

OSMOREGULATION IN MARINE TELEOST EGGS AND LARVAE

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The fact that the eggs and larvae of some marine teleosts can survive and develop in sea water with a very low salt content was established by investigators such as Ford (1929) who incubated herring eggs in a salinity of 4.8‰, and Johansen (1908) who reported newly hatched plaice larvae living in 7‰ in the Baltic. It has also been found that the larvae of some marine fish, for example the clupeids and the rockling, can tolerate salinities considerably greater than sea-water. Battle (1930) reared *Encheilyopus cimbrius* in a salinity as high as 70‰. Kurata (1959) found that the yolk sac larvae of *Clupea pallasii* could tolerate 59.5‰ for 24 hours, and Holliday & Blaxter (1960) found that the same stage of *Clupea harengus* could tolerate 60.1‰. The ability to survive in extremes of salinity was apparently based on the actual tolerance by the embryonic tissues of a change in their water content, coupled with the ability of the developing organism to regulate, at least to some extent, the osmotic concentration of the tissues and tissue fluids.

Among the questions posed by these findings were

- (a) Is this ability found in a wide range of species, and does the age and condition of the fish affect its ability to survive in different salinities?
- (b) How extreme are the changes in water content and are they at the cellular or extracellular level?
- (c) Where are the sites of regulation, and how much does it 'cost' the organism metabolically?

A research program designed to investigate some aspects of these problems is at present being pursued on three species, the Atlantic herring (*Clupea harengus*) the plaice (*Pleuronectes platessa*) and, to a lesser extent, the cod (*Gadus callarias*). These species differ in their adult distribution, spawning habits and developmental pattern. All three are commercially important and two at least have been the subject of hatchery and farming experiments (see Shelbourne et al. 1963 and Bibov, 1960), and basic information on their physiological capabilities is desirable from this point of view.

Much of the material for this work was supplied by, and some of the work done in collaboration or close association with, J.H.S. Blaxter (Aberdeen), A.B. Bowers (Isle of Man), G. Hempel (Hamburg) and J.E. Shelbourne (Lowestoft), and I am grateful to them for the material and for stimulating discussions of the work.

MATERIAL AND METHODS OF INCUBATION

Herring eggs were obtained from autumn spawning fish of the Scottish east coast, and from spring spawning fish of the Scottish Clyde estuary, Norwegian coast and the Baltic. Gametes were collected and stored as described by Blaxter (1955). Plaice eggs were obtained from sea-caught spawners off the Isle of Man and from plaice that had matured during an over-wintering period in outdoor ponds attached to the Marine Biological Station, Isle of Man. In some experiments the plaice eggs were stripped from the ripe parents, in other cases fertilized eggs were skimmed from the surface of the holding ponds. The cod eggs were obtained from a natural spawning of these fish kept in the aquarium of the Marine Biological Station, Isle of Man.

After fertilization in 500 ml jars the eggs were incubated in glass tanks of 5 to 50 liters capacity. Smaller numbers of eggs and larvae were kept in 500 ml jars and the water changed every second day. Glass fibre tanks, capacity 350 liters, were used for long term holding experiments. The water in these tanks was filtered and recirculated.

Low salinity water was made up by adding distilled water to sea water; high salinity water by adding NaCl to sea water. Freezing point depressions ($\Delta^{\circ}\text{C}$) were determined using the apparatus of Ramsay & Brown (1955). Experiments were made at specific temperatures in the range 4–14°C. In some experiments the water was treated with antibiotics (sodium penicillin G and streptomycin sulphate mixture) to keep down bacterial growth (see Shelbourne, 1963).

THE GAMETES

In both plaice and cod the mature gametes were isotonic with the blood of the parent, in the herring the semen was isotonic but the eggs were hypotonic (see Table 1).

Table 1
FREEZING POINT DEPRESSIONS OF FISH BLOOD AND GAMETES ($\Delta^{\circ}\text{C}$)

Species	Adult Blood Δ	Egg Δ	Semen Δ
Plaice.....	0.58	0.60	0.59
Cod.....	0.81	0.80	0.84
Herring.....	0.92	0.75	0.92

Measurements of changes in the osmotic pressure of the peri-vitelline fluid (see fig. 1) showed that the chorion of the egg in all three species was freely permeable, offering no protection against osmotic withdrawal of water in high salinities, but mechanically opposing the entry into the cytoplasm of the egg of more than a certain amount of water in low salinities (Lasker & Theilacker (1962) reported that removal of the chorion resulted in the rapid swelling and bursting of the eggs of *Sardinops caerulea* in low salinities). The sperm have no mechanical barrier to water movement and it is not yet known if any mechanism exists to oppose osmotic change.

