WATER BALANCE IN *CORIXA DENTIPES* (THOMS.) (HEMIPTERA, HETEROPTERA)

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INTRODUCTION

In a previous paper (Staddon, 1963) an investigation of the water balance in the aquatic bugs *Notonecta glauca* L. and *Notonecta marmorea* Fabr. was reported. These bugs, in common with many aquatic insects (Staddon, 1955, 1959), eliminate waste nitrogen mainly in the form of ammonia, undoubtedly in association with the ready availability of water. The water output of starving adults was shown to be considerable, ranging from about 10 to 50% of the total body weight per day, and an increased water output was connected with an increased ammonia output. Water was gained by drinking and evidence was obtained that the osmotic intake of water through the cuticle contributed significantly to the total intake. But these findings are not necessarily generally applicable to aquatic Heteroptera, for the group includes a wide diversity of forms that differ in their structural and physiological adaptations to aquatic life. It seemed desirable, therefore, to carry out further studies on other species from different families. In this paper an investigation of the water balance in *Corixa dentipes* (Thoms.) is reported. These corixids were selected for study as being of conveniently large size and readily collected locally.

MATERIAL AND METHODS

Corixa dentipes adults were collected from Kenfig Pool, Glamorgan, by sweeping in shallow water with a pond net. In the laboratory they were isolated in small jars, each containing from 20 to 30 ml. of de-ionized water, and thereafter starved until required. During this period the water was periodically changed to prevent the accumulation of excretory products. Adults will live for at least 3 weeks in these conditions and may be capable of surviving for a much longer period.

These bugs, which are lighter than water, normally cling to objects below the surface; but they are unable to cling to the smooth surface presented by the bottom of a glass or plastic jar. To overcome this difficulty the floor of each jar was furnished with a grid of 'Tygan'. An adult would dive to the bottom and settle on the grid immediately after being placed in its jar.

Adults were weighed to the nearest o·5 mg. on a 500 mg. torsion balance after being narcotized with CO_2 and dried with filter-paper. Male specimens were on average 8 mg. heavier than females. An illustration of the weight variability in each sex is provided by the results given in Table 1. These specimens had been weighed within 1 day after collection; as will be shown later the body weight is significantly reduced by starvation.

Collection and analysis of rectal fluid and haemolymph

Rectal fluid was squeezed out, gentle pressure being applied to the abdomen, and collected in a capillary pipette as it emerged from the anus. Ammonium was measured by the diffusion-titration method of Shaw & Beadle (1949); bicarbonate by titration of the sample with 0·01 N-HCl that incorporated 5 ml. of B.D.H. '4·5' indicator per 100 ml. (Staddon, 1963). Each measurement required 0·2 μl. of fluid. It was often possible to obtain sufficient fluid from one animal to enable measurements to be made in triplicate by each of the above two methods.

Table 1. *Total body weight*

	Mean total body weight (mg.)	Standard deviation (±mg.)	No. of specimens
Males	94·7	5·2	24
Females	86·3	6·0	44

Adults weighed within 1 day of capture.

Haemolymph was obtained by making a puncture in the wall of the thorax. A small sample was taken up by a capillary pipette which contained liquid paraffin and then deposited under liquid paraffin in a siliconed watch-glass.

Measurements of osmotic pressure were carried out on both haemolymph and rectal fluid by the freezing-point method of Ramsay & Brown (1955). The freezing-point depression was converted into terms of salt concentration by means of the empirical relationship 100 mM/l. NaCl = $\Delta/0\cdot35°$ C.

Water output and ammonia output

The water output was calculated using the relationship $y = x/z$, where y is the water output in μl./day, x the ammonia output in μM/day and z the ammonia concentration in the rectal fluid in μM/μl.

Unfortunately it is not possible to give a quantitative statement of the accuracy of the results obtained by this method. The water output could be overestimated if, for example, the rate of ammonia output increased relative to that of the water output; if the ammonia output decreased relative to that of the water output an underestimate could result. But although the output range may be exaggerated it was assumed that the average of several determinations would yield an unbiased measure of the water output.

