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REVIEW

APPARENT WATER PERMEABILITY AS A PHYSIOLOGICAL PARAMETER IN CRUSTACEANS

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Summary

This article reviews the use of apparent water permeability (AWP) calculated from measurements of isotope-labelled water flux as a physiological estimate of whole-body water permeability in aquatic invertebrates. The rationale and practices of AWP calculations are described in an Appendix.

AWP calculations have provided a wealth of information. However, the validity of the method and therefore also of the information obtained have been questioned. Consequently, the use of AWP data in discussions of osmotic and fluid homeostatic questions in aquatic invertebrates is limited. This article reviews three decades of published experiments in which measurements of isotope-labelled water fluxes were used to estimate water permeability in aquatic invertebrates. Data on 24 species of arthropod, most of them decapod crustaceans, are presented. The combined data indicate that the results obtained by different investigators on the same species show good agreement, even though different tracers and experimental methods have been applied. When available, results from other kinds of studies were used to evaluate the results obtained using the AWP measurements. The various results demonstrate that AWP is influenced not only by natural environmental factors, such as salinity and temperature, and by anthropogenic factors, such as potentially toxic trace metals, but that it is also regulated by intrinsic factors, such as ecdysis and life cycle stage. The results obtained can often be explained as effects of components of the habitat of the animal. Accordingly, studies on variations in AWP contribute to our understanding of the different physiological strategies used by species living in a changing environment.

We conclude that calculations of AWP offer reliable, relevant physiological data in a range of crustacean species, as long as methodological limitations and uncertainties are kept in mind.

In addition, we propose some possible new ways of applying AWP calculations to marine invertebrates other than crustaceans. A major part of this review describes results already obtained for the shore crab *Carcinus maenas* as this species is probably the animal on which most work has been carried out. We suggest topics for future work on this species and review the possibility of using AWP in *C. maenas* as a biomarker of metal exposure.

Key words: aquatic invertebrates, osmoregulation, trace metals, salinity, ecdysis, individuality, *Carcinus maenas*, metal uptake, isotope flux.

Introduction

For about three decades, water fluxes in aquatic animals have been measured with the aid of isotope-labelled water. The isotopes employed have mainly been deuterium (D or ^{2}H) and tritium (T or ^{3}H). In principle, such measurements are extremely simple. External or internal water is isotope-labelled, and the passive diffusion of isotope towards equilibrium is taken as a measure of the organism's permeability to water. If the external compartment is large, the process should follow first-order kinetics. However, as is discussed below, a number of limitations exist with this method. Possible isotope effects and lack of representability of the internal compartment labelled with isotope or used for measuring isotope influx for total organism water are two major parameters limiting the accuracy of measured flux data. Accordingly, water fluxes estimated using isotope-labelled water are usually referred to as 'apparent', both because of the unknown or criticised parameters in the method and because an effect and not a mechanism is observed (Smith, 1970). The method has been criticised for not giving results comparable with those obtained from other types of measurements, such as net water flux determined as urine excretion. Bolt (1989), however, refuted this criticism and stated that calculations of

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apparent water permeabilities (AWP) from measurements of isotope-labelled water fluxes are a valid and reliable method for estimating water permeabilities in marine crustaceans, as long as certain limitations are kept in mind.

Concern about what is actually measured with the AWP method can be divided into two categories: concern about the validity of results obtained using the method and concern about the method's uncertainties. We will deal with these issues one at a time.

Validity of the method

The osmotic permeability coefficient, P_{osm}, may be calculated from net water transfer; the diffusional permeability coefficient, P_{diff}, is calculated from AWP. If both methods are valid, the coefficients should be equal. However, large differences have been observed. The ratio P_{osm}/P_{diff} was reported to be 27.2 using frog skin (Maetz, 1968), 3.16 in the eel *Anguilla anguilla* adapted to fresh water and 1.05 in the same species adapted to sea water (Motais *et al.* 1969). In the crab *Carcinus maenas*, Rudy (1967) observed P_{osm}/P_{diff} ratios ranging between 1 and 2.5. According to Bolt (1989), differences between P_{osm} and P_{diff} could arise because of unstirred layers on either side of the membranes, causing an increase in the diffusion barrier. Bolt (1989) points out that the AWP of *Gammarus duebeni* decreases 10-fold after heart failure, showing that circulation currents minimise unstirred layers and thereby decrease the difference between P_{osm} and P_{diff}, making the ratio closer to unity. Accordingly, the reliability of AWP data depends on fluid movements in both internal and external media to minimise unstirred layers.

Methodological uncertainties

The fluxes of the different unlabelled and isotope-labelled water species (H_2O, THO, DHO, D_2O or $H_2{}^{18}O$) will vary as a result of their different molecular masses. Rankin and Davenport (1981) stated that the difference in diffusion rate between THO (molecular mass 20 Da) and H_2O (molecular mass 18 Da) is measurable, but less than 10 %. Wang *et al.* (1953) found that the diffusion rates of THO and DHO in H_2O did not differ significantly, but that the diffusion rate of $H_2{}^{18}O$ was 14 % slower than the diffusion rate of THO or DHO. The only available investigations of isotope-induced diffusion differences in marine invertebrates were performed by Smith and Rudy (1972) in the crab *Hemigrapsus nodus*. They suggested that water fluxes calculated from methods using THO, DHO or H_2O have relative magnitudes of 91, 95 and 100 %, respectively. From studies of the water permeability of frog skin using D_2O and T_2O, King (1969) concluded that there was no isotopic effect. The molecular mass effects briefly summarised above have been overlooked or ignored by some authors. Even if the influence of isotope on diffusion rates is limited, especially for the hydrogen isotopes, the isotope effect should be kept in mind during the discussion below of results obtained using different isotopes.

