URINE PRODUCTION BY THE ANTENNAL GLANDS OF *PALAEMONETES VARIANS* (LEACH)

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**INTRODUCTION**

The morphology and diversity of the various excretory organs in the invertebrate phyla have frequently been subjects of study, but only rarely have they been investigated from a functional or physiological point of view. This account endeavours to add a little to the present knowledge of excretory organs of the Crustacea and to demonstrate the part played by those organs in osmoregulation. An earlier paper (Parry, 1954) recorded the results of chemical analyses of the excretory fluid of the palaemonid prawn, *Palaemon serratus* (Pennant) when that animal was living in three different salinities. It was established that, although the urine in this species is isotonic with the blood in all conditions of external salinity, the urine serves to remove excess magnesium and sulphate from the body, and this selective excretion is augmented as the external concentration of these ions rises. While the analyses of ions in the blood and urine from animals living in different salinities contributes to our knowledge of the use of the antennal gland, our interpretation of its function as an excretory organ is very incomplete without some estimate of the quantities of salts lost in the excretion. The present inquiry into the volume of urine produced in different conditions by the antennal glands of prawns was undertaken to fill this gap. While *P. serratus* is the best of the available species for chemical analyses of blood and urine since it is the largest, it proved unsuitable for experiments on urine flow since it is very sensitive to handling. The brackish water prawn *Palaemonetes varians* (Leach) is much more amenable to experimental treatment, and was therefore used for this investigation.

The osmotic pressure of the blood and urine in this species under different conditions of salinity were measured by Panikkar (1941). The blood is hypotonic (≈ 2.3% NaCl) to sea water (≈ 3.5% NaCl) when the animal is living in that medium. The urine is isotonic with the blood, or very nearly so. Blood and urine are both isotonic with the medium when it is about 60–70% of sea water (≈ 2.0% NaCl), and at lower salinities both blood and urine maintain their salt concentrations at a level much higher than that of the medium. There is some diminution in the salt content of the body fluids in very dilute media, but even in a medium equivalent to 0.01% NaCl the blood concentration is equivalent to about 1.89% NaCl. In salinities greater than sea water there is similar control of the salt content of the
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body fluids, but it does tend to rise so that in 150% sea water the salt content of the
blood is roughly equivalent to 2.5% NaCl. Throughout this salinity range the
osmotic pressure and chloride content of the urine show only insignificant dif-
ferences from those of the blood. The species has a very wide range of tolerance,
from water that is nearly fresh to concentrated sea water (≈ 5.2% NaCl). Through
this range of c. 5% NaCl, the change in the blood is only c. 1.0% NaCl.

The morphology of the excretory organs of palaemonid prawns has been de-
scribed by Grobben (1880), Weldon (1889, 1891), Marchal (1892), Allen (1892),
Cuénot (1895), and Patwardhan (1937). A brief review is given by Panikkar (1941).
The excretory organs are antennal glands, except in the larval stages where there is a
transitory maxillary gland.

Each antennal gland consists of end-sac, tubular labyrinth, bladder and excretory
pore. The end sac is a small compact coelomic sac lying at the base of the antenna.
Its wall is considerably folded. There is an outer layer of connective tissue with
blood spaces, and an inner convoluted layer which is lined by large epithelial cells
which have conspicuous nuclei and a granular cytoplasm. The blood supply to the
end-sac has been demonstrated by injections of the blood vessels of live animals.
I have dissected injected specimens and reconstructed the arrangement of the blood
vessels from serial sections to confirm these observations. The main branch of the
antennary artery on either side of the thorax leads directly to the end-sac, where it
suddenly splits up into numerous fine vessels which are lost in the walls of the end-
sac. Neither the labyrinth nor any other part of the gland appears to have any direct
blood supply, although the connective tissue of the labyrinth has numerous blood
lacunae, and all the parts of the antennal gland lie within the haemocoele.

The labyrinth is a network of anastomosing tubules which are formed of a single
layer of epithelial cells. It leads to the bladder which is lined by a thin pavement
epithelium. The bladder communicates with the exterior by a short duct to the excre
tory pore which opens at the base of the antennary peduncle on a small papilla.
The most unusual feature of the antennal gland is the presence of two backwardly
projecting arms of the bladders which fuse in development to form a single large
'nephroperitoneal' (Weldon, 1891; Allen, 1892; Patwardhan, 1937; Panikkar, 1941)
or 'renal' (Patwardhan, 1937; Panikkar, 1941) sac, lying dorsally in front of the
heart and gonad and above the stomach. This structure will be referred to as the
'epigastric' sac. It is lined with the same pavement epithelium as the rest of the
bladder; there is no visible histological or structural difference between it and other
parts of the bladder. Neither the bladder nor the epigastric sac appears to have any
intrinsic muscles, but some muscle fibres seem to run from the exoskeleton of the
rostral region to the front of the epigastric sac.

