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Salinity Tolerance and Aspects of Sodium
Regulation in Lake Trout (*Salvelinus namaycush*)

by

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ABSTRACT

As lake trout, *Salvelinus namaycush*, are the only non-anadromous North American salmonid, one objective of this study was to examine the salinity tolerance of lake trout of various age classes as an intolerance to seawater may explain their non-anadromous behaviour. A second objective was to examine the responses of several parameters of ionic regulation to increased salinity.

Sac-fry showed 84% survival after one week in 15 o/oo seawater, but suffered complete mortality in 20 o/oo. Seventy-six percent of the yearling lake trout survived one week of 20 o/oo but could not withstand 25 o/oo. Two-year-olds showed better survival (97%) after one week in 20 o/oo and 20% survived 27 o/oo for one week. It was not possible to acclimate lake trout to any higher salinity. Of the 5 adult lake trout tested, 4 died in 15 o/oo.

Plasma sodium of yearling and two-year-old lake trout was regulated at the freshwater level in 10 o/oo. Two-year-olds regulated at the freshwater level in 15 o/oo but yearlings were unable to regulate at freshwater levels in this salinity and their plasma

sodium showed a significant increase. The plasma sodium of both yearlings and two-year-olds increased to similar values in 20 o/oo. The largest increase occurred in failing yearling lake trout in 25 o/oo. Although plasma sodium increased with salinity, it was regulated below ambient levels in high salinities. The total body-sodium increased with salinity in yearling lake trout as did the sodium space. Body-water of yearlings was elevated above the freshwater value in 10 o/oo but showed dehydration in the hyperosmotic 15 o/oo. A slight rehydration occurred in 20 o/oo. Muscle sodium of yearlings increased with salinity up to 15 o/oo; in 20 o/oo muscle sodium dropped to the 10 o/oo level. Muscle potassium showed a different pattern as it was only elevated above the freshwater value in 20 o/oo. Muscle water was regulated at the freshwater level in 10 o/oo but dehydration occurred in 15 o/oo. A significant rehydration occurred in 20 o/oo, but this did not raise muscle water back to the freshwater level. The efflux rate of exchangeable sodium of yearling lake trout increased with salinity.

Lake trout were concluded to be the least seawater tolerant of all the North American salmonids of all age classes. The intolerance of yearling lake

trout may be due to an intolerance of a high plasma sodium concentration. The main mechanism in limiting the increase in plasma sodium with salinity was expansion of the sodium space, due mainly to increase in the intracellular sodium.

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INTRODUCTION

The lake trout, *Salvelinus namaycush* (Walbaum) 1792 (= *Cristivomer namaycush*) is a non-anadromous salmonid whose present natural range lies almost entirely within the limits of Pleistocene glaciation (Lindsey 1964).

Salmonidae are perhaps the classic examples of diadromous fish; all North American species, with the exception of the lake trout, are anadromous to a greater or lesser degree. Rounsefell (1958) attempted to quantify the anadromous behaviour of salmon, trout and char. He found that *Oncorhynchus* spp. as a group are more anadromous than *Salmo* spp., which similarly are more anadromous than *Salvelinus*. Of the species in the latter genus, he recognized *S. namaycush* as the least anadromous. Thus all species have at least some populations that go to sea, except the lake trout which, according to Dunbar and Hildebrand (1952) is "the most reluctant of all the trouts and chars to enter seawater."

Lindsey (1964) noted their presence in lakes on King William Island and other islands in the Canadian Arctic and stated that, as these islands have appeared

since the disappearance of local glaciation, lake trout must have crossed salt water to get there. Indeed, reports of evidence of their occurrence in brackish water are by no means rare. Lindsey (1964) gave several northern locations where lake trout were captured in brackish water, one of which had the marine capelin (*Mallotus villosus*) in its stomach. Dunbar and Hildebrand (1952) stated that natives reported an occasional lake trout in the brackish water of the George and Koksoak rivers, but they were unable to verify its presence in the salt water of Ungava Bay. Weed (1934) reported that lake trout were occasionally caught in the sea along the eastern coast of Labrador. Blouw and Johnson (pers. comm.) working at Nauyuk Lake on the Kent Peninsula in the Northwest Territories, noted and tagged lake trout moving downstream to Parry Sound (seawater) and a few were caught in brackish water, but they had no salinity records; some tagged fish were caught up to two years later in fresh water. Manning (1953) reported two lake trout at NE point of Castel Bay in brackish water. All these observations, however, are circumstantial as salinity was not reported. Boulva and Simard (1968) reviewing these and their own observations, for which

there existed accurate salinity records, suggested that, in nature, the upper salinity tolerance limit of lake trout is between 11 o/oo (parts per thousand) and 13 o/oo. These values are iso-osmotic or slightly hyperosmotic to freshwater teleost blood.

In view of these records, lake trout cannot be considered a stenohaline fish, but is, at least, slightly euryhaline, which would seem to be a familial characteristic. Thus one objective of this study was to determine the salinity tolerance of lake trout.

Although lake trout never seem to enter the true marine environment, they still occasionally encounter salinities hyperosmotic to their body fluids. Thus, albeit to a reduced degree, they face similar problems as do truly anadromous fish. In view of this, a second objective of this study was to examine responses of various ionic and physiological parameters to increased salinities of yearling lake trout.

Plasma sodium levels were measured as a broad indicator of the extent of regulation in various dilutions of seawater. Total body water was measured to see how yearling lake trout respond to increased osmotic concentrations, and total body sodium was measured to see how they respond to the increased ionic concentrations on a whole body level. As

changes in pool size could mitigate rises in plasma sodium, the responses of the internal distribution of volume of sodium to increased salinity was measured. An increase in the internal distribution volume of sodium could be due to either an increase in the extracellular volume or by an increase in the intracellular compartment, or by a combination of both spaces. The changes in muscle hydration and sodium and potassium levels were measured in order to determine any shift of electrolytes intracellularly. The efflux rate of sodium from yearling lake trout was measured as it is a component of sodium regulation.

LITERATURE SURVEY

The Salmonidae have a variety of life cycles, not all of which involve a marine phase. But, except for lake trout, all species do have some populations that go to sea. In order to assess the salinity tolerance of lake trout of various age classes, it is necessary to review the ontogeny of salinity tolerance in other, anadromous, species.

Salinity Tolerance of Salmonid Eggs

Both normal and dechorionated eggs of the five North American *Oncorhynchus* spp. are unable to withstand full strength seawater two weeks prior to hatching (Weisbart 1968). LD₅₀ values for pink (*O. gorbuscha*) and chum (*O. keta*) eggs were higher than those of coho (*O. kisutch*), chinook (*O. tshawytscha*) and sockeye (*O. nerka*) eggs and in all cases intact eggs had a higher LD₅₀ than the corresponding dechorionated eggs (Weisbart 1968). However, chum show a variable mortality in lower salinities. When exposed to seawater ten days before hatching, hatching was 100% in 25% seawater, 75% in 50% seawater, 50% in 75% seawater and 25% in 100% seawater (Kashiwagi and Sato 1969). Most of the sac-fry hatched in these experimental salinities died.

Atlantic salmon (*Salmo salar*) eggs incubated in various salinities from 24 hr of stripping show lower salinity tolerance. Eggs in 12.5% and 25% seawater have hatching rates of 90% and 78% respectively (Eddy 1968). However, the eggs in 50% seawater became eyed and fully developed embryos were observed but none hatched. No eyed eggs were observed in 100% seawater. In 25% seawater many of the sac-fry were deformed at hatching and there was a subsequent heavy mortality (Eddy 1968). Saunders (1966) reported the results of unpublished experiments in which Atlantic salmon eggs, fertilized in freshwater were successfully incubated in salinities of 3, 6, 8, and 12 o/oo. However, sac-fry hatched in 12 o/oo (34% seawater) were deformed and not vigorously active.

Loeffler and Løvtrup (1970) stated that, as "a general rule, the tonicity of eggs is closely similar to that of the body fluids of the maternal organism." The finding of Eddy (1968) that 9 o/oo (which is approximately iso-osmotic to normal salmon body fluids) causes deformity at hatching must be due to an excess of ions in the medium, rather than the increased osmotic concentration.

Salinity Tolerance of Salmonid Sac-Fry

Weisbart (1968) found that sac-fry of the five Pacific salmon could not withstand full strength seawater, two weeks post hatching. He found the LD₅₀ was greater in pink salmon sac-fry than in the other four species. Prior acclimation to 10 o/oo for 2 days and then 20 o/oo for a subsequent 2 days did not change the LD₅₀ for full strength seawater (31.8 o/oo in his experiments). Kashiwagi and Sato (1959) found that sac-fry of chum salmon which were one day, 10 days, 20 days and 40 days post hatching were able to withstand 25% seawater. One-day-old sac-fry all died in 50% seawater, 10 day olds all died in 75% seawater, and both the 20 and 40 day olds all died in 100% seawater.

Kepshire and McNeil (1972) acclimated chinook salmon sac-fry to 9 o/oo and 18 days post hatch and to 18 o/oo at 35 days post hatch without significant mortality. They did not report if these values were the upper limit of acclimation.

Bailey (1971) found sockeye salmon sac-fry exposed to 29 o/oo for five days suffered complete mortality.

Atlantic salmon sac-fry at one week post hatch and at six weeks post hatch could not withstand 25%

seawater or higher salinities (Parry 1960). The older sac-fry were more resistant ($LD_{50} = 96$ hr) to 25% seawater than the younger animals ($LD_{50} = 45$ hr). Eddy (1968) estimated the LD_{50} of Atlantic salmon sac-fry to be 9 days in 25% seawater but that sac-fry could live indefinitely and develop normally in 12.5% seawater. Saunders (1966) as previously noted, reported that Atlantic salmon sac-fry hatched in 12 o/oo were deformed and not active.

Thus *Oncorhynchus* spp. sac-fry have a higher salinity tolerance than Atlantic salmon sac-fry.

Salinity Tolerance of Salmonid Fry

Oncorhynchus spp. remain in the gravel redd after hatching until the yolk sac is absorbed, where upon they emerge as active, free swimming fry (Scott and Crossman 1973). Pink and chum salmon may immediately migrate to sea upon emergence (Scott and Crossman 1973); their salinity tolerance increased from that of the sac-fry, for Weisbart (1968) found that both species could withstand full strength seawater at this stage. Mason (1974) noted that chum fry moved from freshwater in and out of an estuary daily and so were exposed to marked daily variations in salinity.

Black (1951) found that chum fry when transferred directly from freshwater to seawater were able to ion regulate effectively and that normal Cl^- levels were attained within 12-24 hr. Kashiwagi and Sato (1969) found chum fry able to withstand seawater although they found 60 day old fry only had an 85% survival rate in seawater.

Chinook fry after emerging may proceed directly to sea, but in many British Columbia stocks, they remain in freshwater for a year (Scott and Crossman 1973). The early emigrants may not survive as chinook fry are unable to withstand direct transfer to full strength seawater (Weisbart 1968, Wagner *et al.* 1969).

However, chinook fry are completely tolerant to 15 o/oo from hatching onwards and prior acclimation to this salinity (15-25 o/oo) enable chinook fry to acclimate to full seawater (33 o/oo) with negligible mortality (Kepshire and McNeil 1972, Wagner *et al.* 1969). Fry acclimated to 33 o/oo show a reduced rate of growth (Kepshire and McNeil 1972).