Bolt (1989) offers a detailed description of techniques for calculating AWP from influx and efflux experiments as well as by intrusive sampling. Together, Bolt (1989), Rankin and Davenport (1981) and Motais *et al.* (1969) provide excellent information about the methodology of AWP experiments and calculation of constants such as P_{diff} and P_{osm}. A brief description of the theory and practice of AWP calculations is presented in the Appendix of this review, but we recommend the articles mentioned above for further information.

The technique of AWP calculations has some major advantages compared with other methods used to estimate water flux: (1) the measurements of isotope-labelled water are easy to perform and can potentially be adapted to aquatic organisms other than crustaceans; (2) the cost is low, and experiments can be performed using standard laboratory equipment; (3) the experimental animals are not harmed, the worst case being an injection of tracer into, or a sampling of, the body fluids; (4) measurements can be repeated on the same animal, thus using the animal as its own control, which is especially useful when studying individual responses to stress; and (5) changes in AWP during the course of an experiment may be studied. This, however, requires extensive sampling.

AWP calculations provide information on physiological osmoregulation. Published data will be discussed below and compared both among and within species. However, AWP data additionally offer information on individual responses to stress and on the influence of life cycle on physiology.

AWP may be given with the dimension time^{-1} (h^{-1}, min^{-1}) or as the half-time of water exchange ($T_{1/2}$). An increase in AWP stated as time^{-1} corresponds to an increase in water permeability, whereas an increase in $T_{1/2}$ corresponds to a decrease in permeability. In this review, AWP will be given as h^{-1}. When necessary, $T_{1/2}$ was converted to h^{-1} using the formula: AWP (h^{-1}) = ln2/AWP[$T_{1/2}$ (h^{-1})].

Some authors state salinities as a percentage of full-strength sea water or as mosmol l^{-1} or mmol l^{-1}. For this review, all salinity data have been recalculated to permille (‰) based either on information from the article cited or using 1100 mosmol l^{-1} for full-strength sea water.

Analysis of AWP data

Results obtained by different investigators are collected in Tables 1 and 2. Previous investigations have focused on environmentally relevant factors such as salinity, temperature and ecdysis. The effects of metals have also been investigated, but only at relatively high concentrations probably not relevant to even strongly polluted areas.

Effects of salinity changes and temperature on AWP

Salinity has been the main experimental parameter investigated. In general, animals living in fresh water have lower AWPs than animals living in a brackish or fully saline environments. The freshwater crayfish *Astacus astacus* and *Cherax destructor* both have AWPs of approximately 0.2 h^{-1} compared with a value of approximately 0.8 h^{-1} for equally sized brackish-water decapods such as the crab *Carcinus maenas*. In *A. astacus*, Rudy (1967) using influx of THO and

Table 1. *Apparent water permeability data for the aquatic invertebrates reviewed*

