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REGULATION OF WATER AND SOME IONS IN GAMMARIDS (AMPHIPODA)

II. *GAMMARUS PULEX* (L.)

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INTRODUCTION

Gammarus pulex was investigated by Beadle & Cragg (1940) in their analysis of the process of osmoregulatory adaptation to fresh water. This study was centred on a comparison of the blood and tissue chloride levels in several species of *Gammarus* which were exposed to a wide range of external salinities. Beadle & Cragg concluded that in the euryhaline species *G. duebeni* and *G. locusta* an important part of adaptation to variations in salinity was the ability to regulate or maintain the tissue chloride content at a fairly constant level in the face of large changes in the blood concentration. In contrast the inability of *G. pulex* to tolerate high salinities was associated with its inability to prevent chloride from entering the tissues when the blood concentration was raised above the normal level. This aspect of osmoregulation in crustaceans was later studied in more detail on the large muscles of decapods (Shaw, 1955*a*, *b*, 1958*a*, *b*, 1959; Bryan, 1960*a*, *b*). However, there is still relatively little information about water and ion regulation in the tissues of invertebrates (see reviews by Potts & Parry, 1964; Potts, 1968; Lange, 1968).

The 'tissue' studied by Beadle & Cragg (1940) was in fact the whole animal cut into two parts and pressed between filter papers to remove the blood. Derouet (1952) apparently used the same technique and she obtained very similar results on *G. pulex*. Some blood would undoubtedly remain in this tissue preparation (Sutcliffe, 1971*a*). In the present series of studies on salt and water regulation in gammarids the intracellular concentrations of sodium, potassium and chloride were estimated by an indirect method based on the assumption that the distribution of potassium and chloride in the animal conforms to a Donnan equilibrium between the extracellular and intracellular spaces (Croghan & Lockwood, 1968; Sutcliffe, 1971*a*, *b*). This method is justified in this paper by comparison with the results obtained by Beadle & Cragg and by Derouet, and also by comparison with results based on the extracellular inulin space in *G. pulex* measured by Butterworth (1968).

MATERIAL AND METHODS

Large male specimens of *G. pulex* were obtained locally. The experiments were carried out at a temperature of 9 ± 1 °C. The experimental procedures and methods of analysis were as described previously (Sutcliffe, 1971*a*). That paper also gives the

method employed for the calculation of the blood space, defined as the proportion of the body water in the extracellular space.

RESULTS

Water content

The mean water content remained constant at 79·0–80·3% body wet weight in animals acclimatized to salinities ranging from 0·06 mM/l NaCl to 50% sea water (Table 1). Inspection of the standard errors given in Table 1 shows that variability in the water content of the six animals in each group did not increase at high external salinities. This is surprising in view of the fact that *G. pulex* drinks when in sea-water media (Sutcliffe, 1967) and it was expected that imbibition of salt water might influence water balance in the animal, especially at salinities between 30 and 50% sea water where mortality is high. In this particular case there were no deaths in 10% and 30% sea water at 9 °C, and all of the animals were active and normal in appearance in

Table 1. *Wet weight, water content and blood ion concentrations at various external salinities*

(Mean results from six animals ± 1 standard error.)

Medium	Wet weight (mg)	Water content (% wet wt.)	Blood ions (mM/l) Na_o	Cl_o	K_o	Ratio: body sodium/ chloride
0·06 mM/l NaCl	66·1 ± 0·99	79·8 ± 0·62	106	126	5	1·61
0·25 mM/l NaCl	66·9 ± 1·85	79·3 ± 0·60	131	124	5	1·48
0·25 mM/l NaCl	61·7 ± 1·56	79·4 ± 0·97	—	—	—	1·70
2% SW	72·8 ± 0·97	79·3 ± 0·89	—	—	—	1·45
10% SW	67·1 ± 1·75	79·0 ± 0·65	132	130	6	1·30
10% SW	57·8 ± 1·19	80·3 ± 0·70	—	—	—	1·51
30% SW	65·5 ± 1·99	78·9 ± 0·30	179	188	7	1·08
40% SW	58·4 ± 1·11	79·2 ± 0·67	[200]*	[230]	[8]	1·04
50% SW	54·7 ± 1·99	79·8 ± 0·42	[250]	[280]	[10]	1·00

* [ ] blood concentrations assumed for calculation of blood space and sodium space.

30% sea water. When transferred to 40% sea water they became less active and did not adopt the characteristic attitude of *G. pulex* when at rest, where the telson is curled round to lie beneath the abdomen. Instead the uropod and telson were extended in line with the thorax and abdomen. This is the attitude normally adopted when swimming, and also after death. During a period of 6 days in 40% sea water one-half of the animals died. The remainder were moved to 50% sea water for 24 h. The six animals used for analysis were barely active. The rest were either dead or in a semi-comatose condition.

Sodium and chloride

A few analyses on large pooled samples of blood are given in Table 1. These supplement previous analyses given in the literature (Beadle & Cragg, 1940; Derouet, 1952; Lockwood, 1961; Sutcliffe, 1967; Vincent, 1967). No attempt was made to obtain blood from animals in 40–50% sea water as most of the animals were in a semi-comatose condition. It was assumed that in these animals the sodium concentration in the blood was slightly higher than the sodium concentration in the medium, and that the chloride concentration in the blood was equal to the chloride concentration in the