

OSMOREGULATION IN *LIGIA OCEANICA* AND *IDOTEA GRANULOSA*

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INTRODUCTION

The osmotic relationship between the body fluids of animals and the salinity of the external media is primarily based on their respective total concentrations. In conditions of changing salinity the response of the organism may be a parallel change in the osmotic concentration of its body fluids or some degree of active osmoregulation. Prosser & Brown (1961), who review the subject of osmotic balance, accordingly group animals as osmoconformers and osmoregulators, although these are not necessarily rigid categories.

Ligia oceanica (Linnaeus) and *Idotea granulosa* Rathke occupy natural environments where fluctuations of salinity occur. *Ligia oceanica* is commonly found at or above high-tide level and has been reported up to 450 ft. above high-water mark (Darling, 1947), although it occurs in pools where the surface salinity may become as low as 5% sea water (Naylor & Slinn, 1958). *Idotea granulosa*, on the other hand, is among the intertidal fauna of the sea-weed zone, characteristic of the mid or lower tidal stretches. While *I. granulosa* does not penetrate into estuaries or brackish water to any extent in Britain, it is recorded from the gulf of Finland in salinities of about 19% sea water (Segerstråle, 1947). This species is therefore partly insulated from evaporation and dilution in the intertidal habitat by the salt and water reserves in the sea-weed.

The experiments reported here were 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 the blood of *Ligia oceanica* and *Idotea granulosa* when the animals were exposed to a range of immersion media. What is of immediate importance to the organism as an adaptive response to normal or emergency conditions is its osmotic concentration and not its ionic regulation, and for that reason this study in the first instance is limited to measurements of osmotic concentration.

MATERIAL AND METHODS

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

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. Both *Ligia oceanica* and *Idotea granulosa* were collected on the shores of the Isle of Cumbrae in the Firth of Clyde, Scotland.

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The experimental solutions used as external media ranged from 100 to 25% sea water. The different concentrations were obtained by adding Glasgow tap water (total solids 21.3 mg./l., 1947 analysis, City of Glasgow) to fresh sea water (from Millport, Isle of Cumbrae). 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 percentage sea water at any one time, but such variations are insignificant in terms of the total test range of experimental salinities.

A high temperature ($15 \pm 1^\circ \text{C.}$) and a low temperature ($5 \pm 1^\circ \text{C.}$) were chosen in relation to the recorded average seasonal sea-water temperatures at Millport (Barnes, 1959).

During the period of experiments the animals were starved and kept in darkness in plastic containers with a maximum of thirty-five animals per 3 l. of aerated water at a given salinity and temperature. Any one animal was tested in a single experimental medium.

Blood from the heart was drawn directly into silica glass capillaries since Ramsay (1949) noted that even during short periods there was some diffusion out of hard glass capillaries. Capillary tubes, of a uniform external diameter of about 0.2 mm. were chosen and the sample (about 0.04 microlitres ($\mu\text{l.}$)) was sealed within medicinal paraffin according to Ramsay's technique (1949).

Preliminary experimental results 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 (for example, summer *Ligia oceanica* in 100% sea water at 5°C. , $F = 1.1$, $P > 0.05$) 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 and the blood tests were in this case started on the second day. Usually two or three animals from any one temperature-salinity condition were tested each day. The isopods were weighed after sampling, the sex was noted, and the animals were then discarded.

The osmotic concentration of the sample was determined by the freezing-point depression method described by Gross (1954). Full details have been recorded elsewhere (Todd, 1962). The freezing-point depression in $^\circ\text{C.}$ of the animal's body fluid is indicated by Δ_i , and Δ_e refers to the external experimental medium which was sampled at the same time. Repeated determinations of the freezing-point depression of a standard solution showed the error to be within about $\pm 2\%$. The formula $\Delta/0.6 = \% \text{NaCl}$ (Ramsay, 1949) gives a fairly accurate estimation of the sodium chloride concentration.

RESULTS

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. Groups of equal numbers of animals from the different experimental media (either fifteen or eighteen 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 that of Snedecor (1956, p. 310).

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.

Osmotic balance

Ligia oceanica

Determinations of the freezing-point depression were made on the blood of 190 summer animals and 167 winter animals (Fig. 1). The number tested in each temperature-salinity condition is given in Table 1 with mean values, standard deviations and standard errors of the mean.

