See Table V for analysis of variance

LIGIA OCEANICA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.82	5	2.20*	2.27	2.22
			6	1.98	2.28	2.26
			11	2.02	2.26	2.13
			12	2.24	2.26	2.24
			13	2.33	2.30	2.15
			14	2.21	2.20	2.09
			15	2.14	2.45	2.36
			17	2.17	2.13	—
15°C	100%	1.82	5	2.12	—	2.04
			6	2.01	2.05	2.04
			7	2.02	2.12	2.01
			11	2.04	1.92	1.96
			12	2.02	2.13	2.03
			13	1.99	2.02	2.02
			5	2.06	2.03	2.08
			6	1.98	2.01	2.00
			9	2.23	1.83	—

* Each value represents a different animal. Usually three animals (a, b and c) were sampled on any given day.

LIGIA OCEANICA

Summer

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	75%	1.39	5	2.19	2.10	2.25
			6	—	—	1.81
			9	2.33	2.11	1.90
			10	2.17	2.07	2.14
			11	2.06	2.18	1.96
			12	2.16	—	—
			5	2.18	1.68	2.22
			6	2.32	2.22	1.91
			8	2.25	2.31	2.02
			9	2.30	2.25	2.05
15°C	75%	1.41	5	2.07	2.13	2.08
			6	2.02	1.99	1.89
			9	1.94	1.97	2.00
			10	1.97	1.98	1.96
			11	1.87	1.93	1.99
			12	1.93	2.00	—
			5	2.01	2.09	2.02
			6	1.72	1.73	2.09
			8	2.03	1.82	1.97
			9	2.09	2.04	2.05

LIGIA OCEANICA

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	b	c
5°C	50‰	0.95	5	1.25	1.29	1.24
			7	1.23	—	—
			8	1.14	1.57	1.01
			9	1.31	—	1.04
			10	1.16	1.20	1.48
			11	1.36	1.37	1.13
			7	2.14	2.07	1.87
			8	1.86	1.54	2.42
			9	1.79	2.29	1.36

LIGIA OCEANICA

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	b	c
15°C	50%	0.99	5	1.91	1.61	1.87
			7	1.79	1.82	1.52
			8	1.26	1.56	1.81
			9	—	1.79	1.75
			10	1.83	1.72	1.27
			11	1.46	1.68	—
			12	1.84	—	—

LIGIA OCEANICA

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	b	c
5°C	25‰	0.50	5	1.67	1.17	1.37
			5	1.79	1.49	1.73
			6	1.18	1.62	1.57
			7	1.48	1.08	1.26
			8	1.70	1.68	1.82
			5	1.12	1.59	—
			7	1.62	1.08	1.58

LIGIA OCEANICA

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	b	c
15°C	25‰	0.53	5	1.66	1.65	1.16
			6	0.97	2.06	1.37
			7	1.39	1.42	1.28
			9	1.77	1.41	1.66
			12	1.40	1.85	1.57
			13	1.81	1.84	1.60
			5	1.56	1.33	1.32
			7	1.51	1.43	1.88
			8	—	2.05	1.14

LIGIA OCEANICA

Winter

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	100%	1.77	5	2.59	2.53	—
			7	2.28	2.43	2.47
			8	2.25	2.42	2.26
			11	2.52	2.44	2.33
			5	2.28	2.38	2.52
				2.42	—	—
			6	2.50	2.32	2.30
				2.62	—	—
15°C	100%	1.94	7	2.21	2.03	2.13
			9	2.12	2.15	2.03
			11	2.25	2.06	2.08
			14	2.14	2.01	2.12
			16	2.11	1.89	1.76
			17	2.03	2.04	—
			18	2.02	1.96	2.08
			19	2.08	2.12	2.00
			23	2.06	—	—
			24	2.16	2.17	2.07

LIGIA OCEANICA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.38	5	2.35	2.28	2.31
				2.32	—	—
			6	2.27	2.35	2.33
				2.31	—	—
			7	2.30	2.33	2.42
				2.23	2.33	2.13
15°C	75%	1.42	5	2.07	2.02	1.98
			8	2.04	1.97	2.06
			10	1.99	2.05	1.92
			11	2.03	1.92	1.97
			5	2.04	2.02	2.08
				2.11	—	—
			6	2.05	2.16	2.08
				2.15	—	—

LIGIA OCEANICA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.95	5	2.10	2.13	2.12
			8	2.31	2.15	2.18
			9	2.17	—	2.26
			10	2.03	2.34	2.27
			11	2.23	2.07	2.26
			12	2.29	2.41	2.26
			15	1.97	1.99	1.90
15°C	50%	0.99	5	2.03	1.82	1.98
			6	1.92	2.02	2.03
			7	1.93	1.88	1.67
			14	1.86	1.85	1.83
			15	1.96	1.79	1.58
			16	1.92	1.80	1.53
			18	1.54	1.29	1.36

LIGIA OCEANICA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25%	0.50	5	1.65	1.45	—
			8	1.38	1.76	2.01
			9	1.74	1.72	1.83
			10	2.16	1.38	2.21
			11	1.86	2.07	1.25
			12	1.74	1.83	1.76
			5	1.82	1.15	1.46
				1.90	—	—
			6	1.99	2.15	1.60
15°C	25%	0.48	8	1.83	1.72	1.38
			9	1.85	2.20	1.96
			10	1.45	1.60	1.11
			11	1.82	1.92	1.86
			12	1.97	1.88	—
			5	1.88	2.03	1.16
				1.78	—	—
			6	1.88	1.94	2.07
				1.83	—	—

