THE OSMOREGULATORY ABILITY OF THE LAMPERN (*LAMPETRA FLUVIATILIS* L.) IN SEA WATER DURING THE COURSE OF ITS SPAWNING MIGRATION

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INTRODUCTION

Lampetra fluviatilis (the lampern or river lamprey) undergoes a spawning migration in British rivers during the period between November and February, after which the animals spend from 4 to 6 months in fresh water prior to spawning. Although it is generally held that the earlier stages of the life history of the adult lampern are spent in the brackish water associated with the lower reaches of rivers, there is evidence that the animal can withstand much higher concentrations of sea water at this time, since Meek (1916) records the case of a metamorphosing lampern taken from the North Sea. The fact that lamperns are thus able to exist successfully in both fresh and salt water raises the question of the way in which osmoregulation is achieved in such diverse media.

During the spawning migration, the plasma osmotic pressure of the lampern remains relatively constant, measurements of freezing-point depression varying between -0.46 and $-0.500°$ C. (Galloway, 1933; Dekhuyzen, 1904). Wikgren (1953) has demonstrated that the fresh-water osmoregulating mechanism is essentially similar to that already known for fresh-water teleosts (Krogh, 1939), and recently Robertson (1954) has shown that the ionic constituents of lampern plasma are present in similar proportions to those of a fresh-water teleost.

The only available record suggests that marine osmoregulation is accomplished by a hypotonic mechanism. Burian (1910) obtained a blood freezing-point depression of $-0.58°$ C. from a single specimen of *Petromyzon marinus* (the sea lamprey) caught in the Mediterranean ($\Delta = 2.3°$ C.), and this value approximates to that quoted by Fontaine (1930a) for the same species migrating in fresh water ($\Delta = -0.535°$ C.). However, attempts to return migrating lampreys to sea water have failed to show the existence of a marine osmoregulating system either in *P. marinus* (Fontaine, 1930a, b) or in *L. fluviatilis* (Galloway, 1933). Both investigators found that lampreys showed raised blood osmotic pressures following immersion in sea-water solutions hypertonic to the blood of fresh-water animals, whilst sea-water concentrations of 50 % and above were found to be lethal within 24 hr.

These observations imply that lampreys lose their powers of sea-water osmoregulation as they develop the fresh-water mechanism, the physiological basis for which is certain to be quite different. The present study is concerned with testing this

hypothesis by comparing the marine osmoregulatory powers of lamperns caught as early as possible in their spawning migration (fresh-run animals) with others which had been kept in fresh water for much longer periods of time and were, in consequence, approaching maturity.

MATERIALS AND METHODS

Lamperns were obtained from the River Trent at intervals during the winter in sufficiently large numbers to make possible the comparative study of different populations.

Blood samples were collected from pithed animals by allowing blood to drain from the heart into heparinized hard-glass centrifuge tubes, chilled in ice. Particular care was taken to exclude both pericardial fluid and water from the samples.

The freezing-point depression (Δt) of sea water, plasma and urine samples was measured by Johlin's method (1931, 1933). A micro-Beckmann thermometer was employed to suit the determination of the small amounts of plasma available (0·7–1·2 ml.). Determinations on the same sample agreed within 0·005° C.

Plasma chloride was determined by the method described by Schales & Schales (1941). Using 0·2 ml. of plasma, duplicate samples gave variations of less than 2 %. Urinary chloride was measured by the Volhard-Arnold technique, and sea-water chloride by the Mohr method using potassium chromate as indicator.

The conversion factor recommended by Krogh (1939) (293 mM $\equiv$ 1° C.) has been used to convert chloride concentrations into freezing-point depression (ΔCl). In making this conversion it has been assumed that an equivalent amount of mono-valent cation accompanies the chloride.

A standard technique was used in determining the live weights of animals. Lamperns were taken from water with the bare hands and the water allowed to drain from the gill pouches before transferring the animal into a tared flask containing water. During transfer, the animals were allowed to slide between both hands to remove the water from the body surface.

Urine output was measured in one of two ways. In the first method, the head and trunk of the lampern were enclosed in a horizontal glass tube which opened into an aerated tank of water. The posterior end of the tube was closed by a rubber membrane which fitted around the body of the lampern. Urine was then collected by inserting the hind end of the animal into a glass test-tube closed anteriorly by a rubber membrane.

The second method of urine collection involved cannulating the urinary papilla by means of a fine metal or glass cannula after the animals had been anaesthetized in a saturated solution of chlorbutol. A rubber balloon was then fixed to the open end of the cannula and the whole firmly attached to the tail by means of thread. Lamperns recovered from the effects of the anaesthetic in 5 or 10 min. after being replaced in fresh water and were allowed to swim freely during the collection period.

