J. exp. Biol. 138, 1–15 (1988)
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THE EFFECTS OF EXTERNAL SALINITY ON THE DRINKING RATES OF THE LARVAE OF HERRING, PLAICE AND COD

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Accepted 14 April 1988

Summary

Drinking responses to salinity change in the larvae of herring (*Clupea harengus* L.), plaice (*Pleuronectes platessa* L.) and cod (*Gadus morhua* L.) were measured from the time course of uptake of dextran labelled with tritium, following immersion in solutions of 32‰ and 16‰ sea water. The yolk sac and first feeding larval stages of all three species drink in both salinities. Furthermore, post-yolk sac stages appear to adjust their drinking rates to compensate for different salinities in a manner similar to that of the adults. Drinking rates in 32‰ sea water are approximately double those in 16‰. Mass-related drinking rates of larvae are higher than those in adults, but the differences do not match the differences in surface area to mass ratios, suggesting that larval skin is less permeable to water than is adult gill epithelium. Water absorption is indicated by the evidence of concentration of dextran in the gut. The estimates of drinking rates from tritiated dextran uptake are supported by epifluorescence microscopical measurements of the uptake of fluorescein isothiocyanate dextran.

Introduction

Marine fish larvae are known to be remarkably tolerant of a wide range of salinities, especially in the early post-hatching stages (Holliday, 1969). Species such as Pacific and Atlantic herring (*Clupea pallasii* and *C. harengus*), plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*) can tolerate 60–65‰ (parts per thousand salinity, 'normal' offshore sea water being 34–35‰ ≡ 1·0 osmol kg^{-1}) for 24 h or longer. Herring can also tolerate 1–2‰, plaice 5‰ and cod 10‰ for a similar period. Larvae of flounder *Platichthys flesus* can even withstand 0‰ for a few days (Yin & Blaxter, 1987).

Regulation of the osmotic concentration of the internal body fluids is known to occur (Holliday & Blaxter, 1960; Holliday & Jones, 1967). In fish abruptly

Key words: fish, larvae, drinking, salinity.

transferred between salinities, the osmotic concentration of the body fluids changes accordingly and mass changes can be measured. After a few hours the body fluids return to the 'normal' level, which in an external seawater salinity of about 34‰ (0·995 osmol kg^{-1}) is equivalent to 12‰ (0·35 osmol kg^{-1}) in herring and 15‰ (0·439 osmol kg^{-1}) in plaice. Although the tissues can withstand substantial changes in the osmotic concentration of the body fluids, there are thus well-developed mechanisms to restore the *status quo*.

These mechanisms, such as impermeability of the integument, ion excretion and secretion, urine production and drinking are well known in adult fish (Smith, 1930; Maetz & Skadhauge, 1968; Potts *et al.* 1967). In larvae, chloride cells have been found in the skin, for example in herring by Jones *et al.* (1966) and in sardine (*Sardinops caerulea*) by Lasker & Threadgold (1968). It also seems likely that the integument is relatively permeable and that an active pronephric kidney is functioning. Only Mangor-Jensen & Adoff (1987) have reported active drinking in newly hatched cod held at 5 °C in a salinity of 34‰ using an isotope, [^{3}H]dextran, with a high relative molecular mass.

The present paper explores the drinking activity of three species, herring, plaice and cod held, not only in sea water (32‰) but also in near-isosmotic water of 16‰ salinity (0·468 osmol kg^{-1}), where it might be expected that osmotic problems and, therefore, drinking would be minimal. The isotope technique of Mangor-Jensen & Adoff (1987) was used and, in addition, fluorescein-labelled dextran and epifluorescence microscopy was used as an additional tool. The basis of both these techniques is to place larvae in sea water containing one or other of these substances, to sample the larvae at intervals and to measure the uptake of sea water by the presence of dextran in the gut as shown by its radioactivity or fluorescence. Since dextran is a large molecule and supposedly inert, it should not be digested in the gut. The initial progressive accumulation of dextran in the gut should therefore be a direct measurement of drinking rate.

Materials and methods

Material

The larvae were reared from broodstock caught at sea or near spawning and stripped on board the research vessel or after a holding period in the aquarium. The eggs were hatched in aquarium tanks at temperatures of 7–8 °C and in a salinity of 30–32‰. The larvae were used near or after the end of the yolk sac period which in all species lasted about 1 week. The older larvae were fed rotifers (*Brachionus plicatilis*) or brine shrimp (*Artemia* sp.) nauplii. The larvae were deprived of food overnight before an experiment to ensure the guts were empty. Ages of larvae are given in days post-hatching. Lengths are total lengths of larvae fixed in 4 % formalin in 50 % sea water. Dry masses were obtained by drying fixed larvae to constant mass in an oven at 60 °C and weighing a pooled sample of 10 larvae.

