II. OSMOTIC REGULATIONS IN TELEOST FISHES

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INTRODUCTION

THE problem of regulation of the osmotic concentration of body fluids is common to both marine and fresh-water teleost fishes. Since most teleosts maintain an internal concentration equivalent to freezing point depressions (Δ) between Δ–0.5°C. and Δ–0.8°C., it follows that regulation must occur in these animals, for the osmotic concentration of fresh water is close to 0°C. while that of sea water is usually between Δ–1.5°C. and –2.3°C. For fish in fresh water, therefore, the problem is to maintain body fluids which are hypertonic to the environment; whereas marine teleosts must keep an internal fluid concentration which is hypotonic to that of the external medium. The mechanisms whereby osmotic regulation is accomplished in fish were first coherently assembled and described by Smith (128). Further advances have been discussed by Krogh (94), Baldwin (2), and Scheer (122). A short description of the basic mechanisms will be reviewed in this paper as an introduction to the discussion of some recent work in the physiology of osmotic regulation by teleost fishes. The concentrations of the external and internal media are expressed in the units used by the author to whom reference is being made. Equivalent concentrations are tabulated in table 1 for the convenience of the reader.

TABLE 1

EQUIVALENTS

Δ °C.	Salinity ‰	Chlorinity ‰
–0.4	5	2.8
–0.6	10	5.6
–0.9	15	8.3
–1.2	20	11.1
–1.5	25	13.9
–1.8	30	16.7
–2.1	35	19.4

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Osmotic Regulation in Fresh-water Teleost Fishes

Method of regulation in fresh-water fish

It is generally agreed on the basis of palaeontological and anatomical evidence that the teleosts lived in a fresh-water environment (120). Water regulation in fresh-water teleosts may, therefore, be assumed to be more primitive than in the marine ones.

Since fish in fresh water maintain their body fluids at a concentration higher than that of the surrounding medium, they must be continually taking on water through the tissues by osmosis. The sites of entry are mainly at the gills and oral membranes, the permeability of the rest of the body being relatively low because of the scaly or mucous coats (94). Water is absorbed through the exposed semipermeable membranes and the blood passing through them. The water in the blood is then filtered through the glomerulus of the kidney and a urine hypotonic to the blood is excreted. In this way the water taken on by osmosis is removed by the kidneys. Freezing point depressions of urines of fresh-water fish have been reported by Haywood and Clapp (70). Average value for the catfish (*Ameiurus nebulosus*) was Δ–0.025°C., for the sucker (*Catostomus commersonii*), Δ–0.094°C. The rate of flow was higher for the catfish. Since there is this continuous osmotic inflow of water, the fish does not need to drink water. Smith (127) showed that eels drank no water within the first twenty-four to forty-eight hours in fresh water. More recently Grafflin (64) has also demonstrated that *Fundulus* in fresh water drinks practically no water.

The next problem facing the fresh-water fish is the maintenance of salt content. Some salt will be lost by way of the kidneys even though much is reabsorbed in the kidney tubules. Salts will also be eliminated in the feces. If the fish is to maintain the normal osmotic concentration of the blood and tissues, an intake of salts must occur to replace those lost. Salts will be ingested with food, but fish which normally fast during long periods are also able to replace lost salts. Experiments by Krogh (93) showed that cells in the gills of several species of fresh-water fish were able to take on chloride even though the tissue chloride was much greater than the chloride content of fresh water. It is by this means that fresh-water fish maintain their normal salt concentration. In summary, osmotic regulation in fresh-water teleosts is accomplished by the excretion of a hypotonic urine and the acquisition of salts from food and from the surrounding water.

Recent experimental work on fresh-water fish

Recently Copeland (27) has shown that in *Fundulus heteroclitus* the cells in the gills that are responsible for the excretion of chloride by this euryhaline fish in sea water are the same cells which absorb salts from fresh water. Some experiments by Liu (97) on the paradise fish, *Macropodus opercularis*, indicate that this air-breathing fish can become slowly acclimatized to salt water (NaCl). Liu raised the salt content gradually to 3% over a period of four months. At the end of this time he found well developed "chloride secreting cells" in the gills. Presumably here too the chloride absorbing cell had become a "chloride secreting cell."

Martret (101) found that chloride is excreted in the urine of the carp when the fish is placed in water with a salt concentration up to Δ–1°C. The concentration of the urine increases from Δ–0.07°C. to a maximum Δ–0.88°C. as that of the external medium is increased from Δ–0.15°C. to Δ–1.0°C. Thus the carp seems to be capable of excreting urine with a salt concentration slightly more than a third the concentration of sea water.

The freezing point depressions of blood and muscle of carp increase, respectively, from Δ–0.53 and Δ–0.68 in fresh water to Δ–0.8 and Δ–0.94 after twenty-four hours in water Δ–0.7°C., according to Leövey (96). This increase in concentration of the blood appears to be due to salts alone, for Korzhuev (92) reports that there is no significant change in urea content of the blood of carp and perch after several days in salt solutions up to 1%, though the chloride content doubles.

Determinations of the ions present in the muscle of carp in water of different salinities have been made by Kaplansky and Boldyrewa (84), Drilhon and Pora (37) and Drilhon (32). Their work indicates that the muscles become a reservoir for sodium, calcium, and magnesium, but that potassium tends to decrease rather than increase when the animal is in salt water (salinities up to Δ–2°C.). There appears to be no compensation within twenty-four hours in the carp even in low salinities, although a return towards the normal occurs in the eel after three hours (32), and in chum salmon fry after twelve hours (10b). Viability of carp in salt water decreased from twenty-four hours in dilute salt water (Δ–0.9°C.) to one and a half hours in salt water (Δ-2.0°C.). Kaplansky and Boldyrewa (85) report that carp living for one to two months in 10-15 $^0/_{00}$ NaCl showed an increase of 76% in blood chloride and 62% in muscle sodium but no change in muscle chloride or blood sodium. They conclude that only cations enter tissues, where they are bound in undissociated form.

According to Drilhon (32) the water content of carp tissues decreased from 81% in fresh water to 79.5% after two and a half hours in salt water (Δ-2.0°C.).

Some unpublished experiments on the goldfish (*Carassius auratus*) by Black show that the total body chloride increases 100% (from 50 to 100 milli-equivalents per kilo) within two and a half days after the fish have been placed in 14 $^0/_{00}$ salinity. Further increase does not occur, however, and the fish are capable of living in this salinity for several weeks. According to Pora (114) *C. carassius* can live indefinitely in 15 $^0/_{00}$ salinity. Determinations of water content of *C. auratus* by Black were not satisfactory as the variation among the controls covered the range of variation among fish in 14 $^0/_{00}$ salinity. The fact that many of the fish were mature might have caused the wide range in water content.

