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POTASSIUM TRANSPORT IN THE FRESHWATER BIVALVE *DREISSENA POLYMORPHA*

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Summary

Potassium transport and blood ion composition were examined in the freshwater bivalve *Dreissena polymorpha*. Animals acclimated to artificial pondwater (APW, $[K^+]$=0.05 mmol l^{-1}) for 4 weeks gradually lost Na^+ and Cl^-, but the blood K^+ concentration remained constant near 0.5 mmol l^{-1}. Blood $[K^+]$ in *D. polymorpha* declined by 41 % after 1 week of exposure to K^+-free APW. Conversely, blood $[K^+]$ rose to 1.52±0.05 mmol l^{-1} (mean ± S.E.M.) 24 h after exposure to 0.5 mmol l^{-1} K^+ APW. Total tissue K^+ content remained stable in animals maintained in APW, but fell significantly in animals exposed to K^+-free APW for 2 weeks. The net K^+ flux (J_{net}) for animals incubated in APW, with an average K^+ concentration of 0.07 mmol l^{-1}, was −0.27±0.06 μequiv g^{-1} dry tissue h^{-1}, significantly different from the value of 0.50±0.08 μequiv g^{-1} dry tissue h^{-1} for animals transferred to 0.30 mmol l^{-1} K^+ APW. A transepithelial membrane potential of −3.6±0.7 mV (blood negative compared with the bathing medium) was measured in APW-acclimated mussels. Potassium influx was measured with ^{42}K and displayed Michaelis–Menten saturation kinetics at dilute K^+ concentrations. The K_m was 0.084±0.054 mmol l^{-1} and the J_{max} was 1.74±0.39 μequiv g^{-1} dry tissue h^{-1}. Both the K_m and J_{max} for animals exposed to K^+-free APW for 7 days were unchanged. Using ^{86}Rb, qualitatively similar transport characteristics were observed for animals incubated in K^+-free, Rb^+ APW, but the 22 day K^+ depletion time significantly increased J_{max}. *D. polymorpha* compensated for changes in the ionic composition of the acclimation medium by tolerating alterations in blood solute composition and adjusting ion transport rates.

Key words: potassium transport, rubidium transport, zebra mussel, freshwater bivalve, mussel, *Dreissena polymorpha*.

Introduction

Recent evidence in *Dreissena polymorpha* (zebra mussel) has shown that the ionic profile of its blood and its tolerance towards varying ionic compositions of fresh water are distinct from those of other freshwater bivalves (Horohov *et al*. 1992; Dietz *et al*. 1994). Although it has long been recognized that freshwater bivalves are sensitive to the potassium concentration of fresh water (Imlay, 1973; Daum *et al.* 1979), *D. polymorpha* exhibit a low tolerance towards elevated potassium concentrations (Fisher *et al*. 1991).

In a study of potassium transport in two unionids and a corbiculid, Dietz and Byrne (1990) hypothesized that excess potassium in the blood may depolarize excitable tissue and ultimately determine tolerance limits for freshwater bivalves with respect to environmental potassium concentrations. However, the mode of action of potassium toxicity remains unclear and little information is available regarding changes in zebra mussel ion transport in response to changes in ambient potassium concentrations (Fisher *et al*. 1991).

Several studies have investigated the effects of ion depletion on bivalve physiology and the subsequent changes in ion transport rates (Krogh, 1939; Murphy and Dietz, 1976; Scheide and Dietz, 1982). *Dreissena polymorpha*, however, has an unusually low tolerance for deionized water (Nichols, 1993; Ram and Walker, 1993; Dietz *et al*. 1994) but does respond to salt depletion with characteristic increases in ion transport activity (Krogh, 1939; Vinogradov *et al*. 1993).

This study examines the effects of potassium concentration in the acclimation medium on temporal changes in *D. polymorpha* blood ion composition and total potassium content of the tissue. The effects of potassium and rubidium concentration on the transepithelial membrane potential and unidirectional transport are also investigated. Ultimately, these studies provide insight into the mechanisms of bivalve adaptation to the freshwater environment.

Materials and methods

Animals

Zebra mussels (*Dreissena polymorpha* Pallas) were collected from Lake Erie at the mouth of the Raisin River, Michigan, during May, June and October 1993. Animals were kept, unfed, for 7–14 days before use at 22±2 °C in aquaria

containing aerated artificial pondwater (APW, composition in mmol l^{-1}: 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, 0.05 KCl, 0.2 $MgSO_4$) (Dietz *et al*. 1994). Some of the animals utilized in ion flux studies were kept at 8 °C and were stepwise acclimated to 16 °C and 22±2 °C for a minimum of 5 days at each temperature before use in experiments. Aquarium water was routinely replaced every 2–3 days. Larger specimens (1.5–3 cm shell length, 25–75 mg dry tissue mass) were selected for study and only those animals attached by byssal threads to the container or to another mussel were utilized. All acclimation water and containers were treated with 1 % chlorine bleach for 24 h before being discarded to avoid contaminating local water systems with mussels or veliger larvae.