Measurement of drinking rates

[^{3}H]Dextran solutions

Tritiated dextran, with a mean M_r of 70000 and radioactivity of 9·3 MBq, was purchased in freeze-dried form from Amersham International. The stock solution was made up by dissolving the labelled dextran in 3 ml of sterile distilled water and stored for a maximum period of 48 h before use. The working solutions consisted of 1·5 ml of this stock solution made up to 100 ml of either 16 or 32‰ sea water, which had been passed through 0·22 μm micropore filters to remove micro-organisms. The specific activity of the working solutions was thus approximately 46 kBq ml^{-1}. The larvae were acclimatized to the experimental salinities overnight in a constant-temperature room at 7·5°C in the dark. If the larvae were feeding, this period allowed the guts to be emptied. They were then transferred to the working solutions by wide-mouthed pipette after being washed once in microfiltered water to reduce microbial contamination. The maximum numbers of larvae kept in the 100 ml working solutions were 40 cod, 120 herring or 80 plaice. After 1, 2, 4, 6 and 15 h of exposure to the radiolabelled marker in the dark at 7·5°C in sea water, five samples, containing between two and five of the specimens of herring and plaice, were taken. The sampling procedure for cod differed in the following ways: (1) three samples of 7-day-old yolk sac stage larvae were taken, each containing six individuals removed from 32‰ radioactive solutions; (2) 19-day-old post-larvae were sampled once, each sample containing between six and 18 individuals (see Fig. 4). The larvae were caught with a wide-mouthed pipette and trapped by filtering the discharge from the pipette through a tea strainer, held over the working solution to prevent waste of the radioactive marker. They were then transferred to a wash bath containing 200 ml of water of the same salinity as the working solution but without the marker and left for 2 min. After repeating this washing procedure the sample was placed in 1 ml of soluene-350 (Packard) and allowed to digest for 1–2 h at 45°C. Finally 5 ml of the scintillation fluid Optiphase T was added and the sample mixed thoroughly and left for 16–24 h before measuring radioactivity with a LKB 1219 Rackbeta liquid scintillation counter.

Fluorescein isothiocyanate dextran

Fluorescein-labelled dextran (Sigma) (F-dextran), with a mean M_r of 70000, was made up at a concentration of 3 mg 40 ml^{-1} in working solutions of 16 and 32‰. After overnight acclimation to the test salinities in the dark at 7·5°C, larvae were transferred to the working solutions and held in the same conditions. Individual larvae were removed at intervals during a 5-h period, washed for 2 min, and then transferred to an anaesthetic bath containing benzocaine (500 mg l^{-1}). As soon as they were immobile, individual larvae were placed on a cavity slide and examined by ultraviolet (450–490 nm) epifluorescence microscopy. The length and width of the fluorescence in the gut were measured by micrometry and the intensity of fluorescence was scored using an arbitrary visual scale from 0 (zero fluorescence) to 3 (maximum observed fluorescence in the hindgut after 5 h in

32‰ sea water). The volume of fluorescence in the gut and the total gut volume were calculated using mathematical formulae appropriate to the shape of the gut segment (see Fig. 5). Total gut fluorescence was then estimated by multiplying the appropriate volumes by the appropriate fluorescence scores.

Analyses

Uptake of [^{3}H]dextran. Drinking rate was derived from the time course of increase in radioactivity in whole larvae on the assumption that the uptake of [^{3}H]dextran occurred only *via* the mouth. Mangor-Jensen & Adoff (1987) have suggested, on the basis of autoradiography, that radioactivity is confined to the lumen of the gut of cod larvae similarly exposed to [^{3}H]dextran (see Discussion). Water uptake was calculated using the following equation:

$$\text{water uptake} = (R_s - C)/(R_r)N \text{ nl larva}^{-1},$$

where R_s is the total radioactivity of the sample (counts min^{-1}), C is the background radioactivity obtained from a control sample of larvae, R_r is the specific radioactivity of the working solution (counts $\text{min}^{-1}\,\text{nl}^{-1}$) and N is the number of larvae in the sample. Drinking rates were calculated from the differences between mean estimates of water uptake after 1, 2 and 4 h exposure and, where appropriate, from the slope of the regression lines relating water uptake to exposure time. The significance of the results was tested by *t*-test and regression analysis.

