

WATER BALANCE AND URINE PRODUCTION IN THE AUSTRALIAN ARID-ZONE CRAB *HOLTHUISANA TRANSVERSA*

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SUMMARY

The permeability to tritiated water (hourly exchange fraction = 0.164) and the rate of urine flow (0.47% body weight/day) are lower than recorded in other freshwater decapods. The calculated net osmotic water flux (2.71% body weight/day) is 5 times the water output in the urine, indicating that there is extrarenal excretion of water. Water balance is maintained in the absence of urine production, again indicating an extrarenal excretory site. Water turnover is greater for crabs in burrows than for crabs kept in air of 98% R.H., indicating relatively favourable conditions of moisture availability at the base of the burrow.

INTRODUCTION

Holthuisana transversa Von Martens is a freshwater/land crab belonging to a large group of Asian freshwater crabs (Parathelphusoidea) and occurs widely in arid and semi-arid areas of inland Australia. Dry periods, commonly lasting up to 18 months, are spent in burrows constructed in heavy clay soils. At the base of the burrow the air is at high relative humidity (R.H.) during the day and dewpoint is generally reached at night (Greenaway & MacMillen, 1978). The crabs only become active after rain or flooding when they lead an aquatic existence. Previous papers have considered modifications of the respiratory system for air-breathing (Greenaway & Taylor, 1976; Taylor & Greenaway, 1979), metabolic adjustments to drought (MacMillen & Greenaway, 1978) and salt and water balance in the terrestrial phase of life (Greenaway & MacMillen, 1978). In this and subsequent papers further aspects of water balance are considered.

Representatives of the other two major groups of freshwater crabs, the Potamoidea (Africa and Europe) and Pseudothelphusoidea (S. America), have an exceedingly low permeability to water. In the Potamoidea, urine production is very low (Shaw, 1959; Harris, 1975) but no information is available on the permeability to water. In *Pseudothelphusa jouyi* permeability has been measured, with tritiated water, and found to be lower than in other freshwater decapods. Unfortunately, data on the rate of urine flow are unreliable (Thompson, 1967, 1970), although a very low rate of flow seems probable. Both Shaw (1959) and Potts & Parry (1964) remarked on the very low rate of urine

flow of *Potamon niloticus* and pointed out that unless permeability was 10 times lower than that of other freshwater decapods an extrarenal site of water excretion must be present. In this paper *Holthuisana* is investigated from two different viewpoints. Firstly, data is presented both for permeability to water and for rate of flow of urine. This provides the first complete set of data for a freshwater crab and clarifies the situation with regard to extrarenal water excretion. The crab is also of considerable interest in its own right in view of its unusually harsh environment and additional data is presented on water balance in air and in field situations.

MATERIALS AND METHODS

Materials

Crabs were collected at Rossmore Station near Bourke, N.S.W., and maintained individually in the laboratory in glass jars containing sand and a few cm of water. They were fed on fish, mealworms and vegetable material. Experiments were carried out in artificial tapwater approximating to Sydney tapwater (NaCl 0.5 mM, Ca(HCO₃)₂ 0.2 mM, Mg(HCO₃)₂ 0.1 mM, Mg SO₄ 0.05 mM, KCl 0.05 mM).

Samples for liquid scintillation counting were mixed with 10 ml of a toluene/triton × 100 scintillation cocktail and counted in Packard tricarb liquid scintillation counter.

Methods

Rates of exchange of water for immersed crabs were measured with tritiated water (THO) using a method similar to that of Motais & Isaia (1972). Crabs were loaded with THO in artificial tapwater for 24 h, transferred briefly to unlabelled water to remove THO from the surface and branchial chambers, and then placed in a volume of unlabelled water several times greater than that of the crab (25–100 ml, depending on the size of the crab). One hundred μl samples of the external medium were removed at intervals until equilibrium was reached between THO in internal and external water compartments. The progressive appearance of THO in the external compartment under these conditions (i.e. where THO is introduced into the internal compartment and measured in the external compartment) is described by the equation

$$Q = \text{initial radioactivity in crab} \times \frac{C_2}{C_1} \frac{V_1}{V_2 + V_1} (1 - e^{-kt}), \quad (1)$$