Zebra mussels do not survive in deionized water beyond 3–5 days (Nichols, 1993; Ram and Walker, 1993). To minimize the disruptive effects of overall solute loss on K^+ balance, animals were exposed to media containing ions essential for survival, but selectively deficient in K^+ (Dietz *et al*. 1994). Animals utilized in depletion studies were maintained at a density of 20 animals l^{-1} and the water was changed daily to minimize reabsorption of ions lost by other animals. All acclimation solutions were periodically assayed to ensure consistency of solute composition.

Blood solute and total potassium analyses

Blood samples were obtained by pericardial puncture following the methods of Fyhn and Costlow (1975). All samples were centrifuged at 15 000 ***g*** min^{-1} before use. Total blood osmolarity was determined by freezing point depression. Sodium, potassium and rubidium concentrations were determined by flame emission photometry. Calcium and magnesium concentrations were diluted with $LaCl_3$/HCl and assayed using an atomic absorption spectrophotometer. Chloride concentrations were determined by electrometric titration. The difference between the total solute and the sum of the measured ions was identified as 'other' and may be attributed mostly to bicarbonate (see Horohov *et al*. 1992).

Total potassium content of the tissue was determined from diluted tissue digests. Mussels were dissected from the shell, dried at 95 °C (minimum 12 h) and weighed to the nearest milligram. The dried tissue was digested with concentrated HNO_3, diluted to a standard volume with distilled water and analyzed. Tissue potassium content was normalized to μequiv g^{-1} dry tissue.

Transepithelial potentials

In vivo transepithelial potentials were measured following the methods of Dietz and Branton (1975). The thickness of the valve above the pericardial region was reduced to the nacreous layer by abrasion with a drill bit, and a small glass tube was glued with epoxy resin over the area. The remaining shell was removed and a polyethylene bridge containing 20 mmol l^{-1} KCl in 3 % agar was placed in the blood present in the tube. The other end of the bridge was connected to a calomel electrode attached to a recording potentiometer. A similar reference bridge was placed in the bathing medium and connected to a second calomel half-cell. Potentials were measured with the animal fully immersed and while actively siphoning. Values were corrected for voltage asymmetry between the bridges and are reported with the sign of the voltage being the blood value with the bath as reference. Bathing solutions consisted of APW at various K^+ concentrations, K^+-free APW at various Rb^+ concentrations, Na_2SO_4 and $CaCl_2$, and were exchanged by a flow-through system to reduce disturbance to the animal.

Flux studies

The night before an experiment, the water in the aquaria containing the animals to be studied was replaced. Approximately 30 min before the start of an experiment, animals were removed from their storage containers by severing the byssal threads and rinsed in deionized water to remove any adsorbed ions. Single animals were placed in covered plastic containers with 20 ml of ^{42}K-labelled APW at specific K^+ concentrations. Bath samples were removed after the animals resumed siphoning (30–45 min) and at a timed interval. Net K^+ flux (J_{net}) was calculated from the change in K^+ concentration of the bathing medium. Unidirectional K^+ influx (J_i) was calculated from the disappearance of isotope from the bathing medium (Graves and Dietz, 1982). Sample radioactivity was determined by a liquid scintillation system in a Triton X-114/xylene-based cocktail (Weigman *et al*. 1975). Correction factors for decay were not applied because samples from the same animal were counted consecutively within 10 min. Potassium efflux (J_o) was calculated by difference ($J_o = J_{net} - J_i$). Potassium-deficient animals were acclimated to K^+-free APW for a minimum of 7 days before use in flux experiments. All fluxes were normalized to μequiv g^{-1} dry tissue h^{-1}.

Unidirectional rubidium fluxes were determined with ^{86}Rb by substituting RbCl for KCl in the incubation bath (K^+-free, Rb^+ APW). The notable advantage ^{86}Rb provides over ^{42}K in transport studies is a longer half-life (^{42}K, 12.36 h; ^{86}Rb, 18.65 days) (Walker *et al*. 1989), and *D. polymorpha* tolerated elevated [RbCl] better than elevated [KCl] (S. J. Wilcox and T. H. Dietz, unpublished observations). Rubidium is not detectable in the body fluids of mussels and, therefore, there can be no short-term efflux and the influx should equal the net flux (Dietz and Byrne, 1990).