Three months after the yolk sac is absorbed (150 days of age) chinook fry withstand direct transfer to full seawater (30 o/oo) "a capacity that is not general among salmonids" (Hoar 1976, Wagner *et al.* 1969). In

Wagner's *et al.* (1969) study, no difference was observed in the ontogeny of hypo-osmoregulation between two races of chinook salmon.

Coho salmon fry are similar to chinook in that after emergence from the gravel redd, some may migrate almost immediately to sea, but most remain at least one year in freshwater (Scott and Crossman 1973). Coho fry cannot tolerate direct transfer to 30 o/oo ($LD_{50} = 32$ hr, Weisbart 1968) or 28 o/oo ($LD_{50} = 48$ hr, Conte *et al.* 1966). These LD_{50} values are much lower than that of chinook fry ($LD_{50} = 120$ hr, Weisbart 1968), whose higher resistance was attributed to a tolerance of high plasma chloride (Hoar 1976, Weisbart 1968). Coho fry can tolerate up to 26 o/oo indefinitely with low mortality (Black 1951, Coche 1967a, Conte *et al.* 1966) and acclimation to low salinities (15-20 o/oo) decreased mortality in seawater (Black 1951, Otto 1971).

Sockeye salmon after emerging from the redd usually migrate to a freshwater nursery area and remain in freshwater for 1-3 years (Scott and Crossman 1973). However, the Harrison Rapids race are exceptional in that "the fry migrate to sea as soon as they are free swimming and before the scales have developed" and return at age four (Foerster 1968). These migrant

fry must either spend time in an unknown freshwater or brackish water nursery area or have exceptional osmoregulatory powers, for Weisbart (1968) found sockeye fry to have an LD₅₀ of 18 hr in seawater. Contrary to this, Bailey (1971) found 81 day old sockeye fry able to withstand direct transfer to full seawater for five days. This discrepancy may be due to the different definitions of seawater as Weisbart (1968) used 31.8 o/oo at 11°C as full seawater and Bailey (1971) used 29 o/oo at variable temperature between 7°C and 12.5°C. Both values are well below the salinity of open ocean water of 35 o/oo. Alternatively, these differences may be racial (both authors used hatchery salmon).

Atlantic salmon fry remain in freshwater for 2 or 3 years before migrating to sea (Scott and Crossman 1973). Fry cannot survive transfer to seawater having an LD₅₀ of 10 hr in 28 o/oo and 25.5 o/oo (Huntsman and Hoar 1939, Parry 1960, respectively) nor to the lesser salinity of 20 o/oo (LD₁₀₀ = 30 hr, Huntsman and Hoar 1939). Nine month old fry were able to live indefinitely in 17 o/oo (Parry 1960) but young fry (just after absorption of the yolk sac) were unable to tolerate 15 o/oo (Saunders and Henderson 1969b). Young fry were able to survive indefinitely in 12 o/oo following

abrupt transfer from freshwater and had a higher growth rate than fry raised in freshwater (Saunders and Henderson 1969b), presumably due to the lessening of the osmotic burden. Prior acclimation to 7 o/oo prolonged survival of young fry in 15 o/oo but did not lead to indefinite survival in that salinity.

Saunders and Henderson (1969b) concluded that the lethal level for young Atlantic salmon fry is between 12 and 15 o/oo.

Steelhead trout fry (*Salmo gairdneri*) are unable to tolerate seawater (Conte *et al.* 1966, Wagner 1974b) but are able to survive indefinitely in 20 o/oo (Conte *et al.* 1966). LD₅₀ values for intermediate salinities are 576 hr in 22 o/oo, 144 hr in 24 o/oo, 48 hr in both 26 and 28 o/oo and 24 hr in 30 o/oo (Conte *et al.* 1966). However, Speshilov and Agrba (1970) found one summer old steelhead fry died rapidly when transferred to 6 o/oo.

Cutthroat trout fry (*Salmo clarki*) 3-4 cm survived indefinitely in 20 o/oo but had LD₅₀'s of 268 hr in 22 o/oo, 96 hr in 24 o/oo, 48 hr in 26 o/oo, and 24 hr in both 28 and 30 o/oo; these values are somewhat lower than those for Steelhead fry (Conte *et al.* 1966).

Thus for salmonid fry the survival order is:

Oncorhynchus gorbuscha = *O. keta* > *O. tshawytcha* >
O. kisutch = *O. nerka* > *Salmo gairdneri* >
S. clarki > *S. salar*.

Salinity Tolerance of Salmonid Parr

Chinook salmon parr achieve euryhalinity (tolerance to abrupt transfer to 30 o/oo) at 170-200 days post fertilization, weight 4.0-5.5 g in a spring spawning race (Wagner *et al.* 1969). A fall spawning race achieved euryhalinity at 140 days post fertilization (3.0 g). Prior acclimation to lower salinities (15-25 o/oo) enabled fall chinook to tolerate 30 o/oo after the absorption of the yolk sac (60 days post fertilization). Acclimation enables spring chinook to tolerate 30 o/oo at 100 days post fertilization (3.7 cm, 0.4 g) (Wagner *et al.* 1969).

Coho salmon parr in the size range 7-8 cm (Conte *et al.* 1966) and 9.4-19.7 cm (Coche 1967b) are able to tolerate 30 o/oo indefinitely.

Steelhead trout parr 7-8 cm tolerate 24 o/oo indefinitely but not higher salinities ($LD_{50} = 336$ hr in 26 o/oo, 72 hr in 28 o/oo and 36 hr in 30 o/oo; Conte *et al.* 1966). The salinity tolerance of young steelhead was shown to have a strong seasonal bias and was greatest just prior to, and during the period of normal seaward migration (mid-March to mid-May; Conte and Wagner 1965). However, larger steelhead parr usually achieved euryhalinity (survival on immediate transfer to 30-32 o/oo) at 12-13 cm and 20-30 g (Wagner 1974a). Fish raised in the dark became euryhaline at 8 cm-6 g (Wagner 1974a).

The tolerance of Atlantic salmon parr is similar to that of Steelhead trout parr in that they can live indefinitely in 24 o/oo (Conte *et al.* 1966, calculating from Parry's (1958) data). However, Huntsman and Hoar (1939) found that Atlantic salmon parr 5-10 cm died within 30 hr of exposure to 20 o/oo, with larger parr surviving longer than smaller parr. Similarly, parr 7-8 cm had LD₅₀'s of 9 hr and 72 hr in 33.9 o/oo and 25 o/oo respectively, while smaller parr, 3-4 cm, of the same age (9 months) had LD₅₀'s of 2.5 hr and 10 hr in the same salinities. Saunders and Henderson (1969a) found larger young of the year parr (10-11 cm in December) had low mortality rates from December to May in salinities up to 22 o/oo. About 75% of the parr subjected to 30 o/oo died during the same period.

Cutthroat trout parr cannot tolerate seawater as parr 7-8 cm had an LD₅₀ of 24 hr in 30 o/oo (Conte *et al.* 1966). No data were available for intermediate salinities.

Brown trout (*Salmo trutta*) parr show a lesser tolerance to seawater than cutthroat trout. Parr 8-10 cm, 9 months old, had LD₅₀'s of 7.5 hr and 76 hr in 33.9 o/oo and 25 o/oo respectively. Brown trout 2 years old but not much larger (12-15 cm) had similar LD₅₀'s (11.5 hr and 76 hr) in the same salinities (Parry 1958, 1960). Both size groups could live indefinitely in 17 o/oo (Parry 1960).

Thus the survival order of parr (Parry 1960, Conte *et al.* 1966) is:

Oncorhynchus tshawytscha > *O. kisutch* >
Salmo gairdneri > *S. salar* > *S. clarki* >
S. trutta

Salinity Tolerance of Smolts and Adults

The oncorhynchids as smolts and adults can all tolerate full strength seawater, and normally spend a major part of their life cycle in the ocean, although this period is eliminated in some land-locked populations.

Steelhead trout may reside wholly in freshwater or go to sea, usually after 2 years (1-4) in freshwater (Scott and Crossman 1973). The sea run populations migrate as smolts downstream from late March through May with a peak between mid-April and mid-May (Wagner 1974a) in central Oregon. Although the smolt transformation is not necessary to induce seawater tolerance (Wagner 1974a) holding smolts in fresh water beyond the normal period of migration results in a regression of the hypo-osmoregulatory capability (Conte and Wagner 1965, Hoar 1976, Wagner 1974b).

Atlantic salmon go to sea as smolts when 5-6 inches and 2 or 3 years old (Scott and Crossman 1973). Tolerance to seawater develops abruptly upon transformation into the smolt (Huntsman and Hoar 1939) and size was found to be the most important factor in seawater adaptation (Knutsson and Grav 1976). Parry (1960) found naturally migrating smolts showed an LD₅₀ of between 72-84 hr to 100% seawater, but found in many experiments, more than 50% survived the direct transfer to full strength seawater. Saunders *et al.* (1975) found that 2 year old salmon smolts showed negligible mortality upon overwintering in floating cages in a concrete tide pool.

The cutthroat trout has anadromous populations on the West coast. Scott and Crossman (1973) stated that they go to sea for the first time at about 5 inches in the second or third year. However, cutthroat trout do not undertake the extensive trans-oceanic migrations of the oncorhynchids, but "remain within the influence of the river or go only a very short distance to sea" (Rounsefell 1958).

Parry (1960) found brown trout smolts (19-20 cm, 3 years old) unable to withstand full seawater (33.9 o/oo) having an LD₅₀ of 36 hr but were able to live indefinitely in 75% or less seawater. Conte *et al.*

(1966) calculated from Parry's data that brown trout smolts would survive indefinitely in 22 o/oo, but have an LD₅₀ of 150 hr in 24 o/oo. Some populations, both North American and European, are sea run (Scott and Crossman 1973) but the migrations are mainly coastal, chiefly estuarine and usually short (Rounsefell 1958). Thus the intolerance to seawater may not be disadvantageous if the fish never encounters, for any great time, full seawater.

Rounsefell (1958) considered the extent of Arctic char (*Salvelinus alpinus*) migration to be in the same category as brown trout, although tagged fish have been captured as far as eight miles from the river of origin (Hunter in Scott and Crossman 1973). Adults of the sea run form normally migrate to sea during the spring and return to fresh water to overwinter in lakes by late summer (Moore 1975, Scott and Crossman 1973). Young migrate to sea when 6-8 inches (Scott and Crossman 1973); this may be at ages 5-7 in Frobisher Bay (Grainger in Scott and Crossman 1973). Moore (1975) found that Arctic char younger than 5 years old (shorter than 10 cm) were never found in seawater in the Cumberland Sound area of Baffin Island. Char 5-7 years old were encountered only in the immediate vicinity of the rivers, but those 8-9 years old (19-20 cm) ventured 7 km from

their natal rivers and older fish moved even further from their freshwater source (Moore 1975).

Gjedrem (1975) found that Arctic char smolts placed in nets in the sea (32-35 o/oo) in July showed low mortality until late August, i.e., held past their normal freshwater migration. In one stock, all died by the end of September, while in another stock, 4% were still alive in January. Thus Arctic char seem to show a seasonal ability to hypo-osmoregulate, similar to steelhead.

