OSMOREGULATION IN THE BROOK TROUT, *SALVELINUS FONTINALIS*—II. EFFECTS OF SIZE, AGE AND PHOTOPERIOD ON SEAWATER SURVIVAL AND IONIC REGULATION

Stephen D. McCormick* and Robert J. Naiman

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Abstract—1. Brook trout (*Salvelinus fontinalis*) of a single genetic stock, and hatched at the same time, were raised under two photoperiod and two feeding regimes to obtain fish of the same age but with different sizes and photoperiod experiences. In 11 experiments over 1.5 hrs, fish were gradually exposed to 32 ppt seawater for 20 days to investigate the ontogeny of salinity tolerance.

2. Daily changes in plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, thyroxine, hematocrit and gill Na^+,K^+-ATPase during adaptation to 10, 20 and 32 ppt were examined in one experiment.

3. Size was the primary determinant of seawater survival ($r^2 = 0.77$); the effect of size on seawater survival slowed after fish reached a fork length of 14 cm. The effect of age on seawater survival ($r^2 = 0.65$) was through its covariance with size.

4. Photoperiod affected seawater survival only through its influence on the timing of male maturation, which decreased salinity tolerance.

5. Regulation of plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$ and hematocrit in sea water increased linearly with size over the entire range of sizes (6–32 cm).

6. Gill Na^+,K^+-ATPase activity after 20 days in seawater decreased with increasing size of brook trout, possibly reflecting decreased demand for active ion transport in larger fish.

7. Plasma thyroxine concentrations declined in seawater, but no definitive role of this hormone in seawater adaptation was found.

8. Size dependent survival and osmoregulatory ability of brook trout is compared to other salmonids and a conceptual model is developed.

INTRODUCTION

The economic importance and complex life history of salmonid fishes has led to substantial research regarding their euryhalinity and osmoregulatory ability. Increased size has been shown to be a determinant of hypoosmoregulatory ability in a variety of salmonids, acting directly to influence salinity tolerance (Huntsman and Hoar, 1939; Elson, 1957; Parry, 1958; Houston, 1961; Wagner *et al.*, 1969; Wagner, 1974) as well as the process of smoltification (Clarke *et al.*, 1978). Although size has been implicated as the primary effector of ontogenetic changes in osmoregulatory ability, few studies have attempted to separate the normally covarying properties of size and age. Only Conte and Wagner (1965) and Conte *et al.* (1966), who distinguished only between year classes of salmonids, conclude that chronological age had little influence relative to size in determining hypoosmoregulatory ability.

Most of our knowledge of the ontogeny of salinity tolerance relates to specialized migrating species, particularly Pacific salmon (*Oncorhynchus* sp.), Atlantic salmon (*Salmo salar*) and migratory trout (*Salmo* sp.). Little is known about the osmoregulatory physiology of primitive anadromous salmonids in the genus *Salvelinus* (this genus includes

the charrs and brook trout, while *Salmo* includes trout and Atlantic salmon). The charrs exhibit a lesser degree of anadromy than other salmonids, and have fewer specializations in regard to euryhalinity (Hoar, 1976). Rousnefell (1958) hypothesized that *Salvelinus* were the first salmonids to migrate into seawater; if true, charrs should possess the basic osmoregulatory physiology upon which greater specializations were made by evolutionarily advanced salmonids.

Brook trout (*Salvelinus fontinalis*) are endemic to eastern North America. In the northern distribution of their range, where brook trout have access to the sea, anadromy is characterized by spring emigrations and coastal sea residence lasting for 2–4 months (White, 1940; Wilder, 1952; Castonguay *et al.*, 1982). In the southern portion of their range migrations are more variable, often occurring in autumn (Mullan, 1958). Maximum salinity of coastal environments entered by brook trout range between 28 and 32 ppt. Experimental sea ranching of brook trout has resulted in growth rates which are 4–5 times greater than freshwater populations and return rates between 30% and 60% after 3 months at sea (Whoriskey *et al.*, 1981). Younger and smaller sea-ranched brook trout, however, do not migrate seaward. Size and age dependent migrations are characteristic of all anadromous brook trout (White, 1940; Wilder, 1952; Smith and Saunders, 1958; Castonguay *et al.*, 1982). Brook trout in the Moisie River estuary (Quebec) disappear from regions of intermediate salinity at a

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*Present Address: Fisheries and Environmental Sciences, Department of Fisheries and Oceans, Biological Station, St Andrews, N.B. EOG 2XO, Canada.

size of 15–18 cm, presumably entering coastal waters (~ 28 ppt) at this size. These results suggest that size and/or age may be limiting factors in the salinity tolerance of brook trout. Salinity tolerance will limit not only the natural anadromy of a species, but also its use in seawater for sea ranching, farming or population enhancement.

Hypoosmoregulation in teleosts requires the reversal of passive influx of ions (including $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$) and efflux of water (Parry, 1966). Several mechanisms of active and passive transport, especially gill Na^+,K^+-ATPase, are used to countermand these diffusional fluxes (Kirshner, 1980). There is substantial evidence that plasma thyroxine stimulates smoltification in salmonids (Dickhoff et al., 1978; Grau et al., 1982). Thyroxine may also serve a regulatory function during seawater adaptation and ion transport (Folmar and Dickhoff, 1979; Knoeppel et al., 1982), though a definitive role for this hormone in seawater is lacking (see Leatherland, 1982, for review).

The objectives of this study were: (1) to determine how size, age and photoperiod limit the seawater survival of brook trout; (2) to investigate underlying physiological and hormonal changes accompanying salinity tolerance in order to understand the process of osmoregulatory adaptation in a "primitive" salmonid; and (3) to identify physiological factors which may cause (and can be used to predict) increased salinity tolerance. We have investigated changes in plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, thyroxine, hematocrit, and gill Na^+,K^+-ATPase activity during the process of seawater adaptation of brook trout (up to 20 days), and as hypoosmoregulatory ability changes with size, age and photoperiod.

MATERIALS AND METHODS

Experimental animals

To investigate the ontogeny of brook trout hypoosmoregulatory ability, two photoperiod and two feeding regimes were used to obtain fish of the same age but different sizes and with different photoperiod experiences. 20,000 fertilized brook trout eggs obtained from the Massachusetts State Fish Hatchery at Sandwich were divided into two annually cycling photoperiods; one photoperiod cycle corresponded to the normal calendar date (longest day June 21, shortest day December 21), while the other was 3 months delayed from the norm (longest day September 21, shortest day March 21). Within 1 week after feeding fish were divided randomly, within each photoperiod treatment, into two feeding groups. The high feed group was fed "maximum" rations (percent body weight of feed per day decreased with increasing body size; Leitritz and Lewis, 1976). The low feed group was fed approximately half the amount per unit body weight fed to the high feed group. Details of rearing and photoperiod conditions are reported in McCormick and Naiman (1984a).