where

$$k = \left(\frac{1}{V_1} + \frac{1}{V_2} \right) f_{\text{out}} \quad (2)$$

(Shaw, 1963). Q is the radioactivity in the external compartment at time t , Q_{eq} is the radioactivity in the external compartment at equilibrium, k is the rate constant for water turnover between internal and external water compartments, C_1 , C_2 are the concentrations of water in the internal and external compartments and V_1 , V_2 the volumes of these compartments in ml. f_{out} is the efflux of water from the crab in ml/h. When t is equal to infinity, $Q = Q_{\text{eq}}$, which means that equation (1) can be written as

$$Q = Q_{\text{eq}}(1 - e^{-kt}) \quad (3)$$

and the slope of the regression of $\ln(1 - Q/Q_{eq})$ against time, t , gives k . The rate of renewal of internal water, λ , is defined as

$$\lambda = \frac{f_{out}}{V_1} \quad (4)$$

(Motais & Isaia, 1972) so that substituting for f_{out} from equation (2) into equation (4) gives $\lambda = kV_2/(V_1 + V_2)$.

The internal water pool, measured as the total body water content, was obtained by drying the crabs to constant weight in an oven at 105 °C. Haemolymph volume was calculated from the dilution of [³H]inulin injected into the haemolymph. A period of 1 h was allowed between the injection and taking of the blood sample to allow complete mixing. In several cases inulin clearance was followed and dilution of injected inulin calculated from the regression of concentration of inulin in the haemolymph against time. This method yielded similar results to the simpler technique described above.

To measure water exchange in near-saturated air, crabs were loaded with THO as above, rinsed in unlabelled water and dried with compressed air. They were then set up on mesh trays in plastic containers above a saturated solution of $K_2Cr_2O_7$ that provided air at a R.H. of 98%. Water exchange was measured as the decline in specific activity of THO in 10 μ l samples of haemolymph taken at regular intervals over a period of 120 h. The hourly water exchange fraction (λ) was obtained from the slope of \ln radioactivity of the haemolymph against time. A correction was made for solids in the haemolymph. The half-time for water exchange was calculated from the expression $T_{\frac{1}{2}} = \ln 2/k$. The volume of $K_2Cr_2O_7$ was much greater than that of V_1 (25:1) in order to minimize backflux of THO into the animal and under these conditions $\lambda = k$. Weight loss during the experiment was less than 5% of initial weight.

Artificial burrows were constructed in perspex cylinders as described previously (MacMillen & Greenaway, 1978). The cylinders were placed in an upright position, providing a vertical burrow, and a crab loaded with THO placed in each. Crabs were removed at various time intervals and 10 μ l samples of haemolymph taken. From the specific activity of THO in these samples λ and $T_{\frac{1}{2}}$ were determined as before. \ln radioactivity of the haemolymph bore a linear relationship to time.

Water exchange was measured under field conditions at Rossmore station in February 1977 and 1978. In the experiments of 1977, crabs were captured by filling their burrows with water and catching the animals as they emerged. The burrows were then left vacant for several days to allow re-equilibration with the surrounding soil, which was moist at the time. Crabs were loaded with THO and 10 μ l samples of haemolymph were taken for determination of THO. The crabs were then released down their original burrows and left for 3–4 days, after which time they were dug out and a second haemolymph sample taken. The procedure in 1978 was identical except that burrows were manufactured by boring holes 55 cm deep (a common depth of natural crab burrows) with a soil auger, thereby ensuring that the local soil water balance was not disturbed. In this experiment Wescor PT51 soil water potential probes were buried at 55 cm depth, and water potential of the soil (Ψ_s) and temperature of the soil monitored during the experiment using a Wescor HR 33T dewpoint

meter.

Collection of urine on a routine basis has not been achieved due to anatomical difficulties. The opercula fit extremely tightly over the excretory apertures and cannot be moved without causing damage. Furthermore the exhalant respiratory channels are connected with the excretory channel via the abutment, without fusion, of two portions of the carapace and it is likely that urine normally drains into these exhalant channels. In a few cases it has been possible to seal this abutment without also blocking the excretory duct. Surgical removal of the opercula and implantation of cannulae (after allowing time for wounds to heal) then permitted collection of small volumes of urine. The cation concentrations of these were measured with a Varian 175 AB atomic absorption spectrophotometer. As urine samples were exceedingly difficult to obtain, urine flow could not be measured by the usual techniques involving filtration markers, and a traditional technique, measuring weight increase after blocking the excretory organs, was used instead. The opercula covering the excretory apertures were removed surgically and 1–2 weeks was allowed for the wounds to heal. The apertures were then sealed with superglue. The crabs were kept in artificial tapwater and weighed at 1- or 2-day intervals after careful drying in a stream of dry air.