IDOTEA GRANULOSA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.96	5	1.99	1.93	2.03
			7	2.03	1.92	2.04
			8	2.01	1.93	1.97
			9	1.99	1.94	1.96
			10	2.01	2.05	2.04
			11	2.00	1.98	—
			12	2.00	—	2.06
15°C	100%	1.96	5	1.97	—	1.99
			7	1.93	1.93	2.10
			8	1.91	1.99	2.02
			9	2.06	1.96	1.92
			10	2.01	2.00	2.01
			11	1.80	1.98	—
			12	1.94	1.93	—

IDOTEA GRANULOSA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.37	5	1.46	—	1.58
			6	1.55	—	1.60
			11	1.60	1.48	1.50
			13	1.58	1.47	—
			14	1.55	1.54	1.56
			15	1.43	1.41	1.54
			17	1.51	1.54	1.57
			18	1.59	1.41	1.52
				1.64	—	—
			19	1.44	1.50	1.49
15°C	75%	1.39	7	1.50	1.52	1.51
			12	1.59	1.49	1.43
			14	1.48	1.47	1.49
			15	—	1.39	1.56
			17	1.43	1.48	1.51
			18	1.49	1.44	1.55
				1.49	—	—
			19	1.42	—	—

IDOTEA GRANULOSA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.93	7	1.28	1.55	1.35
			9	1.35	1.33	1.36
			12	1.15	1.41	1.38
			13	1.21	—	—
			15	1.20	1.58	1.33
15°C	50%	0.97	5	1.37	1.01	—
			6	1.03	1.01	1.50
			8	1.03	1.22	—
			5	1.29	1.32	1.26
			6	1.44	1.23	1.25
			9	1.42	1.22	1.33
			12	1.47	—	—
			13	1.50	1.39	1.26
			15	—	1.44	1.63
			16	—	—	1.43

IDOTEA GRANULOSA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25%	0.49	2	0.83	1.05	—
			3	0.73	0.97	—
			5	0.63	0.73	0.67
			6	0.68	0.67	0.67
15°C	25%	0.48	2	0.87	1.14	0.87
			3	0.82	0.97	—
			5	0.95	0.77	0.97
			6	0.78	0.78	—

IDOTEA GRANULOSA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.81	6	1.95	1.79	1.68
			7	1.87	1.86	1.80
			8	1.85	1.83	1.82
			9	1.87	1.96	1.91
			12	1.87	1.81	1.86
			5	1.84	1.95	1.88
				2.02	—	—
15°C	100%	1.83	6	1.84	1.87	1.83
			7	1.90	1.88	1.86
			8	1.87	1.86	1.93
			9	1.81	1.84	1.81
			12	1.88	1.87	1.87
			5	1.94	1.92	1.87
				1.93	—	—

IDOTEA GRANULOSA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.38	11	1.55	1.63	1.64
			12	1.60	1.65	1.64
			15	1.60	1.76	1.75
			16	1.69	1.81	1.74
			6	1.70	1.67	1.42
			7	1.61	1.62	1.66
			8	1.43	1.57	1.57
			9	1.64	1.53	1.65
			11	1.62	1.65	1.54
15°C	75%	1.41	11	1.58	1.54	1.53
			12	1.47	1.50	1.56
			15	1.45	1.57	1.48
			6	1.60	1.60	1.59
			7	1.48	1.65	—
			8	1.50	1.50	1.53
			9	1.43	1.59	1.54
			11	1.50	1.61	—

IDOTEA GRANULOSA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.96	5	1.41	1.45	1.36
			6	1.50	1.54	1.31
			8	1.37	1.46	1.46
			9	1.33	1.42	1.47
15°C	50%	0.93	5	1.37	1.29	1.35
			6	1.34	1.18	1.12
			8	1.25	1.36	1.25
			9	1.25	1.29	1.41
			6	1.12	1.11	1.21

IDOTEA GRANULOSA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25%	0.47	5	0.94	1.19	0.87
			6	0.83	1.03	0.98
			6	—	0.52	—
			4	1.02	1.16	—
			5	1.12	0.84	0.82
				1.14	0.90	0.84
15°C	25%	0.47	5	0.81	0.98	0.88
			6	1.11	0.77	1.16
			8	0.82	1.26	0.76
			9	0.92	0.90	—
			4	1.01	1.27	—
			5	0.83	1.11	1.10
				1.09	1.16	—

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.81	1	1.79	1.76	1.76
			3	—	1.91	—
			4	1.81	1.82	1.80
			10	1.78	1.82	1.77
			12	1.94	1.93	1.81
			13	1.75	1.74	1.74
			14	1.84	1.82	1.83
			15	1.83	1.86	1.80
15°C	100%	1.97	2	1.95	1.89	—
			3	1.93	1.92	—
			4	1.91	1.94	—
			15	2.07	2.06	2.13
			21	2.00	2.08	2.07