Interesting experiments have been done recently by Meyer (104) which indicate that there is a continual gain of chloride at the gills, and loss in the urine in normal goldfish. When 2.5 ml. distilled water or 2.5 ml. of 2% NaCl are injected intraperitoneally the fish respond by decreasing chloride excretion or absorption respectively, rather than by increasing either of these functions. This response appears different from that of the carp (101), but the different methods of administration of salt may have affected the type of response. Meyer's work also indicated that after transfer from one aquarium to another the chloride balance at the gills of the goldfish was temporarily upset (two days). He refers to Selye's review (124), suggesting that a mild form of shock might be the cause. This possibility should be kept in mind, and handling avoided if possible when measurements concerned with tissue permeability are being made.

Four species of fresh-water fish were investigated by Gueylard (67); the minnow (*Cyprinopsis auratus*), perch (*Perca fluviatilis*), roach (*Leuciscus rutilus*) and gudgeon (*Gobio fluviatilis*). In general she found that these fish could not tolerate salinities hypertonic to the body fluids. They did not regain water lost osmotically whereas the euryhaline stickleback did. Correspondingly, the con-

centration of the tissue fluid increased for the fresh-water fish in salt water, though there were no significant changes in the tissue fluids of the stickleback.

Pora (115) found that the salt content of scales in several species of fresh-water fish increased when the fish were placed in 30 $^0/_{00}$ NaCl for twenty-four hours; the water content decreased. He concluded from his experiments that the skin has no role in osmotic regulation and that all changes in scales result from exchange with internal body fluids. Further work by Gradinesco and Pora (57, 58) indicates that an electric current (0.01-0.04 mA/mm^2) decreased the resistance of fresh-water fish to salt water by increasing the permeability of the membranes. Pora also correlated resistance to salinity directly with oxygen consumption per unit weight (113), and inversely with size of the fish (112).

Changes in the density of blood taken from the heart of goldfish in 8, 12, and 19 $^0/_{00}$ Cl showed an initial rise in blood density from 1.042 to about 1.050 within two hours, followed by a decrease in density (103). McLaren found that in 100% sea water (19 $^0/_{00}$ Cl) a gradual increase occurred from two and a half hours until the time of death (three hours). Loss of water from the blood in a hypertonic environment might be responsible for the initial rise in blood density. The attempt at regulation which occurs within three hours may be due to hydration of the blood by swallowing salt water and transferring salts from blood to tissues.

Drilhon (34) carried out experiments which pointed to the medulla of the carp as a significant factor in osmotic regulation of the fish. Carp without the medulla can tolerate salinity of Δ-0.2°C., whereas normal carp tolerate Δ-0.9°C. Nervous and hormonal (109) control of membrane permeability appear to play a part in the responses of stenohaline fish to salinity changes.

Respiratory failure as a cause of death in stenohaline fish was first suggested by Bert (6). Some evidence for this is presented by Fontaine and Firly (42, 53) who found that the increase in serum phosphorous in asphyxiated carp occurred also in carp in salinities above 14 $^0/_{00}$ i.e. lethal salt concentrations.

Pora and Acrivo (116) were unable to find histological evidence for morphological breakdown in gill lamellae of goldfish in 15 $^0/_{00}$ salinity. Pora (114) suggests that death of goldfish transferred from fresh water to salinities higher than 15 $^0/_{00}$ may result in asphyxiation due to the effect of salts on the acid-base balance of the blood, and consequent inability of the hemoglobin to combine with oxygen.

The effect of salts on the oxygen dissociation curves of fish bloods has not yet been described. Pora found, however, that slow adaptation to high salinities decreased mortality, presumably because adjustment of the internal medium to a new ionic equilibrium could take place.

Interesting work has been done recently by Veselov (132) on oxygen consumption of the goldfish (*Carassius carassius*) and carp (*Cyprinus carpio*) in various salinities. In environments between normal fresh water and a medium isotonic (Δ–0.47°C.) to the body fluids of the goldfish, the water content of the tissues and the oxygen consumption of the animal gradually increased. In hypertonic media (Δ–0.5-0.95°C.) water was lost and oxygen consumption decreased to 60% of the normal rate. These results indicate that the mechanism for respiration of the goldfish is impaired by solutions hypertonic to the blood or that, for some reason, the demand for oxygen is not as great.

Although the specific cause of death of fresh-water fish in salt water is uncertain, and probably is not the same for all species, recent work indicates that some of the factors involved are: histology of the gills, extent of gill surface, rate of oxygen consumption, and the control of tissue permeability.

Osmotic Regulation in Marine Teleost Fishes

Method of regulation in marine fish

If marine fish have descended from fresh-water forms, one might expect that the original equipment for osmotic regulation, as seen in fresh-water fish, would become successfully adapted to the new environment. This does, in fact, appear to be the case, for which ample proof is provided in the existence of euryhaline fishes, i.e. fishes that can tolerate a wide range of salinities.

Marine fish continually face osmotic dehydration because their body fluids are hypotonic to the sea water. In order to replace this water loss, sea water is swallowed in large quantities. If swallowing is prevented, water balance is upset and death may follow (65, 72, 88). Conservation of water is brought about by the excretion of small quantities of urine which is slightly hypotonic to body fluids. In some species the glomerulus, which provides a large surface for the filtration of water and salts from the blood of fresh-water fish, has become vestigial. This is presumed to be an evolutionary change resulting from lack of use (61, 129).

Secondly, marine fish must get rid of salt rather than conserve

Smith (127) showed that the sodium, potassium, and chloride in the sea water swallowed were absorbed with the water from the digestive tract; some calcium, magnesium, and sulphate were also absorbed. Most of the magnesium sulphate and calcium is eliminated in the feces; the remainder, i.e. the amount absorbed, is excreted in the urine. However, the chloride content of the urine is low (111, 127). It has been found by Keys (87) that the chloride ion is excreted by cells in the gills of the eel. These cells have been termed "chloride secreting cells" and are well described, with notes on distribution, by Keys and Willmer (89). Since Krogh's work (93, 94) indicates that there is specific ion absorption by the gills of fish in fresh water (Cl^-, Br^-, Na^+, but not K^+), it has been generally accepted that the excretion of salt ions at the gills is also specific. The fact that Copeland has found that the same cell in *Fundulus heteroclitus* is responsible for the intake and outgo of chloride in fresh and sea water respectively, supports this view. As Krogh has suggested (94) there is great scope for the use of radioactive isotopes to determine the movement of ions across the gills of fish during osmotic regulation. According to Krogh it is probable that in fresh-water fish chloride is absorbed by exchange of Cl^- against HCO_3^-. Exchange in the opposite direction may account for the excretion of chloride by the gills of marine fish. Davies *et al.* (28-31) report an exchange of Cl^- and HCO_3^- in the gastric secretion of hydrochloric acid in amphibia. There may be some similarities between the mechanism they describe and the excretion of chloride by cells in the gills of fishes.