All experiments carried out in the laboratory were performed at 25 °C in a temperature-controlled waterbath.

RESULTS

Water exchange in water

The rate of water exchange in adult crabs at the intermoult stage was extremely low, the mean value being 16.4% body weight/h and $T_{\frac{1}{2}}$ 3.80 h (Table 1). The hourly exchange fraction is indeed lower than measured for other freshwater decapods, including the least permeable crabs examined to date, *Metopaulias depressus* and *P. jouyi* (Thompson, 1970; Rudy, 1967; Subramanian, 1975). A few measurements of water turnover in late premoult and early postmoult stages of *Holthuisana* were made and values 2–3 times higher than those of intermoult specimens obtained. No correlation between λ and body weight was found with the adult crabs used.

Water exchange has also been measured in juvenile crabs taken from females in midsummer. The juveniles are carried in a brood pouch formed by the abdomen and released into water if suitable flooding occurs. Each crab weighed approximately 6.8 mg and measured *ca.* 2 mm across the carapace. For experimental convenience, measurements were made on groups, each of ten crabs, placed in 5 ml of water and using a 10 μ l sample size. Turnover of water was an order of magnitude greater than found for adult crabs. The great size difference does not permit direct comparison of water permeability, however, and the juveniles are perhaps better compared with other small crustaceans rather than adults, e.g. *Gammarus pulex* and *Asellus aquaticus* (Table 5).

Water exchange in air

The rate of exchange of water in crabs kept in near-saturated air (98% R.H.) was about 60 times less than the corresponding rate for crabs in water. This reduction in permeability to water probably reflects a reduction in the possible opportunities for water exchange rather than a reduction in permeability *per se* as the amount of water, even in saturated air, is small. It is also possible that back-diffusion of THO from

Table 1. *The rate constant (λ) and half-time for water exchange ($T_{\frac{1}{2}}$) and the water efflux (f_{out}) in immersed adult and juvenile crabs*

Stage	Body weight (g)	$\lambda \pm$ S.E. (h)	$f_{out} \pm$ S.E. (μ l/g.h)	$T_{\frac{1}{2}} \pm$ S.E. (h)	<i>n</i>
Adult - intermoult	4.02-21.36 \bar{x} 10.4	0.164 \pm 0.014	120 \pm 11	3.80 \pm 0.41	10
Adult - late premoult	9.8-9.9	0.311	225	1.96	2
Adult - early postmoult	5.66	0.530	382	1.12	1
Juveniles	0.006	1.880 \pm 0.103	1741 \pm 93	0.37 \pm 0.02	6

Water exchange by juvenile crabs was measured on six groups each of ten individuals.

Table 2. *Exchange of water crabs in air under field and laboratory conditions*

Medium	<i>n</i>	Mean Ψ (J/kg)	λ (h)	Water flux (μ l/g.h)	$T_{\frac{1}{2}}$ (h)	Temp. ($^{\circ}$ C)	Mean body weight (g)
Air (98% R.H.)	9	-2781	0.0026 \pm 0.0002	1.950 \pm 0.169	274.7 \pm 16.7	25	12.22 (10.35-13.76)
Artificial burrows	6	—	0.0067 \pm 0.0005	5.500 \pm 0.45	106.5 \pm 7.4	25-30	11.49 (10.07-13.26)
Field burrows, Feb. 1977	5	-1750	0.0166 \pm 0.0012	12.040 \pm 0.418	42.6	30*	10.03 (5.13-13.02)
Field burrows, Feb. 1978	8	-3986	0.0078 \pm 0.0011	6.100 \pm 0.896	103.5 \pm 17.0	29.5*	17.74 (14.11-22.67)

Values as means \pm S.E.

* Temperature of soil at the same depth as the base of the burrow. Ψ_0 (water potential) at 98% R.H. was calculated. Ψ_s in 1977 was calculated from soil moisture content using data of Greenaway & MacMillen (1978). Ψ_s in 1978 measured with soil probes.

to tissue water caused the exchange of water to be underestimated. Urine draining into the branchial chambers would enhance this effect.