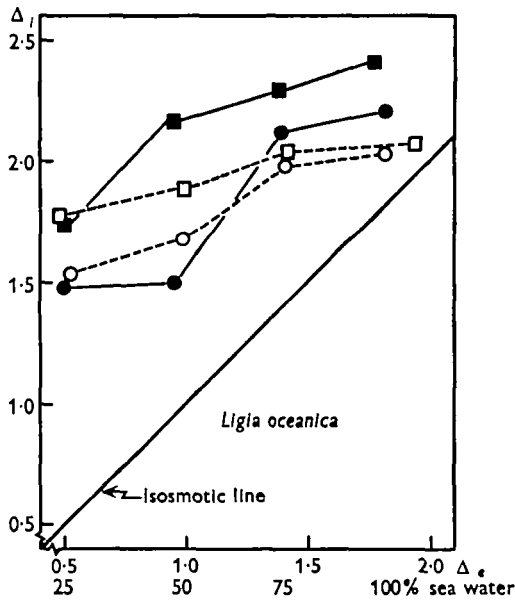


Fig. 1. The relation of the osmotic concentration of the blood of *Ligia oceanica* to the concentration of the medium. Summer animals: 5° C. (—●—), 15° C. (- - ○ - -); winter animals: 5° C. (—■—), 15° C. (- - □ - -).

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° and 15° C.

The osmotic concentration of the blood was influenced by the salinity of the medium at both temperatures and in both seasons ($F = 13.68-42.14$, $P < 0.01$). For example, animals in 50 and 25 % sea water ($\Delta_i = 1.50$ and $\Delta_i = 1.48$) had a significantly lower osmotic concentration than those in 100 and 75 % sea water ($\Delta_i = 2.21$ and $\Delta_i = 2.12$). The values of t from the t -tests 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 of *L. oceanica* compatible with survival is apparently within the range of $\Delta_i = 0.97$ to $\Delta_i = 1.16$ ($\Delta_o = 0.50$ and $\Delta_o = 0.95$); at these values intolerance was shown by oedema and loss of motility. Some animals, however, osmoregulated over prolonged periods in 25% sea water.

Table 1. *Ligia oceanica*

Temp. (°C.)	Sal. (%)	Δ_o (°C.)	N	Δ_i (°C.)	S.D.	S.E.
Summer						
5	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	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	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	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

Temperature and salinity effects

Both temperature and salinity influence the osmotic concentration of the blood in *L. oceanica*, but are interdependent in their effect, as shown by the significance of the temperature-salinity interaction in each season ($F = 4.59$, 5.10 , $P < 0.01$). Table 1, for example, gives a 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 in summer animals. There is a similar effect in winter animals where, 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. ($t = 4.59$, 5.29 , 5.46 , $P < 0.001$). In 25% sea water, however, there was no significant difference between osmotic concentration of the blood in animals adapted to 5° and 15° C.

Seasonal effect

The osmotic concentration of the blood showed a seasonal change ($F = 100.68$, $P < 0.01$) and the total mean values were $\Delta_i = 1.82$ for summer animals and $\Delta_i = 2.05$ for winter animals over the range of salinities, calculated from the analysis of variance data. Seasonally, the osmoregulatory response differed with regard to temperature ($F = 22.73$, $P < 0.01$), salinity ($F = 9.82$, $P < 0.01$), and to the temperature-salinity interaction in summer and winter ($F = 3.42$, $P < 0.05$).

The mean freezing-point depression of the blood differed significantly in summer and winter animals at a temperature of 5° C. ($t = 9.34$, $P < 0.001$) with the winter

animals maintaining a higher osmotic concentration of the blood throughout the whole range of salinities. At a temperature of 15° C. the difference between summer and winter animals narrowed considerably ($t = 3.61, P < 0.001$).

In summer animals the freezing-point depression values levelled off in 50% sea water at 5° C. but in the winter animals in 50% sea water at 15° C. (Fig. 1), indicating that the threshold value is determined by the particular temperature-salinity conditions.

Osmotic balance Idotea granulosa

Determination of the freezing-point depression of the blood was made on 137 summer animals and 147 winter animals (Fig. 2). The number tested in each temperature-salinity combination is given in Table 2, with mean values, standard deviations and standard errors of the mean.

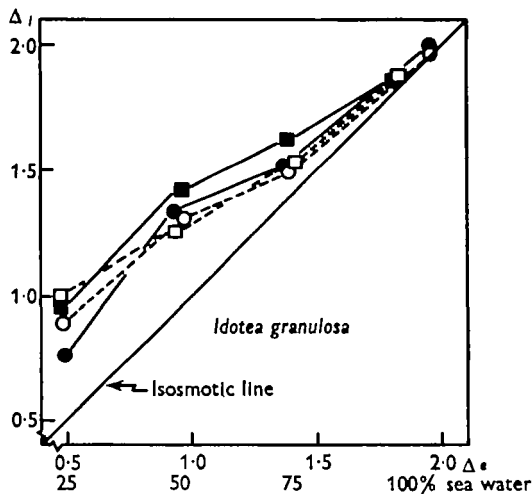


Fig. 2. The relation of the osmotic concentration of the blood of *Idotea granulosa* to the concentration of the medium. Summer animals: 5° C. (—●—), 15° C. (- - ○ - -); winter animals: 5° C. (—■—), 15° C. (- - □ - -).

