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OSMOTIC BALANCE IN *HYDROBIA ULVAE* AND *POTAMOPYRGUS JENKINSI* (GASTROPODA: HYDROBIIDAE)

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INTRODUCTION

Hydrobia ulvae (Pennant) is a common inhabitant of mud flats in estuaries (Rees, 1940) and salt-marsh pools (Nicol, 1935) where, because of rain and tidal fluctuations, there are wide ranges of temperature and salinity. *Potamopyrgus jenkinsi* (Smith, 1889, p. 142, as *Hydrobia*) is also found in estuaries or brackish-water pools and is one of the relatively few pectinibranch snails which has successfully penetrated fresh water (Hunter & Warwick, 1957). There are what appear to be early records of this shell under the name *Rissoa castanea* Jeffreys (Kennard & Woodward, 1899) and shells have been found in Suffolk deposits (Warwick, 1954), so it is possible 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.

MATERIAL AND METHODS

The osmotic concentration of the body fluids, as indicated by the freezing-point depression in °C. (Δ), was obtained over a range of solutions from 100% sea water to fresh water at different temperatures and at different seasons.

Animals collected from November to March were regarded as the winter type and from May to September as the summer type.

Hydrobia ulvae was collected in the Clyde river estuary near Ardoch, and specimens of the giant type (Rothschild, 1936) of the same species from a salt marsh at Tynningham, Scotland.

Potamopyrgus jenkinsi, originally described by Smith (1889), has been separated by Warwick (1952) into three morphological types, A, B, and C, and the osmotic balance of each type was tested. Samples of the A type from fresh water were obtained from Lochend Loch, and from brackish pools at Dunbar, Scotland, with a salinity about 17–19% sea water, but not in direct connexion with the sea. Type B (corresponding to Smith's type specimen) were collected from a brackish ditch at Aldeburgh, England, and the Pembrokeshire type C from fresh-water ditches at Bathesland, Wales. Types B and C were kept at laboratory temperatures in 5% sea water before the experiments were started, but the A type and *Hydrobia ulvae* were placed in the experimental media immediately.

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The experimental solutions ranged from 100% sea water to fresh water. The different concentrations were obtained by adding Glasgow tap water (total solids 21·3 mg.%, 1947 analysis, City of Glasgow) to fresh sea water (from Millport, Scotland). An average value for surface salinity at Millport is 32·1% (Barnes, 1955) and this is taken as the salinity of 100% sea water.

The fresh water used in the experiments came from either Lochend Loch (calcium content about 39·6 mg.%, Hunter, 1953), Loch Lomond (total solids about 45 mg.%, 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 solids about 252·8 mg.%, Weil & Pantin, 1931) was the experimental medium.

A high experimental temperature, 15°C., and a low experimental temperature, 5° C., were chosen in relation to the average seasonal temperatures of sea and fresh water.

During the period of experiments the animals were kept in plastic containers with a maximum of 35 animals per 3 l. of aerated water at a given temperature and salinity.

In fresh water *Hydrobia ulvae* remained retracted within the shell, and in some experiments the dye phenol red, 0·01%, was added to the water to determine how much exchange there was between the body fluid and the external medium under these circumstances. After a test period in the dye solution the shell was removed from the animal, and potassium hydroxide was added to bring up the red colour of the dye.

With experimental animals on average less than 5 mm. in length the small size of the heart made it difficult to collect blood as a routine, and the internal concentration was measured with samples of readily available urine. No marine invertebrate has been shown to excrete a urine which is not approximately isosmotic with the blood, and it is assumed that this applied to the Hydrobiidae. *Hydrobia ulvae* would be expected to have blood and urine isosmotic in fresh water as in saline water. In one group of *Potamopyrgus jenkinsi* in fresh water, blood and urine were collected from the same specimen with ultra-fine capillary tubes (outside diameter 0·1 mm.). The animals were submerged in medicinal paraffin while the samples (about 0·01 μl.) were being collected. The mean concentration of the urine was 83% that of the blood so that the values reported for urine in that species in fresh water may be considered to be 13% less than the value for blood. Silica-glass capillary tubes with an outside diameter of about 0·3 mm. were used to sample the urine, and the determination of the freezing-point depression was made on a volume of about 0·04 μl. The animals were kept in the experimental conditions for at least 24 hr. before samples were taken.