Roberts (1971) found the salinity tolerance of post-spawning Arctic char from a non-migratory population to be 100% in 66‰ seawater, but only 54% were alive after one week in 80‰ (28 o/oo) seawater and 25% were alive after one week in 100‰ seawater.

Dolly Varden (*Salvelinus malma*) show a similar migration pattern to Arctic char, migrating to sea in the spring and early summer and returning to freshwater before winter (Armstrong 1974). Scott and Crossman (1973) stated that the Eva Creek, Alaska, population, which is anadromous, goes to sea after 3 or 4 years in freshwater (8.6-10.6 cm). Armstrong (1974) found freshwater returns to be mainly (61-87%) immature. Rounsefell (1958) considered Dolly Varden to have a similar pattern to Arctic char and brown trout in the

extent of migration in the sea, and their salinity tolerance would probably closely resemble that of the Arctic char, to which they are closely related (McPhail and Lindsey 1970).

Brook trout (*Salvelinus fontinalis*) have a very short migration in the sea and are chiefly estuarine (Rounsefell 1958). Their stay at sea is not very long; Weed (1934) found brook trout in the sea off Labrador from the time of open ice in June to late summer.

Salinity tolerance in brook trout "is approached very slowly as the fish grow" (Sutterlin *et al.* 1976); Wilder (1952) found that of 118 brook trout (wide range of sizes) transferred to 30 o/oo, only 2 (the largest) survived six days, with larger trout lasting longer than smaller trout. Saunders *et al.* (1975) tried to gradually acclimate brook trout (mean length 19 cm) to 30 o/oo and found 48% mortality in January from animals placed in floating net cages in seawater in late November. Sutterlin *et al.* (1976) suggested that direct transfer to full seawater (30 o/oo) with negligible mortality can only be tolerated by brook trout as large as 150 g; they found an LD₆₀ of 4 days in 30 o/oo for brook char weighing 50 g and 15 cm long. However, Wilder (1952) suggested that 30 o/oo is a higher salinity than what anadromous brook char might naturally experience.

Thus in the genus *Salvelinus*, the ranking of salinity tolerance is:

Salvelinus alpinus = *Salvelinus malma* >

Salvelinus fontinalis.

MATERIALS AND METHODS

Lake trout eyed eggs were obtained in the fall of 1975 from wild parents. Eggs were held in an incubator with flowing, dechlorinated water at 4°C until hatching. The resulting sac-fry were allowed to acclimate slowly to 10°C water before use. Yearling lake trout were obtained from the Government of Manitoba's Whiteshell Fish Hatchery and from Environment Canada's Freshwater Institute. These were held until used in running dechlorinated fresh water at 10°C. Two-year-old lake trout were hatchery stock, obtained as yearlings and held one year. The adult fish were gill-netted in the fall of 1975 from Roddy Lake in the Experimental Lakes Area of Northwestern Ontario. These were transported back to Winnipeg and held until spring in running dechlorinated water at 10°C. After death of the adult fish, the pectoral fins were removed so the age of the fish could be determined.

All experiments were performed in a controlled environment room at $9^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and a photoperiod of 12 hr L - 12 hr D. Synthetic sea salt (Instant Ocean, Aquarium Systems Ltd.) was used and salinity measured with a YSI Model 33 Salinity-Conductance-Temperature Meter except in the adult tolerance tests where salinities

were measured by freezing point depression osmometry (Osmette, Model 2001, Precision Systems) and silver nitrate titration (Hoar and Hickman 1975).

Salinity Tolerance

To measure the salinity tolerance of yearlings, two-year-old and adult lake trout fish were starved 24 hr and then placed directly from fresh water into 45-gallon fibreglass tanks containing 10 o/oo seawater for a period of one week. This water was constantly aerated by an airstone, and, for the adult fish tolerance tests only, filtered by an airlift-syphon system causing the water to be passed through a bed of glass wool, crushed oyster-shell and activated carbon. Fish were checked daily and dead or failing fish removed. Fish were considered failing when unable to maintain equilibrium and showed no response to handling. Fish were not fed during the experiment. At the end of one week, survivors from the yearling class were transferred by a dip net to a different tank, containing water of 15 o/oo salt. Two-year-olds were transferred directly to 20 o/oo salt. The filtered system with the adult fish had the salinity raised to 15 o/oo by addition of more synthetic sea salt. This water was not changed.

The same routine was followed for one week in 15 o/oo as for 10 o/oo, and at the end of that time, the salinity was raised to 20 o/oo, then to 25 o/oo (yearlings, adults) or 27 o/oo (two-year-olds) and finally 31 o/oo (two-year-olds). Mortality was tabulated as number of fish dead or failing after one week and percentage survival was calculated as the percentage of fish used at the start of the experiment which survived a particular salinity for one week. This same routine was followed in the acclimation of yearling lake trout to salinities of 10, 15 and 20 o/oo for further experimentation.

The testing of the salinity tolerance of sac-fry (two days after hatching was complete) differed from that of older ages in that five gallon glass aquaria were used and an initial concentration of 5 o/oo was used instead of 10 o/oo. The criterion of death was a milky opaqueness of the yolk sac as opposed to its normal yellow colour.

Plasma Sodium Levels

Acclimated yearling fish were anesthetized in a 1:10,000 solution of tricane methane sulphonate (MS 222) in distilled water and blotted dry. Blood

was removed from the caudal vessels with a 25 gauge needle and collected in a heparinized syringe (ammonium heparin, Sigma). The plasma fraction was drawn off after centrifugation for analysis of sodium content. Haemolysed samples were not used. Sodium was measured with an IL 143 flame photometer (Instrument Laboratories) calibrated to read directly in milli-equivalents/liter.

Total Body-Water and Sodium

Acclimated yearling fish were killed in a lethal concentration of MS 222 and blotted dry and weighed. They were then dried to a constant weight at 100°C. Body-water was calculated as the difference in weight, expressed as a percentage of wet body weight. Each dried fish was transferred to a culture tube and digested with concentrated nitric acid. The volume of the resulting solution was measured in a graduated cylinder and the sodium concentration of a sample was measured by flame photometry.

Sodium Space

The sodium space is defined as the volume which the total exchangeable sodium would occupy when homogeneously distributed at the concentration of the plasma (Mayer and Nibelle 1969).

Sodium space was measured in acclimated yearling fish. The fish were placed in five gallon aquaria; salinity was that of acclimation. This medium was labelled by the addition of ^{22}Na in the form of aqueous $^{22}\text{Na Cl}$. Fish were left for three days, at the end of which time they were removed to unlabelled medium for five minutes to wash external radioactivity from their body surface and buccal cavity. They were anesthetized, blood withdrawn as previously described, killed by a sharp blow to the head, blotted and weighed. After weighing, they were transferred to a culture tube and digested in nitric acid. The blood was centrifuged and a 100 μl sample of plasma was removed to measure radioactivity. Unused plasma and the red cell fraction were returned to the appropriate culture tube; the centrifugation tube was washed several times with nitric acid. After digestion, the volume of the digestate of the fish and unused blood was determined and a 2 ml sample was removed to determine radioactivity. Both the 2 ml digestate sample and the 100 μl plasma sample were made up to 4 ml with deionized water; the activity of each was measured by a Nuclear Chicago Automatic Gamma Well Counter.

Sodium space was determined by:

$$V_i = \frac{Q_t}{(Na_{int})_t}$$

where Q_t is the total quantity (cpm) of ^{22}Na in the fish at time t and $(Na_{int})_t$ the corresponding radioactivity per unit volume of plasma. V_i , the sodium space, is expressed as ml/100 g. (Lahlou and Sawyer 1969, Mayer and Nibelle 1969, Renfro and Hill 1973).

Muscle Water, Sodium and Potassium

Acclimated yearling fish were anesthetized in MS 222 and as much blood as possible was withdrawn. This served two purposes, firstly it enabled plasma electrolytes to be determined and secondly, it reduced the plasma volume, so that error in muscle electrolyte determination due to blood in the muscle would be reduced. The fish were killed by a blow on the head and a fillet of dorsal epaxial muscle was removed. The fillet was skinned and obvious fat removed. The fillet was blotted to remove as much blood as possible. The water and sodium content of the muscle sample were determined as described for whole body levels. Muscle potassium was measured by flame photometry.

Sodium Efflux

Acclimated yearling lake trout were injected intraperitoneally with either 50 μ l of 0.9% ^{22}Na Cl containing approximately 1 μ Ci of radiosodium (Group one) or 100 μ l of 0.9% ^{22}Na Cl containing approximately 2 μ Ci of radiosodium (Group two). The two groups differed in weight as they were from different stocks. They were left for one hr in acclimation medium to allow the labelled sodium to distribute throughout the exchangeable sodium pool and also to allow the injection wound to seal. Each fish was then placed individually in an efflux bath containing 50 ml (Group one) or 100 ml (Group two) of inactive medium. Each bath was constantly aerated to ensure mixing of effluxed ^{22}Na . They were left for one hr, at the end of which time, a 5 ml aliquot of the bath medium was removed and its activity measured. The fish were transferred to glass aquaria containing unlabelled medium for a five minute rinse, then killed in a lethal concentration of MS 222, blotted dry, weighed, and digested with nitric acid. The resulting solution was made up to 250 ml in a volumetric flask and a 5 ml aliquot removed for counting.

The rate constant for sodium efflux (K_e) was determined from:

$$K_e = \frac{1}{t} \ln \frac{Q_o}{Q_t}$$

where t equals time in hr, Q_t equals the radioactivity in the fish at the end of the experiment, Q_o equals the radioactivity in the fish at the start of the experiment, calculated from the final activity plus that of the medium (Evans *et al.* 1973, Potts *et al.* 1967, Potts *et al.* 1970, Potts and Fleming 1970, Potts and Fleming 1971, Roberts 1971). Under these conditions, it is assumed that the backflux of ^{22}Na is negligible. This method of introducing tracer into the internal compartment, i.e., the intraperitoneal injections, was chosen over the alternate form of allowing the fish to influx tracer from labelled medium as the amount of isotope necessary to achieve sufficient activity in salt adapted fish would be truly excessive.

K_e obtained from this calculation is the fraction of exchangeable sodium exchanged per hour. 100 times K_e is percent exchanged per hour.

Statistical Analysis

To ensure homogeneity of variance, Bartlett's test for homogeneity of variance was performed on all data (except tolerance test and efflux data). If

Bartlett's test showed significance at the 5% level, i.e., heterogeneity of variance, Taylor's Power Function was used to determine the transformation. The transformed data were rechecked with Bartlett's test. After homogeneity of variance was achieved, a one-way analysis of variance was performed to determine if any difference was present. There was a significant (5% level) difference in all cases, and Student-Newman-Kuels test was employed to isolate the different means. These statistical tests are described in Snedecor and Cochran (1967) and Steel and Torrie (1960).

RESULTS

Salinity Tolerance

Sac-fry which had hatched in freshwater showed 90% survival after one week in 5 o/oo (Table 1). Survival was not reduced after another week at a higher salinity (10 o/oo) and was only slightly lower in 15 o/oo, but upon transfer to 20 o/oo, complete mortality quickly ensued. Freshwater controls were ambiguous as they all died of a fungus infection soon after the start of the experiment. No fungus was found on any of the experimental animals.

