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The Propagation of Cod *Gadus morhua* L.

WATER BALANCE IN COD EGGS

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ABSTRACT

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The yolk osmolarity of cod eggs decreases from about 400 mOsm 2 days after spawning to 300 mOsm at hatching, as opposed to a sea water osmolarity of c. 1000 mOsm. The osmolarity difference forces an osmotic water loss upon the eggs. The embryos take 3-4 weeks to reach a developmental stage allowing them to compensate water losses by the mechanisms known from the adult fish. In this period the rate of osmotic water loss is minimized by a low water permeability: 0.24×10^{-6} cm^3 cm^{-2} s^{-1} on day one to 0.6×10^{-6} or higher at hatching.

Water content predicted from osmolarities and water permeabilities agrees well with the observed water content until the completion of epiboly, indicating that water balance is governed mainly by the passive, osmotic water loss during this period. The rate of osmotic water loss increases with temperature in nearly the same way as does the rate of embryonic development, thus the total water loss till completion of epiboly may be independent of the water temperature. At later developmental stages the observed water content gradually becomes much larger than predicted, indicating that the water balance after epiboly is not governed exclusively by the passive osmotic water loss.

INTRODUCTION

It has been known for about a century that the adult marine teleost maintains an osmotic concentration different from that of the surrounding sea water (early review by Bottazzi, 1908). The osmolarity in the blood and the tissue fluid of euryhaline teleosts such as cod, plaice, flounder etc. is 350-400 mOsm, as opposed to c. 1000 mOsm in oceanic sea water. Sodium chloride is the major contributor to the osmolarity both in the tissue fluid (150-200 mMol l^{-1}, see e.g. Holmes and Donaldson, 1969) and in sea water (c. 500 mMol l^{-1}).

The concentration difference by itself would create a net diffusion of salt into the fish. An electric potential of 25-30 mV (fish positive) counteracts net influx of the positively charged sodium ions and accelerates uptake of the negatively charged chloride ions. Simultaneously, the fish is subject to a loss of water due to the osmotic difference. As a result of these passive transport processes, the osmolarity of the tissue fluid will tend to approach that of sea water.

The main features of the mechanisms responsible for counteracting the osmotic equilibration were discovered half a century ago. The osmotic water loss is compensated by the fish drinking sea water (Smith, 1930). When the sea water enters the gut, nearly all the sodium chloride is transported from the gut lumen to the surrounding tissues by an active, energy consuming process. This lowers the osmolarity of the gut fluid, so water follows passively, replacing the water lost through the external surfaces.

By compensating the osmotic water loss in this manner, the fish incurs a salt load in addition to the salt entering through the external surfaces. All the excess salt is secreted by the chloride cells located primarily in the gills (Keys, 1931; Keys and Wilmer, 1932), and also this salt transport is an active, energy consuming process.

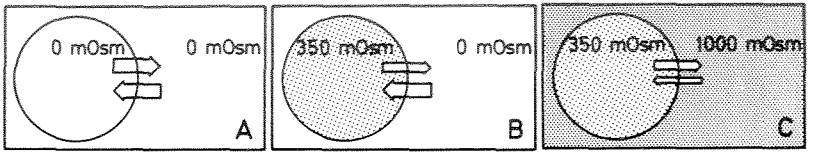

Fig. 1. Flows of water into and out of fish eggs. A: hypothetical situation for determination of water permeability. B: egg spawned in fresh water. C: egg spawned in sea water.

It was shown very early that teleost eggs are isosmotic with the mother fish at spawning (Dakin, 1911; Runnström, 1920). Thus the marine eggs face the same problems concerning loss of water and uptake of salt. Cod eggs require about 3 weeks to hatch in water temperatures of 3-5°C (Westernhagen, 1970), and it takes several days after hatching for the mouth, gut and gills to become functional. Thus the eggs and early larvae will have to survive about four weeks, before they from a morphological point of view can be assumed to replenish water by the mechanism known from the adult fish. The present paper reports on the status of our investigations to elucidate the mechanisms by which the eggs avoid osmotic desiccation during this period.

Fig. 1 describes basic osmotic phenomena in fish eggs. In Fig. 1A, a barrier separates two compartments of pure water. The number of water molecules - most often expressed as the volume of water - diffusing in 1 second through 1 cm^2 of the barrier from a compartment of pure water is by definition the *diffusional water permeability* of the barrier. The permeability is equal in both directions, and water will flow into and out of the egg at equal rates in the hypothetical situation of Fig. 1A.