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 again in 50 and 25% sea water. There was a close relationship between Δ_i and Δ_e throughout the salinity range, in summer and winter animals at both temperatures ($F = 189.36-375.18, P < 0.01$). The mean values for summer animals at 5° C. are $\Delta_i = 1.96, \Delta_i = 1.52, \Delta_i = 1.34, \Delta_i = 0.76$ for the salinity range 100 to 25% sea water, indicating a significant drop in Δ_i for each 25% decrease in Δ_e . The mean values for animals in any two salinities at 5° or 15° C. in both summer and winter are always significantly different (Table 2). Results from the *t*-tests ranged from 4.88 to 33.06 ($P < 0.001$).

I. granulosa survived in the laboratory in the 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. The longest survival time at this salinity was 9 days (winter animals, 15° C.). Summer animals which regulated less efficiently at 25 % had to be tested after 2 days adaptation to obtain sufficient data.

Table 2. *Idotea granulosa*

Temp. (°C.)	Sal. (%)	Δ_o (°C.)	<i>N</i>	Δ_i (°C.)	S.D.	S.E.
Summer						
5	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	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	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	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

Temperature and salinity effects

The temperature-salinity interaction was significant in both summer and winter animals ($F = 4.49, 6.04; P < 0.01$). In winter 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 acclimated to 15° C. had the higher osmotic concentration. This difference in the concentration at the two temperatures becomes significant in salinities of 75 and 50 % sea water ($t = 3.08, 4.10, P < 0.001$). 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 is given in Fig. 2 and Table 2.

Seasonal effect

The osmotic concentration of the blood of *I. granulosa* showed a seasonal change ($F = 6.09, P < 0.05$). The total mean values were $\Delta_i = 1.41$ for summer animals and $\Delta_i = 1.44$ for winter animals over the range of salinities, 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.

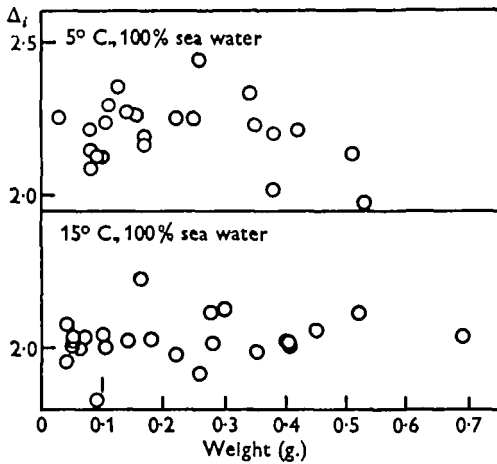


Fig. 3

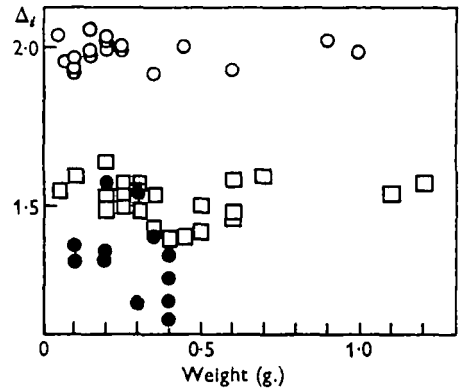


Fig. 4

Fig. 3. Scatter diagram plotting the freezing-point depression values of summer male and female *Ligia oceanica* of different weights. The animals were in 100% ($\Delta_s = 1.82$) sea water at either 5° C. or 15° C.

Fig. 4. Scatter diagram plotting the freezing-point depression values of summer male and female *Idotea granulosa* of different weights. The animals were in 100% (O, $\Delta_s = 1.96$), 75% (□, $\Delta_s = 1.37$) and 50% (●, $\Delta_s = 0.93$) sea water at 5° C.

Table 3. *Ligia oceanica*

Temp. (°C.)	Sal. (%)	N (♂)	Mean Δ_f (°C.)	N (♀)	Mean Δ_f (°C.)
Summer					
5	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	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	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	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			

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° and 15° C. in 100% sea water (Fig. 3) and summer *Idotea granulosa* adapted to 100, 75 and 50% sea water at 5° C. (Fig. 4).

The sex ratio of *Ligia oceanica* and *Idotea granulosa* differed in summer and winter (Tables 3 and 4), but sex was not a factor influencing the osmotic concentration of the blood (Tables 3 and 4), and there was no significant difference in the freezing-point depression of the blood between 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 ($P > 0.05$).

