J. exp. Biol. 147, 125–132 (1989)
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A STUDY OF DIFFUSIONAL PERMEABILITY OF WATER, SODIUM AND CHLORIDE IN YOLK-SAC LARVAE OF COD (*GADUS MORHUA* L.)

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Accepted 9 June 1989

Summary

1. The fluxes of $^{3}H_2O$, ^{22}Na and ^{36}Cl were simultaneously measured in yolk-sac larvae of cod (*Gadus morhua* L.) in 34‰ sea water at 4·5°C.

2. The rates of turnover of all three isotopes were higher than in adult fish. Diffusional permeability coefficients, which relate ion fluxes to surface area, were however lower, indicating that larvae are less permeable than adults. Furthermore, there is close agreement between the diffusional and osmotic permeability coefficients, which supports a previous hypothesis that relatively low drinking rates in marine fish larvae are a consequence of low integumental permeability.

3. Estimates of the sodium and chloride concentrations derived from the equilibrium levels of ^{22}Na, ^{36}Cl and $^{3}H_2O$ indicate that yolk-sac larvae of cod regulate their body fluids hypotonic to sea water. Also, the ionic concentrations of the tissues of yolk-sac cod larvae are similar to those of adults.

Introduction

Tytler & Blaxter (1988*a*,*b*) and Mangor-Jensen & Adoff (1987) have shown that the larvae of marine teleost fish drink as part of their osmoregulation process. The rates of drinking of the larvae of cod (*Gadus morhua*), herring (*Clupea harengus*) and plaice (*Pleuronectes platessa*), when related to body mass, were found to be higher than for adult fish (e.g. Maetz & Skadhauge, 1968; Isaia, 1972) but substantially lower than expected from the surface to mass ratios. Tytler & Blaxter (1988*b*) have suggested that these differences could be explained if larvae were less permeable than adults. In fact, very low water permeability has been measured in marine fish eggs by Potts & Eddy (1973) and Mangor-Jensen (1986). The main barrier to diffusion in eggs is thought to be the vitelline membrane (Loeffler & Lovtrup, 1970). However, this structure no longer forms the integument in larvae and is therefore unlikely to offer the same protection against osmotic stress owing to unfavourable surface area to mass ratios.

The purpose of this work is, therefore, to clarify the situation by measuring the diffusional permeability coefficients of water, sodium and chloride ions in the

Key words: fish larvae, permeability, ion fluxes.

larvae of cod, using radioisotopes, and to compare them with osmotic permeability coefficients derived from earlier measurements of drinking rates.

Materials and methods

The larvae were hatched from spring-spawned eggs of cod (*Gadus morhua* L.) from the west coast of Scotland. On hatching, the larvae were transferred to 2-l glass beakers containing 34 ‰ sea water (SW), which had been passed through 0·22 μm micropore filters and held at 4·5°C in a refrigerator for up to 21 days before use.

Determination of fluxes of water, and sodium and chloride ions

^3H_2O, ^{22}Na and ^{36}Cl were measured simultaneously in 9-day-old yolk-sac stage larvae in SW at 4·5°C. 210 larvae were placed in 40 ml of filtered SW. ^3H_2O (185 MBq ml^{-1}), ^{22}Na (3·7–37 GBq mg^{-1} Na^+, carrier-free) and ^{36}Cl (110 MBq g^{-1} Cl^-), obtained from Amersham International plc, England, were added to produce a working solution containing approximately 1.5×10^7–$3·0\times10^7$ counts min^{-1} ^3H_2O, $0·5\times10^7$ counts min^{-1} ^{22}Na and $0·6\times10^7$ counts min^{-1} ^{36}Cl per ml. ^{22}Na was assayed using a Packard Autogamma 500C instrument. A Canberra Packard 2000 CA scintillation counter was then used to determine ^3H and ^{36}Cl, after subtracting the contribution from ^{22}Na. To measure the time course of influx of the isotopes, the larvae were sampled after 15, 30, 45, 60, 135, 150 and 330 min of exposure to the working solution. At the end of each interval, 15 larvae were removed with a wide-mouthed, fire-polished, bulb pipette into a tea strainer and then passed through three wash baths containing 400 ml of SW over a period of 6 min. The remaining larvae were transferred into 400 ml of SW to measure the effluxes. After 15, 30, 60, 90, 120 and 180 min the larvae were similarly sampled. The larvae were individually removed from the final wash solutions with fine forceps and dabbed dry on a Kleenex medical wipe at the end of both influx and efflux experiments. Three subsamples, each consisting of five larvae, were then placed in 0·5 ml of Soluene (Packard) in a 7 ml plastic vial and solubilized for 15 h overnight at room temperature. Scintillation fluid (Hionic Fluor, Packard, 5 ml) was then added and the radioactivity assayed.