Seawater exposure and blood sampling

Eleven seawater exposure experiments were conducted over 17 months on the four experimental groups (e.g. normal photoperiod: high and low feed groups; 3-month delayed photoperiod: high and low feed groups). Prior to each experiment a subsample of each group was measured for length and weight. Only fish within one standard deviation of the mean length for that group were used for seawater exposures or as freshwater controls. Experimental fish and freshwater controls were placed in 100 l tanks

supplied with freshwater at a constant temperature of 10°C. 32 ± 0.5 ppt seawater pumped from Vineyard Sound (adjacent to the laboratory), passed through a 100 μm filter and preheated or precooled to 10°C was used for all seawater exposures. In this report seawater is functionally defined as having a salinity of 32 ppt. For the first two experiments static tanks with periodic water changes were used for seawater exposures; constant flow-through conditions (six turnovers/day) were used thereafter. In experiments 1 and 2, increased salinities were obtained by removing a given volume of freshwater and replacing it with seawater. Increased salinities in experiments 3–11 were obtained by mixing flowing freshwater and seawater. In experiments 1 and 2 salinity changes were instantaneous, while in experiments 3–11 salinity change took place over 3.5–4.0 hr.

For seawater exposure, 20–32 fish were used in each experimental group. After 4 days of acclimation in freshwater, experimental animals were exposed to 10 ppt for 7 days, 20 ppt for 7 days and finally 32 ppt for 20 days. Gradual acclimation was used to more accurately duplicate the acclimation of brook trout in nature; brook trout spend a relatively longer period of time under estuarine conditions than other anadromous salmonids such as Atlantic salmon (Montgomery et al., unpublished data). Direct transfer of brook trout to 32 ppt seawater results in high mortality (80% within 48 hr, McCormick and Naiman, unpublished data). Salinity was checked regularly and no change greater than ± 1 ppt of the desired salinity was detected. Survival was monitored at least twice daily. Fork lengths of mortalities were measured, fish were sexed and gonad and whole body weight determined.

At 4 and 20 days in 32 ppt seawater brook trout were sampled for changes in blood and gill physiology. Freshwater controls were sampled 1 day before and their data reported in McCormick and Naiman (1984a). At 4 days, six to eight fish were sampled, and at 20 days all surviving fish were sampled. Only non-moribund fish were used. Fish were gently netted, placed in transfer buckets for <2 min, and then placed in 0.4 ml/l phenoxyethanol–seawater anesthetic solution for 30–60 sec. After length and weight were recorded, the caudal fin was severed, blood collected from the dorsal aorta into heparinized capillary tubes and spun for 5 min at 5500 rpm. Hematocrit (% red blood cells) was measured on a microhematocrit capillary tube reader. Plasma was removed for immediate analysis of osmolarity and $[Cl^-]$. Plasma $[Na^+]$, $[K^+]$ and $[Mg^{2+}]$ determinations were made within 48 hr of blood collection. Additional plasma (25–40 μl) was stored in micro-centrifuge tubes at $-17°C$ for later analysis of thyroxine. Immediately after blood withdrawal gill arches were removed, primary filaments trimmed from ceratobranchials, gill tissue placed in a sucrose-EDTA-imidazole buffer solution (Zaugg, 1982) and frozen at $-17°C$ for analysis of gill Na^+,K^+-ATPase activity. Sex was determined and gonads weighed to the nearest 0.01 g.

A single experiment employing more intensive sampling was performed to determine the time course of physiological changes during seawater exposure. Brook trout 1 year of age and 16.5–20.2 cm fork length were maintained in a 1000 l freshwater tank for 30 days prior to exposure to 10 ppt for 8 days, 20 ppt for 7 days and 32 ppt for 60 days. Salinities were changed and monitored in the same manner as those for other flow-through tanks. At each sampling five to six fish were anesthetized and blood and gill samples collected on the following days: 1 day prior to exposure to 10 ppt; 1, 2, 4, 7, 8 days following exposure to 10 ppt; 1, 2, 4, 7 days following exposure to 20 ppt; and 1, 2, 3, 4, 7, and 21 days following exposure to 32 ppt. No physiological sampling occurred after 21 days in seawater. Survival was monitored through 60 days. Only non-moribund females and immature males were used. The experiment was conducted during a declining photoperiod using fish from the normal photoperiod, high feed group.

Analytical techniques

Plasma osmolarity, [Cl⁻], [Na⁺], [K⁺] and [Mg²⁺] were analyzed using a Wescor vapor pressure osmometer, a Buchler–Cotlove chloridometer, and a Perkin–Elmer atomic absorption spectrophotometer used in atomic absorption mode to measure [Mg²⁺] and flame emission mode to measure [Na⁺] and [K⁺]. Gill Na⁺,K⁺-ATPase activity was analyzed using a partial purification method developed by Zaugg (1982). Gill Na⁺,K⁺-ATPase activities based on either protein content of homogenate or wet weight tissue were equally valid; all measurements are reported here as $\mu MP_i \cdot mg \ prot.^{-1} \cdot hr^{-1}$. Plasma thyroxine levels were determined in duplicate using competitive binding radioimmunoassay (Dickhoff et al., 1978). Details of these techniques are reported in McCormick and Naiman (1984a).

Statistical analysis

Seawater survivial is expressed in two ways. Mean survival time is the arithmetic mean of individual survival times (in days), excluding those that were sacrificed after 4 days in seawater or that jumped from tanks ($< 1\%$). Survival time is calculated from the beginning of initial exposure to 32 ppt (i.e. following acclimation and after salinity was changed to 32 ppt). Because of the duration of each experiment, maximum seawater survival time was 20 days. A second expression of seawater survival is the hazard rate, calculated as the number of fish that died in an experiment, divided by the number of days at risk. Days at risk is the number of days that each fish survives before being withdrawn (sacrificed or jumped from tank) or dying, summed for all fish in an experimental group.

Condition factor (CF) is calculated using the formula:

$$CF = [weight/(length)^3] \cdot 100,$$

where weight and length are expressed as wet weight in g and fork length in cm, respectively.

Linear regressions were performed using the least squares method. Standardized residuals (Prescott, 1975) and least squares regression programs were obtained from the BMDP Statistical Software package (Dixon, 1981). Mean values are reported as the arithmetic mean ± 1 standard error of the mean, unless noted otherwise.

Multiple regression analysis of seawater survival was performed using log-linear dichotomous regression (Dumouchel, 1981). Each 20 day experiment was divided into five, 4-day intervals and the number of fish entering, dying, withdrawn and leaving alive were calculated. Fish withdrawn or dying are assumed to have done so half-way through the interval. The outcome (dead or alive) for each individual in each interval is used to calculate the probability of mortality. The probability of mortality is used as a dichotomous dependent variable which is regressed on model specified, log transformed independent variables. This technique allows one to examine the effect of one independent variable while holding all other variables constant. The slope of the regression line, B, and its standard error represent regression coefficients similar to those of ordinary least squares regression. The odds ratio of an independent variable is the change in probability of survival resulting from a change of an independent variable from x_1 to x_2 and is calculated from the formula:

$$odds \ ratio = e^{(x_2 - x_1)} - B$$

The odds ratio was deemed significant when its 95% confidence limit (calculated using the 95% confidence limits of B) did not encompass 1.0. An odds ratio of 1.0 indicates that the chance of survival does not change over the range of values tested; an odds ratio of 2.0 indicates that the chance of survival is doubled when the independent variable moves from x_1 to x_2.