Yearling lake trout survived one week in 10 o/oo and one week in 15 o/oo without significant mortality. Upon transfer to 20 o/oo however, 24% mortality occurred (Table 1). Only 6% survived one week at 25 o/oo and these were failing. Freshwater controls (40 animals) were handled an equivalent amount and showed complete survival over the four weeks of the experiment. In none of the experiments did starvation for four weeks seem to adversely affect the animals; they were all in good condition after this time and were not emaciated. Starved freshwater controls were held for longer than four weeks and showed no ill effects. It is recognized, however, that starvation

Table 1. Percentage survival of various age classes
of Lake trout in differing salinities.

(Sample size in brackets).

Salinity o/oo	Sac-Fry (70)	Yearlings (170)	Two-Year-Old (45)
5	90	-	-
10	89	100	100
15	84	98	-
20	0	76	97
25		6	-
27			20
31			0

is a stress upon the animal and is a less than ideal condition.

Two-year-old lake trout showed complete survival in 10 o/oo and 97% survival in 20 o/oo (15 o/oo was not tested), which is higher than the corresponding values for yearlings (Table 1). Survival dropped at higher salinities; only 20% survived 27 o/oo; these animals did not withstand the transfer to 31 o/oo. These experiments had no freshwater controls.

A limited number of wild caught adult lake trout was available for testing. Four died in 15 o/oo and one died after one day in 25 o/oo (Table 2).

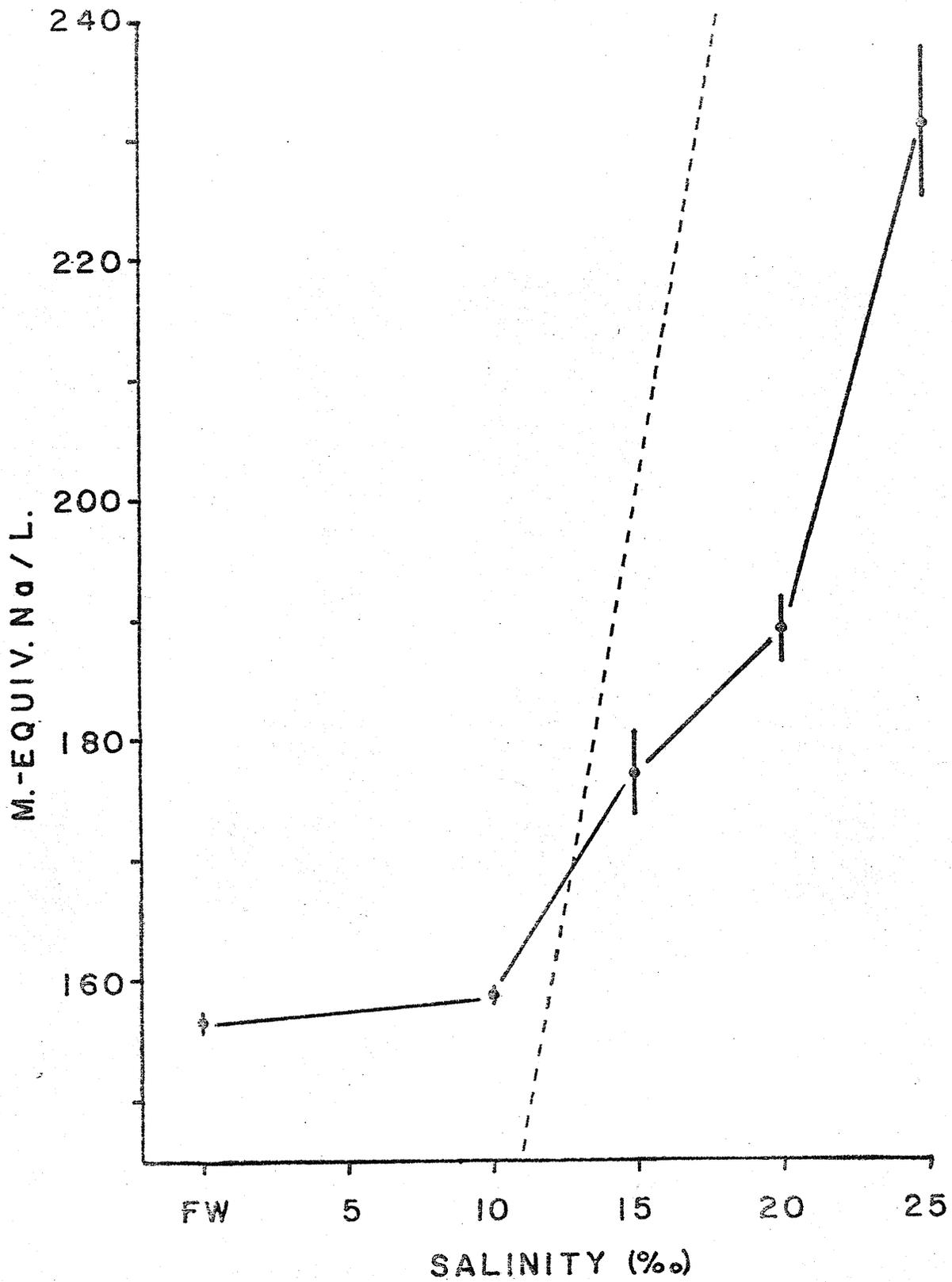
Plasma Sodium

The plasma sodium concentration of freshwater-adapted yearling lake trout was $156 \pm .7$ (10) m-equiv./l (mean \pm one standard error of the mean; sample size) (Fig. 1). After acclimation to 10 o/oo for one week, the plasma sodium concentration increased to $158 \pm .7$ (9) m-equiv./l, not significantly different at the 5% level. A significant 13% increase over freshwater values to 177 ± 3 (7) m-equiv./l occurred in the fish acclimated to 15 o/oo. In 20 o/oo, plasma sodium was 189 ± 3 (8) m-equiv./l, a significant 7% increase from

Table 2. Salinity tolerance of adult Lake trout.

Fish No.	Age (years)	Weight (g)	Salinity at death o/oo
1	11	1002	15
2	12	1289	15
3	7	273	15
4	15	835	15
5	9	519	25

Figure 1. Plasma sodium concentration of yearling lake trout in relation to external salinity. Equal concentration of plasma and medium sodium is represented by the dotted line. Mean \pm one standard error of the mean.



the 15 o/oo level. The largest increase occurred in those few fish which managed to survive one week in 25 o/oo. Their plasma sodium concentration was 231 ± 6 (4) m-equiv./l, a 22% increase from 20 o/oo, and they were failing at the time of sampling.

Although in all salinities above 10 o/oo, plasma sodium was significantly elevated, it was regulated below the environmental sodium concentration even in the fish failing in 25 o/oo.

Two-year-old lake trout showed a somewhat better regulation of plasma sodium than yearlings (Fig. 2). The freshwater value (150 ± 2 (7) m-equiv./l), 10 o/oo value (160 ± 1 (5) m-equiv./l) and 15 o/oo value (157 ± 2 (6) m-equiv./l) are not significantly different. However, in 20 o/oo, the plasma sodium concentration of two-year-olds is 186 ± 4 (7) m-equiv./l, similar to that of yearlings at that salinity.

Total Body-Water

The body-water of yearling lake trout acclimated to freshwater, expressed as a percentage of wet body weight is $79.56 \pm .42\%$ (20) (Fig. 3). It is increased by 1.56% to $80.80 \pm .23\%$ (15) in 10 o/oo, a significant increase. Body-water drops 3.75% from the 10 o/oo value

Figure 2. Plasma sodium concentration of two-year-old lake trout in relation to external salinity. Equal concentration of plasma and external sodium represented by the dotted line. Mean \pm one standard error of the mean.