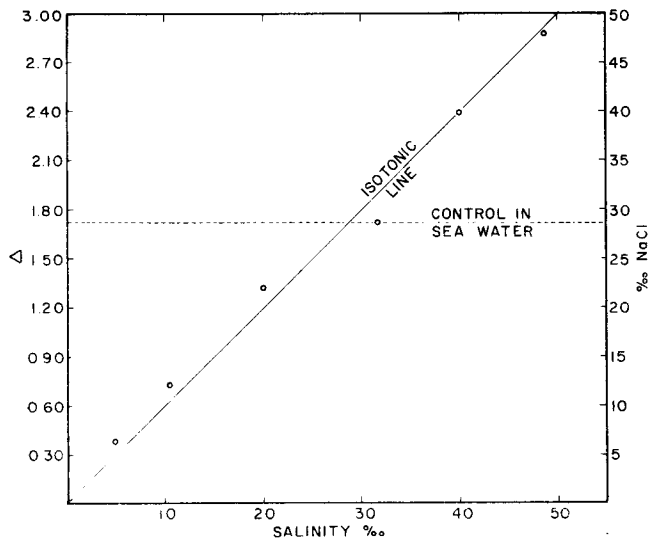


FIGURE 1. Osmotic pressure of the perivitelline fluid of herring eggs, 24 hours after transfer from sea water (32.3‰). Plaice and cod gave almost identical results.

FERTILIZATION

Measurements of percentage fertilization were made on both herring and plaice eggs as a criterion of tolerance of the gametes. Eggs and sperm were stripped directly into two separate 500 ml containers of the test salinity, allowed to stand for 1-2 minutes and then mixed; after a further 10 minutes the water was changed. The results are shown in fig. 2.

Blaxter and Holliday (1963) reported that immediately after the egg of the herring (which is demersal) was shed, the osmotic concentration of the yolk changed rapidly, approaching close to isotonicity with the water into which it was released. Preliminary results suggest that the same trend is not true in the pelagic eggs of the plaice and cod, in these the changes are not so extreme and a large concentration difference is still maintained between the yolk and the external salinity.

EARLY DEVELOPMENT

The size of the developing egg was fixed at or shortly after fertilization. The eggs were larger and heavier in the lower salinities due to their higher water content (see fig. 3).

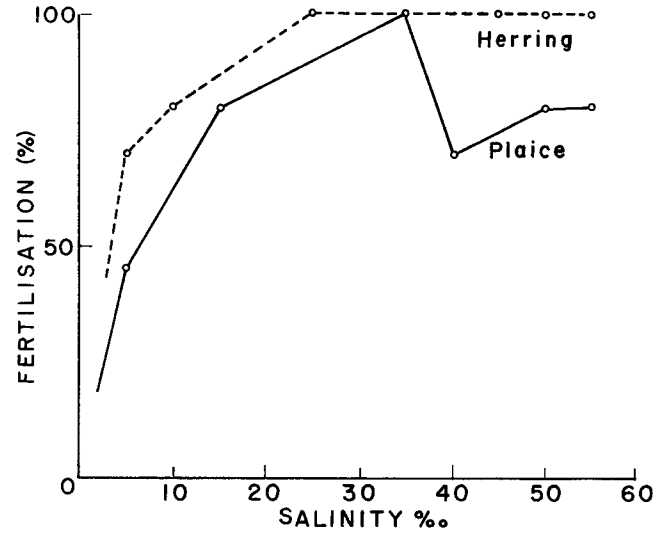


FIGURE 2. Percentage fertilization of herring and plaice eggs. (Data for herring from Holliday & Blaxter 1960).