In marine fish osmotic regulation is accomplished by swallowing sea water and excreting the contained salts. A summary of the mechanisms responsible for osmotic regulation in teleost fishes is presented in table 2.

Recent experimental work on marine fish

Breder (13) made an ecological study of an oceanic fresh-water lake in one of the Bahama Islands and found that all the fish present were brackish water or marine forms, even though the salinity was only 1.45 ‰ in contrast to 33 ‰–37 ‰ in the neighbouring ocean. There was, however, a calcium carbonate froth on the lake and subsequent experiments confirmed the fact that the presence of calcium permitted the existence of these fish in almost fresh water. Other workers have also found that calcium facilitates viability of marine forms in fresh water (9, 12, 74, 136).

classification of migrating fish has been proposed by Myers (106). His terms are defined in table 3.

TABLE 3

MYER'S (106) CLASSIFICATION OF MIGRATING FISH

Term	*Migration*
Diadromous	Between sea and fresh water
Anadromous	From fresh water to sea to breed
Catadromous	From sea to fresh water to breed
Amphidromous	To fresh water or sea, but not primarily for breeding
Potamodromous	In fresh water
Oceanodromous	In sea water

The salmon and eel cannot, however, be considered truly euryhaline fish for they are relatively stenohaline between the time of hatching and entrance into the new environment (94). Eels are euryhaline from the elver stage to maturity. The tissues of the adult eel have a low permeability to water (88), but the gills do not seem to be able to extract chloride from fresh water (93). Hence osmotic regulation by the eel is brought about by practically complete reabsorption of chloride by the kidney and low water permeability. Little is known of the ability of the sea salmon to tolerate fresh water between the time the fish leaves and re-enters the streams. Both salmon (*Salmo*) (39, 100) and eel (*Anguilla*) (62) have kidney structure typical of fresh-water fish.

It is becoming increasingly clear that the endocrine glands play a part in initiating changes which are necessary for the successful penetration of these fish into the new environment. This aspect of the adjustment will be discussed in another section by Hoar (79). However, other aspects of osmotic regulation in anadromous and catadromous fish have been investigated recently.

Eel

During the last ten years work has been done on the eel (*Anguilla vulgaris*) by investigators in France. Fontaine and Callamand (20, 50, 51) report a marked loss of chloride by the eel before migration. The eel does not feed during this period. They also note that this loss is accentuated in warm temperatures. In addition, it has been shown that during starvation the cholesterol/fatty acid ratio of muscles increases, thus favouring the imbibition of water (131). From these facts Fontaine and Callamand have concluded that the summer starvation, loss of chloride or "demineralization," and probably increased imbibition by the tissues have made migration to a more concentrated environment a necessity for the eel. They also recognize the part played by the thyroid gland (47). During the winter migration the "demineralization" diminishes due to the colder water temperature so that in this way the eels survive until they reach the sea. Factors in the migration of the eel have been fully discussed by Callamand (19). The "demineralization" of eels before migration has not, however, been confirmed by other workers, and a later paper by Fontaine and Callamand (52) states that "demineralization" is not necessarily a part of migration since there is no set relation between "demineralization" and development of the gonads.

The cation content of the blood and tissues of the eel in fresh and salt water has been determined by Drilhon (35). When introduced into sea water the sodium in the blood plasma increased slightly during the first four hours, then decreased very slowly over several months; potassium content was probably very small because of absence of red blood cells, and showed no significant change; calcium increased for twenty-four hours, fluctuated considerably for six days and remained slightly higher than in fresh water. In sea water above the normal concentration, Drilhon found that small eels survived better than large ones. This might be correlated with Keys' work on *Fundulus* (86) which indicated that the higher the ratio of head length (gill surface) to body length, the greater the ability of the fish to survive salinity changes. Sodium content of the blood plasma showed the most significant increase in Drilhon's (35) experiments where the salinity of the water corresponded to Δ-2.45°C., i.e. more concentrated than sea water.

The effects of these ions in excess in the external medium showed that the eel could tolerate a 0.6% KCl solution for only twenty-four hours during which time there was abundant mucous secretion and cardiac acceleration (35). The sodium, potassium, and calcium content of the plasma, however, showed no excessive changes so that the toxicity of the potassium chloride solution was probably not a result of salt concentration *per se*. Eels lived in 4% magnesium chloride for fifteen days at which time the sodium content only was above normal. In calcium chloride the results were comparable to those in magnesium chloride. This work by Drilhon indicates that the ability of the eel to maintain a constant total blood concentration during excessive changes in the concentration of the external medium is reflected in the constancy of the individual ions which were measured.

The sodium, potassium, and calcium content of the muscles of the eel, however, increased during the first two and a half hours in sea water, as was the case for the carp; but after a year the ion content had returned to levels approximating those of the ions for eels in fresh water. No analyses were reported between two and a half hours and one year in sea water, but judging from Keys' experiments (88) on the water loss by the eel and Black's (10b) work with chloride content of salmon fry it is quite possible that regulation of muscle ions towards the normal did not occur during the first twelve hours. It seems apparent that in the eel and salmon the tissues supply a temporary storage place for salts until regulation by the gills and kidneys is accomplished. During this time the blood remains only slightly changed in salt concentration.

Osmotic regulation in young migrating eels or "elvers" has been extensively studied by investigators in France. These can adapt to abrupt salinity changes if the mucous coat is intact (40). According to Fontaine and Raffy (55, 118) their oxygen consumption in fresh water is higher than in sea water. Raffy explains this on the basis of change in water content of the tissues. Adult eels show no difference in oxygen consumption in fresh water and sea water, although the migrating silver eels have an increased oxygen consumption when going from fresh water to sea water.

Experiments by Vilter (135) indicate that the pituitary of "elvers" migrating to fresh water is responsible for their halophobic behaviour. Only 30% of the hypophysectomized "elvers" avoided sea water within a half hour period, whereas 100% of the normal fish had vacated the saline area during this time. Histological study by Vilter (134) showed that the activity of the hypophysis increased as the salinity of the environment decreased. Although the hypophysis appears to be active in the anadromous migration of the eel, this gland apparently has no function in controlling the euryhalinity of the adult eel. Fontaine, Callamand, and Olivereau (56) could detect no difference in the ability of hypophysectomized and normal eels to tolerate abrupt changes from sea water to fresh water and back again, even when the concentration of the sea water was doubled (Δ–4°C.).

