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Hypoosmoregulation in an Anadromous Teleost: Influence of Sex and Maturation

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ABSTRACT Salinity tolerance and hypoosmoregulatory ability of anadromous brook trout (*Salvelinus fontinalis*) were investigated in relation to sex and state of maturation. Seawater survival of mature males was significantly poorer than that of females or immature males. Lowered salinity tolerance of adult males became acute during the autumn photoperiod (normal spawning period) when the gonadosomatic index was high. Plasma $[Cl^-]$, $[Mg^{2+}]$, osmolarity and hematocrit were significantly higher in mature males after transfer to seawater, relative to mature females. It is postulated that reduced adult male hypoosmoregulatory ability explains skewed sex ratios in anadromous brook trout populations and may limit the extent of brook trout anadromy.

Physiological differences between sexes of the same species are generally related to primary and secondary sexual characteristics (Naftolin, '81) or to adaptations resulting from differential strategies for reproductive success (Darwin, 1871). Reports of sexual differences in nonsexual physiological processes are rare in lower vertebrates. Such differences occur in mammals and include breathing pattern (White et al., '83), cardiac response (Sebert, '83), water metabolism (Kobayashi and Kawashima, '82), and metabolic rate (Kibler et al., '47). Although these differences are of physiological interest, it is unclear whether they affect the survival or reproductive potential of animals and, therefore, whether they are of ecological significance.

Ontogenetic changes in osmoregulatory ability will determine the extent and life history pattern of diadromy. We have previously investigated size and age-related changes in hypoosmoregulatory ability of anadromous brook trout, *Salvelinus fontinalis* (McCormick and Naiman, '84a,b). The following experiments were designed to examine sexual differences in seawater survival and hypoosmoregulatory ability of brook trout in relation to photoperiod and state of maturation and to examine potential mechanisms underlying these differences. The results indicate that sexual differences in brook trout osmoregulation are physiologically and ecologically important.

MATERIALS AND METHODS

Twenty thousand brook trout eggs, the progeny of several males and females, were divided into two groups with different photoperiod regimes. Both photoperiods cycled annually with daylengths corresponding to 42° N; one photoperiod regime corresponded to normal calendar daylength while the second was 3 months delayed from the norm. After hatching, fish were held in 1,000 l flow-through tanks and were fed commercial trout feed ad libitum. Fresh water rearing and seawater exposures were performed at 10–11°C. Prior to seawater exposure brook trout were anesthetized, weighed to the nearest 0.1 gm and fork length measured to the nearest 0.1 cm. Condition factor was calculated as $(\text{weight}/\text{length}^3) \cdot 100$. Fifteen to twenty fish were killed monthly and gonad weight measured to the nearest 0.1 gm. Gonadosomatic index was calculated as (gonad wt/body wt) · 100.

Three seawater exposure experiments (a, b, c) were conducted during summer, autumn, and spring photoperiods, respectively. Experiments a and b were conducted on the same calendar date (November 1) but under normal and 3-month delayed photoperiod conditions, respectively. Experiment c was conducted 5 months later (spring) under nor-

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mal photoperiod conditions. Brook trout were exposed to seawater in rearing tanks by addition of 32 ppt seawater, preheated or precooled to 10.5°C (± 0.5°C). Salinity was increased in a stepwise manner for a period of 14–30 days. Fish in each experiment were held in 32 ppt seawater for a period of 64 days, which represented the maximum seawater survival time. Survival of fish was monitored twice daily. Sex and state of maturation were determined for all mortalities and survivors at the end of 64 days.

A fourth experiment (d) was performed to determine the physiological basis of differences in salinity tolerance. This experiment was conducted during a declining photoperiod (11.2 hr daylength), just prior to spawning. Mature male and female brook trout were acclimated for 1 week at a salinity of 10 ppt, 1 week at 20 ppt, and finally exposed to 32 ppt seawater. After 4 days in 32 ppt seawater, seawater-exposed and freshwater-control fish were removed from tanks and anesthetized for 30–60 sec in phenoxyethanol-water solution. Fish were then removed from water and blotted dry with a chamois cloth. The caudal peduncle was severed and blood was collected from the dorsal aorta into ammonia heparinized capillary tubes which were centrifuged for 5 min at 5,500 rpm. Brook trout not used for blood sampling were maintained in seawater for 20 days during which survival was monitored twice daily.