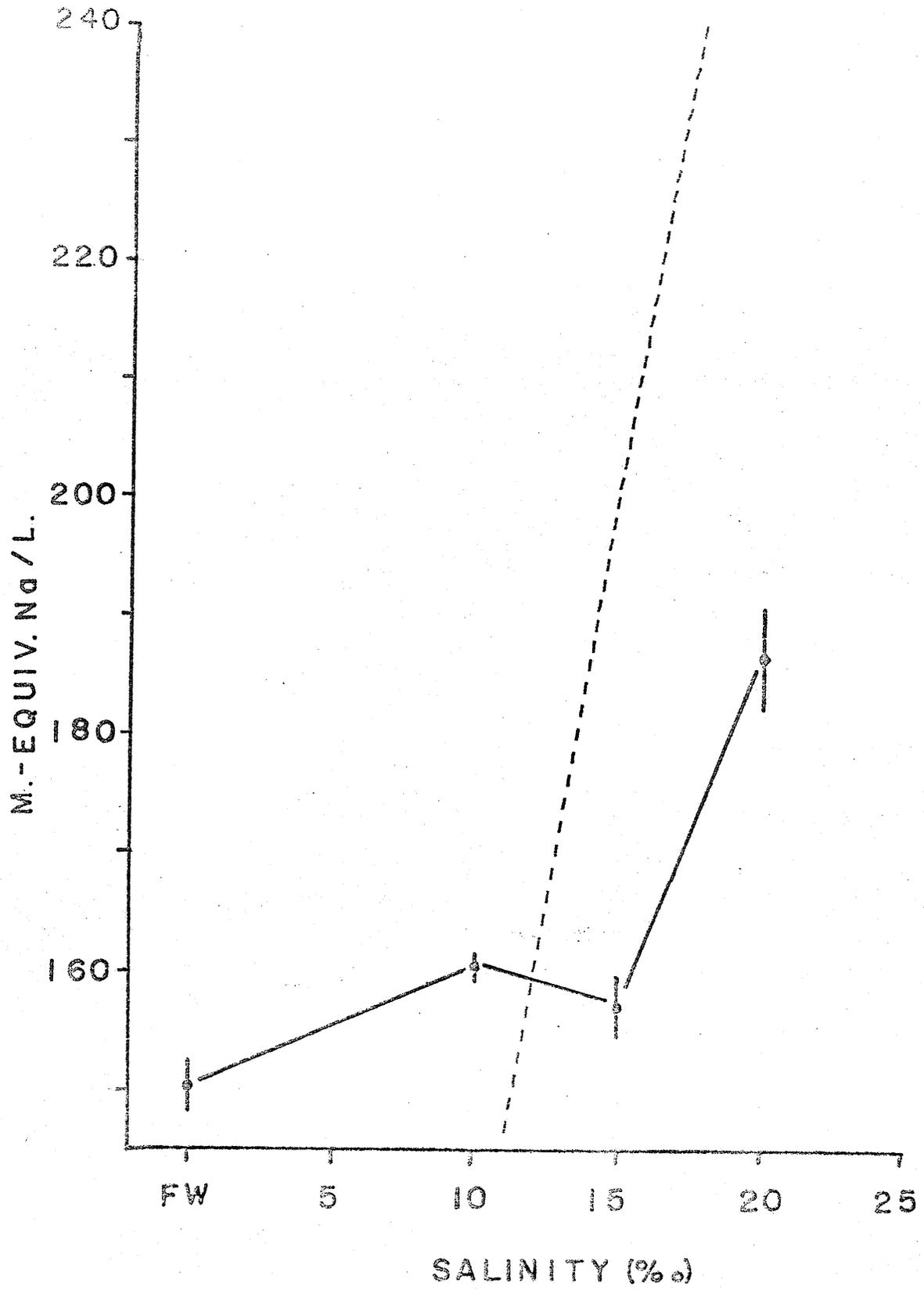
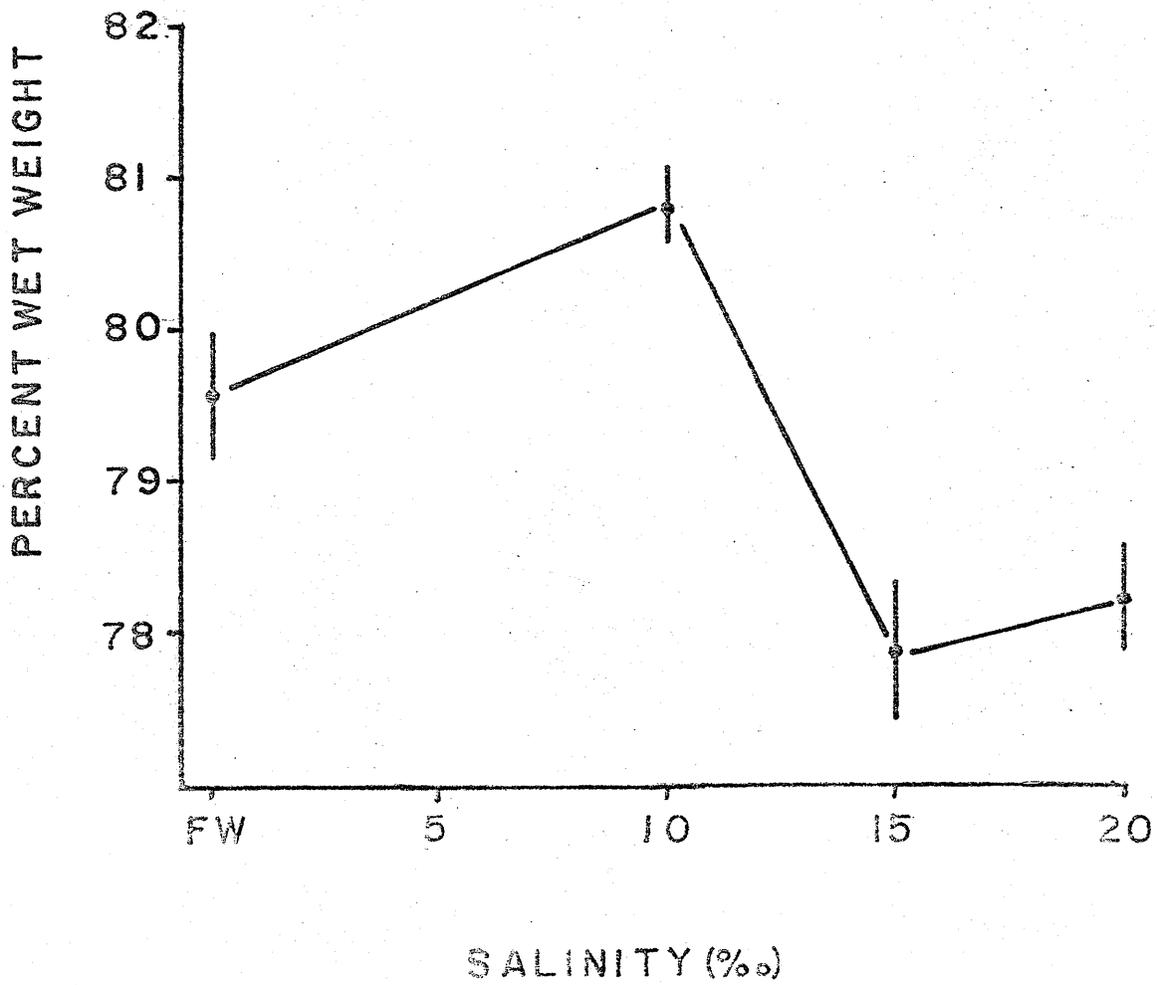


Figure 3. Total body-water of yearling lake trout
in relation to external salinity.
Mean \pm one standard error of the mean.

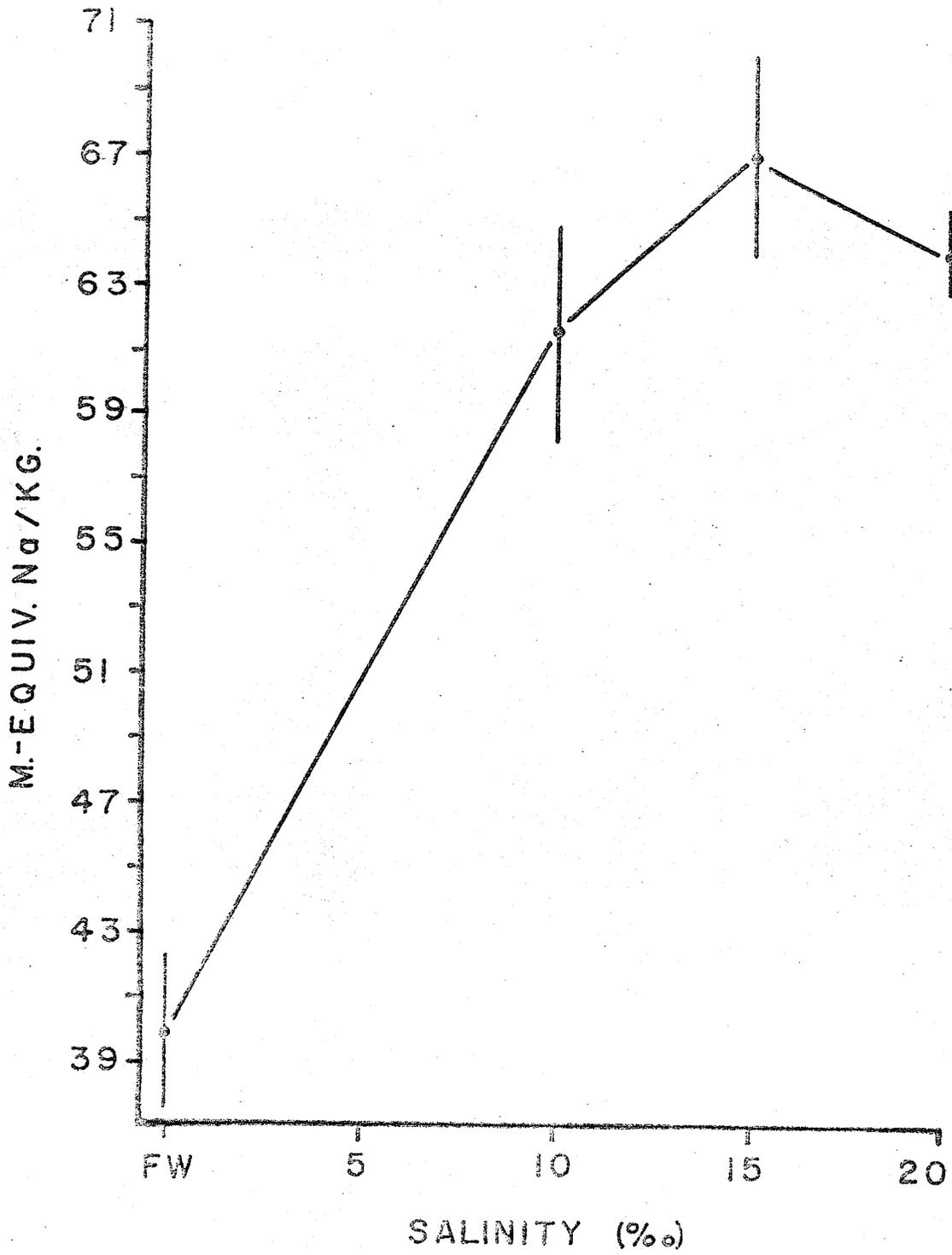


to $77.88 \pm .43$ (10) in the hyperosmotic 15 o/oo, a significant decrease from the freshwater level. The slight increase to $78.21 \pm .32\%$ (19) in 20 o/oo is not significantly different from the 15 o/oo value and is still below the freshwater level. Thus yearling lake trout show an initial slight hydration in the hypo-osmotic 10 o/oo but become dehydrated in the hyperosmotic salinities.

Total Body-Sodium

In freshwater, yearling lake trout had total body-sodium levels of 40 ± 2.3 (10) m-equiv./kg wet weight (Fig. 4). This was increased by 54% in 10 o/oo to 61 ± 3.3 (10) m-equiv./kg wet weight, a medium in which plasma Na^+ was not increased above freshwater values. The increase to 67 ± 3.0 (8) m-equiv./kg wet weight in 15 o/oo is not significant. The 20 o/oo level of 64 ± 1.3 (9) m-equiv./kg wet weight is not significantly different from either 10 o/oo or 15 o/oo body-sodium levels, however plasma Na^+ has increased 19% from the 10 o/oo value in 20 o/oo.

Figure 4. Total body-sodium of yearling lake trout
in relation to external salinity.
Mean \pm one standard error of the mean.



Sodium Space

The sodium space of yearling lake trout adapted to freshwater (Fig. 5) was $23.19 \pm .48$ (10) ml/100 g wet weight. This increased to $27.37 \pm .69$ (9) ml/100 g wet weight in 10 o/oo and $29.48 \pm .53$ (9) ml/100 g wet weight in 15 o/oo. In 20 o/oo, sodium space had increased to 30.8 ± 1.8 (8) ml/100 g wet weight. These are all significantly different.

Sodium space seemed linearly related to environmental salinity, having an 'r' value of .74, significant at the 5% level.

Muscle Water

Muscle water was similar in freshwater and 10 o/oo, being $80.21 \pm .16\%$ (10) and $80.18 \pm .16\%$ (10) wet weight respectively (Fig. 6). Dehydration occurred in the hyperosmotic 15 o/oo, where muscle water content dropped 1.47% to $79.05 \pm .16\%$ (10) wet weight, which is significant. A slight but significant degree of rehydration (0.7%) is evident in 20 o/oo ($79.57 \pm .15\%$ (10) wet weight), but this is still below freshwater levels.

Figure 5. Sodium space of yearling lake trout
in relation to external salinity.
Correlation coefficient = 0.74.
Mean \pm one standard error of the mean.