The epigastric sac is overlain by another smaller sac, termed the 'dorsal' sac
(Allen, 1892) which has been variously interpreted as a blood space (Weldon, 1889)
and as a persistent coelomic space (Allen, 1892). It has no blood corpuscles and no
direct communication with any of the blood sinuses of the body (Allen, 1892). It
appears to have little significance in the osmoregulation of the animal since Panikkar
(1941) measured the osmotic pressure of its contents in Palaemon serratus and failed

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to find any osmotic difference between it and the fluid of the epigastric sac, or of the excretory pores.

A feature which distinguishes this group from the other decapods which live in fresh water is the absence of a tubule between the labyrinth and the bladder. It is this portion of the excretory system in the crayfishes and gammarids which has become modified to function as a salt-resorbing mechanism in the fresh-water forms (Peters, 1935; Schwabe, 1933). In the palaemonids there appears to be no structure in the excretory organ which could be associated with the accommodation of the animal to different external salinities. Schwabe (1933) was unable to find any difference in the size of the gland in fresh-water or brackish-water forms of *Palaemonetes varians*, in contrast to the variable size of the maxillary gland in gammarid species from different salinities.

Among the Caridiidae there is a good deal of variation in the morphology of the gland, although many of them appear to have some form of epigastric sac, as in *Pandalus*, *Hippolyte*, and *Crangon* (Weldon, 1889). In most cases the labyrinth is reduced. It is almost absent in *Pandalus* and *Hippolyte* and completely absent in *Crangon*. The presence or absence of parts has no apparent bearing on the distribution of these species in different salinities.

**MATERIALS**

The animals used in the experiments described here were of the species *Palaemonetes varians* (Leach) and were collected either from salt marshes south of the Thames Estuary at Whitstable, Kent, or from salt marshes bordering the River Stour near Manningtree, Essex. Both environments seemed generally to have a salinity about half that of sea water, although this was variable according to the tides, wind and other climatic conditions.

The animals were acclimatized to salinities not very different from that in which they had been living previously, by placing them in the appropriate salinity 3 or 4 days before experiments. In very high or low salinities they were gradually acclimatized for a period of a week, and then kept for a further week in the salinity of the experiment. Measurements of the chloride content of the blood, and osmotic pressure (Panikkar, 1941) indicated that these animals were acclimatized to the particular salinity within the period allowed.

Media with a salinity less than that of sea water were made from Plymouth sea water (Cl' = 19 % approximately) and distilled water. For salinities greater than sea water, Plymouth sea water was concentrated by boiling to half its original volume, corrected for pH with drops of sodium carbonate and then diluted with Plymouth sea water to the required salinity. In this way the ionic balance was kept similar to that of ordinary strength sea water. Plymouth sea water is referred to as ‘100 % sea water’ and the other salinities referred to as percentages of this standard.
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METHODS AND EXPERIMENTAL RESULTS

In the present investigation several independent techniques have been employed to study the urine flow in *Palaemonetes varians*. Each has certain inherent disadvantages and inaccuracies, but all show the same pattern of urine production in different conditions of external salinity.

(1) Total excretion of injected dye: a qualitative method

It was known to many of the earlier investigators that certain non-toxic dyes were excreted by specific organs when introduced into the body of an animal. Previous investigations of crustacean excretory organs by this means were made by Weldon (1889), Marchal (1892) and Lison (1942). Indigo-carmine was chosen as a suitable dye for experiments with *P. varians* from these earlier accounts. When injected in small quantities it appears to be taken up exclusively by the labyrinth and not by the end-sac, as there is always a colourless patch in the region of the end-sac, while the region of the labyrinth becomes deeply stained. From the labyrinth the blue excretory fluid accumulates in the epigastric sac before being lost through the external openings of the glands. The wall of the epigastric sac does not stain with the dye, contrary to the account of Weldon (1889), since it becomes quite colourless when it empties, and then begins to fill again slowly with the blue fluid. The rate at which the stain is removed from the animal appears to be a function of the rate of excretion and this seems to depend on the external salinity. It seems improbable that the dye is changed to a colourless compound in the body since the usual oxidizing and reducing agents fail to alter it between pH 5.5 and 8.5. After the dye is injected it fills the blood spaces and then is gradually removed to the antennal glands. If the animal is moribund and does not excrete, the blue dye remains distributed throughout the haemocoele until the animal dies.