Uptake of fluorescein isothiocyanate dextran. Drinking rates were estimated by the changes in volume and total fluorescence in the gut with time of exposure, from regression analyses of the linear or second-order hyperbolic relationships between these variables and exposure time.

Results

Uptake of [^{3}H]dextran

Herring larvae

Water uptake of feeding larvae at the 35 days post-hatching stage, derived from the uptake of [^{3}H]labelled dextran, increased linearly with time in both 32 and 16‰ sea water (Fig. 1; Table 1). Using the slope of the regression lines drinking rates were estimated to be 46·6 and 19·2 nl $\text{larva}^{-1}\,\text{h}^{-1}$ in 32 and 16‰ sea water, respectively. In 32‰ sea water, the mean water uptake was higher at all four sampling times, although *t*-tests showed that only at 4 and 6 h were the differences between 32 and 16‰ sea water significant ($P < 0{\cdot}05$ and $<0{\cdot}01$, respectively). Drinking rates, also derived from the regression equations but expressed as a percentage of the gut volume (Table 2), indicate that the times taken to fill the gut with water in 32 and 16‰ sea water are 2·2 and 4·5 h, respectively. A similar difference in drinking rates can be seen in Fig. 1.

Plaice larvae

At 16 days post-hatching, water uptake also increased linearly with time in feeding-stage plaice larvae in both 32 and 16‰ sea water (Fig. 2; Table 1). Mean values for water uptake of larvae in 32‰ sea water at 1, 2 and 4 h were significantly higher than those in 16‰ sea water ($P < 0{\cdot}01$, 0·05 and 0·05, respectively). The drinking rates, derived from the slopes of the regression lines, were 24·1 and 16·6 nl larva^{-1} h^{-1} in 32 and 16‰ sea water, respectively. Drinking rates are expressed as a percentage of the gut volume in Table 2. The time taken to fill the gut is estimated as 2 h and 4·5 h in 32‰ and 16‰, respectively, which is almost identical to the herring.

Cod larvae

The time course of water uptake of 4-day post-hatch, yolk sac stage cod larvae, which was measured at 7°C in 32‰ sea water only, is shown in Fig. 3. The difference between means of water uptake in the first hour and the second hour was not statistically significant. Between the second and fourth hours, however,

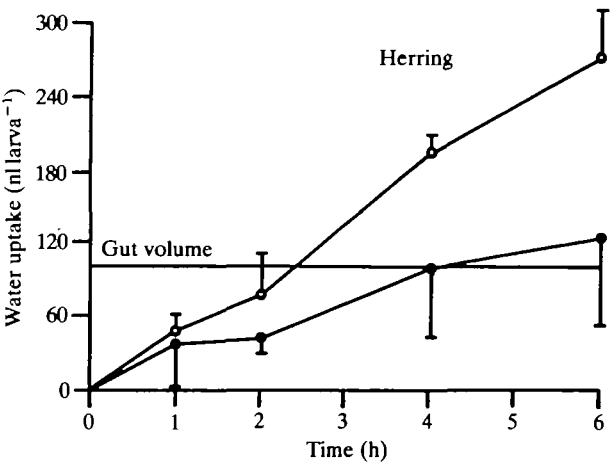

Fig. 1. The uptake of water in nl larva^{-1}, equivalent to the accumulation of [^{3}H]dextran, of 35-day-old herring larvae in 32‰ (○) and 16‰ sea water (●); the vertical bars are 95 % confidence limits; each point represents the mean of five samples each of 2–3 larvae; gut volume was measured in 30-day-old larvae.

Table 1. *Linear regression equations relating water uptake (y) in nl larva^{-1} to exposure time (x), for two species of marine fish larvae*

Species	Salinity (‰)	Regression equation	*r*	*P*	*N*
Herring	16	y = 13·3 + 19·22x	0·69	<0·001	20
	32	y = −4·2 + 46·56x	0·97	<0·001	20
Plaice	16	y = 2·4 + 16·56x	0·74	<0·01	15
	32	y = 12·7 + 24·12x	0·96	<0·001	15

Table 2. *The effect of salinity on the relative drinking rates of herring, plaice and cod larvae with respect to dry mass, wet mass and gut volume*

Species	Age (days)	Total length (±S.D., mm)	Dry mass (mg*)	Wet mass (mg*)	Salinity (‰)	Drinking rate: Relative to dry mass ($\mu l\, g^{-1} h^{-1}$)	Drinking rate: Relative to wet mass ($\mu l\, g^{-1} h^{-1}$)	Drinking rate: Relative to gut volume (% h^{-1}) (volume in nl)
Herring	35	13·15 ±0·82	0·30	1·80	32	155·3	25·9	46·6 (100)
					16	64·7	10·8	19·2 (100)
Plaice	16	7·72 ±0·40	0·23	1·38	32	104·8	17·5	30·2 (80)
					16	72·2	12·1	20·7 (80)
Cod	19	4·04 ±0·34	0·04	0·24	32	670·7	111·6	193·9 (18)
					16	257·5	42·9	57·2 (18)