All collections were made at a temperature of 16–18° C. over periods of time which varied from 6 to 24 hr.

THE RESPONSE TO A GRADUAL INCREASE OF ENVIRONMENTAL OSMOTIC PRESSURE

A gradual increase of sea-water concentration was obtained by allowing artificial sea water (Pantin, 1946) to drip at a constant rate into a tank of fresh water. Thorough mixing was achieved by aerating the tank, and the volume of water was maintained constant by withdrawing liquid from the surface by means of a vacuum system. Under these conditions, the rate of change of sea-water concentration is exponential and can be calculated from the expression

$$T_{(\omega)} = 2 \cdot 303 \, \frac{V}{v} \log_{10} \frac{(1)}{1 - \alpha},$$

where T is the time to reach a fraction α of 100 % sea water, V is the volume of water in the tank (litres), v is the rate of inflow of sea water (litres/hr.).
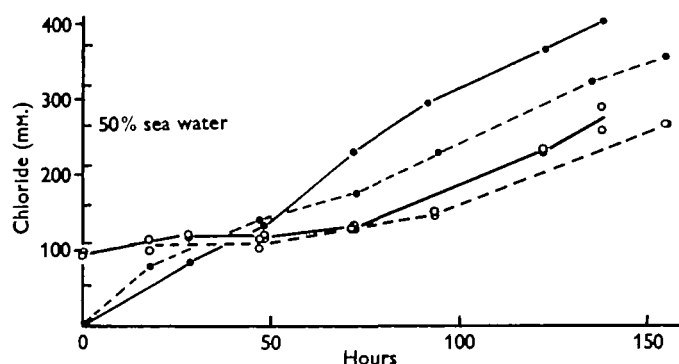

Fig. 1. The effect of gradually increasing sea-water concentration on the plasma chloride of *L. fluviatilis*. November experiment: O—O, plasma chloride; ●—●, sea-water chloride. anuary experiment: O – – – O, plasma chloride; ●– – – –●, sea-water chloride.

To avoid high rates of change of sea water during the early stages of the experiments, the rate of inflow was halved from that calculated to give a concentration of 80 % sea water in 6 days, and was increased to the original value later. In this way approximately straight-line relationships between sea-water concentration and time were obtained (Fig. 1).

Measurements of the level of plasma chloride of individual animals and their ability to survive were used as measures of osmoregulating ability during experiments performed on fresh-run lamperns captured in November 1949 and on lamperns which had been kept in fresh water in the laboratory for 2 or 3 weeks (January 1951). Animals from the same groups, but kept in fresh water, yielded mean values of 96·8 ± 2·5 (from ten observations), 100·5 ± 1·6 (from seven observations) respectively for their plasma chlorides.

Though both experiments demonstrate that lamperns can only maintain their plasma chloride constant in mildly hypertonic environments (Fig. 1), there is

evidence that fresh-run animals are able to withstand the effects of increasing sea-water concentrations better than more mature individuals, because the rapid rise in plasma chloride began at a higher sea-water concentration (230 mM Cl) than was the case in the more mature lamperns (150 mM Cl). In the later stages of both experiments, when the blood chloride was rising at about the same rate as that of the environment, the ventilation rate of the animals began to increase rapidly and they lay on their sides at the bottom of the tank, whilst the skin, fins and eyes showed a diffuse red tinge. The plasma obtained from these animals was heavily haemolysed and the red blood corpuscles were found to be markedly crenated. Those animals which were left at the end of the experiments soon died.

THE URINE OUTPUT AFTER ADAPTATION TO SEA WATER OF DIFFERENT SALINITIES

It was expected that the osmoregulatory ability shown by lamperns in the early stages of sea-water adaptation might be brought about, at least in part, by a reduction of urine output from the high fresh-water level. To investigate this possibility, measurements of urinary output were made on animals adapted to various concentrations of sea water for 2 or 3 days, and these are summarized in Fig. 2. Experiments were conducted mainly on fresh-run animals or animals which had been kept in fresh water for a few weeks after capture.