It has been suggested by Palm (1952) that in some insects the rate of excretion of dyes is proportional to their concentrations in the blood so that other factors besides salinity may influence its excretion in prawns.

The experimental procedure was as follows. Animals previously acclimatized to a particular salinity were injected with a small quantity of the stain (c. 0.001 ml. of a filtered 1% solution of the indigo carmine in sea water isotonic with the blood) into the lateral blood sinuses of the abdomen. Larger doses were not so satisfactory, as the dye is then taken up by the cells of the digestive gland which remains so stained for a considerable period. (This phenomenon was observed by Lison, 1942.) This small quantity of fluid injected into the blood alters the blood very little either in composition or in volume, and the animal certainly appears to be quite unaffected by it. Within 5 min. of the injection the epigastric sac begins to appear blue, pale at first, and then increasing in intensity of colour as the sac expands. After it has reached a certain size (which seems to depend on the size of the animal and on the salinity of the medium) the sac empties and the blue-stained excretory fluid is emitted from the excretory pores. When micturition occurs there is a sudden shrinkage of the central portion of the sac, as though a draw-string had been pulled.
tight, and it shrinks to two finger-like projections lying on each side of the gut; as the sac fills, these swell and the central part pushes back between them so that it forms a large ovoid sac lying above the stomach. The sac is figured in this state by most authors (Allen, 1892; Weldon, 1889; Patwardhan, 1937; Panikkar, 1941). There is some indication in sectioned material of a group of muscles running from the exoskeleton of the rostrum to the front of the sac. These may assist in its contraction, or emptying may be caused by the action of the thoracic muscles. There are no muscle fibres apparent in the walls of the sac itself.

After injection of indigo-carmine the rate of excretion was measured as the time taken for the complete disappearance of the dye. The end-point of this process was necessarily subjective since there must be some concentration of the dye which cannot be detected, but the manner of excretion by concentrating the dye in a small volume minimizes this error. Some of the variation in the results will be caused by variations in the size of the animal and the size of the dose administered, although both were kept approximately constant. Moulting, sex, and other physiological conditions may also add to variability of the results. In Table 1 the results are expressed as the mean time for the total clearance of the dye from the body and as the mean reciprocals of these times of clearance. The reciprocals are plotted against the external concentration in Fig. 1. Since the volume of the injected fluid was not accurately known, nor the volume of the blood, it was not possible to make a quantitative calculation of the clearance of the dye from the blood.

<table>
<thead>
<tr>
<th>Salinity of medium (as % sea water)</th>
<th>Mean time for clearance (hr.)</th>
<th>Mean reciprocal time for clearance (hr.⁻¹)</th>
<th>Standard error</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1:65</td>
<td>0:627</td>
<td>±0:057</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>2:00</td>
<td>0:589</td>
<td>±0:046</td>
<td>11</td>
</tr>
<tr>
<td>50</td>
<td>4:66</td>
<td>0:234</td>
<td>±0:018</td>
<td>20</td>
</tr>
<tr>
<td>70</td>
<td>7:25</td>
<td>0:140</td>
<td>±0:007</td>
<td>8</td>
</tr>
<tr>
<td>100</td>
<td>4:35</td>
<td>0:235</td>
<td>±0:014</td>
<td>11</td>
</tr>
<tr>
<td>125</td>
<td>2:93</td>
<td>0:308</td>
<td>±0:065</td>
<td>7</td>
</tr>
<tr>
<td>150</td>
<td>3:13</td>
<td>0:385</td>
<td>±0:057</td>
<td>11</td>
</tr>
</tbody>
</table>

It appears from these results that the excretion of the dye is slowest when the blood and external medium are isotonic (about 70% of sea water). It increases as the salinity of the medium drops, but it is also increased somewhat as the salinity of the medium rises. Above 125% sea water the speed of excretion seems to be halted, since there is no significant difference between the rate of excretion in 125% and 150% sea water.

(2) Excretion of injected dye: a quantitative method

The injection of indigo-carmine into prawns, described in the previous section, enables the excretory organ, especially the epigastric sac, to be observed during the excretion of the dye. This has been used as a further method of determining the
Urine production by antennal glands of *Palaemonetes varians* urine production in different salinities and of making some quantitative estimate of the urine flow.