Plasma osmolarity and $[Cl^-]$ were measured immediately with a Wescor Vapor Pressure Osmometer and Buchler-Cotlove chloridometer, respectively. Plasma cations were measured within 24 hr using a Perkin-Elmer 403 atomic absorption spectrophotometer set on flame emission mode ($[Na^+]$ and $[K^+]$) or atomic absorption mode ($[Mg^{2+}]$). Intraassay coefficients of variation, including dilutions, were 0.8%, 0.6%, 2.0%, 1.5%, and 1.0% (N = 5) for osmolarity, $[Cl^-]$, $[Na^+]$, $[K^+]$, and $[Mg^{2+}]$, respectively. Interassay coefficients of variation for $[Na^+]$, $[K^+]$, and $[Mg^{2+}]$ were 2.0% (N = 8), 3.8% (N = 7), and 2.8% (N = 9), respectively.

Immediately after blood withdrawal, primary filaments (0.05–0.2 gm wet weight) were trimmed from ceratobranchials and stored in 1 ml sucrose-EDTA-imidazole solution (0.3 M sucrose, 0.02 M disodium ethylenediamine tetraacetate, and 0.1 M imidazole adjusted to a final pH of 7.1 with HCl) at −17°C. Gill Na^+, K^+-ATPase activity was determined by the method of Zaugg ('82). Determination of protein concentrations in gill homogenates was done according to Lowry et al. ('51) as modified by Miller ('59) using bovine serum albumin as standard. Intra- and interassay coefficients of variation were 7% (N = 6) and 21% (N = 4, with five replicates each), respectively.

RESULTS

No significant difference in fork length, weight, or condition factor by sex or state of maturity occurred within experiments a, b, or c ($P > 0.10$, ANOVA). Brook trout in experiments a and b were not significantly different in length, weight, and condition factor (18.9 cm and 18.4 cm, 80.4 gm and 70.3 gm, 1.18 and 1.12, respectively, Student-Newman-Keuls procedure). Though gonadosomatic indexes were low at the time of experiment c, visual inspection of gonads indicated imminent maturation. All brook trout under the same culture conditions as fish used in experiment c became mature in autumn.

Under summer and autumn photoperiods (experiments a and b) mature males had a significantly lower mean survival time in seawater than mature and immature females ($P < 0.01$, Mann-Whitney U-test, see Fig. 1a,b). Survival of mature females after 20 days in seawater in experiment d (also conducted in autumn photoperiod) was significantly greater than that of mature males (17-day and 9-day mean survival times, respectively, N = 11, $P < 0.05$, Mann-Whitney U-test). Although mean survival times of males and females were not significantly different in spring ($P = 0.09$, Mann-Whitney U-test; Fig. 1c), the proportion of surviving females was significantly greater than that of males (31 and 7% survival, respectively, after 64 days in seawater, $P < 0.01$, 2×2 contingency table). Survival of control fish in fresh water was 100% for both sexes, mature and immature. These results indicate that under spring, summer, and autumn photoperiods, mature males have lower salinity tolerance than females.

Mature male salinity tolerance had a seasonal component ($P = 0.02$, Kruskal-Wallis test), with survival in autumn (experiment b) being significantly lower than that in summer or spring (experiments a and c, $P < 0.01$ and 0.04, respectively, Mann-Whitney U-test). Seawater survival of immature males was greater than mature males in autumn ($P < 0.01$, Mann-Whitney U-test) implicat-