The volume of urine collected in fresh water shows marked differences depending upon the particular method employed. The high values of urine output obtained when the membrane method of collection was used ($341 \cdot 9 \pm 41 \cdot 6$ ml./kg./day) are probably the result of damage to the delicate skin of the lampern by the membrane. Not only will this effect water uptake, but the irritation caused by the membrane will also bring about diuresis. Even after very careful handling, most lamperns show a loss of weight (Fig. 3a, 30 hr.; Fig. 4, fresh-water animals) which, in fresh water, can only be due to an increase of urinary output. Cannulation gives much lower and less variable results ($155 \cdot 8 \pm 9 \cdot 9$ ml./kg./day) than the membrane method, indicating that these values are more likely to represent the normal urine output of the fresh-water animal.

The values obtained by other workers for the urine output of fresh-water lamperns are much higher than those obtained by the cannulation method. Wikgren (1953) obtained values of 359 ml./kg./day at 16–18° C., whilst Hardisty (1954) measured a mean increase of weight of 1·1 % per hr. after ligaturing the urinary papillae, a value from which a urine output of 264 ml./kg./day can be calculated. Though these figures resemble those obtained by the membrane method in the present studies and therefore suggest that diuretic responses may have influenced the results, there is a possibility that the higher urinary output may be attributable to an increase in the permeability of the external surfaces which, from evidence given later, appears to take place as the animal matures. Unfortunately, neither Wikgren nor Hardisty specify the particular stage their animals had reached.

The results of urine collections from animals kept in various concentrations of

sea water show that urinary output is reduced in response to increased osmotic pressure (Fig. 2). In a similar series of experiments, Wikgren (1953) found that the volume of urine was reduced to 30–80 ml./kg./day when animals were kept in 100 mM NaCl (osmotically equivalent to 17·4 % sea water), whilst in 200 mM NaCl (osmotically equivalent to 35 % sea water) a urine output of 2·2–13·2 ml./kg./day was obtained. The higher values obtained by Wikgren may be due to the fact that collections were made without previously adapting the animals to the environment. Evidence is presented later that after abrupt immersion in sea water, the urinary output decreases gradually for a period of some hours, so that unless the animal has been adapted, a higher urinary output will be recorded.
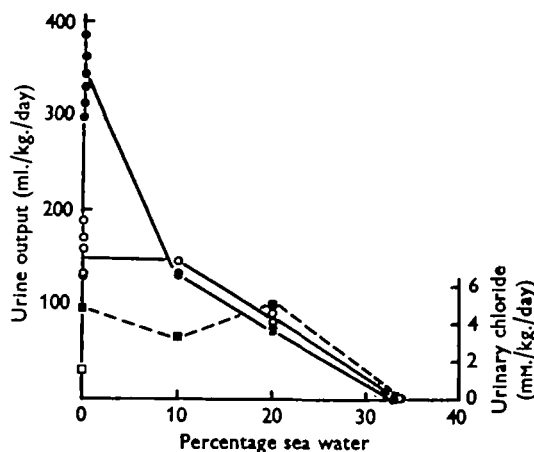

Fig. 2. The urine output and urinary chloride of *L. fluviatilis* kept in various concentrations of sea water. Urine output: O, by cannulation method; ●, by membrane method. Urinary chloride: □, by cannulation method; ■, by membrane method.

A comparison of measurements of urinary chloride from urine collected from fresh-water animals shows that, although the membrane method gives abnormally high results for chloride, there is little evidence that urinary chloride increases markedly with increasing sea-water concentration.

WEIGHT CHANGES AFTER ABRUPT TRANSFER TO SEA WATER
OF DIFFERENT SALINITIES

Serial determinations of weight were made on fresh-run and maturing lamperns in order to compare their ability to maintain a state of water balance in different environments. The migrating lampern does not feed, so that changes of weight are almost entirely due to changes of water content.

The weight changes were recorded from four fresh-run individuals immersed separately in fresh water, 33, 50 and 70 % sea water. This experiment was repeated four times on different individuals within a short period of time, so that, in all, sixteen fresh-run animals were investigated. Later, the same procedure was applied

to other animals which had almost reached sexual maturity in the laboratory (April 1952), although, since no experiments were performed in 70 % sea water, only twelve maturing animals were involved. These lamperns were captured at the same time as those used in the earlier experiments and equal numbers of males and females were used. They were handled very carefully because of the danger of losing part of the content of the gonads.