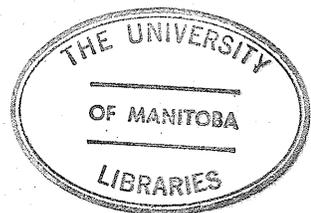
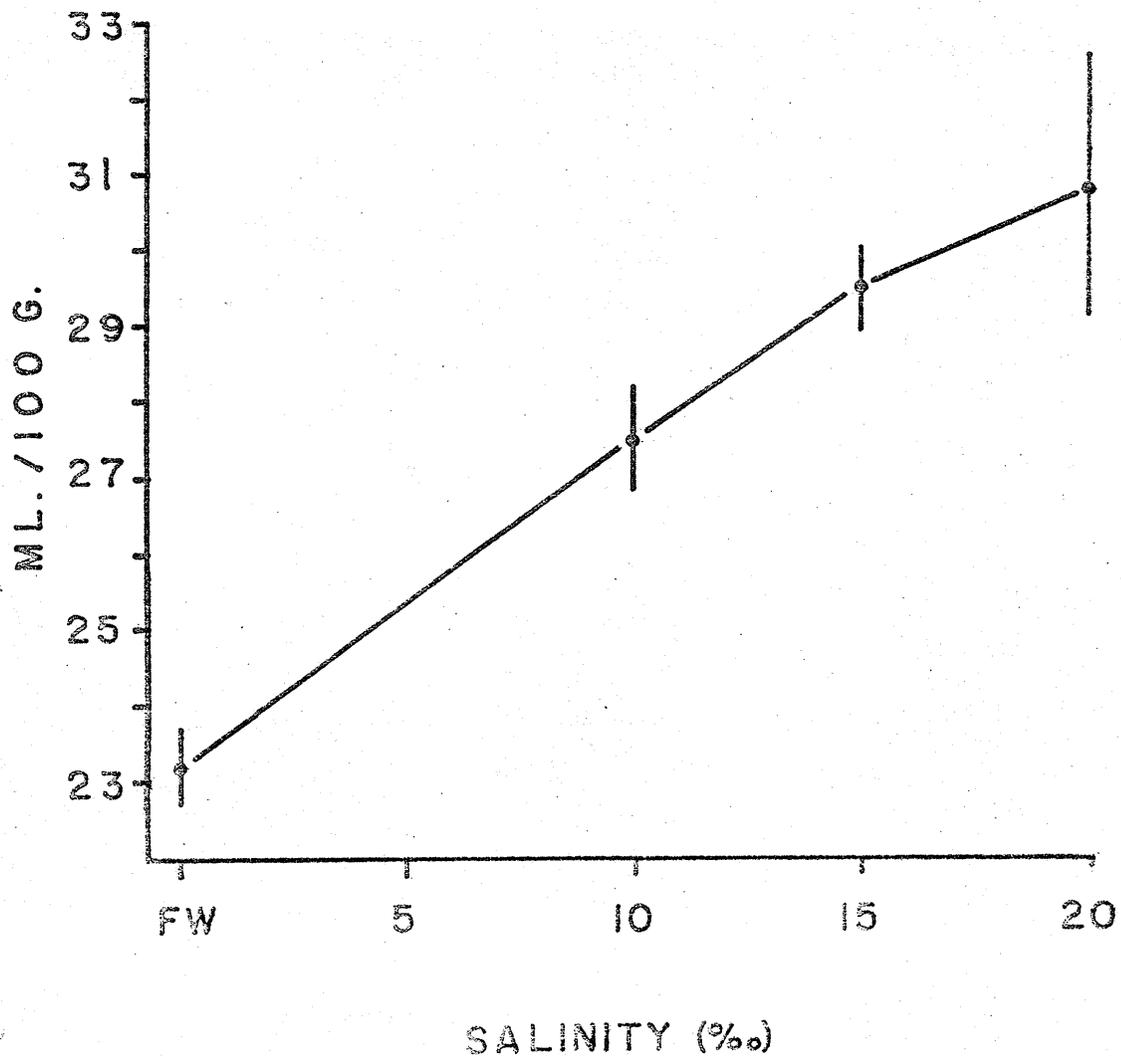
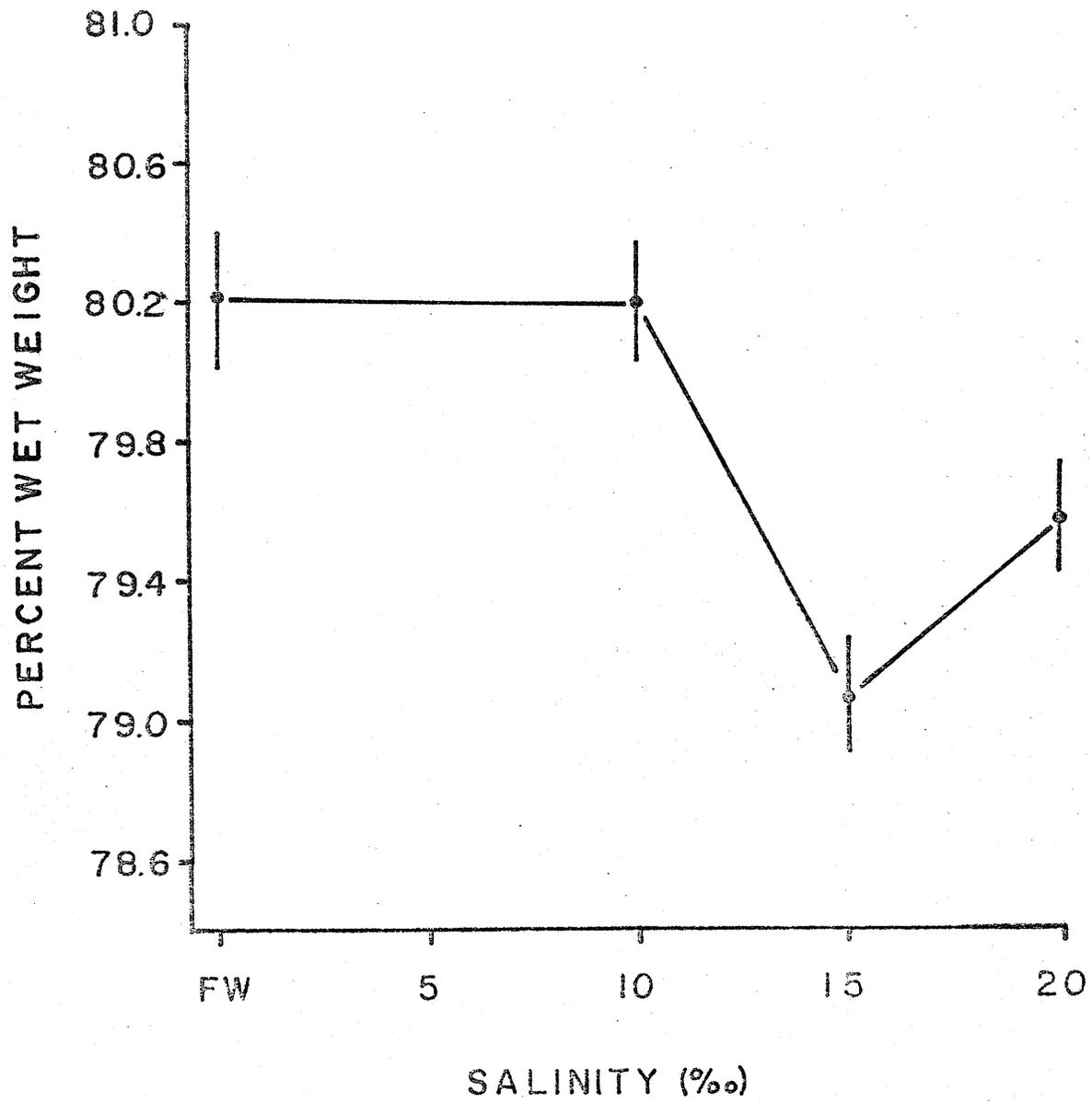


Figure 6. Muscle water content of yearling lake trout in relation to external salinity. Mean \pm one standard error of the mean.



Muscle Sodium

In freshwater, yearling lake trout had muscle sodium of $13.1 \pm .4$ (10) m-equiv./kg muscle water (Fig. 7). This is significantly elevated 17.2% in 10 o/oo to $15.4 \pm .5$ (10) m-equiv./kg muscle water. A further 26.3% increase from 10 o/oo to $19.5 \pm .7$ (10) m-equiv./kg muscle water occurred in 15 o/oo. Muscle sodium content dropped 31% from the 15 o/oo value to $14.8 \pm .4$ (10) m-equiv./kg muscle water in 20 o/oo, a value not significantly different from the 10 o/oo level.

Muscle Potassium

Muscle potassium showed a different pattern from muscle sodium (Fig. 7). Potassium levels were fairly constant in freshwater, 10 o/oo, and 15 o/oo (139 ± 1.3 (10), 139 ± 2.2 (10), 141 ± 1.6 (10) m-equiv./kg muscle water respectively), none of which is significantly different from each other. A large and significant increase in muscle potassium occurred in 20 o/oo to 153 ± 1.2 (10) m-equiv./kg muscle water.

These changes are summarized in Table 3.

Figure 7. Muscle sodium (closed circles) and muscle potassium (open circles) of yearling lake trout in relation to external salinity. Mean \pm one standard error of the mean.

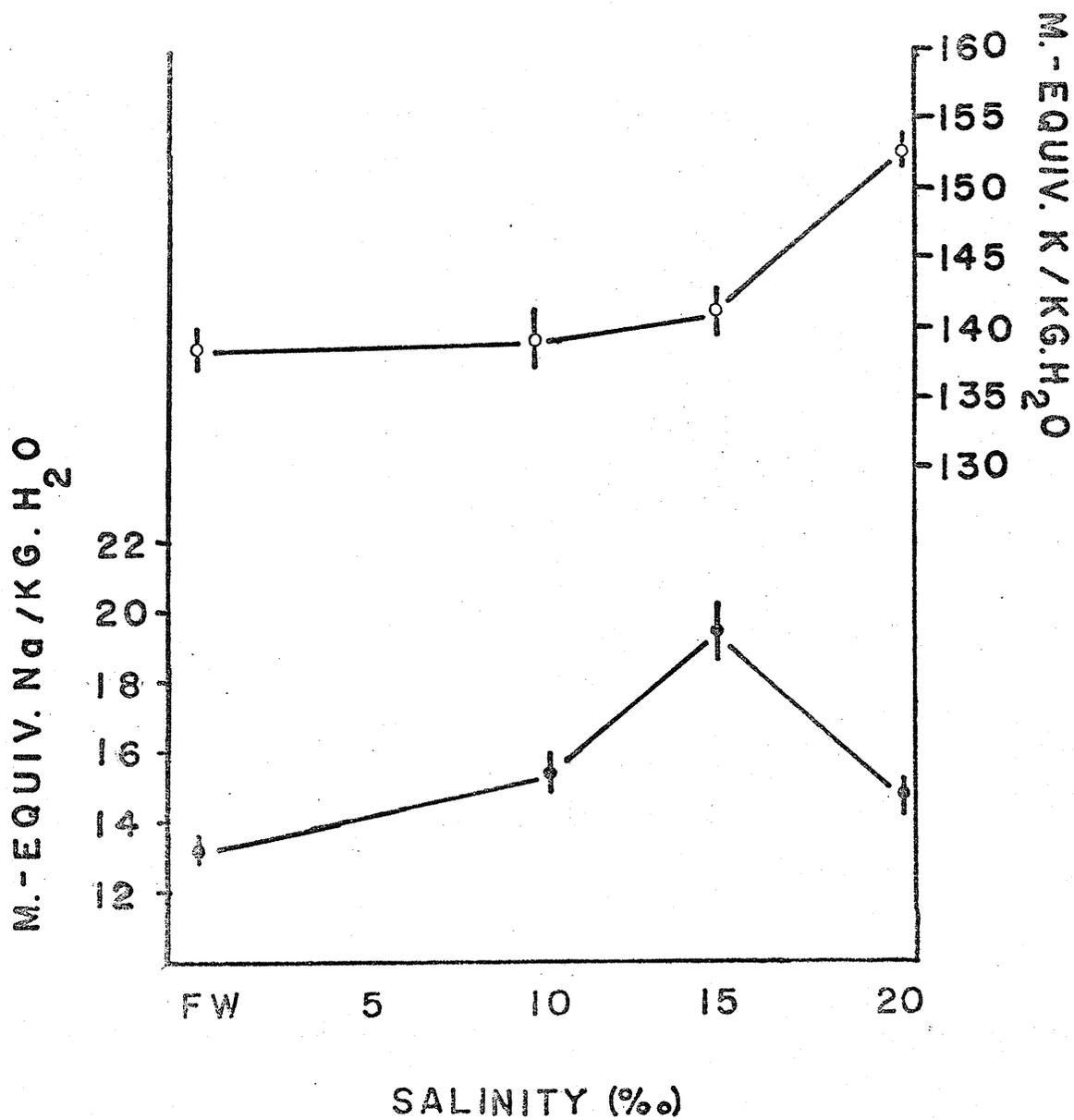


Table 3. Summary of water and ionic changes in yearling lake trout in response to increased salinity.