Species	Order (subphylum)	Medium (salinity stated in ‰)	Temperature (°C)	Factor/Significance	Method	Mean AWP (h^{-1})	Reference
Astacus astacus	Decapoda (Crustacea)	Fresh water	10	–	THO influx	0.20	Rudy (1967)
			15	Ecdysis, $P<0.0001$	THO efflux	0.4, 0.12	Rasmussen (1994)
		0.09, 24 mmol l^{-1} NaCl	15	Salinity, $P<0.0001$	THO efflux	0.16, 0.26	Rasmussen and Bjerregaard (1995)
		$[Ca^{2+}]$ 0, 5 mmol l^{-1}		$[Ca^{2+}]$, $P<0.002$			
Carcinus maenas	Decapoda (Crustacea)	35, 25, 14‰	10	Salinity, NS	THO influx	0.79, 0.78, 0.72	Rudy (1967)
		35, 25, 15, 5‰	15	Salinity, $P<0.0001$	THO efflux	1.19, 0.49	Rasmussen and Bjerregaard (1995)
		$[Ca^{2+}]$ 0, 12.5 mmol l^{-1}	15	$[Ca^{2+}]$, NS			
		15‰		Moult cycle, $P<0.01$		0.42, 0.22	Rasmussen *et al.* (1995)
				[trace metals], $P<0.05$			
				Exposure time, NS			
Chaetogammans marinus	Amphipoda (Crustacea)	31, 1‰	15	Salinity, NS	THO influx	4.62	Bolt (1983)
					THO efflux		
Cherax destructor	Decapoda (Crustacea)	Fresh water	15	Salinity, NS	THO efflux	0.14, 0.17	Rasmussen and Bjerregaard (1995)
		$[Ca^{2+}]$ 0, 5 mmol l^{-1}		$[Ca^{2+}]$, NS			
Corophium volutator	Amphipoda (Crustacea)	29, 5‰	12	Salinity, $P<0.01$	THO efflux	14.9, 10.1	Taylor (1985)
Crangon crangon	Decapoda (Crustacea)	34, 22, 7‰	12	Salinity, $P<0.01$	THO efflux	1.2, 0.95	Campbell and Jones (1990)
		34, 7‰	15–9	Salinity, $P<0.05$	THO efflux	1.7, 0.7	Krag (1994)
				Temperature, $P<0.0001$			
				[trace metals], $P<0.01$			
Crangon franciscorum	Decapoda (Crustacea)	25, 5‰	20	Salinity, $P<0.01$	THO influx	2.6, 1.7	Shaner *et al.* (1985)
Gammarus duebeni	Amphipoda (Crustacea)	33‰	19	Ecdysis, Significant	THO efflux	10.9, 6.3	Lockwood and Inman (1973*a*)
		31, 13, 0.7‰	18	Salinity, Significant	THO efflux	5.7, 3.3, 2.5	Lockwood and Inman (1973*b*)
		31, 1‰	15	Salinity, Significant	THO influx	8.3, 1.7	Bolt (1983)
		31, 10‰	20	Salinity, Significant	THO efflux	6.2, 4.1	Johnson and Jones (1990)
				Trace metals, NS			
Gammarus locusta	Amphipoda (Crustacea)	31, 1‰	15	Salinity, NS	THO influx	9.9	Bolt (1983)
					THO efflux		
Idotea linearis	Isopoda (Crustacea)	33‰	19	Ecdysis, Significant	THO efflux	13.9, 24	Lockwood and Inman (1973*a*)
Libinia emarginata	Decapoda (Crustacea)	34, 27‰	20	Salinity, $P<0.002$	D_2O influx	8.49, 5.96	Cornell (1973)
Limulus polyphemus	Merostomata (Chelicerata)	32, 16, 6‰	24	Salinity, $P<0.01$	THO influx	2.03, 1.40, 1.60	Hannan and Evans (1973)
Macropipus depurator	Decapoda (Crustacea)	35‰	10	–	THO influx	2.4	Rudy (1967)
Palaemon adspersus	Decapoda (Crustacea)	34, 4‰	15–9	Salinity, NS	THO efflux	1.2, 0.6	Krag (1994)
				Temperature, $P<0.005$			
Palaemon elegans	Decapoda (Crustacea)	34, 22‰	12	Salinity, $P<0.05$	THO efflux	1.4, 1.3	Campbell and Jones (1990)
Palaemon longirostris	Decapoda (Crustacea)	43, 34, 22, 7, 0.5‰	12	Salinity, $P<0.001$	THO efflux	1.1, 0.92, 0.89, 0.78, 0.71	Campbell and Jones (1990)
Palaemon squilla	Decapoda (Crustacea)	34, 15‰	15	Salinity, $P<0.05$	THO efflux	1.4, 1.1	Krag (1994)
Palaemonetes varians	Decapoda (Crustacea)	42, 25, 14‰	10	Salinity, NS	THO influx	0.64, 0.64, 0.55	Rudy (1967)
		34, 0.5‰	12	Salinity, $P<0.05$	THO efflux	1.01, 0.88	Campbell and Jones (1990)
Penaeus duorarum	Decapoda (Crustacea)	32‰	24	–	THO influx	0.765	Hannan and Evans (1973)
Uca minax	Decapoda (Crustacea)	32, 16, 1‰	24	Salinity, NS	THO influx	0.33, 0.34, 0.30	Hannan and Evans (1973)
Uca pugilator	Decapoda (Crustacea)	32, 16, 1‰	24	Salinity, NS	THO influx	0.33, 0.36, 0.34	Hannan and Evans (1973)
Uca rapax	Decapoda (Crustacea)	32, 16, 1‰	24	Salinity, NS	THO influx	0.21, 0.22, 0.21	Hannan and Evans (1973)

Data have been converted to hourly exchange rate (h^{-1}).

The method and tracer used are shown in the column Method.

Salinity is the most frequently used experimental parameter in studies of apparent water permeability (AWP).

The parameter tested and the result obtained are shown in the column Factor/Significance; NS, not significant. Note that in some cases the same species has been investigated by more than one author and that different methods may have been applied.

Some of the results in this table are used in Figs 1 and 2.

Table 2. *Data for aquatic invertebrates calculated using methods that do not allow conversion of results to the form used in Table 1*

Species	Order (subphylum)	Medium	Temperature (°C)	Factor/Significance	Method	Reference
Carcinus maenas	Decapoda (Crustacea)	32, 8.5‰	18	Salinity, $P<0.01$	D_2O saturation in 15 min	Smith (1970)
	Decapoda (Crustacea)	34, 8.5‰	21	Salinity, $P<0.001$	THO influx in 15 min	Berlind and Kamemoto (1977)
Hemigrapsus nodus	Decapoda (Crustacea)	29, 18‰	20–10	Salinity, $P<0.001$ Temperature, $P<0.05$ Isotope, NS	DHO/THO saturation in 15 min	Smith and Rudy (1972)
Rhithropanopeus harrisi	Decapoda (Crustacea)	32, 0.3‰	13	Salinity, $P<0.001$	D_2O saturation in 15 min	Smith (1967)
	Decapoda (Crustacea)	26, 3.4‰	18–22	Salinity, $P<0.0005$	D_2O saturation in 30 min	Capen (1972)

NS, not significant.
The method used is briefly explained in the column Method.