The results of experiments performed on animals immersed in 33 % sea water are given as typical examples in Fig. 3 a and b.
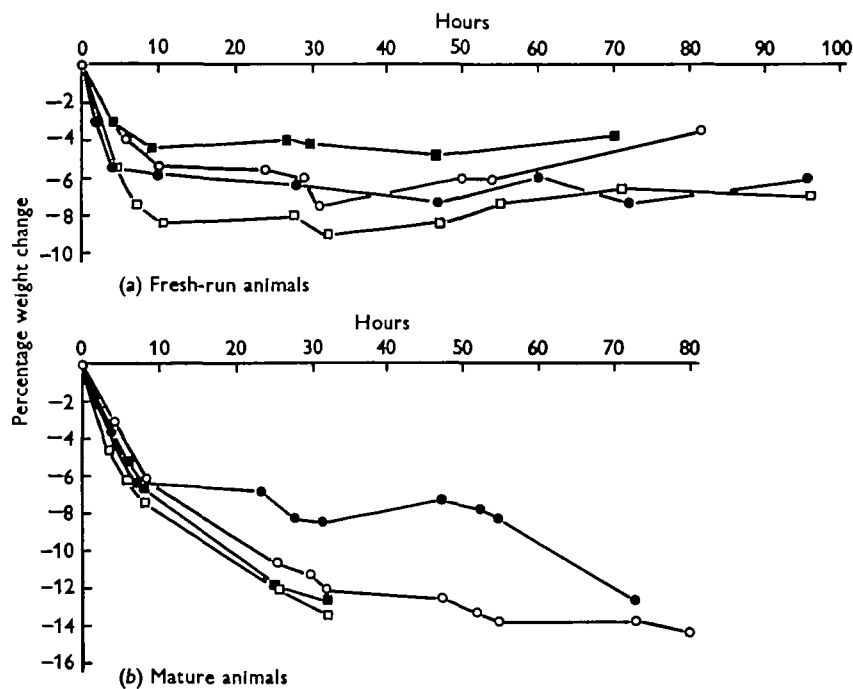

Fig. 3. The change in weight, expressed as a percentage of initial weight, of individual *L. fluviatilis* after immersion in 33 % sea water.

Fig. 4 a and b summarize the whole of the data collected during these experiments, and they have been constructed by interpolating values of percentage weight loss after 5 hr. and at subsequent 10 hr. intervals for each individual. The mean values of the weight change for animals treated in the same way (e.g. fresh-run animals kept in 33 % sea water) have then been calculated for these particular time intervals, so that each line on the graph represents the mean percentage weight change with time for four individuals. The significance of the difference of means for particular time intervals (10 and 20 hr.) has been analysed statistically by the analysis of variance technique, and this has been expressed as the least significant difference and is included in Fig. 4 a and b.

A consideration of Figs. 3 and 4 reveals that the animals can be divided according to the types of response they give. Some, consisting of nearly all fresh-run animals

which had been immersed in 33 % sea water (Figs. 3a, 4a), gradually begin to gain weight after a period of high weight loss, whilst others, comprising the remaining fresh-run animals and almost all maturing animals, continue to lose water.

All animals show some loss in weight, particularly at the beginning of the experiments, for a variety of reasons.
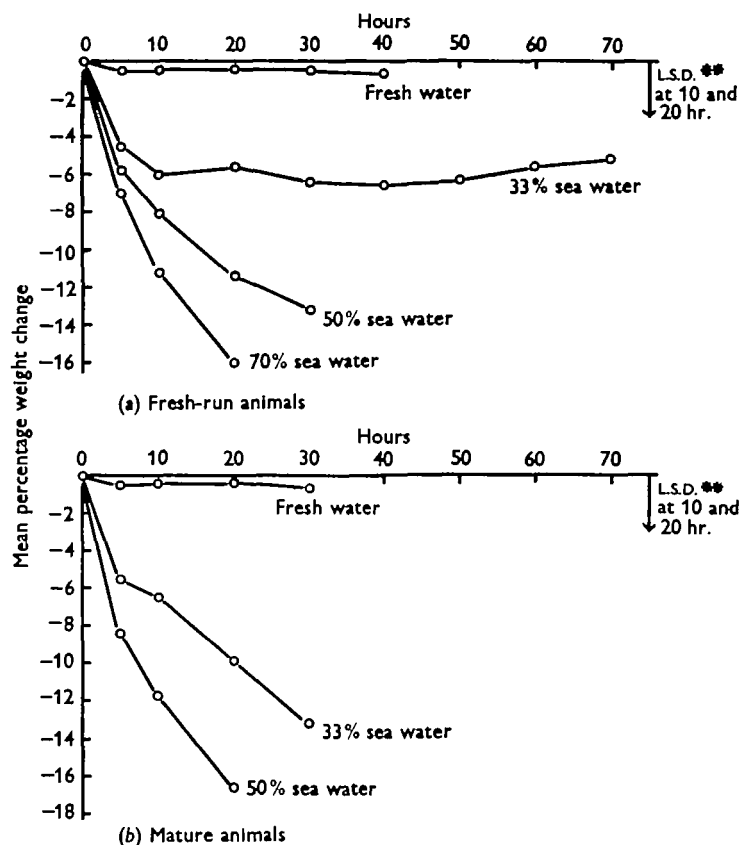

Fig. 4. The mean percentage weight change of *L. fluviatilis* after immersion in various strengths of sea water. (L.S.D.** is least significant difference.)