Samples of equal size were taken, enclosed in medicinal paraffin, and the loaded capillary tube was frozen immediately by placing it on solid carbon dioxide (−78° C.).

The osmotic concentration of the sample was determined by the method first described by Jones (1941) and later modified by Gross (1954). The procedure has been described in detail elsewhere (Todd, 1962).

Repeated determinations of the freezing-point depression of a standard solution showed the error to be within about 2%. The formula $\Delta/0{\cdot}6$ = % NaCl (Ramsay, 1949) gives a fairly accurate estimation of the sodium chloride concentration. Δ_i refers to the internal medium or body fluid and Δ_e to the value for the external medium.

The results from any two groups were analysed by 'Student's' *t*-test for random

samples for groups with unequal numbers (see, for example, Snedecor, 1956, p. 91). The test of significance for paired samples using the *t*-test was employed for blood and urine samples from the same specimen of *Potamopyrgus jenkinsi* (see Snedecor, 1956, p. 49).

RESULTS

Hydrobia ulvae: *estuarine animals*

162 summer animals and 133 winter animals were tested and the results in the different experimental salinities are given in Figs. 1 and 2. The number of animals tested in each group with mean values, standard deviations, and standard errors of the mean are given in Table 1.

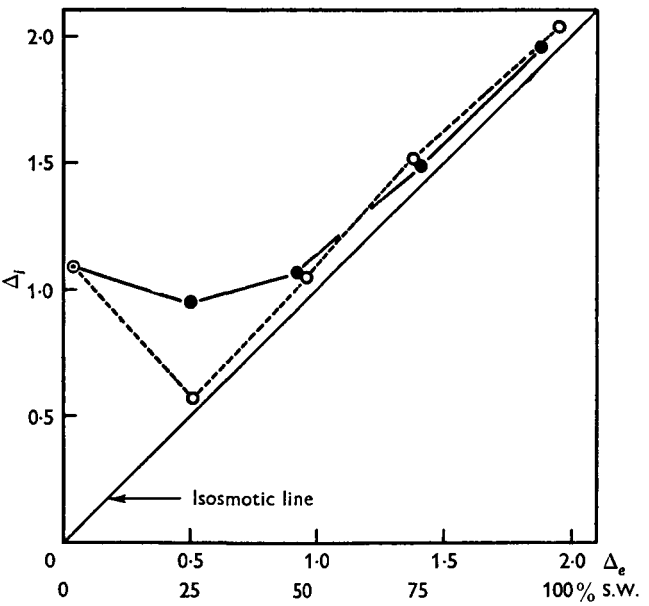

Fig. 1. The relation of the osmotic concentration of the urine of *Hydrobia ulvae*, estuarine animals, to the concentration of the medium. Summer animals: 5° C., --●--; 15° C., --○--.

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 (t = 8·83–17·70, P < 0·001). This species was active except in fresh water. Summer animals in 25% sea water at 5° C. were withdrawn for 8 days (Δ_i 1·19). After 9 days, however, the value (Δ_i 0·67) approximated to that of animals at 15° C. (All results from 1 to 27 days were used to calculate the mean.)

In fresh water the retracted summer animals had similar mean values at 5 and at 15° C., but 75% more survived after 14 days at the lower temperature. Osmotic concentration of the urine of winter animals in fresh water at 5° C. was significantly higher than that of summer animals at 5° C. (t = 4·91, P < 0·001) and at 15° C. (t = 3·30, P < 0·005). In 50 and 100% sea water neither temperature nor season affected the osmotic concentration of the urine.