The Michaelis–Menten kinetics of K^+ and Rb^+ transport were determined from the relationship:

$$J_i = (J_{max} \times [ion])/(K_m + [ion]),$$

where J_{max} is the maximum influx coefficient and K_m is the reciprocal of affinity. The range of Rb^+ concentrations was sufficient for a more complex analysis and a linear component (*y*-intercept held constant at zero) was added to the relationship:

$$J_i = \{(J_{max} \times [ion])/(K_m + [ion])\} + (D \times [ion]),$$

where D is the slope of the diffusive component. Influx values

were subsequently plotted as mean ± standard error of the mean for each concentration.

Statistical analyses

Data were calculated as mean ± S.E.M. Differences between means were tested for homogeneity of variance and compared with the Student's *t*-test at an alpha level of 0.05. One-way analyses of variance (ANOVAs) were performed at an alpha level of 0.05 to examine time-course differences in blood solute composition and total K^+ content of dried tissue. Significant ANOVA values were examined with the Tukey Studentized range procedure at an alpha level of 0.05, or with the Tukey–Kramer modification when sample sizes were unequal (SuperANOVA, Abacus Concepts Inc., Berkeley, CA). Ion flux kinetics were fitted to a rectangular hyperbola by nonlinear regression analyses using the computer program Inplot 4 (GraphPad Software Inc., San Diego, California).

Results

Blood ions

The blood ion concentration of unfed *Dreissena polymorpha* gradually declined with extended acclimation to APW. Na^+ and Cl^- are the principal solutes of mussel blood and decreases in the concentration of these ions contributed to a decline in total solutes (Table 1). K^+ and Mg^{2+} are minor constituents of zebra mussel blood and the concentrations of these ions remained constant with acclimation to APW. Blood K^+ concentrations declined 41 % following 1 week of exposure to K^+-free APW (Table 1). Total solute and ancillary ion concentrations in animals exposed to K^+-free APW declined in a similar fashion to those of APW-acclimated animals. There was virtually no mortality in the animals exposed to K^+-free APW over the 4 week period.

Potassium concentrations in the blood doubled within 8 h in animals acutely transferred from APW to pondwater with $10\times$[KCl] ($0.5\,mmol\,l^{-1}$) (Table 2). A maximum blood concentration of $1.52\pm0.05\,mmol\,l^{-1}$ K^+ was observed after 24 h of exposure. Potassium concentrations remained elevated throughout the 2 week period. Sodium concentration in the blood declined between 8 h and 2 weeks, but [Cl^-] remained constant even though the bath [Cl^-] was elevated from 1.35 to $1.80\,mmol\,l^{-1}$. The initial [Mg^{2+}] was unexplainably low, but stabilized at a more normal concentration by 24 h and remained constant for 2 weeks.

Total tissue potassium content

Over 2 weeks, the total K^+ content of the mussel tissue remained stable in animals acclimated to APW at 22±2 °C (Fig. 1). Conversely, the animals exposed to K^+-free APW lost significantly more tissue K^+ than those animals acclimated in the presence of $0.5\,mmol\,l^{-1}$ K^+ (APW ANOVA, F=3.86, P<0.01, d.f. 5,43; $0.5\,mmol\,l^{-1}$ APW ANOVA, F=10.74, P<0.0001, d.f. 5,36).

Transepithelial potentials

When measured in APW, the *in vivo* transepithelial potential (TEP) for APW-acclimated animals was −3.6±0.7 mV, blood negative compared with the bath (Table 3). The TEP appeared to be independent of K^+ and Rb^+, since increasing the concentration of these cations in the external medium had no effect on the potential difference over the range tested. Elevated Na^+ and Ca^{2+} concentrations significantly increased the TEP (Na^+ ANOVA, F=6.11, P<0.001, d.f. 6,42; Ca^{2+} ANOVA, F=3.58, P<0.05, d.f. 3,24).

Ion transport kinetics

Mussels acclimated to APW were in negative K^+ balance near the K^+ concentration of APW, yet were able to maintain positive K^+ balance at $[K^+]\geqslant0.16\,mmol\,l^{-1}$ (Table 4). Conversely, animals exposed to K^+-free APW for 7 days exhibited a net K^+ uptake when returned to APW containing

Table 1. *Blood ion composition of* Dreissena polymorpha *acclimated to artificial pondwater or K^+-free APW at 22±2 °C for 4 weeks*