RESULTS AND DISCUSSION

Time course of seawater adaptation

Brook trout 1 year of age and 16.5–20.3 cm were gradually exposed to seawater during a declining photoperiod in order to determine the time course of physiological changes during seawater adaptation. Mortalities did not occur until after exposure to 32 ppt. Cumulative mortality of the 21 animals not used for physiological measurements was 19% after 5 days in 32 ppt, 38% after 20 days, and 76% after 60 days.

Plasma ions and osmolarity. Plasma [Na⁺], [Cl⁻] and osmolarity rose 5–15% after 1–2 days exposure to 10 and 20 ppt (Fig. 1) and after 7–8 days were reduced to levels 5–10% higher than initial freshwater levels. Plasma [Na⁺], [Cl⁻] and osmolarity increased within 1 day of exposure to 32 ppt, reaching levels 20% ([Na⁺]), 30% (osmolarity) and 70% ([Cl⁻]) higher than initial freshwater levels after 4 days in seawater. Though some decline in plasma [Na⁺], [Cl⁻] and osmolarity occurred after 7 days in seawater, levels at 21 days were as high as those experienced during the first few days of seawater exposure.

Experiments involving direct transfer of salmonids from freshwater to seawater of various salinities result in peaks of plasma [Na⁺], [Cl⁻] and osmolarity within 1–2 d of exposure (Leray et al., 1981; Bath and Eddy, 1979; Jackson, 1981). We found similar peaks after 1–2 days in seawater, but these remained high for 4 days. Plasma [Na⁺], [Cl⁻] and osmolarity decline after 7 days in seawater, indicating that 16–20 cm brook trout have some ability to regulate plasma ions at this salinity. This ability is clearly incomplete, however, since plasma [Na⁺], [Cl⁻] and osmolarity again increase after 21 days in seawater, and mortalities continue throughout this period. Despite the ability of brook trout to survive an initial 2–3 day period characterized by high plasma and muscle ion concentrations and gill dehydration (Leray et al., 1981), mechanisms for total adaptation are not fully functional for all individuals at this salinity.

Plasma [Mg²⁺] increased less than 15% after exposure to 10 and 20 ppt (Fig. 1). After exposure to 32 ppt, mean plasma [Mg²⁺] rose continuously, reaching a peak 350% higher than freshwater controls after 7 days, and declining to 250% of freshwater controls after 21 days. Although much smaller in absolute magnitude than changes in [Na⁺], [Cl⁻] and osmolarity ([Mg²⁺] constitutes less than 1% of total plasma osmolarity), fluctuations relative to the initial starting value were large. The time course of change in plasma [Mg²⁺] in response to rapid salinity change is similar to that of [Na⁺], [Cl⁻] and osmolarity.

Plasma [K⁺] did not increase immediately after exposure to higher salinity as did other plasma ions (Fig. 1). Instead, plasma [K⁺] declined 50% below freshwater levels after exposure to 10 and 20 ppt. Plasma [K⁺] did not increase above freshwater levels until 3 days after exposure to 32 ppt, and after 21 days in seawater was only 50% of original freshwater concentrations. Bath and Eddy (1979) found only small changes in plasma [K⁺] after seawater exposure of rainbow trout (*S. gairdneri*) to 22 ppt, while Gordon (1959) found significant increases in plasma [K⁺] after exposure of brown trout (*S. trutta*) to 31.5
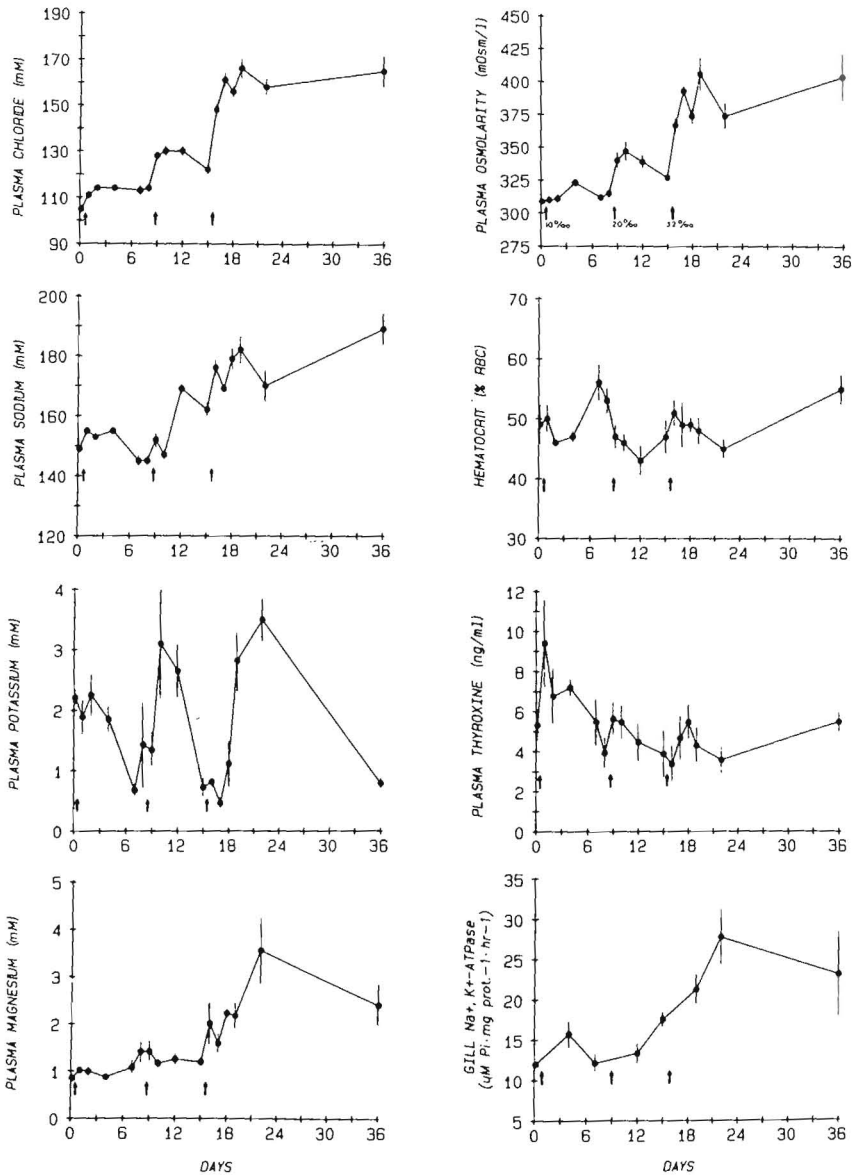
Fig. 1. Time course of changes in plasma and gill physiological parameters after exposure to 10 ppt, 20 ppt and 32 ppt. Arrows indicate the day of salinity change, which was gradual and took 3.5–4 hr. Each physiological variables had significant differences among means during seawater exposure ($P < 0.05$, one-way ANOVA).

ppt. Despite large chemical gradients that exist between the blood and external medium for $[Na^+]$, $[Cl^-]$, $[Mg^{2+}]$ and $[K^+]$, plasma $[K^+]$ is regulated in a fundamentally different manner during seawater adaptation. This may be due, in part, to the existence of high intracellular $[K^+]$.