A comparison of the above investigations by Raffy, Vilter, and Fontaine *et al.* indicates that salinity *per se* does not act as a single

factor affecting oxygen consumption and activity of the hypophysis of eels going from sea water to fresh water or the reverse.

Salmon

Because of its economic importance, the salmon has been the object of much investigation. Most of the work has naturally been done on the applied aspects of life history, habits, and conservation.

Theories regarding the forces involved in migration were based first on the effects of environmental factors, but recent work has indicated that hormones may be active in initiating changes which doubtless cause migration (46, 47, 54, 81). Discussion in this paper will be limited to osmotic regulation from the point of view of interrelationship of the fish and the environment.

Salmon eggs

The eggs of salmon are laid and hatched in fresh water. Pacific coast chum salmon (*Oncorhynchus keta*) lay their eggs in the lower part of the river near the sea where they are sometimes subject to tidal salinity variations. According to Neave (108) the eggs in the tidal area do not appear to hatch successfully because of failure to "harden." A series of unpublished experiments was carried out in 1946 on chum (*O. keta*) and pink (*O. gorbuscha*) salmon eggs by J. E. Moore (105) formerly of the Pacific Biological Station, Canada. Moore's experiments indicated that the upper limit for normal hardening of chum and pink salmon eggs was salinity 3 ‰.

Fisher and Warren (44) have found that hardening of speckled trout eggs will not occur in or above salinity 6 ‰. In a series of solutions they found that non-electrolytes had little or no effect on water entrance or hardening, whereas electrolytes caused imbibition of water by eggs according to the Hofmeister series of sodium salts. These results confirm the work of Bogucki (11) who found that the perivitelline membrane formed on trout eggs in hypertonic solutions of non-electrolytes, but that membrane formation was impeded in hypotonic salt solutions. Bogucki concluded that increase in egg volume, formation of perivitelline membrane, and turgescence of the egg depend on imbibition of colloids eliminated by the egg cell into the perivitelline space.

Extensive experiments by Busnel *et al.* (16) on eggs of the Atlantic salmon (*Salmo salar*) showed that, in 10 ‰ NaCl, 97% hatch; in 15 ‰, 45% hatch; of eggs acclimatized to 20 ‰ only

1% hatch; hatching occurred twenty days later than controls in fresh water, and the alevins lived only about twelve days after hatching. Eggs acclimatized to 25 $^0/_{00}$ NaCl did not hatch. It was observed that the water content of these eggs (in 10 to 25 $^0/_{00}$ NaCl) did not change over an eighteen-day period, but an increase in salt concentration occurred which was proportional to the salt concentration of the environment (from Δ–.59 in fresh water to Δ–1.32 in 25 $^0/_{00}$ NaCl).

Salmon alevins and fry

Rutter (121) reports that the quinnat or spring salmon (*O. tschawytscha*) cannot tolerate sea water until after the yolk sac is absorbed. However, at two months the fry survived in 95% sea water. This species may go to sea after hatching, or remain in fresh water for a year (26). Shepard (125) found that chum alevins (*O. keta*) tended to choose sea water when both sea and fresh water were available. The response to sea water was even more marked among chum fry.

Investigations by Auvergnat and Secondat (1) indicate that the freezing point depression of the yolk sac contents of alevins of *Salmo salar* at the beginning of yolk sac absorption increased above Δ–0.6°C. when the external water was above Δ–0.6°C. At the end of yolk sac absorption, however, the internal concentration remained at Δ–0.6°C. when in water having a concentration, Δ–0.77°C. (12.5 $^0/_{00}$ NaCl); therefore the mechanism for osmotic regulation begins to function at this time. Busnel *et al.* (16) found that *S. salar* alevins tolerate 10 $^0/_{00}$ NaCl but not 15 $^0/_{00}$ NaCl. The water content of alevins in fresh water reached 81.8% thirty-one days after hatching; in 10 $^0/_{00}$ NaCl it was 74.3%. Salt content of alevins in fresh water changed from Δ–0.49°C. to Δ–0.59°C. in the first thirty-one days after hatching; in 10 $^0/_{00}$ NaCl the corresponding change was from Δ–0.69°C. to Δ–0.90°C. Resorption of the yolk sac was slower in alevins in 10 $^0/_{00}$ NaCl.

In previous experiments Fage (39) had reported that twelve-day old *S. salar* alevins tolerated 35% sea water (approximately 12 $^0/_{00}$ NaCl) but not 40% sea water. Viability increased with age so that at sixty-nine days the fry survived in 45% sea water. Respiration and heart rate increased before death in 50% sea water. The branchial epithelium appeared injured in high salinities and gills were pale. These observations support Bert (6) who

concluded that salinity affected the respiratory system of fresh-water fish.

From the experiments described above, one may conclude that salmon eggs develop most favourably in a fresh-water environment.

Salmon fry, parr, and smolt

Investigations of the osmotic regulation of fry and parr of salmon which normally spend their early life in fresh water shows that these fish are relatively stenohaline. Huntsman and Hoar (82) report that Atlantic salmon parr (*Salmo salar*) can only tolerate 10 $^0/_{00}$ salinity. Black (10b) found that Pacific coho salmon fry (*Oncorhynchus kisutch*) lived and fed normally in water of 14 $^0/_{00}$ salinity but could not tolerate 29 $^0/_{00}$ salinity for more than thirty-six hours.

Although some work has been done on the subject of progressive *vs.* abrupt adaptation of parr to sea water the problem has not been conclusively settled. Chaisson (23) indicates that tolerance of salmon parr increases with intermittent exposure to tidal water. Huntsman and Hoar (82) find that apparent increased resistance of salmon parr is a result of the change in relation of body size to exposed tissues and does not involve changes in osmotic regulation. Recent work by Hoar (80) on the Pacific coho salmon does not, however, indicate that size is the significant factor in the ability of coho fry to tolerate sea water.