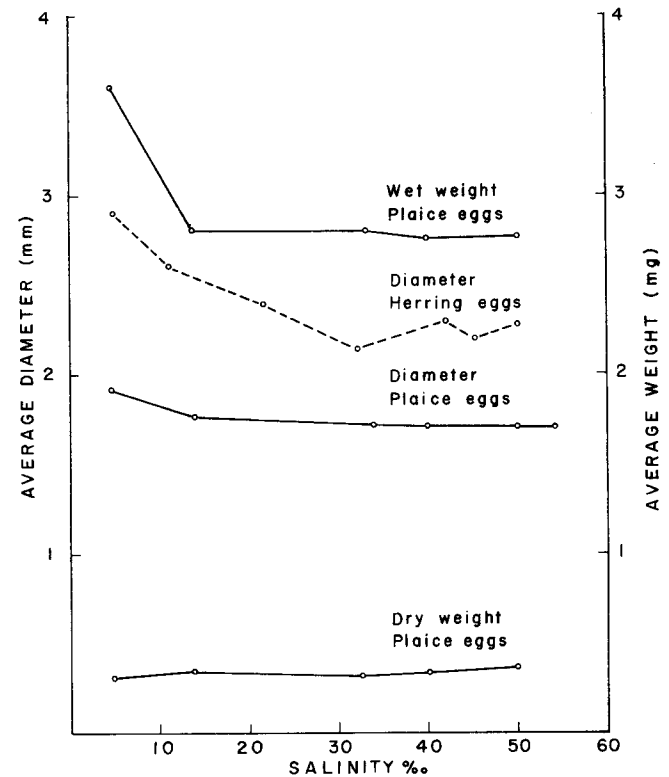


FIGURE 3. Size of developing herring and plaice eggs. (Data for herring from Holliday & Blaxter 1960).

The effects of the low salinities on the size of the developing egg were also apparent in the size of the individual cells of the blastula cap, and the diameter of the whole blastula cap (fig. 4a & b). If plaice and cod eggs, at the 8-16 cell stage, were transferred from sea-water (34‰) to other salinities, changes in the size of the cells and the appearance of the blastula cap occurred within 10 minutes. In the low salinities the cells became bloated, irregular in shape

and tightly pressed together. In the high salinities the cell boundaries became very sharply defined, the cells shrank slightly and became distinctly rounded, remaining in contact with each other only at points on their boundaries.

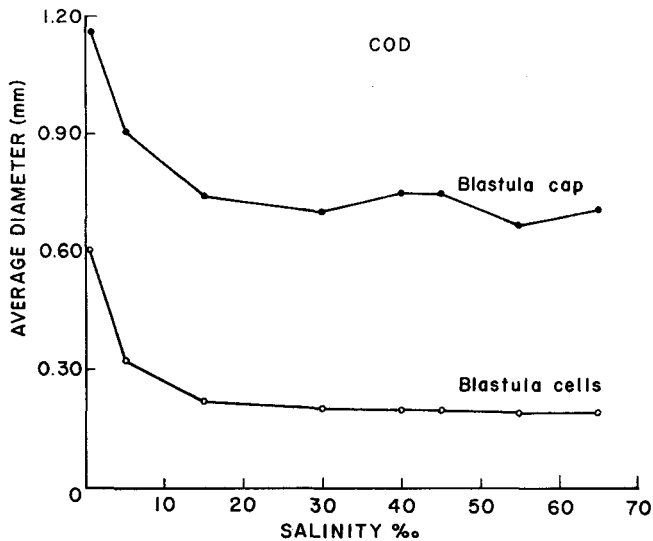


FIGURE 4. Size of the blastula cap and cells of cod and plaice eggs.

In the very low salinities (less than 5‰) the cells of the blastula cap of the plaice showed abnormal cleavage divisions; these divisions appeared to be the result of the tight packing together of the swollen cells. The divisions were sometimes incomplete and often unequal, producing a great range of cell sizes.

The blastula stage in the plaice had little tolerance to low salinities, but after gastrulation the tolerance to the lower salinities was greater (see fig. 5). In the cod heavy mortalities occurred prior to gastrulation in salinities outside the range 35–45‰. McMynn & Hoar (1953), working on the effects of low salinity on the survival of the eggs of *Clupea pallasii*, found that after the closure of the blasto-

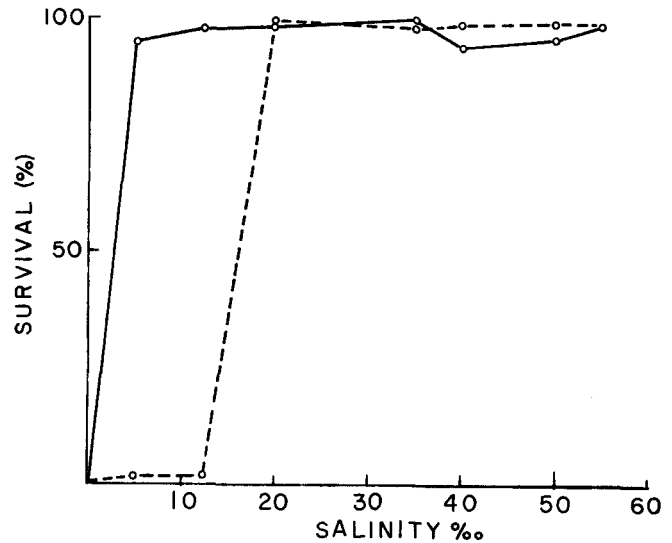


FIGURE 5. The effects of salinity on the survival of plaice eggs. --- blastula stage ——— gastrula stage

pore (i.e. the end of gastrulation) there was a greater ability to tolerate low salinities.