Time (weeks)	Total (mosmol l^{-1})	Concentration (mmol l^{-1})					Other (mosmol l^{-1})
		Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	
Artificial pondwater							
0	43±1[a]	17.1±0.5[a]	3.1±0.3[a]	0.38±0.03[a]	0.49±0.03[a]	16.3±0.4[a]	5.7±0.4[a]
1	43±1[a]	16.9±0.1[a]	2.9±0.2[a]	0.39±0.04[a]	0.51±0.04[a]	16.9±0.5[a]	5.6±0.8[a]
4	36±1[c]	13.0±0.5[b]	3.0±0.5[a]	0.33±0.02[a]	0.48±0.04[a]	14.0±0.3[b]	5.3±0.9[a]
K^+-free APW							
1	40±1[a,b]	17.6±0.5[a]	2.4±0.2[a,b]	0.34±0.03[a]	0.29±0.03[b]	15.7±0.3[a,b]	3.9±1.5[a]
4	37±1[c]	15.3±0.5[a,b]	1.7±0.1[b]	0.37±0.02[a]	0.23±0.02[b]	14.4±0.7[b]	5.4±0.5[a]

Values are mean ± S.E.M. (N=7 for each time interval).
Means within a column having different letters are significantly different by the Tukey Studentized range procedure, P<0.05.
Values for total solute have been rounded to two significant figures.
'Other' represents the difference between the total solute and the sum of the measured ions.
The composition of artificial pondwater is given in Materials and methods.

Table 2. *Blood ion composition of* Dreissena polymorpha *following acute transfer to 0.5 mmol l^{-1} K^+ artificial pondwater*

Time	Total (mosmol l^{-1})	Concentration (mmol l^{-1})					Other (mosmol l^{-1})
		Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	
0 h	43±1[a]	16.4±0.3[a]	3.0±0.3[b]	0.20±0.04[b]	0.52±0.02[c]	16.6±0.4[a]	6.3±0.6[a]
8 h	41±2[a]	15.2±0.7[a,b]	2.7±0.3[b]	0.30±0.03[a,b]	1.17±0.09[b]	16.6±0.8[a]	5.1±1.2[a]
24 h	45±2[a]	15.8±0.8[a,b]	3.0±0.2[b]	0.40±0.02[a]	1.52±0.05[a]	17.7±1.0[a]	6.4±0.5[a]
48 h	42±1[a]	14.6±0.6[a,b,c]	3.3±0.3[b]	0.40±0.04[a]	1.46±0.03[a]	16.9±0.5[a]	5.0±0.4[a]
1 week	43±1[a]	12.4±0.4[c]	4.9±0.5[a]	0.40±0.03[a]	1.38±0.03[a,b]	17.1±0.6[a]	6.3±0.7[a]
2 weeks	40±0[a]	13.3±0.8[b,c]	3.6±0.1[a,b]	0.42±0.04[a]	1.38±0.05[a,b]	16.4±0.3[a]	5.2±1.9[a]

Values are mean ± S.E.M. (N=7 for each time interval).
Means within a column having different letters are significantly different by the Tukey Studentized range procedure, P<0.05.
Values for total solute have been rounded to two significant figures.
'Other' represents the difference between the total solute and the sum of the measured ions.

K^+ concentrations above 0.11 mmol l^{-1}. Potassium efflux did not change significantly in K^+-deficient animals compared with the APW-acclimated mussels. Neither the J_{max} nor the K_m of potassium influx changed significantly after 7 days of K^+ depletion (see Table 6).

Unidirectional Rb^+ fluxes for APW-acclimated animals incubated in K^+-free, Rb^+ APW are shown in Table 5. Net Rb^+ uptake was significantly different from zero at all Rb^+ concentrations examined. As predicted, for Rb^+, J_{net}=J_i and J_o was not significantly different from zero (J_o data not shown). The average net Rb^+ flux for K^+-deficient animals exposed for 22 days to K^+-free APW was 61% greater than the J_i of animals acclimated to APW.

The J_{max} of Rb^+ transport in APW-acclimated animals was significantly higher than the J_{max} of K^+ transport (Table 6). Utilizing the Michaelis–Menten rectangular hyperbolic equation, the J_{max} of Rb^+ transport for K^+-deficient animals was significantly greater than the J_{max} of Rb^+ transport for APW-acclimated mussels and 57% higher than K^+ transport in K^+-deficient animals. The K_m values for both K^+ and Rb^+ influx did not change significantly with K^+ depletion.

A passive diffusion component of Rb^+ influx was tentatively identified in APW-acclimated animals (Fig. 2). When the [Rb^+] of the bath was increased to 0.73 mmol l^{-1}, the influx values of the three highest Rb^+ concentrations appear to have a linear relationship rather than approaching J_{max}