No marked permanent change in salt content of salmon migrating either to the sea or to fresh water has been recorded. Analyses were made by Black (10b) of the body chlorides and density of chum and coho salmon fry, two to three months after hatching. Transfer from fresh water to sea water (28 $^0/_{00}$–30 $^0/_{00}$ salinity) induced a rise in both density and chloride content. The density of these salmon increased from approximately 1.003 to 1.028 within twelve hours and then fell to a level close to that of the sea water (1.020) at about twenty-four hours. The body chloride increased at about the same rate in both species for twelve hours (from 30 to 70 milli-equivalents chloride per kilo). Between twelve and twenty-four hours, however, the coho fry continued to acquire chloride until death occurred, whereas the chum fry began to lose chloride, returning to their normal level, or slightly above, within thirty-six hours. In 50% sea water (14 $^0/_{00}$ salinity) a milder but similar response of body chloride occurred without lethal effect for either species. The ability of chum fry to survive in sea water is thought to be due to the development of "chloride secreting cells" in the gills of these fish. The survival of chum salmon fry in sea water is not surprising in view of the fact that this species enters the sea soon after hatching, whereas the coho spends at least a year in fresh water (26). Hoar (80) has observed "chloride secreting cells" in coho yearlings about to migrate to sea, but was unable to locate these cells in the gills of coho fry.

Attempts were made to acclimatize chum and coho salmon fry by leaving them in 50% sea water for varying lengths of time before transferring them to sea water for twenty-four hours. The effect of acclimatization was similar for both species, i.e. six days of acclimatization effected a decrease of 10 m.eq. chloride per kilo in the body chloride after twenty-four hours in sea water. In the coho, however, the change was from 80 to 70, in the chum from 62 to 52 m.eq. per kilo. This change in the coho indicates that slow acclimatization may be possible.

Experiments were also carried out by Black (10b) to determine the changes in density and chloride when chum and coho fry were returned to fresh water after twelve hours in sea water. At twelve hours the body chloride and density were high. Subsequent decrease in fresh water was relatively fast, attaining normal level in five or six hours. It is interesting to note in this connection that the return adjustment to the normal sea water environment made by *Fundulus heteroclitus* was also much more rapid than adjustment to fresh water (8). Apparently the kinetics of regulation in each case favours the normal environment, indicating that the concentration difference between the internal and external environment is not the only factor involved.

At the time of smolt metamorphosis before the salmon goes to sea, many changes occur which undoubtedly enable the salmon smolt to enter sea water. The shape of the fish changes resulting in a decrease in the weight-length ratio (78). The exposed surface is covered by a coat of guanine, and the fat content of fish tissues is low (80). According to Lovern (98) the type of fat of *Salmo salar* also changes at this time from the type typical of most fresh water fish to that peculiar to salmon. This depletion of fat probably raises the cholesterol/fatty acid ratio of the tissues and so increases imbibition. This tendency to imbibe water would be counteracted by penetration of a more concentrated environment, and may be a factor in migration, as suggested for the eel. Huntsman and Hoar

(82), and Vibert (133) find that salmon smolts can survive direct transfer to sea water. According to Jones (83), however, a period of ten hours acclimatization is necessary. Vibert (133) reports that salmon smolts which have spent two weeks in sea water may be returned to fresh water without ill effects. Black (10b) also found that chum salmon fry could be transferred from fresh water to sea water and back again without apparent harm. However, chum salmon fry which had been kept in fresh water for four months began to die at a high rate. The density and dry weight of these dying fish tended to be below the normal. Kuroda (95) likewise reports a rise in water content of the blood of chum fry between 100 and 150 days after hatching. Chin and Kuroda (25) were able to raise chum salmon in fresh water for two years after hatching by controlling diet, light, temperature, and sound. Normally, however, chum salmon fry go to sea soon after hatching.

Adult salmon

The life of the adult salmon in the sea has been difficult to follow. With the exception of determinations of the freezing point depression of the blood made by Greene (66) for the chinook or spring salmon (*O. tschawytscha*) and Benditt *et al.* (5) for the Atlantic salmon (*S. salar*) little has been done. These investigators report freezing point depressions of –0.76°C. and –0.79°C. respectively, for salmon in the sea; –0.63°C. and –0.64°C. for salmon which had returned to fresh water. It is not known whether sea salmon which are not ready to spawn are euryhaline.

The skin of the quinnat or spring salmon (*O. tschawytscha*) becomes very slimy and thick when the adults return to fresh water (121). Vibert (133) reports that he has reacclimated mature male Atlantic salmon in fresh water to full strength sea water. This is not surprising since this species may return to the sea after spawning.

Summary

A few general statements may be made on changes in osmotic regulation in the migrating salmon, but the physiological mechanisms whereby these changes are brought about are not necessarily the same for all species (80). Salmon eggs cannot develop normally in saline solutions. Young salmon entering the sea must have adequately developed "chloride secreting cells" in the gills. In addition, changes occur in the body covering and fat content which probably assist in the adjustment. On returning to the rivers as adult salmon the concentration of the blood is only slightly lowered in Pacific spring salmon and Atlantic salmon. Probably failure to regulate to osmotic changes is not the primary cause of death in the Pacific salmon. The Atlantic salmon remains euryhaline and returns to the sea after spawning.

"Rainbow trout"

Investigators in France have done work on the rainbow trout (*Salmo irideus* = *S. shasta* + *S. gairdneri* (15)) in an attempt to re-stock their rivers and make this fish more abundant. Sornay (130) made the first critical statement of the problem in 1934 and since then Busnel *et al.* (14–16) have carried out a series of experiments to determine the euryhalinity of the rainbow trout (*S. irideus*).

In one of his papers (15) Busnel states that the Service des Eaux et Forêts find better and more rapid hatching of rainbow trout eggs in brackish water and that the adults thrive well in a saline environment because their parasites are killed by salt water. However, Busnel *et al.* (16) found that alevins after hatching sustained mortality as follows:

⁰/₀₀ NaCl	% Mortality	No. of days
0	10	50
5	80	45
10	100	31
15	100	13

Some water was lost and salt absorbed during the first two days, in Δ–0.52°C. to Δ–0.78°C. but water was regained within forty-one days as development progressed. After the absorption of the yolk sac (three weeks) osmotic regulation was effective in salinities up to 15 ⁰/₀₀ NaCl but at 19 ⁰/₀₀ NaCl the freezing point depression of the tissue fluid increased and mortality ensued. At three months *S. irideus* still tolerated only 15 ⁰/₀₀ NaCl whereas when two months old the Atlantic salmon (*S. salar*) can live in 17 ⁰/₀₀ NaCl (39) and the quinnat salmon (*O. tschawytscha*) withstands 26 ⁰/₀₀ NaCl (121).