The ability of the developing egg to regulate the osmotic concentration of the yolk and tissues is still being investigated; preliminary results on the herring (Blaxter & Holliday, 1963) suggest that at the blastula stage there is little regulation of the yolk. After gastrulation, when the entire surface of the egg is covered by ecto and endodermal layers, there is developed the power to regulate, and the osmotic content of both the yolk and the tissues are, at the primitive streak stage, regulated to the level found at hatching. The cells of the ecto and endoderm appear to be the site of this regulation, and osmotic and ionic exchange between the egg and the medium takes place over the whole of the exposed ectodermal surface.

HATCHING

The percentage of eggs hatching after incubation in a range of test salinities may be used as an index of the tolerance of the most susceptible stage in development. Figure 6 shows this data for the herring, plaice and cod. The developing eggs of the herring are more tolerant of the low salinities than either cod or plaice, and the plaice eggs are more tolerant of high salinities than the herring or cod.

Ford (1929) and McMynn & Hoar (1953) reported an increase in mortality of Atlantic and Pacific herring eggs at, or just prior to, hatching in low salinities. Larvae were found dead partly emerged from the chorions. This phenomenon was also found in the experiments on cod and plaice. It varied both from year to year and with the origin of the eggs. For example, eggs from pond-wintered plaice in 1963 showed heavier mortalities from this cause than eggs from pond wintered plaice in 1962 or sea wintered fish in 1962 or 1963. An inability to completely wriggle free of the chorion may be due to poorly developed tail musculature in the low salinities (as suggested by Battle (1930) in the rockling). However this was not espe-

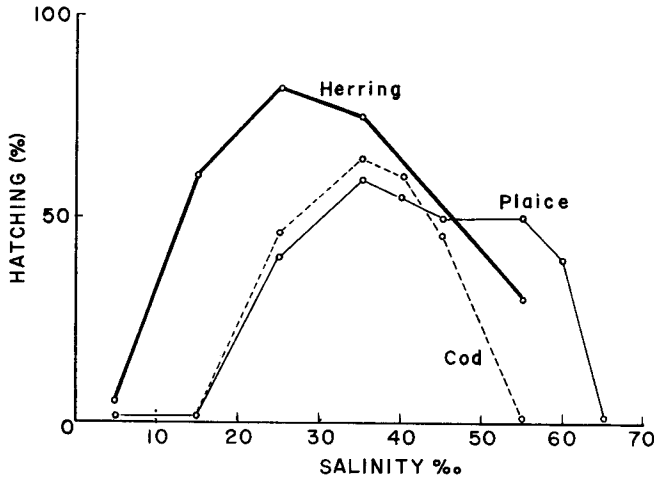


FIGURE 6. Percentage hatching of herring, cod and plaice eggs. (Data for herring from Holliday & Blaxter 1960).

cially obvious and it is possible that the low specific gravity of the water made it more difficult for the larvae to get free. It may also be that the chorions do not rupture so easily in low salinities, and differences in 'toughness' of the chorion might account for year to year variations.

There are several reports of structural abnormalities of larvae hatched in unnatural salinities (e.g. Battle, 1930 and Kryzhanovski, 1956). The abnormalities were usually either skeletal or distortions of the body cavities. About 2 per cent of the cod larvae hatched in 45‰ had deformed tails. No deformities were found in either plaice or herring larvae, although there was a clear difference in size between different

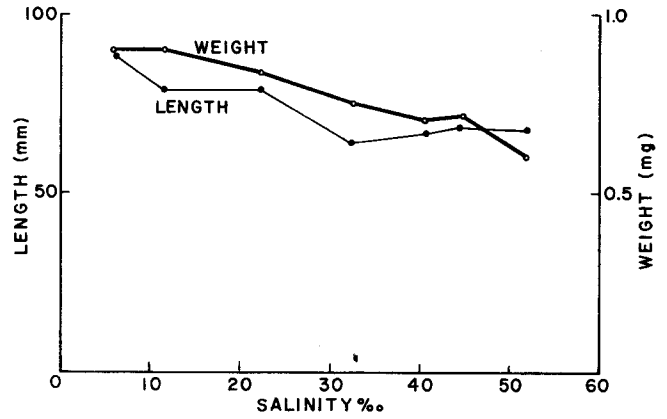


FIGURE 7. Size of newly hatched herring larvae.

salinities, this is shown for herring larvae in fig. 7. Plaice and cod showed very similar trends.

The size difference is almost certainly due to the greater water content of the larvae hatched in the lower salinities. The yolk sacs of larvae produced in salinities greater than 50‰ were shrunken, bright yellow in color and firm when touched. In salinities below 15‰ the yolk sacs were turgid and pale yellow in color.