Rasmussen and Bjerregaard (1995) using THO efflux both calculated an AWP of $0.2\,h^{-1}$. This result indicates that the passive diffusion of water both into and out of animals is large compared with the net water flux (see also Bolt, 1989). The very low AWPs of freshwater crayfish are usually explained as a strategy for minimising osmotic influx of water due to the osmotic difference of around 10‰ between the internal and external media. When adapted to 1‰ salinity, the crabs *Uca minax*, *Uca pugilator* and *Uca rapax* all have AWPs of $0.21–0.36\,h^{-1}$. This corresponds closely to the AWP of freshwater crayfish. Hannan and Evans (1973) suggested that these low AWPs were an adaptation to the terrestrial environment (*U. rapax*) or to the freshwater environment (*U. minax*) that are the preferred habitats for these animals. It must be noted, however, that these three crab species have the same low permeability when acclimated to 32‰ salinity. Hannan and Evans (1973) do not comment on this but it can be speculated that the crabs have lost the ability to change their AWP owing to the lifestyle mentioned above.

The amphipod *Gammarus duebeni* is an intensively studied species. There is general agreement that this organism lowers its AWP significantly when exposed to a dilute medium. When the animal is transferred from 31 to 1‰ salinity, the AWP (calculated from THO influx) decreases from 8.3 to $1.7\,h^{-1}$ (Bolt, 1983). This is in good agreement with the decrease from 5.7 to $2.5\,h^{-1}$ (calculated from THO efflux) reported by Lockwood and Inman (1973*b*) after transfer of the same organism from 31 to 0.7‰ salinity. These results correspond well in spite of the fact that different methods were employed. The decapod *Crangon crangon* also reduces its AWP when exposed to dilute media. Krag (1994) and Campbell and Jones (1990) found decreases from 1.7 to $0.7\,h^{-1}$ and from 1.2 to $0.95\,h^{-1}$, respectively, when this species was transferred from 34 to 7‰ salinity.

AWP data obtained in *C. maenas* indicate extensive interexperimental variation. Rudy (1967) observed no reduction in AWP of this organism when the salinity was reduced from 35‰ (below which the animal becomes hyperosmotic) to 14‰ salinity. However, Rasmussen and Bjerregaard (1995) observed a statistically significant ($P<0.0001$) reduction of AWP in *C. maenas* when the salinity was reduced from 35 to 5‰. Also, Smith (1970) and Berlind and Kamemoto (1977) found a significant reduction in AWP ($P<0.01$ and $P<0.001$, respectively) when they moved this species from 32 to 8.5‰ salinity and from 34 to 8.5‰ salinity, respectively. Smith (1970) and Berlind and Kamemoto (1977) used different methods (see Table 2) for calculating AWP from those used by Rudy (1967) and Rasmussen and Bjerregaard (1995), so even though the responses can be compared, the actual results cannot. The discrepancy between the results obtained by Rudy (1967) and those obtained by Rasmussen and Bjerregaard (1995) may be explained in part by the experimental design, in part by the natural habitat and in part by the size of the animals used in the experiments. Rudy (1967) collected his crabs in an area of high and fairly constant salinity, whereas Rasmussen and Bjerregaard (1995) obtained their crabs from an area of intermediate and changing salinity. Also, Rudy (1967) used larger crabs than those used by Rasmussen and Bjerregaard (1995). Size has been demonstrated to influence several physiological parameters in the shore crab (see the discussion on intraspecific variation in AWP below). Also, the results of Smith (1970) indicate that the water permeability of *C. maenas* decreases with increasing size. It must be noted that, despite the differences in response to salinity changes, the AWP was estimated to be approximately $0.8\,h^{-1}$ by both Rudy (1967) and Rasmussen and Bjerregaard (1995).

The discrepancy between the various results published may also be due to temperature differences: Rudy (1967) performed his experiments at 10 °C whereas Rasmussen and Bjerregaard (1995) used 15 °C, Smith (1970) used 18 °C and Berlind and Kamemoto (1977) used 21 °C. Temperature effects on AWP have been reported by several investigators. Temperature-induced changes in heart rate are thought to be responsible for these differences. A decreased heart rate will reduce the blood flow through the gills and thereby conceivably reduce the area-to-volume ratio of the ventilated gills or increase the unstirred layer (Campbell and Jones, 1990). As noted by Lockwood *et al.* (1973), this is not a real change in cuticle permeability; however, it still has biological significance for the animal. Another explanation may be a rectification of blood flowing through the gills and consequently a change in effective gill area. In *G. duebeni*, the gill lamellae are wider when the animal is acclimated to a salinity of 33‰ than when it is exposed to 7‰ salinity (Lockwood *et al.* 1973). The gills of *C. maenas* contain valves that enable the animal to change its

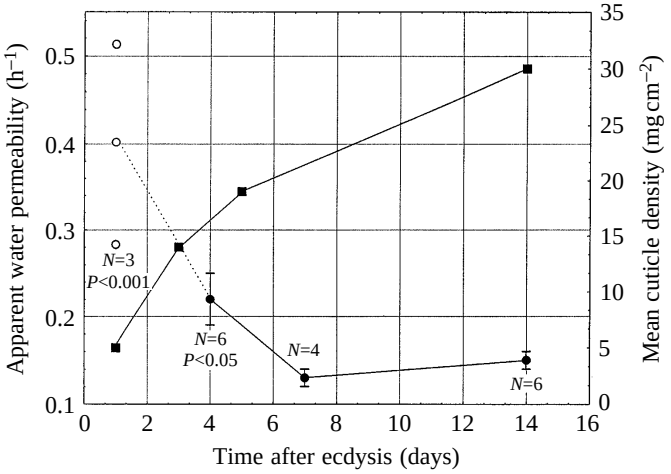


Fig. 1. Apparent water permeability (AWP) of the freshwater crayfish *Astacus astacus* during and shortly after moulting (Rasmussen, 1994). Significant differences from the intermoult control group (14 days after ecdysis) are stated as well as the number of animals investigated. Values are means ± S.E.M. (filled circles for AWP) or single measurements (open circles for AWP). The mass of 1 cm^2 of carapace from crayfish at 1, 3, 5 and 14 days after moulting (cuticle density) is also shown (filled squares). Data from Welinder, 1975.