Diuresis caused by handling brings about some weight loss. This is shown clearly in both groups of fresh-water animals (Fig. 4a, b), where the loss of weight, in such a short time interval, can only be accounted for by an immediate increase in urine output. Individuals immersed in 33 % sea water (Fig. 3, 30 hr.) also show the same response.

Animals immersed in sea water also lose weight for other reasons. In all concentrations of sea water, animals show an initially high rate of weight loss which starts to decline between 5 and 10 hr. Although the initial rates of weight loss appear to be faster in more concentrated environments, the differences in the rates seem disproportionately small considering the wide range of sea-water concentrations in which

the animals were immersed. The reason for this seems to be that urine output continues at the high fresh-water rate for some time and only gradually declines as the animal loses water. During an experiment designed to show this more directly, a lampern in fresh water gave a urine output of 170 ml./kg./day. For the first 12 hr. following immersion in 33% sea water, the average urine output was 52·2 ml./kg./day, whilst immediately after this the urinary output amounted to 0·4 ml./kg./day. The expected weight loss after 12 hr. therefore becomes 2·61% from this source, and this is within the limits of loss over this period for a fresh-run animal (Fig. 3).

Weight loss is also caused by the removal of water from the animal by the hypertonic external environment, and the rate at which this takes place will depend not only upon the osmotic gradient existing between the animal and the environment,
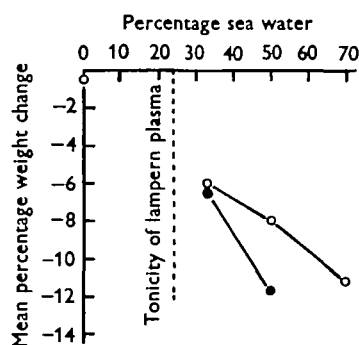

Fig. 5. The mean percentage weight loss of *L. fluviatilis* after 10 hr. immersion in various strengths of sea water. O, fresh-run animals; ●, mature animals.

but also upon the permeability of the tissues separating the two fluids. The effect of the osmotic gradient on the rate of water loss can be seen from Fig. 5. In this graph, the mean weight losses after 10 hr., for both fresh-run and nearly mature lamperns, have been plotted against the concentration of sea water responsible for the loss, and the figure will therefore include weight loss due to urine output as well as that caused by osmosis.

One must conclude from these experiments that only fresh-run animals immersed in 33% sea water show any signs of maintaining a state of water balance, and that even in these animals this response is a variable one (Fig. 3a). The fact that in most of these cases the animal gains some weight in the face of so many factors tending to promote weight loss, indicates that some active process must exist which results in water being taken up by the animal.

Mature animals kept in the same environment fail to balance water in the same way (Fig. 3b), and, although they can exist for long periods of time in 33% sea water, they eventually die. One lampern from this group actually gained weight at one stage of the experiment, but it eventually lost as much weight as the others.

CHANGES IN PLASMA CHLORIDE AND FREEZING-POINT DEPRESSION FOLLOWING IMMERSION IN 33% SEA WATER

After it had been established that some fresh-run lamperns could only balance their water content in 33% sea water, complementary experiments designed to assess ion balance were carried out on animals kept in this environment. The experiments were performed on groups of fresh-run and almost mature animals, which were removed in pairs from tanks of aerated 33% sea water after a suitable length of time and measurements made of the levels of freezing-point depression and chloride in the plasma taken from each individual. In the case of fresh-run animals, the changes in weight brought about by immersion were also recorded.

The results of these experiments are illustrated graphically in Fig. 6a, b and c. In these figures the values for plasma chloride have been converted into freezing-point depression, and because of the need to relate plasma osmotic pressure (Δt), plasma chloride (ΔCl) and percentage weight change with time, a similar symbol has been used for each of the variables measured for any individual. The lines joining the points in Fig. 6a and b have been used as a means of grouping animals which appeared to respond in a similar manner and therefore may be without significance when used as an indication of the way in which a particular type of individual might behave with time.