THE LARVAE

The behavior of the newly hatched larvae depended on the salinity. Herring and plaice larvae in sea water (34‰) swam actively almost the whole time. Cod larvae tended to remain quiescent, floating near the surface and occasionally swimming in rapid bursts of

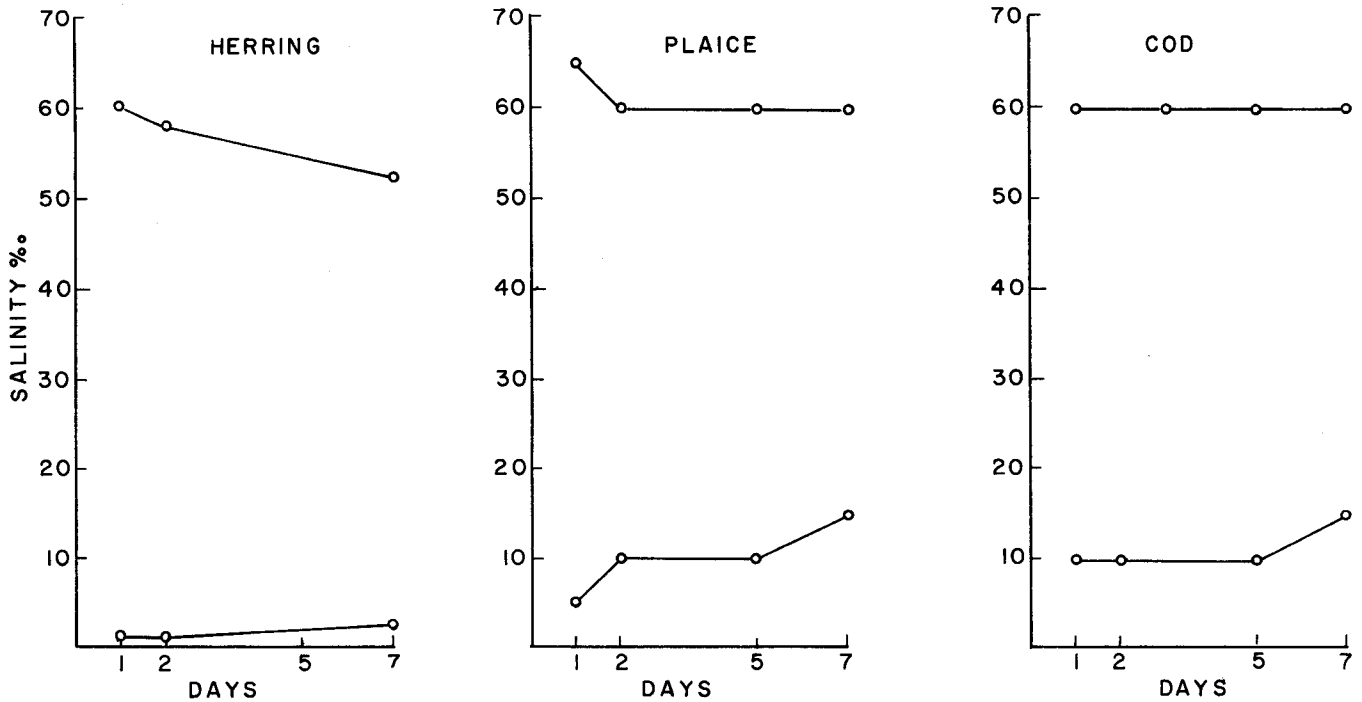


FIGURE 8. Salinity tolerance of newly hatched larvae. Criterion of tolerance was that 50% of the larvae should survive and remain active for 7 days in the test salinity.

activity. In salinities less than 12‰ all the larvae swam near to the bottom, and often lay there for long periods. In salinities from 12‰ to 45‰ activity was the same as in sea water. In salinities above 45‰ the herring and plaice larvae spent a good deal of time trying to swim down; cod larvae remained very quiescent on the surface, moving violently if disturbed. These differences in activity are discussed later in relation to energy expenditure and survival in different salinities.

The results of salinity tolerance experiments on the yolk sac larvae are shown in figs. 8a, b & c. Clearly at this stage all three species are capable of withstanding a wide range of salinities, for the test periods of up to 7 days. Again an interesting difference between the species was the greater tolerance of yolk sac herring larvae to the lower salinities, and the greater tolerance of plaice and cod larvae to higher salinities. It has been shown (Holliday & Blaxter, 1960) that the survival in high and low salinities in the herring is based on tissue tolerance and body fluid regulation. This is also true in plaice and preliminary results on the cod do not suggest any different mechanism involved. Figures 9 and 10 show changes in total body weight and in the freezing point of the tissue fluids which indicate the osmotic movement of water into the tissues in low salinities and from the tissues in high salinities. There is a short term tolerance by the tissues of internal osmotic concentrations equivalent to