* Individual masses from a pooled sample of 10 larvae.

the water uptake increased significantly ($P<0{\cdot}01$) giving a drinking rate of 7·5 nl larva^{-1} h^{-1}. Larvae at a later stage (19 days post-hatch) had higher rates in 32‰ sea water (Fig. 4). Although the relationship between water uptake and exposure time, in larvae exposed to 16‰ sea water, was approximately linear, the relationship in 32‰ sea water was hyperbolic. Drinking rates based on the uptake of water in the initial 2 h of exposure were estimated to be 26·8 and

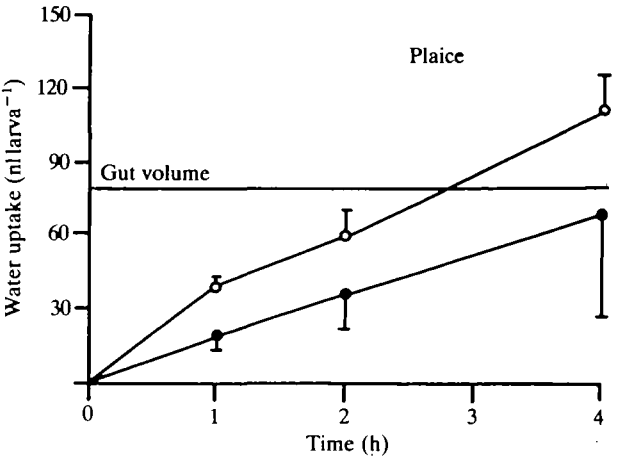

Fig. 2. The uptake of water in nl larva^{-1}, equivalent to the accumulation of [^{3}H]dextran, of 16-day-old plaice larvae in 32‰ (○) and 16‰ sea water (●); vertical bars are 95 % confidence limits; each point represents the mean of five samples each of five larvae; gut volume was measured in 16-day-old larvae.

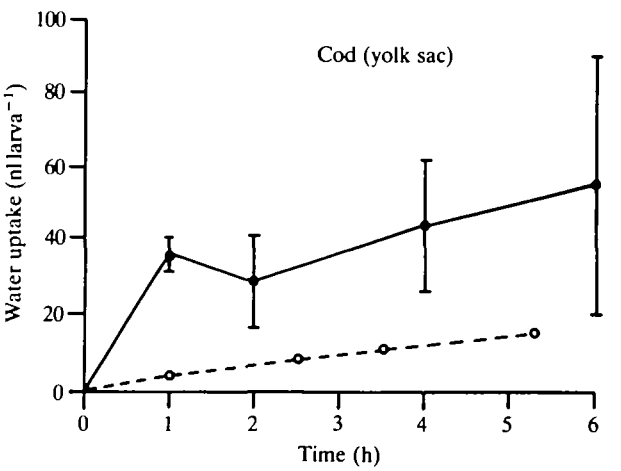

Fig. 3. The uptake of water in nl larva^{-1}, equivalent to [^{3}H]dextran accumulation, of 7-day-old cod larvae in 32‰ sea water; vertical bars are 95 % confidence limits; the mean water uptake (○) obtained using the method of Mangor-Jansen & Adoff (1987) for 5-day-old cod larvae is also included; each point (●) represents the mean of three samples each of six larvae.

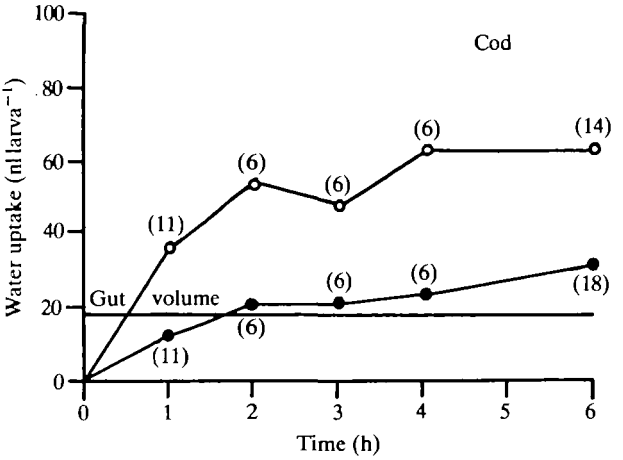

Fig. 4. The uptake of water, equivalent to [^{3}H]dextran accumulation, of 19-day-old cod larvae in 32‰ (○) and 16‰ sea water (●); each point represents a single sample; the number of larvae in each sample is given in parentheses; the gut volume was measured in 19-day-old larvae.