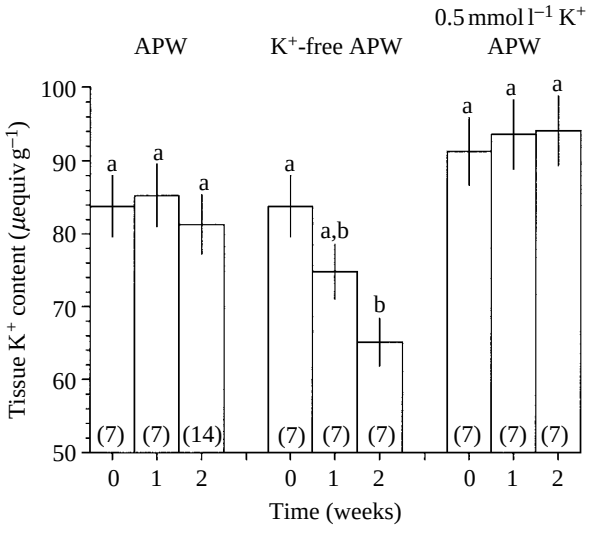

Fig. 1. Mean total tissue K^+ content of *Dreissena polymorpha* acclimated at 22±2 °C to artificial pondwater (APW), K^+-free APW and 0.5 mmol l^{-1} K^+ APW with the sample size in parentheses. Error bars are ± 1 S.E.M. Means with different letters within a group are significantly different by the Tukey–Kramer Studentized range procedure, P<0.05. Values for animals exposed to K^+-free APW are significantly different from both the APW and 0.5 mmol l^{-1} K^+ APW groups (see text).

Table 3. In vivo *transepithelial potentials between the blood and the bathing medium for* Dreissena polymorpha

Cation concentration (mmol l^{-1})	Transepithelial potential difference (mV)*			
	K^+	Rb^+	Na^+	Ca^{2+}
0	–3.2±0.7[a]			
0.05	–3.6±0.7[a]	–3.3±0.6[a]		
0.3	–3.6±0.6[a]	–3.3±0.6[a]		
0.4				–3.0±0.6[b]
0.5	–3.4±0.5[a]	–3.0±0.5[a]	–4.0±0.7[b,c]	
1.0	–3.4±0.5[a]	–3.3±0.5[a]	–5.3±0.7[c]	–2.5±0.8[a,b]
1.5	–3.3±0.6[a]	–3.1±0.5[a]		
2.0			–4.9±0.6[c]	–1.3±0.7[a,b]
5.0			–4.1±0.6[b,c]	0.2±0.9[a]
10.0			–2.6±0.7[a,b,c]	
20.0			–1.5±0.8[a,b]	
50.0			–0.6±0.9[a]	

The effects of K^+ and Rb^+ were measured in artificial pondwater.
*Sign of the blood compared with the bathing medium; values are mean ± S.E.M. (N=7).
Means within a column having different letters are significantly different by the Tukey Studentized range procedure, P<0.05.

Table 4. *Unidirectional K^+ fluxes for APW-acclimated and K^+-deficient* Dreissena polymorpha *incubated in APW at various K^+ concentrations*

	Unidirectional fluxes (μequiv g^{-1} dry tissue h^{-1})						
	APW-acclimated				K^+-deficient		
Average bath [K^+] (mmol l^{-1})	J_{net}	J_i	J_o	Average bath [K^+] (mmol l^{-1})	J_{net}	J_i	J_o
0.07	–0.27±0.06*	0.83±0.10	1.10±0.13	0.06	0.33±0.20	0.98±0.22	0.65±0.11
0.13	–0.01±0.05	1.05±0.14	1.06±0.12	0.11	0.26±0.14	1.20±0.13	0.95±0.16
0.16	0.11±0.05	0.97±0.15	0.86±0.12	0.14	0.62±0.12*	1.48±0.20	0.87±0.13
0.30	0.50±0.08*	1.43±0.21	0.93±0.18	0.26	0.57±0.12*	1.80±0.25	1.23±0.17
				0.51	1.15±0.19*	2.10±0.21	0.95±0.19

K^+-deficient animals were exposed for 7 days to K^+-free APW.
Values are mean ± S.E.M. (N=14–15).
All J_i and J_o values are significantly different from zero with the Student's t-test, $P<0.05$.
*J_{net} significantly different from zero with the Student's t-test, $P<0.05$.
APW, artificial pondwater.

asymptotically. Separating the diffusive component from the total influx resulted in a significantly better-fitting curve than using the rectangular hyperbola alone (F=11.4, $P<0.01$, N=1,65):

$$J_i = \{(1.12\pm0.42[Rb^+])/(0.005\pm0.022 + [Rb^+])\} + (3.84\pm0.82[Rb^+]).$$

At Rb^+ concentrations above 0.3 mmol l^{-1}, the diffusive component exceeded that of the saturable transport component that we consider to be due to active transport.