In Fig. 1B the water in one compartment is replaced by a 350 mOsm solution, equivalent to the yolk of a fish egg. The presence of dissolved matter decreases the tendency of the water molecules to leave this compartment, without interfering with the tendency of external water molecules to

enter: there will be a net diffusion of water into an egg spawned in fresh water.

Finally, the water in the second compartment is replaced by a 1000 mOsm solution corresponding to sea water (Fig. 1C). Again the net diffusion of water will be from the compartment of lower osmolarity into the compartment of higher osmolarity, which in the case of a marine teleost egg is out of the egg: The egg will shrink.

According to the above description, an osmotic water loss can be calculated as the difference between diffusion into and out of the egg, given the internal and external osmolarities and the water permeability of the surface barrier. If the calculated water loss agrees well with the observed water loss, we may assume that the water balance of the eggs is governed exclusively - or nearly so - by the passive loss of water. If not, other possibilities must be considered.

The external egg envelope, the chorion, is freely permeable to both water and salts, thus we may assume the osmotic surface barrier to be the cell membrane in early stages, and the embryonic epithelium in later stages.

MATERIALS AND METHODS

The present investigations involved four batches of eggs, being the offspring of separate pairs of female and male cods. The eggs were incubated in sea water of 34‰ salinity at 5±1°C.

Yolk osmolarity and embryonic water.

Both the yolk osmolarity and the water content of embryo + yolk were determined on each of two batches of eggs. Measurements were performed as described for halibut eggs (*Hippoglossus hippoglossus* (L.)) by Riis-Vestergaard (1982a), except that samples of 20 cod eggs were used for water

determinations.

Water exchange

Water exchange before and after fertilization was investigated on two separate egg batches. Eggs were preloaded with radioactively labelled water (THO) by incubation at 5°C in Ringer's (unfertilized eggs) or sea water (fertilized eggs) containing c. 50 mCi THO l^{-1}, washed briefly to remove activity from the perivitelline fluid, and then transferred singly to containers with unlabelled medium. The activity lost from each egg was monitored by samples taken from the external media. At the termination of the experiments, four diameters were quickly measured at different angles. Then the activity still present in the egg was measured, allowing the calculation of the initial activity, M^0, and the fractional activity within the egg, M/M^0, during the experiment.

Calculations

The rate constants describing the transport of labelled water molecules out of the eggs were estimated by nonlinear curve fitting computer programmes.

The diffusion coefficient D, accounting for the resistance to diffusion of water molecules within the yolk, is required for the calculation of water permeabilities (Løvtrup, 1963) but has not been measured in cod eggs. In plaice eggs (*Pleuronectes platessa* (L.)) with a water content of 95%, the D-value was 95% of the value for pure water (Riis-Vestergaard, 1983). By analogy, the value for cod eggs with 92-93% water content was taken to be 92.5% of the value for pure water.

Water permeabilities were calculated under the assumption that water passes the surface barrier by simple diffusion. It was neglected that the embryonic water is not a compartment of pure water. This introduces an error of less than 1% in the absolute permeability values, but no error in the

calculated water loss.

The rate of osmotic water loss was calculated by

eq. 1: $dV/dt = P^d \times A \times (p^{out} - p^{in})/p^{in}$

(slightly modified from Loeffler and Løvtrup, 1970), P^d being the diffusional water permeability, A the area of the surface barrier, and p^{out} and p^{in} the vapour pressures outside and inside the surface barrier. The osmolarity, O, is substituted for vapour pressure by 'Raoult's law' on reduction of the vapour pressure, after which eq. 1 takes the form

eq. 2: $dV/dt = P^d \times A \times (O^{in} - O^{out})/(55.6 + O^{out})$

55.6 being the number of moles per kg of pure water.

The expected water volume at any stage of development was approximated by iteration of

eq. 3: $V^{t + \Delta t} \simeq V^t + dV/dt \times \Delta t$

starting with an initial volume and inserting the time dependent, observed values for P^d and O^{in}. The initial volume was that calculated for the time of the first actual observation by a linear regression fitted to the values observed during the first 7 days of incubation. It was assumed that P^d and O^{in} varied linearly between the actual observation points. A was calculated as $4\pi(3V^t/4\pi)^{2/3}$. The accuracy of the approximation depends on the choice of Δt. Intervals of 1 h were used in Fig. 6; 24 h intervals causes the volume calculated for the day of hatching to be only 2.5% smaller than with 1 h intervals.