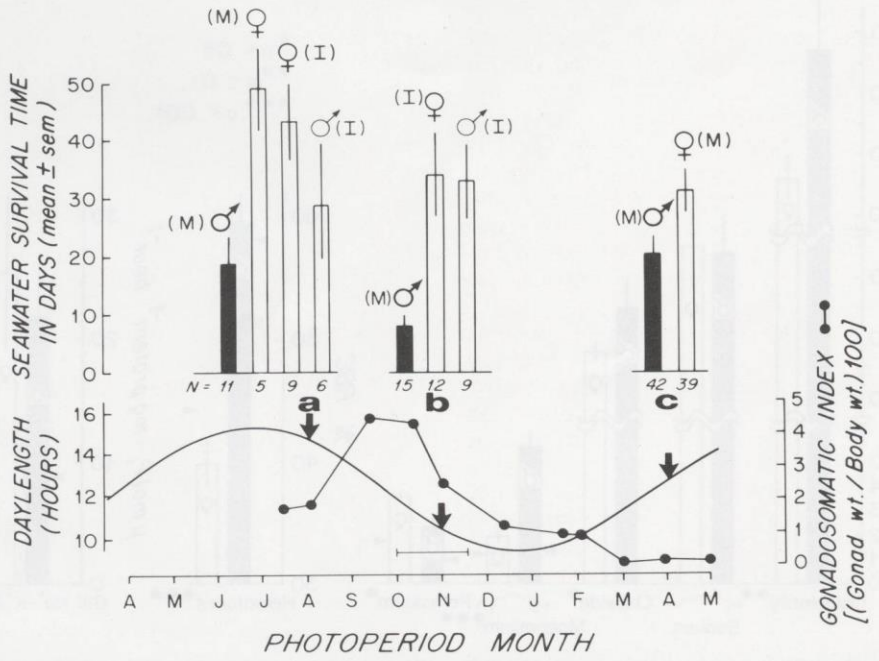

Fig. 1. Upper: Mean seawater survival time of mature (M), and immature (I) male and female brook trout gradually acclimated to 32 ppt seawater. Experiments a and b began on the same calendar date (November 1) but under photoperiod conditions of summer and autumn, respectively. Experiment c began the following spring under normal photoperiod conditions. Lower: Daylength conditions and mean mature male gonadosomatic index (GSI). Photoperiod month (ordinate) is calendar month normally corresponding to the shown daylength. Photoperiod determined timing of maturation under both normal and 3-month delayed photoperiod. Gonadosomatic indexes shown here are mean values of mature males in 3-month delayed photoperiod, corresponding to the shown photoperiod cycle. Final maturation, when sperm could be exuded from males by gently compressing body wall, occurred during October–November photoperiod (11.2 declining to 9.4 hr daylength) in each photoperiod regime and is represented by a horizontal bar. Arrows at daylength curve correspond to photoperiod conditions under which seawater exposure began for experiments a, b, and c.

ing maturation as a factor in male seawater survival. No such decline in female survival time occurred as a result of season ($P = 0.34$, Kruskal-Wallis test) or maturation (summer mean survival time of mature and immature females was 49 days and 43 days, respectively, $P = 0.21$, Mann-Whitney U-test).

Plasma ion and osmotic concentrations after seawater exposure (experiment d) show that hypoosmoregulatory and ionoregulatory abilities differed between mature males and females (Fig. 2; Table 1). Male brook trout had a significantly higher plasma osmotic concentration, higher plasma $[Cl^-]$ and $[Mg^{2+}]$, and a higher hematocrit than mature females of the same size ($P < 0.05$, Student's t-test). Plasma $[K^+]$ was significantly lower in males than females. We found $[K^+]$ to be regulated in a manner different from other plasma ions; freshwater levels of $[K^+]$ were maintained for 2–4 days after exposure to seawater, while plasma $[Na^+]$, $[Cl^-]$, $[Mg^{2+}]$, and osmolarity increased within 12 hr and peaked in 2–4 days (McCormick and Naiman, '84b). With the exception of hematocrit, there were no significant differences in freshwater osmoregulatory physiology of mature males and females in autumn ($P > 0.10$, Student's t-test; freshwater hematocrit values during autumn were 58% for males and 44% for females, $P < 0.01$, Student's t-test).

TABLE 1. Mean (± standard error of the mean) length, weight, and gonadosomatic index (GSI) of brook trout in experiment d[1]

	N	Fork length (cm)	Weight (gm)	GSI
Male	4	21.4 (0.4)	109.2 (10.4)	2.8 (0.2)
Female	4	21.2 (0.3)	105.3 (8.5)	9.0 (2.4)

[1]See Figure 2.