Two experiments were actually performed in April 1952, only one of which is shown (Fig. 6c). Almost the same responses were obtained in both cases, i.e. the plasma freezing-point depression and chloride rose at the same rate until at 40 hr. a steady state was attained.

Although it is clear that the osmoregulatory capacities of fresh-run animals are very variable (Fig. 6a, b), it is impossible to decide in the early stages of an experiment of this type whether low plasma osmotic pressure can be attributed to osmoregulatory ability, or to the short length of time during which the animals were immersed in sea water. For this reason, a second type of experiment was designed in which animals were immersed in 33% sea water for a minimum period of 40 hr.; the length of time taken for animals to reach a steady state (Fig. 6b, c). Fig. 7 summarizes the results of several experiments of this pattern and also includes observations from fresh-run animals which were used in the previous experiments and which satisfy the same conditions. In this figure, the values of total osmotic pressure (Δt) and osmotic pressure attributable to chloride and its accompanying cation (ΔCl, shaded area) are plotted against the initial weight of the animal, which has been used as a measure of the size of the animal and hence its blood volume.

The figure emphasizes the fact that some fresh-run lamperns possess marked powers of osmoregulation in hypertonic environments, and also shows that this faculty is correlated with the maintenance of low blood chloride and slight water loss. In other animals caught at the same time, the ability to osmoregulate is present in different degrees, and some of these show large weight losses associated with plasma osmotic pressures which are hypertonic to their environment, so that they exhibit similar behaviour to mature animals kept under the same conditions.

Hours

Percentage weight change

−2
−4
−6
−8

(a) The weight change of individual fresh-run animals expressed
as a percentage of the initial weight

Plasma Δ(°C.)

−0·8
−0·7
−0·6
−0·5
−0·4
−0·3

Δ_t 33% sea water

Δ_{Cl} 33% sea water

Δ_t

Δ_{Cl}

Hours

(b) The plasma freezing-point depression (Δ_t) and plasma chloride
(Δ_{Cl}) of the same fresh-run animals

Plasma Δ(°C.)

−0·8
−0·7
−0·6
−0·5
−0·4
−0·3

Δ_t 33% sea water

Δ_{Cl} 33% sea water

Δ_t

Δ_{Cl}

Hours

(c) The plasma freezing-point depression (Δ_t) and plasma
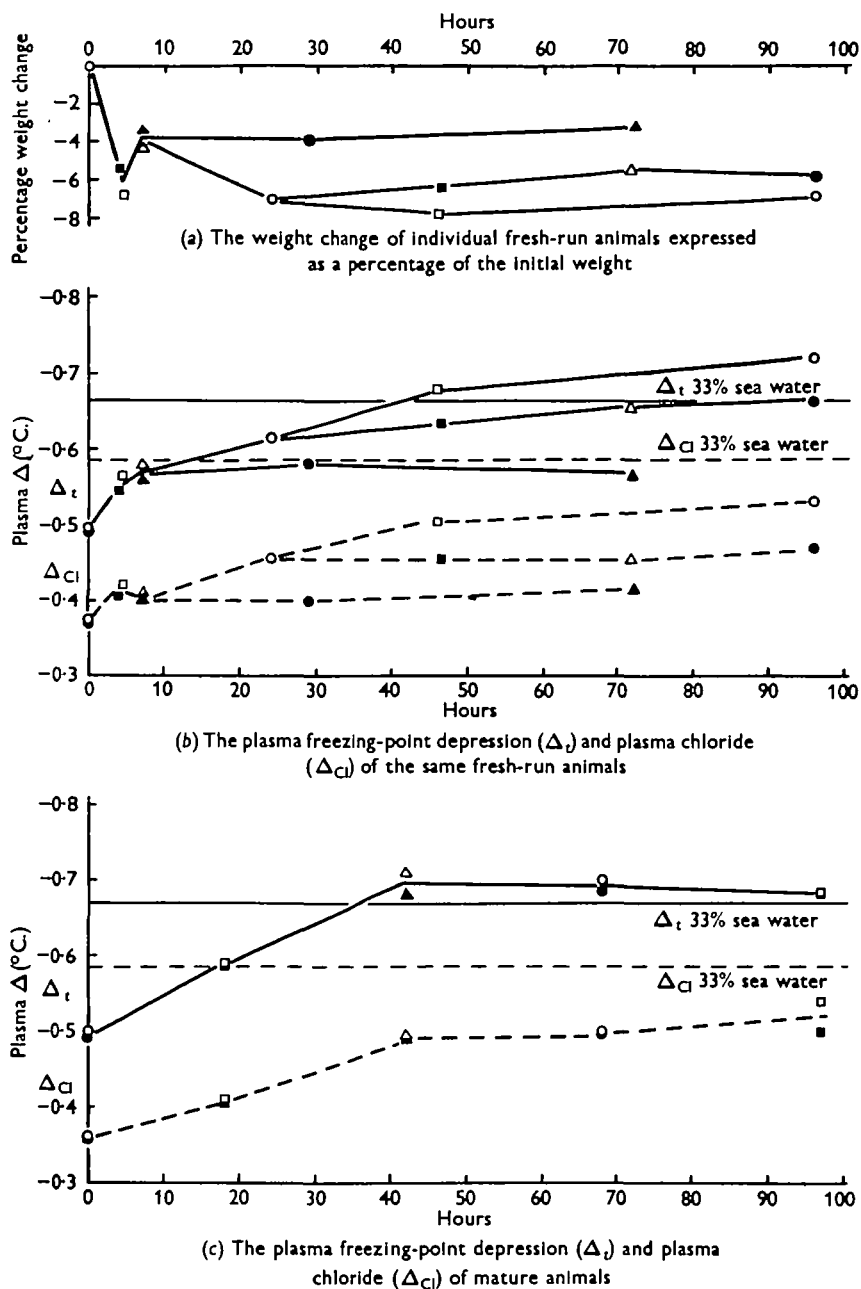chloride (Δ_{Cl}) of mature animals