Mean values of water loss were calculated from mean values of P^d and O^{in} (Fig. 6, heavy curves). P^d - 2 standard deviations were combined with O^{in} + 2 standard deviations for the estimation of minimum losses, and vice versa for maximum losses (Fig. 6, light curves).

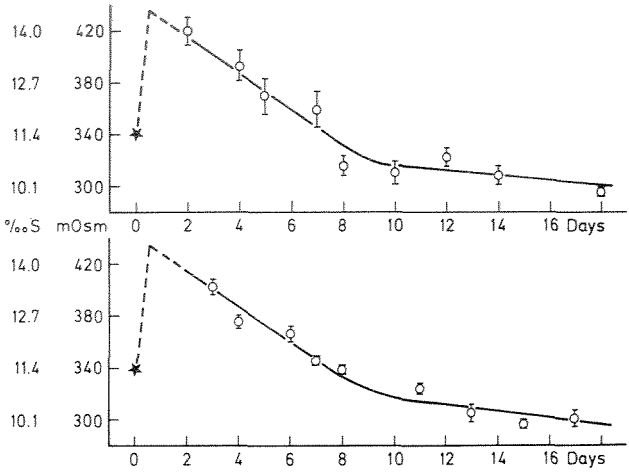

Fig. 2. *Gadus morhua:* Yolk osmolarity and salinity of isosmotic sea water in two batches of eggs incubated in 34‰ salinity at 5°C. ✱ : unfertilized eggs. o: fertilized eggs. Symbols represent mean values of 4-8 eggs; bars indicate 2 standard deviations. (Data by A. Mangor-Jensen, University of Bergen, 1982).

RESULTS

Yolk osmolarities from two batches of eggs are presented in Fig. 2. An initial rise in osmolarity is presumably taking place within the first few hours. The relatively small increment seems to indicate 'good-quality' eggs (Kjørsvik et al., 1984). After the initial increase, the eggs are able to decrease their osmolarity in spite of the hyperosmotic sea water, especially during the first week, after which the osmolarity tends to stabilize around 300 mOsm. A slower but more prolonged decrease has been observed in halibut eggs (Riis-Vestergaard, 1982a).

The diffusion of labelled water out of cod eggs before and one day after fertilization is shown in Fig. 3. The results fit well to straight lines in a semilogarithmic plot, indicating that the embryonic water (yolk + blastodisc)

exchanges essentially as one kinetic compartment. Therefore, the diffusion is best described by a monoexponential equation:

eq. 4: $M/M^0 = K \exp(-kt)$

in which k is the rate constant, K the intercept on the ordinate axis, and t the experimental time. The water permeabilities, being essentially proportionate to the slopes, are calculated according to Løvtrup (1963, Løvtrup's eq. 5). The results show that, at equal temperatures, the water permeability of unfertilized eggs is much higher than that of fertilized ones. At 5°C, the ratio of the permeabilities before and one day after fertilization is 25 in cod eggs, as compared to a ratio of 10 in eggs of pike (*Esox lucius* L., 9°C, Loeffler, 1971) and plaice (5°C, Riis-Vestergaard, 1982b), 15 in eggs of salmon (*Salmo salar* L., 3°C, Potts and Rudy, 1969), and about 50 in eggs of a killifish (*Epiplatys dageti* Poll, 20°C, Riis-Vestergaard, unpublished data).

It is also evident from Fig. 3 that the water permeability after fertilization, and hence the rate of osmotic water loss, is very temperature dependent. This is also illustrated in Table 1, which furthermore shows the apparent activation energy for water permeation. This quantity is proportionate to the slope of a semilogarithmic plot of water permeability against reciprocal temperature in Kelvin-degrees (a so-called Arrhenius plot), and thus is a measure of the temperature dependence of water permeation. Activation energies for water exchange in cod eggs before and after fertilization are comparable to those of plaice eggs (20 and 28 kcal/mol, respectively (Riis-Vestergaard, 1982b)). It should be noted that the activation energies for fertilized fish eggs are the highest ones known so far for water permeation across cell membranes, values for other cell types ranging from 5 to 15 kcal mol^{-1}.