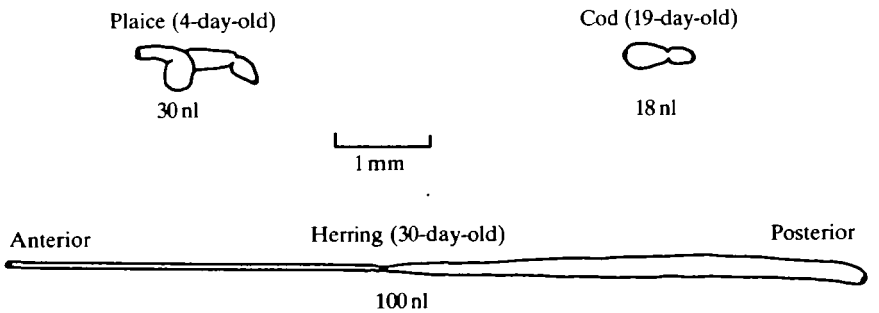

Fig. 5. Scale diagrams of the guts of 30-day-old herring, 4-day-old plaice and 19-day-old cod.

10·3 nl larva^{-1} h^{-1} in 32 and 16‰ sea water, respectively. The time taken for larvae to fill their guts in 32‰ (0·62 h) was 31 % of that for larvae in 16‰ sea water (2·0 h) (Fig. 4; Table 2).

Uptake of fluorescein isothiocyanate dextran

Herring larvae

The gut in herring larvae at the 41-day post-hatching stage is a straight tube, composed of a narrow foregut (volume = 22 nl) which is separated by a constriction from the hindgut (volume = 248 nl) (Fig. 5). In the early stages of exposure to fluorescein-labelled dextran solutions fluorescence first appeared in the pharynx and foregut. The timing of the appearance of fluorescence in the hindgut, although

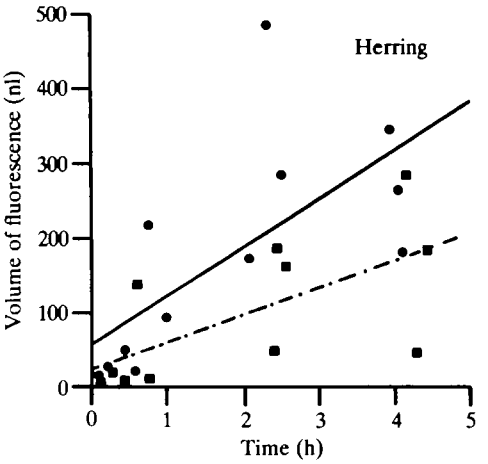

Fig. 6. The rate of change in the volume of fluorescence in the guts of 41-day-old herring larvae at two salinities; 32‰ (●) and 16‰ (■). Also shown are the regression lines for 32‰ (solid line) and 16‰ (broken line).

Table 3. *Regression equations relating the volume of fluorescence (V_t, nl) and the total fluorescence (T_f) in the gut of 41-day post-hatch herring larvae to the time of exposure (x, h)*

Salinity (‰)	Regression equation	*r*	*P*	*N*
16	$V_t = 25{\cdot}3 + 35{\cdot}9x$	0·5	NS	12
32	$V_t = 59{\cdot}8 + 65{\cdot}1x$	0·72	0·01	12
16	$T_f = 53{\cdot}0 + 102{\cdot}0x$	0·23	NS	12
32	$T_f = 145{\cdot}0 + 198{\cdot}0x$	0·75	0·01	12

NS, not significant.

primarily influenced by the salinity of the test solutions, was also subject to individual variation. The volume of fluorescence in the gut increased in an irregular fashion in both 16 and 32‰ sea water (Fig. 6). Linear regression analyses of the data for both salinities are summarized in Table 3. The drinking rates, based on the slopes of the lines of best fit were 65·1 and 35·9 nl larva^{-1} h^{-1} in 32 and 16‰ sea water, respectively (Table 4). Although the larvae at 41 days were larger than the 35-day post-hatching stage larvae used in the [^{3}H]dextran experiments and the estimated volumes of water uptake were higher, the order of difference in drinking rates in 32 and 16‰ sea water was similar. Most of the change in gut fluorescence occurred in the hindgut. The measure of the volume of fluorescence does not take account of the concentration of dextran in the gut. The estimate of total gut fluorescence (see Materials and methods), however, goes some way towards