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.40	3	1.45	1.50	1.42
			4	1.47	1.46	1.46
			5	1.38	1.36	1.43
			6	1.43	1.37	1.42
			8	1.46	1.41	1.44
			9	1.36	1.36	1.44
			10	1.30	1.36	1.35
			11	1.42	1.42	1.42
			12	1.25	1.37	1.34
15°C	75%	1.42	4	1.46	1.43	1.42
			5	1.43	1.43	1.41
			6	1.42	1.39	—
			7	1.42	1.40	1.42
			22	1.47	1.40	1.41
			35	1.53	1.55	—
			36	1.47	1.46	—
			40	1.48	1.52	1.47

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.93	1	1.00	1.06	0.84
			2	0.92	0.93	0.94
			3	0.93	0.92	0.90
			11	0.90	0.92	0.93
			12	0.92	0.89	0.91
			13	0.92	0.93	0.92
			14	0.95	0.93	0.98
			15	0.96	0.92	0.92
			17	0.93	0.94	0.94
15°C	50%	0.97	1	0.99	0.95	0.88
			3	0.97	—	1.00
			5	0.98	0.97	—
			6	1.01	0.98	0.95
			7	0.93	—	0.96

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	45‰	0.88	8	1.04	1.00	1.25
			14	0.89	—	0.96
			15	0.87	1.22	0.88
			16	0.88	1.26	0.89
			17	0.86	0.87	0.98
			18	0.88	0.91	1.06
			19	0.90	0.88	1.21
			21	0.98	0.84	0.93
15°C	45‰	0.84	1	1.14	1.08	1.00
			2	0.87	0.82	0.84
			3	0.90	0.86	0.83
			4	0.85	0.84	0.83
			5	0.83	0.82	0.82

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	40%	0.77	2	1.22	1.18	0.88
			4	0.82	1.07	0.79
			5	0.77	1.14	—
			6	0.95	0.89	1.32
			7	0.86	1.00	1.14
			8	0.84	0.90	0.78
			9	0.82	0.98	0.93
			11	0.81	1.09	0.85
			21	0.81	0.80	0.80
			22	0.85	0.78	1.05
			23	0.82	0.81	—
15°C	40%	0.78	3	1.03	—	0.90
			4	1.02	1.05	1.53
			5	0.91	1.09	0.91
			6	1.12	0.97	—
			8	1.11	—	1.26

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	35‰	0.68	1	1.02	1.15	1.60
			3	1.17	1.22	1.23
			5	0.84	1.11	1.41
			6	0.98	1.33	1.16
			7	1.18	1.18	0.82
			9	1.15	1.00	0.99
			12	1.10	0.76	1.14
			13	0.68	0.78	1.38
			15	1.02	—	0.96
			16	0.75	1.15	0.77
			19	0.63	0.65	1.06
			20	—	1.38	1.26
			21	0.77	1.40	—
			22	0.73	0.76	0.95
			31	0.84	1.10	0.72
15°C	35‰	0.69	1	1.14	1.19	1.13
			2	0.98	1.22	1.26
			3	1.16	0.97	0.76
			4	1.11	1.14	1.09
			5	0.96	0.64	0.68
			6	0.87	1.01	0.75
			7	0.82	0.86	0.78
			10	1.10	0.87	0.88
			11	0.70	0.70	0.97

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	30‰	0.60	2	1.42	1.45	1.48
			3	1.32	1.59	1.55
			4	1.39	0.92	1.41
			5	—	1.17	1.45
			7	0.91	1.12	1.38
			8	1.02	1.23	1.48
			15	0.75	1.46	0.71
			16	0.71	1.29	0.67
			17	1.38	1.24	—
15°C	30‰	0.60	1	1.27		
			2	1.05		
			3	0.76		
			4	0.83		
			5	0.75		
			2	0.83		
			3	1.17		
				0.97		
			5	0.73		
			6	0.90		
			7	1.35		
				0.96		
				0.68		

LITTORINA LITTOREA

Summer

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	25%	0.53	1	1.19	1.12	1.42
			3	1.32	1.23	1.31
			4	—	1.27	—
			5	1.32	1.17	1.44
			7	0.72	1.36	1.43
			8	0.78	0.62	—
			10	1.26	1.09	1.08
			11	0.62	0.62	1.36
			12	0.76	1.32	—
			13	1.12	0.94	1.15
15°C	25%	0.48	1	1.44	0.97	1.36
			2	1.38	1.37	1.36
			3	1.20	0.86	1.21
			4	—	1.50	1.23
			5	0.85	1.05	1.00
			6	0.63	0.90	1.37
			7	1.16	1.10	0.80

LITTORINA LITTOREA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	150%	2.78	1	2.73	2.82	2.76
5°C	125%	2.35	1	2.32	—	2.38
			2	2.31	2.38	2.34
			3	2.38	2.41	2.38
			4	2.47	2.47	2.41
			9	2.40	2.37	2.36
			10	2.43	2.46	2.38
			15	2.39	2.42	2.44
			1	—	—	2.35
			2	2.31	2.36	2.34
			4	2.29	2.26	2.25
			11	2.40	2.41	2.34
			12	2.38	2.49	2.28
			13	2.34	—	—
15°C	125%	2.41	1	2.37	2.43	2.42
			2	2.31	2.27	2.31
			3	2.43	2.43	2.40
			4	2.51	2.43	2.49
			9	2.42	2.28	2.43
			10	2.37	2.51	2.46
			15	2.50	2.49	2.52
20°C	125%	2.46	1	—	2.35	—
			2	2.43	2.40	2.43
			3	2.36	2.37	2.41
			11	2.62	2.64	2.62
			12	—	2.67	2.53
			13	2.69	—	—