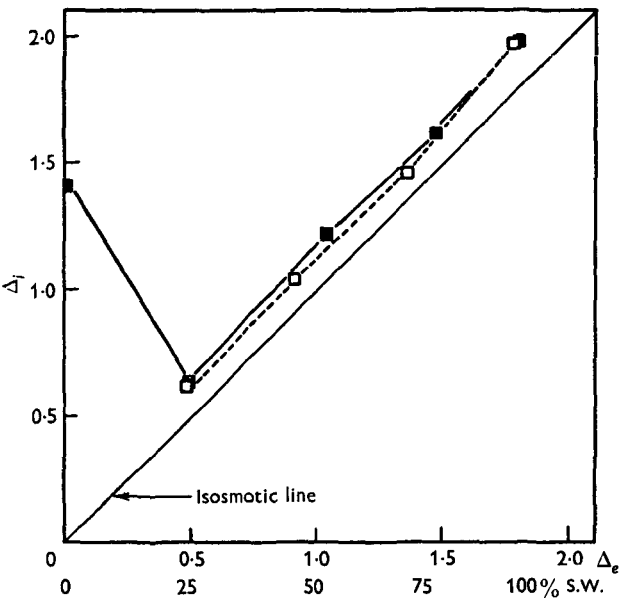

Fig. 2. The relation of the osmotic concentration of the urine of *Hydrobia ulvae*, estuarine animals, to the concentration of the medium. Winter animals: 5° C., --■--; 15° C., --□--.

Table 1. Hydrobia ulvae, *estuarine animals*

Temperature (°C.)	Salinity (%)	Δ_e° C.	No.	Δ_i° C.	S.D.	S.E.
			Summer			
5	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	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	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	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.

Hydrobia ulvae: *salt-marsh animals*

164 summer animals were tested and the results are given in Fig. 3 and Table 2. The urine of the animals tested at 5 and 15° C. was slightly hyperosmotic relative to the medium down to 25% sea water (except animals in 25% at 5° C.) and thereafter was markedly hyperosmotic (t = 11·88 and 16·16, P < 0·001). At 5° C. the animals were withdrawn in 50% sea water for 8 days and for 29 days in 25%. The salt-marsh

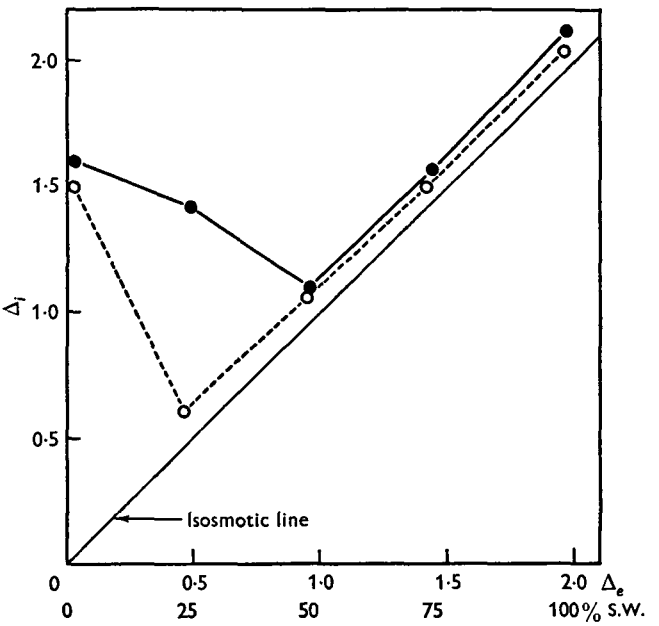

Fig. 3. The relation of the osmotic concentration of the urine of *Hydrobia ulvae*, salt-marsh animals, to the concentration of the medium. Summer animals: 5° C., --●--; 15° C., - -○--.