Fig. 6. The osmotic responses of fresh-run and mature *L. fluviatilis* after abrupt immersion in
33 % sea water. (For further explanation see text.)

DISCUSSION

These studies show that there are marked differences in the marine osmoregulatory capacities of fresh-run and mature lamperns, and that even fresh-run animals may vary considerably in their powers of osmoregulation.

Mature lamperns are incapable of maintaining ion or water balance in sea water hypertonic to their blood and, in consequence, are unable to osmoregulate. These animals show similar responses to those obtained by Keys (1933) in his work on the eel. He prevented eels from osmoregulating by blocking their swallowing mechanism, after which the animals lost water continuously and eventually died when they had lost 11–14 % of their total body weight. The lampern usually dies after losing 14–19 % of its total weight.
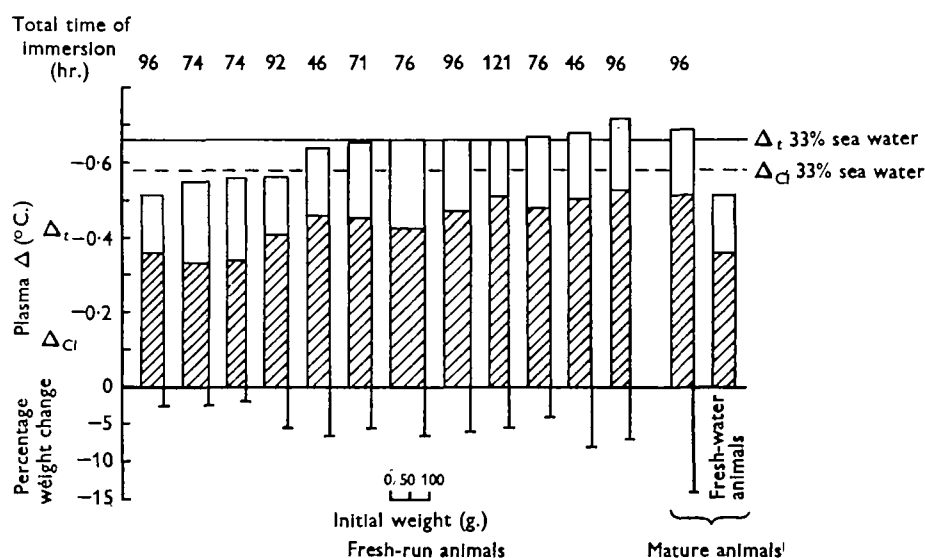

Fig. 7. The osmotic responses of individual *L. fluviatilis* after abrupt immersion in 33 % sea water for a minimum period of 40 hr. (For explanation see text.)

In 33 % sea water the curves relating plasma chloride and osmotic pressure with time (Fig. 6c) and weight loss with time (Fig. 4b) are similar in character, though inversely related; both indicate that a steady state is reached in about 40 hr. These observations suggest that water loss from the kidney and from the gills and integument is mainly responsible for raising the plasma osmotic pressure. As a steady state is approached, the plasma osmotic pressure eventually becomes higher than that of the surrounding sea water, whilst the plasma chloride remains somewhat lower. Osmotic water loss alone is insufficient to explain this situation, and though this condition could be brought about by the excretion of a hypotonic urine, the very small amount of urine produced by fresh-run animals in 33 % sea water (Fig. 2) would indicate that this is improbable.