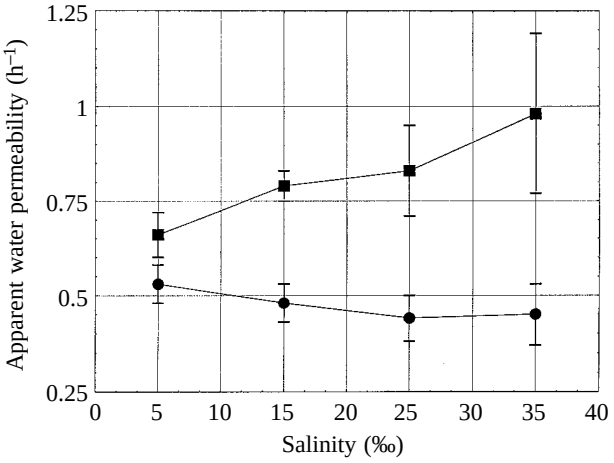


Fig. 2. The effect of salinity on apparent water permeability (AWP) of green (squares) and red (circles) individuals of the shore crab *Carcinus maenas*. The AWP of green crabs increases significantly with increasing salinity (ANOVA, P<0.01), whereas the AWP of red crabs shows no significant change when salinity increases. Total N=55, 6–8 animals in each group; values are means ± S.E.M. In this experiment, artificial sea water containing a calcium concentration of 5 mmol l^{-1} was used. Modified from Rasmussen (1994) and Rasmussen and Bjerregaard (1995).

haemolymph flow (Taylor and Taylor, 1986). Whether these mechanisms contribute to changes in AWP is not known; however, as AWP can change abruptly, the mechanism regulating water homeostasis must respond rapidly to the need for adjustment to a changing environment.

Life-cycle induced changes in AWP

Apart from the external factors salinity and temperature, endogenous factors have been found to influence AWP. Fig. 1 shows an extensive increase in AWP in *A. astacus* during moulting. Previously, Lockwood and Inman (1973*a*) and Morris *et al.* (1987) reported that AWP almost doubled during moulting in *Gammarus duebeni* and *Idotea linearis*. The increased AWP can be correlated with the formation of a new exoskeleton. Data on exoskeleton formation in freshwater crayfish from Welinder (1975) have been included in Fig. 1. The fully developed exoskeleton of crustaceans is considered to be impermeable to water because of the matrix of chitin, proteins and calcium salts. This indicates that AWP may be considered to be a measure of gill permeability in intermoult animals, as stated by Capen (1972). This assumption is supported by regional studies of permeability in crustaceans using dyes (Mary, 1986) and by Bergmiller and Bielawski (1970), who calculated that 90 % of the water excreted as urine in the crayfish *Astacus leptodactylus* has been taken in by the gills. Animals trying to keep their uptake of water to a minimum must be expected to limit oral water uptake. In *Limulus polyphemus*, drinking was estimated to contribute less than 0.5 % of total water influx (Hannan and Evans, 1973) and therefore to have almost no influence on net water flux, although a somewhat higher value was found for *Uca pugilator* (Hannan and Evans, 1973). The modest size of the change in AWP during moulting can be ascribed to the fact that a new exoskeleton has been partially formed beneath the old skeleton before the old one is shed, and possibly to a change in gill lipid composition rendering the gills less permeable to water. Ecdysis-induced changes in lipid composition have been observed in *G. duebeni* (Morris *et al.* 1987). At 7 days after ecdysis, the AWP of *A. astacus* was already as low as or even lower than the AWP of intermoult animals (Fig. 1), indicating that the carapace rapidly becomes water-impermeable. Crustaceans take up water before and after moulting in order to increase their size prior to tanning and mineralization of the new exoskeleton. The assumptions used for calculating AWP may therefore be violated as net water flux may no longer be negligible compared with diffusional water flux. Water uptake in moulting decapods starts some hours before, and is completed within a few hours after, ecdysis (Cameron, 1989; Mykles, 1980), however, and calculation of AWP will thereafter again be valid.

Apart from the rapid changes in AWP induced by moulting, the life cycle of crustaceans may influence the AWP over the long term. Most individuals of *C. maenas* going through the normal moult cycle live in the intertidal zone, where salinity and other environmental factors may vary extensively. These crabs have a green exoskeleton and are better osmoregulators than red crabs in prolonged intermoult. Red crabs constitute the bulk of the larger crabs, are more aggressive and mostly live in the sublittoral zone (Kaiser *et al.* 1990; Reid *et al.* 1989). The data in Fig. 2 indicate that this age-induced change in life mode has a major influence on AWP. Green crabs are capable of extensively changing their AWP with changing salinity, but red crabs seem to have lost this ability, possibly as a response to living in a more stable environment or as a consequence of

an extensively increased intermoult period (Rasmussen, 1994; Rasmussen and Bjerregaard, 1995).