Discussion

Dreissena polymorpha blood K^+ concentration is maintained near 0.5 mmol l^{-1} for extended periods by active epithelial K^+ transport opposing high renal or extra-renal K^+ losses. The maximum calculated rate of K^+ influx into APW-acclimated animals is 1.74±0.39 μequiv g^{-1} dry tissue h^{-1}, but 7 days of K^+ depletion did not elevate J_{max}. Although Na^+ and Cl^- transport by zebra mussels is markedly greater than that by other freshwater bivalves (Horohov *et al.* 1992), the rate of K^+ influx is approximately the same as in *Corbicula fluminea* (Dietz and Byrne, 1990). In contrast, potassium efflux does not appear to be reduced in *D. polymorpha* exposed to K^+-deficient waters compared with that in other freshwater bivalves.

To demonstrate that an ion is actively transported, it is necessary to show that movement is against an electrochemical gradient. The measured *in vivo* transepithelial potential of *D. polymorpha* in APW is −3.6±0.7 mV (blood negative compared with the bath). Ussing's (1949) passive flux ratio

Table 5. *Unidirectional Rb^+ fluxes for APW-acclimated and K^+-deficient* Dreissena polymorpha *incubated in K^+-free APW at various Rb^+ concentrations*

	Unidirectional fluxes (μequiv g^{-1} dry tissue h^{-1})						
	APW-acclimated				K^+-deficient		
Average bath [Rb^+] (mmol l^{-1})	N	J_{net}*	J_i*	Average bath [Rb^+] (mmol l^{-1})	N	J_{net}*	J_i*
0.04	12	0.96±0.09	1.03±0.12	0.03	14	1.34±0.15	1.53±0.16
0.07	7	1.26±0.29	1.46±0.32	0.08	15	2.32±0.23	2.27±0.26
0.13	13	1.58±0.11	1.77±0.11	0.14	14	2.30±0.35	2.14±0.34
0.26	9	1.92±0.26	2.21±0.34	0.22	14	2.96±0.23	2.85±0.25
0.36	10	1.94±0.12	2.10±0.23	0.36	15	3.48±0.38	3.68±0.34
0.50	9	2.56±0.25	2.72±0.29				

K^+-deficient animals were exposed to K^+-free APW for 22 days.
Values are mean ± S.E.M.
*All values are significantly different from zero with the Student's t-test, $P<0.05$.
APW, artificial pondwater.

Table 6. *Michaelis–Menten kinetic coefficients of K^+ and Rb^+ influx for* Dreissena polymorpha *acclimated to APW and K^+-free APW*

Acclimation medium	J_{max} (μequiv g^{-1} dry tissue h^{-1})	K_m (mmol l^{-1})
Potassium influx		
APW	1.74±0.39	0.084±0.054
$-K^+$	2.47±0.34	0.095±0.038
Rubidium influx		
APW	2.82±0.2*	0.071±0.023
$-K^+$	3.88±0.43†	0.064±0.023

Values are calculated from a rectangular hyperbolic equation using influx measurements obtained at K^+ and Rb^+ concentrations below 0.51 mmol l^{-1}.

*Significantly different from APW potassium J_{max} with the Student's *t*-test, $P<0.05$.

†Significantly different from APW rubidium and $-K^+$ J_{max} for potassium with the Student's *t*-test, $P<0.05$.

APW, artificial pondwater; $-K^+$, K^+-free artificial pondwater.

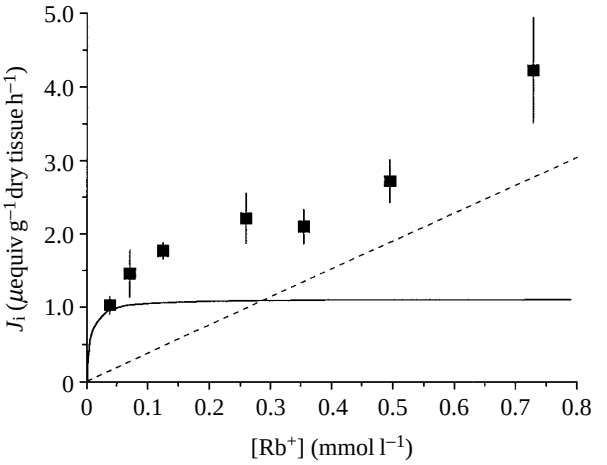

Fig. 2. Partitioning of the mean total Rb^+ influx (filled symbols) into a diffusive component (dashed line) and a rectangular hyperbolic Michaelis–Menten component (solid line). Data are from the APW-acclimated mussels described in Table 5, with an additional group incubated at 0.73 mmol l^{-1} Rb^+ (N=72). Error bars are ± 1 S.E.M.

equation may be applied to determine the diffusive component of ion influx:

$$J_i/J_o = (c_o/c_i)e^{zFE/\boldsymbol{R}T},$$

where c_o and c_i are the external and internal ion concentrations respectively, z is the valence, F is Faraday's constant, E is the transepithelial voltage, $\boldsymbol{R}$ is the gas constant and T is the absolute temperature. On the basis of the blood K^+ concentration in APW from Table 1 and the measured potential, the predicted diffusion flux ratio is 0.12. Assuming that renal loss accounts for about 50 % of K^+ efflux in APW (Table 5) (Murphy and Dietz, 1976), the observed epithelial flux ratio would be 1.5, an order of magnitude higher than passive J_i/J_o. Passive diffusion, therefore, may account for approximately 8 % of the observed K^+ influx when animals are in APW. Thus, an active epithelial K^+ transport system is likely to be present in zebra mussels.