The ammonia output was estimated by depositing the specimen in a 1·8 × 7·4 cm. glass tube containing 5·0 ml. of de-ionized water; the tube was then covered and left undisturbed for 24 hr. in a constant-temperature chamber maintained at 18° C As shown in Fig. 1 the specimen clung to the grid that had been fitted to the bottom of the tube. The ammonia content of the water was estimated by the diffusion-titration method of Shaw & Beadle (1949). Measurements were made in triplicate on 50 μl. samples but before these were taken the tube was shaken to ensure that the rectal fluid had thoroughly mixed with the water.

The ammonia output was also estimated by depositing specimens each in a 100 ml. beaker containing 25 ml. of de-ionized water and 24 hr. later measuring the ammonia content of the water colorimetrically after direct Nesslerization of 5 or 10 ml. samples.

The diffusion-titration and colorimetric methods were compared by carrying out simultaneous estimations of the ammonia content of water containing excretory fluid. The colorimetric method yielded results which were on average 7% higher than those obtained by the diffusion method. A similar discrepancy was found on estimating by both methods the ammonia concentration of pooled samples of rectal fluid. In view of these results it would seem desirable to measure the ammonia concentration
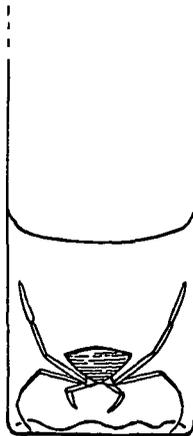

Fig. 1. *Corixa dentipes* in glass tube.

of the rectal fluid and the output ammonia by the same method. However, although the small quantities of fluid available made it necessary to measure the ammonia concentration of the rectal fluid by the diffusion-titration method, the colorimetric method was found to be very convenient in terms of speed and simplicity for measuring output ammonia. The disadvantage of slightly less accurate results seemed to be outweighed by these technical considerations in making a large series of output determinations.

Oral intake of water

This was estimated as before (Staddon, 1963) by measuring the uptake of amaranth from a 0·01 M solution of the dye. The adults were left in the dye for 8 hr.; a longer period appeared to be unsuitable for then there was the danger of losing amaranth by evacuation in the rectal fluid.

In one series the intake of water by the mouth was measured simultaneously with the water output. The change in total body weight was also recorded to provide a measure of the gain or loss of water from the body. The method entailed weighing the specimen under CO_2 narcosis and then depositing it in a glass tube containing 5·0 ml. of the amaranth solution. The specimen soon recovered and clung to the grid, after diving to the bottom, as shown in Fig. 1. Eight hours later the specimen was exposed by tilting the tube, narcotized, removed with the aid of forceps and reweighed. The

ammonia concentration of the rectal fluid, amaranth uptake and ammonia output were then measured in that order.

This work was commenced in September and concluded in the December of 1963.

RESULTS

Water content of the body

The question of whether adults maintain a constant water content under conditions of starvation in de-ionized water was investigated by following the changes in total body weight. Six specimens were weighed within one day after capture and thereafter
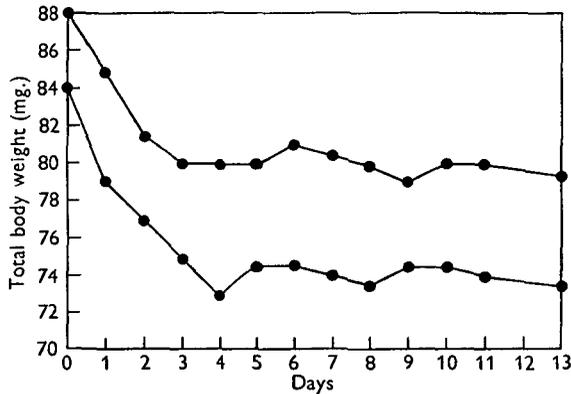

Fig. 2. Weight changes under conditions of starvation in de-ionized water.

daily for a total period of 13 days. The weight changes of all six specimens were essentially similar and two of these, which were selected as being typical, are shown in Fig. 2. These adults showed a sharp loss of weight during the first few days but the total body weight was reduced only slightly during further starvation, on average by about 0·25 mg./day. The weight changes have not been analysed but the initial sharp loss of weight could result from the emptying of the midgut, which is distended with food in freshly captured specimens, from a loss of body water or from a combination of the two. The slow loss of weight associated with further starvation could result from a loss of body water or might be the result of the gradual oxidation of the food reserves of the body.