The approximate volume of urine produced was measured by estimating the maximum size attained by the epigastric sac and the times of micturition. The size of the sac was measured with a squared eyepiece and the measurements converted to cubic millimetres. The sac was observed continually, and the length and breadth of the sac was measured every few minutes until micturition occurred. The volume
of urine excreted in a certain time was estimated and expressed in terms of body weight. The figure used for 'volume' was obtained as the product of length, breadth and depth of the epigastric sac, and so will give a somewhat higher value than the true one since its shape is that of an ovoid sac and not a rectangle. As this shape was constant in all animals the estimated volumes are comparable with each other and proportional to the true volume. The animals were weighed immediately after each experiment. They all appeared healthy and survived handling in the experiments without adverse effects.

The estimated urine production in several different salinities is given in Table 2 and plotted against the salinity of the medium in Fig. 1. There appears to be a minimal flow of urine in a medium of about 50% sea water and a rapid rise in the volume excreted as the salinity of the medium is lowered. In the more saline media the flow appears to increase somewhat and is thereafter approximately constant in a medium between 70 and 100% sea water.

Table 2. Rate of urine flow in Palaemonetes varians: measurement of volume of epigastric sac after injection of indigo-carmine

<table>
<thead>
<tr>
<th>Salinity of medium (% sea water)</th>
<th>Urine as % body weight per hour</th>
<th>Standard error</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.63</td>
<td>±0.09</td>
<td>34</td>
</tr>
<tr>
<td>15</td>
<td>1.06</td>
<td>±0.04</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>0.94</td>
<td>±0.09</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>0.15</td>
<td>±0.01</td>
<td>10</td>
</tr>
<tr>
<td>67</td>
<td>0.45</td>
<td>±0.04</td>
<td>14</td>
</tr>
<tr>
<td>85</td>
<td>0.40</td>
<td>±0.05</td>
<td>13</td>
</tr>
<tr>
<td>100</td>
<td>0.42</td>
<td>±0.04</td>
<td>12</td>
</tr>
<tr>
<td>120</td>
<td>0.11</td>
<td>±0.01</td>
<td>6</td>
</tr>
</tbody>
</table>

(3) Weight changes after excretory blockage

The third method of investigation was the classical one of stopping the excretory organs and measuring the subsequent weight changes. This method was used for comparison with the published data of excretion in other decapods. The most satisfactory material for blocking the pores was found to be dental cement, which was mixed freshly to a thin suspension and then pipetted into the opening. If the cement is used to cover the surface of the excretory pore, the mouthparts remove it very quickly. The method is unsatisfactory in that it is impossible to be sure that the pores are adequately blocked until the expected changes begin to take place. The method assumes that the changes in weight are all due to the accumulation of unexcreted urine, but there may be other explanations. The increase in weight could be produced by swallowed water. Although swallowing has been observed by adding nigrosin to the medium and by some previous authors (Panikkar, 1941; Fox, 1952) quantitative and consistent observations could not be made to check this.

The animals were weighed immediately after stopping the openings of the glands, and thereafter at hourly intervals. The results were variable, although
each animal showed a steady trend of gain or loss in weight. In Table 3, the mean percentage gain in weight per hour is given. Measurements were made every hour for a period of up to 8 hr., but the results were calculated from the first 4 hr. The column of figures marked 'E.P.O.' in the table represents animals with open excretory pores; these are the controls of the experiments. From 5 to 100 % sea water there are only very insignificant changes in the weight of these control animals. In these experiments some of the control animals were found to gain, some to lose a little weight, perhaps due to defaecation or drinking. The column of figures marked 'E.P.Bl.' refers to animals with sealed excretory pores. Although animals in media up to 125 % sea water will survive for at least 24 hr. with blocked excretory pores, in higher salinities they do not survive well and no satisfactory measurements could be made on animals living in media more concentrated than 125 % sea water.