LITTORINA LITTOREA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.86	2	1.86	1.87	1.88
				1.88	—	—
			3	1.87	1.92	1.76
				1.86	1.93	2.09
			2	1.66	1.94	—
			3	1.88	—	—
15°C	100%	1.85	11	1.89	1.97	—
			18	—	1.87	—
			2	1.83	—	—
			2	1.71	1.88	—
			3	1.87	1.93	—
			9	1.87	1.84	—

LITTORINA LITTOREA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.40	2	1.39	1.44	1.41
				1.44	—	—
			3	1.39	1.40	1.26
				1.37	1.49	1.42
			3	1.46	—	—
			4	1.41	1.46	—
15°C	75%	1.40	2	—	1.28	—
			5	1.39	—	—
			8	1.40	1.44	1.46
			10	1.25	1.22	1.46
			11	1.40	1.42	1.42
			3	1.45	—	—
			4	1.49	—	—
			6	1.49	—	—

LITTORINA LITTOREA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50‰	0.93	2	0.96	0.98	0.95
				0.98	—	—
			3	0.97	0.98	0.95
				0.89	0.95	—
			7	1.02	0.99	—
			4	0.96	0.98	—
15°C	50‰	1.00	1	1.11	1.04	1.08
			4	1.05	—	—
			5	1.12	1.02	1.10
			6	0.98	0.97	0.90
			7	1.00	1.01	—
			18	1.33	0.98	0.93
			2	0.93	0.93	—

LITTORINA LITTOREA

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25‰	0.49	2	1.18	1.43	1.39
				1.32	—	—
			3	1.18	1.06	1.24
				1.07	—	—
			7	1.24	1.18	1.15
15°C	25‰	0.48	2	1.26	1.17	0.98
				1.07	—	—
			3	0.94	1.12	1.11
				1.04	—	—
			7	1.25	1.11	0.96

LITTORINA LITTORALIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	100%	1.86	2	1.92	—
			3	1.89	1.89
			4	1.93	—
			2	1.94	—
			3	1.74	—
			4	1.80	—
			7	1.93	—
			8	1.90	—
			1	1.85	—
			2	1.91	1.89
			3	1.87	1.91
			5	1.81	1.83
15°C	100%	1.86	1	1.79	1.78
			2	1.79	1.97
			3	1.88	1.94
			6	1.75	1.83
			7	1.88	1.84
			1	1.92	—
			2	1.87	—
			4	1.90	—
			7	2.03	—
			8	1.85	—

LITTORINA LITTORALIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.43	8	1.41	1.38	1.42
			9	1.19	1.13	—
			21	1.47	1.50	1.51
			23	1.43	—	1.54
			41	1.54	—	—
15°C	75%	1.38	1	1.38	1.37	—
			2	1.33	1.43	—
			3	1.38	1.39	—
			6	1.37	1.42	—
			7	1.43	1.45	—
			8	1.35	1.38	—
			13	1.43	1.35	—

LITTORINA LITTORALIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.94	4	1.02	1.02	0.93
			6	0.96	0.90	0.97
			7	0.97	0.95	0.99
			8	—	0.95	0.98
			9	1.39	0.97	1.00
			2	0.87	0.88	0.97
			3	0.97	0.96	0.96
			4	0.94	—	0.93
			22	—	1.06	—
15°C	50%	0.95	1	0.93	0.93	—
			2	0.97	0.98	—
			3	0.90	1.00	—
			6	0.95	0.94	—
			7	0.93	0.93	—
			8	0.98	0.93	—

LITTORINA LITTORALIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25%	0.48	2	0.90	0.95	—
			4	0.53	0.85	1.11
			5	0.69	0.94	0.88
			6	0.97	0.62	—
			7	0.62	0.64	0.91
			8	—	0.72	0.57
			9	0.56	—	—
			1	1.47	1.46	—
			2	1.22	1.22	—
			3	1.28	0.78	—
			4	0.62	1.11	—
			7	0.99	0.69	—
			8	0.79	1.07	0.72
15°C	25%	0.47	1	1.59	1.43	—
			2	1.29	1.00	—
			3	1.28	1.38	—
			6	0.97	0.98	—
			1	1.26	1.48	—
			2	1.10	0.96	—
			3	0.77	1.02	—
			4	0.92	0.85	—
			7	0.82	0.98	—
			1	1.35	1.43	—
			2	0.70	—	—
			3	0.93	0.64	—
			6	1.11	1.04	—
			7	1.06	—	—

LITTORINA LITTORALIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.84	1	1.83	2.02	—
			1	1.94	2.01	1.97
				2.04	2.10	—
			2	—	—	1.96
			2	2.03	1.93	—
			3	2.10	—	—
			9	1.87	1.92	2.28
15°C	100%	1.79	1	1.82	1.79	1.85
			2	1.92	—	1.99
				1.82	1.82	—
			4	1.83	—	—
				1.90	2.06	—
			7	1.98	1.87	—
			8	1.87	—	—
			9	1.89	1.93	—
			2	1.88	1.95	—
			3	1.88	—	—

LITTORINA LITTORALIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.41	1	1.28	1.43	—
			2	—	1.43	1.51
			7	1.46	1.43	1.46
			6	1.47	1.43	—
			9	1.46	1.50	—
15°C	75%	1.37	2	1.30	1.33	—
				1.35	1.39	—
			4	1.43	1.46	—
				1.37	1.43	—
			7	1.33	1.43	—
			8	1.38	1.54	—
			9	1.43	1.44	—
			3	1.45	—	—
			4	1.33	—	—
			6	1.51	—	—