	Plasma Na ⁺ Yearlings	Plasma Na ⁺ two-year- olds	Total Body Water	Total Body Na ⁺	Na ⁺ Space	Muscle Water	Muscle Na ⁺	Muscle K ⁺
FW-10 o/oo	0	+	++	++++	+++	0	+	0
10 o/oo-15 o/oo	++	-	---	++	++	---	++	0
15 o/oo-20 o/oo	++	+++	+	-	++	++	---	++
20 o/oo-25 o/oo	++++							

0 = no change upon increase in salinity; + = slight increase; ++ = medium increase; +++ = large increase; ++++ = very large increase; - = slight decrease; -- = medium decrease; and --- = large decrease.

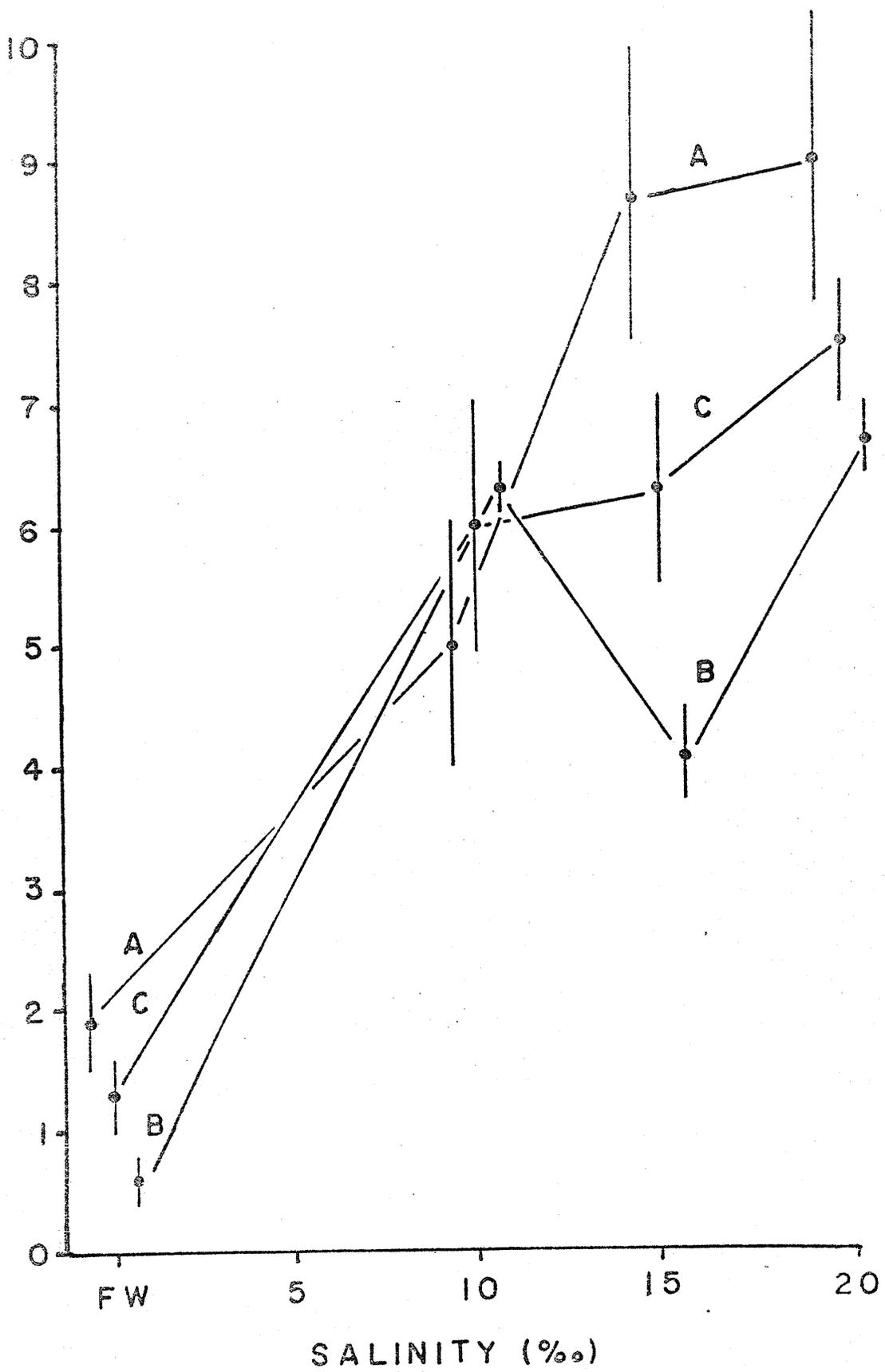
Sodium Efflux

The rate of loss of internal exchangeable sodium from yearling lake trout is shown in Fig. 8. Two groups of fish were used in this experiment; One from the Whiteshell Fish Hatchery (WPH) and the other from the Freshwater Institute (FWI), which originated from Northern Saskatchewan. The main difference between them is size; Group two (FWI) averaged 21.5 g and Group one (WPH) averaged 12.9 g. As these two groups behaved differently, with respect to sodium efflux, their efflux rates are treated separately. Listed below are the efflux rates of the two groups and the mean value, expressed as fraction/hr.

FW	10 o/oo	15 o/oo	20 o/oo
Group One			
.019 ± .004	.05 ± .01	.087 ± .012	.090 ± .012
Group two			
.006 ± .002	.063 ± .002	.041 ± .004	.067 ± .002
Mean			
.013 ± .003	.06 ± .01	.063 ± .008	.075 ± .005

Figure 8. Efflux rates of ^{22}Na from acclimated yearling lake trout in relation to external salinity. Line 'A' represents Group one animals; line 'B' represents Group two animals and line 'C' represents the mean of the two groups. Mean \pm one standard error of the mean.

% / hr



DISCUSSION

Salinity Tolerance

Lake trout sac-fry which were tested 2 days post hatching showed tolerance to dilute salinity up to 15 o/oo, but 2 days after transfer (21 days post-hatch at time of transfer) to 20 o/oo, there was complete mortality. This is a greater salinity tolerance than exhibited by Atlantic salmon which could not withstand 25% seawater (8.75 o/oo) (Eddy 1968, Parry 1960). However their experiments were of longer duration; lake trout sac-fry might show reduced tolerance if held for longer periods in the dilute salinities.

The salinity tolerance of yearlings, two-year-olds and adults is lower than all other North American salmonids of the appropriate age class. There was some evidence of an increased ability to hypo-osmoregulate in two-year-olds from the yearlings as two-year-olds showed a greater survival in 20 o/oo and 27 o/oo than yearlings. This could be due to the greater size of the two-year-olds. Also, as this is the time that anadromous salmonids normally smoltify, a phenomenon associated with increased salinity tolerance, this increased tolerance may be a family characteristic, which is expressed to a far lesser degree in lake trout.

There was no evidence that smoltification occurred in the experimental animals.

The sample size of the adult fish was too small to enable conclusions to be drawn. However, the majority (4 of 5) died when transferred to the hyperosmotic 15 o/oo and this would tend to support Boulva and Simard's (1968) hypothesis that adult lake trout can withstand only 11-13 o/oo.

Plasma Sodium Regulation

The freshwater sodium levels of yearling (156.6 m-equiv./l) and two-year-old lake trout (159.4 m-equiv./l) are close to that reported by Hammond (1969) of 157.45 m-equiv./l. Arctic char have a freshwater plasma sodium level of 151 m-equiv./l (Roberts 1971); splake (*Salvelinus fontinalis* X *S. namaycush*) have an identical value. These are similar to plasma sodium levels of other salmonids, which range from 117 m-equiv./l for freshwater Atlantic salmon parr (Parry 1961) to 163 m-equiv./l for juvenile Chinook salmon (Snodgrass and Halver 1971). The lowest freshwater value published is 110 m-equiv./l for Pink salmon that had spawned, were in an advanced state of degeneration, and were too weak to maintain headway in moderate stream currents (Triplett and Calaprice 1974).

Good regulation is shown by both yearling and two-year-old lake trout in 10 o/oo. Their plasma sodium levels (158 and 160 m-equiv./l, respectively) are not elevated much above the freshwater level, and are still within normal plasma levels. In 15 o/oo the pattern changes; two-year-old trout are still able to regulate plasma sodium at freshwater levels (157 m-equiv./l) in spite of a 2000-fold increase in the external concentration. Yearling lake trout were unable to regulate their plasma sodium at freshwater levels in this salinity. Their value of 177 m-equiv./l indicates they have some capacity to hypo-osmoregulate, as it is below the environmental level of 200 m-equiv./l.

In 20 o/oo, however, the capacity of two-year-olds to regulate has reached a maximum, and their plasma sodium level rises to 186 m-equiv./l, similar to yearlings (189 m-equiv./l). Arctic char (post-spawning adults) in a similar salinity (17.5 o/oo) showed better regulation, as their plasma sodium was regulated at 172 m-equiv./l (Roberts 1971). Gordon (in Holmes and Donaldson 1969) found brook trout had plasma sodium of 163 m-equiv./l in 17.5 o/oo. Thus lake trout plasma sodium, while high, is not excessive. At this salinity, yearlings show 24% mortality; the animals

unable to survive presumably had a higher plasma sodium level, or were unable to tolerate a plasma level of 189 m-equiv./l. Two-year-olds, with similar plasma levels, showed negligible mortality in this salinity.

The few (6%) yearlings able to survive one week in 25 o/oo, showed a very large increase to 231 m-equiv./l in their plasma sodium; they were all failing at the time of sampling.

This plasma level is very much larger than normal salmonid plasma sodium levels in seawater which are 166 m-equiv./l for brown trout, 182 m-equiv./l for coho salmon (Gordon, in Holmes and Donaldson 1969, Conte, in Holmes and Donaldson 1969), 170 m-equiv./l in chinook salmon (Snodgrass and Halver 1971) and 159 m-equiv./l for Atlantic salmon smolts (Parry 1961). However, other fish survive with plasma sodium concentrations as high as the yearling 25 o/oo value for Arctic char had plasma sodium of 233 m-equiv./l in seawater; a medium in which they exhibited 25% survival (Roberts 1971) and *Fundulus olivaceus*, the black spotted top minnow, following direct transfer from freshwater to 50% seawater, a medium in which they can live indefinitely, has plasma sodium levels of 235 m-equiv./l, two days after transfer (Duff and

Fleming 1972b). Lake trout yearling and two-year-olds thus only have a limited ability to regulate their plasma sodium, unlike a true diadromous regulator. The limiting factor in the salinity tolerance of yearling lake trout may be an intolerance to high plasma sodium levels.

