AN IMPROVED LORAN-C DRIFTING BUOY AND DROGUE FOR COASTAL APPLICATIONS

by

William Joseph Burke

Submitted on May 13, 1983 in partial fulfillment of the requirements for the degrees of Civil Engineer from the Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution and Master of Science in Civil Engineering from the Massachusetts Institute of Technology.

ABSTRACT

The engineering development of a second generation Loran-C drifting buoy system is presented. This system is designed for use in the coastal ocean environment and has the capability to take Loran-C position fixes hourly and to transmit these fixes back to a shore station on a daily basis.

A custom surface buoy was designed for use with the system and was found to have favorable hydrodynamic drag and stability characteristics while providing a secure, watertight and protected platform for the system electronics and power supply.

An improved drogue was also developed and tested on both a scale model and full scale basis. It was found to have a drag coefficient on the order of 2.15 ± .14, to be free from error-inducing behavior such as kiting or towing at an angle to the current, and to have minimal ballast requirements.

The system was field tested and a sample experiment performed, from which it was determined that the system is relatively simple to deploy and recover and has a somewhat uncertain positioning error of approximately 100 feet.

A hydrodynamic analysis of a drifting buoy in wind, waves and a current was performed, indicating that surface waves do have an important effect on drifting buoy performance.

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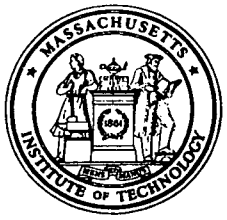

JOINT PROGRAM IN OCEANOGRAPHY AND OCEAN ENGINEERING

Doctoral Dissertation

Effects of Size, Age and Photoperiod on Hypoosmoregulation in Brook Trout, Salvelinus fontinalis

by

Stephen D. McCormick

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DOCTORAL DISSERTATION

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EFFECTS OF SIZE, AGE AND PHOTOPERIOD ON HYPOOSMOREGULATION IN BROOK TROUT, *Salvelinus fontinalis*

by

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B.S., Bates College
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SUBMITTED IN PARTIAL FULFILLMENT
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DEDICATION

to my mother and father

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EXPERIMENTAL DATA: A copy of raw data (computer print-out) is available from Stephen D. McCormick or the Education Office, Woods Hole Oceanographic Institution.

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EFFECTS OF SIZE, AGE AND PHOTOPERIOD ON HYPOOSMOREGULATION IN BROOK TROUT, *Salvelinus fontinalis*

by

Stephen Daniel McCormick

Submitted to the Woods Hole Oceanographic Institution-Massachusetts Institute of Technology Joint Program in Biological Oceanography on August 24, 1983, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

Brook trout (*Salvelinus fontinalis*) raised from eggs under two photoperiod and two feeding regimes were tested for physiological changes preparatory for transition from freshwater to seawater. Size, age, growth rate, photoperiod, and diel rhythms were examined for possible influences on plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, thyroxine concentration, hematocrit, and gill Na^+,K^+-ATPase activity of brook trout in freshwater. Significant diel cycles were found in plasma osmolarity, $[Na^+]$ and thyroxine concentration. Significant size and/or age related changes occurred for plasma osmolarity, $[Na^+]$, $[K^+]$ and hematocrit, but could explain little of their total variation ($0.02 < r^2 < 0.18$). A sexually dimorphic response to photoperiod was observed in hematocrit for both mature and immature fish, with hematocrit of mature females declining in autumn and hematocrit of immature males increasing in autumn. Gill Na^+,K^+-ATPase activity did not respond to photoperiod or feeding treatment and showed no change with size or age. Plasma thyroxine levels responded to feeding and photoperiod treatment. There was a significant correlation between the percent mean difference in plasma thyroxine and the mean difference in growth rate between high and low feed fish ($r^2 = 0.51$), suggesting a relationship between thyroxine and growth.

In 11 experiments over 1.5 yrs, brook trout were gradually exposed to 32 ppt seawater for 20 d to investigate the ontogeny of salinity tolerance. A single experiment examined daily changes in plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, thyroxine, hematocrit and gill Na^+,K^+-ATPase during adaptation to 10 ppt, 20 ppt and 32 ppt. 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. Photoperiod affected seawater survival only through its influence on the timing of male maturation, which decreased salinity tolerance.

Hypoosmoregulation of plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$ and hematocrit increased linearly with size over the entire range of sizes (6-32 cm). Gill Na^+,K^+-ATPase activity after 20 d in seawater decreased with increasing size of brook trout, possibly reflecting decreased demand for active ion transport in larger fish. Plasma thyroxine concentrations generally declined in seawater, but no definitive role of this hormone in seawater adaptation was found. Size dependent survival and osmoregulatory ability of brook trout is compared to other salmonids and a conceptual model is developed.

Decreased salinity tolerance and hypoosmoregulatory ability was found in mature male brook trout and was not found in females or immature males. Lowered salinity tolerance of adult males becomes acute during autumn photoperiod when normal spawning occurs. Plasma $[Cl^-]$, $[Mg^{2+}]$, osmolarity and hematocrit are 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 populations, limits the extent of anadromy, and was a significant phase in the evolution of extended salmonid migration.

Anadromous brook trout of Riviere a la Truite, Quebec, were examined for physiological changes associated with salmonid smoltification, and compared to non-anadromous brook trout of the Matamek River. There were no significant differences in plasma thyroxine concentration, gill Na^+,K^+-ATPase activity, hematocrit or osmoregulatory ability of anadromous and non-anadromous brook trout. Moisture content was significantly different between fish from the two river systems, but had the same pattern of declining moisture content as summer progressed. Silver coloration of brook trout in Riviere a la Truite was significantly associated with larger fish and higher gill Na^+,K^+-ATPase activity, but not with changes in plasma thyroxine, moisture content, hematocrit or condition factor. Silver coloration was absent in Matamek River brook trout. Brook trout at high salinity estuarine sites had significantly greater gill Na^+,K^+-ATPase activity and hypoosmoregulatory ability than brook trout at low salinity sites. Atlantic salmon (Salmo salar) in high salinity estuarine sites had significantly higher plasma thyroxine and gill Na^+,K^+-ATPase activity than brook trout. The results indicate that smoltification is relatively undeveloped in brook trout and that estuarine residence is important in salinity adaptation and eventual seaward migration.

Thesis Supervisor: Robert J. Naiman, Associate Scientist

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INTRODUCTION

Most multicellular animals maintain a degree of separation from the external environment through the possession of extracellular fluid (blood and lymph). For some animals the blood offers a medium in which to maintain homeostasis under changing environmental conditions, a means by which to regulate 'le milieu interior'. In the words of Potts and Parry (1958, pg. 2): "In more complex animals the tissues are no longer in direct contact with the ambient medium, but are bathed in blood or some other extracellular fluid. The fluids form a private pond supplying oxygen and food to the cells and receiving their waste products. The volume of extracellular fluid is usually smaller than that of the cells it surrounds, and in these crowded conditions many complex systems have developed to regulate the composition of the fluids. Of these regualtory processes, respiration supplies oxygen and carbon dioxide, digestion maintains the level of nutrients, and osmoregulation controls the volume and composition of the body fluids." The inability to control "the volume and composition of the body fluids" under changing conditions will limit the distribution of many groups and species of animals, while others that possess this ability are free to exploit many different habitats.

Most marine multicellular invertebrates maintain their extracellular fluids at concentrations that are the same as the external medium. These animals are known as osmoconformers. Under conditions of high or increasing salinity, low intracellular ion concentrations and cell volumes are maintained by mobilizing organic osmolytes which are usually free amino acids (or their derivatives),

polyhydric alcohols or urea and methylamines (Yancey et al., 1982). All freshwater invertebrates are osmoregulators, maintaining their extracellualr fluids at higher osmotic concentrations than freshwater by decreasing passive water loss and actively transporting ions.

Aquatic vertebrates possess a variety of osmoregualtory mechanisms. With the exception of the hagfish (Myxinidae), which are osmoconformers and live only in seawater, all marine aquatic vertebrates maintain plasma salt concentrations at lower levels than exist in seawater. Marine elasmobranchs (sharks and rays) and the coelacanth are generally isosmotic with their seawater environs, maintaining a high total plasma osmolarity through high concentrations of urea and trimethylamine oxide (Potts and Parry, 1958).

Teleosts in both freshwater and seawater maintain their plasma osmolarity within a relatively narrow range, approximately 1/3 that of seawater (300-350 mOsm/l). In seawater, therefore, teleosts hypoosmoregulate by reversing the passive loss of water and actively removing salts from the blood. In contrast, freshwater teleosts hyperosmoregulate, maintaining plasma salt concentrations up to 2 orders of magnitiude higher than ambient. It is evolutionarily interesting that freshwater lamprey (Petromyzonidae), elasmobranchs and teleosts, though phyletically disparate, have arrived at the same solution for maintaining osmotic equilibrium in freshwater.

Most teleosts are stenohaline, living either in freshwater or seawater, but not in both. The development of euryhalinity has allowed some teleosts to exploit habitats and life history strategies not available to stenohaline fish. However, movement from freshwater to seawater presents formidable physiological problems. Freshwater

teleosts actively take up salts and excrete water, while seawater teleosts take up water and excrete salts. Euryhaline teleosts must possess the osmoregulatory mechanisms to do both (Parry, 1966). Limitations on the capability of euryhaline fish to reverse ion and water transport will limit their ability to move between fresh and salt water.

Salmonids display remarkable diversity in their exploitation of freshwater and marine habitats. Pink and chum salmon (Oncorhynchus gorbuscha and O. keta) migrate to sea immediately after hatching and die if kept in freshwater. Other Pacific salmon and Atlantic salmon spend 1-4 yr in freshwater as 'parrs', then undergo a seasonally cued transformation to the seaward migratory 'smolt'. Still other species, such as land-locked salmon and many trout populations, never migrate to sea.

Brook trout, Salvelinus fontinalis, are endemic to eastern North America ranging from 35 to 60 ^{o}N latitude (Scott and Crossman, 1973). Most populations of brook trout are strictly freshwater (Power, 1980). However, anadromous populations exist above 41 ^{o}N in streams and rivers that enter coastal waters. Seaward migration in northern latitudes is characterized by downstream movement in spring, residence in estuarine or coastal waters for 2-4 mo, followed by upstream migration in autumn (White, 1940; Castonguay et al., 1982). Montgomery et al. (unpublished data), studying sea-run brook trout of Riviere a la Truite (a tributary of the lower Moisie River, Quebec), found seaward migration to be highly synchronized among individuals. In the southern portion of their range, the timing and duration of seaward migration is more variable (Mullan, 1958; Smith and Saunders,

1958). In addition to seasonal aspects of migration, size dependent migration has been reported for all sea-run brook trout populations (White, 1940; Wilder, 1952; Smith and Saunders, 1958; Dutil and Power, 1980; Castonguay et al., 1982).

Recent experiments conducted at Woods Hole Oceanographic Institution's Matamek Research Station in Quebec indicate that non-anadromous brook trout, removed from the Matamek River and transplanted to the estuary, migrated into salt water. Brook trout spent 2-3 mo in coastal waters, with 30-60^{o}/o returning to the Matamek to spawn. Growth rate was 4-5 times greater than brook trout that remained in freshwater (Whoriskey et al., 1982). The limited migration and consequential high returns of the brook trout, coupled with high growth rates, make it a potentially important species for sea ranching, farming and enhancement programs.

The experimental program at Matamek demonstrated that younger (and smaller) fish released into the estuary moved immediately back to freshwater where they grew slowly. It appeared that these juveniles preferred freshwater over seawater, indicating that they may not possess the ability to osmoregulate in salt water. It was not known whether the osmoregulatory ability of brook trout in salt water is influenced by size, age, or some combination.

All teleosts regulate the osmotic concentration of their blood which acts as a buffer to most of the body's tissues. The ability to osmoregulate in media of varying salinity, however, exists for only a limited number of teleosts (Gunter, 1956). These euryhaline fish presumably use the same mechanisms for hypoosmoregulation and hyperosmoregulation that are used by marine and freshwater fishes,

respectively. The transition from fresh to salt water will, therefore, result in the following: 1) an increase in the drinking rate which is accompanied by increased influx of ions and water through the intestines, 2) a reversal in the net flux of monovalent ions across the gill epithelium from one of net uptake to net loss, 3) a decrease in glomerular filtration rate, production of an isotonic urine (instead of dilute urine) and excretion of divalent ions by the kidneys (Parry, 1966). Accompanying these functional changes in ion transport are changes in membrane composition, enzyme activities and morphology of gills, kidney, gut and skin (Evans, 1981).

The osmoregulatory mechanisms outlined above act to prevent the passive loss of water and influx of ions during the transition from freshwater to seawater. Inability to prevent high plasma ion concentrations results in mortality (Gordon, 1959; Jackson, 1981). The ability to maintain low plasma ion concentrations during the first few days after seawater exposure has been used to measure hypoosmoregulatory ability and to predict seawater survival (Parry, 1958; Conte and Wagner, 1965; Conte et al., 1966; Clarke et al., 1978; Jackson, 1981).

The fact that "larger" salmonids can adapt to sea water more readily than "smaller" salmonids has been known for some time (Parry, 1958). Size and age are both incorporated in the term "large", yet these parameters have rarely been separated when investigating osmoregulatory ability. Conte and Wagner (1965) and Conte et al. (1966) attempted to distinguish size and age effects on salinity adaptation in juvenile steelhead trout (Salmo gairdneri) and coho salmon (Oncorhynchus kisutch), respectively, and concluded that

chronological age has no effect on salinity adaptation. These studies distinguished only between year classes. It is important to resolve differences between size and age as controlling factors in salinity tolerance, because if size is indeed responsible for its control, accelerated growth will allow faster introduction of brook trout into salt water.

Certain species of the subfamily Salmoninae (which includes the genus Salvelinus) undergo a parr-smolt transformation that precedes or accompanies migration into seawater. The physiology of smoltification has been reviewed recently by several authors (e.g. Hoar, 1976; Folmar and Dickhoff, 1980; Wedemeyer, 1980). For the purposes of this investigation a smolt is defined as a salmonid in freshwater which has undergone all of the morphological and physiological changes which are common to all known smolting salmonid species. Included in the metamorphic aspects of smoltification are deposition of guanine and hypoxanthine in skin and scales (silvering), fin darkening, reduced body lipid and increased moisture content (Wedemeyer et al., 1980). Several physiological changes have been shown to accompany increased hypoosmoregulatory ability of smolts. Among the most prominent of these are increases in gill Na^+,K^+-ATPase activity (an enzyme involved in active ion transport), a surge in plasma thyroxine concentrations, and changes in plasma ion concentrations (Houston and Threadgold, 1963; Zaugg and McLain, 1972; Dickhoff et al., 1978). These changes may also occur in brook trout and may be preparatory to seawater entry.

The seasonal occurrence of many salmonid migrations (including that of brook trout) indicate that environmental cues may affect salt

water tolerance in these species. Photoperiod and photoperiod experience have a profound effect on smoltification and osmoregulatory ability of sockeye (O. nerka) and coho salmon (O. kisutch) (Clarke et al., 1978). However, Ewing et al.(1979) report that although photoperiod has some effect on gill Na^+,K^+-ATPase activity in chinook salmon, endogenous rhythms have an even greater effect.

Exogenous and endogenous rhythms in physiological functions are most often controlled through the actions of hormones. A variety of hormones have been implicated in the control of teleost osmoregulation (Johnson, 1973). Pickford and Phillips (1959) have shown prolactin to be a key factor for freshwater survival in hypophysectomized mummichog, Fundulus heteroclitus, and this hormone seems to be important in most, though not all teleosts (Lam, 1972). Growth hormone facilitates sea water adaptation in salmonids, but this hormone may work by acting on general metabolic pathways rather than acting on specific osmoregulatory organs (Clarke et al., 1977). The proven role of thyroid hormone in amphibian metamorphosis (Regard et al., 1978) has influenced the interpretations of thyroxine surges that precede the parr-smolt transformation in salmonids (Dickhoff et al., 1978). To date, the true role of this hormone in osmoregulatory changes remains unknown. Cortisol may also play an important role in ion regulation by teleosts. Corticosteroids have been shown to affect ion fluxes in isolated teleost intestines (Utida et al., 1972) and to increase the activity of gill Na^+,K^+-ATPase in eels (Butler and Carmichael, 1972). The role of cortisol in salmonid regulation remains unexplored, in part due to the sampling difficulties that

result from the rapid change in circulating levels of this hormone in response to stress (Schreck, 1982).

Most of our knowledge of the ontogeny of salinity tolerance relates to specialized migrating species, particularly Pacific salmon (Oncorhynchus spp.), Atlantic salmon (Salmo salar) and steelhead trout (Salmo gairdneri). 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). Charrs exhibit a lesser degree of anadromy than other salmonids (Hoar, 1976). Morphological evidence clearly indicates that Salmo is intermediate between Oncorhynchus and Salvelinus (Jordan and McGregor, 1925). The freshwater and seawater origins of salmonids has been the subject of some debate (see Thorpe, 1982 for review). Tchernavin (1939) presents compelling evidence for the freshwater origins of salmonids, implying a more 'primitive' origin for charrs. Rounsefell (1959) hypothesized that Salvelinus were most like the earliest anadromous salmonids; if this is true, charrs should possess the basic osmoregulatory physiology upon which greater specializations were made by more advanced salmonids. In addition, the outward signs of smolting (silvering and seaward migration) occur in northern populations of brook trout (White, 1940; Wilder, 1952; Black, 1981; Castonguay et al., 1982). Smoltification may have arisen in the charrs, and it follows that the phylogeny of smoltification is incomplete without an understanding of smolting in this pivotal group.

The present series of experiments were designed to reveal the factors which limit anadromy in natural populations of brook trout,

and which would affect the culture and stocking of brook trout in salt water for natural enhancement, sea ranching and farming. In addition, the physiological constraints of a less specialized anadromous species such as brook trout represent a 'primitive archetype' from which we can interpret specializations made by more advanced salmonids. Specifically, I have investigated the role of size, age and photoperiod in determining the osmoregulatory ability of brook trout.

Brook trout were reared from eggs and maintained under two photoperiod and two feeding regimes to obtain fish of the same age but with different sizes and photoperiod experiences. Chapter 1 examines physiological changes associated with salmonid smoltification. The effects of size, age, growth rate, photoperiod, and diel rhythms on plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$ and thyroxine concentration, hematocrit and gill Na^+,K^+-ATPase activity of brook trout in freshwater were investigated.

In 11 experiments over 1.5 yrs, fish were gradually exposed to 32 ppt seawater for 20 d to investigate the ontogeny of salinity tolerance. Chapter 2 reports the effects of size, age and photoperiod on seawater survival and hypoosmoregulation of plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$. The role of gill Na^+,K^+-ATPase activity and plasma thyroxine in size dependent osmoregulatory ability was determined. In addition, a single experiment examined daily changes in plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$ and thyroxine concentration, hematocrit and gill Na^+,K^+-ATPase activity during adaptation to 10 ppt, 20 ppt and 32 ppt.

Sex and maturation related differences in seawater survival and hypoosmoregulation are reported in Chapter 3. Evidence is presented which indicates these differences affect the population dynamics of anadromous brook trout populations.

Experiments on natural populations of brook trout (Chapter 4) were undertaken to verify and supplement laboratory results. Anadromous and non-anadromous brook trout populations from the North Shore of Quebec were compared for physiological changes associated with smoltification. Freshwater and estuarine brook trout were examined for seasonal and size related changes in silvering, plasma thyroxine concentration, gill Na^+,K^+-ATPase activity, moisture content, hematocrit and hypoosmoregulatory ability. Estuarine Atlantic salmon were also examined for comparative purposes, since this species is known to undergo smoltification.

Each of the chapters addresses a unique question relating to brook trout osmoregulation, and each is a self-contained manuscript. In combination, they give a broad view of the factors which limit brook trout salinity tolerance and of the physiological factors which are involved in hypoosmoregulation. In Chapter 2 (Perspectives on Salmonid Osmoregulation) the results are placed in context of the comparative physiology and evolution of salmonid osmoregulation. The summary recounts the major results of each chapter and briefly discusses their ecological implications, and their application to sea ranching, farming and natural enhancement of brook trout, and discusses the importance of this work to our understanding of the evolution of euryhalinity in salmonids.

REFERENCES

Black, G. A. 1981. Metazoan parasites as indicators of movements of anadromous brook charr (Salvelinus fontinalis) to sea. Can. J. Zool. 59, 1892-1896.

Butler, D. G. and F. J. Carmichael. 1972. Na^+-K^+ ATPase activity in eel (Anguilla rostrata) gills in relation to changes in environmental salinity: role of adrenocortical steroids. Gen. Comp. Endocrinol. 19, 421-427.

Castonguay, M., G. J. Fitzgerald and Y. Cote. 1982. Life history and movements of anadromous brook charr, Salvelinus fontinalis, in the St. Jean River, Gaspe, Quebec. Can. J. Zool. 60, 3084-3091.

Clarke, W. C., S. W. Farmer and K. M. Hartwell. 1977. Effect of teleost pituitary growth hormone on growth of Tilapia mossambica and on growth and sea water adaptation of sockeye salmon (Oncorhynchus kisutch). Gen. Comp. Endocrinol. 33, 174-178.

Clarke, W. C., J. E. Shelborne and J. R. Brett. 1978. Growth and adaptation to sea water in underyearling sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon subjected to regimes of constant or changing day length. Can. J. Zool. 56, 2413-2421.

Conte, F. P. and H. H. Wagner. 1965. Development of osmotic and ionic regulation in juvenile steelhead trout Salmo gairdneri. Comp. Biochem. Physiol. 14, 603-620.

Conte, F. P., H. H. Wagner, J. Fesler and C. Gnose. 1966. Development of osmotic and ionic regulation in juvenile salmon (Oncorhynchus kisutch). Comp. Biochem. Physiol. 18, 1-15.

Dickhoff, W. W., L. C. Folmar and A. Gorbman. 1978. Changes in plasma thyroxine during smoltification of coho salmon (Oncorhynchus kisutch). Gen. Comp. Endocrinol. 36, 229-232.

Dutil, J. D. and G. Power. 1980. Coastal populations of brook trout, Salvelinus fontinalis, in Lac Gillaume-Delisle (Richmond Gulf) Quebec. Can. J. Zool. 58, 1828-1835.

Evans, D. H. 1981. Chapter 6. Fish. In: Compararive Physiology of Osmoregulation in Animals. (edited by Maloiy, G. M. O.). Academic Press, N. Y.

Ewing, R. D., S. L. Johnson, H. J. Pribble and J. A. Lichatowich. 1979. Temperature and photoperiod effects on gill Na^+,K^+-ATPase activity in chinook salmom (Oncorhynchus tshawytscha). J. Fish. Res. Bd. Can. 36, 1347-1353.

Folmar, L. C. and W. W. Dickhoff. 1979. Plasma thyroxine and gill Na^+-K^+ ATPase changes during seawater acclimation of coho salmon (Oncorhynchus kisutch). Comp. Biochem. Physiol. 63A, 329-332.

Gordon, M. S. 1959. Ionic regulation in the brown trout (Salmo trutta L.). J. Exp. Biol. 36, 227-252.

Gunter, G. 1956. A revised list of euryhaline fishes of North and Middle America. Am. Midland Nat. 56, 345-354.

Hoar, W. S. 1976. Smolt transformation: evolution, behavior and physiology. J. Fish. Res. Bd. Can. 33, 1234-1252.

Houston, A. H. and L. T. Threadgold. 1963. Body fluid regulation in smolting salmon. J. Fish. Res. Bd. Can. 33, 1233-1252.

Jackson, A. J. 1981. Osmotic regulation in rainbow trout (Salmo gairdneri) following transfer to sea water. Aquaculture, 24, 143-151.

Johnson, D. W. 1973. Endocrine control of hydromineral balance in teleosts. Am. Zool. 13, 799-818.

Jordan, D. S. and E. A. McGregor. 1925. Family Salmonidae. In : Records of fishes obtained by David Starr Jordan in Japan, 1922 (Jordan, D. S. and C. L. Hubbs). Mem. Carn. Mus. 10, 93-346.

Lam, T. J. 1972. Prolactin and hydromineral regulation in fishes. Gen. Comp. Endocrinol. (Supplement) 3, 328-338.

Mullan, J. W. 1958. The sea-run or "salter" brook trout (Salvelinus fontinalis) fishery of the coastal streams of Cape Cod, Massachusetts. Mass. Div. Fish and Game. Bull. No. 17.

Parry, G. 1958. Size and osmoregulation in salmonid fishes. Nature 181, 1218-1219.

Parry, G. 1966. Osmotic adaptation in fishes. Biol. Rev. 41, 392-444.

Pickford, G. E. and J. G. Phillips. 1959. Prolactin, a factor promoting survival of hypophysectomized killifish in freshwater. Science 130, 454-455.

Potts, W. T. W. and G. Parry. 1964. Osmotic and ionic regulation in animals. Macmillan Company, New York.

Power, G. 1980. The brook charr, Salvelinus fontinalis. In: Charrs (edited by Balon, E. K.), pp. 141-203. Dr. W. Junk bv Publishers, The Hague.

Regard, E., A. Taurog and T. Nakashima. 1978. Plasma thyroxine and triiodothyronine levels in spontaneously metamorphosing Rana catesbiana tadpoles and in adult anuran amphibians. Endocrinol. 102, 674-684.

Rousnefell, G. A. 1958. Anadromy in North American Salmonidae. U.S. Fish Wildl. Serv. Fish. Bull. 58, 171-185.

Schreck, C. B. 1982. Stress and rearing of salmonids. Aquaculture, 28, 241-249.

Scott, W. B. and E. J. Crossman. 1973. Freshwater Fishes of Canada. Fish. Res. Bd. Can. Bull. 184.

Smith, M. W. and J. W. Saunders. 1958. Movements of brook trout, Salvelinus fontinalis (Mitchill) between and within fresh and salt water. J. Fish. Res. Bd. Can. 15, 1403-1449.

Tchernavin, V. 1939. The origin of salmon. Salmon Trout Mag. 95, 1-21.

Thorpe, J. E. 1982. Migration in salmonids, with special reference to juvenile movements in freshwater. In: Proceedings of the Salmon and Trout Migratory Behavior Symposium (edited by Brannan, E. L. and E. O. Salo), pp. 86-97. Univ. of Washington, Seattle.

Utida, S., T. Hirano, H. Oide, M. Ando, D.W. Johnson and H.A. Bern. 1972. Hormonal control of the intestine and urinary bladder in teleost osmoregulation. Gen. Comp. Endocrinol. (Supplement) 3, 317-327.

Wedemeyer, G. A., R. L. Saunders and W. C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42, 1-14.

White, H. C. 1940. Life-history of sea-running brook trout (Salvelinus fontinalis) of Moser river, Nova Scotia. J. Fish. Res. Bd. Can. 5, 176-186.

Whoriskey, F. G., R. J. Naiman and W. L. Montgomery. 1981. Experimental sea ranching of brook trout, Salvelinus fontinalis, Mitchill. J. Fish Biol. 19, 637-651.

Wilder, D. G. 1952. A comparative study of anadromous and freshwater populations of brook trout (Salvelinus fontinalis (Mitchill)). J. Fish. Res. Bd. Can. 9, 169-203.

Yancey, P. H., M. E. Clark, S. C. Hand, R. D. Bowlus and G. N. Somero. 1982. Living with water stress: evolution of osmolyte systems. Science 217, 1214-1222.

Zaugg, W.S. and L.R. McLain. 1972. Changes in adenosine triphosphatase activity associated with parr-smolt transformation in steelhead trout, coho and spring chinook salmon. J. Fish. Res. Bd. Can. 29, 167-171.