Despite the superior osmoregulatory ability of mature female brook trout, activity of

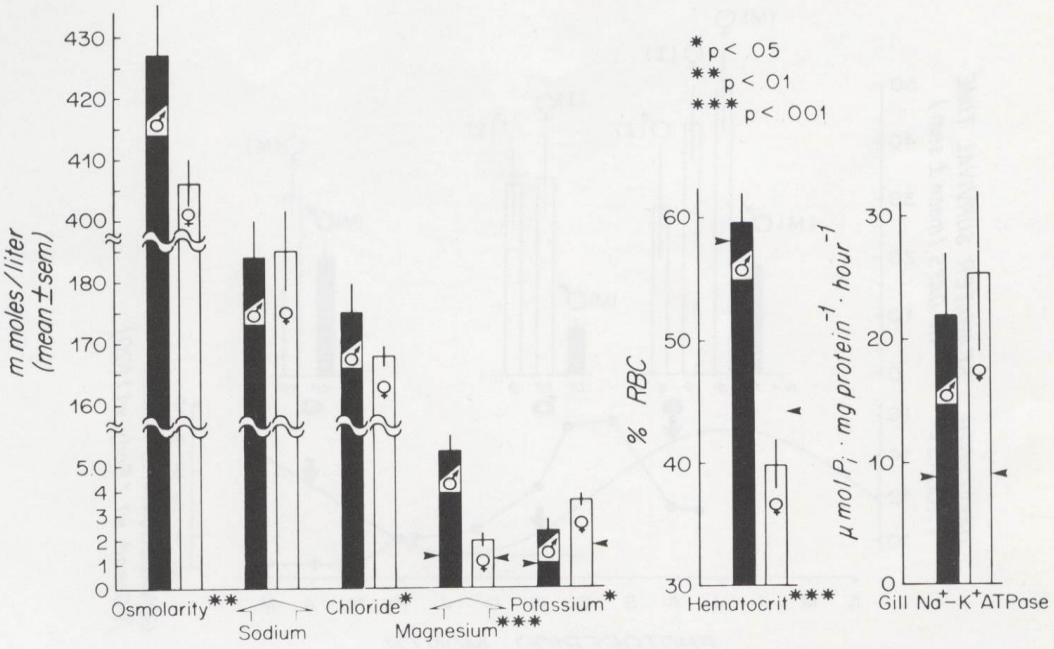

Fig. 2. Physiological comparison of mature male and female brook trout 4 days after exposure to 32 ppt seawater. Fish were first acclimated for 7 days in 10 ppt and 7 days in 20 ppt. Student's t-test was used to determine statistically significant differences between means (N = 4). Values for male and female freshwater controls are depicted by arrows at the side of each histogram. Plasma osmolarity, $[Na^+]$, and $[Cl^-]$ of freshwater controls are not shown because they are below their respective scales as drawn here.

gill Na^+, K^+-ATPase in seawater was not significantly higher in females (Fig. 2). Freshwater values ranged between 5 and 12 $\mu molP_i \cdot mg\ protein^{-1}hr^{-1}$ and did not differ by sex during autumn ($P = 0.49$, Student's t-test). Elevated activity of gill Na^+, K^+-ATPase in mature males indicates that this seawater adaptation mechanism is operative; other ion transport or permeability properties must be responsible for the observed differences between mature males and females.

DISCUSSION

These results establish two separate but related phenomena: 1) salinity tolerance of adult male brook trout is lower than adult females during spring, summer, and autumn; and 2) adult male salinity tolerance and hypoosmoregulatory abilities reach their lowest levels in autumn and are related to sexual maturation. The sexual differences in salinity tolerance found in spring, summer, and autumn may be caused by competing physiological demands specific for mature males or by a direct link between hormonal control of maturation in males and hormonal control of ion and water transport. Competing physiological demands for ion transport occur in teleosts; for example, increased oxygen transport and acid-base regulation are made at the expense of ionic homeostasis (Randall et al., '72; Booth et al., '82).