Effects of metal cations on AWP

Other indications that changes in AWP are biologically meaningful responses to changes in the environment stem from exposure experiments using metal cations. In natural waters, these cations may form various mixed complexes with the ligands available; we use the term X^{2+}, where X is a metal, to signify a number of different chemical species. Ecotoxicological experiments are often more or less acute using high concentrations of a single metal and it is difficult to assess whether the results can be extrapolated to natural conditions where concentrations are lower, where exposure time is longer and where several uncontrolled factors may have influence on the toxicity due to metal exposure.

Both *C. maenas* and *C. crangon* exposed to high levels of potentially toxic trace metals lower their AWP within hours and maybe even minutes after exposure (Rasmussen *et al.* 1995). When exposed to Zn^{2+}, *C. crangon* decreased its AWP, but in contrast to *C. maenas* exposed to Cd^{2+} this decrease was reversible (even though exposure continued), and AWP returned to the normal level within 5–6 days (Rasmussen *et al.* 1995). This is in agreement with the results of Johnson and Jones (1990), who found that the AWP of *G. duebeni*, also a euryhaline species known to be able to change its AWP in response to salinity changes (Bolt, 1983), was unchanged after 7 days of Zn^{2+} exposure. Of course, this does not reveal whether this organism lowered its AWP at the beginning of the exposure period. *C. maenas* did not restore its AWP to a normal level during a 10 day period of exposure to Cd^{2+} (Rasmussen *et al.* 1995).

The mechanism responsible for a metal-induced decrease in AWP is not known, but it may be speculated that the animals detect the metals and try to avoid interaction with an unfavourable environment by decreasing the exposed gill area. Another explanation could be that the metals exert a toxic effect directly on the permeable area, as perhaps seen in the isopod *Jaera nordmanni*, which produces mucus on the gills when exposed to metal cations (Bubel, 1976). Spicer and Weber (1992) reported a respiratory impairment in the crab *Cancer pagurus* exposed to Cu^{2+} or Zn^{2+}. This appeared to be caused by an increase in the diffusion barrier thickness of the gills which was reversible during continued exposure. Alternatively, a mechanism responsible for general changes in osmoregulation, such as the osmotic-pressure mechanism regulated by sensors situated in antenna of the lobster *Panulirus japonicus* (Tazaki, 1975), could play a role.

Some authors have speculated about the possible effect of AWP on the uptake of metals. Chan *et al.* (1992) suggested that differences in metal uptake between two populations of *C. maenas* could be explained in part by differences in AWP. In a review on metal uptake in crustaceans, Rainbow (1995) stated that, even though physicochemical conditions beyond physiological control (e.g. salinity and organic chelating agents) can often explain metal uptake in crustaceans, some experimental data indicate that physiological parameters (e.g. AWP) must be taken into account. In a study of the effect of salinity on passive Cd^{2+} uptake in *Artemia franciscana* (Blust *et al.* 1992), Cd^{2+} uptake increased with increasing salinity of the pre-exposure acclimation medium, but decreased with increasing salinity of the Cd^{2+} exposure medium. The data available so far do not allow firm conclusions to be drawn as to whether AWP has an effect on metal uptake or whether other medium-induced changes in the animals are responsible for the observed results. The bioconcentration factor for $^{65}Zn^{2+}$ in red and green crabs exposed for 6 days is shown in Fig. 3 (the factor is explained in the figure legend). In this experiment, red crabs appeared to take up less Zn^{2+} than did green crabs at both 5 and 25‰ salinity, but the differences were not statistically significant (P=0.07). This could be related to the generally lower AWP in red crabs. In this experiment, the effect of salinity on the uptake of Zn^{2+} overrides any possible influence of AWP within colour groups. If future studies show a correspondence between AWP and metal uptake, then this will probably be because the individuals taking up most metal somehow have gills that also facilitate water diffusion.

We conclude that, although metal cations significantly reduce the AWP of some marine invertebrates, it is questionable whether this decrease has any measurable effect on metal uptake within species. This does not exclude the possibility that differences in metal uptake among species can be correlated with differences in AWP. The interactions are complex because salinity (and other factors) influences both AWP and the bioavailability of metals, and because metal exposure influences AWP.

Future applications of AWP calculations

As AWP calculations seem to offer sound estimates of the

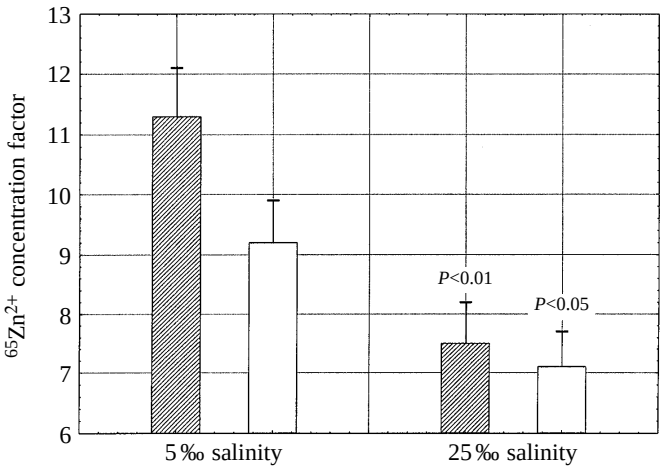


Fig. 3. Whole-body concentration factor [(cts min^{-1} g^{-1} fresh mass)/(cts min^{-1} ml^{-1} seawater)] after 6 days of exposure to carrier-free $^{65}Zn^{2+}$ in green (hatched columns) and red (open columns) specimens of the shore crab *Carcinus maenas* acclimated to 5 or 25‰ salinity. Total N=29, 7–8 animals in each group; values are means + S.E.M. Significant differences between salinities within colour groups are indicated (two-way ANOVA).