CHAPTER 1

Osmoregulation in the brook trout, Salvelinus fontinalis. I. Diel, photoperiod and growth related physiological changes in freshwater.

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ABSTRACT

1. Brook trout (Salvelinus fontinalis) raised from eggs under two photoperiod and two feeding regimes were tested for physiological changes preparatory for transition from freshwater to seawater. Size, age, growth rate, photoperiod, and diel rhythms were examined for possible influences on plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, thyroxine concentration, hematocrit, and gill Na^+,K^+-ATPase activity of brook trout in freshwater.
2. Significant diel cycles were found in plasma osmolarity, $[Na^+]$ and thyroxine concentration.
3. Significant size and/or age related changes occurred for plasma osmolarity, $[Na^+]$, $[K^+]$ and hematocrit, but could explain little of their total variation ($0.02 < r^2 < 0.18$).
4. A sexually dimorphic response to photoperiod was observed in hematocrit for both mature and immature fish, with hematocrit of mature females declining in autumn and hematocrit of immature males increasing in autumn.
5. Gill Na^+,K^+-ATPase activity did not respond to photoperiod or feeding treatment and showed no change with size or age.
6. Plasma thyroxine levels responded to feeding and photoperiod treatment. There was a significant correlation between the percent mean difference in plasma thyroxine and the mean difference in growth rate between high and low feed fish ($r^2 = 0.51$), suggesting a relationship between thyroxine and growth.

INTRODUCTION

Anadromous salmonids are truly euryhaline over only a limited portion of their life cycle. A variety of physiological and morphological changes associated with transformation from freshwater parr to the migratory smolt occur wholly in freshwater (Gorbman et al., 1982) and are presumably adaptive to the fishes existence in seawater. These changes, which include guanine deposition in skin and scale, increased hypoosmoregulatory ability, increased activity of gill Na^+,K^+-ATPase and a surge of plasma thyroxine, are responsive to internal changes such as size and growth (Wedemeyer et al., 1980) and environmental cues such as lunar and seasonal rhythms (Grau et al., 1982). While smoltification occurs in migratory species of Oncorhynchus and Salmo, little is known regarding adaptations to euryhalinity in the charrs, which comprise the salmonid genus Salvelinus. This genus is regarded as a primitive group relative to Salmo and Oncorhynchus and displays a more generalized and restricted pattern of seaward migration (Hoar, 1976).

Although most populations of the brook trout (Salvelinus fontinalis) are restricted to freshwater, many coastal rivers in northeastern North America contain anadromous brook trout. In northern latitudes brook trout migrations are characterized by spring emigrations and coastal sea residence which lasts for 2-4 months (White, 1940; Wilder, 1952; Dutil and Power, 1980; Castonguay et al., 1982). In the southern portion of its range migrations are more variable, often occurring in the fall (Mullan, 1958). Sea ranching experiments resulted in spring emigrations and fall returns in which migrating fish obtained growth rates 4 to 5 greater than those of fish

that remained in fresh water, and returned at rates between 30 $^{o}/_{o}$ and 60 $^{o}/_{o}$ (Whoriskey et al., 1981).

The studies reported here were designed to reveal the factors which limit anadromy in natural populations of brook trout, and which would affect the culture and stocking of brook trout for natural enhancement, sea ranching and farming. In addition, the physiological adaptations of a less specialized anadromous species such as brook trout represent a 'primitive archetype' from which we can interpret specialization made by more advanced salmonids. In conjunction with a study of factors which limit salinity tolerance and hyppoosmoregulatory ability in brook trout (Chapter 2), we have investigated the preparatory physiological adaptations for euryhalinity that are characteristic of smolting salmonids which may occur in the facultatively anadromous brook trout. Specifically, we report here the effect of size, age, growth and photoperiod on gill Na^+,K^+-ATPase and plasma thyroxine levels of brook trout in freshwater. Plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$, and hematocrit were also measured to determine possible ontogenetic changes in hyperosmoregulation which may signal a physiological change relating to preparatory adaptations for hyppoosmoregulation. Diel cycles of blood parameters were examined to verify their existence as reported for other teleosts (Hannah and Pickford, 1981; Eales et al., 1981; Spieler and Noeske, 1979; Osborn et al., 1978), and to analyze their possible effects in determining seasonal cycles.

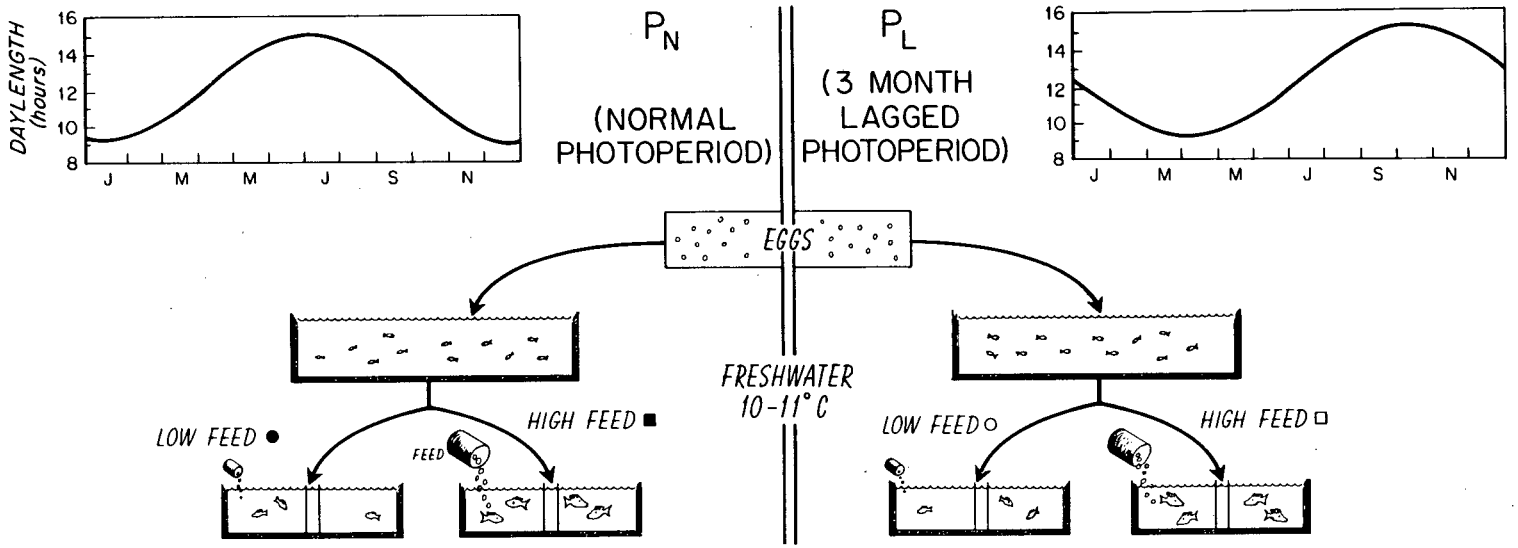
MATERIALS AND METHODS

Experimental Animals

20,000 fertilized brook trout eggs were obtained from the Massachusetts State Fish Hatchery at Sandwich. Although the breeding stock has been exclusively freshwater for the last 30 years (Lloyd Raymond, Hatchery Manager, pers. comm.), studies indicate the strain displays anadromy similar to wild stocks when released into coastal rivers (Mullan, 1958). Fertilized eggs were transported to the Woods Hole Oceanographic Institution's Shore Lab facility and supplied with 10-11 C well water. Eggs were randomly divided into two annually cycling photoperiods corresponding to a latitude of $^{o}42$ N; one photoperiod cycle corresponded to the normal calendar date (longest day June 21, shortest day December 21), while the other was 3 mo delayed from the norm (longest day September 21, shortest day March 21, Fig. 1). Daylength was changed every 5 d. Sunrise and sunset were simulated each day by a 15 min period of gradual illumination or dimming of incandescent bulbs. Beginning and end of daylight period were delayed 2 hr from Eastern Standard Time. Vita-Lite spiralux fluorescent bulbs and incadescent bulbs were used to simulate daylight.

Eggs were hatched in 250 l flow-through hatchery troughs. After first feeding fish were transferred to 1,000 l flow-through tanks which received supplemental aeration. Within one week after feeding fish were divided randomly, within each photoperiod treatment, into two feeding groups. For 4 wk after first feeding, fish in each group were fed equal amounts. Afterward, one group was fed commercial fish pellets ad libitum following common hatchery procedures (Leitritz and Lewis, 1976). The low feed group was fed approximately

Figure 1. Experimental design and fish culture conditions consisting of two photoperiod regimes (one normal, one 3-mo delayed) and two feeding regimens (high feed and low feed).


DAYLENGTH (hours)
16
14
12
10
8
J M M J S N
P_N
(NORMAL PHOTOPERIOD)
P_L
(3 MONTH LAGGED PHOTOPERIOD)
EGGS
FRESHWATER 10-11° C
LOW FEED ●
HIGH FEED ■
FEED
LOW FEED ○
HIGH FEED □

half the amount, per unit body weight, fed the high feed group. Each group was fed 4 to 5 times daily during daylight hours for the first several months after hatching, 1 to 3 times daily thereafter. Some fish, which were used as control fish in a related study of salinity tolerance (Chapter 2), were maintained for 3 to 6 wk in 100 l flow-through tanks prior to sampling. Feeding behavior of fish in these tanks was attentuated for a period of 4 to 5 d after transfer and then returned to normal.

Every 6-8 wk at least 25^{o}/o of the fish from a 1,000 l tank in each feeding group in the normal photoperiod were weighed. Fish were dip-netted, anesthetized, blot-dried on a moist chamois cloth, fork length was measured to the nearest mm and fish were weighed to the nearest 0.01 g.

Specific growth rates (G_w) were calculated using the following formula:

$$G_w' = \frac{\log_e W_t - \log_e W_o}{t} \cdot 100,$$

where W_t is the weight at time t, W_o is the weight at time 0, and t is time in days. In order to compare growth rates of animals of different sizes, the $\log_e G_w$ of a fish of unit size was calculated (Jobling, 1983) using the following equation:

$$a' = \log_e G_w' - b \log_e W_t,$$

where a is the $\log_e G_w$ of a fish of unit size and b is the slope of the linear relationship between $\log_e G_w$ and $\log_e W_t$. An experimentally derived value of b = -0.49 was used in all calculations of a. A generalized value of b = -0.41 for salmonids was reported by Brett (1979).

Blood and Gill Tissue Sampling

Fish were starved overnight prior to blood collection which occurred between one hour after dawn and one hour prior to sunset. Brook trout were removed from tanks, placed in aerated transfer buckets for < 2 min, and transferred immediately to a 0.4 ml/l phenoxyethanol-water solution for 30-60 s. Anesthetized animals were blotted with a damp chamois cloth, fork length was measured and fish were weighed. After severing of the caudal fin, blood from each fish was collected from the dorsal aorta into two heparinized micro-hematocrit tubes, which were then sealed at one end with vinyl plastic putty. Hematocrit tubes were centrifuged for 5 min at 5500 rpm, hematocrits ($^{o}/o$ red blood cells) read, and plasma removed for later analysis (see below). Gill arches were removed immediately after blood collection for gill Na^+,K^+-ATPase activity, and gonads were removed and weighed.

Analytical Techniques

Plasma was withdrawn from hematocrit tubes with a positive displacement Hamilton syringe. Osmolarity and $[Cl^-]$ were measured immediately with a Wescor Vapor Pressure Osmometer and Buchler-Cotlove Chloridometer, respectively. A 5 µl plasma sample was diluted in 495 µl deionized water in a 2.5 ml acid-washed, polyethylene vial and stored in the dark for a maximum of 48 hr. Reference standards were diluted and stored in the same manner. $[Na^+]$ and $[K^+]$ were measured using flame emission spectrophotometry; $[Mg^{2+}]$ was measured using atomic absorption spectrophotometry. An additional dilution of 1:250 was made in duplicate vials for measuring $[Na^+]$.

It was found that Na^+ had a small but detectable interference with K^+ that was constant over the physiological range of plasma $[Na^+]$. To correct for this error, 150 mmole $[Na^+]$/l was added to all $[K^+]$ standards. Intraassay coefficients of variation, including dilutions, were 0.8°/o, 0.6°/o, 2.0°/o, 1.5°/o, and 1.0°/o (N = 5) for osmolarity, $[Cl^-]$, $[Na^+]$, $[K^+]$ and $[Mg^{2+}]$, respectively. Interassay coefficients of variation for $[Na^+]$, $[K^+]$, and $[Mg^{2+}]$ were 2.0°/o (N = 8), 3.8°/o (N = 7) and 2.8°/o (N = 9), respectively.

For thyroxine determination 25 to 40 µl of plasma was removed from hematocrit tubes, placed in a 250 µl polyethylene microcentrifuge vial and stored at -17 C for up to 6 mo. For fish less than 8.5 cm fork length (high feed group in June 1981 and low feed group from June 1 to August 11, 1981), plasma from 2 to 4 fish was pooled. For statistical analysis the value of a pooled sample was treated as that of a single fish (e.g., for N = 5, up to 20 fish were actually used). Samples were thawed and duplicate 10 µl samples withdrawn and analyzed using a competitive binding radioimmunoassay (Dickhoff et al., 1978). Charcoal stripped brook trout plasma was used to make all standards. Sensitivity of the thyroxine radioimmunoassay was approximately 0.25 ng/ml. Intraassay variation was $\pm$ 10°/o (N = 5), interassay variation was $\pm$ 13°/o (N = 3, with 4 replicates each).

Primary gill filaments (0.05-0.2 g wet weight) were trimmed from ceratobranchials and stored in 1 ml Sucrose-EDTA-imidazole (SEI) solution (0.3 mmole/l sucrose, 0.02 mmole/l disodium ethylenediamine tetraacetate and 0.1 mmole/l 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 (1982). Protein determinations were done according to Lowry et al. (1951) as modified by Miller (1959) using bovine serum albumin as standard. Gill Na^+,K^+-ATPase activity is expressed as μM inorganic phosphate per mg protein per hr ($\mu MP_i \cdot$ mg prot.$^{-1} \cdot hr^{-1}$). To determine the reproducibility of the assay and assess the effect of storage, primary filaments from several fish were pooled and then separated into vials containing 1 ml SEI solution. Intra- and interassay coefficients of variation were 7°/o (N = 6) and 21°/o (N = 4, with 5 replicates each), respectively. The mean activities of 5 samples decreased 9.6°/o after 40 d and an additional 13.8°/o after 80 d. Maximum storage time of gill tissue was 50 d.

Statistical Analysis

The experimental design (Fig. 1) is a 2 x 2 matrix, such that feeding treatment, which affects growth and size simultaneously, is tested twice (once in normal photoperiod, once in 3-mo delayed photoperiod), and photoperiod treatment is tested twice (once on high feed fish, once on low feed fish). To analyze our results, four seperate two-way analyses of variance for each physiological variable were used to assess the effect of feeding and photoperiod treatment. Data from each day were entered in columns and two feeding or photoperiod treatments entered as rows. A photoperiod or feeding effect was deemed significant when the group (row) effect was $p < 0.05$. This method avoided the false influence of day-to-day variations in fish response to uncontrolled stimuli and day-to-day variations in analytical techniques.

RESULTS AND DISCUSSION

Size and Growth Rate

Length and weight of fish in high and low feed groups under normal photoperiod conditions are shown in Fig. 2A,B and were similar for high and low feed groups, respectively, in 3-mo delayed photoperiod. Growth rates in each feeding treatment (Fig. 2C) were similar at low body weight (shortly after first feeding), were lower in the low feeding group at intermediate body weight, then became similar at higher body weights. Growth rate per unit size, as a function of time (Fig. 2D), shows similar growth in the two feeding treatments in March, 1981, corresponding to first feeding. Growth rates from April to December 1981 are much lower for the low feeding group, after which growth in the two groups became more similar, though still lower in the low feed group except for the last weighing. Mean condition factor ($[\text{weight} \cdot (\text{length}^3)^{-1}] \cdot 100$) in each group at every time interval was greater than 0.95 (range: 0.96-1.36, low feed group; 1.09-1.41, high feed group)

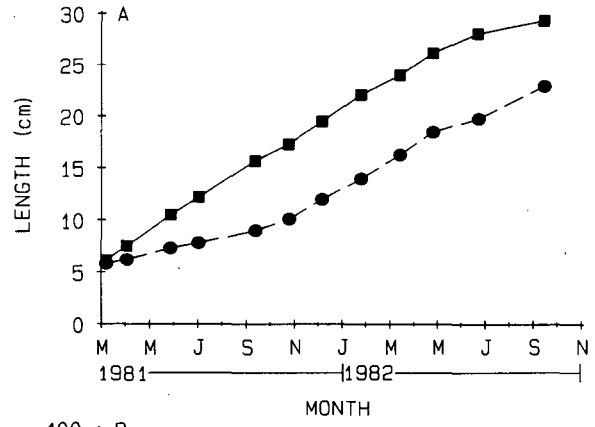
Similarity of growth rates in high and low feed groups in March 1981 reflects the equal feeding rates given the two groups just prior to this period. For an eight month period, smaller ration size in the low feed group drastically reduced growth rate. Similarity of growth rates, from December 1981 on, may reflect a growth-ration relationship which changes with body size, though little is known of this function (Ricker, 1979). Reduction in growth rate of high feed fish at large body size may result from a combination of maturation and tank size which may act more strongly in larger fish to inhibit growth.

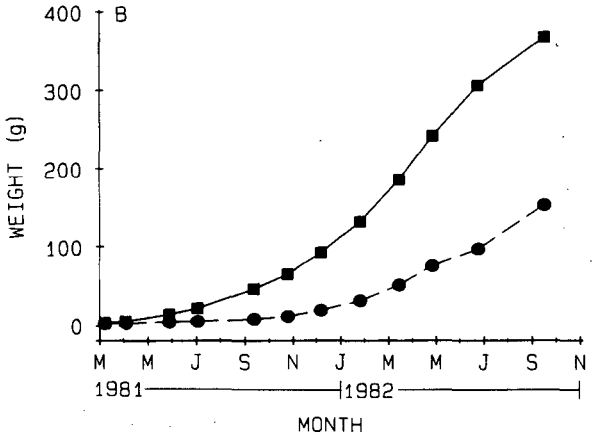
Diel cycles

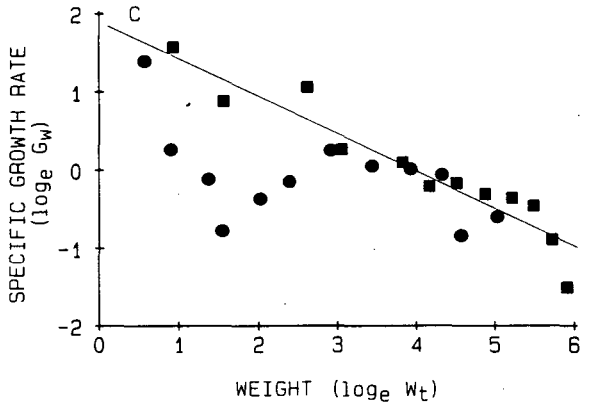
Plasma [Cl^-], [K^+], [Mg^{2+}] and hematocrit showed no significant diel cycle ($p > 0.10$, ANOVA, Fig. 3). Plasma osmolarity, [Na^+] and thyroxine concentration, however, significantly changed over a 20-h period ($p < 0.01$, ANOVA, Fig. 3). Plasma osmolarity peaked after 8.5 hr of light. Both [Na^+] and osmolarity declined by the first night sample (Fig. 3). Thyroxine levels were highest during the light period, declined prior to dusk, and reached their lowest levels during darkness. There was no significant difference in variance over the 20-h period of any of the plasma variables ($p > 0.10$, Bartlett's test), indicating that no change in variability occurred as a result of diel cycles.

Diel cycles of plasma ions have been found in other teleosts and are considered to be rhythmic responses to changes in activity or light levels. Hannah and Pickford (1981) found 'afternoon' peaks of plasma [Na^+] in killifish, Fundulus heteroclitus, which did not occur for [Cl^-] and [K^+]. They also determined a daytime rise in hematocrit, which was not observed in brook trout or juvenile sockeye salmon, Oncorhynchus nerka (Leatherland et al., 1974). Although our results show that peaks in [Na^+] and total osmolarity were concurrent, increases in 'afternoon' [Na^+] are not large and cannot account for the more substantial increases in total osmolarity (see Fig. 3). An unmeasured plasma constituent must make up the remaining portion. Hannah and Pickford (1981) hypothesized that 'afternoon' increases and 'post-sunset' declines in [Na^+] may be due to locomotor activity which for killifish and brook trout is high during daylight and low at night. Wood and Randall (1973) have shown that

Figure 2. Effect of feeding treatment on size and growth rate. (A) Length and (B) weight of normal photoperiod fish in high feed (squares) and low feed (circles) groups as a function of time. Mean value of 40-75 fish per sampling date. (C) Log_e specific growth rate (G_w) as a function of the natural logarithm of fish weight (g) in high and low feed groups. Regression line is for high feed fish only ($Log_e\ G_w = 1.97 + -0.49\ Log_e\ W_t$). (D) Log_e of specific growth rate of fish of unit size (a comparative measure of growth rate which is independent of body size, see text for explanation), as a function of time for high and low feed groups under normal photoperiod conditions.


A
LENGTH (cm)
30
25
20
15
10
5
0
M M J S N J M M J S N
1981
1982
MONTH


B
WEIGHT (g)
400
300
200
100
0
M M J S N J M M J S N
1981
1982
MONTH


C
SPECIFIC GROWTH RATE
(log$_e$ G_w)
2
1
0
-1
-2
0 1 2 3 4 5 6
WEIGHT (log$_e$ W_t)

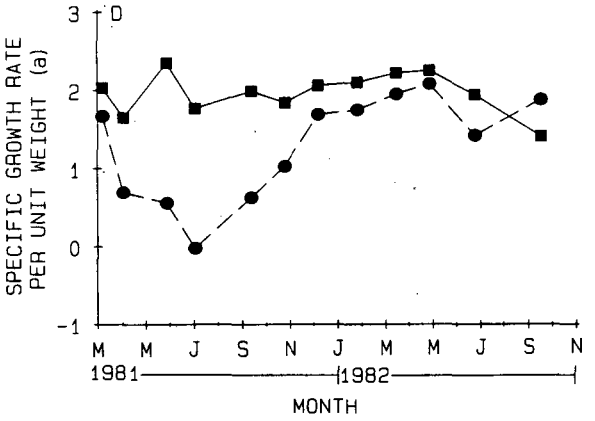
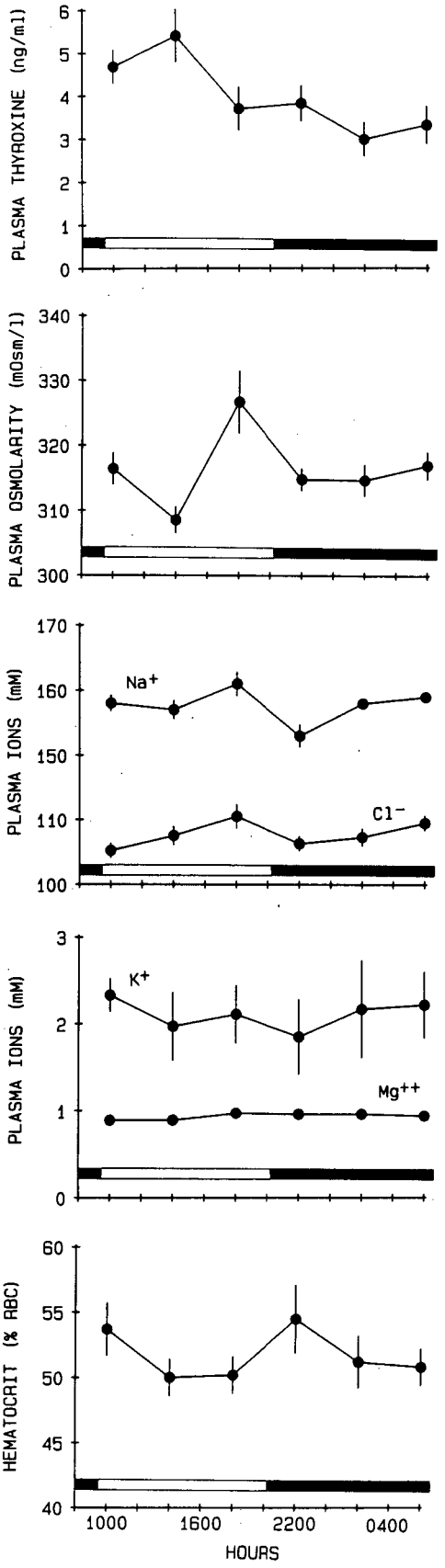

D
SPECIFIC GROWTH RATE
PER UNIT WEIGHT (a)
3
2
1
0
-1
M M J S N J M M J S N
1981
1982
MONTH

Figure 3. Diel cycles of plasma thyroxine, osmotic and ionic concentrations, and hematocrit (mean $\pm$ 1 standard error of the mean) over a 20 hr period. Only plasma thyroxine, osmolarity and $[Na^+]$ had significant diel cycles ($p < 0.05$, one-way ANOVA). Experiment was conducted on February 12 under normal photoperiod conditions (10.4 hr daylight, 13.6 hr darkness). The clear horizontal bar indicates daylight period, darkened bar indicates darkness. Sample size was 6 fish for each time interval, except for the 1200 and 0200 intervals when only 4 samples for $[Na^+]$, $[K^+]$ and $[Mg^{2+}]$ were used.


PLASMA THYROXINE (ng/ml)
6
5
4
3
2
1
0
PLASMA OSMOLARITY (mOsm/l)
340
330
320
310
300
PLASMA IONS (mM)
170
160
150
110
100
Na+
Cl−
PLASMA IONS (mM)
3
2
1
0
K+
Mg++
HEMATOCRIT (% RBC)
60
55
50
45
40
1000
1600
2200
0400
HOURS

plasma [Na^+] increases with activity in rainbow trout. Alternatively, daily [Na^+] and osmolarity changes may be related to feeding activity. Although our animals were not fed during the day of sampling, ionic and osmotic cycles may still be due to rhythms associated with daytime feeding. Using an experimental design similar to ours, in which fish were starved overnight, Leatherland et al. (1974) found that plasma free fatty acids of juvenile sockeye salmon peaked and declined during daylight. Other nutrients and waste products may cycle in a similar fashion and result in the observed 'afternoon' peak in plasma osmolarity.

Diel cycles of thyroxine, which in the present study peaked during daylight, have been observed in other teleosts. White and Henderson (1977) reported levels of thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) in brook trout that were higher at midday and evening than at dawn. Similar diel patterns in T_4, and possibly T_3, have been reported for rainbow trout and goldfish (Eales et al., 1981; Spieler and Noeske, 1979). In contrast, Osborn et al. (1978) described diel cycling of T_4 and T_3 in rainbow trout, in which lowest values were observed during daylight and highest values at night. Since other investigators have failed to find diurnal variations in plasma thyroxine in rainbow trout (Leatherland et al., 1977; Brown et al., 1978), it seems clear that experimental conditions are involved in diel variations. Eales et al. (1981) have shown that starvation for 72 hr eliminates diel variations in T_4. They also demonstrate that it is not the time of feeding which determines the timing of T_4 and T_3 peaks. From their results it appears that feeding stimulates the diel thyroxine cycle,

while some other factor (possibly the light-dark period, or the animals locomotor response to it) acts to sychronize it.