LITTORINA LITTORALIS

Winter

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	b	c
5°C	50%	0.93	2	1.00	0.97	1.03
			7	0.97	0.95	0.97
			2	0.98	0.95	—
			3	0.91	—	—
			6	0.93	0.87	—
15°C	50%	0.96	7	0.93	—	1.00
			4	0.87	0.99	—
				0.87	0.87	—
			7	0.97	—	—
			8	1.04	1.10	—
			9	1.10	1.00	—

LITTORINA LITTORALIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25‰	0.48	10	0.94	0.66	1.00
			13	0.95	0.67	0.74
			17	1.03	0.49	0.64
			1	1.64	1.95	—
			1	1.42	1.42	1.18
				1.26	1.47	1.58
			2	1.50	1.07	1.44
			6	0.80	1.29	—
			7	0.70	0.60	1.23
15°C	25‰	0.45	1	1.45	1.00	—
			2	0.96	1.18	1.02
				1.15	1.16	—
			4	1.14	1.42	—
				1.12	1.00	—
			7	0.95	1.22	—

LITTORINA SAXATILIS

Summer

Temp.	Sal.	Δ_e °C	Days in med.	Δ_i °C a	Δ_i °C b
5°C	100%	1.85	2	1.94	1.92
			3	1.90	1.87
			6	1.98	—
			7	1.88	—
			8	1.97	1.93
			13	2.06	2.19
15°C	100%	1.86	1	1.79	1.94
			2	1.88	1.92
			3	1.88	1.94
			4	1.75	1.94
			7	1.94	2.02

LITTORINA SAXATILIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b
5°C	75%	1.40	1	1.41	—
			2	1.44	1.42
			3	1.41	1.41
			6	1.42	1.35
			7	1.49	1.54
			8	1.51	1.48
			13	1.41	1.57
15°C	75%	1.34	1	1.37	1.37
			2	1.42	1.38
			3	1.38	1.39
			4	1.35	1.59
			7	1.51	1.53
			8	1.44	1.52

LITTORINA SAXATILIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.93	3	1.07	0.98	—
			6	0.93	0.92	—
			7	1.12	1.06	—
			8	0.98	0.99	—
			13	0.97	1.00	0.84
			22	1.07	—	—
15°C	50%	0.90	3	0.92	0.92	1.02
			4	1.00	0.98	0.98
			7	0.97	1.11	1.03
			8	1.19	0.90	1.12

LITTORINA SAXATILIS

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25%	0.48	1	1.84	1.44	—
			2	1.85	1.93	—
			3	1.60	1.89	—
			6	1.56	1.40	—
			7	1.44	1.50	—
			8	1.41	1.40	—
			13	1.31	1.33	1.08
15°C	25%	0.48	1	1.42	1.46	—
			2	1.73	1.83	—
			3	1.23	1.32	1.38
			4	1.56	1.08	1.57
			7	1.32	0.97	1.27
			8	1.19	—	—

LITTORINA SAXATILIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	100%	1.86	15	1.92	1.93
			16	1.89	1.88
			17	1.93	—
			19	1.91	1.93
			2	1.94	1.95
			3	1.92	—
			4	2.00	2.00
			6	1.91	1.91
15°C	100%	1.90	1	2.16	2.33
			2	—	2.09
			13	1.87	2.00
			14	1.99	2.15
			19	2.21	2.08
			20	2.08	2.40
			2	1.89	1.91
			3	1.92	—
			4	—	1.83
			6	1.87	1.92
			4	1.99	2.05
				2.04	2.11
			6	2.08	—

LITTORINA SAXATILIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.41	4	1.50	—	1.60
			5	1.42	1.48	1.54
			6	1.59	1.57	1.43
			8	1.50	1.26	—
			13	1.37	1.56	1.66
			3	1.47	—	—
15°C	75%	1.42	19	—	1.59	1.69
			20	1.76	—	—
			1	1.52	—	1.63
			2	1.38	1.39	1.42
			4	1.46	1.48	1.62
			3	1.45	—	—
			4	1.58	1.43	1.52
				1.63	1.65	1.57
			6	1.64	1.58	—
				1.58	1.72	1.65

LITTORINA SAXATILIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.93	4	0.93	0.92	1.13
			5	0.98	1.08	—
			6	0.97	0.94	0.97
			8	0.92	1.03	1.18
			10	—	0.97	1.06
			13	0.99	0.91	1.02
			2	0.99	0.99	—
			3	0.96	0.94	—
15°C	50%	0.94	2	0.97	—	0.95
			13	0.97	0.98	1.02
			19	1.04	1.13	1.14
			1	0.99	1.00	—
			2	1.14	1.11	—
			4	0.94	0.93	1.02

LITTORINA SAXATILIS

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25%	0.48	4	1.08	1.69	1.21
			5	1.22	1.13	—
			6	1.28	—	1.42
			7	1.20	1.18	1.37
			8	1.33	1.33	1.35
			10	—	0.99	1.04
			13	1.28	1.33	1.30
			17	1.05	1.17	—
15°C	25%	0.50	1	1.73	—	1.53
			2	1.38	1.54	1.62
			1	1.98	1.33	1.62
			2	0.93	0.92	1.36
			4	1.63	1.19	1.21
			7	1.15	1.37	1.12
			11	1.29	—	—
			12	1.00	—	—