Although it would appear that the water content of the body is being maintained approximately constant during prolonged starvation a negative water balance could be masked by the compensatory retention of water in the rectum. This possibility was investigated by comparing the rectal fluid weights of freshly collected adults and of adults that had been starved in de-ionized water for 14 days. From the fact that there was no significant difference between the two groups it can be concluded that there is no trend towards increased retention of fluid in the rectum under conditions of starvation of up to 14 days.

The rectal fluid contributes comparatively little to the total body weight. The average rectal fluid weight was found to be 1·9 mg. and the standard deviation ±1·1 mg.; the highest value was 3·2 mg.

The total body water was measured by weighing specimens before and after drying in an oven maintained at 105° C. From the results given in Table 2 it can be seen that the amount of body water in adults of both sexes is about 75% of the total body weight.

Table 2. *Water content of body*

	Water content (% total body weight)	Standard deviation (± %)	No. of specimens
Males	74·3	1·6	7
Females	74·3	2·6	10

Adults starved between 8 and 13 days.

Table 3. *Composition of rectal fluid*

Measurement	Sample no. 1	Sample no. 2	Average
Freezing-point depression (° C.)	0·28	0·12	0·20
Osmotic pressure (mM/l. NaCl)	80	34	57
Ammonium (mM/l.)	83	29	56
Bicarbonate (mM/l.)	72	34	53

Osmotic pressure of the haemolymph and composition of the rectal fluid

Samples of haemolymph were taken from seven specimens that had been starved in de-ionized water for about 10 days. The mean osmotic pressure was found to be 149 mM/l. NaCl and the standard deviation was ± 8·8 mM/l.

The results of the analysis of two samples of rectal fluid are given in Table 3. From these results it would appear that the greater part of the total osmotic pressure of the rectal fluid can be attributed to ammonium bicarbonate. A fluid of similar composition has been shown to be produced by larvae of *Sialis lutaria* (Shaw, 1955) and by adults of *Notonecta* (Staddon, 1963).

The rectal fluid was invariably strongly hypotonic to the haemolymph. The osmotic pressure of the rectal fluid of starving adults, as indicated by measurements of its ammonia concentration, ranged from 20 to 90 mM/l. NaCl and the highest value amounted to only 60% of the osmotic pressure of the haemolymph. It is possible that adults are incapable of separating a solution of ammonium bicarbonate isotonic with the haemolymph but no attempt has been made to verify this experimentally. In the previous work on *Notonecta* there was some evidence to suggest that the ceiling concentration for ammonium bicarbonate in the rectal fluid was of the order of 120 mM/l., or about 70% of the osmotic pressure of the haemolymph.

The rectum is a uniformly thin-walled sac and presumably its main function is to store excreta, rather than to modify it. Some support for this assumption appeared to be provided by the fact that samples of excretory fluid collected after normal discharge showed the same variability in ammonia concentration as samples collected prematurely by applying pressure to the abdomen. The opportunity for making this comparison was provided by the fact that *C. dentipes* adults, by being lighter than water, are compelled to rest on the surface if deprived of suitable support, such as that provided by the grid. Some adults were kept under these conditions and those resting

ventral-side uppermost were seen to eject fluid from the anus. The clear fluid, in volume between 2 and 3 μl., was ejected fairly rapidly to form a droplet on the hydrofuge undersurface of the abdomen. Several samples of fluid were collected immediately after discharge, by touching each droplet with a capillary pipette, and the specimens were in no way disturbed. This method of collecting rectal fluid was not used other than for the purpose described, but is clearly the ideal one if a critical analysis of the excreta be required.