Plasma thyroxine concentrations varied 40^{0}/o over 24 hr, and approximately 31 ^{0}/o over the daytime period in which our sampling for annual cycles occurred. These variations probably did not affect our ability to detect seasonal cycles since sampling occurred during the day when thyroxine levels were highest, and because the seasonal variability (ranging over an order of magnitude) was 3 to 4 times greater than that of the diel cycle. Changes in the magnitude and timing of diel cycling of thyroxine, however, could vary with season. Meier's (1975) review of circadian prolactin and cortisol rhythms in birds has shown that seasonal changes in diel cycles exist and possess a regulatory function. The role of diel thyroxine cycles in teleosts has yet to be established.

Ontogenetic Changes in Freshwater

Plasma Osmotic and Ionic Concentrations - Range, mean and standard deviation of plasma osmolarity, $[Na^{+}]$, $[Cl^{-}]$, $[K^{+}]$ and $[Mg^{2+}]$ are shown in Table 1. Brook trout used for this analysis were between 6.0-30.5 cm fork length, 6-30 mo old and contained both mature and immature individuals. Plasma osmolarity, $[Na^{+}]$ and $[K^{+}]$ were significantly correlated with length and age (Table 1), but length and age explained little of the variation in these plasma constituents ($0.02 < r^2 < 0.12$, Table 1). Plasma $[Mg^{2+}]$ was not significantly correlated with length or age.

Plasma $[Cl^{-}]$ was significantly correlated with length and age, but in 100 l tanks the relationship was positive ($r^2 = 0.02$ and

Table 1. Size, age, photoperiod and feeding effects on plasma parameters and gill Na^+,K^+-ATPase. Sample size (N), range, mean, standard deviation (S.D.), and slope (b), y-intercept (y-int.), coefficient of determination (r^2), and significance of regression slope (p), are given for each physiological variable and their regression on length and age. Brook trout were 6.0 and 30.5 cm fork length and 180 to 700 d old. Feeding and photoperiod effect were determined using two-way ANOVA (Yes, $p < 0.05$; No, $p > 0.05$). Yes(H) indicates that photoperiod effect was only significant in high(H) feeding group.

	N	Range	Mean	S.D.	LINEAR REGRESSION Independent Variable	b	y-int.	r^2	p	Feeding Effect	Photoperiod Effect
Osmolarity	793	272-362	307	12.4	Length	0.64	296	0.12	< 0.01	YES	NO
					Age	0.022	298	0.07	< 0.01		
$[Na^+]$	535	126-186	152	8.0	Length	0.33	146	0.06	< 0.01	NO	NO
					Age	0.013	147	0.06	< 0.01		
$[K^+]$	607	0.10-7.12	1.99	1.24	Length	-0.037	2.68	0.03	< 0.01	YES	NO
					Age	0.0009	2.42	0.01	< 0.01		
$[Mg^+]$	605	0.64-2.23	1.04	0.22	Length	-	-	-	0.47	NO	YES(H)
					Age	-	-	-	0.12		
$[Cl^-]$ (100 l)	352	96-133	108	5.5	Length	0.13	105	0.02	0.02	-	-
					Age	0.012	102	0.08	< 0.01		
$[Cl^-]$ (1,000 l)	311	103-142	124	6.6	Length	-0.15	127	0.02	0.02	-	-
					Age	-0.008	128	0.03	< 0.01		
Hematocrit	940	30-72	48	7.2	Length	0.47	40	0.18	< 0.01	-	-
					Age	0.01	44	0.04	< 0.01		
Na^+,K^+-ATPase	687	1.3-21.2	7.9	3.4	Length	-	-	-	0.52	NO	NO
					Age	-	-	-	0.60		
Thyroxine	728	0.0-10.1	3.01	1.87	Length	0.14	0.48	0.20	< 0.01	YES	YES(H)
					Age	0.0045	1.05	0.10	< 0.01		

0.08, respectively) while in 1,000 l tanks it was negative (r^2 = 0.02 and 0.03, respectively). Plasma [Cl^-] of fish kept in 100 l and 1,000 l tanks were significantly different ($p < 0.01$, student's t-test). These differences were observed within 24 hr of transfer from 1,000 to 100 l tanks. pH of water was greater in smaller tanks (6.2-6.4) than in the larger tanks (5.8-6.1), and was possibly due to increased aeration in small tanks. Plasma [K^+] showed inconsistent differences under the two culture conditions (being sometimes higher and sometimes lower in 100 l tanks), while plasma osmolarity, [Na^+], [Mg^{2+}], thyroxine concentration, hematocrit, and gill Na^+,K^+-ATPase activity showed no significant differences between tanks ($p > 0.05$, student's t-test).

Exposure of fish to lethal and sublethal acidic conditions results in decreased plasma [Na^+] and [Cl^-] (Packer and Dunson, 1970; Neville, 1979; McDonald et al., 1980; Holeton et al.,1983). Plasma [Na^+], however, did not differ between 1,000 l and 100 l tanks. The relatively small differences in pH may account for the absence of plasma [Na^+] reduction. It is interesting to note that while plasma [Cl^-] of fish in 100 l tanks decreased an average 16 mmole/l, no decrease in total osmolarity occurred, nor was there a substantial change in other plasma ions. An unmeasured plasma constituent, probably anionic, must substitute for decreased plasma [Cl^-].

Feeding regime had a significant effect on plasma osmolarity and [K^+], but not on other plasma ion concentrations (Table 1). When significant differences between high and low feeding groups were observed ($p < 0.05$, student's t-test), plasma osmolarity was always higher in the high feed group, and plasma [K^+], with one exception,

was always higher in the low feed group. Significant differences in plasma osmolarity and $[K^+]$ between high and low feed groups occurred even for the largest and oldest fish in the low feed group, indicating that meal size itself was exerting an influence.

Photoperiod treatment had a significant effect on plasma $[Mg^{2+}]$ (in high feed group only, Table 1), but not on other plasma ions. No consistent difference in $[Mg^{2+}]$ due to daylength was found.

Plasma ion and osmotic concentrations of brook trout in freshwater are typical of those reported for other freshwater teleosts (Holmes and Donaldson, 1969). Size and age related adjustments in plasma ion and osmotic concentrations can explain only a small amount of the variation of these parameters. Size related changes in blood ions may not be due to osmoregulatory changes per se, but rather to other impinging physiological responses that vary with size. For example, the degree of digestion of the previous days meal is size dependent (Jobling et al., 1977) and could result in nutrient transport related changes in plasma ionic and osmotic concentrations. Despite these alternative explanations, size related changes in hyperosmoregulation may exist.

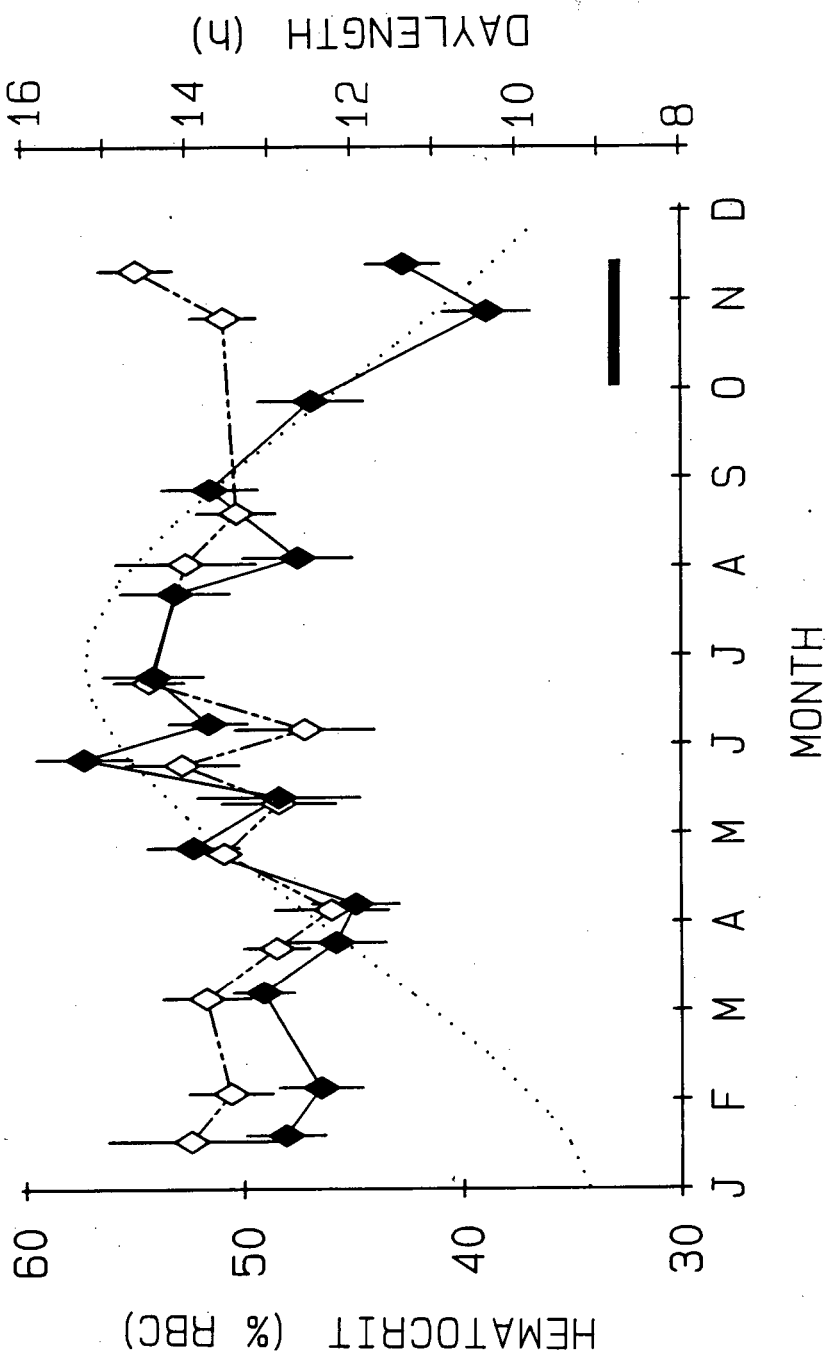
Significant seasonal changes in plasma ion levels that might signal preparatory physiological adapations were not found in the present experiment. Seasonal changes in plasma ions have been found in rainbow trout (Lane, 1979; Houston and Smeda, 1979). We found seasonal changes in $[Mg^{2+}]$ only, and this effect occured only in the high feed group. There was no clear trend of variations in plasma $[Mg^{2+}]$ with changing daylength in either photoperiod. Lack of photoperiod effect on plasma ions in our experiments, which were

conducted at constant temperature, indicates that temperature (or synergy between photoperiod and temperature) may play a more important role than photoperiod alone in determining seasonal ion changes reported by other researchers. Alternatively, brook trout may not display seasonal cycles in ion concentration that are seen in other salmonids.

Hematocrit - Hematocrit increases with increasing length which can explain a portion of the variation in hematocrit (r^2 = 0.18, Table 1), while age can explain little of the variation (r^2 = 0.04). This relationship held true when mature fish were excluded (r^2 = 0.13 and 0.09 for length and age, respectively). Adult male and female hematocrits of 1+ yr fish are the same for much of the year (Fig. 4) and do not differ until autumn when male hematocrit rises slightly and female hematocrit drops significantly from the spring and summer average ($p < 0.01$, student's t-test). Changes in hematocrit occur simultaneously with final maturation, when sperm is running freely and egg diameters are at a maximum. Significant male-female differences in hematocrit, however, were also observed in immature fish; immature males and females in autumn photoperiod (11.5-9.5 hr daylength) had mean hematocrits of 50°/o (N = 15) and 44°/o , respectively (N = 13; $p < 0.01$, student's t-test). Mean hematocrits of immature males and females during winter photoperiod (9.1-10.2 hr daylength) were not significantly different (44°/o and 46°/o, N = 15 and 11, respectively, $p > 0.25$).

Hematocrit levels and their variability as reported here are typical of those reported elsewhere for brook trout and other salmonids (Sniezko, 1960; Sano, 1980). Since size explains a greater

Figure 4. Annual variation in hematocrit ($^{o}/o$ red blood cells) for males (open diamonds) and females (closed diamonds) under normal photoperiod conditions. Mean value of 6-10 fish per sample $\pm$ 1 standard error of the mean. Daylength ($^{\cdot}$) and spawning time (horizontal bar) are also shown. Fish in this experiment were from high feed group, all of which became mature at age 1^{+} yr in their second autumn (November 1982). Mean female hematocrit in autumn in significantly lower than the non-autumn mean ($p < 0.05$, student's t-test).


DAYLENGTH (h)
16
14
12
10
8
HEMATOCRIT (% RBC)
60
50
40
30
J F M A M J J A S O N D
MONTH

portion of the variation in hematocrit than age, age is probably significant only to the extent that it covaries with size. Adult male rainbow trout, pike (Esox lucius) and largemouth bass (Micropterus salmoides) have higher hematocrit than females (Sano, 1960; Mulcahy, 1970; Steuke and Atherton, 1965) indicating that sexual differences in teleost hematocrit are common. Sano (1960) also reported a sharp reduction in hematocrit of adult rainbow trout of both sexes that was correlated with gonadal development. In brook trout, only the female hematocrit declines during the onset of spawning. Further work is necessary to determine the mechanistic control of hematocrit and how this control may be related to sex, spawning and photoperiod cycles.

Gill Na^+,K^+-ATPase - Individual gill Na^+,K^+-ATPase activities ranged from 1.3 to 21.2 $\mu MP_i \cdot mg\ prot.^{-1} \cdot hr^{-1}$, with a mean value of 7.9 (Table 1). Gill Na^+,K^+-ATPase activity (and its log transformation) were not significantly correlated with size or age. Mean value of gill Na^+,K^+-ATPase did not rise above 13.0 $\mu MP_i \cdot mg\ prot.^{-1} \cdot hr^{-1}$ for any sampling period. Feeding treatment had no effect on gill Na^+,K^+-ATPase levels in either photoperiod (Table 1), nor did the two photoperiod treatments differ in their effect on gill Na^+,K^+-ATPase activity.

None of the experimental manipulations of the present study affected gill Na^+,K^+-ATPase activity of brook trout. In contrast, gill Na^+,K^+-ATPase activity in migratory Pacific salmon (Oncorhynchus sp.), Atlantic Salmon (Salmo salar) and steelhead trout (Salmo gairdneri) exhibit a seasonal cycle (usually peaking in spring or autumn, and corresponding to the period of seaward migration) which is synchronized by photoperiod (Zaugg and McLain, 1970; Zaugg and

Wagner, 1973; Saunders and Henderson, 1978). Ewing et al. (1979) found that although chinook salmon displayed a seasonal cycle of gill Na^+,K^+-ATPase activity under simulated normal photoperiod conditions, a 3 mo advanced photoperiod did not significantly alter the cycle, indicating the controlling influence of endogenous rhythms. In the present study there was no daylength-related rhythm of gill Na^+,K^+-ATPase activity in either photoperiod treatment, and no evidence of endogenous rhythms.

Ewing et al. (1979) found that growth rate of chinook salmon (altered by changes in temperature) affected the cyclic annual change in gill Na^+,K^+-ATPase activity and that size was positively correlated with this activity. Size, growth and photoperiod did not alter gill Na^+,K^+-ATPase activity of brook trout in the present study, possibly due to the less specialized nature of its seaward migration. Brook trout, and charr in general, show a less anadromy than other salmonids (Hoar, 1976), and there are more non-migratory than migratory populations of brook trout (Power, 1980). Anadromous brook trout spend long periods in estuaries (Montgomery et al., unpublished manuscript) where gill Na^+,K^+-ATPase activity increases (Chapter 4), indicating that adaptations for seawater entry may have an important behavioral component.

It is possible that possession of preparatory changes in gill Na^+,K^+-ATPase activity is genetically determined in brook trout. However, the migratory pattern of the hatchery stock used in these experiments did not differ from that of natural populations (Mullan, 1958). Furthermore, gill Na^+,K^+-ATPase activities of an anadromous population of brook trout, which show external signs of

smolting (silvering), were not significantly different from a nearby non-anadromous population (Chapter 4).

Plasma Thyroxine - Plasma thyroxine concentration was significantly correlated with both size and age ($p < 0.01$) and explained $20^{0}/o$ and $10^{0}/o$ of the thyroxine variation, respectively (Table 1). The significance of these correlations may be explained, in part, by changes in growth rate. Within each photoperiod, feeding treatment resulted in significant differences in thyroxine levels (Table 1 and Fig. 5). Fish in high feed groups have significantly higher plasma thyroxine at most sampling times starting from the first sample period (June 1981) and continuing until December 1981. This is the same time that growth rates in the high feed group were much greater than in the low feed group (Fig. 2D). After this period, growth rates and thyroxine levels were similar for both feeding groups until April 1982, when high feed fish again attained higher plasma thyroxine levels. This pattern was similar for both photoperiods (Fig. 4A,B). Under normal photoperiod conditions the percent mean difference in plasma thyroxine (between the high and low feed group) and the mean difference in specific growth rate per unit body weight (a) 1-2 wk later were significantly correlated ($r = 0.71$). (Sufficiently accurate growth rate measurements were not determined in the 3-mo delayed photoperiod). These results indicate that higher growth rates in brook trout are associated with higher levels of plasma thyroxine.

Under normal photoperiod conditions (Fig. 4A) there was a strong pattern of high thyroxine levels in the 'spring' (increasing photoperiod), low 'summer' levels which rose to a secondary 'autumn'

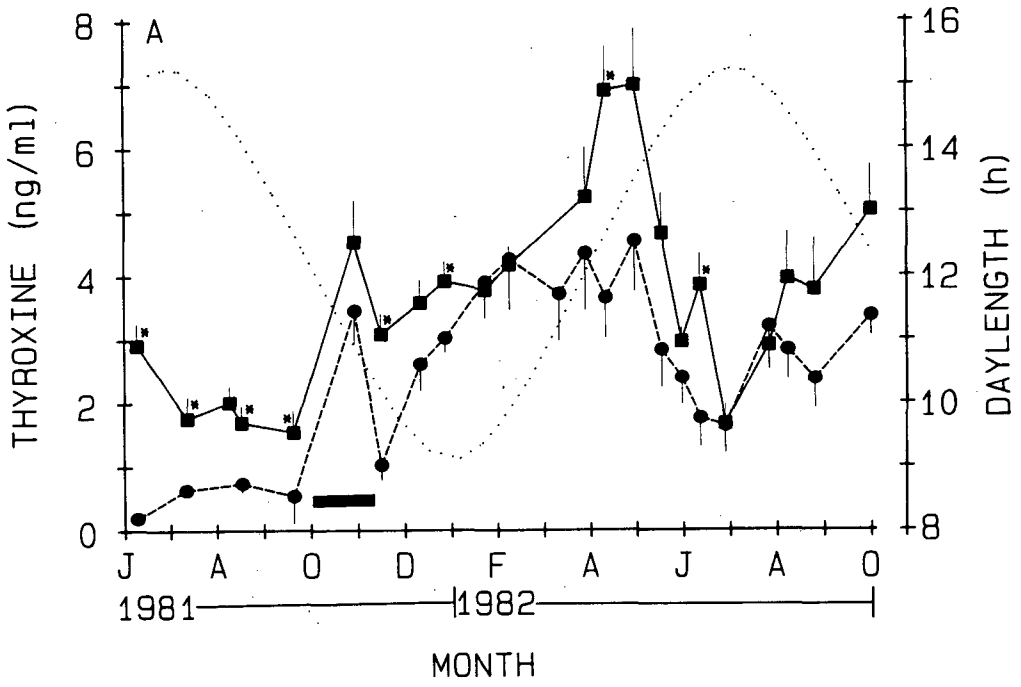
peak. This pattern was consistent in both high and low feed fish, with the exception that the 'spring' peak was attenuated in the low feed group.

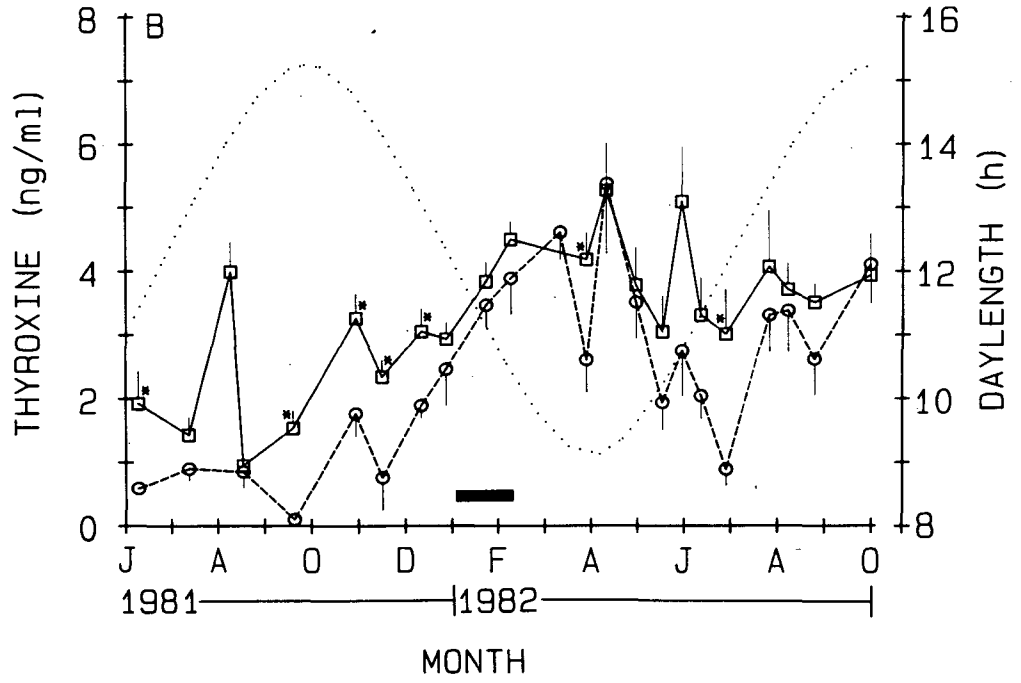
Although a single 'spring' peak occurred under the 3-mo delayed photoperiod for the high feed fish, there was no rise in thyroxine levels in either group during the second 'spring', and there was no subsequent 'summer' decline (Figure 5B). As such, there was no clearly discernible daylength pattern in thyroxine levels under 3-month delayed photoperiod.

Photoperiod treatment had a signficant effect only on fish in the high feed group (Table 1). There was no clear pattern in the differences between normal and delayed photoperiods for the high feed group (Fig. 5A,B), and it was clear that a simple 3 mo shift in the thyroxine cycle did not occur as a result of treatment with a 3-mo delayed photoperiod.

The significant effect of feeding level on circulating thyroxine concentrations in brook trout is probably related to growth, since significant differences in thyroxine levels in high and low feeding groups occurred when growth rates of the two groups were most different. It is unlikely that insufficient iodine in the low feeding group resulted in lower thyroxine levels since under normal laboratory conditions less than 20^{o}/o of the iodine needed by rainbow trout is obtained from the diet (Hunt and Eales, 1979), and only 5^{o}/o of the total iodine is used by the thyroid. In addition, brook trout deprived of food for several weeks increase their plasma iodine (Higgs and Eales, 1971), while rainbow trout show no detectable change in T_4 after up to 40 d of starvation (Leatherland et al., 1977; Milne

Figure 5. Annual cycles of plasma thyroxine in high feed (squares) and low feed (circles) groups in normal photoperiod (A, solid symbols) and 3 month delayed photoperiod (B, open symbols), as a function of calendar date of sampling. Mean value of 5-16 fish per sample $\pm$ standard error of the mean. An asterisk (*) next to mean of high feed group indicates a significantly ($p < 0.05$, student's t-test) higher mean plasma thyroxine levels than the low feed group at that sampling period. Each point represents samples taken on a single day except for three instances (June, July and August 1981) when samples taken within 2-4 days were combined. Daylength (°) and time of spawning (horizontal bar) are shown for each photoperiod. Feeding treatment had a significant effect on thyroxine levels in both photoperiods ($p < 0.01$, two-way ANOVA). Photoperiod treatment significantly affected thyroxine levels in the high feed group ($p < 0.01$, two-way ANOVA), but not the low feed group ($p = 0.43$).


A
THYROXINE (ng/ml)
DAYLENGTH (h)
8
6
4
2
0
16
14
12
10
8
J A O D F A J A O
1981
1982
MONTH


B
THYROXINE (ng/ml)
DAYLENGTH (h)
8
6
4
2
0
16
14
12
10
8
J A O D F A J A O
1981
1982
MONTH

et al., 1979). Thyroid hormones, particularly triiodothyronine, administered exogenously can stimulate growth in a variety of teleosts and most salmonids (Higgs et al., 1982). Thyroxine may act in synergy with other anabolic hormones, particularly growth hormone, to stimulate somatic growth (Donaldson et al., 1979). Our findings of increased circulating thyroxine levels associated with higher growth rates support such a model of thyroid influence on growth.

A seasonal pattern of circulating thyroxine similar to those reported here was found in adult brook trout by White and Henderson (1977), with the one exception that a secondary fall peak was not found. This seasonal pattern in brook trout thyroxine levels is similar to that found for smolting salmonids (Dickhoff et al., 1978; and reviewed by Dichoff et al., 1982). The magnitude of the springtime peak, however, is generally greater in smolting salmonids. The lack of preparatory seawater-entry adaptations in brook trout (such as increases in gill Na^+,K^+-ATPase) suggests that spring thyroxine increases activate other physiological functions. The thyroxine cycle displayed in primitive salmonids such as brook trout has perhaps been sequestered by specialized migrators to synchronize migration and smoltification.

Winter flounder (Pseudopleuronectes americanus) displayed peak thyroxine concentrations in spring and low concentrations in autumn (Eales and Fletcher, 1982), while high winter concentrations and low summer concentrations were observed in rainbow trout (Osborn et al., 1978). Constant temperatures used in our experiments indicate that temperature changes are not necessary to ellicit seasonal thyroxine cycles. Although feeding activity increased during spring photoperiod

in our experiment (personal observation), we did not detect increased growth rates under increasing photoperiod and therefore cannot ascribe higher spring thyroxine levels to increased growth during this period.

The delayed photoperiod regime did not shift thyroxine cycles 3 mo from the normal regime; in low feed fish photoperiod had no effect on plasma thyroxine, while in high feed fish the effect seemed to be a dampening of the normal cycle. These results raise the possibility that an endogenous cycle, or an exogenous cycle synchronized by an environmental factor other than photoperiod or temperature, exists in brook trout. Other photoperiod cued cycles, in particular maturation, did respond to the photoperiod treatment; final maturation under our experimental conditions was delayed 3 mo in the delayed photoperiod (Chapter 3). These results are somewhat conflicting, especially in light of other evidence associating thyroxine changes with the maturation cycle (see Leatherland, 1982, for review). Nonetheless, it appears that an annual cycle of thyroxine with a spring peak is not necessary to begin or synchronize the maturation cycle of brook trout.

SUMMARY

Significant diel cycles were observed in plasma osmolarity, $[Na^+]$ and thyroxine concentration, and were not detected in plasma $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$ and hematocrit. Plasma osmolarity, $[Na^+]$ and thyroxine concentrations were highest during daylight and lowest at night. This cycle may be caused by feeding and locomotor activity which are highest during periods of light.

Plasma osmolarity, $[Na^+]$, $[K^+]$ and hematocrit increased with increasing size and/or age of brook trout, and can explain a small portion of their variation. Plasma osmolarity and $[K^+]$ were also influenced by feeding level. The effect of size on plasma ions may be explained by a more favorable surface area to volume ratio which, other things being equal, will result in lower net water influx, lower plasma water, higher plasma ions and higher hematocrit with increasing size. We cannot, however, rule out other factors which may also covary with size and/or age.