Table 3. Rate of urine flow in Palaemonetes varians: measured by weight changes after blocking the excretory pores

<table>
<thead>
<tr>
<th>Salinity of medium (% sea water)</th>
<th>Mean percentage increase in weight per hour</th>
<th>Urine as % body weight per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.P.O.</td>
<td>E.P.Bl.</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>1.12-2.23</td>
<td>1.68 (6)</td>
</tr>
<tr>
<td>5-10</td>
<td>0.06-0.06</td>
<td>0.06 (6)</td>
</tr>
<tr>
<td>50</td>
<td>0.03-0.17</td>
<td>0.03 (3)</td>
</tr>
<tr>
<td>70</td>
<td>0.03-0.17</td>
<td>0.03 (4)</td>
</tr>
<tr>
<td>100</td>
<td>0.29-0.36</td>
<td>0.10 (5)</td>
</tr>
<tr>
<td>125</td>
<td>-0.12-0.50</td>
<td>-0.27 (3)</td>
</tr>
<tr>
<td>150</td>
<td>-1.22-1.85</td>
<td>-1.43 (3)</td>
</tr>
<tr>
<td>200</td>
<td>-3.84-4.26</td>
<td>-4.08 (3)</td>
</tr>
</tbody>
</table>

The number of measurements upon which the mean is based is given by the figure in parentheses.

The rate of urine production has been calculated from the two sets of observations in the columns E.P.O. and E.P.Bl. For the range 5-70 % sea water the urine produced is taken as the difference between the E.P.O. and the E.P.Bl. figures. In the hypertonic solutions the 'open' animals lose weight presumably as a result of an osmotic outflow of water, so that the E.P.Bl. figure represents the weight of urine produced, less the osmotic water loss. The sum of E.P.O. and E.P.Bl. is taken to represent the weight of urine in such circumstances. The urine production is calculated only for the range 5-125 % sea water as outside this range there is only limited survival after blocking the excretory organs. The E.P.O. figures above and below these salinities show that there must be a considerable osmotic flux of water in markedly hypo- or hypertonic conditions.

It appears from these results that there is little difference to be observed in the quantity of urine produced between 50 and 100 % sea water, but this may be a reflexion of the inadequacy of the method. An increased rate of urine production in hypo- and hypertonic media is indicated by this method.
Direct measurement of urine production by cannulation

The final method to be described was a more direct one than any of the previous ones and consisted of cannulating one of the excretory pores. Although its directness has advantages, it was difficult to execute, and many possible errors were involved. Cannulation of the excretory pore may lead to stimulation of micturition, and the amount of urine present in the epigastric sac at the beginning and end of each experiment cannot be taken into account. As no measures were taken to prevent the escape of urine from the ‘open’ excretory pore, the volume collected from the one was doubled to account for both. These sources of error made it unfruitful to continue these experiments which serve only to amplify the evidence provided by other means. Some collections of urine were made from *Palaemon elegans* (Rathke) (= *Leander squilla* (L.)) and *P. longirostris* (Milne-Edwards), which are included here for comparison.

Table 4. Rate of urine flow in *Palaemonetes varians*: measured by cannulating one excretory opening

<table>
<thead>
<tr>
<th>Salinity of medium (% sea water)</th>
<th>Species</th>
<th>Urine as % body weight per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td><em>P. varians</em></td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td><em>P. varians</em></td>
<td>0.84</td>
</tr>
<tr>
<td>25</td>
<td><em>P. varians</em></td>
<td>0.21</td>
</tr>
<tr>
<td>70</td>
<td><em>P. varians</em></td>
<td>0.02</td>
</tr>
<tr>
<td>100</td>
<td><em>P. varians</em></td>
<td>0.50</td>
</tr>
<tr>
<td>100</td>
<td><em>P. varians</em></td>
<td>0.46</td>
</tr>
<tr>
<td>75</td>
<td><em>P. varians</em></td>
<td>0.55</td>
</tr>
<tr>
<td>75</td>
<td><em>P. longirostris</em></td>
<td>1.82</td>
</tr>
<tr>
<td>75</td>
<td><em>P. elegans</em></td>
<td>0.10</td>
</tr>
<tr>
<td>75</td>
<td><em>P. elegans</em></td>
<td>0.35</td>
</tr>
</tbody>
</table>

The figures in the last column are calculated to include both glands.