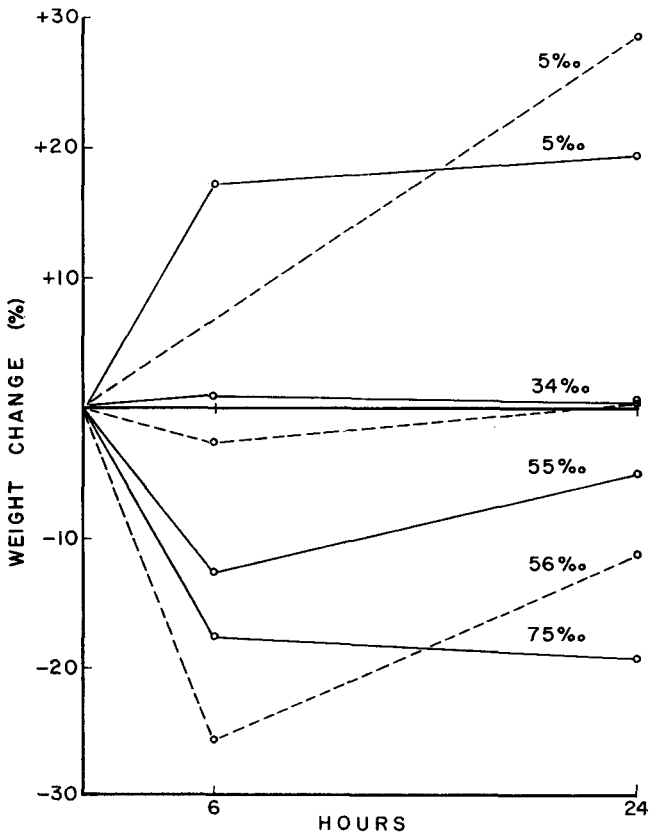


FIGURE 9. Weight changes of larvae after transfer from sea water (34‰) to the test salinity. ----- herring (data from Holliday & Blaxter 1960). ——— plaice.

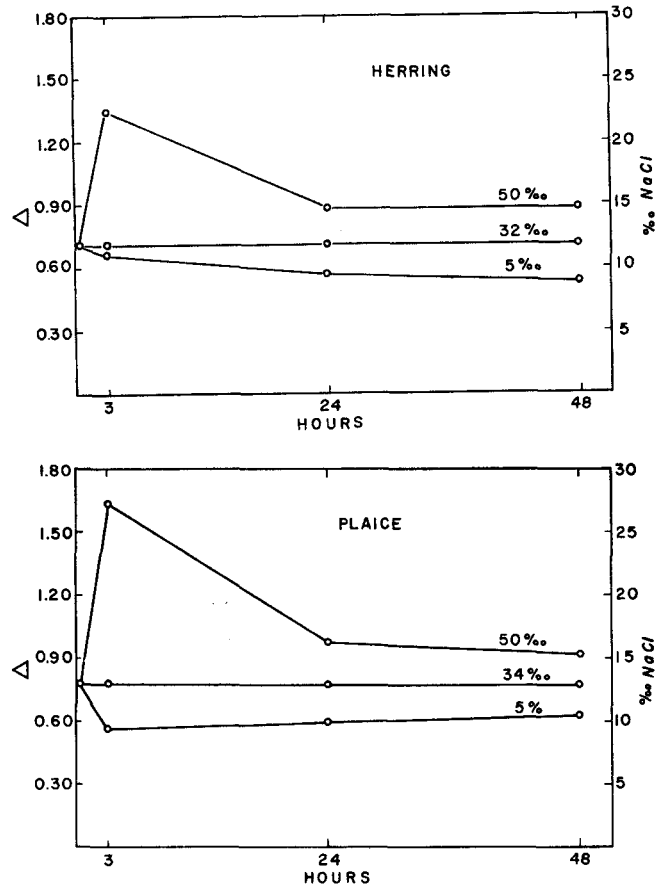


FIGURE 10. Osmotic concentrations of larval body fluids. Plaice larvae transferred from 34‰ . . . Herring larvae transferred from 32‰.

salinities of 30‰ in the plaice and 22.5‰ in the herring. Regulation takes place within 24 hours, returning the body fluid concentration to a level equivalent to about a third of the external environment in salinities of 35‰ and above, and at a level equivalent to about 10‰ in salinities below sea water.

The observations of changes in size of the blastula cells are a strong indication that changes in body water brought about by osmosis take place at the intra-cellular level. So far there is no evidence for this hypothesis in the larval stages, but Gordon (1959) concludes, for adult *Salmo trutta*, that tissue concentration changes were attributable to changes in intra-cellular water.