Hematocrit. Although some changes in hematocrit (% red blood cells) occurred during seawater adaptation (Fig. 1) there is no apparent response pattern to changing salinity. None of the values for mean hematocrit after exposure to increasing salinity are outside the range of initial freshwater values (39–63%). These results agree with those of Bath and Eddy (1979), who found that hematocrit of juvenile rainbow trout (13.3 g mean weight) fluctuated in the

first 24 hr of exposure to 22 ppt, but remained constant thereafter.

Gill Na^+,K^+-ATPase. Initial freshwater levels of gill Na^+,K^+-ATPase activity were $12.0 \pm 0.6 \, \mu MP_i \cdot mg \, prot.^{-1} \cdot hr^{-1}$ (Fig. 1). These levels remain constant through exposure to 10 ppt, increased steadily after 7 days in 20 ppt, and leveled off after 7 days in 32 ppt. Activity of gill Na^+,K^+-ATPase after 21 days in seawater was $23.1 \pm 5.1 \, \mu MP_i \cdot mg \, prot.^{-1} \cdot hr^{-1}$.

With few exceptions, euryhaline teleosts increase gill Na^+,K^+-ATPase activities 2 to 5 times when transferred to seawater (Kirschner, 1980). Towle *et al.* (1976) found that mummichog (*Fundulus hetero-clitus*) in 16 ppt seawater had lower gill

Na^+,K^+-ATPase activity than fish in freshwater or seawater. Brook trout display no such adaptation in gill Na^+,K^+-ATPase activity at intermediate salinities. Gill Na^+,K^+-ATPase activities increase immediately upon transfer of mummichog (Towle, 1977) and striped mullet, *Mugil cephalus* (Hossler, 1980) to seawater, whereas in salmonids (Zaugg and McLain, 1970) and in the American eel, *Anguilla rostrata* (Forrest *et al.*, 1973) gill Na^+,K^+-ATPase activities increase 2–5 days after transfer to seawater. The timing and magnitude of increases in gill Na^+,K^+-ATPase activity reported here for brook trout are similar to those of other diadromous teleosts.

Plasma thyroxine. Mean plasma T_4 was highest 1 day after exposure to 10 ppt (80% increase over freshwater levels). Plasma T_4 levels dropped 20% below freshwater levels after 8 days in 10 ppt. Exposure of fish to 20 and 32 ppt caused plasma T_4 to increase 25–30% within 2–3 days, followed by decreasing levels after 4 and 7 days exposure. These changes should be considered only as trends, however, since within group variances are large relative to changes in mean value. Plasma T_4 after 21 days in 32 ppt seawater was 5.5 ng/ml, compared to an initial freshwater level of 5.3 ng/ml.

The role of thyroxine during the process of adaptation of teleosts to seawater is unclear. Knoeppel *et al.* (1982) found that a functional thyroid or supplemental T_4 was necessary for mummichog to regulate $[Na^+]$ and survive after transfer to seawater. Folmar and Dickhoff (1979) found transient peaks in circulating T_4 coincided with increasing gill Na^+,K^+-ATPase activity in coho salmon (*Oncorhynchus kisutch*) after transfer to seawater. It has been suggested that regulation of Na^+,K^+-ATPase is the basic mechanism by which thyroxine performs its physiological functions in vertebrates (Ismail-Beigi and Edelman, 1970). In the present study, highest T_4 levels occurred after exposure to 10 ppt, 2 weeks before significant increases in gill Na^+,K^+-ATPase were observed. If the thyroid does play a role in regulating seawater gill Na^+,K^+-ATPase activity (or the seawater adaptation process) of brook trout, it is not a simple response to change in circulating levels of thyroxine.

The time course of brook trout seawater adaptation reported here is in substantial agreement with research on other teleosts indicating that the first 4 days following changes in external salinity are associated with large changes in plasma ions and osmolarity. Measurement of plasma variables after 4 days in seawater will, therefore be an accurate indicator of hypoosmoregulatory ability. We did not expect plasma ions and osmolarity after 20 days in seawater to show such large variations and values substantially greater than freshwater levels. Measurements after 20 days in seawater must also be viewed as a period of osmotic disequilibrium for brook trout adapting to seawater.

Seawater survival

Size and age. Fish in the high feed group in all experiments were larger than fish in the low feed group (Fig. 2(A)), with the absolute difference in length increasing over time. Mean seawater survival
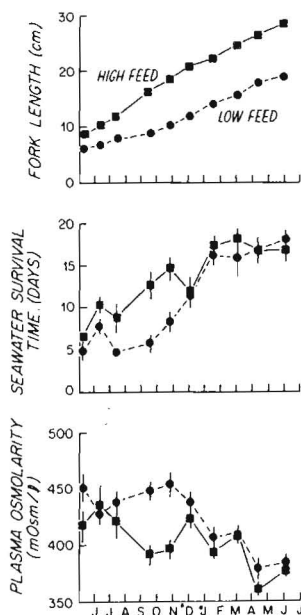

Fig. 2. Size (fork length) at time of seawater exposure, mean survival time in seawater, and plasma osmotic concentration after 4 d in seawater as a function of time of year of seawater exposure. Experiments were conducted under normal photoperiod conditions. Closed squares represent high feed (fast growing) fish, closed circles represent low feed (slow growing) fish.

time was higher for the high feed group in each of the first five experiments (Fig. 2(B)); a decline in seawater survival time of high feed fish occurred in Experiment 6 which was conducted during normal spawning time and included several mature males. We have shown poor seawater survival and hypoosmoregulatory ability in mature males, which becomes acute during autumn spawning (McCormick and Naiman, 1984b). There were no mature males in the low feed group, which had mean seawater survival time equal to the high feed group. For Experiments 7–10 mean seawater survival times of high and low feed groups are similar, having plateaued at 16–18.5 days (38–81% survival). The smallest brook trout to reach this plateau were 14 cm. Failure of brook trout to achieve 100% survival may be the result of the rapid transfer from 20 to 32 ppt, a relatively high salinity for the normal distribution of brook trout. Small increases in salinity (28 to 32 ppt) have been found to radically alter the survival of rainbow trout (Jackson, 1981).