The turnover of water has also been measured in crabs in artificial burrows in the laboratory and in burrows in the field at Bourke (Table 2). In field experiments carried out in February 1977 the soil was relatively moist and a brief thundershower further moistened the soil during the experiment, although it is doubtful that the crabs encountered free water. By contrast, conditions in the field were much drier in 1978 and this is reflected in the lower Ψ_s at this time (Table 2). The rates of turnover of water in both artificial and field burrows were similar but 3-6 times greater than in near-saturated air. This is probably due to diurnal fluctuations in temperature in the field habitat which are believed to produce condensation at night at the base of the burrow (Greenaway & MacMillen, 1978; P. Greenaway & P. Ikin, unpublished data). Condensation has also been observed on crabs in artificial burrows but not on crabs in the humidity chambers.

Total body water and inulin space

Values for male and female crabs are given in Table 3 and have been expressed as % body volume as well as % body weight. Body water as a percentage of body weight was constant over the range of body size used but the mean value for male crabs was significantly lower than found for females ($0.02 > P > 0.01$). The haemolymph

Table 3. *Inulin space and total body water in crabs maintained in water*

	Males	Females	All crabs
Total body water as % wet wt.	73.1 (21)	76.8 (23)	74.7 ± 0.77 (51)
Total body water as % body volume	77.4 (21)	81.3 (23)	79.1 (51)
Inulin space as % wet wt.	32 (7)	35.6 (26)	34.8 ± 0.72 (33)
Inulin space as % body volume	33.9 (7)	37.7 (26)	36.9 (33)

Values as mean ± s.e. (n).

Table 4. *Cation concentrations in the urine of Holthuisana kept in water*

Crab	Na (mM)	Ca (mM)	Mg (mM)
665 L	56	—	—
R	246	12.6	5.9
654 L	205	—	—
R	234	—	—
658 R	49.2	3.4	1.2
648 L	2.5	—	—
R	135.2	—	—

L and R represent left and right excretory organs.

volume, measured as inulin space, represented a constant proportion of body weight over the size range examined (13.5–30.6 g). Male crabs again had a significantly lower haemolymph volume than female crabs ($0.05 > P > 0.02$). Body volume was found to be linearly related to body weight. Body volume = $0.034 + 0.944$ body weight ($r = 0.997$, $P < 0.001$): volumes given in Table 3 are calculated from this relationship. The regression was identical in both male and female crabs.

Urine composition and rate of flow

Fluid was collected from cannulae implanted in the excretory apertures of crabs in which an attempt had been made to seal the connection between respiratory and excretory channels with 'Supaglu' (Selleys Astrabond). In many crabs this procedure also blocked the excretory organ but in a few crabs fluid was collected and analysed (see Table 4). The data show large discrepancies in Na^+ concentration between individuals and between left and right excretory organs of the same individual. It is probable, therefore, that the low values are caused by dilution of urine with artificial tapwater, due to incomplete blockage of the connection between respiratory and excretory channels. Thus the highest values obtained are likely to be the most correct. The normal haemolymph concentration of Na^+ is about 270 mM (Greenaway & MacMillen, 1978) and it is likely that the urine is of similar concentration to the haemolymph, a situation common to freshwater crabs (de Leersnyder, 1967; Shaw, 1959; Harris & Micallef, 1971; Thompson, 1970).

An estimate of the rate of production of urine was made by measuring the increase in body weight after the excretory openings had been sealed. To check the efficiency of the technique used to seal the openings crabs were injected with [^3H]inulin before the start of the experiment. This substance is excreted only in the urine (Greenaway, in preparation) by *Holthuisana* and its appearance in the medium would indicate leakage of urine. No significant amount of inulin appeared in the water during the