Table 4. *Comparison of drinking rates established by [^{3}H]dextran and F-dextran in the larvae of herring, plaice and cod*

Species	Salinity (‰)	F-Dextran (nl larva^{-1} h^{-1})	Age (days)	[^{3}H]Dextran (nl larva^{-1} h^{-1})	Age (days)
Herring	16	35·9	41	19·2	35
	32	65·1		46·6	
Plaice	16	34·7	4	16·6	16
	32	26·8		24·1	
Cod	32	18·7	19	26·8	19

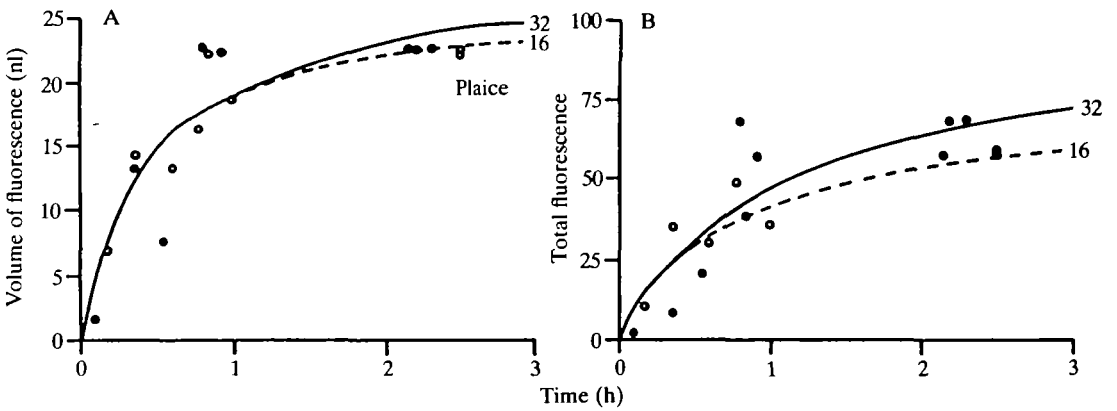

Fig. 7. (A) The change in volume of fluorescence in the gut of 4-day-old plaice in 32‰ (○) and 16‰ sea water (●). (B) The change in total fluorescence score in the gut of 4-day-old plaice exposed to 32‰ (○) or 16‰ sea water (●). The lines are based on regression analyses of the relationships between $\log_e(V_g - V_t)$ and time (t), where V_g is full gut volume and V_t is the volume of fluorescence in the gut at time (t).

correcting this error. The rate of change in total fluorescence based on linear regression analysis (Table 3) was 1·94 times higher in 32 than in 16‰ sea water.

Plaice larvae

In 4-day post-hatching stage plaice larvae, four distinct sections of the gut were identified; oesophagus (volume = 2·95 nl), stomach (13·8 nl), intestine (8·8 nl) and rectum (4·6 nl) (Fig. 5). As a result, it was easier to follow the progress of the fluorescence down plaice gut than herring gut, which is a long, straight cylinder. The volume of the fluorescence increased hyperbolically with time in both 32 and 16‰ sea water (Fig. 7A). The times taken to half fill the gut were not significantly different (0·5 and 0·4 h in 32 and 16‰ sea water, respectively), but the theoretical asymptotic volume was higher in 32‰ (29·6 nl) than in 16‰ sea water (26·3 nl). Drinking rates based on the half times were 27 and 35 nl larva^{-1} h^{-1} in 32 and 16‰ sea water, respectively. Analyses of the total gut fluorescence showed similar

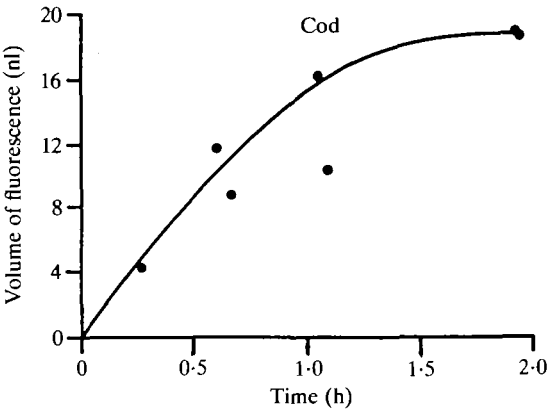

Fig. 8. The rate of change in the volume of fluorescence in the guts of 19-day-old cod larvae in 32‰ sea water. The line is based on regression analysis of the relationship between $\log_e(V_g - V_t)$ and time (t) (see Fig. 7).

uptake kinetics (Fig. 7B), from which the estimated times taken to reach an arbitrary asymptotic total fluorescence score of 58 in 32 and 16‰ sea water were 1·5 and 2·5 h, respectively.