THE EFFECTS OF AGE ON SALINITY TOLERANCE

Kurata (1959) showed that salinity tolerance in *Clupea pallasii* varied with age, 10-day old larvae were more tolerant, than yolk sac larvae, while 20-day old larvae were less tolerant. Holliday & Blaxter (1960) found that post-metamorphic *Clupea harengus* had a much narrower tolerance than the yolk sac larvae (Fig. 11a and b). Difficulties in rearing herring larvae to the stage of metamorphosis prevented the determination of the time when this change in tolerance oc-

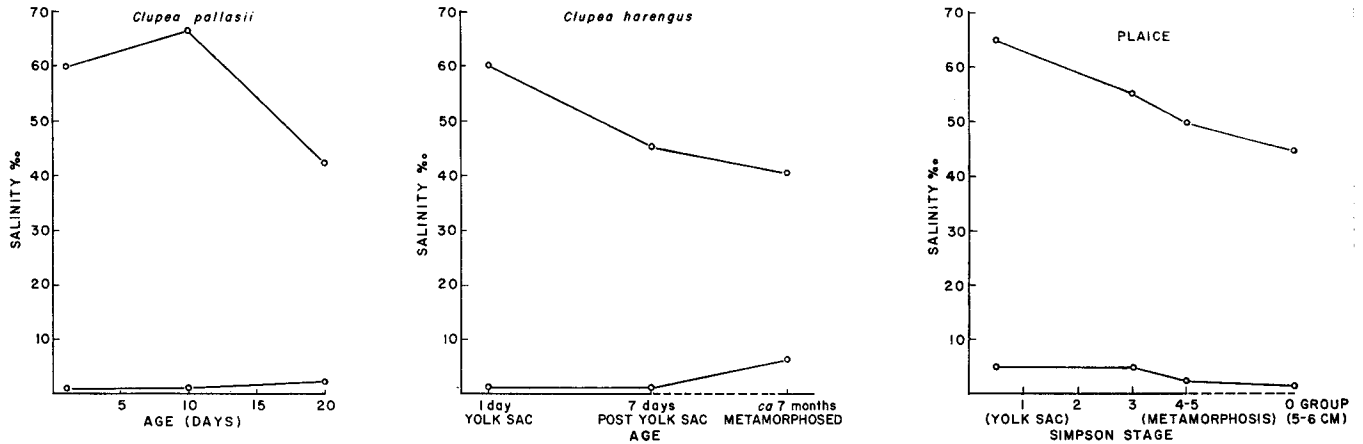


FIGURE 11. The effects of age on salinity tolerance. (Data for *Clupea pallasii* from Kurata 1959).

curred. This difficulty did not arise in the case of the plaice. Figure 11 c shows the salinity tolerance of plaice larvae at different stages in development.

Clearly the change in tolerance is a gradual process completed just after metamorphosis, when the typical adult pattern is established. In the plaice the tolerance to high salinities decreased, but it increased to low salinities. In the herring after metamorphosis, tolerance decreases to both high and low salinities.

At metamorphosis the epidermis thickens, develops scales and becomes chiefly protective in function. This is incompatible with the previous respiratory and regulatory functions and these processes are restricted to the gill epithelium.

Nothing is known of the osmo-regulatory functions of the pro-nephric kidney found in the early larvae of the herring, plaice and cod. The part played by the gut in osmo-regulation in the larvae is also unknown. It is normally not fully formed until a short time after hatching. It seems likely, at least from the structural point of view, that it is at metamorphosis that the adult pattern of regulation, based on the balance between gut absorption and renal and extra-renal excretion, is established.

SALINITY AND METABOLISM

The metabolic cost of osmotic regulation has been assessed as insignificant for the larvae of *Sardinops caerulea* (Lasker & Theilacker, 1962), and considerable for young and adult *Plotosus anguillaris* Job, (1959). It is not possible to generalize, as such factors as the state of adaptation, the sensitivity of the tissues of different species and the age and 'fitness' of the individual fish will almost certainly affect the metabolic response to different salinities.

Recent work by Holliday, Blaxter and Lasker (1964) on oxygen uptake of herring larvae showed that if the larvae were reared in a wide range of salinities (5-55‰) oxygen uptake did not vary with the salinity. However if the larvae were subjected to an abrupt change in salinity then oxygen uptake oscillated during the period of osmotic change, returning to its original level after regulation had taken place. It was found necessary to obtain a standard degree

of activity by anaesthetizing the larvae. As described earlier the difference in buoyancy of the larvae in different salinities resulted in activity (and hence oxygen uptake) being less in the low salinities. For this reason low salinities probably make less demands on the metabolism of the larvae. The ability to survive in a given salinity may also depend on the available food supply, a factor which may vary considerably in the sea. Shelbourne (1957) reported starving plaice larvae associated with poor plankton patches in the North Sea. He suggested that death might be due to a breakdown of osmo-regulatory ability. The results of experiments on the survival of starving larvae in different salinities are shown in Figure 12.