Ammonia output

The ammonia output of starving adults ranged from 0·33 to 2·7 μM/day but rarely exceeded 1·5 μM/day. The modal output was about 1 μM/day in adults that had been starved for about 7 days. The output showed no apparent connexion with the sex or total body weight of the specimens.
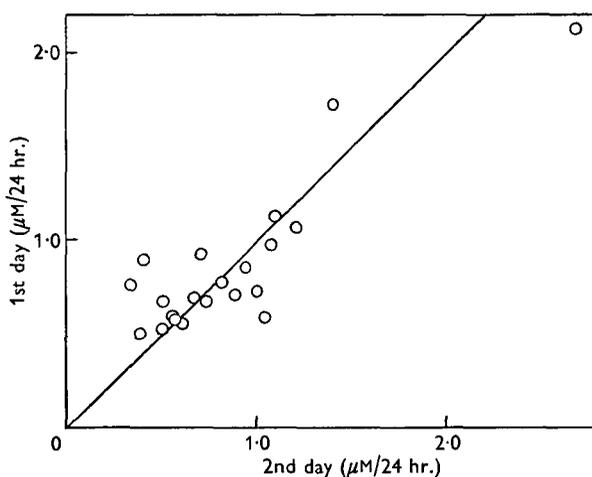

Fig. 3. Output of ammonia on 2 successive days (vol. water 25 ml.; ammonia output estimated by using Nessler's reagent).

Irregularities in the ammonia output were revealed by making measurements on 2 successive days. Such irregularities as are shown by the results plotted in Fig. 3 could lead to inaccuracies in the estimation of the water output from measurements of ammonia output and ammonia concentration of the rectal fluid.

The ammonia concentration of the rectal fluid ranged from 20 to 90 mM/l., the majority of results lying apparently at random within the range 25–70 mM/l.

Water output

The water output, as estimated by measuring the ammonia output and ammonia concentration of the rectal fluid, was found to be variable. Table 4 gives a typical series of results for which the water output ranged from 9 to 30 μl./day. The average output was 16·6 μl./day, or about 19% of the total body weight per day.

A possible relationship between the water output and ammonia output was investigated by making a more extensive series of measurements of ammonia output and ammonia concentration of the rectal fluid. The results were used to construct the

Table 4. *Water output*

Specimen no.	Body weight (mg.)	Rectal fluid NH₄ concentration (mM/l.)	NH₄ output (µM/24 hr.)	Water output (µl./24 hr.)
1	69	21	0·63	30
2	75	28	0·45	16
3	88	85	1·98	23
4	87	67	0·90	13
5	97	41	0·42	10
6	93	62	0·57	9
7	95	47	0·59	13
8	89	33	0·69	21
9	88	55	0·81	15
Av.	87	49	0·78	16·6

Adults starved 15 days; vol. water 5·0 ml.; ammonia output estimated by diffusion-titration.
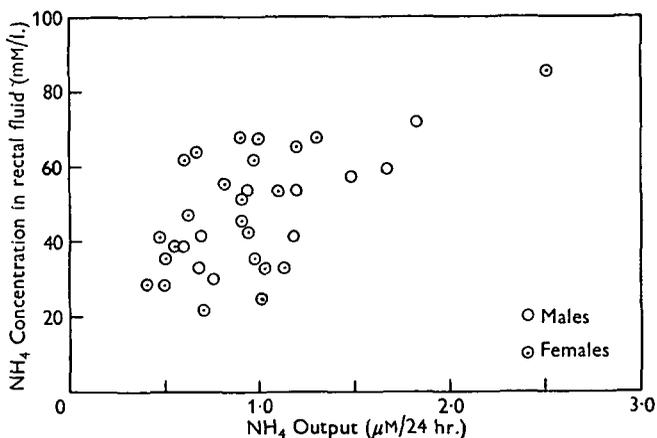

Fig. 4. Ammonia concentration of the rectal fluid and ammonia output (vol. water 25 ml.; ammonia output estimated by using Nessler's reagent).
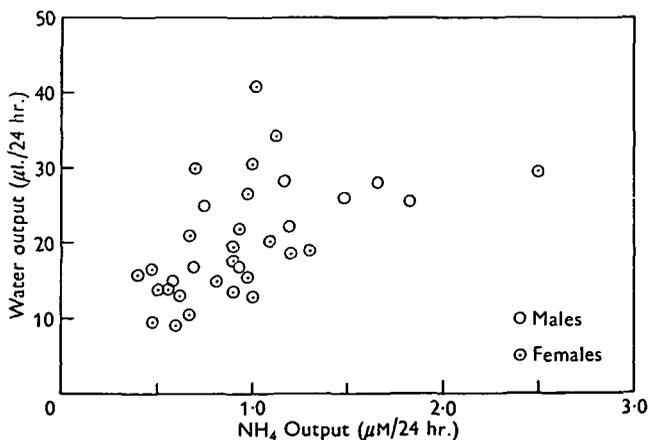