The animal was laid on its back on a bed of cotton-wool between two banks of sealing wax, fastened down with cotton threads and wax, and then placed in a bath of the appropriate salinity (to which it had been acclimatized) beneath a binocular microscope. The cannulae used were of ordinary soda-glass, drawn to a steep taper, with the fine tip softened in a micro-flame so that it should have no jagged edges. The cannula was held in a small clamp. By the application of a slight pressure the fluid in the bath was prevented from rising by capillarity while the cannula was arranged. The presence of the cannula did not appear to harm the animal in any way, as most animals survived the experiment indefinitely. In many cases the experiment failed because the cannula became blocked or because the animal moved and displaced the cannula, so allowing the external medium to leak into it. The fluid collected was tested for osmotic pressure or chloride, since there should always be some concentration difference between it and the medium, except in those media in which blood, urine and medium are isotonic. The expected concentration of the urine could be calculated from Panikkar’s (1941) osmotic pressure data, or from chloride analyses of the blood.
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The results obtained by this cannulation method are given in Table 4, the urine flow being expressed as the percentage body weight excreted per hour. Although the results are few in number, they do confirm those of the other three methods, namely that the urine flow is high in hypotonic media (as in brackish water c. 5% sea water), minimal in isotonic media and high again in a hypertonic media. The urine flow measured by this method is similar in magnitude to that estimated by the other quantitative methods.

DISCUSSION

The general pattern of excretion shown by these diverse methods indicates a slow flow of urine in media isotonic, or nearly isotonic, with the blood. If the medium becomes hypotonic the urine flow is increased—a tenfold decrease in the salinity of the medium apparently inducing a tenfold increase in the volume of urine produced. Thus, in 50% sea water, the urine flow has been estimated as 0.15% body weight per hour; while in 5% sea water it has risen to 1.63% body weight per hour (from the observations of dye excretion, method 2). In changing the medium from 50 to 5% sea water, the flow of urine appears to have increased progressively. In media more concentrated than isotonic there is a tendency for the urine flow again to be increased, although the rise is a comparatively small one and after the minimal flow has been approximately doubled, it appears to be kept at a relatively steady level while the medium continues to increase in salinity (again using the observations of the second dye excretion method).

The salinity of the medium at which the urine flow is minimal is variable between samples of animals and may reflect differences in the method of estimating urine flow, or it may reflect different physiological states, different races acclimatized to different habitats, or the different times of the year when the experiments were made. The minimal urine production in the second set of experiments appears at a lower salinity than in the previous dye-injection method. This may be associated with the different environments in which the animals were found (the first were from salt marshes at Whitstable, Kent; the second from salt marshes near the River Stour, Essex), or perhaps with the different times of the year when the experiments were made (the first during May-June, 1953; the second during March-April, 1954). This shift in the minimum might thus be attributed to a seasonal change in the animals (such as the drop in osmotic pressure of the blood in summer recorded by Panikkar, 1941) or to the results of a long-term acclimatization to different environments. In spite of these differences, however, the general pattern of excretion is so similar in the different methods employed that some conclusions may be based upon this estimate of urine production.

It is difficult to compare these results with those recorded for other decapods (Table 5) since, with the exception of the figures for Eriocheir, all the previous records refer to fresh-water animals (such as Cambarus and Potamobius) or to marine animals (such as Maia, Cancer and Carcinides). Only for Carcinides are there experiments indicating a varying flow following a change in the salinity of the medium. Nagel's results (1934) indicate that the urine flow is roughly doubled
when the salinity of the medium is reduced from 100 to 50% sea water—a gradient of change in the urine flow which appears very similar to that found in the present investigation for *Palaemonetes varians*, when transferred from an isotonic medium to dilute brackish water (from about 50% sea water to 5% sea water).

Table 5. *Rate of urine flow in some decapod Crustacea*

<table>
<thead>
<tr>
<th>Species</th>
<th>Medium</th>
<th>Author</th>
<th>Urine as % body weight per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Maia</em></td>
<td>Sea water</td>
<td>Bialaszewicz (1932)</td>
<td>0.125</td>
</tr>
<tr>
<td><em>Cancer</em></td>
<td>Sea water</td>
<td>Robertson (1939)</td>
<td>0.125-0.416</td>
</tr>
<tr>
<td><em>Eriocheir</em></td>
<td>Fresh water</td>
<td>Scholles (1933)</td>
<td>0.175</td>
</tr>
<tr>
<td><em>Carcinides</em></td>
<td>Sea water</td>
<td>Nagel (1934)</td>
<td>0.416</td>
</tr>
<tr>
<td><em>Carcinides</em></td>
<td>½ sea water</td>
<td>Bethe et al. (1935)</td>
<td>0.300</td>
</tr>
<tr>
<td><em>Carcinides</em></td>
<td>½ sea water</td>
<td>Nagel (1934)</td>
<td>0.708</td>
</tr>
<tr>
<td><em>Cambarus</em></td>
<td>Fresh water</td>
<td>Lienemann (1938)</td>
<td>0.217</td>
</tr>
<tr>
<td><em>Potamobius</em></td>
<td>Fresh water</td>
<td>Herrmann (1931)</td>
<td>0.158</td>
</tr>
</tbody>
</table>