HYDROBIA ULVAE

Estuarine Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	100%	1.88	1	—	—
			2	2.03	2.02
			5	1.83	—
			6	—	1.94
			8	2.00	1.88
			12	1.95	2.11
			15	1.89	—
			16	1.95	—
15°C	100%	1.95	1	1.84	1.86
			2	1.88	—
			5	1.97	2.09
			6	2.03	2.26
			8	1.92	2.02
			12	1.88	2.09
			14	2.22	2.08
			15	2.08	2.36
			16	2.05	2.10

HYDROBIA ULVAE

Estuarine Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	75%	1.41	1	1.53	1.41
			2	1.63	1.45
			5	1.45	1.46
			6	1.48	1.56
			8	1.28	1.36
			14	1.73	1.62
			15	1.55	1.44
			16	1.40	1.50
			27	1.46	1.48
15°C	75%	1.38	1	1.50	1.42
			2	1.71	1.40
			5	1.46	1.52
			6	1.57	1.61
			8	1.75	1.41
			12	1.44	1.39
			14	1.44	1.74
			15	1.43	1.53
			16	1.67	1.45

HYDROBIA ULVAE

Estuarine Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	50%	0.92	1	—	—
			2	0.97	1.03
			5	1.05	1.08
			6	1.23	1.06
			8	1.00	0.95
			12	1.37	1.12
			14	1.08	1.06
			15	0.93	1.12
			16	1.03	1.02
			27	1.02	1.06
				1.08	—
15°C	50%	0.96	1	0.98	0.99
			2	1.00	0.94
			5	0.93	1.02
			6	0.96	1.05
			8	0.98	0.94
			12	1.08	1.07
			14	1.14	1.06
			15	1.11	1.24
			16	1.39	1.04

HYDROBIA ULVAE

Estuarine Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	25‰	0.50	1	1.24	1.27
			2	1.33	1.34
			5	0.82	1.37
			6	1.28	1.06
			8	1.23	1.01
			12	0.58	0.62
			14	0.61	0.49
			15	1.18	0.70
			16	0.64	0.63
			27	0.62	—
15°C	25‰	0.51	1	—	—
			2	0.52	0.55
			5	0.54	0.50
			6	0.50	0.52
			8	0.50	0.54
			12	0.78	0.69
			14	0.62	0.57
			15	0.62	0.60
			16	0.52	0.62
			27	0.53	0.63

HYDROBIA ULVAE

Estuarine Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	b
5°C	F.W.	0.04	1	0.89	1.39
			2	1.21	1.29
			5	0.95	—
			6	1.21	1.25
			8	0.97	1.03
			12	1.07	0.99
			14	0.92	0.97
			15	1.31	1.08
			16	0.97	1.03
15°C	F.W.	0.04	1	1.33	—
			2	1.37	—
			5	1.38	1.29
			6	0.84	—
			8	0.92	1.13
			12	1.06	1.00
			14	0.59	—

HYDROBIA ULVAE

Estuarine Animals

Winter

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	100%	1.80	2	1.98	2.03
			5	2.13	1.99
			6	2.03	1.99
			20	—	1.95
			21	1.86	—
			22	1.88	1.85
			23	—	2.07
15°C	100%	1.78	1	1.99	1.93
			2	2.07	2.06
			3	2.00	2.04
				1.96	2.02
			5	1.87	1.99
				1.88	—
			8	1.87	—
			9	2.00	1.95
			10	1.88	1.96

HYDROBIA ULVAE

Estuarine Animals

Winter

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	75%	1.48	2	—	1.50
			5	1.44	1.43
			6	1.59	1.46
			20	1.71	1.66
			21	1.59	1.55
			22	1.63	1.80
			23	1.83	1.89
15°C	75%	1.36	1	1.48	1.47
			2	—	1.28
			3	1.63	1.42
				1.47	1.57
			5	1.33	1.47
				1.47	1.40
			8	1.60	1.36
			9	1.53	1.49
			10	—	1.47

HYDROBIA ULVAE

Estuarine Animals

Winter

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	50%	1.04	2	1.12	1.04
			6	0.98	1.17
			20	1.20	1.17
			21	1.15	1.17
			22	1.47	1.56
			23	1.19	1.37
15°C	50%	0.91	1	1.05	1.17
			2	1.01	1.11
			3	0.92	0.92
				0.94	1.03
			5	1.05	0.96
				1.07	0.97
			8	1.07	1.09
			9	1.08	1.18
			10	1.09	1.06

HYDROBIA ULVAE

Estuarine Animals

Winter

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	25%	0.49	2	0.52	—
			5	0.57	0.61
			6	—	0.58
			20	0.58	0.56
			21	0.71	0.66
			22	0.64	0.69
			23	0.74	0.69
15°C	25%	0.48	1	0.62	0.53
			2	0.63	0.77
			3	0.63	0.60
				0.54	0.57
			5	0.74	0.66
				0.52	0.57
			8	0.67	0.62
			9	0.66	0.68
			10	0.57	0.61
			9	0.67	0.60
			10	0.60	0.59
				0.60	—

HYDROBIA ULVAE

Estuarine Animals

Winter

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	F.W.	0.01	2	1.67	1.73
			3	1.37	1.56
			17	1.31	1.24
			18	1.17	1.36
			19	1.28	1.67
			20	1.32	1.22