Fig. 5. Water output and ammonia output (vol. water 25 ml.; ammonia output estimated by using Nessler's reagent).

scatter diagram shown in Fig. 4. From these same results the water output was calculated and plotted against ammonia output as shown in Fig. 5. It would appear from the results that the water output has little or no relation to the ammonia output, and this conclusion was unaffected by taking into account the variability in the body weights of the adults. It cannot be concluded, however, that there is no connexion between water output and ammonia output for the methods used in the present work may not have been sufficiently sensitive to reveal one. Furthermore, the majority of specimens exhibited a narrow range of ammonia output. In the previous work on *Notonecta* it was found possible to investigate the water output over a relatively wide range of ammonia output (0·5–4 μM/day) and in these adults the water output was increased at the highest rates of ammonia output.

Water intake

Table 5 gives the results of simultaneous measurements of water output, oral intake, and gain or loss of total body water.

The total output refers to that calculated by measuring the ammonia output and ammonia concentration of the rectal fluid. The output results showed no unusual features.

Table 5. *Intake and output of water (results in μl./8 hr.)*

Measurement	Specimen no.										
	1	2	3	4	5	6	7	8	9	10	11
Initial body weight (mg.)	94	89	93	84	81	94·5	83	96	71	86	87
Total output	3·2	1·9	1·7	5·5	2·7	2·1	2·6	10·8	4·6	2·9	10·5
Water gained	0·0	0·0	0·0	1·0	1·0	0·5	—	—	—	—	—
Water lost	0·0	0·0	0·0	—	—	—	1·0	5·0	3·0	1·0	8·0
Total intake	3·2	1·9	1·7	6·5	3·7	2·6	1·6	5·8	1·6	1·9	2·5
Oral intake	2·6	0·1	0·1	4·7	0·3	0·7	1·6	3·7	2·8	0·7	0·9
Intake deficit	0·6	1·8	1·6	1·8	3·4	1·9	0·0	2·1	−1·2	1·2	1·6

Adults starved 3 weeks in de-ionized water.

The specimens have been grouped, solely for convenience, according as to whether water was gained or lost from the body, and the weight changes in mg. have been given in μl. Specimens 1–3 showed no measurable change in the total water content of the body; specimens 4–6 gained water; a loss of body water was shown by specimens 7–11; the loss was appreciable in specimens 8, 9 and 11.

To provide an estimate of the total water intake the water gained by the body was added to the total output; water lost was subtracted from the output. In most specimens a deficit was apparent on the intake side as shown by subtracting the oral from the total intake, specimens 7 and 9 providing the exceptions.

The value of attempting to balance the results in this way may be questioned in view of the uncertain accuracy of the water output and low accuracy of the measured gain or loss of total body water. But it is encouraging that although the oral intake shows little correspondence to the total water output the correspondence between the oral intake and total intake (which takes into account the change in water content) is quite good as shown by the fairly constant deficit on the intake side. The intake deficit was found to average 1·35 μl./8 hr. and the 95% confidence limits were ±0·81 μl.

These limits correspond to 0·54 and 2·61 μl./8 hr., or about 1·9–7·4% of the total body weight per day. Although the intake deficit could result from a systematic error in technique just such a deficit is to be expected if water is taken up through the cuticle.

It was hoped to obtain some confirmation of the intake deficit by measuring the osmotic intake of water through the cuticle by the method of Holdgate (1956) as modified by Staddon (1963). This method entails killing the adults and measuring the weight increase after a definite period of time in water. The average of seven determinations by this method corresponded to an uptake of 36% of the body weight per day and the standard deviation was ±8·8%. From these results it seems clear that the method grossly overestimates the osmotic uptake in *Corixa* adults. The reason for this is not known. The osmotic uptake through the cuticle of *Notonecta* adults was found by the same method to be of the order of 7% of the total body weight per day, and the results agreed quite well with those obtained by other methods (Staddon, 1963).