Gill Na^+,K^+-ATPase activity in brook trout did not respond to feeding or photoperiod treatment, nor was there evidence of size, age or daylength related changes. Brook trout therefore do not possess preparatory physiological adaptations in gill Na^+,K^+-ATPase that are characteristic of other migratory salmonids. It would appear that the more variable and opportunistic nature of brook trout migrations has not resulted in sufficent selection pressure for the development of preparatory, photoperiod-controlled changes in gill Na^+,K^+-ATPase.

Plasma thyroxine concentrations were higher in high feed fish and were directly correlated with size. Significant diffences in plasma

thyroxine concentration between high and low feed groups occurred when differences in growth between the two groups was greatest. These results are best explained by an interaction between growth rate and plasma thyroxine. Under normal photoperiod conditions, plasma thyroxine exhibited a seasonal cycle consisting of high levels in spring, low summer levels and a secondary peak in autumn. Three-month delayed photoperiod did not result in a shift of the thyroxine cycle. Since seasonal changes of brook trout gill Na^+,K^+-ATPase activity and hypoosmoregulatory ability did not occur (Chapter 2), the annual cycle of plasma thyroxine does not stimulate these physiological functions as it is presumed to in smolting salmonids. The seasonal thyroxine cycle which exists in the more primitive charrs must exert its influence through other seasonally occurring physiological functions such as growth, activity or appetite.

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REFERENCES

Brett, J. R. (1979) Environmental factors and growth. In: Fish Physiology, Vol. VIII (edited by Hoar, W. S., Randall, D. J. & Brett, J. R.), pp. 599-675. Academic Press, New York

Brown, J., Fedoruk, K. & Eales, J. G. (1978) Physical injury due to injections or blood removal causes elevations of plasma thyroxine in rainbow trout, Salmo gairdneri. Can. J. Zool. 56, 1998-2003.

Castonguay, M., Fitzgerald, G. J. & Côté, Y. (1982) Life history and movements of anadromous brook charr, Salvelinus fontinalis, in the St. Jean River, Gaspé, Québec. Can. J. Zool. 60, 3084-3091.

Dickhoff, W. W., Darling, D. S. & Gorbman, A. (1982) Thyroid function during smoltification of salmonid fish. In: Gunma Symposia on Endocrinology, Vol. 19, Phylogenic aspects of thyroid hormone actions. (edited by Institute of Endocrinology, Gunma Univ.), pp. 45-61. Center for Academic Publications Japan, Tokyo.

Dickhoff, W. W., Folmar, L. C. & Gorbman, A. (1978) Changes in plasma thyroxine during smoltification of coho salmon, Oncorhynchus kisutch. Gen Comp. Endocrinol. 36, 229-232.

Donaldson, E. M., Fagerlund, U. H. M., Higgs, D. A. & McBride, J. R. (1979) Hormonal enhancement of growth in fish. In: Fish Physiology, Vol VIII. (edited by Hoar, W. S., Randall, D. J. & Brett, J. R.), pp. 456-597. Academic Press, New York.

Dutil, J. D. & Power, G. (1980) Coastal populations of brook trout, Salvelinus fontinalis, in Lac Gillaume-Delisle (Richmond Gulf) Quebec. Can. J. Zool. 58(10), 1828-1835.

Eales, J. G. & Fletcher, G. L. (1982) Circannual cycles of thyroid hormones in plasma of winter flounder (Pseudopleuronectes americanus Walbaum). Can. J. Zool. 60, 304-309.

Eales, J. G., Hughes, M. & Uin, L. (1981) Effect of food intake on diel variation in plasma thyroid hormone levels in rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol. 45, 167-174.

Ewing, R. D., Johnson, S. L., Pribble, H. J. & Lichatowich, J. A. (1979) Temperature and photoperiod effects on gill Na+,K+-ATPase activity in chinook salmom (Oncorhynchus tshawytscha). J. Fish. Res. Bd. Can. 36, 1347-1353.

Gorbman, A., Dickhoff, W. W., Mighell, J. L., Prentice, E. F., & Waknitz, F. W. (1982) Morphological indices of developmental progress in the parr-smolt coho salmon, Oncorhynchus kisutch. Aquaculture 28, 1-19.

Grau, E. G., Specker, J. L., Nishioka, R. S. & Bern, H. A. (1982) Factors determining the occurence of the surge in thyroid activity during smoltification. Aquaculture 28, 49-57.

Hannah, S. G. & Pickford, G. E. (1981) Diurnal peaks in hematocrit and in serum sodium in the killifish, Fundulus heteroclitus, and their absence in serum potassium and chloride: a lack of correlation. Comp. Biochem. Physiol. 70A, 157-159.

Higgs, D. A. & Eales, J. G. (1971) Iodide and thyroxine metabolism in the brook trout, Salvelinus fontinalis (Mitchill) during sustained exercise. Can. J. Zool. 49, 1255-1269.

Higgs, D. A., Fagerlund, U. H. M., Eales, J. G. & McBride, J. R. (1982) Application of thyroid and steroid hormones as anabolic agents in fish culture. Comp. Biochem. Physiol. 73B, 143-176.

Hoar, W. S. (1976) Smolt transformation: evolution, behavior and physiology. J. Fish Res. Bd. Can. 33, 1234-1252.

Holeton, G. F., Neumann, P. & Heisler, N. (1983) Branchial ion exchange and acid-base regulation after strenuous exercise in rainbow trout (Salmo gairdneri). Resp. Physiol. 51, 303-318.

Holmes, W. N. & Donaldson, E. M. (1969) The body compartments and the distributions of electrolytes. In: Fish Physiology, Vol. I, (edited by Hoar, W. S. & Randall, D. J.), pp. 1-90. Academic Press, New York.

Houston, A. H. & Smeda, J. S. (1979) Thermoacclimatory changes in the ionic microenvironment of hemoglobin in the stenothermal rainbow trout (Salmo gairdneri) and eurythermal carp (Cyprinus carpio). J. exp. Biol. 80, 317-340.

Hunt, D. W. C. & Eales J. G. (1979) Iodide balance in rainbow trout, Salmo gairdneri and effects of testosterone proprionate. J. Fish. Res. Bd. Can. 36, 282-285.

Jobling, M. (1983) Growth studies with fish - overcoming the problems of size variation. J. Fish Biol. 22, 153-157.

Jobling, M., Gwyther, D. & Grove D. J. (1977) Some effects of temperature, meal size and body weight on gastric evacuation time in the dab, Limanda limanda (L.). J. Fish Biol. 10, 291-298.

Lane, H. C. (1979). Progressive changes in hematology and tissue water of sexually mature trout (Salmo gairdneri) Richardson, during autumn and winter. J. Fish Biol. 15, 425-436.

Leatherland, J. F. (1982) Environmental physiology of the teleostean thyroid gland: a review. Env. Biol. Fish. 7, 83-110.

Leatherland, J. F., Cho, C. Y., & Slinder, S. J. (1977) Effects of diet, ambient temperature, and holding conditions on plasma thyroxine levels in rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Can. 34, 677-682.

Leatherland, J. F., McKeown, B. A. & John, T. M. (1974) Circadian rhythm of plasma prolactin, growth hormone, glucose and free fatty acid in juvenile kokannee salmon, Oncorhynchus nerka. Comp. Biochem Physiol. 47A, 821-828.

Leitritz, E. & Lewis, R. C. (1976) Trout and Salmon Culture. Calif. Fish & Game Bull. 164. pp. 220.

Lowry, O. H., Roseborough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

McDonald, D. G., Hobe, H. & Wood, C. M. (1980) The influence of calcium on physiological responses of the rainbow trout, Salmo gairdneri, to low environmental pH. J. exp. Biol. 88, 259-267.

Meier, A. H. (1975) Chronoendocrinology of vertebrates. In: Hormonal correlates of behavior, Vol. 2. (edited by Eleftherious, B. E. & Sprout, R. L.), pp. 469-549. Plenum Press, New York.

Miller, G. L. (1959) Protein determination for large numbers of samples. Anal. Chem. 31, 964.

Milne, R. S., Leatherland, J. F., & Holub, B. J. (1979) Changes in plasma thyroxine, triiodothyronine and cortisol associated with starvation in rainbow trout (Salmo gairdneri). Env. Biol. Fish. 4, 185-190.

Mulcahy, M. F. (1970) Blood values in the pike Esox lucius L. J. Fish Biol. 2, 203-209.

Mullan, J. W. (1958) The sea-run or "salter" brook trout (Salvelinus fontinalis) fishery of the coastal streams of Cape Cod, Massachusetts. Mass. Div, Fish and Game. Bull. No. 17,

Neville, C. M. (1979) Influence of mild hypercapnia on the effects of environmental acidification on rainbow trout (Salmo gairdneri). J. exp. Biol. 83, 345-349.

Osborn, R. H., Simpson, T. H. & Youngson, A. F. (1978) Seasonal and diurnal rhythms of thyroidal status in the rainbow trout, Salmo gairdneri Richardson. J. Fish Biol. 12, 531-540.

Packer, R. K. & Dunson, W. A. (1970) Effects of low environmental pH on blood pH and sodium in brook trout. J. exp. Zool. 174, 65-71.

Power, G. (1980) The brook charr, Salvelinus fontinalis. In: Charrs (edited by Balon, E. K.), pp. 141-203. Dr. W. Junk bv Publishers, The Hague.

Ricker, W. E. (1979) Growth rates and models. In: Fish Physiology, Vol. VIII (edited by Hoar, W. S., Randall, D. J. & Brett, J. R.), pp. 678-737. Academic Press, New York.

Sano, T. (1960) Haematological studies of the culture fishes in Japan. 3. Changes in blood constituents with growth of rainbow trout. J. Tokyo Univ. Fisheries 46, 77-87.

Saunders, R. L & Henderson, E. B. (1978) Changes in gill ATPase activity and smolt status of Atlantic salmon (Salmo salar). J. Fish. Res. Bd. Can. 35, 1542-1546.

Snieszko, S. F. (1960) Microhematocrit as a tool in fishery research and management. U.S. Fish and Wildl. Serv. Spec. Rep. Fish. 341, 1-15.

Spieler, R. E. & Noeske, T. A. (1979) Diel variations in circulating levels of triiodothyronine and thyroxine in goldfish, Carrassius auratus. Can. J. Zool. 57, 665-669.

Steucke, E. E. & Atherton, C. R. (1965) Use of microhaematocrit values to sex largemouth bass. Prog. Fish Cult. 27, 87-89.

Sutterlin, A. M., Harmon, P. & Barchard, H. (1976). The culture of brook trout in salt water. Can. Fish. & Mar. Serv. Tech. Rept. No. 636.

White, H. C. (1940) Life-history of sea-running brook trout (Salvelinus fontinalis) of Moser river, Nova Scotia. J. Fish. Res. Bd. Can. 5, 176-186.

White, B. A. & Henderson, N. E. (1977). Annual variation in the circulating levels of thyroid hormones in the brook trout, Salvelinus fontinalis, as measured by radioimmunosassay. Can. J. Zool. 55, 475-481.

Whoriskey, F. G., Naiman, R. J. & Montgomery, W. L. (1981) Experimental sea ranching of brook trout, Salvelinus fontinalis, Mitchill. J. Fish Biol. 19, 637-651.

Wilder, D. G. (1952) A comparative study of anadromous and freshwater populations of brook trout (Salvelinus fontinalis (Mitchill)). J. Fish. Res. Bd. Can. 9, 169-203.

Wood, C. M. & Randall, D. J. (1973) The influence of swimming activity on sodium balance in the rainbow trout (Salmo gairdneri). J. Comp. Physiol. 82, 207-233.

Zaugg, W. S. (1982) A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aq. Sci. 39, 215-217.

Zaugg, W. S. & McLain, L. R. (1970) Adenosine triphosphatase activity in gills of salmonids: seasonal variation and salt water influence in coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. 35, 587-596.

Zaugg, W. S. & Wagner, H. H. (1973) Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (Salmo gairdneri): influence of photoperiod and temperature. Comp, Biochem. Physiol. 45B, 955-965.

CHAPTER 2

Osmoregulation in the brook trout, Salvelinus fontinalis. II. Effects of size, age and photoperiod on seawater survival and ionic regulation.

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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 create fish of the same age but with different sizes and photoperiod experiences. In 11 experiments over 1.5 yrs, fish were gradually exposed to 32 ppt seawater for 20 d 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 d 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). Rounsefell (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 mo (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 $30^{o}/o$ and $60^{o}/o$ after 3 mo 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 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 d), 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 were obtained from the Massachusetts State Fish Hatchery at Sandwich. Although the breeding stock has been exclusively freshwater for 30 yr (Lloyd Raymond, Hatchery Manager, pers. comm.), studies indicate the strain displays anadromy similar to wild stocks when released into

coastal rivers (Mullan, 1958; J. Bergin, Mass. Div. Fish and Wildlife, unpublished data). Fertilized eggs were transported to the Woods Hole Oceanographic Institution's Shore Lab facility and supplied with 10-11 C well water. Eggs were divided into two annually cycling photoperiods corresponding to a latitude of 42 ^{o}N; one photoperiod cycle corresponded to the normal calendar date (longest day June 21, shortest day December 21), while the other was 3 mo delayed from the norm (longest day September 21, shortest day March 21). Daylength was changed every 5 d. Sunrise and sunset were simulated each day by a 15 min period of gradual illumination or dimming of incandescent bulbs. Beginning and end of daylight period were delayed 2 hr from Eastern Standard Time. Vita-Lite spiralux fluorescent bulbs and incadescent bulbs were used to simulate daylight.

Eggs were hatched in 250 l flow-through hatchery troughs. After first feeding fish were transferred to 1,000 l flow-through tanks which received supplemental aeration. Within 1 wk after feeding fish were divided randomly, within each photoperiod treatment, into two feeding groups. Fish in each group were fed equal amounts for the first 4 wk after first feeding . Afterward, one group was fed of commercial fish pellets ad libitum following common hatchery procedures (Leitritz and Lewis, 1976). The low feed group was fed approximately half the amount, per unit body weight, fed to the high feed group. Each group was fed 4 to 5 times daily during daylight hours for the first several months after hatching; 1 to 3 times daily thereafter. Care was taken to prevent overcrowding which might inhibit growth. Ammonia concentration of rearing and experimental tanks was checked periodically.

Specific growth rates (G_w) were calculated using the following formula:

$$G_w' = \frac{\log_e W_t - \log_e W_o}{t} \cdot 100,$$

where W_t is the wet weight at time t, W_o is the wet weight at time 0 and t is time in days. In order to compare growth rates of animals of different sizes, the $\log_e G_w$ of a fish of unit size was calculated (Jobling, 1983) using the following equation:

$$a' = \log_e G_w' - b \log_e W_t,$$

where a is the $\log_e G_w$ of a fish of unit size and b is the slope of the linear relationship between $\log_e G_w$ and $\log_e W_t$. An experimentally derived value of b = -0.49 was used in all calculations of a. A generalized value of b = -0.41 for salmonids was reported by Brett (1979). Growth rates of high and low feed groups are reported in Chapter 1.

Condition factor (CF) is calculated using the formula:

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

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

Seawater Exposure and Blood Sampling

Eleven seawater exposure experiments were conducted over 17 mo on the 4 experimental groups (e.g., normal photoperiod: high and low feed groups; 3-mo 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 $\pm$ 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 (6 turnovers/d) 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 d of acclimation in freshwater, experimental animals were exposed to 10 ppt for 7 d, 20 ppt for 7 d and finally 32 ppt for 20 d. 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^{0}/o within 48 hr, McCormick and Naiman, unpublished data). Salinity was checked regularly and no change greater than $\pm$ 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 d in 32 ppt seawater brook trout were sampled for changes in blood and gill physiology. Freshwater controls were sampled 1 d before and their data reported in Chapter 1. At 4 d, 6-8 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 s. 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 ($^{o}/o$ 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 concentration. 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 adaptation. Brook trout 1 yr of age and 16.5 - 20.2 cm fork length were maintained in a 1,000 l freshwater tank for 30 days prior to exposure to 10 ppt for 8 d, 20 ppt for 7 d and 32 ppt for 60 d. Salinities were changed and monitored in the same manner as those for

other flow-through tanks. At each sampling 5-6 fish were anesthetized and blood and gill samples collected on the following days: 1 d prior to exposure to 10 ppt; 1, 2, 4, 7, 8 d following exposure to 10 ppt; 1, 2, 4, 7, d following exposure to 20 ppt; and 1, 2, 3, 4, 7, and 21 d following exposure to 32 ppt. No physiological sampling occurred after 21 d in seawater. Survival was monitored through 60 d. 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 $[Mg^{2+}]$ were analyzed using a Wescor vapor pressure osmometer, a Buchler-Cotlove chloridometer, and a Perkin Elmer atomic absortion spectrophotometer used in atomic absorption mode to measure $[Mg^{2+}]$ 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 (Appendix A); 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, 1978). Details of these techniques are reported in Chapter 1 and Appendix A.

Statistical Analysis

Seawater survival 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 d in seawater or that jumped from tanks (< $1^{o}/o$). 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 d. 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.

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 $\pm$ 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 d experiment was divided into 5, 4-d 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:

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

The odds ratio was deemed significant when its 95^0/o confidence limit (calculated using the 95^0/o 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 yr of age and 16.5-20.2 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. Of the 21 animals not used for physiological measurements, 19^0/o had died after 5 d in 32 ppt, 38^0/o after 20 d in 32 ppt, and 76^0/o after 60 d.

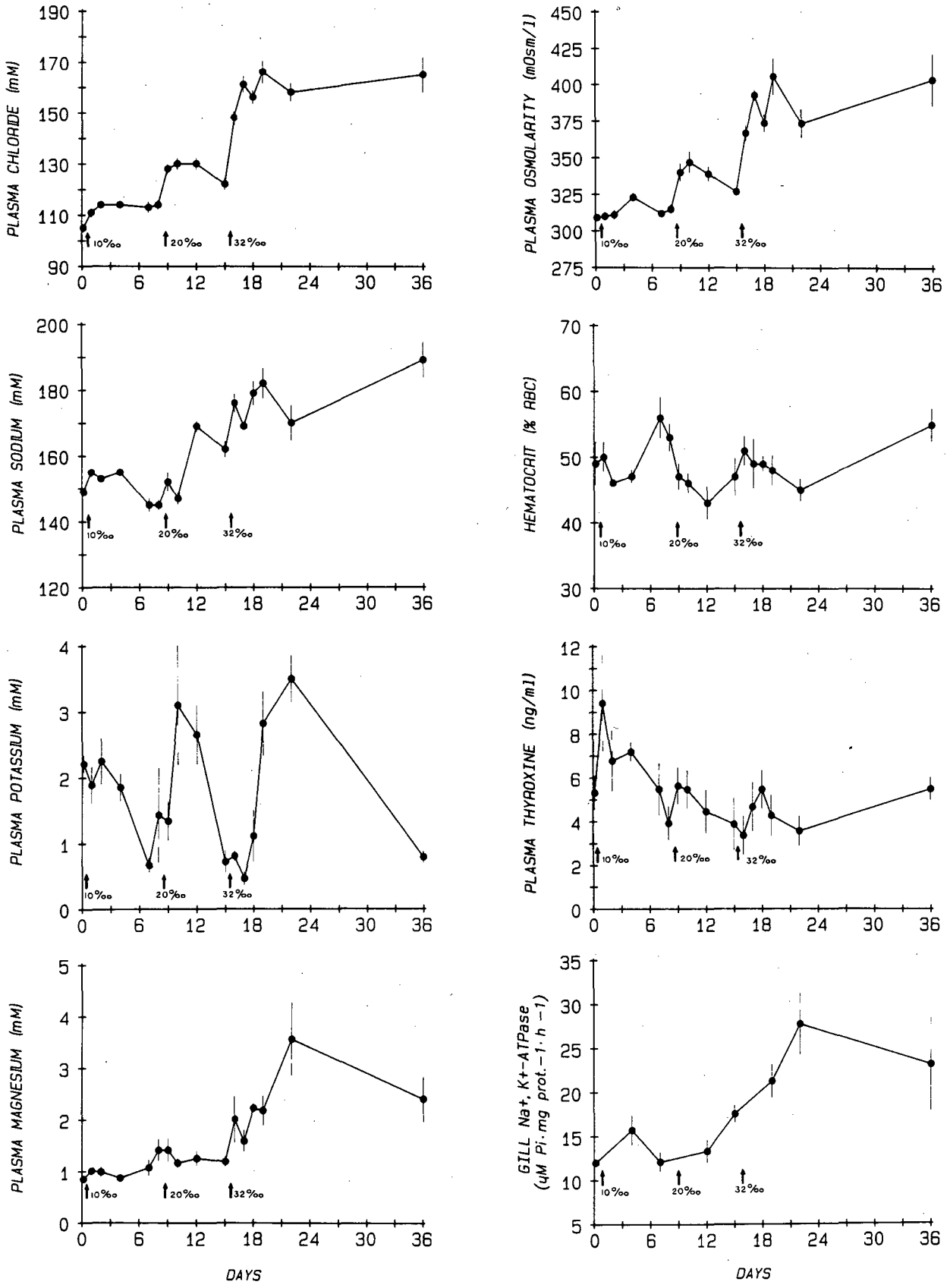
Plasma ions and osmolarity - Plasma [Na^+], [Cl^-] and osmolarity rose 5-15^0/o after 1-2 d exposure to 10 and 20 ppt (Fig. 1) and after 7-8 d were reduced to levels 5-10^0/o higher than initial freshwater levels. Plasma [Na^+], [Cl^-] and osmolarity increased within 1 d of exposure to 32 ppt, reaching levels 20^0/o

($[Na^+]$), 30°/o (osmolarity) and 70°/o ($[Cl^-]$) higher than initial freshwater levels after 4 d in seawater. Though some decline in plasma $[Na^+]$, $[Cl^-]$ and osmolarity occurred after 7 d in seawater, levels at 21 d 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 to 2 d of exposure (Leray et al., 1981; Bath and Eddy, 1979; Jackson, 1981). We found similar peaks after 1 to 2 d in seawater, but these remained high for 4 d. Plasma $[Na^+]$, $[Cl^-]$ and osmolarity decline after 7 d 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 d in seawater, and mortalities continue throughout this period. Despite the ability of brook trout to survive an initial 2-3 d 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 $[Mg^{2+}]$ increased less than 15°/o after exposure to 10 and 20 ppt (Fig. 1). After exposure to 32 ppt, mean plasma $[Mg^{2+}]$ rose continuously, reaching a peak 350°/o higher than freshwater controls after 7 d, and declining to 250 °/o of freshwater controls after 21 d. Although much smaller in absolute magnitude than changes in $[Na^+]$, $[Cl^-]$ and osmolarity ($[Mg^{2+}]$ constitutes less than 1°/o of total plasma osmolarity), fluctuations relative to the

Figure 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).


PLASMA CHLORIDE (mM)
PLASMA OSMOLARITY (mOsm/l)
PLASMA SODIUM (mM)
HEMATOCRIT (% RBC)
PLASMA POTASSIUM (mM)
PLASMA THYROXINE (ng/ml)
PLASMA MAGNESIUM (mM)
GILL Na+, K+-ATPase (µM Pi.mg prot.-1.h-1)
10‰
20‰
32‰
DAYS

initial starting value were large. The time course of changes in plasma [Mg^{2+}] 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^o/o below freshwater levels after exposure to 10 and 20 ppt. Plasma [K^+] did not increase above freshwater levels until 3 d after exposure to 32 ppt, and after 21 d in seawater was only 50^o/o 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 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 (^{o}/o 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^o/o). 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 d in 20 ppt, and leveled off after 7 d in 32 ppt. Activity of gill Na^+,K^+-ATPase after 21 d 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 heteroclitus) 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 d 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 d after exposure to 10 ppt (80^o/o increase over freshwater levels). Plasma T_4 levels dropped 20^o/o below freshwater levels after 8 d in 10 ppt. Exposure of fish to 20 and 32 ppt caused plasma T_4 to increase 25-30^o/o within 2-3 d, followed by decreasing levels after 4 and 7 d 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 d 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^+-ATPases are the basic mechanisms 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 wks 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 changes in circulting 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 d following changes in external salinity are associated with large changes in plasma ions and osmolarity. Measurement of plasma variables after 4 d in seawater will, therefore, be an accurate indicator of hypoosmoregulatory ability. We did not expect plasma ions and osmolarity after 20 d in seawater to show such large variations and values substantially greater than freshwater levels. Measurements after 20 d in seawater must also be viewed as a period of osmotic disequilibruim 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 (Figure 2A), with the absolute difference in length increasing over time. Mean seawater survival time was higher for the high feed group in each of the first five experiments (Fig. 2B); 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 (Chapter 3). 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 d (38-81^{o}/o survival). The smallest brook trout to reach this plateau were 14 cm.

The seawater hazard rate is a measure of the probability of mortality in seawater over 20 d. 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 ^{o}/o of the fish were mature males, 2) gonadosomatic indexes were > 1.5^{o}/o, 3) experiments were conducted during autumn photoperiod, and 4) mean survival time was significantly lower than that of immature males, mature and immature females ($p < 0.025$, Mann-Whitney U-test).

Figure 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.