Cod larvae

The gut in 19-day post-hatching cod larvae consists of two ellipsoidal sections. The larger anterior part (volume = 13·8 nl) was separated from the posterior part (4·1 nl) by a sphincter-like constriction (Fig. 5). The uptake of fluorescein-labelled dextran by 19-day post-hatch cod larvae was measured in 32‰ sea water only (Fig. 8). The rate of change in the volume of fluorescence was hyperbolic, with the major changes occurring in the anterior section. The estimated time taken to half fill the gut was 0·66 h which gives a drinking rate of 18·7 nl $larva^{-1}$ h^{-1} during this period.

Discussion

One of the major factors enabling fish larvae to survive changes in salinity is the ability to regulate the contents of their body fluids (Holliday, 1969). In water hyperosmotic to body fluids the process of regulation in larvae may be the same as in adults, which involves drinking and salt secretion (Smith, 1930). Maetz & Skadhauge (1968) found that adult eels, *Anguilla anguilla*, increased drinking as the salinity of the water to which they were acclimated increased. They found, for example, that the drinking rate in sea water was approximately half that in double-strength sea water. Potts *et al.* (1967) showed that *Tilapia mossambica* in 40 % sea water drank at 40 % of the rate in sea water.

The first evidence of drinking in fish larvae, presented by Mangor-Jensen & Adoff (1987), showed that newly hatched cod larvae in sea water (34‰, 5°C)

increased their rate of drinking with age so that at 7 days post-hatching they were swallowing sea water at a rate of 0·5 % body mass h^{-1}, which was similar in relative terms to that in adult fish (Smith, 1930; Maetz & Skadhauge, 1968). In the present study, the estimated drinking rate for 4-day-old cod larvae, also based on the uptake of [^{3}H]dextran, was 7·5 nl $larva^{-1}$ h^{-1} at 7·5°C in 32‰ sea water, which is higher than the mean drinking rate of 2·93 nl $larva^{-1}$ h^{-1} measured by Mangor-Jensen & Adoff (1987) for 5-day-old cod.

To assess drinking in aquatic animals, the accepted practice is to place the animal in a solution containing a marker substance that is taken into the alimentary canal but not absorbed across its wall. After an interval, sufficient to allow the substance to enter the gut but not to be defaecated, the amount contained in the gut is measured and divided by its concentration in the solution. Mangor-Jensen & Adoff (1987) assert that dextran is a suitable marker because it is 'inert'. They also showed, by autoradiography, that [^{3}H]dextran is only found in the lumen of the gut of larval cod. In the present study, fluorescein-labelled dextran was also found only in the gut of all three species of larvae. However, dextran, in common with all organic markers, is biodegradable. As a carbohydrate it is ultimately degraded to carbon dioxide and water by micro-organisms in aerated sea water. In effect, [^{3}H]dextran becomes, in natural sea water, a diminishing component of the total radioactivity of the marker solution, whereas tritiated water (HTO) becomes an increasing component. The tritium released by microbial degradation, as HTO, will exchange rapidly with the larvae; a half-time of 13·5 min for HTO efflux from yolk sac herring larvae at 4·6°C in 34‰ sea water has been measured (P. Tytler, in preparation). Also, the radioactivity taken in this form will tend to be lost from the larvae during the washing procedure. Microbial degradation of the dextran marker was minimized by using working solutions which were freshly made up with sterile sea water, larvae washed in sterile sea water, lower temperatures and exposure times of not more than 6 h to estimate drinking rates. This may explain the differences between our estimates of drinking in yolk sac cod larvae and those of Mangor-Jensen & Adoff (1987).

The method of observing the uptake of fluorescein isothiocyanate dextran was used as a visual check of the time course of uptake of radiolabelled dextran. It confirmed that larvae drink in both salinities and that dextran uptake is confined to the gut (Tytler & Blaxter, 1988). The variability in the estimates of drinking rates (Fig. 6) and the differences in the time course of uptake (Figs 2 & 7) indicate that this method is too insensitive and unreliable on its own. However, in spite of these criticisms, the estimates of drinking rates by the two methods are similar. In all three species the drinking rates, based on [^{3}H]dextran uptake, in 16‰ sea water were approximately half those in 32‰ sea water (Table 1), which is a similar response to that of adult fish (Maetz & Skadhauge, 1968; Potts *et al.* 1967). Clearly herring, plaice and cod larvae can alter their drinking rate as part of the process of regulating their body fluids.