HYDROBIA ULVAE

Salt Marsh Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	100%	1.97	1	1.96	2.03
			4	2.03	2.06
			8	—	—
			12	2.01	2.22
			13	2.22	2.21
			16	2.11	2.20
			18	2.26	2.10
			19	2.36	2.12
			20	2.02	2.03
15°C	100%	1.96	1	—	—
			4	2.00	2.14
			8	1.95	2.03
			12	2.04	2.00
			13	2.01	1.98
			16	2.00	2.12
			18	2.04	2.00
			19	2.06	2.16
			20	2.03	2.02

HYDROBIA ULVAE

Salt Marsh Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	75%	1.44	1	1.86	—
			4	—	—
			8	1.39	—
			12	1.77	1.63
			13	1.38	1.52
			16	1.43	1.46
			18	1.75	1.68
			19	1.52	—
			20	1.50	1.50
15°C	75%	1.42	1	1.45	—
			4	—	1.47
			8	1.42	1.50
			12	1.65	1.70
			13	1.34	1.43
			16	1.47	1.58
			18	1.42	1.46
			19	1.64	1.48
			20	1.51	1.46

HYDROBIA ULVAE

Salt Marsh Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	50%	0.96	1	1.14	1.12
			4	1.72	1.10
			8	0.99	1.07
			12	0.96	1.03
			13	1.02	1.20
			16	0.98	1.00
			18	1.16	1.17
			19	0.98	1.05
			20	0.97	1.06
15°C	50%	0.95	1	0.99	1.07
			4	1.05	0.86
			8	0.93	1.30
			12	1.01	0.97
			13	0.98	1.14
			16	1.08	1.08
			18	1.05	1.01
			19	0.99	1.24
			20	1.16	1.13

HYDROBIA ULVAE

Salt Marsh Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	Δ_i °C b
5°C	25%	0.49	1	1.71	1.65
			4	1.77	1.76
			8	1.55	—
			12	1.40	1.39
			13	1.94	1.42
			16	1.36	1.23
			18	1.36	1.31
			19	1.47	1.50
			20	1.17	1.52
			43	0.69	0.71
15°C	25%	0.46	1	—	—
			4	—	—
			8	0.77	0.49
			12	0.56	0.69
			13	0.63	0.54
			16	0.49	0.58
			18	0.55	0.70
			19	0.71	0.61
			20	0.57	0.61
				0.55	—

HYDROBIA ULVAE

Salt Marsh Animals

Summer

Temp.	Sal.	Δ_e °C	Days in Med.	Δ_i °C a	b
5°C	F.W.	0.03	1	1.78	1.63
			4	1.48	1.79
			8	1.18	1.74
			12	1.55	1.97
			13	0.60	1.07
			16	1.68	1.54
			18	0.90	1.54
			19	1.58	1.74
			20	1.54	1.62
15°C	F.W.	0.03	1	1.79	1.70
			4	1.73	1.85
			8	1.27	2.14
			12	1.23	1.51
			13	1.81	1.29
			16	1.71	1.61
			18	1.63	1.06
			19	1.77	1.49
			20	—	—

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	100%	1.86	1	1.71	1.84
			2	2.16	2.02
			5	—	—
			6	2.17	2.17
			7	2.08	—
			8	—	—
15°C	100%	1.86	1	1.93	1.88
			2	2.10	1.95
			5	—	—
			6	2.04	—
			7	—	—

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	75%	1.40	1	1.03	1.47
			2	1.66	1.50
			5	—	1.67
			6	1.61	1.55
			7	1.57	1.62
15°C	75%	1.39	1	1.44	1.38
			2	1.51	1.62
			5	1.53	1.52
			6	1.83	1.47

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	50%	0.95	1	0.75	1.05
			2	1.13	1.09
			5	1.15	1.12
			6	1.16	1.08
			7	1.09	1.00
			8	1.13	1.22
			9	1.10	1.19
			11	1.09	1.09
			12	1.21	—
			13	1.15	—
15°C	50%	0.94	2	1.02	1.00
			5	1.11	1.12
			6	—	1.13
			7	1.06	1.02
			8	1.11	1.03
			9	1.07	1.15
			11	1.37	1.04
			12	1.17	1.13
			13	1.20	1.32

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	25%	0.50	1	0.50	0.72
			2	0.57	0.71
			5	0.66	0.66
			6	0.60	0.59
			7	0.67	0.58
			8	0.60	0.53
			9	0.59	0.57
			11	0.51	0.58
			12	0.52	0.65
			13	0.58	0.72
			23	0.53	0.71
15°C	25%	0.50	1	0.50	0.52
			2	0.49	0.64
			5	0.43	0.62
			6	0.70	0.62
			7	0.54	0.62
			8	0.51	0.57
			9	0.56	0.56
			11	0.70	0.58
			12	0.55	0.57
			13	0.57	0.62
			23	0.82	0.64

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Summer

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	F.W.	0.03	1	—	0.10
			2	—	0.20
			5	0.32	0.29
			6	0.24	0.25
			7	0.22	0.25
			8	0.17	0.24
			9	0.18	0.17
			11	0.21	0.24
			12	0.22	0.19
			13	0.23	0.19
			23	0.18	0.20
15°C	F.W.	0.03	1	0.20	0.19
			2	0.23	0.23
			5	—	0.27
			6	—	0.26
			7	0.26	0.21
			8	0.21	0.22
			9	0.20	0.16
			11	0.21	0.23
			12	—	—
			13	0.27	0.21