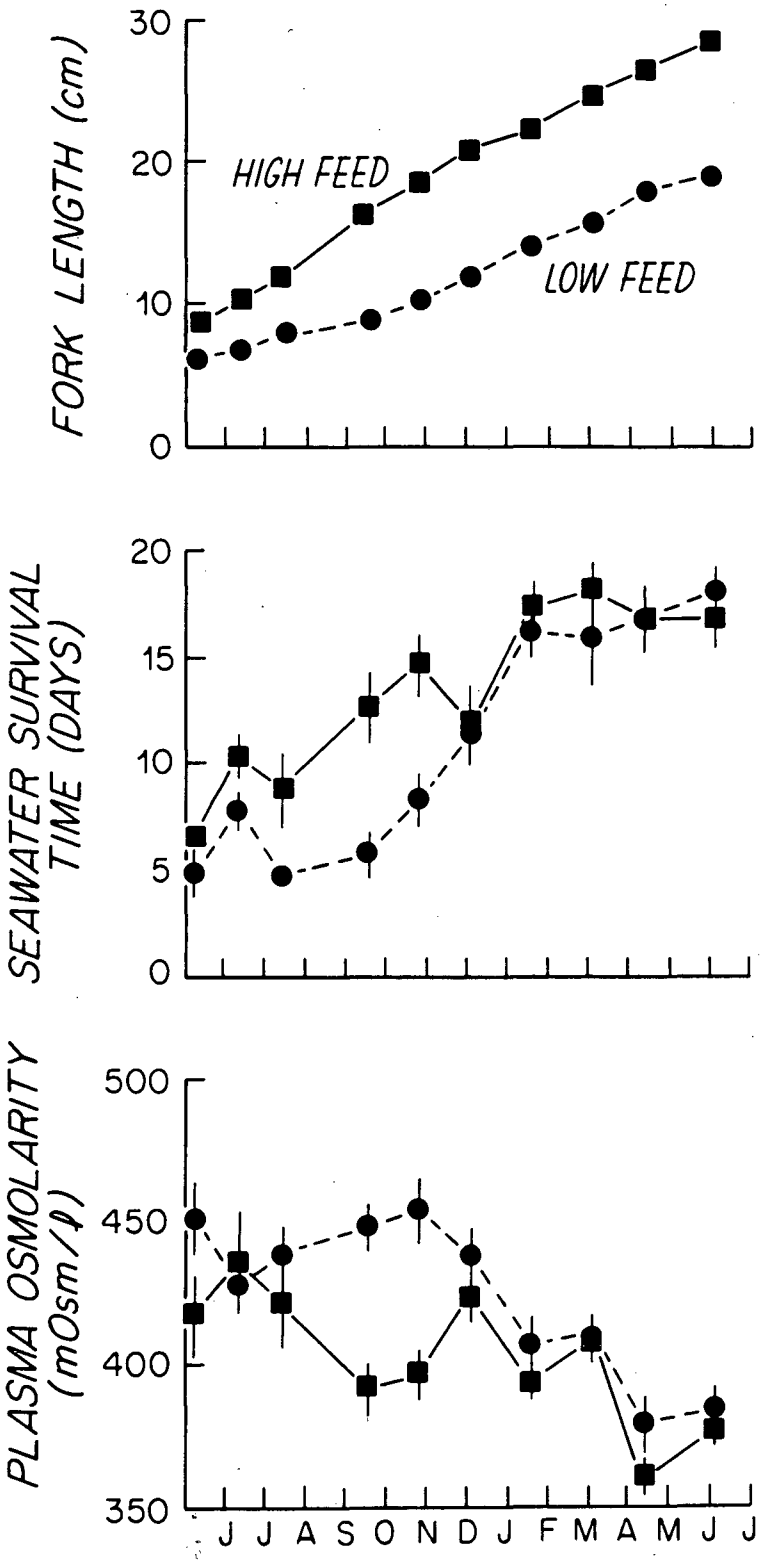
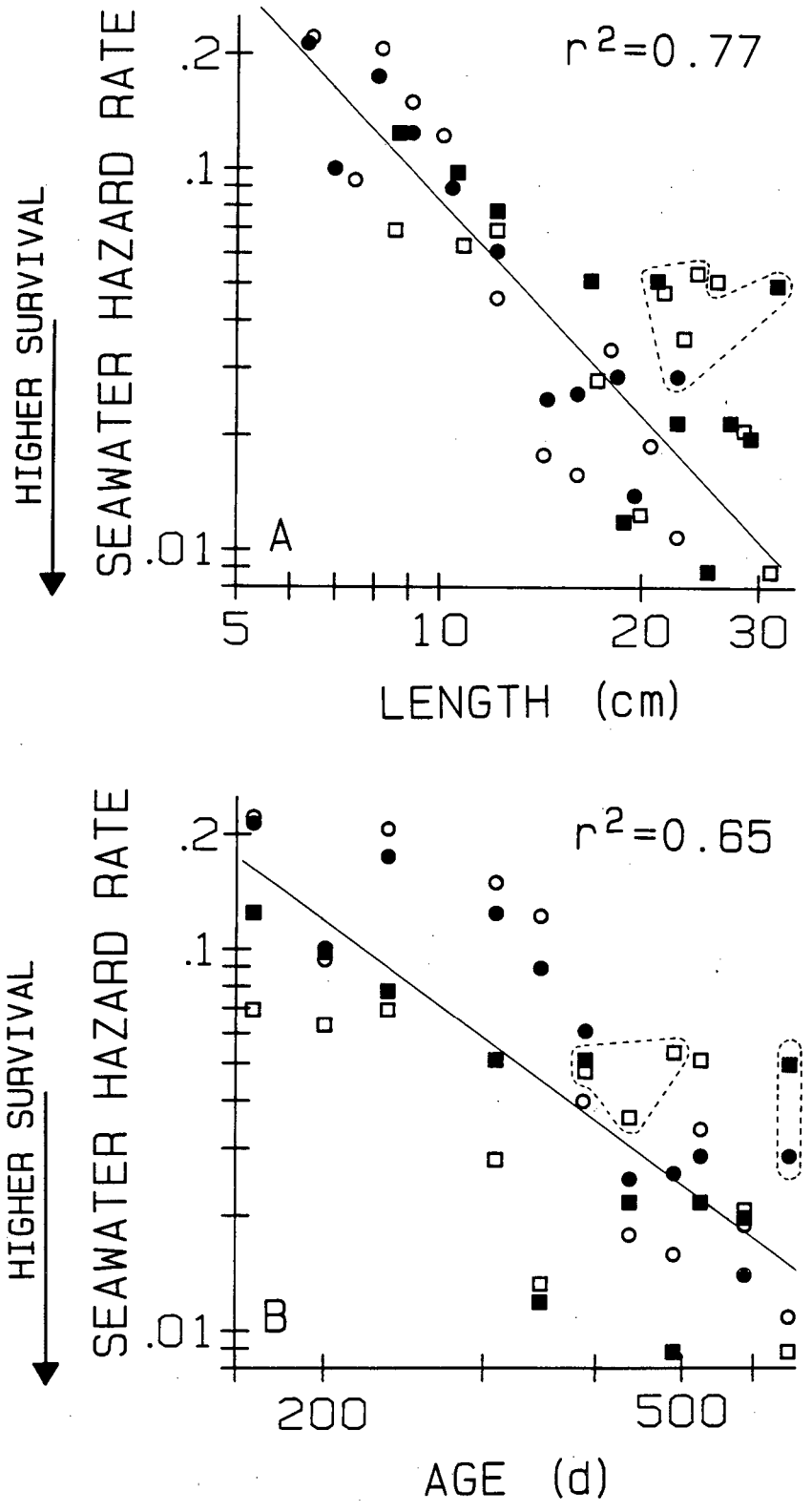

FORK LENGTH (cm)
30
20
10
0
HIGH FEED
LOW FEED
SEAWATER SURVIVAL TIME (DAYS)
20
15
10
5
0
PLASMA OSMOLARITY (mOsm/ℓ)
500
450
400
350
J J A S O N D J F M A M J J

Figure 3. Seawater survival as a function of size and age. Log of seawater hazard rate versus (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). 11 experiments (4 groups per experiment) were conducted over 17 mos. 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.


HIGHER SURVIVAL
SEAWATER HAZARD RATE
.2
.1
.01
A
r^2=0.77
5
10
20
30
LENGTH (cm)
HIGHER SURVIVAL
SEAWATER HAZARD RATE
.2
.1
.01
B
r^2=0.65
200
500
AGE (d)

Fish surface-area-to-volume ratios, as estimated by length^2/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. 2B).

Age also had a significant effect on seawater hazard rate (r^2 = 0.58, Fig. 3B). It is clear, however, that at ages < 1 yr there is a clear distinction in seawater hazard rate between high and low feed groups (Fig. 3B). This is most likely caused by size differences, since growth rate 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. 3A), despite differences in growth rate and age between the two 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 d of each experiment were used in this analysis because the last 8 d fit all models poorly (75^o/o of the mortalities

Table 1. Dichotomous log-linear regression of length, age and male maturity on survial in 32 ppt seawater for 12 d. 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 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	-.61	.06	-
2	.73	.09	-
3	.52	.13	-
Length	-2.90	.33	106.6 *
Age	-0.89	0.28	1.1
Male Maturity	.006	.002	.169 *

occurred in the first 12 d). The statistical model shown in Table 1 is the simplest model (fewest variables) with a highly significant Chi-square (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 chi-square 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 suvival 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 trout show no effect of age on seawater surival. Our results also indicate that size dependent seawater survival is a basic characteristic of the sub-family salmoninae.

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 (Chapter 1), 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.

Ontogeny of Hypoosmoregulatory Ability

Plasma ions and osmolarity - Plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{2+}]$ after 4 d in seawater decreased with increasing length of brook trout (Table 2). Plasma osmolarity showed the

Figure 4. Standardized residuals of the regression of log 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.

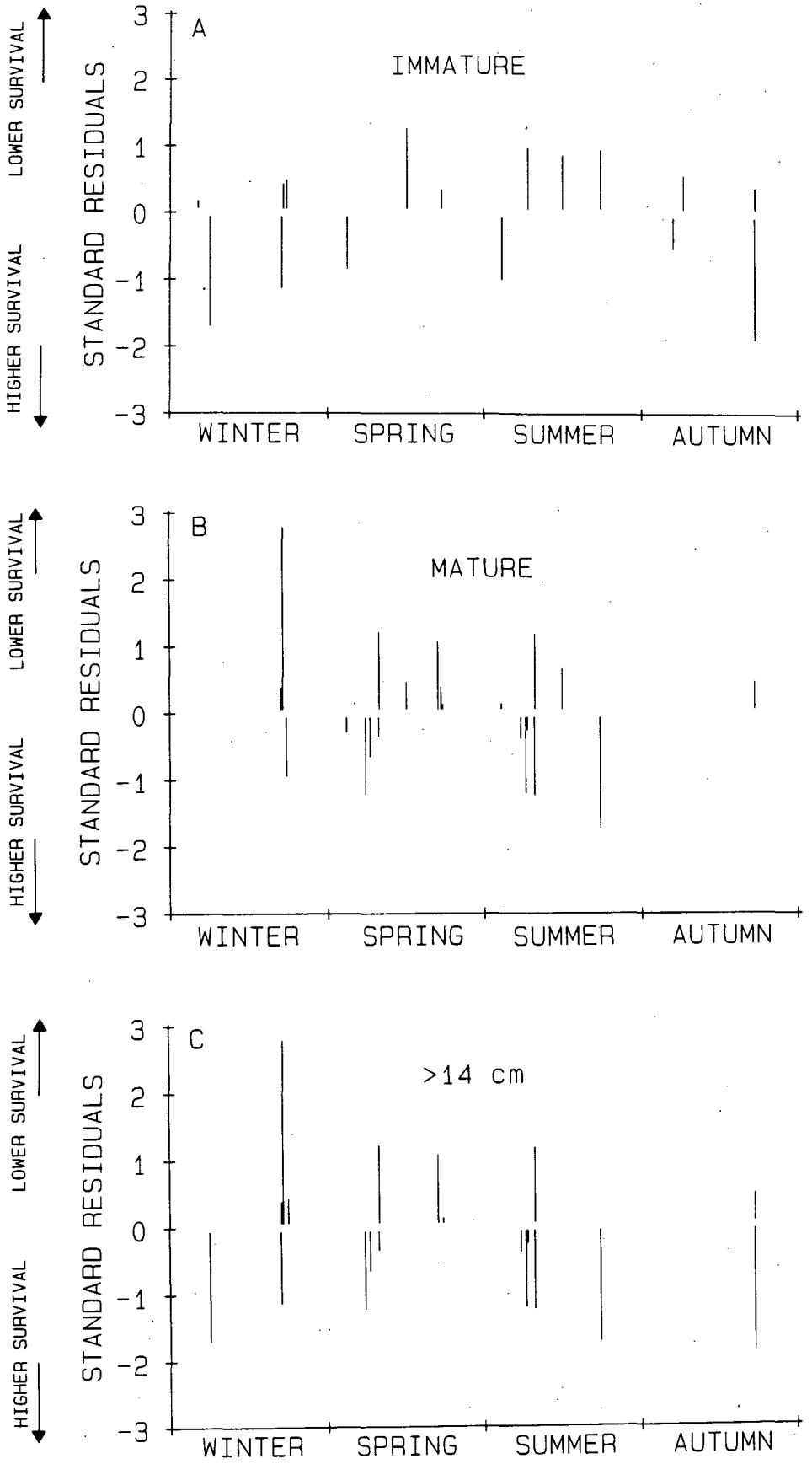

A
IMMATURE
B
MATURE
C
>14 cm
STANDARD RESIDUALS
LOWER SURVIVAL
HIGHER SURVIVAL
3
2
1
0
-1
-2
-3
WINTER
SPRING
SUMMER
AUTUMN

Table 2. Physiological variables after 4 d 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 6-8 individuals. All listed correlation coefficients are significant ($p < 0.05$).

	LENGTH					
Dependent variable	r	y-int.	b	S_b	p	N
Osmolarity	-.80	465.7	-3.27	0.37	0.001	44
$[Cl^-]$	-.40	188.9	-0.77	0.27	0.010	38
$[Na^+]$	-.46	215.2	-1.15	0.38	0.004	38
$[K^+]$	-.70	5.42	-0.129	0.022	0.001	38
$[Mg^{2+}]$	-.64	4.45	-0.081	0.016	0.001	38
Hematocrit	.44	42.9	0.285	0.095	0.005	40
Na^+,K^+-ATPase	-	-	-	-	0.101	40
Thyroxine	.48	0.98	0.083	0.026	0.003	36

	LOG SEAWATER HAZARD RATE					
Independent variable	r	y-int.	b	S_b	p	N
Osmolarity	.70	-5.04	.009	.001	0.001	44
$[Cl^-]$	.42	-3.47	.012	.004	0.008	38
$[Na^+]$	.49	-3.24	.009	.003	0.002	38
$[K^+]$	.53	-1.87	.141	.037	0.001	38
$[Mg^{2+}]$	.65	-2.18	.251	.048	0.001	38
Hematocrit	-.39	-0.05	-.030	.012	0.013	40
Na^+,K^+-ATPase	-	-	-	-	0.219	40
Thyroxine	-.46	-1.03	-.140	.047	0.003	36

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°/o, 21°/o, 49°/o and 41°/o of the variation in plasma [Cl^-], [Na^+], [K^+] and [Mg^{2+}], respectively, after 4 d in seawater. Age could explain less of the variation than length in each of the plasma ions (6°/o, 20°/o, 38°/o and 31°/o, respectively).

Plasma osmolarity and [Mg^{2+}] after 20 d in 32 ppt were significantly correlated with length (Table 3). The levels of plasma ions after 20 d in seawater for any given size are, in most cases, lower than those after 4 d in 32 ppt. These results indicate that smaller fish that survive for 20 d 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 d 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

Figure 5. Plasma osmolarity and gill Na^+,K^+-ATPase in seawater as a function of brook trout size. (A) Plasma osmolarity after 4 d in seawater, (B) plasma osmolarity after 20 d in seawater , and (C) gill Na^+,K^+-ATPase activity after 4 d (open triangles) and 20 d (closed triangles) in seawater, versus 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).

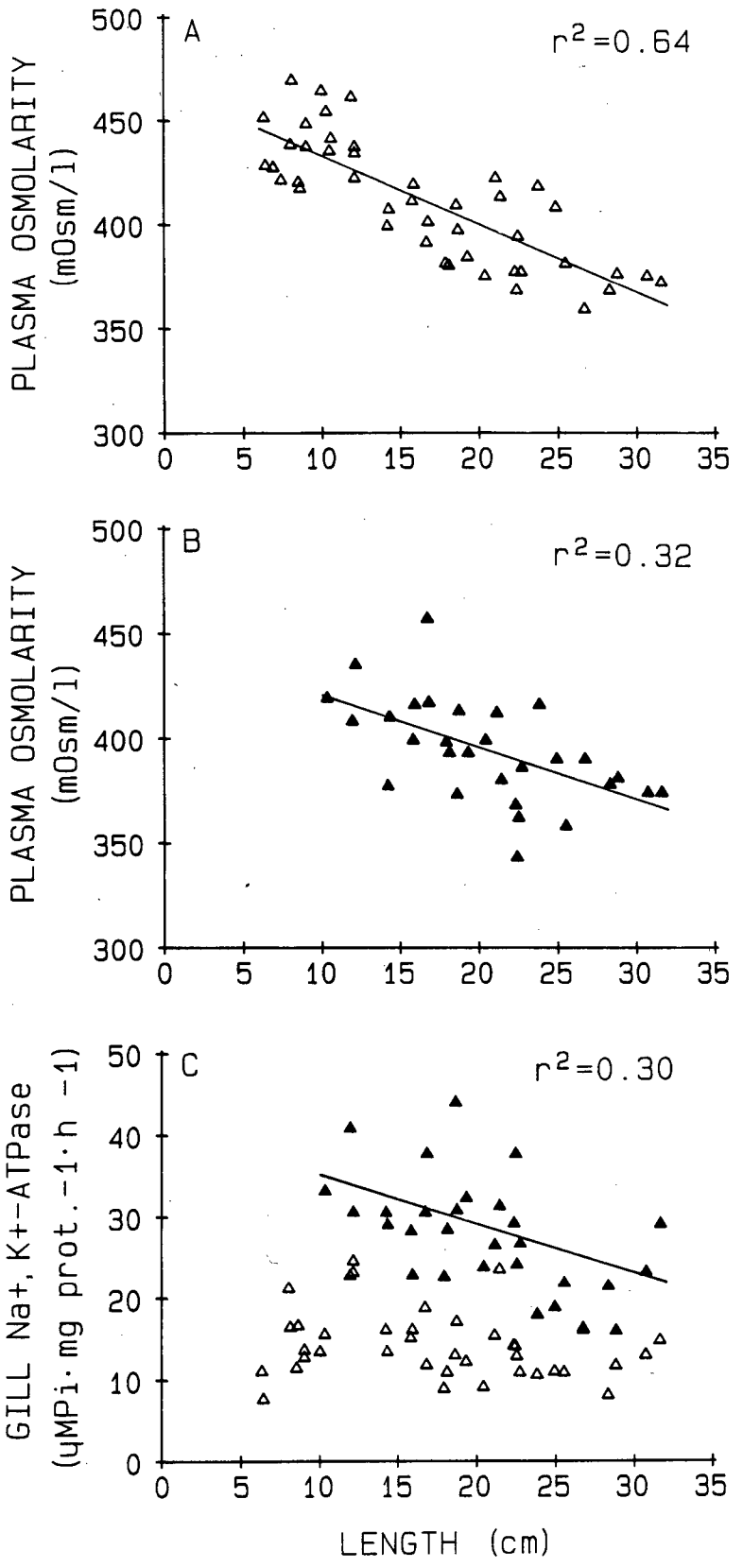

A
r^2=0.64
PLASMA OSMOLARITY (mOsm/l)
500
450
400
350
300
0
5
10
15
20
25
30
35
B
r^2=0.32
PLASMA OSMOLARITY (mOsm/l)
C
r^2=0.30
GILL Na+, K+-ATPase (µMPi·mg prot.-1·h-1)
50
40
30
20
10
0
LENGTH (cm)

Table 3. Physiological variables after 20 d 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 ($p < 0.05$).

	LENGTH					
Dependent variable	r	y-int.	b	S_b	p	N
Osmolarity	-.57	445.5	-2.48	0.69	0.001	29
$[Cl^-]$	-	-	-	-	0.076	29
$[Na^+]$	-	-	-	-	0.190	20
$[K^+]$	-	-	-	-	0.345	28
$[Mg^{2+}]$	-.49	4.51	-0.079	0.028	0.008	28
Hematocrit	-	-	-	-	0.160	29
Na^+,K^+-ATPase	-.55	42.1	-0.687	0.190	0.002	29
Thyroxine	-	-	-	-	0.776	24

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 d in seawater ranged between 31 $^{o}/o$ and 73 $^{o}/o$ and mean hematocrit was positively correlated with length (Table 2). Hematocrit of brook trout in freshwater also increases with size (Chapter 1). 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. Hematocrit after 20 d in seawater ranged between $42^{o}/o$ and $58^{o}/o$ (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 ± 0.13 $\mu MP_i \cdot mg\ prot.^{-1} \cdot hr^{-1}$ ($N = 687$). After adaptation at intermediate salinities and 4 d in seawater, gill Na^+,K^+-ATPase activities were higher than freshwater levels, and higher still after 20 d in seawater (Fig. 5C). Gill Na^+,K^+-ATPase activity after 4 d in seawater did not significantly correlate with size ($p = 0.10$, Table 2). Gill

Na^+,K^+-ATPase activity after 20 d in seawater decreased with increasing length of brook trout (r^2 = 0.30, Fig. 5C, 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 d in seawater, however, are not significantly correlated ($p > 0.50$). 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 d 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 (Chapter 1), 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 6-10 brook trout in freshwater, sampled one day prior to sampling of fish after 4 d 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 d in 32 ppt from this initial value. The mean net change in T_4 after 4 d 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 d in 32 ppt were not significantly correlated with length ($p = 0.08$).

Plasma thyroxine levels after 20 d in seawater were not significantly correlated with length (Table 3). Mean net change in thyroxine after 20 d 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 d in seawater were the same as initial freshwater levels. Net change in plasma thyroxine after 20 d 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 (Chapter 1). 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 controls (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 conditons) 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*.

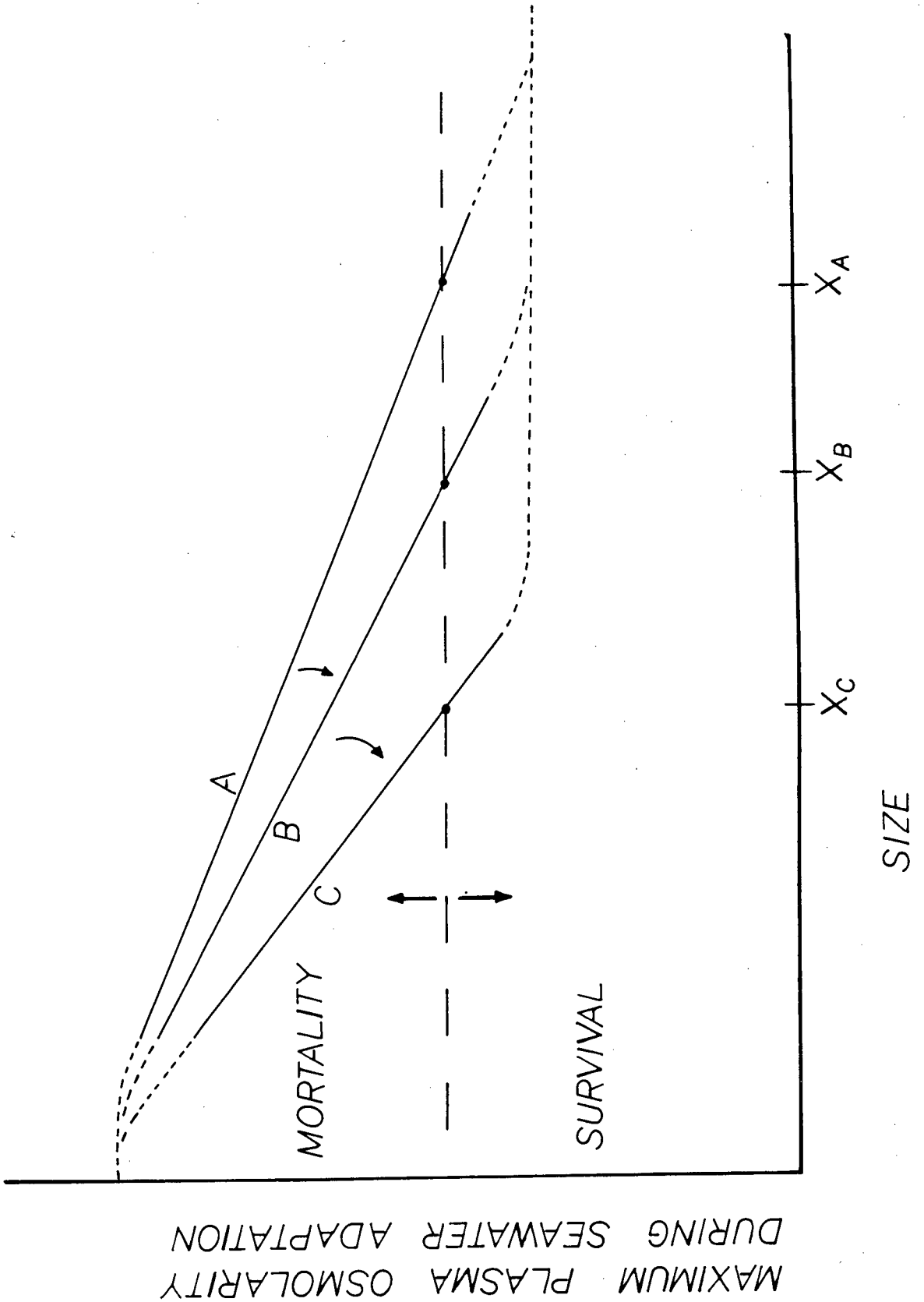
Within genera there may exist subgroups with significantly different sizes at which high seawater survival is attained. For instance, pink and chum salmon (*O. gorbuscha* and *O. keta*) survive in seawater at smaller sizes than other species of *Oncorhynchus* (Table 4; Weisbart, 1968).

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 osmoregualtion in a species of *Salvelinus*, line B *Salmo*, and

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

Species	Salinity Tolerance: Salinity (ppt)	Salinity Tolerance: Method of Acclimation	Salinity Tolerance: Duration (d)	Salinity Tolerance: Size at 75°/o Survival (cm)	Size at Seaward Migration(cm)	References
Oncorhynchus						
Pink Salmon	32	direct	> 14	< 6.0	2.5-4.0	(Weisbart, 1968; Scott and Crossman, 1973)
Chum Salmon	32	direct	> 14	< 6.0	3.2-7.0	(Weisbart, 1968; Scott and Crossman, 1973)
Chinook Salmon	30	direct	30	6.5	6.0-10.0	(Wagner et al., 1969; Healy, 1980)
	30	gradual	30	4.2		
Coho Salmon	30	direct	30	7.0	10.0-11.0	(Conte et al., 1966; Healy, 1980)
Salmo						
Atlantic Salmon	29	direct	44	13.8	12.7-15.2	(Johnston and Saunders, 1981; Scott and Crossman, 1973)
Steelhead Trout	30	direct	30	16.0	15.0-20.0	(Conte and Wagner, 1965; Wagner et al., 1963)
Salvelinus						
Brook Trout	32	gradual	20	19.0	15.0-18.0	(present study; Montgomery et al., unpublished manuscript)

Figure 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.


MAXIMUM PLASMA OSMOLARITY
DURING SEAWATER ADAPTATION
MORTALITY
SURVIVAL
A
B
C
X_A
X_B
X_C
SIZE

line C Oncorhychus; 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 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 hypoosomoregulatory 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 hypoosmoregulation.

The phylogenetic comparison of salmonid osmoregualtory physiology made here is in substantial agreement with that proposed by Rousnefell

(1958) and Hoar (1976). This phylogeny implies that increased exploitation of the sea by more advanced salmonids was acheived, in part, through changes in size dependent osmoregulatory ability. Physiological changes due to smoltification, desmoltification and maturation 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, unpublished data) 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 is salmonids can therefore be viewed as a succession of adaptations made to overcome size dependent ion transport capabilities.

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REFERENCES

Bath, R. N. & Eddy, F. B. (1979) Salt and water balance in rainbow trout (Salmo gairdneri) rapidly transferred from fresh water to sea water. J. exp. Biol. 83, 193-202.

Black, G. A. (1981) Metazoan parasites as indicators of movements of anadromous brook charr (Salvelinus fontinalis). Can. J. Zool. 59, 1892-1896.

Brett, J. R. (1979) Environmental factors and growth. In: Fish Physiology, Vol. VIII (edited by Hoar, W. S., Randall, D. J. & Brett, J. R.), pp. 599-675. Academic Press, New York

Castonguay, M., Fitzgerald, G. J. & Côté, Y. (1982) Life history and movements of anadromous brook charr, Salvelinus fontinalis, in the St. Jean River, Gaspé, Quebec. Can. J. Zool. 60, 3084-3091.

Clarke, W. C., Shelbourn, H. E. & Brett, J. R. (1978) Growth and adaptation to sea water in 'underyearling' sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon subjected to regimes of constant and changing temperature and daylength. Can. J. Zool. 56, 2413-2421.

Conte, F. P. & Wagner, H. H. (1965) Development of osmotic and ionic regulation in juvenile steelhead trout (Salmo gairdneri). Comp. Biochem. Physiol. 14, 603-620.

Conte, F. P., Wagner, H. H., Fesler, J. & Gnose, C. (1966) Development of osmotic and ionic regulation in juvenile salmon (Oncorhynchus kisutch). Comp. Biochem. Physiol. 18, 1-15.

Dickhoff, W. W., Folmar, L. C. & Gorbman, A. (1978) Changes in plasma thyroxine during smoltification of coho salmon, Oncorhynchus kisutch. Gen. Comp. Endocrinol. 36, 229-232.