Immature *S. irideus* between 15 and 18 cm. (fifteen months old) tolerate salinities up to Δ–1.6°C.; adults, Δ–1.9°C. When curves are drawn showing changes in freezing point depression of the internal medium with increase in the salinity of the external medium the line for the rainbow trout is intermediate between the steno-

haline carp and the euryhaline eel (14). Freezing point depressions of both blood and muscle of *S. irideus* are compared at different salinities (14, 16). No typical "chloride secreting cells" were found in the gills of the rainbow trout, but masses of mucous cells were present (14).

The question of oxygen consumption in fresh and salt water was also investigated (16). Alevins and fry showed an oxygen consumption about 8% lower in 5 ⁰/₀₀ NaCl than in fresh water. In 10 ⁰/₀₀ NaCl oxygen consumption decreased 35%.

According to Neave (107), there are on the west coast of Canada two distinct races of *Salmo gairdneri* which show hereditary differences in scale counts and in migratory habits. Those that go to the sea (steelheads) migrate before their third year and may return to fresh water as adults; the non-sea-run group (rainbow trout) remain in fresh water and tend to move upstream rather than downstream at the migratory period.

Shad

Observations have been made on the blood of the shad (*Alosa*?) by Fontaine (45, 46). This species enters the rivers in March and April and spawns in May and June. Some fish regain the sea after spawning, but according to Fontaine most of the fish succumb on the spawning grounds. As the fish progress upstream salt is lost from the body causing a marked decrease in the salt concentration of the blood.

Euryhaline Teleost Fishes

Euryhaline fish discussed in the following section are those species which can tolerate a fairly wide salinity range, but in which this tolerance is not so strongly associated with migration as was the case for the anadromous and catadromous fish. Migrations to fresh or brackish water do occur, however, in many euryhaline species at the time of spawning. Although low salinity is doubtless a factor, other conditions such as temperature, oxygen content, current, and bottom conditions are probably equally important to the spawning fish. Light as well as temperature may be a factor, the former affecting the endocrine glands, the latter affecting the permeability of the tissues. External conditions causing migration have been discussed by Chidester (24) for the killifish (*Fundulus heteroclitus*), and by Rogers (119) for the killifish, stickleback (*Gasterosteus aculeatus*), and silverside (*Menidia*).

Distribution of euryhaline fishes

Although studies have been made of the distribution of animals in brackish water, probably one of the first thorough investigations of euryhaline fish in a specific area on this continent was made recently by Gunter (69) on the Texas coast. He reports a large number of euryhaline species of marine origin, of which forty were taken below $5\,^0/_{00}$ salinity. Gunter records seasonal movements of many of these fish and presents data to show that the smaller members of the species penetrate the brackish waters more persistently than the large ones. This fact bears out the work of Keys (86) on *Fundulus parvipinnis*. Keys found that small fish, with longer head length in relation to total length, survived dilution better than large fish. He attributes the survival to greater gill surface and hence greater ability to cope with temporary respiratory stress resulting from the dilute environment.

In Canada, a study of the flora and fauna of Lost Lagoon, a brackish water area cut off from Vancouver harbour, was made by Carl (21). Only four species of fish were reported: the stickleback (*Gasterosteus aculeatus*), the sculpin (*Cottus asper*), the cutthroat trout (*Salmo clarkii*), and the starry flounder (*Platichthys stellatus*). The flounder was not thriving in the brackish water environment.

Water balance

1. *From concentrated to dilute environment.* A marine fish entering a more dilute medium tends to take on water by osmosis. The problem is to decrease osmotic imbibition and dispose of the water absorbed. An integument which has a low permeability for water will help to decrease water absorption while the activity of the kidneys serves to excrete any excess water.

Experiments by Henschel (72) on two species of flounder indicate the importance of protection by the integument. One species succumbed to diluted sea water more readily than the other. The more resistant species, however, showed signs of distress when the skin was injured. Henschel concludes from these observations that the body covering is an important factor in salinity tolerance. Similar experiments are reported by Krogh (94). Black (10b) found that serial weights could not be made on coho and chum salmon fry because frequent drying caused death. *Fundulus heteroclitus*, however, survived the same treatment with no apparent ill effects (8).

Recent work on glomerular and aglomerular kidneys of fish in-

dicates that the kidney tubule is the essential structure, capable of carrying out all normal kidney functions (38, 63, 99). The glomerulus, however, with its large capillary surface, assists in the withdrawal of water from the blood in an efficient manner, and is, therefore, a great asset to fish in fresh water. Most euryhaline fish have glomerular kidneys and can therefore take care of the increased urine flow when in fresh water. The relation of the kidney structure to habitat has been discussed by Marshall and Smith (100) and Grafflin (63). The former investigators find a correlation between the number of glomeruli and habitat for sixty-seven species of fish. Grafflin, however, describes the aglomerular kidney of the fresh-water pipefish (*Microphis boaja*) and indicates that kidney structure is not the limiting factor for survival in a dilute medium.

Keys (88) showed that the eel transferred to fresh water took on water at a rate which increased the weight 2%-3% in twelve to twenty-four hours. After this time, however, weight decreased again to the normal level in three days. Black (8) found an increase in weight of 4%-5% in *Fundulus heteroclitus* under the same conditions, followed by a marked decrease between seven and twenty-four hours. The decrease in weight is presumably brought about by the activity of the kidneys in removing water.

2. *From dilute to concentrated environment.* When, on the other hand, a fish goes from a less to a more concentrated environment, the possibility of osmotic dehydration of the tissues becomes the problem. Here again the protection afforded by the integument is important. The lateral plates of the stickleback have for some time been considered a protective adaptation to cope with changes in temperature and salinity (7). Gueylard (67) found that a species of stickleback without plates (*Gasterosteus pungitius*) was less resistant to sea water than *G. aculeatus* with lateral plates. The correlation is not perfect, however, and Heuts (76, 77) presents evidence for two physiological races of stickleback, one found largely in fresh water, the other in a more saline environment. He finds, however, that natural selection operates in survival; that is, when the fresh-water type is reared in sea water the individuals having the most lateral plates survive (77).

The change in activity of the kidney towards a less voluminous urine flow is explained by Keys (88) as follows: "if water is withdrawn from the blood by passive diffusion the resulting increase in colloid osmotic pressure will reduce the glomerular filtration and urinary water loss; the reverse effect will take place following the transfer of the fish from a concentrated to a dilute environment." In the eel, Keys (88) reports a 4%-5% loss of weight in twelve hours with a return to the normal in forty-eight hours. Black (8) showed that *Fundulus heteroclitus* returned to sea water lost weight rapidly within the first eight hours. This weight loss was not regained within the four days of the experiment. Experiments by Gueylard (67) indicated a sharp loss of weight by the stickleback within the first half hour in sea water, but a rapid levelling off occurred over a two day period in immature fish in $20\ {}^0/_{00}$ NaCl. During the summer breeding season, however, osmotic regulation by sticklebacks in varying salinities was greatly diminished. This fact was confirmed by Black (10a) on sticklebacks of the Cowichan river in British Columbia during August. The body water content decreased when fish were placed in sea water ($28\ {}^0/_{00}$–$30\ {}^0/_{00}$ salinity). Black found that the failure to retain water was true of immature as well as mature fish during this period.