Determination of rate constants

The rate constant of influx (K_1) was derived from the time course of uptake of each radioisotope using the equation:

$$Q = Q_{eq}(1 - e^{K_1 \cdot t}) , \quad (1)$$

(Motais & Isaia, 1972), where Q is the quantity (counts min^{-1}) of the isotope in the larva as a function of time (t), Q_{eq} is the equilibrium quantity and K_1 is the rate constant of influx.

The rate constant for efflux was estimated in two ways. First, from the regression line which fitted the data best and is described by the equation:

$$Q = ae^{K_2 \cdot t}, \tag{2}$$

where K_2 is the rate constant of efflux and a is a constant. Second, the rate of turnover during first hour of efflux (k) was also calculated using the equation:

$$k = 2{\cdot}3/(t_1 - t_0)\log_e(\text{counts min}^{-1} \text{ in larva at } t_0/\text{counts min}^{-1} \text{ in larva at } t_1) \tag{3}$$

(Evans, 1967), where k is the alternative rate constant of efflux, t_0 is the start of the experiment and t_1 is any time after t_0, which was taken to be 60 min.

Determination of permeability coefficients

Diffusional permeability coefficients (P_{diff}) were calculated from the equation (after Motais & Isaia, 1972):

$$P_{diff} = F_{H_2O}/A[H_2O] \text{ (cm s}^{-1}), \tag{4}$$

where F_{H_2O} is the ionic efflux (mmol s^{-1}), A is the total body area (cm^2, calculated from de Silva, 1973) and $[H_2O]$ is the internal water concentration (mmol cm^{-3}).

The osmotic permeability coefficient (P_{osm}) was calculated using the equation:

$$P_{osm} = V/A\alpha S, \tag{5}$$

in which V is the net water flow (mmol s^{-1}) which is calculated, in adult fish, from the difference between drinking rate and rate of urine flow. For cod larvae, since urine flow was not measured, net water flux was assumed to be the drinking rate previously measured by Tytler & Blaxter (1988*b*). Isaia (1972) found that net water flux in *Serranus scriba* was between 50 and 72 % of the drinking rate, depending on the acclimation temperature, which suggests that the P_{osm} for cod larvae may be overestimated. A is the total body area (cm^2), α is the reflexion coefficient which was assumed to be 1·0 and S is the concentration gradient of solutes (mmol l^{-1}).

Determination of water content and the concentrations of sodium and chloride

The water, sodium and chloride content of cod yolk-sac larvae were estimated from the asymptotic levels obtained by extrapolating from the exponential functions for each component. Thus, the tritiated water equilibrium level in 9-day-old cod larvae (Q_{eq} = 5654 counts min^{-1}, Fig. 1) obtained after exposure to a working solution with a specific activity of external bath of 17 441 counts min^{-1} translates into a water content of 0·32 ± 0·03 mg (efflux data). The concentrations of sodium and chloride were estimated from their respective equilibrium levels and the above estimate of the water content.

The wet mass was obtained by weighing a pooled sample of 10 larvae.

Results and discussion

The ^3H_2O, ^{22}Na and ^{36}Cl influxes in 9-day-old yolk-sac larvae of cod are shown

in Figs 1A, 2A and 3A. The lines describing the time courses of uptake of radioactivity are regression lines derived from equation 1.