The seawater hazard rate is a measure of the probability of mortality in seawater over 20 days. Mean survival time and hazard rate for the seawater exposure experiments were significantly correlated ($r = 0.97$, $P < 0.01$, $N = 44$). Size can explain a large portion of the variation in seawater hazard rate ($r^2 = 0.64$), which is increased to 0.77 when experiments with fully mature males are excluded (Fig. 3). Experiments with fully mature males had the following common properties: (1) greater than 25% of the fish were mature males, (2) gonadosomatic indexes were $> 1.5\%$, (3) experiments were conducted during autumn photoperiod, and (4) mean survival time was significantly lower than that of immature males,
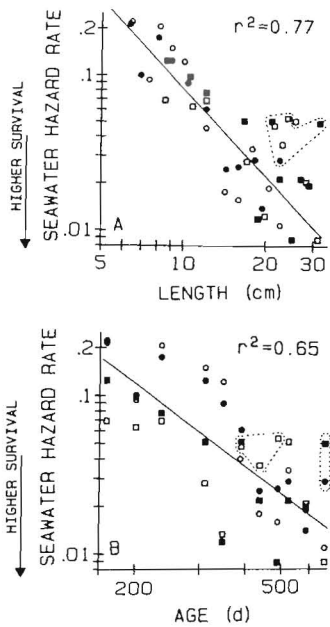
Fig. 3. Seawater survival as a function of size and age. Log of seawater hazard rate vs (A) log of fork length and (B) log of age for fish in high feed (squares) and low feed (circles) fish in normal photoperiod (closed figures) and 3-month delayed photoperiod (open figures). Eleven experiments (four groups per experiment) were conducted over 17 months. Points encircled with dashed lines represent experiments with mature males exposed to seawater during autumn photoperiod. Linear regression of log hazard on log size had r^2 of 0.64 and 0.77 for experiments with and without fully mature males, respectively. Regression lines do not include experiments with fully mature males. Linear regression of log hazard on log age had r^2 of 0.58 and 0.65 for experiments with and without fully mature males, respectively.

mature and immature females ($P < 0.025$, Mann–Whitney U-test).

Fish surface-area-to-volume ratios, as estimated by length²/weight, also had a strong ability to predict survival in seawater ($r^2 = 0.79$, excluding experiments with fully mature males). Log transformation of length explained a greater portion of the variation in seawater hazard ($r^2 = 0.77$) than untransformed length ($r^2 = 0.69$), indicating the effect of size on

survival decreases with increasing size. This effect corresponds to the plateau in seawater survival time of brook trout at sizes greater than 14 cm (Fig. 2(B)).

Age also had a significant effect on seawater hazard rate ($r^2 = 0.58$, Fig. 3(B)). It is clear, however, that at ages < 1 year there is a clear distinction in seawater hazard rate between high and low feed groups (Fig. 3(B)). This is most likely caused by size differences, since growth per unit size explains less of the variation in seawater hazard ($r^2 = 0.59$) than either size or age. In addition, there is no consistent difference in seawater survival of high and low feed fish as a function of length (Fig. 3(A)), despite differences in growth rate and age between the groups at any given length.

The relative roles of size and age can be further clarified by examining the results of dichotomous regression on seawater survival. This statistical modelling procedure allows one to examine the effects of a variable on seawater survival while holding all other independent variables constant. We chose models which included all combinations of the following independent variables: length, age weight, male maturity, growth rate, feeding group and rate of change in daylength. Only the first 12 days of each experiment were used in this analysis because the last 8 day fit all models poorly (75% of the mortalities occurred in the first 12 days). The statistical model shown in Table 1 is the simplest model (fewest variables) with a highly significant χ^2 (377.4, d.f. = 5, $P < 0.001$). This model shows a significant effect of size and male maturation on seawater survival. The effect of age was not significant. Inclusion of other variables such as growth rate or feeding group will increase χ^2 but are difficult to interpret because of their covariance with both size and age. All models which include the variables listed above showed length to be the most significant determinant of seawater survival.

Although several researchers have concluded that size is the primary factor determining seawater survival in salmonids (Huntsman and Hoar, 1939; Parry, 1958; Houston, 1961; Wagner et al., 1969; Wagner, 1974) and striped mullet, *Mugil cephalus* (Nordlie et al., 1982), few have attempted to separate the effects of size and age. Conte and Wagner (1965) and Conte et al. (1966) determined that size, rather than age determined seawater survival in rainbow trout and coho salmon, although they did not manipulate growth rates to control for age. Similarly, brook

Table 1. Dichotomous log-linear regression of length, age and male maturity on survial in 32 ppt seawater for 12 days. B is the slope of the regression line. Index of male maturity for an experiment was determined by multiplying the percent mature males in an experiment by their mean gonadosomatic index. The odds ratio was calculated for the range of values in our experiments and represents the change in probability of survival for that range. Odds ratio greater than one indicates increasing chance of survival (e.g. an odds ratio of 100 for size indicates the probability of survival increases 100 times when brook trout go from 6 to 32 cm). An asterisk indicates the odds ratio is significantly different from 1.0 ($P < 0.05$). Chi-square for this model was 377.4 with 5 degrees of freedom ($P < 0.001$)

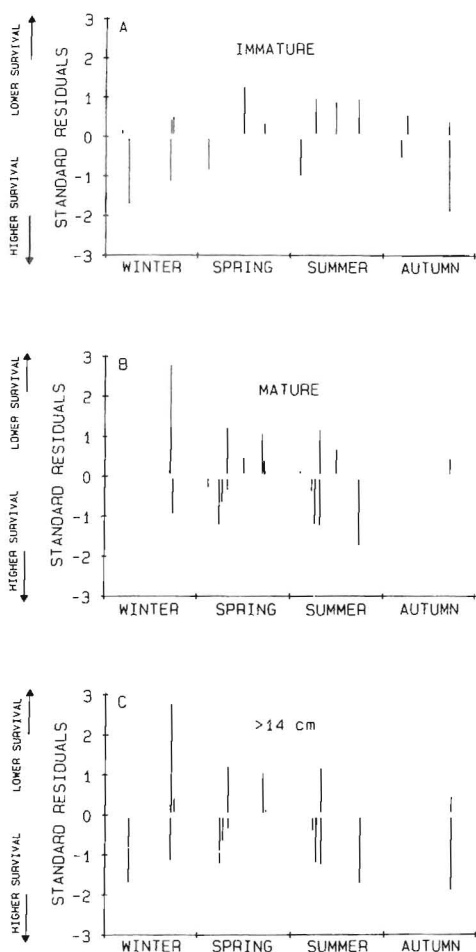| Independent Variables	B	Standard Error, B	Odds Ratio
Interval			
1	−0.61	0.06	—
2	0.73	0.09	---
3	0.52	0.13	—
Length	−2.90	0.33	106.6*
Age	−0.89	0.28	1.1
Male Maturity	0.006	0.002	0.169*

Fig. 4. Standardized residuals of the regression of log seawater hazard rate on log length, as a function of season for (A) immature brook trout, (B) mature brook trout and (C) brook trout greater than 14.0 cm. Season corresponds to the photoperiod conditions fish experienced just prior to and during seawater exposure.

trout show no effect of age on seawater survival. Our results also indicate that size dependent seawater survival is a basic characteristic of the sub-family Salmonidae.