Salt balance

1. *From concentrated to dilute environment.* For marine fish entering a dilute medium there is a tendency to lose salts by diffusion through the gill and oral membranes as well as by way of the kidneys and feces. This loss has been clearly shown for *Fundulus heteroclitus* in fresh water (8). Although rapid at first the loss of body chloride becomes more gradual from the second to the fourth day after which time the chloride content appears quite stable at 55%-60% of the normal. In these experiments the fish were not fed so that the only means of gaining chloride would have been through the chloride-absorbing cells in the gills. Scott (123) reports the following changes in the blood of *F. heteroclitus* in fresh water: decrease in specific gravity from 1.051 to 1.047 in eight hours; fewer red blood corpuscles and less hemoglobin than in sea water fish.

Sticklebacks re-entering fresh water after five hours in sea water lost the chloride they had absorbed from the water (10a). This loss was probably the result of diffusion. It is interesting to note that chloride was gained and lost at approximately the same rate in the stickleback. For the coho salmon fry the rate of loss was greater than the rate of initial absorption. The difference in rate was even more marked in the chum salmon fry. Specific variations in membrane permeability are probably responsible for these results.

Although the sticklebacks used by Black were not euryhaline when transferred directly to sea water, they survived as well as coho or chum fry in 50% sea water ($14\ {}^0/_{00}$ salinity) and changes in body chloride were the same.

Extensive experiments by Koch and Heuts (90, 91) on two subspecies of *Gasterosteus aculeatus* (*gymnurus* and *trachurus*) provide data for changes in chloride and osmotic pressure of the blood of mature and immature sticklebacks. Their work shows that changes in chloride in the blood play only a small part in the total osmotic pressure. Bateman and Keys (3) found this to be true of the eel as well. Koch and Heuts suggest that the spawning migration of the sticklebacks may be due to the effect of hormones on neuromuscular activity.

It has been found that calcium is effective in diminishing exchange of water and salts by animals transferred to a dilute environment. Breder (12) reported that calcium had a protective value for scup entering dilute sea water. He found that this applied to other marine fish also (see p. 59 of this paper). Experiments by Black (9) showed that calcium in the water decreased the extent of chloride loss and water gain in *Fundulus heteroclitus* in fresh water. Only 40% of the body chloride was lost in six days in water saturated with calcium salts, whereas 60% was lost in soft tap water. Work by Heuts (74) indicates that calcium in the water is a factor influencing the distribution of the stickleback (*Gasterosteus aculeatus*).

Temperature is also significant. Heuts (75) found that two subspecies of *G. aculeatus* had a geographical distribution which corresponded with his results on the effect of temperature on salt balance.

Some attempts were made by Black (9) to acclimatize *F. heteroclitus* to fresh water by keeping fish in 1/3 or 2/3 sea water for twenty-four hours before transferring them to fresh water. The loss of chloride in fresh water at the end of this time decreased by 15 m.eq./kilo (from 25 to 40) during the first five days spent in dilute sea water. The fish were not fed, and after five days the results were extremely variable. The experiments indicated, however, that loss of salt by *Fundulus* in fresh water was less after acclimatization in dilute sea water.

2. *From dilute to concentrated environment.* The penetration of fish into a more saline environment seems to be less general than movements from the sea to fresh water. Gunter (68) observed that for every fresh-water fish that has been taken in sea water in North

America, nine marine fishes have been taken in fresh water. The reason for this may well lie in the problem of salt excretion. The "chloride secreting cell" must be sufficiently developed to effect the extrarenal excretion of chloride swallowed by the fish in sea water. Copeland (27) reports complete development of "chloride secreting cells" within twenty-four hours in *Fundulus heteroclitus* that have been accommodated for one or two weeks to tap water. The cells may reverse their polarity within two days. In *Fundulus*, then, the adaptability of the cells in the gills to fresh or sea water is comparatively rapid. The ability of the cell membrane to work against a chloride gradient is the essential property of the "chloride secreting cell." In both sea and fresh water it transfers chloride from a less concentrated chloride "solution" to one more concentrated.

The adjustment of the "chloride cells" is probably not as rapid in most fish as in *Fundulus* though the only evidence for this is found in the work of Liu (97) where a fresh-water fish was acclimatized to 3% sodium chloride over a four month period. There is, however, no other evidence so far that the ability of fish to adapt to sea water is dependent for the most part on "chloride secreting cells." Nor is there evidence that cells capable of absorbing chloride in fresh water can invariably secrete it in sea water. The eel can excrete chloride but cannot absorb it effectively (93).

Gueylard (67) found rapid adaptation to sea water by the stickleback. There was no significant change in the freezing point depression of the tissue fluid over a two day period, whereas for the roach and gudgeon, stenohaline fresh-water fish, there was a marked increase. During the breeding season, however, the stickleback also had a high tissue fluid concentration after being transferred to sea water. Black (10a) found an average increase of 100% in chloride content of the stickleback within twenty-four hours during the summer season.

Experiments on the effect of osmotic pressure, *per se*, similar to those reported by Drilhon (35) for the eel were made in 1903 by Siedlecki (126) on the stickleback. The fish tolerated 10% sugar solution but at 15% they did not feed normally and died in three days. A 6% glycerine solution was viable but not 7%. This chemical seemed to affect the nervous system. Fish died within twenty-four hours in 1 ‰ KCl, 35 to 40 ‰ NaCl, 50 to 60 ‰ Na_2SO_4, and 60 to 70 ‰ $MgSO_4$. Sticklebacks lived normally in distilled water. Small, mature, and starving fish were less resistant to adverse conditions. He concluded from his experiments that osmotic pressure

was not necessarily the determining factor in the survival of the fish, and that certain solutions were more toxic than others. It is well known that tissues and animals survive better in "balanced" saline solutions such as sea water and Ringer's solution than in solutions of single salts having the same osmotic pressure (71). The effect of the salts on the permeability and imbibition properties of the tissues appears to be the significant factor. Some experimental evidence for the effect of unbalanced solutions on fish is reported by Young (138).