The herring larvae, which were older and larger than the other larvae, had the highest drinking rates in both salinities (Table 1). However, when the drinking

Table 5. *A comparison of the relative surface areas and wet masses of the larval stages before the development of gills, with post-metamorphosis stages of herring and plaice*

	Larvae		Post-metamorphosis	
Species	Surface area ($mm^2 mg^{-1}$)	Wet mass (mg)	Gill area ($mm^2 mg^{-1}$)	Wet mass (g)
Plaice	21·58*	0·218	0·165	1
			0·083	100
Herring	19·26*	0·249	0·88	1
			0·34	100

The data are derived from regression equations relating surface area to mass during development (De Silva, 1973).

* Stages used in [^{3}H]dextran uptake measurements.

rates were related to body mass the differences were reduced (Table 2). In terms of wet mass, drinking rates of the larvae of herring and plaice ranged from 17·5 to 25·9 $\mu l\, g^{-1}\, h^{-1}$ which are higher than for adults. Potts *et al.* (1967) suggested that size had an influence on relative drinking rates in adult fish, in order to explain the high drinking rates (11 $\mu l\, g^{-1}\, h^{-1}$, 34‰) in *Tilapia mossambica* weighing less than 3 g compared with larger eels (3·3 $\mu l\, g^{-1}\, h^{-1}$, 34‰) weighing in excess of 100 g. In view of the adverse surface area to mass ratio (Table 5) it is not surprising to find high relative drinking rates in larvae. However, the differences in relative drinking rates do not match the enormous differences in the area to mass ratio, which points to interesting differences in the permeability of adult gills compared with larval skin. In the present study none of the larvae investigated had developed gills (De Silva, 1973). It seems likely that the appearance of gills represents an interesting stage in the ontogeny of osmoregulation in fish larvae. When drinking rates are related to gut volume the size effect should also be reduced. In fact, herring and plaice larvae took similar times to fill their guts in 32‰ sea water (2·2 and 2 h, respectively). Cod larvae, which are substantially smaller than either herring or plaice larvae (Table 2), had the highest relative drinking rates, in terms of both body mass and gut volume. The high rate of gut filling may explain the hyperbolic nature of the relationship between water uptake and time (Fig. 4). At 32‰ the probable residence time of fluid in the gut is 2–4 h. The time course of uptake of [^{3}H]dextran by herring and plaice larvae is also hyperbolic, but the time taken to reach saturation is longer than for cod larvae. In 36-day-old herring larvae, adapted to 32‰ sea water, the maximum level of [^{3}H]dextran was reached after 9 h.

When the volume of imbibed [^{3}H]dextran solution exceeds the volume of the gut, the implication is that dextran is being concentrated, as water is absorbed in the gut. The major change in the fluorescein-labelled dextran content of the herring gut occurred in the hindgut, where the volume and intensity of fluor-

escence increased with exposure time. In some specimens, following long exposure to F-dextran, circular areas of bright fluorescence were seen in the gut wall in the anal region immediately after defaecation. These spots suggest the presence of pits or folding of the gut epithelium where F-dextran is concentrated and trapped. Generally a level of fluorescence higher than ambient fluorescence in 32‰ F-dextran solutions would indicate water absorption in this region. The sites of water absorption were similarly located in the stomach of plaice and the foregut of cod. It is clear, nonetheless, that the processes and structures involved in water absorption in the larval gut are largely unknown and require further investigation.

The clearance of both forms of dextran from saturated herring and cod followed an irregular defaecation pattern in which at least 7 h was required for complete emptying of the gut. Since the saturation level, in 32‰ sea water, indicates a volume of fluid imbibed which is greater than the gut volume in all species, it is reasonable to assume that drinking is a continuous process and that the saturation level is set by the rates of water absorption and defaecation.

We are grateful to Professor J. R. Sargent, University of Stirling, and Dr R. M. Stagg, Heriot-Watt University, for reading this paper in manuscript, to Dr J. Gamble and Mr D. Seaton, DAFS Marine Laboratory, Aberdeen for supplying cod eggs, and to Dr J. Parkes and Mr W. Buckingham of the SMBA for help in using the scintillation counter and epifluorescence microscope. Financial support by the Royal Society from the Browne, Hill and Murray research fund and by the University of Stirling's internal research fund is gratefully acknowledged. Finally special thanks are due to Professor Sargent for his enthusiasm for the project and his encouragement.

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