Photoperiod. Seasonal patterns of seawater survival, other than those imposed by male maturation, may exist. Standardized residuals from the regression of log length against log hazard have no photoperiod-related pattern for mature, immature, or fish greater than 14.0 cm (corresponding to the size at which the effect of size on seawater survival begins to level off; Fig. 4). The only significant outlier (standard residual > 2.0) was also the only experiment conducted under winter photoperiod conditions with large numbers of post-spawning adults. This may be the result of general post-spawning weakness which caused a generalized increase in susceptibility to osmotic stress. Dichotomous regressions did not show any significant effect of daylength, rate of change in daylength or season when these variables were included in models of seawater survival.

Specialized migrating salmonids, particularly those which undergo smoltification, show seasonal, photoperiod-induced changes in seawater survival (Conte and Wagner, 1965), hypoosmoregulatory ability (Conte and Wagner, 1965; Clarke *et al.*, 1978) and freshwater levels of gill Na^+,K^+-ATPase activity (Zaugg and Wagner, 1973; Ewing *et al.*, 1979). Silvering (caused by guanine deposition in skin and scales) and increases in gill Na^+,K^+-ATPase activity normally associated with smolting, do not occur in this strain of brook trout (McCormick and Naiman, 1984a), in spite of its seaward-migrating behavior. Silvering has been found in other anadromous populations of brook trout but is not indicative of imminent seawater entry as it is in Pacific and Atlantic salmon (Black, 1981). Our results indicate that there is no seasonal change in seawater survival, except for that associated with male maturation. Lack of seasonality in brook trout may reflect the lower degree of anadromy and greater opportunism displayed by this and other species in the genus *Salvelinus*.

Table 2. Physiological variables after 4 days in seawater regressed on length, and log hazard as a function of physiological variables. Linear regression statistics include correlation coefficient (r), y-intercept (y-int), slope (b), standard error of the slope (S_b), probability of rejecting the null hypothesis that the slope of the regression line is 0 (P), and the number of experiments (N). Each experiment is the mean of six to eight individuals.

Dependent variable	r	Length y-int.	b	S_b	P	N
Osmolarity	−0.80	465.7	−3.27	0.37	0.001	44
[Cl⁻]	−0.40	188.9	−0.77	0.27	0.010	38
[Na⁺]	−0.46	215.2	−1.15	0.38	0.004	38
[K⁺]	−0.70	5.42	−0.129	0.022	0.001	38
[Mg²⁺]	−0.64	4.45	−0.081	0.016	0.001	38
Hematocrit	0.44	42.9	0.285	0.095	0.005	40
Na⁺,K⁺-ATPase	—	—	—	—	0.101	40
Thyroxine	0.48	0.98	0.083	0.026	0.003	36

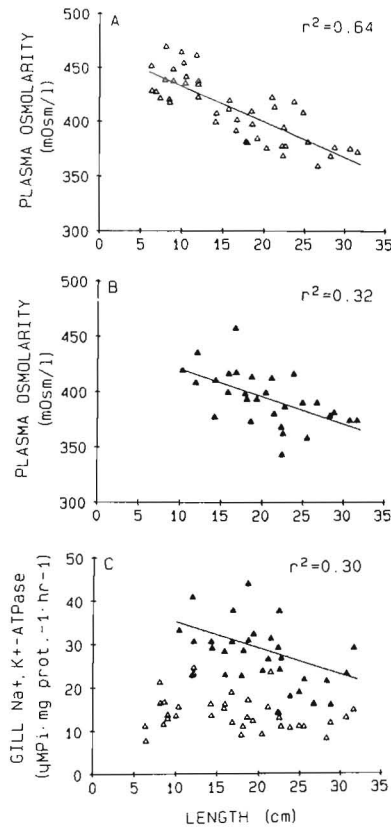| Independent variable	r	Log seawater hazard rate y-int.	b	S_b	p	N
Osmolarity	0.70	−5.04	0.009	0.001	0.001	44
[Cl⁻]	0.42	−3.47	0.012	0.004	0.008	38
[Na⁺]	0.49	−3.24	0.009	0.003	0.002	38
[K⁺]	0.53	−1.87	0.141	0.037	0.001	38
[Mg²⁺]	0.65	−2.18	0.251	0.048	0.001	38
Hematocrit	−0.39	−0.05	−0.030	0.012	0.013	40
Na⁺,K⁺-ATPase	—	—	—	—	0.219	40
Thyroxine	−0.46	−1.03	−0.140	0.047	0.003	36

Fig. 5. Plasma osmolarity and gill Na$^+$,K$^+$-ATPase in seawater as a function of brook trout size. (A) Plasma osmolarity after 4 days in seawater, (B) plasma osmolarity after 20 days in seawater, and (C) gill Na$^+$,K$^+$-ATPase activity after 4 days (open triangles) and 20 days (closed triangles) in seawater, vs fork length of brook trout. Regression lines, where drawn, have slopes which are significantly different from zero ($P < 0.05$, see Tables 2 and 3).

Ontogeny of hypoosmoregulatory ability

Plasma ions and osmolarity. Plasma osmolarity, [Na$^+$], [Cl$^-$], [K$^+$] and [Mg^{2+}] after 4 days in seawater decreased with increasing length of brook trout (Table 2). Plasma osmolarity showed the strongest correlation with length ($r^2 = 0.64$, Fig. 5) and was the best physiological predictor of mortality in seawater (Fig. 5, Table 2). Length can explain 16%, 21%, 49% and 41%, of the variation in plasma [Cl$^-$], [Na$^+$],

[K$^+$] and [Mg^{2+}], respectively, after 4 days in seawater. Age could explain less of the variation than length in each of the plasma ions (6%, 20%, 38% and 31%, respectively).

Plasma osmolarity and [Mg^{2+}] after 20 days in 32 ppt were significantly correlated with length (Table 3). The levels of plasma ions after 20 days in seawater for any given size are, in most cases, lower than those after 4 days in 32 ppt. These results indicate that smaller fish that survive for 20 days in seawater cannot regulate blood osmolarity and [Mg^{2+}] to the same extent as larger fish surviving the same length of time.