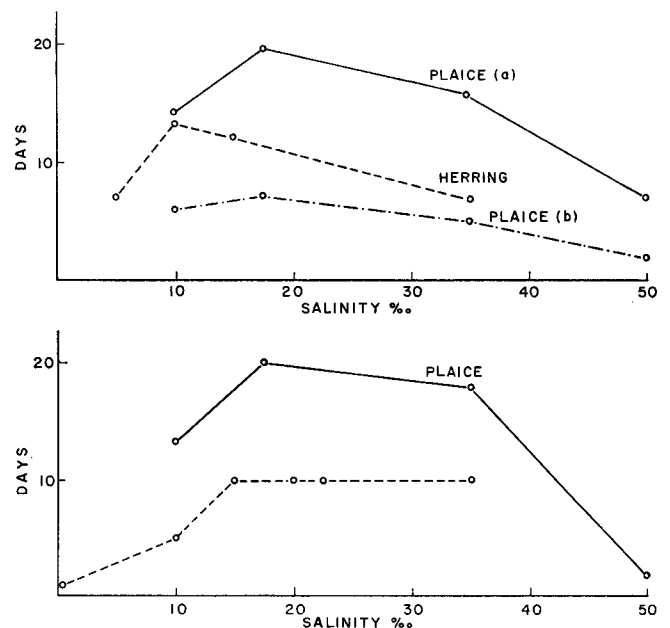


FIGURE 12. The survival of starving herring and plaice. a. Yolk sac larvae. Herring and plaice (a) starved in the test salinities. Plaice (b) starved for 10 days in sea water (34‰) then transferred to test salinity. b. ———, Plaice larvae, Simpson stages III and IV. - - - - - O Group plaice 38-50 mm. (Data for herring and O group plaice from Bishai 1961 at temperatures of 13-17° C; other experiments at 8° C).

Survival is best in salinities from 10‰ to 17.5‰. These salinities are not far from isotonic with the body fluids and it might be argued that conservation of osmotic energy and therefore longer survival was possible for this reason. It seems more likely however that the beneficial effects of these salinities again lies in the effects of their specific gravity on the activity of the larvae. The favorable effects of salinities approximately isotonic with the body fluids were also reported by Gilson & Hirst (1955) on *Lebistes*. Growth rate was fastest in 9‰ sea water. Shanklin (1954) demonstrated in *Fundulus* the dependence of osmotic regulation on specific metabolic pathways. In the presence of suitable inhibitors the embryo would only tolerate isotonic salinities, when normally they would survive in 70‰.

GENERAL CONCLUSIONS

It would appear that the ability to tolerate a wide range of salinities is a characteristic of the tissues of marine fish larvae, and it may be an indication of the unspecialized nature of the embryonic cells. Some stages are more tolerant than others, and the question of the long term effects of salinity changes is still to be investigated. Although survival for a given time period has been the criterion of tolerance adopted in this work, the absolute criterion of tolerance perhaps ought to be that the organism should survive and produce normal offspring. Preliminary experiments indicate that young herring and plaice can survive many months in water of 6–10‰ and grow apparently normally.

It is unlikely that extremes of salinity will be met with by eggs of most marine teleosts, although in areas such as the Baltic low salinities are usual; Brandhorst (1959) observed spawning herring in the Kiel canal in a salinity of 5‰ and some Baltic herring spawn in fresh water. Zaitsev (1955) reported that salinities as high as 60.25‰ occurred naturally in bays of the Black Sea. However the eggs of the Black Sea flounder did not hatch at all in salinities greater than 50.15‰, and percentage hatching was low in salinities above 39.75‰.

It would appear unlikely that minor salinity changes in rearing tanks would be harmful to larvae, although until long term experiments are done this cannot be certain. The larvae appear to be well adapted to meet any naturally occurring osmotic conditions.

SUMMARY

Some effects of salinity on the developing eggs and larvae of *Clupea harengus*, *Pleuronectes platessa* and *Gadus callarias* are described. Measurements were made of percentage fertilization, size of developing eggs, percentage hatching and the salinity tolerance of various stages in development. Measurements of the freezing points of the tissue fluids indicated the osmotic movements of water into and from the organism. The effects of age, starvation, oxygen uptake and activity in relation to salinity were also investigated. The results are discussed with reference to survival at sea and in rearing tanks.

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