Attempts to acclimatize the stickleback by putting fish in 50% sea water for varying periods before transferring them to full strength sea water (28 ‰–30 ‰ salinity) showed that the response of the fish was variable but on the whole acclimatization, as measured by body chloride content after twenty-four hours in sea water, occurred at a faster rate than for salmon fry. However, mortality was very high if the transfer was made before the fish had been in 50% sea water for four days (10a).

Experiments (already described) by several investigators have clearly confirmed the fact that the muscles become a reservoir for salts absorbed by the fish in sea water until some mechanism, such as the "chloride secreting cells," is developed to enable excretion of salts. Stimulation for the development of the "chloride secreting cells," however, is thought by Keys (88) and Copeland (27) to result from the increase in the chloride content of the blood by absorption of salts from the digestive tract. Copeland did some experiments to show that fish (*Fundulus*) in fresh water were capable of developing "chloride secreting cells" when salt solution was injected into the alimentary tract.

Internal factors

Internal factors are also involved in osmotic regulation. Koch and Heuts (73, 90) found that when thyroid was administered via the alimentary tract a decrease in ability to tolerate sea water resulted. This change in tolerance also occurs in sexually mature sticklebacks which show a preference for brackish or fresh water.

Another internal change which appears to have some importance is that of physical imbibition of water by the tissues. Gueylard (67) did some interesting experiments with the stickleback which showed that imbibition of water resulting from an increase in the cholesterol/fatty acid ratio in the tissues (102) may be significant for the stickleback entering salt water. The spleen which, according to

Gueylard, is a source of cholesterol decreases in weight in the stickleback in salt water, but no significant change occurs in the spleen weight of the typical fresh water fish examined (perch and roach). Gueylard concludes that the mobilization of spleen cholesterol in the stickleback increases the cholesterol/fatty acid ratio of the tissues and is responsible for the maintenance of normal water content of the tissues of this species in sea water. The water lost osmotically is thus regained by physical imbibition of the tissues. There is some substantiation of this theory in the fact that the normal spleen weight of "summer" sticklebacks is about half that of "winter" fish. Presumably one reason for the poor adaptation of "summer" fish is the fact that the cholesterol available from the spleen is much less. Gueylard found, however, that the eel showed no loss in spleen weight when transferred to sea water, and could tolerate changes in salinity after splenectomy. Imbibition properties described above apply to dead fish also. Dead sticklebacks killed in concentrated sea water, ether, chloroform, acetone, or carbon disulfide showed a gain in weight when in sea water and a loss in weight in fresh water. The reverse was true for the fresh-water fish, *Cyprinopsis auratus*. When killed by strychnine and saponin, however, the stickleback behaved the same as *Cyprinopsis*. Gueylard concludes that the two latter poisons affect the cholesterol lipids. Experiments by Veselov (132) also show that the water content of the tissues of dead fish depends on the killing solution. Dead goldfish (*Carassius carassius*) in a sodium chloride solution, Δ–0.95°C., lost weight within two hours to 91% of the normal weight; fish killed by formalin, instead of salinity, gained weight in sodium chloride solutions, Δ–0.90°C. The gain was only 1% of the normal weight in two hours, 7% in twenty-nine hours. Goldfish killed by formalin and kept in fresh water gained 11% of the normal weight in twenty-nine hours.

Graetz (59, 60) believes that water regulation in the stickleback (*G. aculeatus*) is controlled by the physical properties of the cells rather than by osmotic forces or kidney function. He found that the mucous coat of the fish was not effective in preventing water exchange, although it did hinder the passage of salts (chloride).

Biochemical Characteristics of Fish in Fresh Water and Sea Water

Biochemical characteristic such as alkaline reserve, hydrogen ion concentration, protein content, isoelectric point, and sedimenta-

tion rate of corpuscles have been determined for the blood of stenohaline and euryhaline fish.

In general, the alkaline reserve of fish in sea water is lower than in fresh water (36, 49).

The hydrogen ion concentration of the blood of the carp and the tench increases (pH 7.8 to 7.34) after three and a half hours in a saline environment, whereas the pH of the blood of the eel shows no significant change (18). Busnel's (15) work on rainbow trout shows that the pH of the blood increases from 7.95 in water, Δ–0.02°C., to 7.5 in water, Δ–1.6°C., whereas the pH of the muscle decreases from 6.2 to 6.62.

In a survey of protein content of stenohaline and euryhaline fish, Fontaine and Boucher-Firly (48) found that the migrating eel had the highest serum protein, and they suggest that the proteins increase the solubility of calcium by the formation of non-ionizable or weakly ionizable complexes. Additional work has shown that the protein and lipid content of the blood serum of eels is less in sea water than in fresh water (17, 41, 43). Some work on the effect of salinity on protein content and the isoelectric point of the blood of fishes is reported by Drilhon and Florence (36) and Drilhon and Pora (37).

The sedimentation rate of the blood of the eel increases after transfer from fresh water to sea water, whereas in the stenohaline carp in about half sea water (Δ–0.9°C.) the rate decreases (33). The sedimentation rate of the blood of the rainbow trout also shows a marked decrease after the fish has been in a saline environment (Δ–1.5°C.) (15). These experiments show that the viscosity of the plasma of stenohaline fresh-water fish is much greater in sea water than in fresh water. It has been shown that the viscosity of dog blood rises as the concentration of the cells is increased (137). The apparent increase in viscosity of the blood of fresh-water fish in sea water may be due to an increased cell volume arising from loss of water.

The work cited in this section indicates that the biochemical changes resulting from a change in the concentration of the external environment are different in stenohaline and euryhaline fish.

Summary

Mechanisms have been reviewed whereby teleost fishes can maintain the salt concentration of the body fluids at a constant level, although the natural habitat is much less concentrated (fresh water) or much more concentrated (sea water).

The death of fresh-water fish in sea water appears to be due to the accumulation of salts in the tissues until a lethal concentration is reached. In some species injury to the gills and consequent respiratory difficulties may be an important cause of death.

Marine fish in fresh water, on the other hand, suffer an excess tissue water content that cannot be relieved by the kidneys before death. The permeability of the tissues to water can, however, be reduced by the addition of calcium to the fresh water, so that survival of marine fish in fresh water may be increased by this means.

Osmotic regulation of anadromous and catadromous fish such as the salmon and eel is subject to change at different stages in the life history. These changes are probably associated with hormonal factors, which may, in some cases, be stimulated by external conditions.

Euryhaline fish are able to withstand variations in the salinity of the environment because of low permeability of the body surface, adaptability of the kidney, and ability to develop "chloride secreting cells." External factors such as calcium content and temperature of the water influence the permeability of the tissues and hence the tolerance of the fish for salinity changes.

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