Dixon, W. J. (1981) BMDP Statistical Software 1981. pp. 725. University of California Press, Berkeley.

Donaldson, E. M., Fagerlund, U. H. M., Higgs, D. A. & McBride, J. R. (1979) Hormonal enhancement of growth in fish. In: Fish Physiology, Vol VIII. (edited by Hoar, W. S., Randall, D. J. & Brett, J. R.), pp. 456-597. Academic Press, New York

Dumouchel, W. H. (1981) Documentation for DREG. Massachusetts Institute of Technology, Tech. Rept. No. 27.

Elson, P. F. (1957) The importance of size in the change from parr to smolt in Atlantic salmon. Can. Fish Cult. 21, 1-6.

Ewing, R. D., Johnson, S. L., Pribble, H. J. & Lichatowich, J. A. (1979) Temperature and photoperiod effects on gill Na^+,K^+-ATPase activity in chinook salmom (Oncorhynchus tshawytscha). J. Fish. Res. Bd. Can. 36, 1347-1353.

Farmer, G. J., Ritter, J. A. & Ashfield, D. (1978) Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, Salmo salar. J. Fish. Res. Bd. Can. 35, 93-100.

Folmar, L. C. & Dickhoff, W. W. (1979) Plasma thyroxine and gill Na^+,K^+-ATPase changes during seawater acclimation of coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. 63A, 329-332.

Forrest, J. N., Cohen, A. D., Schon, D. A. & Epstein, F. H. (1973) Na transport and Na-K ATPase in gills during adaptation to seawater - effects of cortisol. Am. J. Physiol. 224, 709-713.

Gordon, M. S. (1959) Ionic regulation in the brown trout (Salmo trutta L.). J. exp. Biol. 36, 227-251.

Grau, E. G., Specker, J. L., Nishioka, R. S. & Bern, H. A. (1982) Factors determining the occurence of the surge in thyroid activity during smoltification. Aquaculture 28, 49-57.

Healey, M. C. (1980) The ecology of juvenile salmon in Georgia Strait, British Columbia. In: Salmonid Ecosystems of the North Pacific. (edited by McNeil, W. J. & Himsworth, D. C.) Oregon State University Press, Corvallis.

Hoar, W. S. (1976) Smolt transformation: evolution, behavior and physiology. J. Fish. Res. Bd. Can. 33, 1234-1252.

Hossler, F. E. (1980) Gill arch of the mullet, Mugil cephalus. III. Rate of response to salinity change. Am. J. Physiol. 238, R160-R164.

Houston, A. H. (1961) Influence of size upon adaptation of steelhead trout (Salmo gairdneri) and chum salmon (Oncorhynchus keta) to seawater. J. Fish. Res. Bd. Can. 18, 401-415.

Huntsman, A. G. & Hoar, W. S. (1939) Resistance of Atlantic salmon to seawater. J. Fish. Res. Bd. Can. 4, 409-411.

Ismail-Beigi, F. & Edelman, I. S. (1970) Mechanism of thyroid calorigenesis: role of active transport. Proc. Nat. Acad. Sci. U.S.A. 67, 1071-1078.

Jackson, A. J. (1981) Osmotic regulation in rainbow trout (Salmo gairdneri) following transfer to sea water. Aquaculture, 24, 143-151.

Jobling, M. (1983) Growth studies with fish - overcoming the problems of size variation. J. Fish Biol. 22, 153-157.

Kirschner, L. B. (1980) Comparison of vertebrate salt secreting organs. Am. J. Physiol. 238, R219-R223.

Knoeppel, S. J., Atkins, D. L. & Packer, R. K. (1982) The role of the thyroid gland in osmotic and ionic regulation in Fundulus heteroclitus acclimated to freshwater and seawater. Comp. Biochem. Physiol. 73A, 25-29.

Leatherland, J. F. (1982) Environmental physiology of the teleostean thyroid gland: a review. Env. Biol. Fish. 7, 83-110.

Leitritz, E. & Lewis, R. C. (1976) Trout and Salmon Culture. Calif. Fish & Game Bull. 164. pp. 220.

Leray, C., Colin, D. A. & Florentz, A. (1981) Time course of osmotic adaptation and gill energetics of rainbow trout (Salmo gairdneri) following abrupt changes in external salinity. J. Comp. Physiol. 144, 175-181.

Mullan, J. W. (1958) The sea-run or "salter" brook trout (Salvelinus fontinalis) fishery of the coastal streams of Cape Cod, Massachusetts. Mass. Div, Fish and Game. Bull. No. 17,

Nordlie, F. G., Szelistowski, W. A. & Nordlie, W. C. (1982) Ontogenesis of osmotic regulation in striped mullet, Mugil cephalus L. J. Fish Biol. 20, 79-86.

Parry, G. (1958) Size and osmoregulation in salmonid fishes. Nature 181, 1218-1219.

Parry, G. (1966) Osmotic adaptation in fishes. Biol. Rev. 41, 392-444.

Prescott, P. (1975) An approximate test for outliers in linear regression. Technometrics 17, 129-132.

Rousnefell, G. A. (1958) Anadromy in North American Salmonidae. U.S. Fish Wildl. Serv. Fish. Bull. 58, 171-185.

Savage, J. D. & Robinson, G. D. (1983) Inducement of increased gill Na^+,K^+-ATPase activity by a hemolymph factor in hyperosmoregulating Callincetes sapidus. Comp. Biochem. Physiol. 75A, 65-69.

Scott, W. B. & Crossman, E. J. (1973) Freshwater Fishes of Canada. Fish. Res. Bd. Can. Bull. 184.

Smith, M. W. & Saunders, J. W. (1958) Movements of brook trout, Salvelinus fontinalis (Mitchill) between and within fresh and salt water. J. Fish. Res. Bd. Can. 15, 1403-1449.

Stagg, R. M. & Shuttleworth, T. J. (1982) Na^+-K^+ ATPase, ouabain binding and ouabain-sensitive oxygen consumption in gills from Platichthys flesus adapted to seawater and freshwater. J. Comp. Physiol. 147, 93-99.

Towle, D. W. (1977) Sodium pump sites in teleost gill: unmasking by detergent. Am. Zool. 17, 869.

Towle, D. W., Gillman, M. E. & Hempel, J. D. (1976) Rapid modulation of gill Na^+,K^+-ATPase activity during acclimation of the killifish, Fundulus heteroclitus. J. Exp. Zool. 202, 179-186.

Wagner, H. H. (1974) Seawater adaptation independent of photoperiod in steelhead trout (Salmo gairdneri). Can. J. Zool. 52, 805-812.

Wagner, H. H., Conte, F. P. & Fesler, J. L. (1969) Development of osmotic and ionic regulation in two races of chinook salmon, Oncorhynchus tshawytscha. Comp. Biochem. Physiol. 29, 325-341.

Wagner, H. H., Wallace, R. L. & Campbell, H. K. (1963) The seaward migration and return of hatchery-reared steelhead trout in the Alsea River, Oregon. Trans. Am. Fish. Soc. 92, 202-210.

Weisbart, M. (1968) Osmotic and ionic regulation in embryos, alevins, and fry of the five species of Pacific salmon. Can. J. Zool. 46, 385-397.

White, H. C. (1940) Life-history of sea-running brook trout (Salvelinus fontinalis) of Moser River, Nova Scotia. J. Fish. Res. Bd. Can. 5, 176-186.

Whoriskey, F. G., Naiman, R. J. & Montgomery, W. L. (1981) Experimental sea ranching of brook trout Salvelinus fontinalis Mitchill. J. Fish Biol. 19, 637-651.

Wilder, D. G. (1952) A comparative study of anadromous and freshwater populations of brook trout (Salvelinus fontinalis (Mitchill)). J. Fish. Res. Bd. Can. 9, 169-203.

Zaugg, W. S. (1982) A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aq. Sci. 39, 215-217.

Zaugg, W. S. & McLain, L. R. (1970) Adenosine triphosphatase activity in gills of salmonids: seasonal variation and salt water influence in coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. 35, 587-596.

Zaugg, W. S. & Wagner, H. H. (1973) The effect of photoperiod and temperature on gill adenosine triphospatase activity as related to parr-smolt transformation in steelhead trout (Salmo gairdneri). Comp. Biochem. Physiol. 45, 955-965.

CHAPTER 3

Hypoosmoregulation in an anadromous teleost: influence of sex and maturation.

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ABSTRACT

We report here decreased salinity tolerance and hypoosmoregulatory ability in mature male anadromous brook trout (Salvelinus fontinalis) that does not occur in females or immature males. Lowered salinity tolerance of adult males becomes acute during autumn photoperiod when normal spawning occurs. Plasma $[Cl^-]$, $[Mg^{2+}]$, osmolarity and hematocrit are 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 populations, limits the extent of anadromy, and was a significant phase in the evolution of extended salmonid migration.

Physiological differences between sexes of the same species are generally related to primary and secondary sexual characteristics[1] or to adaptations resulting from differential strategies for reproductive success[2]. Reports of divergence in non-sexual physiological processes are rare in lower vertebrates. Such differences occur in mammals and include breathing pattern[3,4], cardiac response[4,5], gastric secretion[6], metabolic rates[7,8], and oxygen consumption[7,8]. The ecological significance of this type of sexual dimorphism has not been established. We report here a decrease in hypoosmoregulatory ability of mature males of facultatively anadromous brook trout, Salvelinus fontinalis, which affects survival and can result in significant changes in the population dynamics of anadromous stocks.

Experiments exploring the effects of size, age and photoperiod on hypoosmoregulation in brook trout were conducted from 1980 to 1982 at the Woods Hole Oceanographic Institution. Twenty thousand brook trout eggs, the progeny of several males and females, were divided into two 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 mo behind normal daylength. Fish were fed ad libitum and all fresh water rearing and seawater exposures were performed at 10-11°C. Under these conditions maturation occurred in approximately 41°/o and 78°/o of the males and 5°/o and 38°/o of the females in the normal and lagged photoperiods, respectively, during their first autumn (age 0+ yr). All fish became mature during their second autumn (age 1+ yr).

Under summer and autumn photoperiods mature males had a significantly lower mean survival time in seawater than females ($p < 0.01$, Mann-Whitney U-test, see Fig. 1a,b). 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°/o and 7°/o survival, respectively, after 64 d in seawater, $p < 0.01$, 2x2 contingency table). Survival of control fish in freshwater was 100°/o 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 being significantly lower than that in summer or spring ($p < 0.01$ 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) implicating 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 d and 43 d, respectively, $p = 0.21$, Mann-Whitney U-test).

To determine the physiological basis of differences in salinity tolerance, mature male and female brook trout were acclimated for one week at a salinity of 10 ppt, one week at 20 ppt and finally exposed to 32 ppt seawater. This experiment was conducted during a declining photoperiod (11.2 hr daylength), just prior to spawning. After 4 d in 32 ppt seawater, fish were removed from tanks and anesthetized with

phenoxyethanol. After 30-60 s of anesthetization, blood was collected from the dorsal aorta and hematocrit, plasma [Na^+], [Cl^-], [K^+], [Mg^{2+}] and osmolarity measured. Gill arches were removed, frozen at -17°C and analyzed within 40 d for Na^+, K^+-ATPase activity[10].

Plasma ion and osmotic concentrations analyses indicated that hypoosmoregulatory and ionoregulatory abilities differed between sexes during maturation. Male brook trout of comparable length and weight had a significantly higher plasma osmotic concentration, higher plasma [Cl^-] and [Mg^{2+}], and a higher hematocrit than mature females ($p < 0.05$, student's t-test; Fig. 2). Plasma [K^+] was significantly lower in males than females. We found [K^+] to be regulated in a manner different from other plasma ions; fresh water levels of [K^+] were often maintained for 4 d after exposure to seawater, while plasma [Na^+], [Cl^-], [Mg^{2+}] and osmolarity increased within 12 hr and peaked between 3-4 d. With the exception of hematocrit, there were no significant sexual differences in freshwater physiology ($p > 0.10$, student's t-test; freshwater hematocrit values during final maturation were 58‰ for males and 44‰ for females, $p < 0.01$ student's t-test).

Despite the superior osmoregulatory ability of mature female brook trout, activity of gill Na^+, K^+-ATPase was not significantly higher in females (Fig. 2). Freshwater values ranged between 5 and 12 $\mu MP_i \cdot$ mg protein^{-1} $\cdot hr^{-1}$, and did not differ by sex during autumn ($p = 0.49$, student's t-test). Elevated levels of gill Na^+, K^+-ATPase activity in mature males indicates that this seawater adaptation mechanism is operative; other ion transport or permeability

Figure 1. Upper: Mean seawater survival time of mature and immature male and female brook trout gradually acclimated to 32 ppt seawater Fish were maintained in 1,000 L tanks from first feeding (age 30 d). At the time of seawater acclimation, fish were exposed in the same tanks by addition of seawater. Salinity was increased in a stepwise manner for a period of 14-30 d. Fish in each experiment were held in 32 ppt seawater for a period of 64 d, which represented the maximum seawater survival time. Experiments a and b were conducted on the same calendar date; experiment a was under normal photoperiod conditions; photoperiod in experiment b cycled 3 mo behind the norm. 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 experiment a and b were not significantly different in length, weight and condition factor (18.9 cm and 18.4 cm, 80.4 g and 70.3 g, 1.18 and 1.12, respectively, Student-Newman-Keuls procedure). Fish in experiment c were larger, heavier and had slightly greater condition factor (24.2 cm, 185.4 g, and 1.26 respectively, $p < 0.05$, 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 fully mature during autumn photoperiod. Other experiments conducted during autumn photoperiod have shown that seawater survival of mature females did not decline during spawning period and was significantly greater than seawater survival of mature males.
Lower: Daylength conditions and mean mature male gonadosomatic index (GSI = [gonad weight/body weight] ·100). Photoperiod month (ordinate) is calendar month normally corresponding to the shown daylength. Photoperiod determined timing of maturation under both normal and 3 mo delayed photoperiod. Gonadosomatic indexes shown here are mean values of mature males in 3 mo 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.

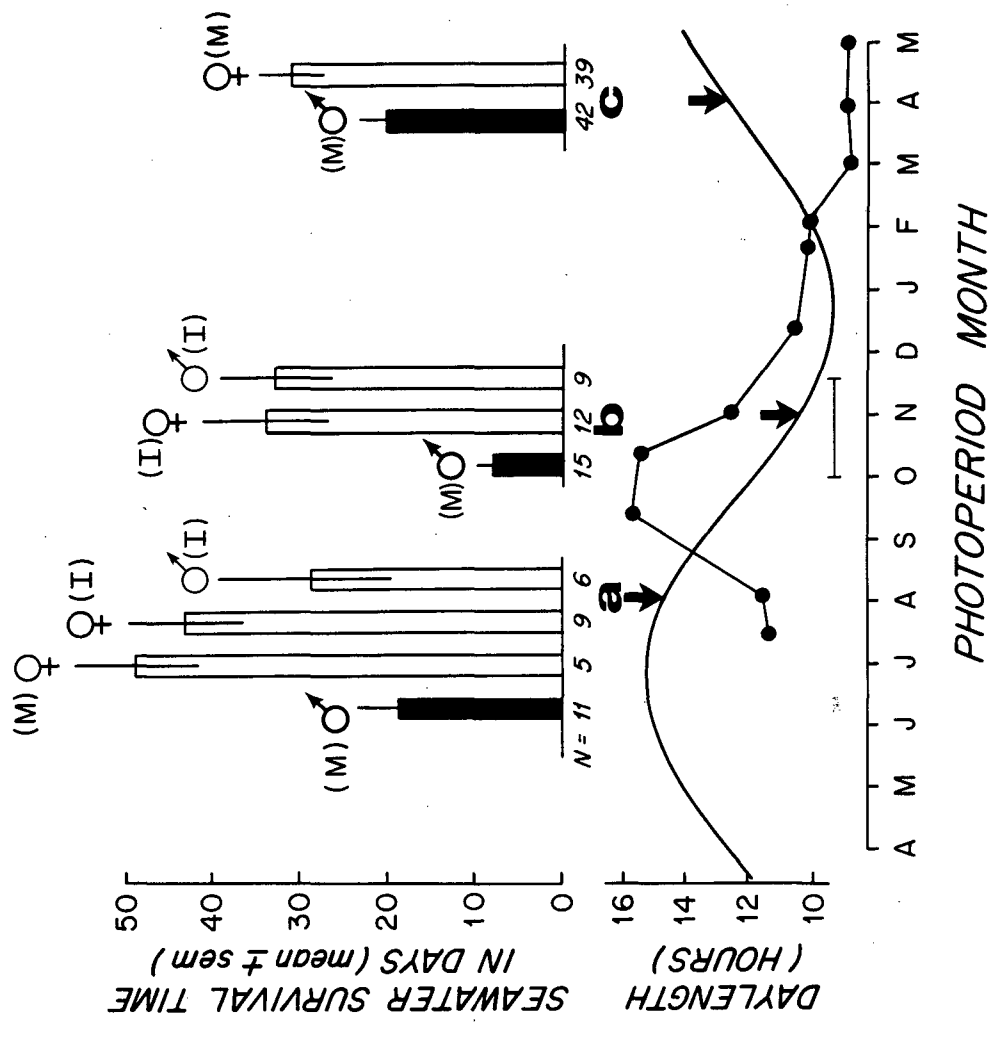
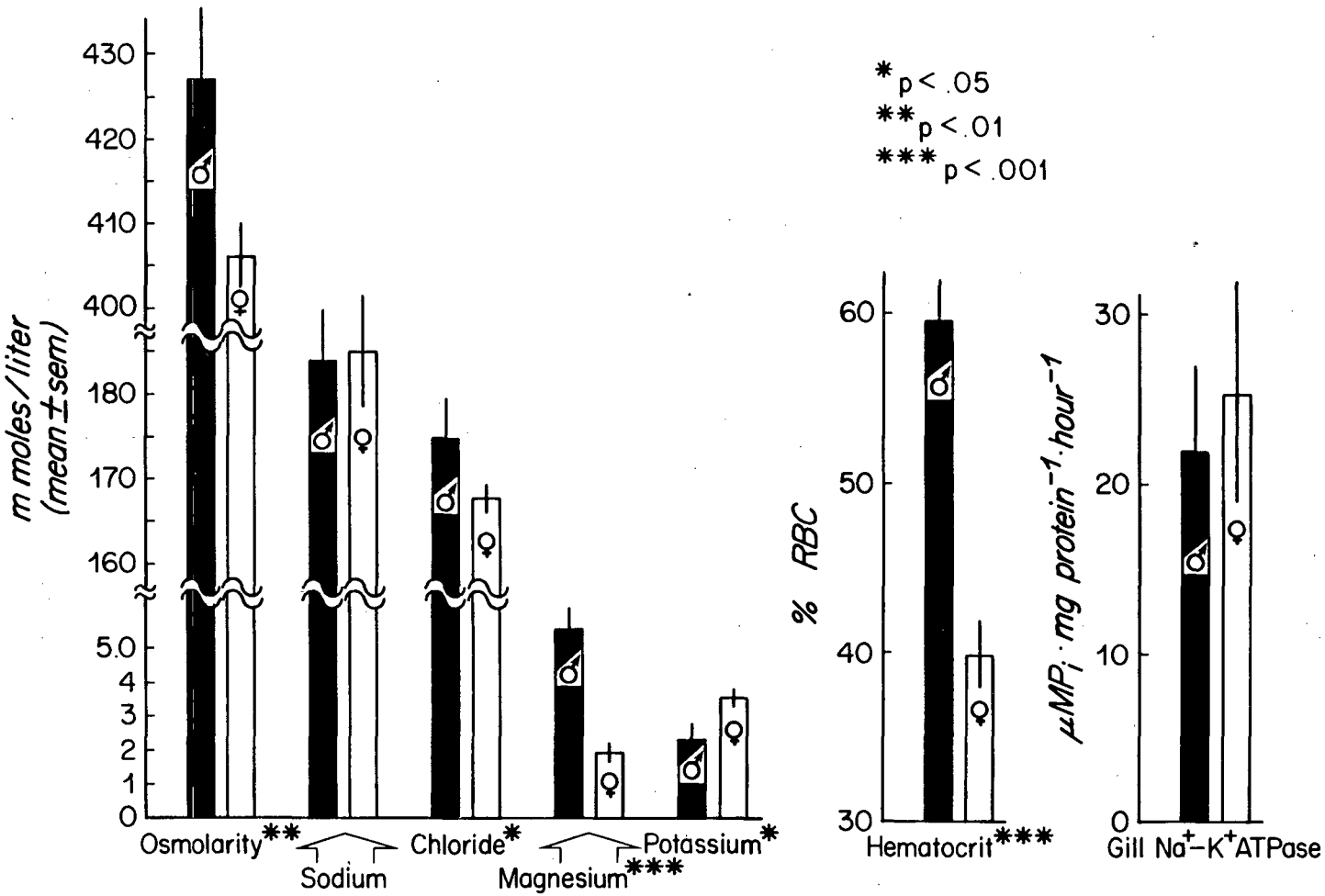

GONADOSOMATIC INDEX
[(Gonad wt./Body wt.)100]
0 1 2 3 4 5
PHOTOPERIOD MONTH
A M J J A S O N D J F M A M
DAYLENGTH (HOURS)
16 14 12 10
SEAWATER SURVIVAL TIME IN DAYS (mean ± sem)
50 40 30 20 10 0
N = 11 5 9 6
15 12 9
42 39
a
b
c
♂(M)
♀(M)
♂(I)
♀(I)

Figure 2. Physiological comparison of mature male and female brook trout 4 d after exposure to 32 ppt seawater Fish were first acclimated for 7 d in 10 ppt and 7 d in 20 ppt. Student's t test was used to determine statistically significant differences between means. Mean (± 1 standard error of the mean) length, weight and gonadosomatic index (GSI) of fish in this experiment are as follows:

	Sample Size	Fork Length (cm)	Weight (g)	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)


m moles/liter (mean ± sem)
430
420
410
400
180
170
160
5.0
4
3
2
1
0
Osmolarity**
Sodium
Chloride*
Magnesium***
Potassium*
* p < .05
** p < .01
*** p < .001
% RBC
60
50
40
30
Hematocrit***
μMPi · mg protein⁻¹ · hour⁻¹
30
20
10
0
Gill Na+–K+ATPase

properties must be responsible for the observed differences between mature males and females.

These results establish two separate but related phenomena: 1) adult male salinity tolerance is lower than adult females during spring, summer and autumn; 2) adult male salinity tolerance and hypoosmoregulatory abilities reach lowest levels in autumn and are related to sexual maturation. Hydration of sperm during this period may present a larger water demand for males that cannot be met under saline conditions. It was also noted that mature, fully ripe males in both freshwater and seawater had "stickier" skin than females or immature males, indicating the possible reduction of mucous production by mature males. The epidermis of mature male migratory sea trout (Salmo trutta L.) has only a single layer of epithelial cells and no mucous cells[11], which may result in altered skin permeability and impose hardships under hyperosmotic conditions. Red sores on the skin of moribund mature males in seawater indicate that the skin may be involved in osmotic failure of these animals. Sperm hydration and changes in the skin-scale-mucus complex are possibly under gonadotropin control, implicating an indirect role of the pituitary in hypoosmoregulation.

The proximate cause of reduced salinity tolerance of adult males in spring and summer is less tractable. Several hypotheses seem plausible of which we consider two to be most suitable: (1) there is a competing physiological demand specific for mature males, or (2) hormonal control of maturation in males is linked to hormonal control of ion and water transport. These hypotheses are not mutually exclusive and possibly relate to the cause of autumnal decreases in

mature male hypoosmoregulation. Competing physiological demands for ion transport occur in teleosts and include trade offs with oxygen transport[12] and pH balance[13].

It seems unlikely that sex steroids directly control osmoregulation throughout the year. Effects of sex steroids on other hormonal systems, however, could result in different ion transport properties between males and females. Alternatively, maturation and osmoregulatory ability need not be immediately connected, but could have a common and early cause. For example, it has been demonstrated that smolting (which is accompanied by increases in hypoosmoregulatory ability) and precocious male maturity are incompatible in the same year for Atlantic salmon (Salmo salar), and that the decision to smolt or to mature is made within a few months of hatching[14].

Seaward migration of anadromous brook trout in northern latitudes of North America occurs in late spring[15-18]. 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 (ref. 15-18 and Montgomery, W. L. et al., unpublished manuscript), four have sex-ratios skewed towards a greater number of females. Low coastal salinity may explain the equal sex ratio observed in one anadromous population[18]. Skewed sex-ratios do not occur in nearby freshwater populations of brook trout[17]. That female-dominated sex ratios occur on the spawning grounds[15], as well as among seaward migrators[15-17] and freshwater returning migrators[15-16], suggests that male-female behavioral and physiological differences occur in nature.

Physiological tolerances of critical life-history stages limit population sizes and spatial distribution of species[20]. 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 established a 'primitive' level for Salvelinus relative to other salmonids[19]. Sex and maturational differences in hypoosmoregulatory ability by brook trout may represent a characteristic of early salmonids which 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 are, in part, a solution to conflicting physiological demands of maturation and hypoosmoregulation.

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REFERENCES

1. Naftolin, F. Science 211, 1263-1264 (1981).
2. Darwin, C. R. The Decent of Man, and Selection in Relation to Sex (J. Murray, London, 1871).
3. White, D. P. et al. J. Appl. Physiol. 54, 874-879 (1983).
4. Sebert, Ph. Eur. J. Appl. Physiol. 50, 421-428 (1983).
5. Sanchez, J. and Ph. Sebert. Eur. J. Appl. Physiol. 50, 429-444 (1983).
6. Grossman, M. I. et al. Gastroenterology 45, 14-26 (1963).
7. Kibler, H. H. and S. Brody. J. Nutr. 33, 331-338 (1947).
8. Dubois, E. F. Basal Metabolism in Health and Disease (Lea and Febiger, Philadelphia, 1936).
9. Krebs, J. R. and N. B. Davies. An Introduction to Behavioral Ecology (Singer Assoc. Inc., Sunderland, MA., 1981).
10. Zaugg, W. S. Can. J. Fish. Aquat. Sci. 39, 215-217 (1982).
11. Stoklosowa, S. Copeia 1966, 613-614 (1966).
12. Randall, D. J. et al. Comp. Biochem. Physiol. 41A, 629-637 (1972).
13. Booth, J. H. et al. Can. J. Zool. 60, 1123-1133 (1982).
14. Saunders, R. H. et al. Aquaculture 28, 211-230 (1982).
15. White, H. C. J. Fish Res. Bd. Can. 5, 176-186 (1940).
16. Wilder, D. G. J. Fish. Res. Bd. Can. 9, 169-202 (1952).
17. Castonguay, M. et al. Can. J. Zool. 60, 3084-3091 (1982).
18. Dutil, J. D. and G. Power. Can. J. Zool. 58, 1828-1835 (1980).
19. Hoar, W. S. J. Fish. Res. Bd. Can. 33, 1233-1252 (1976).
20. Braum, T. in Ecology of Freshwater Fish Production (ed Gerking, S. D.) 102-131 (John Wiley a Sons, New York, 1978)

CHAPTER 4

The Physiology of Smoltification in Anadromous and Non-anadromous Brook Trout (*Salvelinus fontinalis*) and Atlantic Salmon (*Salmo salar*) from the Matamek River and Rivière à la Truite, Québec.