The gradual loss of total solute by unfed zebra mussels acclimated to APW in the laboratory suggests that osmotic regulation may be compromised. Dietz *et al*. (1994) showed that K^+ is a critical factor affecting Na^+ and Cl^- balance in *D. polymorpha* and, although APW deficient in K^+ will support osmotically stressed animals for longer than 7 weeks, total solute levels decline. It was suggested that decreased blood K^+ concentrations could disrupt the electrochemical gradients necessary for Na^+ and Cl^- transport. Chloride transport is partially dependent upon Na^+ in *D. polymorpha*, and decreases in Na^+ influx may consequently affect blood Cl^- levels (Horohov *et al*. 1992). The decline in *D. polymorpha* blood solutes with acclimation to APW has been reported previously (Dietz *et al*. 1994), but the animals survived for months in the laboratory. This species rapidly loses ions (especially Mg^{2+}) when in ion-deficient media. The reduction in blood solute concentrations observed in this study, in the presence of K^+, suggests that additional factors may be involved in zebra mussel osmoregulation. Perhaps mineral dietary supplements are important for ion balance in this freshwater bivalve, or the 0.05 mmol l^{-1} K^+ in the APW is too low a concentration for long-term maintenance of unfed zebra mussels.

Zebra mussels exposed to K^+-free media experienced a significant decline in blood K^+ concentration after 1 week. These results differ from those measured in the larger unionid mussel *Ligumia subrostrata*, where the concentration of extracellular K^+ remained unchanged after 60 days of acclimation to deionized water (Murphy and Dietz, 1976). The decline in blood K^+ concentration while exposed to K^+-deficient water was not due to a low K^+ transport capacity in *D. polymorpha*, because it is equal to or greater than unionid K^+ transport (Dietz and Byrne, 1990). Thus, the critical difference in zebra mussel K^+ balance, when compared with other freshwater bivalves, may be the limited ability to reduce rather high K^+ losses (renal and/or extra-renal).

The 22 % decrease in tissue K^+ content after 2 weeks of exposure to K^+-free APW is comparable to the 15 % decline in intracellular K^+ concentration observed in a unionid after 30 days of exposure to deionized water (Murphy and Dietz, 1976). These data suggest that *D. polymorpha* have a limited ability to conserve K^+, an ion that is critical for cell volume regulation.

The blood ion composition and total tissue K^+ content remain stable in animals exposed to APW with a high K^+ concentration. The rise in blood K^+ levels to 300 % of that observed for APW-acclimated animals could be due, in part, to an increase in passive diffusion across the epithelia when the K^+ concentration in the bathing medium is elevated. This inference also is supported by the low transepithelial potential, which suggests that the epithelia are relatively permeable to ions.

Although Na^+ concentrations in the blood of zebra mussels

exposed to APW with 0.5 mmol l^{-1} KCl decreased in parallel with those of APW-acclimated animals, the stability of Cl^- levels at concentrations similar to those in initial APW control animals may be indicative of a secondary Cl^- transport system associated with elevated K^+ concentrations. However, the animals exposed to a high K^+ concentration were also bathed in a higher Cl^- concentration (1.80 mmol l^{-1} *versus* 1.35 mmol l^{-1} in APW), and the elevated Cl^- concentration of the bath may have contributed to the apparent stability of blood Cl^- level.

D. polymorpha are similar to other freshwater bivalve species in that the *in vivo* transmembrane potential is directly dependent upon the concentration of Ca^{2+} in the external medium (Dietz and Branton, 1975). Although the possibility remains that excess K^+ in the bath may disrupt intracellular potentials of excitable tissues, increasing the K^+ concentration of the bath had no apparent effect on the epithelial membrane potential.