rate of diffusional water exchange between extracellular water and the surroundings, and thus of the permeability of whole animals, this parameter has potential applicability to a host of experimental questions. The main question addressed so far, whether salinity changes induce changes in AWP, has been discussed in detail above. Other questions, such as the influence of metals on AWP and the influence of AWP on metal uptake, have received some attention. Although experimental data indicate that exposure to metals affects the AWP, the effect this influence has on the overall physiology of the animal as well as the animal's strategy for coping with this effect is unknown. Intraspecific variations in AWP have been investigated only in the shore crab; the results indicate that AWP calculations offer an efficient tool for studying physiological differences between the life stages of this organism.

An obvious way to expand the use of AWP as a physiological parameter is to adapt the methods hitherto used almost exclusively in crustaceans to other aquatic invertebrate phyla. This may help clarify invertebrate strategies not only towards osmotic stress, but also towards toxic stress. The AWP of the annelid *Arenicola marina* (the lugworm) has been studied in our laboratory and was found to be somewhat larger than that of crustaceans of equal size. This was to be expected because polychaetes have a more permeable body than do crustaceans.

Cadmium exposure influenced the AWP of *A. marina* in a manner similar to that reported for crustaceans (A. D. Rasmussen and O. Andersen, unpublished observations), indicating that AWP calculations might offer an avenue for more generally applicable studies on defensive strategies of aquatic organisms against xenobiotics. If AWP studies are to be extended to phyla other than arthropods, the experimental design should be developed to take into account the lifestyle of the experimental animals chosen.

Perhaps a promising application of AWP calculations would be its use as a biomarker of metal exposure. Mayer *et al.* (1992) list five criteria for the selection and development of useful biomarkers. Briefly stated, these are: (1) the biomarker should be easy to apply; (2) a dose- or time-dependent response should be present; (3) sensitivity should be higher than that of traditional endpoints; (4) variability of the biomarker due to other factors should be understood; (5) the biomarker must have biological significance. Conceivably, all these criteria could be met during employment of AWP calculations in *C. maenas*. In this species, the isotope flux data necessary for AWP calculations are easy to obtain, a dose–response relationship seems to be present (Rasmussen *et al.* 1995), the sensitivity is probably higher than traditional endpoints (even though this point awaits further clarification) and the influence of some exogenous (salinity, temperature) and endogenous (developmental stage, size) factors has been established (Rasmussen and Bjerregaard, 1995; Rasmussen, 1994; Smith, 1970). Thus, variations in the biomarker are likely to indicate biologically relevant events. It must be kept in mind, however, that habitat-based differences in AWP have been observed in this species and that the effects of a variety of other exogenous factors are unknown.

AWP as a biomarker of metal exposure would probably be most sensitive for the most toxic metals, because Cd^{2+}, Hg^{2+} and Pb^{2+}, in particular, lower the AWP of *C. maenas* as opposed to Co^{2+} and Zn^{2+}, which have no effect on AWP during short-term exposure (Rasmussen *et al.* 1995). No attempt has been made to link changes in AWP to the general physiological status of the organism. It is therefore difficult to estimate the magnitude of the effect of a permanent change in AWP on the function of organisms. This link was also considered important when developing biomarkers (Mayer *et al.* 1992). Bjerregaard (1991) determined several characteristics in *C. maenas* that indicate the physiological condition of the animal during exposure to Cd^{2+}. It would be important to relate individual AWP changes induced by Cd^{2+} exposure to the biomarkers of 'condition' employed by Bjerregaard (1991).

It is the hope of the authors that the present review will stimulate increased use of AWP as a physiological marker. Much information would be obtained and many questions answered if AWP were calculated together with relevant individual parameters such as heart rate, respiration and ion fluxes.

Appendix: theory and practice of AWP calculation

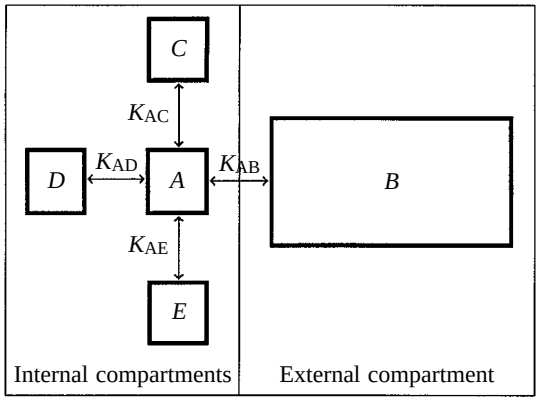
Theory

A schematic compartmental model of a crustacean is shown in Fig. 4A. The compartments represent extracellular fluids in the animal's circulation (e.g. haemolymph) (*A*), the external medium (e.g. sea water) (*B*) and fluids in different tissues connected to the systemic fluid (e.g. hepatopancreas, muscle tissue, alimentary tract) (*C–E*). Water diffusion will occur between these compartments with rate constants K_{AB}, K_{AC}, etc., depending on diffusion barriers.