The time courses of efflux of the three isotopes are also described by regression lines in Figs 1B, 2B and 3B which are based on equation 2. The rate constants for

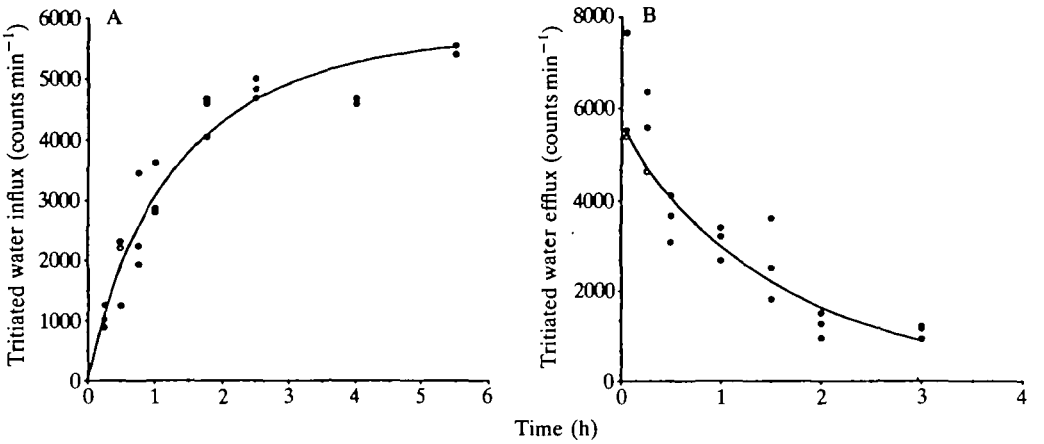

Fig. 1. The time course of the influx (A) and efflux (B) of tritiated water in 9-day-old cod larvae. The lines are based on the regression equations:

(A) $\log_e(Q_{eq} - Q) = 8{\cdot}61 - 0{\cdot}79t, \quad r^2 = 91{\cdot}8\,\%$,

(B) $\log_e Q = 8{\cdot}61 - 0{\cdot}61t, \quad r^2 = 85{\cdot}9\,\%$,

(see Materials and methods for key to variables).

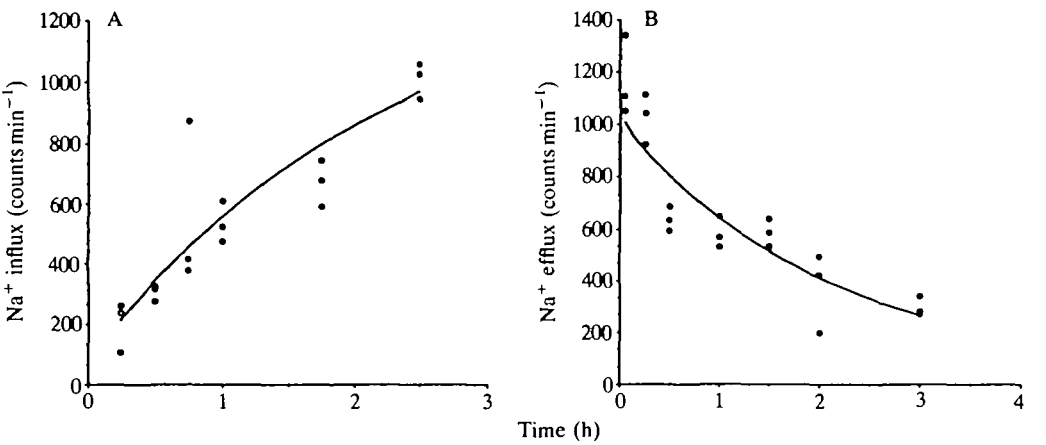

Fig. 2. The time course of influx (A) and efflux (B) of ^{22}Na in 9-day-old cod larvae. The lines are based on the regresssion equations:

(A) $\log_e(Q_{eq} - Q) = 7{\cdot}19 - 0{\cdot}45t, \quad r^2 = 79{\cdot}1\,\%$,

(B) $\log_e Q = 6{\cdot}91 - 0{\cdot}45t, \quad r^2 = 78{\cdot}9\,\%$.