Zebra mussels transport ions faster than freshwater unionid species (Horohov *et al.* 1992). Potassium influx in APW-acclimated *D. polymorpha*, at a K^+ concentration similar to that of APW, is approximately double that reported for *Ligumia subrostrata* and *Carunculina texasensis* and equal to that of *Corbicula fluminea* (Dietz and Byrne, 1990). The positive correlation between J_{net} and the K^+ concentration of the water indicates that unfed zebra mussels are able to maintain K^+ balance by way of epithelial transport at concentrations typical of many fresh waters. From an analysis of blood from animals acclimated to media deficient in K^+, it appears that increased transport rates occurred when blood K^+ levels fell to less than 50 % of normal values. The K^+ influx kinetic coefficients derived from regression analyses were not significantly different for K^+-deficient and APW-acclimated animals, suggesting that the animals utilized in the K^+-deficient influx study were not sufficiently depleted after 7 days to stimulate uptake. This inference is supported by the significant elevation of Rb^+ influx in mussels exposed to K^+-free APW for over 3 weeks.

Zebra mussels are unusually intolerant of renal and extra-renal ion loss when maintained in deionized water (S. J. Wilcox and T. H. Dietz, unpublished observations; Nichols, 1993; Ram and Walker, 1993, Dietz *et al.* 1994). The present study confirms that the high K^+ loss is due to prolonged and relatively constant K^+ efflux in K^+-deficient zebra mussels. The freshwater bivalve *Ligumia subrostrata* decreases renal Na^+ efflux when acclimated to Na^+-free water (Scheide and Dietz, 1982). Perhaps *D. polymorpha* lacks the ability to increase renal reabsorption of K^+ when exposed to K^+-deficient water. In deionized water, zebra mussels die (50 % mortality in 2–3 days) before compensatory mechanisms are employed (Dietz *et al.* 1994). Isotopic Rb^+ is often substituted for K^+ in tracer studies under the premise that both cations are transported by similar mechanisms at similar rates (Grubb *et al.* 1988; Sanders and Kirschner, 1983). However, Dietz and Byrne (1990) estimated the kinetics of K^+ and Rb^+ influx in two freshwater bivalve species and observed quantitative differences in transport between the two ions. The maximum rate of Rb^+ transport for *Corbicula fluminea* and *Carunculina texasensis* was significantly less (approximately 55 %) than that of K^+ influx, yet both species exhibited apparently higher affinities for Rb^+. The results of the present study indicate that Rb^+ influx in APW-acclimated *D. polymorpha* is 60 % greater than K^+ influx at low ion concentrations (<0.13 mmol l^{-1}) but that the transport system may have the same affinity for both Rb^+ and K^+.

The significant difference in J_{max} for K^+ and Rb^+ in *D. polymorpha* acclimated to APW may be due to high rates of passive diffusion. An analysis of mean Rb^+ influx values at elevated Rb^+ concentrations indicated that the passive diffusion and active transport components of Rb^+ influx are equal at a relatively low Rb^+ concentration (0.3 mmol l^{-1}) (see Fig. 2). The combination of active epithelial K^+ transport and high passive epithelial permeability in freshwater bivalves may be the basis for their inability to survive an excessive elevation of $[K^+]$ in fresh water (Dietz and Byrne, 1990).

Lack of sufficient influx values at lower K^+ and Rb^+ concentrations, however, prevent more thorough analysis of transport kinetics when the diffusive component is excluded. Therefore, these data suggest the calculated J_{max} values may be overestimates of active transport. The K^+ transport studies were restricted to lower K^+ concentrations to avoid the lethal effects of elevated $[K^+]$ in fresh water.

We have noted remarkable variability in the transport rates of specific ions and blood composition between different groups of zebra mussels and this may be due to numerous factors (seasonal variations in temperature, food availability, reproductive stress, age and animal size). Ion uptake in freshwater bivalves was strongly influenced by diurnal rhythms (McCorkle-Shirley, 1982; Graves and Dietz, 1980). Although the gill is the primary site of Na^+ and Cl^- uptake in freshwater unionid bivalves (Dietz and Graves, 1981; Dietz and Hagar, 1990), K^+ uptake has not been localized. Indeed, the contribution of dietary constituents or K^+ absorption by the gut to blood ion balance in *D. polymorpha* has yet to be quantified. Nevertheless, these animals can maintain blood and tissue K^+ balance without feeding.

The classification of *D. polymorpha* as a recent immigrant of fresh water is based on fossil records and life history characteristics common to marine or estuarine forms (pelagic larvae, byssus attachment) (McMahon, 1991). *D. polymorpha* are similar to other freshwater bivalves in having low blood and tissue solute concentrations that minimize ion loss and water gain from the external medium, representing a unique adaptation to the problems of the osmotic gradient existing between the freshwater external medium and animal body fluids. However, the adaptation of *D. polymorpha* to fresh water may be incomplete, as shown by the rapid ion turnover and the inability to reduce ion loss in dilute fresh water (this study; Dietz *et al.* 1994; Horohov *et al.* 1992) and may be considered as a transitional stage in bivalve evolution as a freshwater species.

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