Salinity tolerance of mature males reached its lowest point in autumn when gonadosomatic index was high. Exudation of sperm by gentle abdominal pressure during this period (Fig. 1) indicates that hydration of semen (spermiation) had occurred (Billard et al., '82). Hydration of sperm may present a larger water demand for males that cannot be met under saline conditions. Clemens and Grant ('65) found that gonadotropin induces spermiation in carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*). By implication, autumnal decreases in hypoosmoregulatory ability of male brook trout may be under direct control of gonadotropins.

Spermiation may also be related to the high hematocrit and low plasma water of freshwater mature males in autumn (Fig. 2). High hematocrit has been found in mature males for a variety of teleosts (Sano, '60; Steuke and Atherton, '65; Mulcahy, '70). Hematocrit did not change significantly, however, in

either males or females after 4 days of exposure to seawater. A similar result was found by Bath and Eddy ('79) for rainbow trout. Nonetheless, low plasma water volume already present in freshwater may be detrimental to normal processes of hypoosmoregulation.

Gill Na^+, K^+-ATPase activities in freshwater and seawater were not significantly different between males and females and cannot account for male-female differences in hypoosmoregulatory ability. Other mechanisms of active transport (e.g., $Cl^- - HCO_3^-$ exchange) or resistance to passive influx may differ between the sexes. Poor regulation of Mg^{2+} by mature males is probably due to differences in renal capabilities since the kidney is the only means by which teleosts can regulate this divalent action under hypersaline conditions (Smith, '30).

Hormonal control of teleost osmoregulation involves differentiation (e.g., of chloride cells) and activation of ion transport capacities (see review by Foskett et al., '83). Little is known, however, regarding the direct or indirect effects of gonadotropins and sex steroids on osmoregulatory processes. Oral administration of 17α-methyltestosterone to coho salmon (*Oncorhynchus kisutch*), while increasing growth, had a negative effect on salinity tolerance (Fagerlund and McBride, '75). Osmoregulatory changes that must occur during spawning migrations of anadromous fishes indicate a possible role of sex hormones in regulating such changes.

Seaward migration of anadromous brook trout in northern latitudes of North America occurs in late spring (Power, '80). Reduced salinity tolerance of adult males, which we have demonstrated to occur under both increasing and decreasing photoperiods, should affect this migratory pattern. Of the five well-studied populations of anadromous brook trout, four have sex ratios skewed toward a greater number of females (White, '40; Wilder, '52; Castonguay et al., '82; W.L., Montgomery, unpublished observations). Low coastal salinity may explain the equal sex ratio observed in one anadromous population (Dutil and Power, '80). Skewed sex ratios do not occur in nearby freshwater populations of brook trout (Castonguay et al., '82). That female-dominated sex ratios occur on the spawning grounds, as well as among seaward migrators and freshwater returning migrators, suggests that male–female behavioral and physiological differences occur in nature.

Further evidence for the ecological impact of sexual differences in salinity tolerance can be derived from experimental sea ranching of brook trout (Whoriskey et al., '81). Upstream, nonmigratory brook trout from the Matamek River, Québec, were transferred into the freshwater portion of the Matamek River estuary. In experiments duplicated in consecutive years, brook trout that returned from seaward migration had greater female:male sex ratios than brook trout that remained in freshwater.

Physiological tolerances of critical life-history stages limit population sizes and spatial distribution of species (Braum, '78). Critical life-history stages are often the larval and reproductive stages of development. Our results indicate that sexual differences in adult physiological tolerances may limit exploitation of habitats by a species and may be a more widespread phenomenon than currently thought.

Finally, Hoar ('76) established a "primitive" level for *Salvelinus* relative to other salmonids. Sex and maturational differences in hypoosmoregulatory ability of brook trout may represent a characteristic of early salmonids that limited the length of the migratory period and the number of possible migrations over an animal's lifetime. Perhaps the complex and structured life histories of specialized seaward-migrating species is, in part, a solution to conflicting physiological demands of maturation and hypoosmoregulation.

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