It is assumed that net water flux is small compared with diffusional water flux so that no changes in water content of compartments occur. For calculating AWP, it is usually assumed that only compartments *A* and *B* exist (single-compartment model). That is, movement of water within the organism is faster than movement of water between the organism and the external medium, making the latter the rate-limiting step. Accordingly, passive diffusion of water from *A* to *B* will follow simple first-order kinetics. It is further assumed that compartment *B* is large compared with compartment *A*, so that the net flux of tracer will be a good estimate of unidirectional flux. These assumptions are shown in Fig. 4B (single-compartment model). When measuring the appearance of tracer in compartment *B* and plotting the results as amount of tracer (Q_t) *versus* time (t), a curve equivalent to the one shown in Fig. 4C will result. When equilibrium is reached, Q_t will be equal to Q_{eq}. The results can now be transformed and plotted as $\ln(Q_{eq}-Q_t)$ *versus* time. This results in the regression line also shown in Fig. 4C. The slope of this line ($-K_e$) gives the AWP.

When performing this data transformation, it is our experience that the regression almost always has an r^2 value

A



B

Model	Assumptions
Single compartment	• Compartment *B* large compared with internal compartments so that backflux of tracer is negligible. • $K_{AB} \ll K_{AC}$, K_{AD} and K_{AE}; K_{AB} is rate-limiting (equals K_e)
Two compartments	• Compartment *B* large compared with internal compartments so that backflux of tracer is negligible. • $K_{AC} < K_{AB}$; $K_{AB} < K_{AD}$; K_{AC} soon becomes rate-limiting (equals K_e)

C

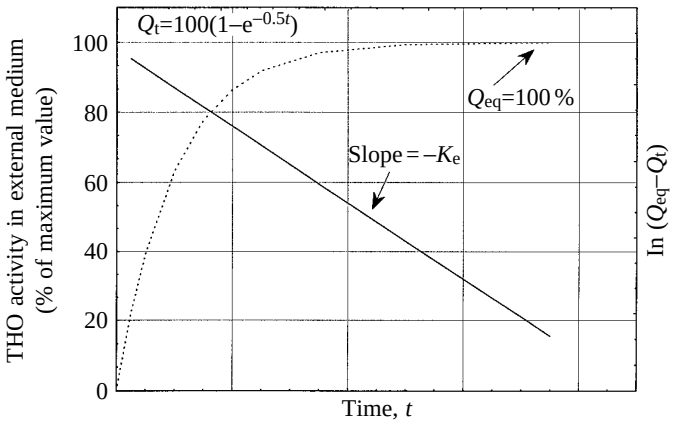


Fig. 4. The theory and practice of apparent water permeability (AWP) calculations. Please refer to the Appendix for a more detailed description. (A) A multi-compartment model representing, for example, a crustacean. (B) Assumptions for two types of submodels derived from the multi-compartment model. (C) Plots of labelled water flux (dotted line) and the log transformation (solid line) for calculation of AWP. Data confirm a single-compartment model. Q_t, amount of tracer at time *t*; Q_{eq}, equilibrium value of Q_t; the slope, K_e, gives AWP; K_{AB}, K_{AC}, etc. are rate constants.

of approximately 1, thereby validating the assumptions made in Fig. 4B (single-compartment model) and indicating that, in this case, AWP was constant during the sampling period.

The efflux of water f_{out} is given by:

$$f_{out} = \mathrm{AWP}[(V_A V_B)/(V_A + V_B)],$$

where V_A and V_B are the volumes of compartments *A* and *B*, respectively. If $V_A \ll V_B$, then $(V_A V_B)/(V_A+V_B)$ approaches 1 and f_{out} approaches AWP.

If one more internal compartment (e.g. *C*) has a sizeable exchange rate (i.e. K_{CA}), thereby invalidating the assumption that K_{AB} is the rate-limiting step, this will result in the situation shown in Fig. 4B (two-compartment model). It is assumed that tracer in compartments *A* and *C* is in initial equilibrium and also that compartment *C* is small compared with compartment *B*.

The two-compartment model can be used, for instance, when examining ionic efflux from fish (Rankin and Davenport, 1981). The two-compartment model was reported by Rudy (1967) to describe water diffusion in *Carcinus maenas*, but this was not confirmed in the same species by other authors. Efflux of $^{22}Na^+$ from *C. maenas* was found to be in accordance with the single-compartment model (Rasmussen, 1994).

Practice

Prior to performing flux measurements for calculating AWP, organisms are acclimated to the experimental conditions (salinity, temperature, etc.). The organisms are then loaded with tracer in one of two ways: either by injecting tracer directly into the systemic fluid and then allowing it to distribute, or by placing the organism in a medium containing the tracer and allowing it to stay there for sufficient time to equilibrate. The test organism is then transferred to tracer-free medium, and the release of tracer to the medium is monitored over time by taking samples of the medium. Alternatively, the organism can be placed in a medium containing the tracer and samples can be drawn from the systemic fluid at given time intervals. If only one sample is taken after a pre-set time, kinetic data will not be available and AWP cannot be calculated. In this case, only relative differences between experimental conditions can be observed. If one sample is taken at approximately $T_{1/2}$ and another after equilibrium has been reached, AWP can be calculated as described by Potts and Fleming (1970).

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