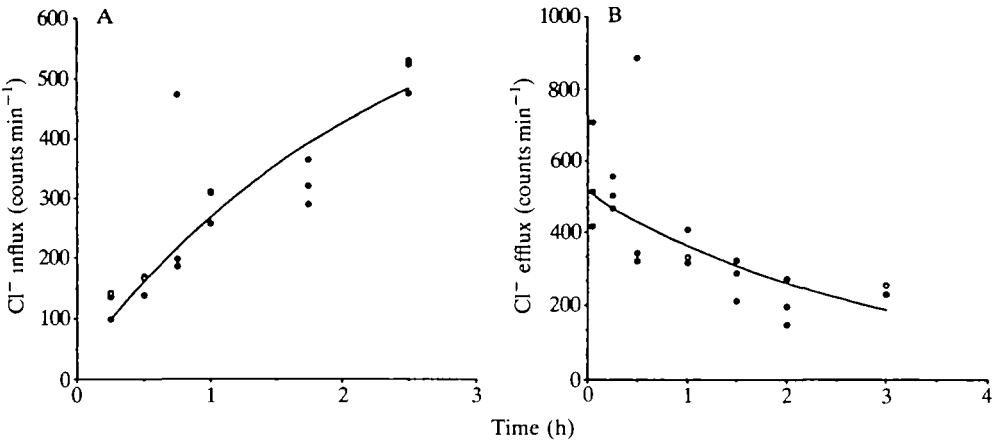

Fig. 3. The time course of influx (A) and efflux (B) of ^{36}Cl in 9-day-old cod larvae. The lines are based on the regression equations:

(A) $\log_e(Q_{eq} - Q) = 6{\cdot}53 - 0{\cdot}34t$, $r^2 = 71{\cdot}9\,\%$,

(B) $\log_e Q = 6{\cdot}23 - 0{\cdot}33t$, $r^2 = 58{\cdot}2\,\%$.

influx and efflux, K_1 and K_2, respectively, and the rates of turnover in the first hour (k), calculated using equation 3, of the isotopes are presented in Table 1.

There is good agreement between K_1 and K_2 rate constants, except in the case of ^3H_2O where the rate constant of influx ($K_1 = 0{\cdot}79\,h^{-1}$) appears to be higher than that for efflux ($K_2 = 0{\cdot}61\,h^{-1}$), but the difference was found not to be significant (t-test). The rate constants k and K_2 for efflux for ^{22}Na (0·51 and $0{\cdot}45\,h^{-1}$, respectively) and ^{36}Cl (0·29 and $0{\cdot}33\,h^{-1}$, respectively) are also very similar, but k for ^3H_2O was significantly lower than K_2. Rate constants for ions fluxes in adult fish have been found to be highly temperature-dependent, decreasing with decreasing temperature with Q_{10} of between 2·1 and 2·5 (Motais & Isaia, 1972). Since diffusional processes vary with absolute temperature, these flux changes are attributed to temperature-dependent changes in branchial blood flow. In 9-day-old yolk-sac cod larvae the gills are not yet formed and diffusion exchange is

Table 1. *Rate constants for ^3H_2O, ^{22}Na and ^{36}Cl during influx (K_1) and efflux (K_2 and k) in 9-day-old cod larvae in 34‰ sea water at 4·5°C*

	Rate constants (h^{-1})		
	Influx	Efflux	
Isotope	K_1	K_2	k
^3H_2O	0·79 ± 0·06	0·61 ± 0·05	0·46
^{22}Na	0·45 ± 0·06	0·45 ± 0·05	0·51
^{36}Cl	0·34 ± 0·05	0·33 ± 0·07	0·29

Values are mean ± S.D.

Table 2. *The ^3H_2O permeability coefficients of diffusion (P_{diff}) and osmosis (P_{osm}) for eggs, larvae and adults of teleost fish*

Species	P_{diff} ($cm\,s^{-1}\times10^6$)	Temperature (°C)	P_{osm} ($cm\,s^{-1}\times10^6$)	Temperature (°C)	Source
Eggs					
Cod	0·2–1·2	5	–	–	Mangor-Jensen (1986)
Plaice	0·2	5	–	–	Riis-Vestergaard (1984)
Larvae					
Cod	2·4	4·5	5·7	7·5	Present study and
Herring	1·0	7·0	3·1	7·5	Tytler & Blaxter (1988*b*)
Adults					
Serranus sp.	9·0	5·0	8·0	5·0	Isaia (1972)
Anguilla anguilla	9·0	5·0	1·5	5·0	Motais & Isaia (1972)