Though fresh-run animals vary in the way in which they respond to sea water, some animals manage to osmoregulate efficiently in 33 % sea water; an environment which is hypertonic to the blood of the fresh-water lampern. The responses of these animals are characterized by a low blood osmotic pressure ($\Delta = -0.55^\circ$ C.) which may be brought about by the active maintenance of the low blood chloride associated with it. There is thus no doubt that the lampern can carry out hypotonic regulation in 33 % sea water. This finding is in agreement with the measurement made on *Petromyzon marinus* by Burian.

Table 1. *A comparison of weight loss in the lampern and the eel*

Animal	Environment (% sea water)	Mean percentage weight loss after 10 hr.
Eel (Keys, 1932)	100	3–6
Lampern (fresh-run)	33	3·9–8·4
Lampern (fresh-run)	70	10·9–11·4

Those animals capable of osmoregulation in 33 % sea water respond in a similar manner to the eel when it is transferred from fresh water to full-strength sea water (Keys, 1933). Both animals show a passive phase of water loss which is followed by an uptake of water after a period of 30–40 hr. has elapsed. The most important difference between the fresh-run lampern and the eel lies in the strength of sea water in which they are able to survive, lamperns appearing unable to withstand sea-water concentration greater than 50 %, either after abrupt transfer or when gradually acclimatized to this concentration under the conditions of the present experiments. One of the reasons for the difference is that the lampern loses much more water than the eel before the active process responsible for water uptake intervenes (Table 1). These figures, though not directly comparable, indicate a marked dissimilarity between the water permeability of the external surfaces of the animals, since it seems unlikely that the disparity in the surface volume/weight relationship between the animals, or the differences of the rates of urine output, could account for such a large difference in the rates of initial water loss.

There is evidence that the external surfaces of the lampern become more permeable to water as the animal matures (Fig. 5), and it is interesting to speculate that this situation may be the end result of a process which starts at the very beginning of the migration, so that even in fresh-run animals capable of osmoregulating in 33 % sea water, the external surface permeability may have already increased sufficiently to make survival in more concentrated environments impossible. This argument receives some support from the fact that fresh-run animals show marked differences in the rates at which they lose water during the early stages of weight-loss experiments (Fig. 3a), indicating that these animals may have reached different stages in the general trend toward increased permeability.

One of the most striking features of nearly all the experiments conducted on fresh-run animals is that they show gradations of osmoregulatory ability which can only be explained by assuming that a gradual breakdown of the mechanisms which

control sea-water osmoregulation takes place. The causes of this breakdown process cannot be analysed from the present experimental data, but it should be noted that at least three separate mechanisms may be involved. Besides the increase of water permeability already considered, there are signs that the mechanism responsible for the water uptake is also undergoing a marked change, because some fresh-run animals show little or no gain in weight (Figs. 3*a*, 7), whilst in the case of the mechanism controlling ion balance, there is a similar variability which is reflected in the levels of the plasma chloride of various individuals (Fig. 7). The details of the mechanism of marine osmoregulation and the causes of its breakdown will form the subject of a later communication.

SUMMARY

1. Although fresh-run lamperns are able to withstand the effects of increasing sea-water concentration better than maturing animals, they can only maintain their plasma chloride constant in environments more dilute than 50 % sea water. This is achieved, in part, by gradually reducing the urine output from the normal fresh-water level (155·8 ml./kg./day) to a negligible rate in solutions which are mildly hypertonic to the blood (33 % sea water).

2. Studies on the rate of change of weight loss, of plasma chloride and of plasma osmotic pressure following abrupt immersion in dilute sea water show that mature lamperns cannot osmoregulate and can only survive in 33 % sea water by tolerating a raised blood osmotic pressure caused by water loss.

3. Similar experiments on fresh-run animals suggest that the external surfaces of their bodies are less permeable to water than mature animals. Unlike mature animals, they also show considerable variation in the way in which they respond to 33 % sea water. Some are able to maintain their plasma osmotic pressure and chloride well below that of the environment. These animals also show little loss in weight, and this indicates that water is taken up actively, since this process has been shown to occur in some animals. Other fresh-run animals show raised plasma osmotic pressures in varying degrees and these are associated with larger losses of weight. These facts suggest that the hypotonic regulating mechanism gradually degenerates as the lampern enters fresh water.

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