In general the figures for the urine flow in *Palaemonetes varians* in the middle range of its habitat (25–100% sea water) are of similar magnitude to those recorded for other marine decapods which are approximately isotonic with the sea water in which they live. *Carcinides* in 50% sea water has a blood concentration considerably higher than that of the medium, and its urine flow is apparently much faster than when it is in an isotonic medium. There are no estimates of urine production in lower salinities, and indeed they would be difficult to make since *Carcinides* will not live successfully in salinities lower than this. We may perhaps assume that, as the salinity is lowered, the urine flow of such an animal will increase, as it appears to do in *Palaemonetes*. But the fresh-water Crustacea, and even *Eriocheir* which may be regarded as a successful marine invader of fresh water, have generally a much lower urine flow, in contrast to the greatly augmented flow in *Palaemonetes* in water which is nearly fresh. The urine flow of this prawn in 5% sea water is nearly 10 times as great as that recorded for *Eriocheir* in fresh water. If the urine flow of *Palaemonetes* acclimatized to even lower salinities were measured, it seems probable that the flow would be even greater than that recorded for 5% sea water. This leads one to expect that in *Eriocheir* in fresh water some further means of osmotic control has been brought into play, which perhaps reduces the salt loss through the antennal glands or reduces the osmotic influx of water.

Whether some other mechanism is present in the fresh-water variety of *Palaemonetes* we do not know, but the evidence suggests that this must be so. The fresh-water variety of *Palaemonetes* has been accorded racial status as *P. varians* var. *macrogenitor* on the basis of morphological studies (Sollaud, 1923, 1932), and the only record of the osmotic pressure of the blood of the fresh-water variety (Viali, 1925) indicates that this may be very much lower (Δ = 0.54° C.) than that recorded for *P. varians* from Britain (Δ = 1.28–1.40° C.) (Panikkar, 1941). The lowest value recorded by Panikkar for animals gradually acclimatized to almost fresh water (0.01% NaCl = Δ 0.006° C.) was for blood, 1.982% NaCl = Δ 1.18° C. The methods
Urine production by antennal glands of Palaemonetes varians

used for measuring osmotic pressure were very different (Vialli used Monti's thermo-electric method; Panikkar a Hill-Baldes thermocouple; I have confirmed Panikkar's figures using Ramsay's freezing-point apparatus (1949)) but even allowing for some degree of error in Vialli's results there still seems to be a significant difference between these figures.

This possible difference between two varieties of *P. varians* is interesting in the light of the energy required for osmotic work in such conditions. A marine *Eriocheir* put into fresh water would use about 11% of its total available energy for osmotic work (Potts, 1954), but a specimen of *Eriocheir* adapted to fresh water only uses 0.54% of its total energy since the blood concentration is considerably reduced. The urine in a fresh-water *Eriocheir* remains isotonic with the blood after transfer from sea to fresh water, but if it were of lower concentration than the blood, a further saving of energy would be achieved. Truly fresh-water Crustacea, such as the crayfishes, have a urine which is very dilute, thus using a minimum of energy for the maintenance of the osmotic difference between internal and external media.

It is clear from this that the variety of *Palaemonetes varians* found in Britain could only extend into fresh water at considerable energetic expense, if the blood and urine concentrations are to be maintained at the level shown by animals in 0.01% NaCl (the lowest medium for which Panikkar (1941) recorded a determination of the osmotic pressure of the blood). On the other hand, the fresh-water variety of *P. varians* from southern Europe appears to have a very low osmotic pressure of the blood, so that even if the urine is still isotonic with the blood, the animal will be saving a considerable amount of energy in comparison with a brackish water specimen in a very dilute medium. *P. varians* seems never to have been recorded from completely fresh water in Britain, and indeed seems restricted to certain rivers of southern Europe (Boas, 1898). The geographical separation and the considerations of the energy necessary to maintain the brackish water variety in fresh water seems to support the concept of two distinct races for this species, previously based upon morphological and embryological observations. The fresh-water variety of *P. varians* should thus provide an interesting study in osmoregulation with some of the modern methods of investigation.