Size related increases in [Na$^+$], [Cl$^-$] and water transport capabilities during seawater acclimation have been found in Atlantic salmon (Parry, 1958; Houston, 1961), rainbow trout (Conte *et al.*, 1966; Wagner, 1974; Jackson, 1981) coho and chinook salmon (Clarke *et al.*, 1978) and mullet (Nordlie *et al.*, 1982). Our results show that [K$^+$] and [Mg^{2+}] are also regulated in a size dependent manner by brook trout. Increased ionic regulatory ability with length may reflect decreasing surface-area-to-volume ratios that accompany increased length. In our experiments, log transformations did not improve correlations between length and ionic or osmotic concentrations after 4 days in seawater, nor are there any indications of a decrease in the effect of length on these plasma constituents. As length increases, an eventual decrease in the effect of size on plasma ions and osmolarity must occur, since ions are unlikely to be regulated below freshwater levels. The length corresponding to such a size threshold, however, was not reached in our experiments which used animals up to 32.0 cm. In contrast, length did show a decline in its effect on seawater survival. This may reflect the reduction of plasma ions below a "critical" survival level such that, while size may still act to decrease ion levels during seawater adaptation, survival will be close to maximum and increases in survival will not be detectable. Such a phenomenon has been reported by Jackson (1981) for rainbow trout.

Hematocrit. Hematocrit after 4 days in seawater ranged between 31% and 73% and mean hematocrit was positively correlated with length (Table 2). Hematocrit of brook trout in freshwater also increases with size (McCormick and Naiman, 1984a). Failure to find a response of hematocrit to salinity change during the time course of adaptation indicates that hematocrit is either conserved (within limits) or has little to do with osmoregulatory phenomenon.

Table 3. Physiological variables after 20 days in seawater regressed on length. Linear regression statistics include correlation coefficient (r), y-intercept (y-int.), slope (b), standard error of the slope (S_b), probability of rejecting the null hypothesis that the slope of the regression line is 0 (P), and the number of experiments (N). Each experiment is the mean of 3–8 individuals. All listed correlation coefficients are significant ($P < 0.05$)

Dependent variable	r	Length y-int.	b	S_b	p	N
Osmolarity	−0.57	445.5	−2.48	0.69	0.001	29
[Cl$^-$]	—	—	—	—	0.076	29
[Na$^+$]	—	—	—	—	0.190	20
[K$^+$]	—	—	—	—	0.345	28
[Mg^{2+}]	−0.49	4.51	−0.079	0.028	0.008	28
Hematocrit	—	—	—	—	0.160	29
Na$^+$,K$^+$-ATPase	−0.55	42.1	−0.687	0.190	0.002	29
Thyroxine	—	—	—	—	0.776	24

Hematocrit after 20 days in seawater ranged between 42% and 58% (values typical for freshwater), and was not significantly correlated with length ($P > 0.40$).

Gill Na$^+$,K$^+$-ATPase. Mean value of freshwater gill Na$^+$,K$^+$-ATPase activity in brook trout was $7.9 \pm 0.13 \mu MP_i \cdot mg$ prot.$^{-1} \cdot hr^{-1}$ ($N = 687$). After adaptation at intermediate salinities and 4 days in seawater, gill Na$^+$,K$^+$-ATPase activities were higher than freshwater levels, and higher still after 20 days in seawater (Fig. 5(C)). Gill Na$^+$,K$^+$-ATPase activity after 4 days in seawater did not significantly correlate with size ($P = 0.10$, Table 2). Gill Na$^+$,K$^+$-ATPase activity after 20 days in seawater decreased with increasing length of brook trout ($r^2 = 0.30$, Fig. 5(C), Table 3). Plasma osmolarity after 20 d in seawater is decreasing at a similar rate (Fig. 5). Gill Na$^+$,K$^+$-ATPase activity and plasma osmolarity after 20 days in seawater, however, are not significantly correlated ($P > 0.05$). Declining gill Na$^+$,K$^+$-ATPase activity with length may reflect decreased demand for active transport in larger animals due to a more favorable surface-area-to-volume ratio. (An alternative explanation is that surface-area-to-volume ratios of the gills themselves may be smaller in larger fish; since gill Na$^+$,K$^+$-ATPase is surface area dependent and homogenate protein content is volume dependent, a smaller gill Na$^+$,K$^+$-ATPase activity would be measured in larger fish). Gill Na$^+$,K$^+$-ATPase activity may in fact be more responsive to changes in internal osmotic conditions than external ones. Savage and Robinson (1983) have shown gill Na$^+$,K$^+$-ATPase activity of blue crab (*Callinectes sapidus*) to be responsive to a hemolymph factor induced by changes in external salinity. The mechanism by which teleost gill Na$^+$,K$^+$-ATPase activities are regulated during seawater adaptation remains largely unexplored.

Plasma Thyroxine. Plasma T$_4$ levels after 4 and 20 days exposure to seawater increased with increasing size of brook trout (Table 2 and 3). Our inability to find a distinct pattern of plasma thyroxine changes during hypoosmotic adjustment makes it difficult to interpret these changes. Initial freshwater T$_4$ values were changing with growth rate and season (McCormick and Naiman, 1984a), which would tend to obscure size related changes that occur after seawater exposure. To determine the net effect of seawater exposure on T$_4$ levels, the mean value of thyroxine of six to ten brook trout in freshwater, sampled 1 day prior to sampling of fish after 4 days in 32 ppt, was used as an initial value. Net changes in T$_4$ were calculated by subtracting mean plasma T$_4$ levels at 4 and 20 days in 32 ppt from this initial value. The mean net change in T$_4$ after 4 days in seawater was -0.71 ± 0.22 ($N = 37$ experiments). Although this value is significantly different from zero ($P < 0.01$, student's t-test), in 13 out of 37 seawater exposures the mean thyroxine level increased from freshwater levels. Mean net change in thyroxine levels after 4 days in 32 ppt were not significantly correlated with length ($P = 0.08$).

Plasma thyroxine levels after 20 days in seawater were not significantly correlated with length (Table 3). Mean net change in thyroxine after 20 days in 32 ppt was -1.43 ± 0.25 ($N = 26$), and was lower than freshwater levels in 24 out of 26 cases. These results are in apparent contrast to our experiment ($N = 1$) on the time course of seawater adaptation (Fig. 1) in which levels of plasma thyroxine after 20 days in seawater were the same as initial freshwater levels. Net change in plasma thyroxine after 20 days in 32 ppt did not significantly correlate with length ($P > 0.10$, $N = 24$).

Thyroxine levels in salmonids are thought to affect growth by interacting with direct stimulators such as growth hormone (Donaldson *et al.*, 1979). We have shown that a growth related function can explain much of the variation in freshwater plasma thyroxine (McCormick and Naiman, 1984a). Declines in plasma thyroxine following seawater exposure may be related to changing growth; feeding stops for at least a week after exposure to 32 ppt, and growth over the period of seawater exposure is less than freshwater control (McCormick and Naiman, unpublished data).

PERSPECTIVES ON SALMONID OSMOREGULATION

We have found that increased size of brook trout results in greater osmoregulatory ability and survival after exposure to seawater. This is a common feature of salmonid osmoregulation (Parry, 1958; Conte and Wagner, 1965; Wagner *et al.*, 1969). The size at which high survival in seawater is attained (under similar exposure conditions) is species dependent, and is near the size at which migration into seawater occurs (Table 4). The size at which high seawater survival occurs, grouped by genera, falls in the following order:

Oncorhynchus < Salmo < Salvelinus.