Table 2. Hydrobia ulvae, *salt-marsh animals*

		Summer				
Temperature (°C.)	Salinity (%)	Δ_e° C.	No.	Δ_i° C.	S.D.	S.E.
5	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	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

animals therefore took longer to adapt to a low salinity/low temperature combination than the estuarine group. The temperature of the pools from which the summer animals were collected was often over 25° C. and it is probable that the initial with-

drawal and inactivity at 5° C. was a temperature response pending adaptation to the low temperature, as it did not occur in winter estuarine animals.

While temperature did not cause a significant difference in internal concentration in fresh water, after 20 days there was 100% survival at 5° C. and complete mortality at 15° 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. ($t = 4{\cdot}48$, $P < 0{\cdot}001$) and at 15° C. ($t = 4{\cdot}66$, $P < 0{\cdot}001$). There are no significant differences due to temperature within the salinity range 100 to 50% sea water.

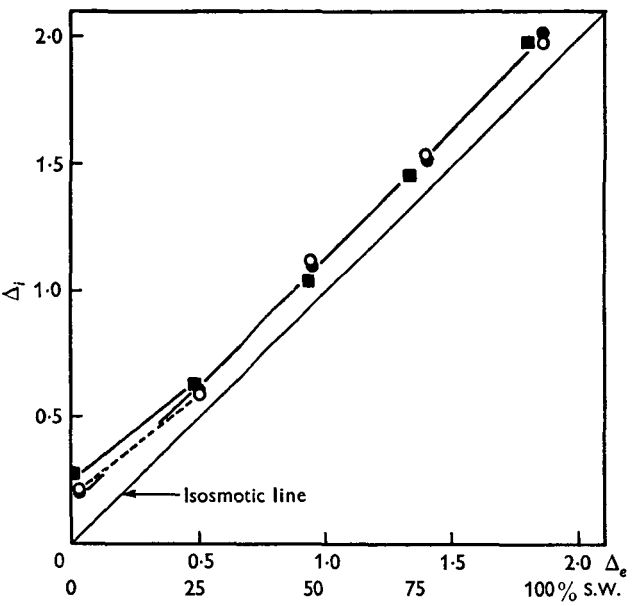

Fig. 4. The relation of the osmotic concentration of the urine of *Potamopyrgus jenkinsi*, type A, from fresh water, to the concentration of the medium. Summer animals: 5° C., --●--; 15° C., --○--. Winter animals: 5° C., --■--.

The experiments with 0·01% phenol red were carried out on estuarine *Hydrobia ulvae* in retracted animals in fresh water. Controls in phenol red in 100% sea water showed no abnormality. The dye penetrated into the tissues, mainly the kidney, within 48 hr. at both temperatures. Because of the small size of the animals, no quantitative estimate was attempted.

Potamopyrgus jenkinsi

All the types of *Potamopyrgus jenkinsi*, including those from fresh water, lived 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 to the test medium. Conditioning, however, had no effect on osmotic concentration of the urine, which reached a steady state within 24 hr. when the animals were transferred from a lower to a higher salinity.

Type A: fresh water

144 summer animals were tested at 5 and 15° C. and 52 winter animals at 5° C. (Fig. 4; Table 3) over the range from fresh water to 100% sea water. The urine was hyperosmotic relative to the medium over the whole range of salinities and also in

Table 3. Potamopyrgus jenkinsi *type A, fresh water*

Temperature (°C.)	Salinity (%)	Δ_e° C.	No.	Δ_i° C.	S.D.	S.E.
			Summer			
5	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	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	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

Table 4. Potamopyrgus jenkinsi *type A, brackish water*

Temperature (°C.)	Salinity (%)	Δ_e° C.	No.	Δ_i °C.	S.D.	S.E.
			Summer			
5	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	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.

fresh water (t = 9·76–32·30, $P < 0·001$). In 75 and 100% sea water, summer and winter animals died with 8 days. In 50% at 15° C. they lived indefinitely whereas at 5° C. summer animals all died in 19 days, in contrast to winter animals, which survived. In 25% sea water and fresh water all the animals were active immediately and lived indefinitely. There was a significantly higher osmotic concentration of the urine in winter animals than in summer animals in fresh water both at 5° C. (t = 3·69; $P < 0·001$) and at 15° C. (t = 3·37; $P < 0·005$).