TABLE 1

Diffusional water permeability P^d of cod eggs before and 1 day after fertilization. Mean values±standard deviation (n=3)

	$P_d \times 10^6$ ($cm^3\ cm^{-2}\ s^{-1}$)	
°C	Unfertilized	Fertilized
2	4.60±0.06	0.15±0.01
5	5.81±0.33	0.24±0.01
8	9.43±1.06	0.39±0.01
11	12.70±1.15	0.69±0.03
14		1.08±0.02
17		1.86±0.06
Apparent activation energy:	(kcal mol^{-1})	
for water permeation	18.30±1.73	26.56±0.52
for embryonic development*		22.21±1.25

*: based on results by Westernhagen (1970)

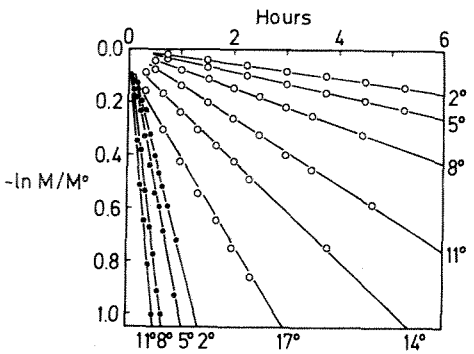

Fig. 3. *Gadus morhua:* Efflux of radioactively labelled water from fertilized (o) and unfertilized (●) eggs. M/M^0: fractional activity remaining in the egg.

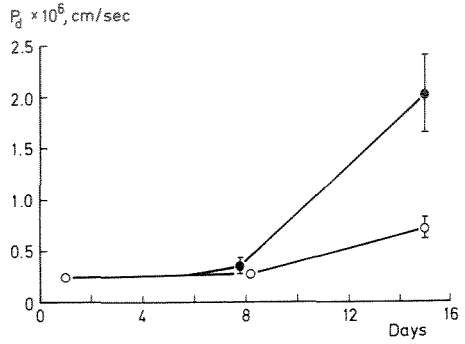

Fig. 5. *Gadus morhua:* Diffusional water permeabilities (5°C) of the surface barrier of eggs at different developmental stages, calculated according to a one-compartment model (o) and a two-compartment model (●). Mean values ±standard deviations (n=3). The eggs were fertilized day zero and incubated in 34‰ at 5°C.

model.

The smaller permeability values from the one-barrier model in cod eggs were used to estimate the mean values, and upper and lower limits, of the expected water content for each of the two batches of eggs of known internal osmolarity. The results are depicted by the continuous curves in Fig. 6. The individual data points represent the observed volumes. There is a good agreement between observed and predicted volumes until day 7 or 8, at which time the epiboly is complete. The water loss is relatively rapid during this period. After that the observed rate of water loss decreases, whereas the predicted rate increases, as both the permeability and the osmotic gradient increases. In eggs of halibut (Riis-Vestergaard, 1982a) and plaice (Riis Vestergaard, in preparation) the observed water loss proceeds at a rather constant rate throughout all egg stages.

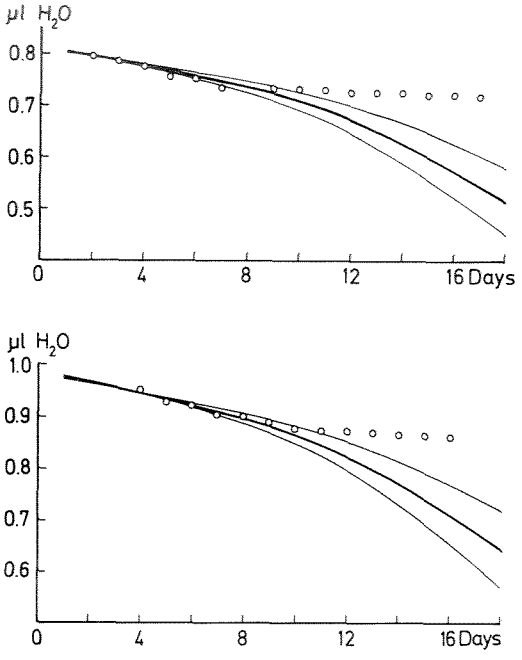

Fig. 6. *Gadus morhua:* Water content in embryos (including yolk) incubated in 34‰ at 5°C. Continuous curves: predicted mean values, and upper and lower limits calculated from mean ±2 standard deviations of water permeability and yolk osmolarity. Data points: observed water content in two batches of eggs. Data by A.Mangor-Jensen, University of Bergen, 1982).