Freshwater Body-Water and Body-Sodium

The freshwater body-water content of yearlings is similar to other salmonids, 75% body weight in Arctic char (Roberts 1971), 81.1% in Atlantic salmon (Potts *et al.* 1970) but higher than freshwater teleosts in general, which have an average value of 71.4% body weight (Thorson, in Holmes and Donaldson 1969).

The freshwater total body-sodium is somewhat higher than other salmonids. Roberts (1971) found Arctic char and brown trout in freshwater had total body-sodium levels of 32.18 m-equiv./kg wet weight and 37.5 m-equiv./kg wet weight respectively. Potts *et al.* (1970) gave a value of 30.3 m-equiv./kg wet weight for Atlantic salmon smolts. These salmonid values are lower than those found for other freshwater fish (all in mm/kg wet weight) e.g., 45.9 in *Tilapia*

mossambica (Fam. Cichlidae, Potts *et al.* 1967), 65.67 in *Perca fluviatilis* (Fam. Percidae, calculated from Lutz 1972b), 69.2 in *Amia calva* (Fam. Amiidae, Hanson *et al.* 1976), 95.3 in *Cyprinodon rubrofluviatilis* (Fam. Cyprinodontidae, Renfro and Hill 1973), 55 in *Fundulus catenatus* (Fam. Cyprinodontidae, Duff and Fleming 1972a) and 58 in *F. olivaceus* (Duff and Fleming 1972b).

Regulation in Salinities Above Freshwater

After transfer to 10 o/oo, the total body-water of yearling lake trout increases by 1.56%. This represents an increase in extracellular fluid as muscle water does not change. As 10 o/oo (286 m Osm) is still hypo-osmotic to normal lake trout blood (328.6 m Osm, Hammond 1969), a reduction or stoppage in renal function would cause an osmotic net influx of water, leading perhaps, to this hydration. Hammond (1969) found lake trout to have urine flow rates between .736-3.16 ml/kg/hr.

Total body-sodium is increased by 54% in 10 o/oo to 61 m-equiv./kg wet weight. Atlantic salmon smolts in seawater show only a 47.8% increase in total body-sodium, an absolute value of 44.8 m-equiv./kg wet weight (Potts

et al. 1970). Arctic char in seawater however, show a 138.3% increase in total body-sodium to a value of 76.7 mM/kg body weight. This is 19.8% higher than the lake trout value in only 20 o/oo.

Though there is a 54% increase in total body levels, the plasma sodium concentration does not change (Fig. 1). To accomplish this, lake trout expand their sodium space by 18.6% in 10 o/oo to counteract the increase in body-sodium and prevent a rise in plasma sodium concentration.

Sodium space is defined as "the volume occupied by sodium when uniformly distributed at the same concentration as that of the plasma" (Mayer and Nibelle 1969) and is a composite of extracellular and intracellular spaces. Mayer and Nibelle (1969) also stated that "it is reasonable to suppose that an augmentation of the distribution space is a means by which the animal can limit the increase in a hypertonic medium, the excess saline being distributed throughout a larger space". This is shown in eels, *Anguilla anguilla*, which do not increase the chloride space upon acclimation to seawater (Kirsch and Mayer-Gostan 1973) but do enlarge the sodium space (Mayer and Nibelle 1969); equilibrium plasma chloride is much higher in seawater than in freshwater while plasma sodium is

regulated at a level close to freshwater values (Kirsch and Mayer-Gostan 1973).

Cyprinodon rubrofluviatilis, the Red River pupfish, has an increased sodium space upon acclimation to hyperosmotic medium, but neither the extracellular (inulin space) nor the intracellular volumes were increased (Renfro and Hill 1973). As the increase in sodium concentration of the plasma was not sufficient to account for the increase in total body sodium, it was concluded that the intracellular sodium concentration increased greatly upon acclimation (Renfro and Hill 1973).

Other cyprinodonts, *Fundulus olivaceus* (Duff and Fleming 1972b) and *F. catenatus* (Duff and Fleming 1972a) showed expanded sodium spaces in high salinity. The sodium space of *F. olivaceus* was related to the adaptation medium, and increased with increasing salinity, similar to lake trout. This strategy of increasing sodium space in response to increased salinity is also shown in goldfish, *Carassius auratus*, which can be acclimated to half strength seawater and show an increased sodium space in that salinity over freshwater. However, this is not sufficient to allow plasma regulation of sodium as plasma sodium concentrations equalled the environmental concentrations (Lahlou *et al.* 1969).

Leatherland *et al.* (1974) considered that an expansion of sodium space in *Tilapia alcalica* upon adaptation to freshwater from high sodium environments, may, in part, account for the reduction of plasma sodium observed in that species. However, neither sodium space nor total body sodium were measured.

Finally, increase of sodium space in salinities greater than freshwater is a strategy not limited to teleosts, for Kooistra and Evans (1976) found that the Green Sea Turtle, *Chelonia mydas*, had a sodium space 20% larger in seawater than freshwater.

As sodium is mainly extracellular in distribution, the freshwater sodium space can be assumed to equal the freshwater extracellular volume (Holmes and Donaldson 1969). (This is not truly valid in this case as the technique used to measure sodium space takes no account of the heterogenous distribution of ions).

As total body-water increases by 1.56% in 10 o/oo with muscle water showing no change, then the extracellular volume would have increased by 5.3% in 10 o/oo (cannot use 10 o/oo sodium space to represent extracellular volume as muscle sodium has increased significantly and would indicate an expression of intracellular sodium space). The sodium content of the extracellular fluid in freshwater is then 36.29

m-equiv./kg (sodium space (= extracellular volume) X plasma concentration) and 38.60 m-equiv./kg in 10 o/oo. This only accounts for 11.2% of the 21.61 m-equiv./kg increase in total body sodium in 10 o/oo from freshwater. Thus 89% of the increased sodium content of yearling lake trout is intracellular.

This raises muscle sodium 17.2% over the freshwater value. If muscle sodium content is representative of the intracellular sodium concentration, then this rise would mean that the intracellular pool size in a one kilogram fish is 8.45 litres (19.18 (increase in intracellular sodium content) ÷ 2.27 (increase in muscle sodium concentration)). Thus the intracellular pool is heterogenous in sodium distribution.

Lutz (1972a, c) found that sodium spaces of muscle, liver, and gut of perch (11.78, 26.71, and 62.20 g/100 g tissue water respectively) were quite different, suggesting a heterogenous intracellular distribution of sodium. In response to 1/2 seawater, both muscle and liver increased their sodium space, but the gut sodium space was reduced.

The above calculations for lake trout do not allow for the sodium contained in the bone crystal. Lutz (1972b) found that perch bone, after being treated to remove the cellular and extracellular components,

still contained 170.3 mM Na⁺/kg dry weight, 40% of the whole bone value. However, Houston (1964) stated that uptake of sodium by bone is insignificant in reducing extracellular electrolyte levels.

After the transfer to 15 o/oo, total body-sodium is increased by 5 m-equiv./kg wet weight over the 10 o/oo value, but this is not statistically significant. However, the total body-sodium concentration increases as the total body-water shows a 3.74% decrease from the 10 o/oo level. This water loss in the hyperosmotic medium is both extracellular and intracellular as muscle water is decreased 1.47% from freshwater and 10 o/oo values.

The rise in plasma sodium concentration is ameliorated by a rise in muscle sodium of 26.3%, greater than that which can be accounted for by a cellular dehydration of 1.47%, although a heterogenous intracellular accumulation of sodium cannot be ruled out. This rise in muscle sodium is reflected in the 7.2% increase in sodium space from 10 o/oo. Muscle potassium is regulated to a greater extent, as it is not increased in 10 o/oo or 15 o/oo from the freshwater values.

Total body-water and total body-sodium do not change upon transfer to 20 o/oo but plasma levels do rise. Thus the source for the increased extracellular

sodium must be intracellular. Muscle sodium shows a 31.7% drop, returning it to 10 o/oo levels. Of this 31.7%, only .7% can be accounted for by the .6% increase in muscle water found in this salinity. Thus muscle sodium shows a real drop of 31%. This sodium from muscle and other intracellular sources is distributed within a sodium space which has increased 4.4% from 15 o/oo. The extracellular value in 20 o/oo must not have increased from 15 o/oo as muscle exhibits some hydration and total body-water does not increase significantly. Thus the increased sodium space in 20 o/oo does not result from an increase in extracellular volume. The intracellular source for the increased sodium may be heterogenous, with muscle contributing a considerable quantity, and some other intracellular pool expanding.

Another possible explanation is that the lake trout in 20 o/oo, a hyperosmotic medium, is faced with osmotic water loss to the environment. Most fish drink their medium to replace the osmotic water loss (Potts and Parry 1964). That the lake trout is osmoregulating effectively is seen by the fact that total body-water in 20 o/oo shows a slight, but non-significant, increase from 15 o/oo. Thus

any water which is lost osmotically is being replaced, probably by drinking. However, sodium and chloride are absorbed more rapidly than water (Maetz 1974); in rainbow trout, 99% of the ingested sodium and 94-98% of the ingested chloride, but only 80% of the ingested water is absorbed (Shehadeh and Gordon 1969). Even so, the possibility exists that, due to the presence of radiosodium tracer in the external medium, there may be tracer in the gut contents which would be measured in sodium space. Without knowledge of the drinking rate of lake trout, it is impossible to measure the extent of this error.

The 31% drop in muscle sodium, in the face of increased plasma sodium levels would suggest that this is an active extrusion of sodium. That muscle potassium shows an 8% rise (Na^+ drop/ K^+ rise = 2.4) is also suggestive of active removal of sodium. This may be necessary for the continued functioning of the muscle cell, due to possible upsets in the cell resting potential or other electrical events.

Sodium Efflux

The efflux rate of exchangeable sodium is increased in salinities above freshwater in the two groups tested. Group one showed an increase

in every salinity except 20 o/oo. Group two showed a decreased efflux in 15 o/oo and an increase in 10 o/oo and 20 o/oo. It is not known why these animals differed, but could be due to the size differences, stock differences or seasonal differences as Group one was tested in the spring and Group two was tested in the summer.

CONCLUSIONS

1. The data support the view that lake trout are the least salt tolerant of all North American salmonids. Sac-fry, yearlings and two-year-olds are able to adapt to hyperosmotic salinities. Of 5 adults tested, 4 died in the hyperosmotic 15 o/oo.
2. Yearling lake trout are able to regulate their plasma sodium concentrations in salinities up to 20 o/oo and in the hyperosmotic 15 and 20 o/oo, regulate plasma sodium below environmental concentrations.
3. An expansion of the sodium space is the main mechanism by which yearling lake trout control the rise of plasma sodium in dilute seawater environments.
4. The main expansion of sodium space is intracellular in 10 and 15 o/oo and accounts for 95% of the increased sodium load in 10 o/oo. In 20 o/oo, sodium is extruded from muscle, and it was postulated that another intracellular pool expanded to account for the increased sodium space.
5. The intolerance of yearling lake trout to high salinities is perhaps due to an intolerance of high plasma sodium levels.

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