In the brackish water variety of *P. varians* with which this study is concerned, the osmotic work done by the animal is dependent on the permeability of the integument to the various constituents of the medium. From the results of the experiments described in this paper, and from some recorded briefly in the Appendix (p. 420), certain facts are established. First, it is clear from the experiments involving weight changes of animals with open excretory pores in distilled water and in media more concentrated than 125% sea water, that water is gained or lost with facility in markedly hypo- or hypertonic media. Secondly, experiments with heavy water (see Appendix, p. 420) suggest a rapid exchange of water in hypo- (5% sea water), iso- (70% sea water) and hypertonic (120% sea water) salinities. The half-time of penetration of heavy water in these salinities was between 0.43 hr. and 0.73 hr. Thirdly, experiments with an isotope of sodium, $^{24}$Na (see Appendix, p. 421), suggest a rapid exchange of sodium in different salinities. The half-time of exchange (outflow) was
about 2–3 hr. in 5 % sea water, 1–1½ hr. in 70 % sea water and 1½–2 hr. in 120 % sea water.

From these conclusions we must assume that in the salinities in which the animal normally regulates without prolonged acclimatization, i.e. from 1 % sea water to 120 % sea water, there must be some mechanism compensating for these influxes and outfluxes of water and ions. The production of urine cannot be considered instrumental in maintaining the ‘steady state’ of the animal. While the progressively increased flow of urine in hypotonic media may reflect and counteract the inward osmotic flow of water, much essential salt is lost at the same time. In hypertonic media, the apparent increase in the flow of urine loses water from the animals as well as salts, and this loss must be made good elsewhere.

**Summary**

1. Four methods for estimating the rate of urine flow in *Palaemonetes varians* are described.
2. The rate is minimal when the external medium is approximately isotonic with the blood. All methods indicate that the rate increases progressively with increasing dilution of the external medium below 50 % sea water. There is some evidence to suggest that the rate increases in hypertonic external media.
3. These results are discussed in relation to estimates of the urine production in some other Crustacea and in relation to the ecology of the genus *Palaemonetes*.

I should like to thank Prof. Sir James Gray, F.R.S., and Prof. H. Munro Fox, F.R.S., for the facilities offered by their departments.

**Appendix**

Experiments to determine the exchange rates of water and sodium ions were planned using D₂O and Na. These experiments did not provide sufficiently precise data for a calculation of the exchange rates, but did demonstrate the permeability of *Palaemonetes varians* to heavy water and the sodium isotope. A brief summary of these experiments is appended.

1. **Heavy-water experiments**

Animals were placed in solutions of heavy water (c. 20 %) and different salinities (5, 70 and 120 % sea water) and the rate of penetration of the heavy water measured. This was done by taking blood from the animals at half-hourly intervals, distilling the water from this sample, and estimating its heavy water content by measuring the density of the distillate (using a simple modification of the method of Fenger-Eriksen, Krogh & Ussing, 1936).

The mean half-time of penetration in the three salinities was as follows:

- 5 % sea water, \( t_1 = 0.53 \pm 0.14 \) hr. \((n = 12)\),
- 70 % sea water, \( t_1 = 0.73 \pm 0.28 \) hr. \((n = 8)\),
- 120 % sea water, \( t_1 = 0.43 \pm 0.15 \) hr. \((n = 8)\).
Urine production by antennal glands of Palaemonetes varians

(2) Sodium isotope experiments

Animals were left in a solution of $^{24}$Na and various salinities (5, 70 and 120 % sea water) for 24 hr. After this time the radioactive count of the animal did not rise appreciably, and it was assumed that all the unbound sodium had been exchanged. The animal was then placed in front of the window of a GM 4 tube and washed in a constant current (3 ml./min.) of the non-active medium. The radioactivity of the animal was counted at half-hourly intervals until it was reduced to an insignificant level. Some typical measurements of the half-time of washing-out were as follows:

<table>
<thead>
<tr>
<th>Medium</th>
<th>Half-time (hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 % sea water</td>
<td>3.25</td>
</tr>
<tr>
<td>5 % sea water</td>
<td>2.30</td>
</tr>
<tr>
<td>70 % sea water</td>
<td>1.50</td>
</tr>
<tr>
<td>70 % sea water</td>
<td>1.40</td>
</tr>
<tr>
<td>70 % sea water</td>
<td>0.85</td>
</tr>
<tr>
<td>120 % sea water</td>
<td>1.65</td>
</tr>
<tr>
<td>120 % sea water</td>
<td>1.90</td>
</tr>
</tbody>
</table>

REFERENCES