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ABSTRACT

Anadromous brook trout, Salvelinus fontinalis, of Rivière à la Truite, Québec, were examined for physiological changes associated with salmonid smoltification, and compared to non-anadromous brook trout of the adjacent Matamek River. There were no significant differences in plasma thyroxine concentration, gill Na^+,K^+-ATPase activity, hematocrit or osmoregulatory ability between anadromous and non-anadromous brook trout. Moisture content was significantly different between fish from the two river systems, but the same pattern of declining moisture content as summer progressed existed in both groups. Silver coloration of brook trout in Rivière à la Truite was significantly associated with larger fish and higher gill Na^+,K^+-ATPase activity, but not with changes in plasma thyroxine concentration, moisture content, hematocrit or condition factor. Silver coloration was absent in Matamek River brook trout. Brook trout at high salinity estuarine sites had significantly greater gill Na^+,K^+-ATPase activity and hypoosmoregulatory ability than brook trout at low salinity sites. Silvering of Atlantic Salmon (Salmo salar) in Rivière à la Truite was significantly associated with larger fish, higher gill Na^+,K^+-ATPase activity, higher plasma thyroxine and lower hematocrit. Gill Na^+,K^+-ATPase activity of highly silvered Atlantic salmon was greater than that of highly silvered brook trout. Atlantic salmon in high salinity estuarine sites had significantly higher plasma thyroxine concentration and gill Na^+,K^+-ATPase activity than brook trout. The results indicate that smoltification is relatively undeveloped in brook trout and that estuarine residence is important in their salt water acclimation and eventual seaward migration.

INTRODUCTION

The physiology of the parr-smolt transformation in anadromous salmonids has received much attention in recent years due, in part, to the realization that production of quality smolts is limiting returns of hatchery reared salmon (Wedemeyer et al., 1980). Research has concentrated on Pacific salmon (Oncorhynchus spp.), Atlantic salmon (Salmo salar) and steelhead trout (Salmo gairdneri), which are regarded as the most advanced salmonids (Rousnefell, 1958). The outward signs of smolting (silvering and seaward migration), however, occur in brook trout, Salvelinus fontinalis (White, 1940; Wilder, 1952; Black, 1981; Castonguay et al., 1982). The charrs (genus Salvelinus) are thought to be most like the earliest anadromous salmonids (Rousnefell, 1958). Smoltification may have arisen in the charrs, and it follows that the phylogeny of smoltification is incomplete without an understanding of smolting in this pivotal group.

Brook trout are endemic to eastern North America ranging from 35 to 60 ^{o}N latitude (Scott and Crossman, 1973). Anadromous populations exist above 41 ^{o}N in streams and rivers that have access to coastal waters. Seaward migration in northern latitudes is characterized by downstream movement in spring, residence in estuarine or coastal waters for 2-4 mo, followed by upstream migration in autumn (White, 1940; Castonguay et al., 1982). Montgomery et al. (1983, and unpublished data), studying sea-run brook trout of Rivière à la Truite (a tributary of the lower Moisie River, Québec), found seaward migration to be highly synchronized between individuals. In the southern portion of their range, the timing and duration of seaward migration is more variable (Mullan, 1958; Smith and Saunders, 1958).

In addition to seasonal aspects of migration, size dependent migration has been reported for all sea-run brook trout populations (White, 1940; Wilder, 1952; Smith and Saunders, 1958; Dutil and Power, 1980; Castonguay et al., 1982). Size dependent and seasonally cued seaward migratory activity are characteristic of all smolting salmonids. Smoltification involves environmentally induced, hormonally regulated changes in morphology, behavior, biochemical composition and osmoregulatory physiology which are presumably adaptive to seawater entry (see Hoar, 1976; Folmar and Dickhoff, 1980; Wedemeyer et al., 1980, for review). For the purposes of this investigation , a smolt is defined as a salmonid in freshwater which has undergone all of the metamorphic and physiological changes which are common to all known smolting salmonid species. Prominent among these common characteristics are increases in hypoosmoregulatory ability, gill Na^+,K^+-ATPase activity, plasma thyroxine, skin and scale deposition of guanine and hypoxanthine (silvering), body moisture content, and decreases in condition factor and body lipid levels (Wedemeyer et al., 1980).

Previous work has shown that physiological changes associated with smoltification do not occur in a Massachusetts hatchery stock of anadromous brook trout (Chapter 1). However, this strain does not exhibit silvering and migratory synchrony characteristic of northern populations. The possibility remains that northerly, non-hatchery stocks undergo smoltification.

Anadromous brook trout in eastern Canada currently support a valuable recreational fishery (Yvon Côté, personal communication). Experimental sea ranching of brook trout has resulted in 30-60^{o}/o

return rates and growth rates of 4-5 times that of their cohorts remaining in freshwater (Whoriskey et al., 1981). Knowledge of preparatory physiological adaptations for seawater entry in brook trout would aid our understanding of the natural anadromy of brook trout as well as the use of this species for natural enhancement, sea ranching and farming.

The objectives of this study were to determine whether physiological changes associated with smoltification occur in northern anadromous brook trout, and whether these changes are prepartory for seawater entry. To this end we examined an anadromous population of brook trout from Rivière à la Truite, Québec, for changes in plasma thyroxine concentration, gill Na^+,K^+-ATPase activity, silvering, moisture content, hematocrit, condition factor and hypoosmoregulatory ability, and compared them to non-anadromous brook trout from the adjacent Matamek River. Brook trout in both freshwater and the respective estuaries were examined. Brook trout in the Matamek river estuary have been washed over the 1^{st} Falls and do not contribute reproductively to the river population. Our ability to detect the process of smoltification under natural conditions was verified by examining the physiology of Atlantic salmon. Smoltification under artificial rearing conditions has been well-studied (Wedemeyer et al., 1980), and includes changes in the morphological and physiological characteristics listed above. Atlantic salmon parr and smolt captured in Rivière à la Truite and the Matamek River estuary were examined for comparative purposes.

STUDY SITES

The Moisie and Matamek rivers empty into the Gulf of St. Lawrence approximately 22 and 36 km east of Sept-Isles, Québec, respectively (Fig. 1). Rivière à la Truite is a 4th order stream entering the Moisie River 14 km upstream of the Gulf of St. Lawrence. Rivière à la Truite has an average width of 10 m, and a maximum mid-summer depth of 2 m. Our study site on Rivière à la Truite was located 0.4 km upstream of its confluence with the Moisie River.

The Moisie River broadens into a 2 km wide estuary. Sandbars restrict confluence with the Gulf of St. Lawrence to a width of 0.25 km. Two sites were chosen in the Moisie River estuary: one was located 2 km upstream of the Gulf of St. Lawrence; the second was at the confluence of the river with the Gulf (Fig. 1). The Moisie River estuary mouth site is characterized by higher salinities than the upstream site (Table 1; Montgomery, 1980).

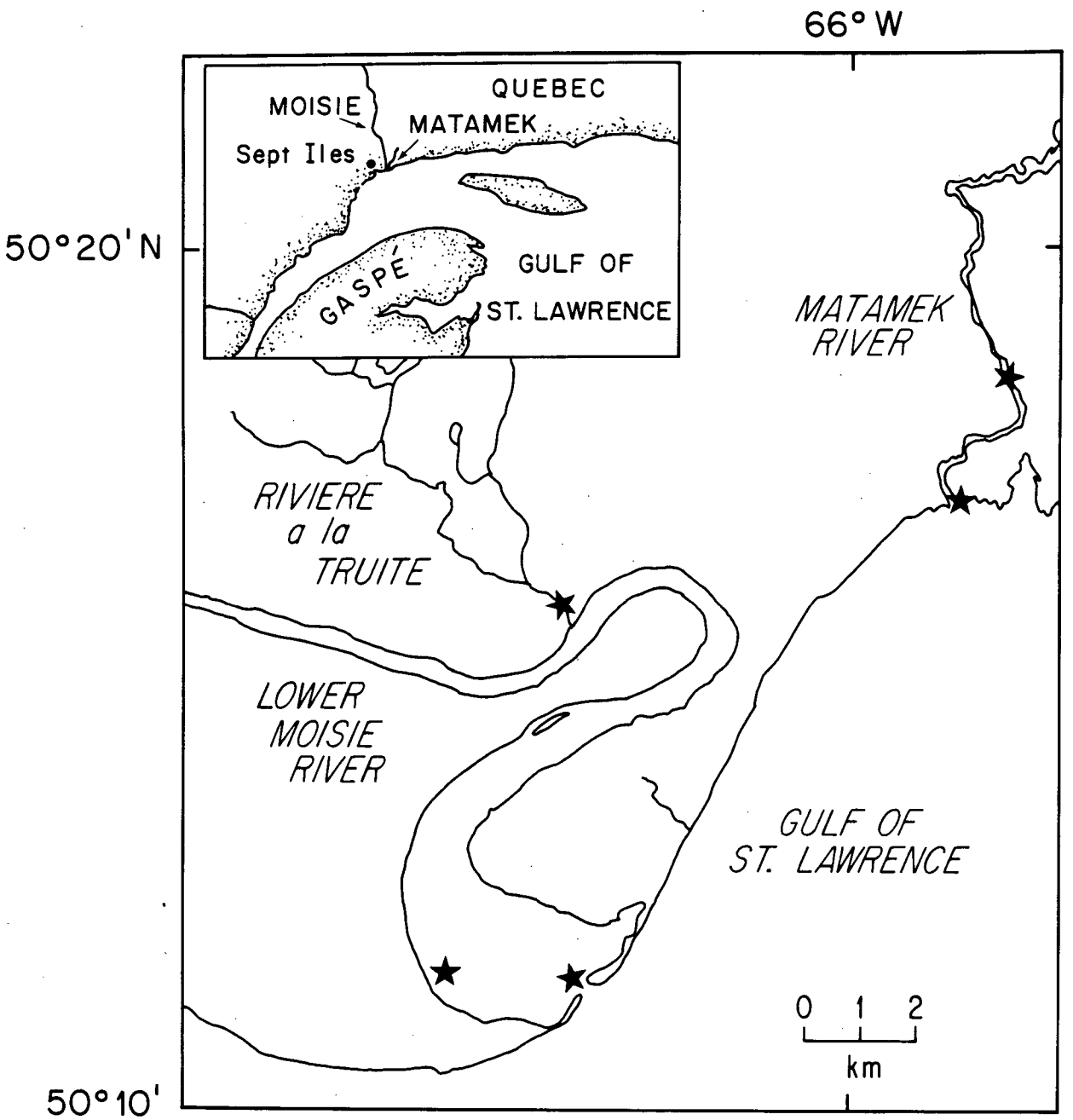
The Matamek River (6th order) drains a watershed separate from that of the Moisie River. The Matamek River averages 50 m wide and passes over 5 waterfalls from Matamek Lake to the Gulf of St. Lawrence (9.6 km). The 2^{nd} Falls is a barrier to upstream migration of brook trout (Naiman, unpublished data). Sampling of brook trout in the Matamek River occurred at the base of the 3^{rd} Falls.

The Matamek River estuary averages 80 m wide; saline water can intrude nearly to the base of the 1^{st} Falls. Brook trout in the Matamek River estuary are considered to be a non-reproducing population that is replenished by fish that are washed over the 1^{st} Falls. This is due to the rare migration of brook trout over the

Table 1. Physical characteristics of Moisie River estuary and Matamek River estuary sampling sites. Moisie and Matamek River estuaries were sampled from July 2 to August 31, and June 4 to July 29, respectively. Although no salinity change was detected at the Moisie River estuary upstream site, some salt water intrusion occurs at this site and beyond (Montgomery, 1980).

	Moisie River estuary		Matamek River estuary
	Upstream	Mouth	
Temperature (C)	12-17	10-16	10-15
Salinity (ppt)	0	0-27	5-27
Maximum Depth (m)	6-7	6-7	5-6
Tidal Influence	yes	yes	yes

Figure 1. Freshwater and estuarine sampling sites. Anadromous brook trout were captured at Rivière à la Truite and at two sites in the Moisie River estuary. Non-anadramous brook trout were captured above the second Falls of the Matamek River, a barrier to upstream brook trout migration.


66° W
50°20'N
50°10'
QUEBEC
MOISIE
MATAMEK
Sept Iles
GASPÉ
GULF OF
ST. LAWRENCE
MATAMEK
RIVER
RIVIERE
a la
TRUITE
LOWER
MOISIE
RIVER
GULF OF
ST. LAWRENCE
0 1 2
km

1st Falls, and the lack of suitable spawning areas below the Falls (Naiman, umpublished data).

MATERIALS AND METHODS

Sampling Methods

In Rivière à la Truite, two Fyke nets spanning the river were placed so that one faced upstream and one faced downstream. Nets were checked and emptied daily. Details of capture methodology are reported in Montgomery et al., (1983). Fish in the Matamek River were captured by Fyke nets (checked and emptied daily or every other day) or beach seine. Physical characteristics of Rivière à la Truite and Matamek River are detailed in Fig. 2. Sampling of brook trout in the Moisie River estuary was done with beach seines and occurred during the day within 2 h of high tide. Atlantic salmon smolts were not captured in the Moisie River estuary, presumably due to their early migration and brief residence in the estuary. In the Matamek River estuary brook trout and Atlantic salmon were sampled with beach seine at night within 2 h of high tide.

After capture, fish were examined for degree of silvering, fork length was measured to the nearest mm, and fish were weighed to the nearest 0.1 g. Conditon factor (CF) was calculated as follows:

$$CF = [\text{weight} \cdot (\text{length}^3)^{-1}] \cdot 100$$

where weight is wet weight in g and length is fork length in cm. Degree of silvering was determined by inspection using the following criteria: (1) No silvering; (2) Partial silvering, many scales reflective and silver, but parr marks and/or vermiculation pattern on dorsal surface clearly visible; (3) Full silvering, entire body

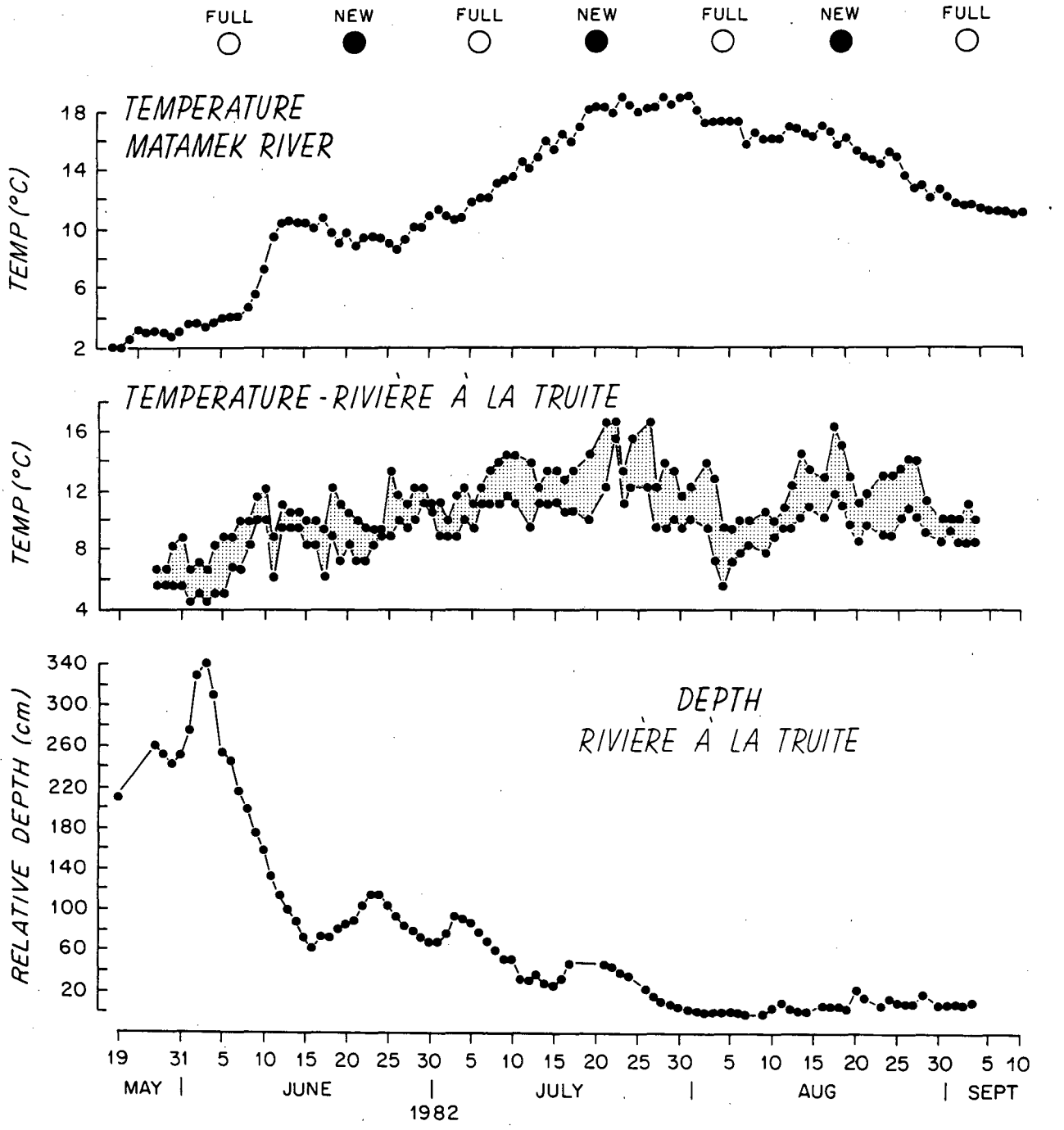
surface is reflective and silver, parr marks and vermiculation pattern not clearly visible.

Analytical Techniques

Blood and gill sampling occurred in the field within 15 min of capture. Due to the difficulty of getting sufficient blood from small animals, only fish 8.5 cm fork length were used in physiological analysis. Fish were anesthetized in 0.4 ml/l phenoxyethanol solution for 30-60 s. After anesthetization, the caudal fin was severed and blood collected into two ammonium heparinized capillary tubes which were sealed at one end. Gill arches were removed and 0.05-0.2 g (wet weight) primary gill filament was trimmed from ceratobranchials and placed in 1 ml Sucrose-EDTA-imidazole (SEI) solution (0.3 mmole/l sucrose, 0.02 mmole/l disodium ethylenediamine tetraacetate and 0.1 mmole/l imidazole adjusted to a final pH of 7.1 with HCl). Blood and gill samples and fish carcasses were placed on ice and transported within 30 min to the laboratory. Hematocrit tubes were centrifuged for 5 min at 5500 rpm, hematocrit (^{o}/o red blood cells) read, and plasma removed. Duplicate 25 1 plasma samples and gill samples were stored at -17 C for later analysis of thyroxine concentrations and gill Na^{+},K^{+}-ATPase activity.

Plasma osmolarity was measured immediately after centrifugation using a Wescor Vapor Pressure Osmometer (intraassay coefficient of variation $\pm$ 1.0^{o}/o, N = 5). Body moisture content (^{o}/o water) was determined by drying the central portion of the body (excluding head and tail and including viscera) at 60 C to a constant weight. Plasma thyroxine was analyzed by competitive binding radioimmunoassay

Figure 2. Temperature and depth chatacteristics of Rivière à la Truite and the Matamek River from May 19 to September 3, 1982. Ice-off occurred on May 13 and May 3 for Rivière à la Truite and the Matamek River, respectively.


FULL
NEW
FULL
NEW
FULL
NEW
FULL
TEMPERATURE
MATAMEK RIVER
TEMP (°C)
18
14
10
6
2
TEMPERATURE - RIVIÈRE À LA TRUITE
TEMP (°C)
16
12
8
4
DEPTH
RIVIÈRE À LA TRUITE
RELATIVE DEPTH (cm)
340
300
260
220
180
140
100
60
20
19 31 5 10 15 20 25 30 5 10 15 20 25 30 5 10 15 20 25 30 5 10
MAY | JUNE | JULY | AUG | SEPT
1982

(Dickhoff et al., 1978). Gill Na^+,K^+-ATPase activity was determined by the method of Zaugg (1982). Details of these techniques can be found in Chapters 1 and 2.

Frozen blood and gill samples were transported to Woods Hole Oceanographic Institution for analysis of plasma thyroxine concentration and gill Na^+,K^+-ATPase activity. During transit (8 h), partial thawing of some samples occurred. Repeated freezing and thawing does not affect the concentration of plasma thyroxine (Reimers et al., 1982). To determine the possible affect of thawing and refreezing on measurement of gill Na^+,K^+-ATPase activity, duplicate gill samples from 5 laboratory reared brook trout were frozen in SEI solution at -17 C. After 2 wk of storage, one set of duplicates was kept at 4 C for 16 h and refrozen. Gill Na^+,K^+-ATPase activity of the thawed and refrozen samples decreased an average of 8.3 o/o ($\pm$ 3.6 o/o) from their unfrozen duplicates. This effect is smaller than the interassay variation for this technique (coefficient of variation: 21 o/o).

Seawater Challenge

Clarke and Blackburn (1978) used a 24 h seawater challenge test to measure the hypoosmoregulatory ability and degree of smoltification of chinook (*O. tshawytscha*) and coho salmon (*O. kisutch*), so as to judge the correct time for release of hatchery stocks. Fish were transferred directly from freshwater to seawater and plasma [Na+] measured 24 h later. We adopted a similar technique to measure the hypoosmoregulatory ability of brook trout. Gulf of St. Lawrence seawater (28 ppt) was supplemented with Instant Ocean salt to a

salinity of 32 ppt in order to provide sufficient salinity stress. Seawater challenges were conducted in a 400 l aquarium maintained at 10 ± 0.5 C, an average spring temperature for both rivers and their estuaries. To avoid over-crowding, no more than 12 fish were used in the aquarium at one time. Ammonia levels were checked periodically. Fish used in seawater challenge tests were transported to the laboratory within 15-45 minutes of capture and placed directly in the seawater aquarium. Care was taken to prevent temperature changes (± 1 C) or oxygen depletion during transport. After 24 h (± 15 min), fish were removed from the aquarium, anesthetized in phenoxyethanol-seawater solution, and blood samples taken and analyzed as previously described.

In Rivière à la Truite, downstream migrating brook trout between 9.5 and 19.5 cm (13.5 ± 1.0, mean ± 1 S.E.) fork length (captured between June 8-24) were subjected to a 24 h seawater challenge. Brook trout from the Matamek River were 14.0-20.2 cm (17.5 ± 0.7) fork length (captured between August 2-29) and were also seawater challenged. Length and date of capture of estuarine fish used in seawater challenge are reported in the text.

Statistical Methods

Two-way Analysis of Variance (ANOVA) was used to determine the significance of physiological differences between Rivière à la Truite and Matamek River brook trout. One-way ANOVA was used to determine the significance of within river changes in brook trout physiology over time, differences among fish based on silvering characteristics, and differences between estuarine brook trout and Atlantic salmon. The F_{max} test was used to determine the homogeneity of sample

variances (Sokal and Rohlf, 1981). In cases where variances were heterogeneous, the data were log transformed to reduce variance heterogeneity. The Scheffé method was used in a posteriori tests of differences among means. Confidence level for all statistical tests of significance was 95 ^{o}/o, unless otherwise stated. Means are reported as the arithmetic mean $\pm$ 1 standard error of the mean.

RESULTS

Freshwater Studies: Rivière à la Truite and Matamek River

There was no silvering in any of the brook trout sampled in the Matamek River; significantly greater silvering occurred among fish in Rivière à la Truite ($p < 0.01$; Fig. 3). Moisture content was also significantly greater in Rivière à la Truite brook trout ($p < 0.01$). There were no significant differences, however, in plasma thyroxine, gill Na^+,K^+-ATPase activity or hematocrit between fish from the two rivers ($p > 0.10$, Fig. 3).

Plasma thyroxine levels of brook trout in Rivière à la Truite were significantly higher during periods of downstream movement (June) than during upstream movement (July and August). Matamek River brook trout did not display a significant change in plasma T_4 over time. Moisture content and hematocrit had a similar pattern for fish in each river; moisture content declines with time while hematocrit increases with time (Fig. 3). We did not make comparisons of gill Na^+,K^+-ATPase activities within rivers over time; use of high temperature incubations of gill homogenates (e.g. 36 C in our method) will underestimate gill Na^+,K^+-ATPase activities of fish

acclimated to high temperatures, such as those that exist in mid-summer (McCarthy and Houston, 1977).

Brook trout from Rivière à la Truite captured during the period of peak downstream migration (May 20 to June 30) were examined for physiological smolt characteristics (Fig. 4). Brook trout possessing more silvering were larger and had higher gill Na^+,K^+-ATPase activity ($p < 0.02$). There were no significant differences in plasma thyroxine, moisture content, hematocrit or condition factor among brook trout grouped by silvering ($p > 0.10$). Hematocrit and moisture content of downstream migrating fish were correlated with date of capture (day 1 = May 20, day 41 = June 30; $r = 0.61$ and -0.31, $p < 0.01$ and 0.05, respectively) and were significantly correlated with one another ($r = -0.28$, $p < 0.05$, $N = 51$). With the exception of silvering, fork length did not significantly correlate with any of the measured physiological variables.

Atlantic salmon captured in Rivière à la Truite were also examined for physiological smolt characteristics (Table 2). Atlantic salmon with high silvering were significantly larger and had significantly greater gill Na^+,K^+-ATPase activity and plasma thyroxine than fish with intermediate and no silvering (these latter two groups were combined because of small sample sizes). Hematocrit was significantly lower in highly silvered Atlantic salmon. Moisture content and condition factor were not significantly different between the two groups.

Figure 3. Movements and physiology of anadromous brook trout in Rivière à la Truite and non-migratory brook trout of the Matamek River. Downstream and upstream movements of brook trout are daily captures of upstream and downstream facing nets. Dashed lines indicate periods when net was washed away by high water. Fish captured were divided into 7 time intervals by date of capture (four 10 d, one 20 d and two 25 d intervals), so that each time interval had approximately equal sample size. Brook trout sampled in Rivière à la Truite from late May to mid-July were moving downstream, those sampled in late July and August were moving upstream. Catches in the Matamek River did not vary greatly over the 3.5 mo of sampling, averaging 1 fish per d. p values (in parenthesis) are the significance of physiological differences between brook trout from the two rivers (two-way ANOVA). Horizontal bars represent a posteriori differences among time interval means within rivers (one-way ANOVA, Scheffé test). Because the Scheffé test uses 'experiment-wise' errors (and is therefore a stricter test than ANOVA), significant differences found by one-way ANOVA were not always found by the Scheffé test. Time intervals not connected by horizontal bars are significantly different from one another at the 95^{o}/o confidence level. For example, Rivière à la Truite brook trout plasma thyroxine levels were significantly higher in intervals 3 and 4 (June 9-29) than in intervals 5 and 6 (June 30 - July 19); all other time intervals were not significantly different from one another. Sample size for each time interval are listed in length histogram. Values are reported as mean + 1 standard error of the mean.