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	50%	0.93	1	0.91	—
			2	0.98	1.28
			3	0.98	1.17
			17	1.06	1.11
			18	0.98	1.02
			19	0.93	1.00
			20	1.04	1.03
5°C	75%	1.33	1	1.38	1.44
			2	1.47	—
			3	1.58	1.44
5°C	100%	1.80	1	1.94	1.85
			2	2.17	2.14
			3	1.93	1.88

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	25%	0.48	1	0.56	0.53
			2	0.62	0.58
			3	0.54	0.60
			17	0.60	0.54
			18	0.75	0.62
			19	0.75	0.79
			20	0.63	0.67

POTAMOPYRGUS JENKINSI

Type A, Fresh water

Winter

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	Δ_i°C b
5°C	Loch W.	0.01	2	0.23	0.16
			3	0.26	0.18
			4	0.30	0.38
			18	0.29	0.37
			19	0.34	0.33
			20	0.28	0.29
			21	0.29	0.27

POTAMOPYRGUS JENKINSI

Type A, Brackish Water

Autumn

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	100%	1.81	1	1.84	1.83	—
			2	1.91	2.03	—
			7	1.99	2.01	2.13
			8	2.08	2.09	2.03
			9	2.05	2.12	2.13
15°C	100%	1.80	1	1.82	1.91	—
			2	2.02	2.17	—
			1	1.98*	1.88*	1.90*
				1.95*	2.19*	—
			28	—	—	2.46*
			10	1.91*	2.61*	—

* Active

POTAMOPYRGUS JENKINSI

Type A, Brackish Water

Autumn

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	75%	1.31	1	1.65	1.27	—
			2	1.39	1.48	—
			7	1.55	1.60	1.69
			8	1.42	1.52	1.50
			9	1.53	1.49	—
15°C	75%	1.32	1	1.45	1.34	—
			2	1.40	1.56	—
			7	1.40	1.53	1.64
			8	1.40	1.46	1.43
			9	1.47	1.49	1.66

POTAMOPYRGUS JENKINSI

Type A, Brackish Water

Autumn

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	50%	0.90	1	1.10	1.08	—
			2	1.02	0.96	—
			7	0.98	1.03	—
			8	1.03	1.01	1.06
			9	0.98	1.10	—
15°C	50%	0.89	1	0.96	1.00	—
			2	1.04	0.95	—
			7	1.02	0.95	1.08
			8	0.97	1.06	0.97
			9	0.92	0.94	1.07

POTAMOPYRGUS JENKINSI

Type A, Brackish Water

Autumn

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	25‰	0.46	1	0.53	0.57	—
			2	0.53	0.61	—
			7	0.57	0.56	0.58
			8	0.59	0.68	0.47
			9	0.57	0.54	—
15°C	25‰	0.45	1	0.54	0.53	—
			2	0.59	0.51	—
			7	0.51	0.57	0.54
			8	0.49	0.52	0.54
			9	0.54	0.53	0.53

POTAMOPYRGUS JENKINSI

Type A, Brackish Water

Autumn

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
5°C	F.W.	0.01	1	0.26	—	—
			2	0.23	0.16	—
			7	0.19	0.14	—
			8	0.21	—	—
			9	0.17	0.16	0.14
15°C	F.W.	0.01	1	0.17	0.19	—
			2	0.18	0.15	—
			7	—	0.12	—
			8	0.15	0.14	0.17
			9	0.17	0.22	0.19
15°C	C.T.W.	0.01	1	0.26	0.24	—
			2	0.24	0.25	—
			7	0.21	0.22	0.22
			8	0.24	0.27	0.23
			9	0.23	0.24	0.25
				0.22	—	—

POTAMOPYRGUS JENKINSI

Type B

Temp.	Sal.	Δ_e°C	Days in med.	a	Δ_i°C b	c
15°C	100%	1.82	12	1.94	—	—
			2	2.06	1.92	2.44
15°C	75%	1.39	21	1.41	1.58	—
			2	1.58	1.49	1.44
15°C	50%	0.92	21	0.92	0.95	1.08
			2	1.25	1.09	1.00
15°C	25%	0.46	21	0.52	0.61	0.53
			23	0.51	0.53	0.53
15°C	F.W.	0.02	21	0.21	0.15	0.20

POTAMOPYRGUS JENKINSI

Type C

Temp.	Sal.	Δ_e°C	Days in med.	Δ_i°C a	b	c
15°C	100%	1.79	26	1.95	2.16	—
15°C	75%	1.40	5	1.50	1.71	—
			6	1.40	1.55	—
			1	1.53	1.62	—
			21	1.42	—	—
			15	1.68	—	—
15°C	50%	0.94	5	0.99	0.96	—
			6	0.95	1.32	—
			1	0.98	0.98	—
			15	1.03	1.13	—
15°C	25%	0.47	5	0.49	0.48	—
			6	0.62	0.57	—
			7	0.54	—	—
			1	0.60	—	—
			15	0.61	0.68	—
15°C	5%	0.13	5	0.29	0.24	—
15°C	F.W.	0.01	5	0.13	0.14	—
			1	0.17	0.17	—
			15	0.20	0.20	—
			26	0.21	0.15	0.20