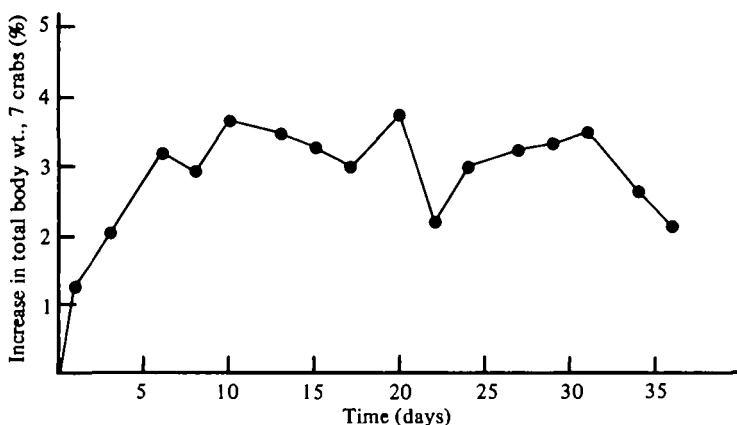


Fig. 1. Changes in mean body weight of seven crabs following blockage of their excretory organs.

experiment. An initial increase in body weight occurred after blocking the excretory apertures (Fig. 1) and a paired t test was used to test for difference between the values of body weight at times 0 and 6 d. The two sets of data were found to be significantly different ($0.01 > P > 0.05$, $n = 7$). Assuming this increase to be due to retained urine, the mean rate of urine production was $0.196 \mu\text{l/g.h}$ equivalent to $4.7 \mu\text{l/g.day}$ or 0.47% body weight/day. In the freshwater crayfish, urine flow is much higher at *ca.* 5% /day (Greenaway, 1972; Bryan, 1960) and a similar value of 4.4% /day has been obtained for the freshwater grapsid crab *Metopaulias* (Thompson, 1970). In crabs of the Potamoidea and Pseudothelphusoidea, urine flow is of a similar order to that found for *Holthuisana*, although technical difficulties have often prevented precise measurement of such low rates of flow; *P. niloticus* $0.05\text{--}0.6\%$ body weight/day (Shaw, 1959), *Potamon edulis* 0.58% body weight/day (Harris, 1975), *Sudano-nautes africanus ca.* 0.005% body weight/day (Lutz, 1969) and *P. jouyi* $0.4\text{--}8.4\%$ body weight/day (Thompson, 1970). Flow of urine in the parathelphusoid *Holthuisana*, then, is at the lower end of this range. After 8 days the increase in body weight ceased and body weight was maintained around the new level until the experiment was terminated (Fig. 1). As no leakage of urine occurred it follows that osmotic uptake of water was eliminated by an extrarenal mechanism.

DISCUSSION

The net osmotic flux of water into *Holthuisana* has been calculated to be 0.92% of the total water flux (Table 1) (using a value of 525 m osmol for the concentration of the haemolymph (Greenaway & MacMillen, 1978) and 2 m-osmol for artificial tapwater). The net flux of water, then, is $26.6 \mu\text{l/g.day}$, equivalent to 2.71% body weight/day or 3.63% total body water/day, a value 6 times the measured rate of flow of urine. In previous investigations of freshwater animals the calculated net flux, based on THO or D_2O measurements, has frequently been found to underestimate urine production (Potts & Parry, 1964; Smith, 1976). In *Holthuisana*, however, the net flux greatly exceeds urine flow and an extrarenal site of water excretion is indicated.

Table 5. *Rates of exchange of water in some freshwater crustaceans*

Species	λ corrected to 20 °C (h)	Temperature of measurement (°C)	Reference
<i>Holthuisana transversa</i>	0.12	25	This paper
<i>Metopaulias depressus</i>	0.24	ca. 20	Thompson (1970)
<i>Pseudothelphusa jouyi</i>	0.24	ca. 20	Thompson (1970)
<i>Astacus fluviatilis</i>	0.40	10	Rudy (1967)
	0.39	13	Subramanian (1975)
<i>Procambarus clarki</i>	0.46	ca. 20	Born (1970)
<i>Astacus leptodactylus</i>	0.30	15	Ehrenfeld & Isaia (1974)
<i>Gammarus pulex</i>	3.59	20	Lockwood (1961)
<i>Asellus aquaticus</i>	1.66	—	Lockwood (1959)

Values were corrected to 20 °C assuming a Q_{10} of 2.