DISCUSSION

It seems to be a general feature of marine teleost eggs, shared also by cod eggs, that they are able to maintain an osmolarity much lower than that of the surroundings. Due to this osmotic gradient the eggs inevitably incur an osmotic water loss.

The fundamental factor responsible for minimizing this water loss is an exceptionally low water permeability of the surface of the embryo. The water permeabilities of fertilized

fish eggs are the lowest known for any cell membrane. At comparable temperatures, the permeability of the fertilized fish egg is 10-50 times lower than before fertilization. It is two orders of magnitude lower than that of frogs eggs and sea urchin eggs and three or more orders of magnitude lower than that of red blood cells, muscle cells, mammalian egg cells and most other cell types.

It is not known how the low water permeability is achieved. It is presumably dependent upon a special composition and structure of the lipoid cell membrane. If so, detergents and lipofile molecules dissolved or dispersed in the sea water are expected to involve a potential danger to the water balance, as they may be able to interfere with the structural integrity of the membrane and thus increase the water permeability.

It also seems common to teleost eggs that the apparent activation energy (i.e., the temperature dependence) for water permeation is high already before fertilization and still higher afterwards. Physically, the activation energy may be interpreted as the amount of kinetic energy, which one mol of water molecules must possess to penetrate the surface barrier. At fertilization the barrier seems to undergo changes which exclude a larger fraction of low-energetic water molecules from passing it.

Data on the time to hatching for cod eggs at several temperatures have been published by Westernhagen (1970). From a plot of the logarithm of the reciprocal number of days to hatching versus the reciprocal temperature, we obtain the temperature dependence of the rate of embryonic development, equivalent in form to the activation energy (Table 1), though in this case the term cannot be ascribed to any definite physical process. The temperature dependence of the rate of development is about the same as that of the rate of osmotic water loss. Thus we may expect that the total amount of water lost in the period from spawning till a certain stage of development, will be rather independent of the ambient temperature, at least until the completion of epiboly (see

below).

The water permeability after the completion of epiboly is 3-10 times higher than in the earlier stages (Fig. 5) - depending on the interpretation of the curvilinear results (Fig. 4). In either interpretation, the epithelium of the embryo is more water tight than most living, biological structures and thus essential to minimize water loss. If the curvilinear plots (Fig. 4) are to be interpreted as an initially elevated permeability due to mechanical stress, then we may expect the water balance of the eggs to be affected also by the impact of waves in rough weather, as the eggs at least at intervals will be near the surface, and by water jets in incubation systems with running sea water. In this interpretation the apparent activation energy remains around 26 kcal mol^{-1}, and therefore the total water losses may be temperature independent also in the older egg stages.

The water balance of cod eggs incubated at 5^0C can be described as a simple passive, osmotic loss of water governed by the diffusional water permeability and the osmolarity difference between yolk and sea water until the completion of epiboly. Fig. 6 shows that this is not the case in later egg stages, as the true values for water content are not confined within the limits of the light curves (see Calculations). Clearly the difference between observed and expected volumes would have been much larger if the higher permeabilities from the two-compartment model had been used.

Deviations between predicted and observed water flows are often encountered in biological systems and have been discussed for decades; however, in all other cases the observed volumes are smaller than the predicted ones in 'shrinking experiments', (see e.g., House, 1974). To account for this observation, it has been suggested that the barriers possess water-filled channels or pores. In this case the diffusional water permeability is not an appropriate parameter to describe volume changes, because a bulk flow through the pores will proceed at a faster rate than predicted. In contrast, in the case of cod eggs, the osmotic volume changes

seem smaller than predicted - no explanations to comply with this fact have been suggested.

It is possible to make a correct prediction of the volume changes from the two-compartment model by assuming that the subdermal space is nearly isosmotic with the sea water and thus strongly hyperosmotic to the yolk sac. This assumption seems unlikely for two reasons. Firstly, the cells comprising the embryonic body must then also be assumed to have this high osmolarity, contrary to the cell osmolarity in all subsequent life stages, secondly, the assumption ascribes a large osmotic gradient to the internal barrier, so that the yolk sac should shrink much faster than is actually the case.

The differentiation of cells and tissues begins well before the incongruity appears day 8. A possible cause of the difference is, therefore, that the older egg stages may have developed an ability to replenish the osmotic water loss in a way different from that applied by the adult fish. So far, however, we have no hints as to the underlying mechanism.

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