Type A: brackish water

133 summer animals (Table 4) were tested in solutions ranging from fresh water to 100% sea water at 5 and 15° C. and in Cambridge tap water at 5° C. 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). In contrast to the fresh-water group, the brackish-water animals showed much greater tolerance in 75 and 100% sea water. At a temperature of 5° C. the animals were all

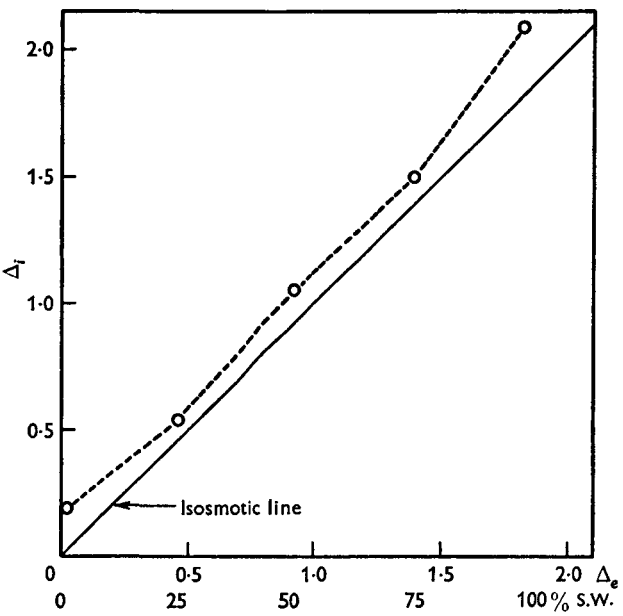

Fig. 5. The relation of the osmotic concentration of the urine of *Potamopyrgus jenkinsi*, type B, to the concentration of the medium. Summer animals: 15° C., --O--.

alive on the 10th day, whereas at 15° C. they had all died after 7 days. In a salinity of 75% sea water the animals lived indefinitely. There were no significant differences in osmotic concentration of the urine at different temperatures in any salinity, but the mean osmotic concentration in Cambridge tap water was always significantly higher than that of animals in Lochend Loch water (t = 2·17–7·86; P < 0·005 and 0·001).

Type B and type C

24 type B and 37 type C animals were tested in summer in solutions ranging from fresh water to 100% sea water at 15° C. (Fig. 5; Tables 5 and 6). They were immediately active and survived indefinitely in 75% and lower salinities. Samples from animals in 100% sea water were obtained from those transferred from 75% since they died within 5 days after direct transfer into 100% from 5% sea water.

The urine was significantly hyperosmotic in both types in fresh water (t = 4·54 and 10·20; P < 0·05 and 0·01) but was not significantly different in fresh-water as compared with brackish-water animals of type A over the salinity range. Type C

showed a significantly lower mean value than fresh-water type A at 15° C. when the two values were compared in animals from fresh water ($t = 3{\cdot}96$; $P < 0{\cdot}001$).

The freezing-point depression of both blood and urine of 8 brackish-water type A and 3 type B *P. jenkinsi*, in fresh water at 15° C., were tested using the ultra-fine capillary tubes. The mean values for 12 animals were Δ_i 0·18 for the blood and Δ_i 0·15 for the urine. The urine was hypo-osmotic, with a concentration 83% that of the blood ($t = 3{\cdot}35$; $P < 0{\cdot}01$), indicating that in fresh water *P. jenkinsi* maintains the osmotic balance in part by the excretion of a hypo-osmotic urine.