Within genera there may exist subgroups with significantly different sizes at which high seawater survival is attained. For instance, pink and chum salmon (*O. gorbuscha* and *O. keta*) survive in seawater at smaller sizes than other species of *Oncorhynchus* (Table 4; Weisbart, 1968).

There is a large body of evidence indicating that size dependent survival of a salmonid species, following exposure to seawater, is related to size dependent ion transport capabilities (Conte and Wagner, 1965; Conte *et al.*, 1966; Wagner *et al.*, 1969; Farmer *et al.*, 1978; present study), and that a critical level of [Na$^+$], [Cl$^-$] or total plasma osmolarity determines survival (Gordon, 1959; Jackson, 1981). It follows that generic or species differences in seawater survival also relate to differences in ion transport capability (Parry, 1958; Weisbart, 1968; Clarke *et al.*, 1978). This concept is shown schematically in Fig. 6, where line A may represent size dependent osmoregulation in a species of *Salvelinus*, line B *Salmo*, and line C *Oncorhynchus*; sizes X_A, X_B and X_C correspond to a size (or size range) at which high seawater survival occurs for each species. Support for the relative shape of these lines (similar intercepts and decreasing slopes) is scarce. Farmer *et al.* (1978) found that plasma osmolarity of Atlantic salmon (6–15 cm) after exposure to ~ 32 ppt seawater was a decreasing function of size. Comparison of their results with those reported here for brook trout indicates that small Atlantic salmon and brook trout (6 cm) had similar

Table 4. Phylogenic comparison of size-dependent salinity tolerance and seaward migration in salmonids

	Salinity tolerance					
Species	Salinity (ppt)	Method of acclimation	Duration (days)	Size at 75% survival (cm)	Size at seaward migration (cm)	Sources
Oncorhynchus						
Pink Salmon	32	Direct	>14	<6.0	2.5–4.0	1.2
Chum Salmon	32	Direct	>14	<6.0	3.2–7.0	1.2
Chinook Salmon	30	Direct	30	6.5	6.0–10.0	3.4
	30	Gradual	30	4.2		
Coho Salmon	30	Direct	30	7.0	10.0–11.0	5.4
Salmon						
Atlantic Salmon	29	Direct	44	13.8	12.7–15.2	6.2
Steelhead Trout	30	Direct	30	16.0	15.0–20.0	7.8
Salvelinus						
Brook Trout	32	Gradual	20	19.0	15.0–18.0	9.10

Sources: (1) Weisbart, 1968; (2) Scott and Crossman, 1973; (3) Wagner *et al.*, 1969; (4) Healy, 1980; (5) Conte *et al.*, 1966; (6) Johnson and Saunders, 1981; (7) Conte and Wagner, 1965; (8) Wagner *et al.*, 1963; (9) present study; (10) Castonguay *et al.*, 1982.

plasma osmolarity after exposure to seawater, but that osmoregulatory ability of Atlantic salmon increased more rapidly with size.

Although the ability to regulate plasma ions accounts for some differences in seawater survival, the ability to tolerate higher plasma ion levels may also affect survival (Fig. 6, horizontal survival/mortality line). Weisbart (1968) has shown that increased survival of chinook salmon alevins, relative to coho and sockeye salmon alevins, is due to increased tissue tolerance of high plasma ionic and/or osmotic concentrations. A comparison of tissue tolerances among salmonid genera has yet to be made.

The existence of size dependent hypo-osmoregulatory ability among salmonids should not imply that there is a single "critical size" resulting in salinity tolerance for a given species. Although a critical plasma ion level may exist, the attainment of this level after seawater exposure will depend on salinity, method of acclimation, temperature and other environmental variables affecting hypo-osmoregulation.

The phylogenetic comparison of salmonid osmoregulatory physiology made here is in substantial
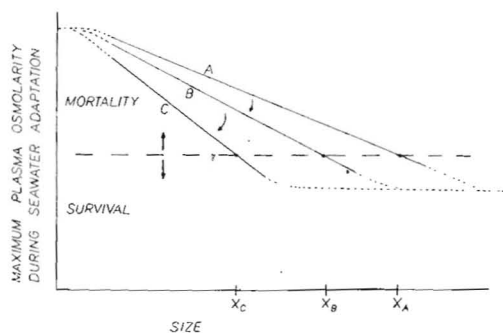

Fig. 6. Salmonid phylogeny of size dependent ion transport ability and resulting size dependent seawater survival. Lines A, B and C may represent *Salvelinus*, *Salmo*, and *Oncorhynchus*, respectively, and X_A, X_B and X_C the size (or size range) at which seawater survival occurs. The horizontal dashed line represents tissue tolerance of high plasma osmolarity. Tissue tolerance may also differ among genera and species, and will affect the size at which seawater survival occurs.

agreement with that proposed by Rousnefell (1958) and Hoar (1976). This phylogeny implies that increased exploitation of the sea by more advanced salmonids was achieved, in part, through changes in size dependent osmoregulatory ability. Physiological changes due to smoltification, desmoltification, maturation and regressions in osmoregulatory ability will alter the shape of size-survival curves. These processes, however, are themselves size dependent and do not substantially alter the underlying size dependent survival nor the resultant phylogenetic relationships.

SUMMARY

We have demonstrated that the regulation of plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$ during seawater adaptation is size dependent throughout the size range of animals tested. Seawater survival is also strongly tied to size, and is not dependent on age. These results are most easily explained by hypothesizing a constant set of permeability barriers and transport capabilities which act more effectively to reduce influx of plasma ions as surface-area-to-volume ratios decrease with increasing size. Though intuitively appealing, there has been little experimental work on the effect of surface-area-to-volume ratios, *per se*, on seawater adaptation in teleosts.

Size dependent salinity tolerance in brook trout indicates that accelerated growth will allow earlier seawater adaptation, an important economic consideration in potential sea ranching and sea farming. Brook trout maturation, however, is also size dependent (McCormick and Naiman, 1984c) and in males has a negative effect on seawater survival. Significant mortality occurred even in the largest experimental groups (>32 cm). Variability in the effect of size on both salinity tolerance and maturation allows opportunities for artificial selection. In light of the high growth rates and high return rates of this migratory salmonid, such an investment in artificial selection may prove worthwhile.

The opportunistic nature of seaward migration in brook trout and their less advanced status in salmonid phylogeny (Rousnefell, 1958; Hoar, 1976) allows consideration of this species as a "primitive

archetype" of early salmonid migrators. As such, the demonstration of size related salinity tolerance and osmoregulatory ability in salt water establishes size as a basic physiological constraint to euryhalinity in this group. More advanced salmonids also display size dependent hypoosmoregulatory ability; the size at which salinity tolerance of a species is achieved is related to their degree of anadromy. The evolutionary pattern of euryhalinity in salmonids can therefore be viewed as a succession of adaptations made to overcome size dependent ion transport capabilities.

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