A further series of measurements of the oral intake of water was carried out independently of the water output. The intake ranged from 0·1 to 5·9 μl./8 hr. Although the intake was never zero it was frequently less than 1 μl./8 hr. as shown by the distribution of 35 measurements given in Table 6. This feature of the results was unexpected in view of the seemingly high rate of water output. But the adult behaviour may be influenced by the experimental conditions and it is clearly desirable to measure the two variables simultaneously if attempting to correlate them.

Table 6. *Intake of water by the mouth*

Water intake (μl./8 hr.)...	0·1–1	1–2	2–3	3–4	4–5	5–6	Total
No. of specimens	16	6	6	2	1	4	35

Metabolic water

An approximation was arrived at by measuring the oxygen consumption and for simplicity of calculation assuming that fat only was being oxidized. Two determinations were made using a Dixon constant-pressure respirometer (Dixon, 1934). Each adult was provided with a grid and the water level was so adjusted that the air film surrounding the animal was in communication with the air in the flask. Measurements were taken when the oxygen consumption was proceeding at a steady rate. The average rate of uptake was 572 μl./100 mg. body weight/24 hr. at N.T.P. Now the oxidation of 1 μg. fat (palmitin) requires 2 μl. O_2 and yields 1·1 μg. water. The uptake of 572 μl. O_2 thus corresponds to the oxidation of 286 μg. fat and the production of 315 μg. of water, i.e. approximately 0·3 μl./100 mg./day. The production of water by the oxidation of the food reserves is clearly very small in relation to the total water turnover.

DISCUSSION

An attempt has been made to give a complete picture of the water balance in *Corixa dentipes* adults, using comparatively simple methods, but several questions remain unanswered. Of these one of the most urgent is that of the importance of the cuticle as a route of water intake. Evidence was obtained that the osmotic intake of

water contributed significantly to the total intake, but an examination of the problem by more critical methods is clearly desirable. A further question of interest is that of the highest concentration of ammonia that can be reached in the rectal fluid, for this could determine the minimum, obligatory output of water at high rates of ammonia output. It is hoped to examine this question more closely in a future paper in this series.

In *Notonecta* adults the water output was found to be broadly related to the ammonia output; ammonia appeared to be implicated in the regulation of the water output (Staddon, 1963). It was tacitly assumed that water was ingested to offset the potential dehydration resulting from a copious output of water. It seems doubtful, however, whether this conception of the water balance can be applied to *Corixa dentipes* adults. In these adults the water output, as judged by the very low concentration of ammonia in the rectal fluid, frequently appeared to be greatly in excess of that required to eliminate ammonia.

If the high output of water is not explicable in terms of a high osmotic intake of water through the cuticle then it must be attributed to the copious ingestion of water. Unfortunately, the evidence was far from conclusive on this point. It was shown that adults may ingest a great deal of water under experimental conditions but unexpectedly the measured intake was frequently very low.

It may be of significance that whereas other aquatic Heteroptera are carnivorous fluid feeders the Corixidae are omnivorous and may suck in solid as well as liquid food (Sutton, 1951). Their main source of food appears to be the organic ooze at the bottom of lakes and ponds, and Parsons (1959) has suggested that corixids must feed nearly continuously to compensate for the low nutritive value of the material ingested. If this be so a high intake of water incidental to feeding would not be surprising and it is possible that the bugs continue to ingest water automatically even when no food material is present. An investigation of the feeding mechanism in corixids and of the associated water intake would form an interesting extension of the present work.

SUMMARY

1. The water balance in *Corixa dentipes* (Thoms.) has been investigated under conditions of starvation in de-ionized water.

2. The rectal fluid was found to contain almost sufficient ammonium bicarbonate to account for the total osmotic pressure. It was invariably strongly hypotonic to the haemolymph.

3. The water output, as estimated by measuring the ammonia output and ammonia concentration of the rectal fluid, was shown to be appreciable but no connexion was found between the output of ammonia and of water.

4. Adults were shown to gain water by the mouth and some evidence was obtained that the cuticle may be an important route of water intake.

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