cutaneous. Rate constants in adults are also species-specific, but generally they tend to be lower than those for cod larvae (Table 1). Isaia (1972) found influx rate constants (K_1) for ^3H_2O and ^{22}Na to be 0·24 and $0{\cdot}22\,h^{-1}$, respectively, for *Serranus scriba* adapted to SW at 15 °C. A slightly higher ^3H_2O influx rate constant of $0{\cdot}35\,h^{-1}$ was measured by Motais & Isaia (1972) for SW-adapted eels (*Anguilla anguilla*) at 15 °C. In cod larvae the equivalent K_1 influx rate constants for ^3H_2O and ^{22}Na were 0·79 and $0{\cdot}45\,h^{-1}$, respectively, which are not only higher than for adults but K_1 for water is higher than that for sodium. The rate constants for ^{36}Cl in cod larvae are lower than that for ^{22}Na. Evans (1967) also found that the K_1 influx rate constant for ^{36}Cl was 65 % of that for ^{22}Na in *Xiphister atrapurpureus*.

Diffusion permeability coefficients (P_{diff}) for ^3H_2O of cod larvae, which relate ion flux to surface area, are much lower than in adult marine fish but higher than for eggs (Table 2). The low permeability of eggs has been attributed to the physical properties of the vitelline membrane (Potts & Eddy, 1973). It would appear that low permeability in larvae may also be attributable to the structure and composition of the integument. The relatively higher permeability in adults results from the high level of diffusion exchange which occurs across the gills. Isaia *et al.* (1979) measured an osmotic permeability coefficient of $30\times10^{-6}\,cm\,s^{-1}$ for gills in isolated head preparations of SW-adapted trout (*Salmo gairdneri*), which is considerably higher than that of intact animals (Table 2). It would be of considerable interest to examine the changes in permeability during the development of larvae, when the site of diffusion moves from skin to gills. The ratio of osmotic to diffusional permeability in yolk-sac cod larvae was found to be 1:2·4 (Table 2). In view of the likely 62 % overestimate of P_{osm} (see Materials and methods), P_{diff} and P_{osm} in cod larvae are similar. Evans (1967) and Motais *et al.* (1969) have also found the ratio of the permeability coefficients to be near unity in the adults of euryhaline species. The close match of the permeability coefficients in larvae verifies the estimates of drinking rates made by Mangor-Jensen & Adoff

(1987) and Tytler & Blaxter (1988*b*). It also supports the hypothesis that the relatively low drinking rates reported by Tytler & Blaxter (1988*b*) are a function of low skin permeability.

The water content of cod larvae was estimated, from the equilibrium level of ^3H_2O, to be $0{\cdot}32 \pm 0{\cdot}03$ mg, which is very close to the wet mass of 0·30 mg. This method is an alternative and may be a more reliable method of measuring body water content than conventional weighing techniques which rely on extrapolation to account for evaporative water loss. Pelagic larvae, which tend to be buoyant, have a high water content, for example Craik & Harvey (1984) found the water content of the tissues of cod yolk-sac larvae to 92 %. The ^{22}Na equilibrium level (1260 counts min^{-1} in a working solution of 20·9 counts min^{-1} $mmol^{-1}$ Na^+) gave a Na^+ concentration in the larva of 189 mmol l^{-1}. In adult marine fish the plasma Na^+ concentration is generally around 180 mmol l^{-1} (Rankin & Davenport, 1981), although Evans (1967) found a lower plasma Na^+ concentration of 160 mmol l^{-1} in *Xiphister atrapurpureus*, a small intertidal species. From the equilibrium radioactivity of ^{36}Cl of cod larvae the Cl^- concentration was estimated to be 148 mmol l^{-1}. According to Rankin & Davenport (1981), the typical plasma chloride concentration is 150 mmol l^{-1}. Evans (1967) has found a value of 156 mmol l^{-1} in *X. atrapurpureus*. It seems that cod larvae regulate body fluid hypo-osmotic to SW with sodium and chloride concentrations similar to that in adult fish.

We wish to thank Professor John Sargent for his generous support and invaluable advice. We are also indebted to the staff of the DAFS Marine Laboratory, Aberdeen, for the cod larvae.

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