Table 5. Potamopyrgus jenkinsi *type B*

Summer

Temperature (°C.)	Salinity (%)	Δ_e° C.	No.	Δ_i° C.	S.D.	S.E.
15	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

Table 6. Potamopyrgus jenkinsi *type C*

Summer

Temperature (°C.)	Salinity (%)	Δ_e° C.	No.	Δ_i° C.	S.D.	S.E.
15	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

DISCUSSION

The experimental results have to be considered along with the range of salinity and temperature tolerated by *Hydrobia ulvae* and *Potamopyrgus jenkinsi*. Within the viable salinity range the Hydrobiidae adapted better to media at the higher temperature, but survival was prolonged at 5° C. outside the range. Ellis (1925) observed that *Hydrobia ulvae* from a salt marsh were all inactive in 25% sea water at temperatures of 12–18° C., but at temperatures of 18–25° C. were active in 20% sea water for more than 10 days. MacMillan (1948) reported that animals from a brackish ditch and from an estuary were not active in a salinity below 25% whereas salt-marsh animals were active in 7·5% sea water, and on the basis of her results she suggested that the two groups could be regarded as different biological races.

The change in the osmotic concentration of the urine of *Potamopyrgus jenkinsi* closely following that of the medium reflects an ability to adapt to a wide range of natural conditions. This species inhabits stretches of the River Leven, Scotland, where salinity fluctuations of from 1·5 to 78% sea water over 24 hr. have been recorded, and in the Randjers Fjord it survives variations from 3 to 65% sea water (Johansen, 1918). Boycott (1936) reported that no difficulty was experienced in keeping *P. jenkinsi* alive in solutions ranging from fresh water to 100% sea water.

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. For example, the freezing-point depression of the blood of *Theodoxus fluviatilis* in fresh water at 20° C. was given as Δ_i 0·15 (Neumann, 1960), of the same order as the mean value obtained for the blood of *Potamopyrgus jenkinsi*, Δ_i 0·18 at 15° C.

The freezing-point depression of the fresh-water pulmonate gastropods has been reported at Δ_i 0·17 for *Viviparus viviparus* (Monti, 1914), Δ_i 0·21 for *Viviparus fasciatus* (Obuchowicz, 1958) and 0·26 for *Lymnaea peregra* (Picken, 1937). Of these, only *L. peregra* has been shown to have a hypo-osmotic urine, with a concentration about 60% that of the blood (Picken, 1937), compared to 83% in *Potamopyrgus jenkinsi* in fresh water. This confirms with the relatively undifferentiated kidney in *P. jenkinsi*, similar to the urinary apparatus in *Hydrobia ulvae* and other marine gastropods, whereas fresh-water invertebrates, such as *Lymnaea peregra*, excreting a urine markedly hypo-osmotic relative to the blood, have evolved a tubular kidney.

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. The latest determination of the osmotic concentration of the blood of *Anodonta cygnea* by Potts (1954*a*) gives a value of Δ_i 0·08. Picken (1937) demonstrated that *A. cygnea* excretes a urine hypo-osmotic relative to the blood and, as Potts (1954*b*) points out, there is, with the penetration into fresh water, an initial selective advantage in the reduction of the osmotic concentration of the body fluids.

Both *P. jenkinsi* and *Hydrobia ulvae* tolerated wide variations of internal osmotic concentration and maintained a hyperosmotic concentration in brackish water. Hyperosmotic regulation even in higher salinities is a useful adaptation for survival in very low salinities, and it is possible that organic as well as inorganic constituents contribute to the hyperosmotic condition. Since *Potamopyrgus jenkinsi* maintains an hyperosmotic concentration when transferred from fresh water to saline solutions, this implies something more than the 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 to diminished total osmotic concentration of body fluids is kept to a minimum by the relative increase in hyperosmotic regulation. *P. jenkinsi* only inhabits hard waters, that is, those with a fairly high calcium content (Nicol, 1936), 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 softer water.