Table 6. *Body water and haemolymph volume in some aquatic and terrestrial decapods*

Species/habitat	Total body water (% body wt)	Haemolymph vol. (% body wt)	Reference
<i>Holthuisana transversa</i> T/FW	74.7	34.8 I	This paper
<i>Sudanonautes africanus</i> FW/T	58.3	—	Lutz (1969)
<i>Potamon sidneyi</i> FW/T	65.7	—	Dandy & Ewer (1961)
<i>P. depressus</i> FW/T	65.8	—	Dandy & Ewer (1961)
<i>P. warreni</i> FW/T	67.4	—	Dandy & Ewer (1961)
<i>Pseudothelphusa jouyi</i> FW	67.4	22.9 I	Thompson (1970)
<i>Metopaulias depressus</i> FW	64.8	29.7 I	Thompson (1970)
<i>Gecarcinus lateralis</i> T/M	66.2	22.0 S	Flemister (1958), Skinner (1965)
<i>Ocypode albicans</i> T/M	69.9	21.5 I	Flemister (1958)
<i>Carcinus maenas</i> M	65.4	19.4 I	Robertson (1960) Binns (1969)
<i>Astacus leptodactylus</i> FW	77.0	—	Ehrenfeld & Isaia (1974)
<i>Austropotamobius pallipes</i> FW	74.0	26.0 EB	Greenaway (unpublished), Bryan (1960)

I = inulin space, S = [¹⁴C]sucrose space, EB = Evans-blue space.

The maintenance of water balance, despite cessation of urine formation, similarly indicates an extrarenal site of water elimination. It appears that water balance is normally maintained by elimination of incoming water both renally and extrarenally but the extrarenal system alone is capable of coping with the entire net influx of water. Extrarenal water excretion was suggested for *P. jouyi* (Thompson, 1970) and *P. niloticus* (Shaw, 1959) and may well be a characteristic of freshwater crabs generally. In grapsid crabs the gut has been implicated as a site of water elimination (Heeg & Cannone, 1966), whilst the foregut of *Gecarcinus lateralis* is also involved in salt and water transport (Mantel, 1968). Further studies are necessary to determine the site of extrarenal water elimination in *Holthuisana*.

The permeability of *Holthuisana* to water is lower than measured in other freshwater decapods, although it is of the same general order of magnitude (Table 5). Blood osmolalities are also similar between freshwater crabs and crayfish and it is reasonable to suppose that net osmotic influx of water is also of the same order. The chief difference between these two groups of freshwater decapods lies not in permeability to water but in the route by which water is eliminated. In crayfish, osmotic uptake

of water is compensated for by excretion in the urine, whilst in *Holthuisana*, and probably also in other freshwater crabs, the major route is extrarenal (Potts & Parry, 1964; Shaw, 1959; Thompson, 1970).

The low permeability to water shown by *Holthuisana* is reflected in a rate of evaporative water loss to air lower than in other terrestrial crustaceans examined (Greenaway & MacMillen, 1978). This is of obvious adaptive value for a crab leading a predominantly terrestrial life. Water turnover in crabs in field burrows was considerably greater than for crabs kept in air of 98% R.H. (Table 2), although Ψ_s of the former was lower than Ψ_a in the humidity chambers. The explanation of this anomaly lies in the temperature regimes in the two experiments. The humidity chambers were maintained at 25 °C whilst temperature at the base of the burrows fluctuates considerably on a diurnal basis. In fact the temperature drop at night produces saturated conditions and causes dew formation in the lower part of the burrow from sunset to sunrise (Greenaway & MacMillen, 1978), and the resultant exchange of THO with water condensing on the body surface and with saturated air ($\Psi_a = 0$) would be considerably greater than in the constant R.H. experiments. This again emphasises that the burrow plays an important role in the water balance of this species. Dehydrated crabs have, however, been collected in the field in summer (Greenaway & MacMillen, 1978) and this condition is possibly due to a combination of unfavourable weather conditions (high winds accompanied by high temperature) causing excessive water loss during the day. It is interesting, therefore, to consider the water reserves within the crab. The values given for total body water and inulin space are higher than reported in the literature for other aquatic and terrestrial crabs (Table 6) and it is possible that this represents an adaptation to arid conditions. It is difficult to make strict comparisons, though, as the water content expressed as percentage of body weight is affected by the weight of the exoskeleton. The exoskeleton in *Holthuisana* is relatively lightly calcified (personal observation) and may, therefore be responsible in part for the high proportion of body water. To enable strict comparisons it is necessary to express haemolymph and body water volume as a percentage of body volume rather than weight as this, to a large extent, nullifies the variable contribution of the skeleton and eliminates much of the associated error.

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