Table 4. *Idotea granulosa*

Temp. (°C.)	Sal. (%)	N (♂)	Mean Δ_t (°C.)	N (♀)	Mean Δ_t (°C.)
Summer					
5	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	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	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	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			

DISCUSSION

Considering their respective ecology the difference in the responses of *Ligia oceanica* and of *Idotea granulosa* with regard to osmotic concentration of the blood and survival in the test range of salinities was to be expected.

In 25% sea water *Ligia oceanica* and *Idotea granulosa* maintained mean blood concentrations equivalent to $\Delta_t = 1.63$ and $\Delta_t = 0.90$, respectively, compared with $\Delta_t = 2.18$ and $\Delta_t = 1.92$ in 100% sea water. To drop to those levels merely by passive dilution would require a water intake that would be evident as an increase in volume, particularly in *I. granulosa*. In *Ligia oceanica* this did not happen in the animals kept in 25% sea water until 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 since there was no weight change there must be an exchange of salt in *Carcinus maenas* in dilute sea water, and Croghan (1958*a*) suggested that the main changes in blood concentration of *Artemia salina* were due more to movements of sodium chloride than to movements of 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 (1958*b*) demonstrated localized staining of cells of the branchiae of *Artemia salina* by silver salts which may be interpreted as indicating ion exchange in this area.

Temperature

Osmoregulation is influenced by temperature in both *Ligia oceanica* and *Idotea granulosa*. Temperature response in the form of a change in concentration of body fluids is known to occur in a number of animals in a variety of conditions and accounts for differences in the estimations of ionic and osmotic concentrations. Dakin (1908), for example, reported a higher osmotic concentration of the blood of *Pleuronectes platessa* (a difference of $\Delta_i = 0.08$) when measured in the field (Heligoland) and in the laboratory, probably due to the different temperatures in the natural and laboratory media, and there are many reports of temperature differences in other animals.

There are many explanations of the effect of temperature on osmoregulation, and certainly some of the conflicting results can be explained by the differences in the experimental media. Lockwood (1960) suggested that the increase in the sodium-ion concentration of the blood 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, which is difficult to accept. Riegel (1959) compared the weights of sphaeromid isopods at temperatures of 5° 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 temperatures.

Temperature and salinity

Although the temperature and salinity of the medium influence survival and osmotic concentration of the blood independently, in certain experiments the organism responded to the combined effect of temperature and salinity. 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, make it difficult to explain this interdependence of temperature and salinity as a simple relation. It can be assumed that the maintenance of a hyper-

osmotic state involves metabolic work, and the association of a higher osmotic concentration with a higher temperature in the medium which demands the most osmotic work could be related to the initial increase in the metabolic rate (a Q_{10} of 2 to 3 is generally found) and to an increase in cell permeability.

Crangon vulgaris tested at 4° and 21° C. (Broekema, 1941) also had the higher osmotic concentration of the blood at 4° C. in 100% sea water and at 21° C. in salinities less than 65% sea water. 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° and 8.8° C.) are compared.

The data obtained so far with regard to the effect of the temperature-salinity factor on the osmotic concentration of body fluids in the Crustacea can be related to their range of ecological adaptation. For example, *Gammarus duebeni*, Kinne (1952) and *Rithropanopaeus harrisi* (Kinne & Rotthauwe, 1952) 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, either saline or fresh water, the lower temperature is associated with the higher osmotic concentration of the blood and there is some support for the concept of an optimum temperature for adaptive response to a change in the osmotic concentration of the medium (Kinne, 1956; Schlieper, 1958).

Season

Temperature affects most metabolic processes and seasonal adaptations are often associated with temperature differences.

Seasonal differences in the physiological processes of animals cannot, however, 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* have a different response to the interdependent effect of temperature and salinity; summer animals have a higher osmotic concentration of the blood at 15° C. in 50 and 25% sea water, while the winter animals have the same osmotic concentration at 5° and 15° C. in 25% sea water. The seasonal effect is also evident in Parry's (1953) figures of $\Delta_4 = 2.15$ for *L. oceanica* tested in April to June and $\Delta_4 = 2.28$ for animals tested in October to December, with both groups kept at 18–20° C. in wet sand. Again, experiments on tolerance to high temperatures in *Hemigrapsus nudus* and *H. oregonensis* after adaptation to various temperature-salinity combinations (Todd & Dehnel, 1960) showed that summer and winter animals were physiologically different.

Another seasonal effect independent of temperature is recorded by Woodhead & Woodhead (1959) who found that summer *Gadus morrhua* 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. 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 hyperosmotic.
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, $\Delta_t = 1.65$, compared with $\Delta_t = 0.90$ in *Idotea granulosa*.
5. In both species the osmotic concentration of the blood was influenced by season, by temperature and by a temperature-salinity interaction.
6. Neither size nor sex of the animal influenced osmotic concentration of the blood.

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