The results for *Hydrobia ulvae* were similar to those for other marine molluscs which have been tested. Garrey (1905) reported on several species, all of which were isosmotic in 100% sea water. *Busycon canaliculatum* tested in 50% sea water (Δ_e 1·02) showed a decrease in osmotic concentration of the blood (Δ_i 1·07) within 30 hr. *Scrobicularia plana* (Freeman & Rigler, 1957), tested at 15° C., gave results similar to those obtained in *Hydrobia ulvae* in 100–50% sea water, but was significantly hyperosmotic after 60 hr. in 30% sea water. *Littorina littorea*, *L. littoralis*, and *L. saxatilis*

(Todd, 1964) also showed a close correspondence of blood and media down to 50% sea water and a markedly hyperosmotic blood in 25%. Tests on *L. littorea* demonstrated that between 50 and 25% the degree to which the blood was hyperosmotic increased the lower the salinity.

Bethe (1930) showed that in sea water as low as 50% there was a ready exchange of water and salts through the body wall of *Aplysia limacina*, and this also occurs in *A. uliana* with a test solution of 95% sea water (Weel, 1957). Presumably a similar exchange of water and salts occurs in the Hydrobiidae.

The withdrawal of *Hydrobia ulvae* in fresh water so that the shell prevents osmotic swelling, thereby delaying dilution of the blood, could explain the maintenance of a hyperosmotic condition. There is no mechanical barrier to initial slight shrinking with a hyperosmotic external solution (e.g. transfer from 5 to 100%) with loss of water and subsequent inflow of what would be an isosmotic solution to maintain the normal body turgor.

The withdrawal response occurred in these gastropods and in the Littorinidae (Todd, 1964) before the condition of body fluids could have been appreciably affected, certainly before an emergency state was reached, and thus is probably triggered off by a peripheral salinity receptor. Arnold (1957) found that only the cephalic tentacles and mantle fringe of *Patella vulgata* contracted when tested with fresh water, implying that the salinity receptors were in those areas.

There is no ready explanation of the better survival at the lower temperature in adverse conditions in the two species. This has also been demonstrated in the Littorinidae (Todd, 1964). Unlike many crustaceans, (Broeckema, 1941; Kinne, 1952; Todd, 1963) the internal concentration in the gastropods is not directly correlated with temperature. Temperature alone is not the significant factor, as 5–15° C. is within the seasonal range for the species. 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 reported here. An alternative explanation is some kind of physiological temperature–salinity interaction, perhaps influenced by season.

SUMMARY

1. Osmotic balance was studied in *Hydrobia ulvae* and *Potamopyrgus jenkinsi* over the range 100% sea water to fresh water, by determining the freezing-point depression of the urine in the different solutions.

2. *Hydrobia ulvae* was slightly hyperosmotic from 100 to 50% sea water, and sometimes initially markedly hyperosmotic in 25% sea water at 5° C. The urine was always markedly hyperosmotic relative to fresh water, and the animals were withdrawn. Experiments with phenol red indicated that the tissues were not shut off from the medium.

3. *Potamopyrgus jenkinsi* was hyperosmotic from fresh water to 100% sea water. Osmotic balance in fresh water is maintained in part by the excretion of a urine hypo-osmotic relative to the blood.

4. There was some variation in the reaction of different ecological groups of *Hydrobia ulvae* and *Potamopyrgus jenkinsi* to the experimental conditions.

5. In the Hydrobiidae, whether transformed from a lower to a higher salinity or

vice versa, survival outside the viable range was longer at 5° C. than at 15° C., although, within the range, activity occurred more rapidly at 15° C.

6. In fresh water, winter animals of both species had a higher osmotic concentration of the urine than summer animals, but no differences in osmotic concentration correlated with temperature were demonstrated.

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