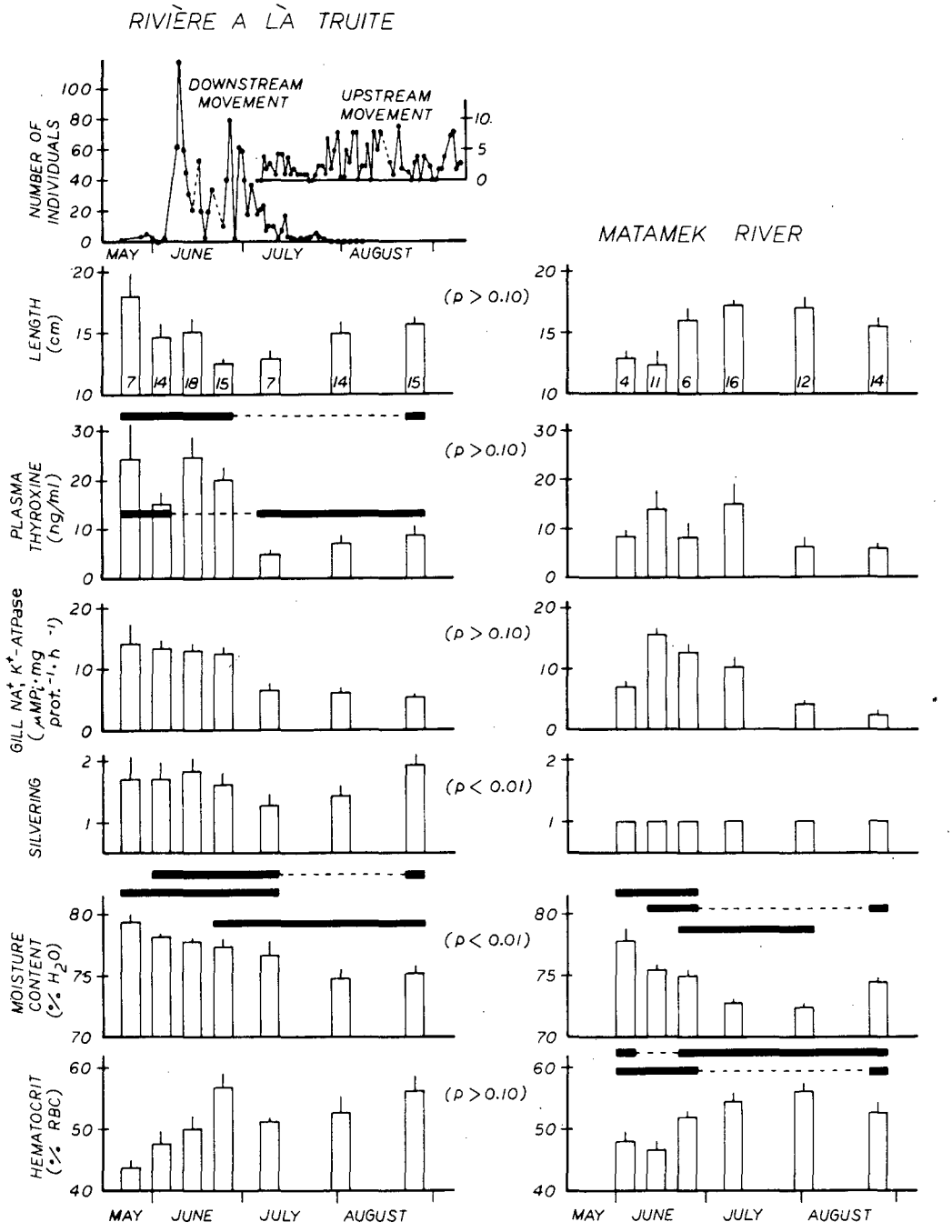

RIVIÈRE A LÀ TRUITE
MATAMEK RIVER
NUMBER OF INDIVIDUALS
DOWNSTREAM MOVEMENT
UPSTREAM MOVEMENT
LENGTH (cm)
PLASMA THYROXINE (ng/ml)
GILL NA+; K+-ATPase (µMPi·mg prot.-1·h-1)
SILVERING
MOISTURE CONTENT (% H2O)
HEMATOCRIT (% RBC)
(p > 0.10)
(p > 0.10)
(p > 0.10)
(p < 0.01)
(p < 0.01)
(p > 0.10)
MAY
JUNE
JULY
AUGUST

Figure 4. Physiological comparison of brook trout migrating downstream in Rivière à la Truite from May 20 to June 30. Fish were divided into three classes on the basis of silvering and compared using one-way ANOVA. Sample size is listed in length histogram. Values are reported as mean ± 1 standard error of the mean.

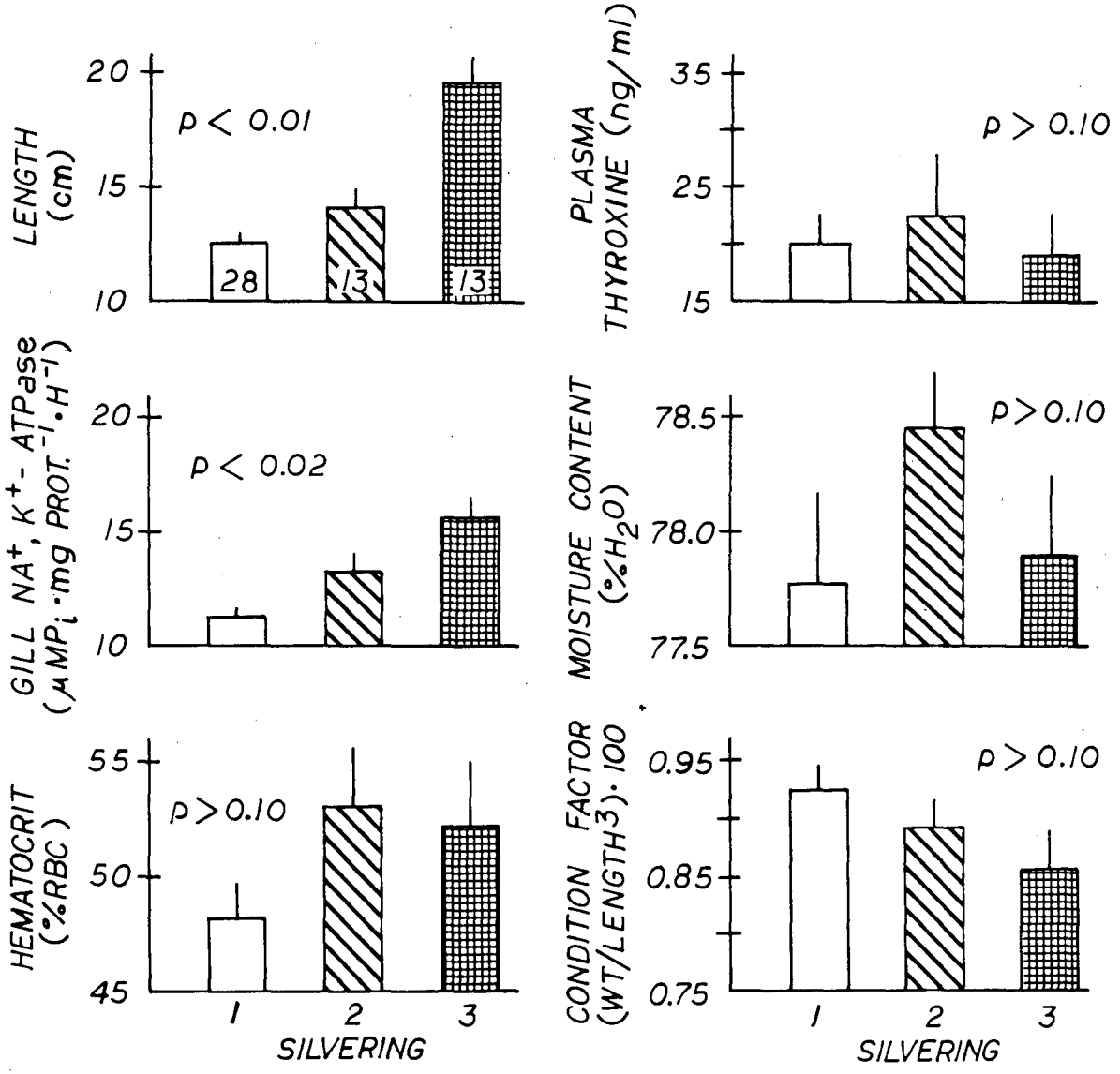

LENGTH (cm)
20
15
10
p < 0.01
28
13
13
PLASMA THYROXINE (ng/ml)
35
25
15
p > 0.10
GILL NA+, K+- ATPase (μMPi·mg PROT.-1·H-1)
20
15
10
p < 0.02
MOISTURE CONTENT (%H2O)
78.5
78.0
77.5
p > 0.10
HEMATOCRIT (%RBC)
55
50
45
p > 0.10
CONDITION FACTOR (WT/LENGTH3)·100
0.95
0.85
0.75
p > 0.10
1
2
3
SILVERING
1
2
3
SILVERING

Table 2. Physiological comparison of Atlantic salmon captured in Riviere a la Truite between June 6 and 29. Fish are divided into high (3) and low (1 and 2) silvering on the basis of visible inspection. Values are reported as mean (± 1 standard error). Asterisk indicate significant differences between means of high and low silvering groups at $p \leq 0.05$ (*) and $p \leq 0.01$ (**) using student's t-test.

Degree of Silvering	Length (cm)	Gill Na^+,K^+-ATPase (μMPi mg prot-1 hr-1)	Plasma Thyroxine (ng/ml)	Hematocrit (o/o RBC)	Moisture Content (o/o H_2O)	Condition Factor (W/L^3)·100
Low (n=8)	10.3 (0.7)	13.2 (2.4)	7.7 (3.1)	57 (1.6)	77.66 (0.33)	0.943 (0.018)
High (n=6)	12.9 * (0.6)	26.3 ** (2.4)	22.7 * (6.1)	49 ** (2.4)	77.94 (0.36)	0.928 (0.008)

Seawater Challenge - Freshwater Fish

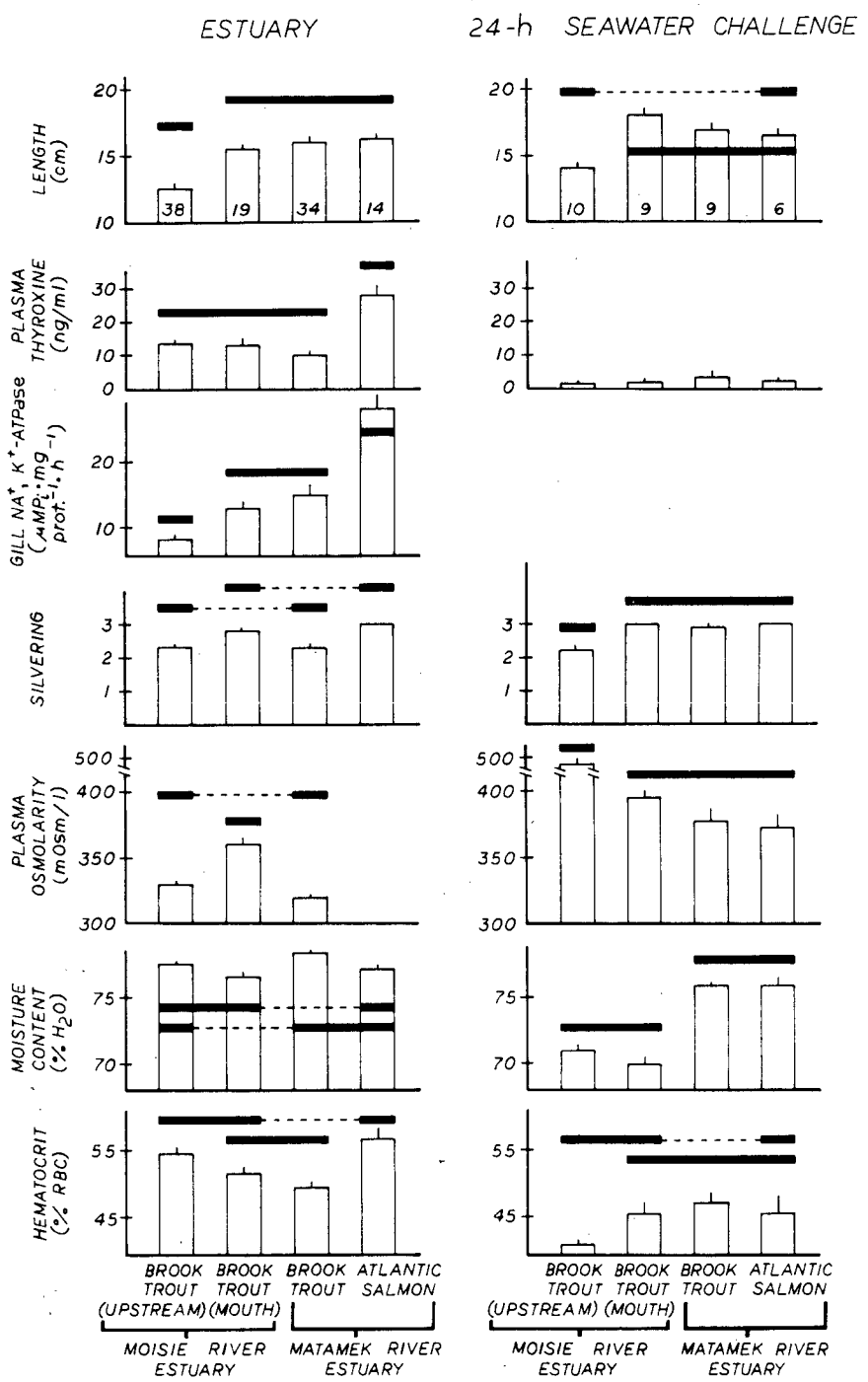
Mean plasma osmolarity of Rivière à la Truite brook trout after 24 h in seawater was 442 ± 15.7 mOsm/l (N= 10). This level of plasma osmolarity was not significantly different from seawater challenged Matamek River fish (459 ± 1.35 mOsm/l; N = 9). Fork length and plasma osmolarity after seawater challenge were not significantly correlated ($p > 0.10$) in either river.

Estuarine Studies - Moisie and Matamek River Estuaries

Brook trout at the upstream site of the Moisie River estuary were significantly smaller than brook trout from the downstream site (Fig. 5). This is the result of size dependent migration in the Moisie River estuary (Montgomery et al., unpublished data). Brook trout at the mouth of the Moisie River estuary, and brook trout and Atlantic salmon in the Matamek River estuary were the same size (range 10.9 - 23.0; Fig.5). All fish captured at estuarine sites had silvering (either category 2 or 3); there was no significant difference in brook trout silvering between estuarine sites.

Plasma thyroxine concentrations were the same for brook trout from all estuarine locations, but were significantly lower than those of Atlantic salmon from the Matamek River estuary (Fig. 5). Similarly, gill Na^+,K^+-ATPase activity was 2 - 3 times higher in Atlantic salmon than estuarine brook trout. Gill Na^+,K^+-ATPase activity of brook trout captured at high salinity sites (e.g. Matamek River estuary and mouth of the Moisie River estuary) were greater than at the upstream Moisie River estuary site. Plasma osmolarity of fish at the mouth of the Moisie River estuary was significantly higher than

Figure 5. Physiological comparison of brook trout and Atlantic salmon captured at estuarine sites. Brook trout were captured at one low salinity site (Moisie River estuary - upstream) and two high salinity sites (Moisie River estuary - mouth, Matamek River estuary). Atlantic salmon were captured in the Matamek River estuary only. Panels on left half of figure represent sampling immediately after capture. Panels on right half of figure report physiological changes after 24 h exposure to seawater. Horizontal bars represent a posteriori comparison among means (one-way ANOVA, Scheffé test). Groups not connected by horizontal bars are significantly different from one another at the 95^{0}/o confidence level. For example, moisture content of brook trout in the Matamek River estuary was significantly higher than brook trout at the Moisie River estuary mouth; moisture content of other groups were not significantly different from one another. Sample size is listed in length histogram. Values are reported as mean $\pm$ 1 standard error of the mean.


ESTUARY
24-h SEAWATER CHALLENGE
LENGTH (cm)
PLASMA THYROXINE (ng/ml)
GILL NA+; K+-ATPase (μMPi·mg prot.-1·h-1)
SILVERING
PLASMA OSMOLARITY (mOsm/l)
MOISTURE CONTENT (% H2O)
HEMATOCRIT (% RBC)
38
19
34
14
10
9
9
6
BROOK TROUT (UPSTREAM)
BROOK TROUT (MOUTH)
BROOK TROUT
ATLANTIC SALMON
MOISIE RIVER ESTUARY
MATAMEK RIVER ESTUARY

brook trout at the upstream Moisie River estuary site, or the Matamek River estuary. Moisture content was significantly lower in brook trout at the Moisie River estuary mouth compared to brook trout in the Matamek River estuary. These results suggest that brook trout at the Moisie River estuary mouth were undergoing seawater adaptation. The fact that salinities in the Matamek River estuary were always above 5 ppt during our sampling (and were similar to those at the mouth of the Moisie River estuary) suggests that seawater adaptation had occurred to a greater extent in brook trout from the Matamek River estuary.

Seawater Challenge - Estuarine Fish

Mean plasma thyroxine concentrations of brook trout and Atlantic salmon after 24 h in seawater were 75-90^{o}/o lower than fish sampled immediately after capture (Fig. 5). Lack of freshwater culture facilities prevented us from conducting 24 h freshwater controls. There was no significant difference in plasma thyroxine levels after seawater challenge between species or among estuarine sampling locations. Plasma osmolarity of brook trout after seawater challenge at the upstream site of the Moisie River estuary was significantly higher than brook trout and Atlantic salmon from the mouth of the Moisie River estuary and the Matamek River estuary. Plasma osmolarity after seawater challenge did not significantly correlate with length for any of the estuarine groups ($p > 0.10$). Length was significantly correlated with plasma osmolarity after 24 h seawater challenge when fish from all freshwater and estuarine sites were considered ($r = 0.34$, $p < 0.01$, $N = 55$). This may reflect, in part, the fact that larger fish were found at high salinity sites. Moisture content after

seawater challenge of brook trout at both Moisie River estuary sites was significantly lower than brook trout and Atlantic salmon from the Matamek River estuary.

DISCUSSION

Wilder (1952) found that freshwater and anadromous brook trout have few meristic and morphological differences (with the notable exception of skin coloration), and considered the two a single taxonomic unit. McGlade and MacCrimmon (1979) studied electrophoretic, meristic and morphometric differences of several brook trout populations in Québec, including anadromous brook trout of the Moisie River and non-anadromous brook trout of the Matamek River. Their results indicate that, while Matamek and Moisie River fish were genetically distinct, they had greater genetic similarity than freshwater populations of brook trout examined over a broader geographical area. While these studies addressed general taxonomic characters, physiological factors associated with seaward migration may undergo stronger selection pressure, possibly resulting in genetic or environmentally induced differences related to smolt physiology between anadromous and freshwater brook trout.

Physiological changes involved in smoltification and seawater migration of salmonids include increases in silvering, plasma thyroxine concentration, gill Na^+,K^+-ATPase activity, moisture content and osmoregulatory ability. These changes are characteristic of smolting Pacific salmon, Atlantic salmon and steelhead trout (Salmo gairdneri) and in most cases are synchronous with entrance into seawater (Wedemeyer et al., 1980). These physiological changes do not

always occur simultaneously (for instance, increased osmoregulatory ability can precede smolting (Wagner, 1974)), and their interrelationships are not fully known. It is clear, however, that smoltification occurs without seawater induction through the influence of environmental cues (e.g. photoperiod and lunar rhythms), resulting in hormonal activation of the parr-smolt transformation (Komourdjian et al., 1976; Folmar and Dickhoff, 1980; Grau et al., 1982).

The bulk of the evidence cited here indicates that there is little difference in smolting physiology between anadromous and non-anadromous brook trout. Seasonal changes in plasma thyroxine concentration with high springtime values coinciding with migration occurred in anadromous brook trout, but these changes were not significantly different from non-anadromous brook trout. Hypoosmoregulatory ability of downstream migrating brook trout was not different from non-anadromous brook trout.

Among the biochemical changes involved in smoltification is an increase in moisture content which coincides with decreased lipid levels (Komourdjian et al., 1976; Farmer et al., 1978; Saunders and Henderson, 1978). Although moisture content was significantly different in brook trout from Rivière à la Truite and the Matamek River, each had a similar trend of decreasing moisture content with time. Furthermore, moisture content was lower (though not significantly so) in migratory Rivière à la Truite brook trout which possessed the greatest silvering. Changes due to smoltification are probably not responsible for the between river differences in moisture content. Falling moisture content and increasing hematocrit indicate that both intracellular and extracellular compartments have lower

water content. Since moisture content of teleosts is inversely related to lipid levels (Phillips, 1969), reduction in moisture content with time in the two rivers may be the result of greater fat deposition as food supply increases.

A seasonal cycle with high spring levels of plasma thyroxine has been found in brook trout which do not undergo visible signs of smoltification (White and Henderson, 1977; Chapter 1). Spring peaks of these cycles are lower than those of smolting salmonids (Dickhoff et al., 1978). The absence of differences in plasma thyroxine concentration between anadromous and non-anadromous brook trout suggests that the seasonal cycle of this hormone regulates functions other than smolting in brook trout. White and Henderson (1977) hypothesized that the seasonal thyroxine cycle is involved in maturation. We have found, however, that a 3 mo delayed photoperiod caused a 3 mo shift in maturation but not in the thyroxine cycle (Chapter 1). Thyroxine levels were higher in fish fed maximally, and were positively correlated with differences in growth rate. Spring increases of plasma thyroxine concentrations in nature may play a role in, or result from, increased somatic growth during this period.

Of the physiological parameters investigated, only the degree of silvering showed a clear distinction between brook trout in the Matamek River and Rivière à la Truite. Greater silvering in Rivière à la Truite brook trout is associated with greater size and increased gill Na^+,K^+-ATPase activity, characteristics which are typical of smolting salmonids (Zaugg and McLain, 1972; Lasserre et al., 1978; Buckman and Ewing, 1982). The difference in gill Na^+,K^+-ATPase activity between high and no silver groups was only 25^0/o, a small

amount relative to those found between salmon parr and smolt (Folmar and Dickhoff, 1980; Table 2). Black (1981), used the marine trematode, Brachyphallus crenatus, as an indicator of seawater residence. Brook trout captured upstream in the Moisie River estuary, though highly silvered, had only a 3^{o}/o incidence of infection. Fish from the Moisie River estuary mouth had an 86^{o}/o infection rate. Silvering of brook trout was concluded to be an unreliable indicator of eventual seawater entry. In the present study, brook trout captured at the upstream Moisie River estuary site had significant silvering, but low gill Na^{+},K^{+}-ATPase activity and hypoosmoregulatory ability. We cannot rule out the possibilty that silvering (and perhaps increases in gill Na^{+},K^{+}-ATPase activity) is acquired during residence in the Moisie River estuary and is retained over the winter. Silvering was induced in a Matamek River brook trout maintained in 32 ppt for 2 mo (personal observation), and is apparently induced in brook trout washed over the 1st Falls into the Matamek River estuary.

In contrast to the findings for brook trout, Atlantic salmon captured in Rivière à la Truite and divided on the basis of silvering showed very clear indications of smoltification (Table 2). Gill Na^{+},K^{+}-ATPase activity in highly silvered Atlantic salmon was approximately 2 times greater than Atlantic salmon of intermediate and no silvering, while plasma thyroxine was 3 times greater. Plasma thyroxine, however, was as high for all silvering categories of brook trout as it was for highly silvered Atlantic salmon. Perhaps increased growth or activity of brook trout, irrespective of degree of

silvering, can explain high plasma thyroxine of all brook trout during this period.

Although moisture content is higher and condition factor is lower in highly silvered Atlantic salmon than in less silvered Atlantic salmon, the differences are not significant (Table 2). Failure to detect significant differences in these smolt characteristics may reflect differences in feeding or other environmental variables which were constant in other investigations of smolting, but which cannot be controlled under natural conditions.

Interpretation of the estuarine physiology of brook trout must be made in light of the size dependent migration that occurs in the Moisie River estuary. Brook trout at the upstream site are significantly smaller than at the downstream site (Fig. 5). Montgomery et al. (unpublished data) have found that brook trout 15 cm are rare in the Moisie River estuary and there are none 18 cm. Larger brook trout leave the estuary and enter the Gulf of St. Lawrence, returning to spawn in autumn. Salinity tolerance of brook trout is size dependent (Chapter 2). Size dependent salinity preference and salinity tolerance coincide in other salmonids (McInerny, 1964; Weisbart, 1968). A similar phenomenon in brook trout may explain the size dependent distribution of brook trout found in the Moisie River estuary.

Our results indicate that the estuary is an important site of adaptation of brook trout to salt water which permits their eventual seaward migration. Residence time in the estuary is size dependent; larger fish spend little or no time in the estuary while smaller fish reside for longer periods of several weeks before leaving the estuary

(Montgomery et al., unpublished data). Gradual adaptation to seawater significantly increases seawater survival of brook trout (Chapter 2). Activities of gill Na^+,K^+-ATPase are elevated and hypoosmoregulatory ability is greater at high salinity sites, suggesting that seawater adaptation is occurring through exposure to seawater. It would appear that a major adaptation of anadromous brook trout populations to existence in seawater is both behavioral and physiological; movement and extended residence in estuaries permits gradual seawater acclimation which in turn results in greater salinity tolerance.

In this context it is instructive to compare the estuarine physiology of brook trout and Atlantic salmon. Tagging studies have shown that Atlantic salmon residence in the Matamek River estuary averages only 3-4 d (Gibson, 1978). Visible inspection of coloring and body form indicates that these fish are fully smolted as they first enter the estuary. Plasma thyroxine concentrations and gill Na^+,K^+-ATPase activities of Atlantic salmon are significantly higher than estuarine brook trout. The preparatory physiological adaptations associated with smoltification, and displayed by estuarine Atlantic salmon, appear adaptive for rapid seawater acclimation and short estuarine residence. The ability to directly enter seawater without gradual estuarine acclimation may represent a fundamental advancement of smolting salmonids, an adaptation which appears to be absent in brook trout.

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REFERENCES

Black, G. A. 1981. Metazoan parasites as indicators of movements of anadromous brook charr (Salvelinus fontinalis) to sea. Can. J. Zool. 59: 1892-1896.

Buckman, M. and R. D. Ewing. 1982. Relationship between size and time of entry into the sea and gill (Na^+-K^+)-ATPase activity for juvenile spring chinook salmon. Trans. Am. Fish. Soc. 111: 681-687.

Castonguay, M., G. J. Fitzgerald and Y. Côté. 1982. Life history and movements of anadromous brook charr, Salvelinus fontinalis, in the St. Jean River, Gaspé, Québec. Can. J. Zool. 60: 3084-3091.

Clarke, W. C. and J. Blackburn. 1978. Seawater challenge tests performed on hatchery stocks of chinook and coho salmon in 1977. Can. Fisheries and Mar. Serv. Tech. Rep. No. 761.

Dickhoff, W. W., L. C. Folmar and A. Gorbman. 1978. Changes in plasma thyroxine during smoltification of coho salmon, Oncorhynchus kisutch. Gen Comp. Endocrinol. 36: 229-232.

Dutil, J. D. and G. Power. 1980. Coastal populations of brook trout, Salvelinus fontinalis, in Lac Gillaume-Delisle (Richmond Gulf) Quebec. Can. J. Zool. 58: 1828-1835.

Farmer, G. J., J. A. Ritter and D. Ashfield. 1978. Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, Salmo salar. J. Fish. Res. Bd. Can. 35: 93-100.

Folmar, L. C. and W. W. Dickhoff. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21: 1-37.

Gibson, R. J. 1978. Salmon Studies. In: Matamek Annual Rept. for 1977. (ed.: Gibson, R. J.). Woods Hole Oceanographic Institution Tech. Rept. 78-92.

Grau, E. G., J. L. Specker, R. S. Nishioka and H. A. Bern. 1982. Factors determining the occurence of the surge in thyroid activity during smoltification. Aquaculture 28: 49-57.

Hoar, W. S. 1976. Smolt transformation: evolution, behavior and physiology. J. Fish Res. Bd. Can. 33: 1234-1252.

Komourdjian, M. P., R. L. Saunders and J. C. Fenwick. 1976. Evidence for the role of growth hormone as a part of a 'light-pituitary axis' in growth and smoltification of Atlantic salmon, (Salmo salar). Can. J. Zool. 54: 544-551.

Laserre, P., G. Boeuf and Y. Harache. 1978. Osmotic adaptation of Oncorhynchus kisutch Walbaum. I Seasonal variations of gill Na+,K+-ATPase activity in coho salmon, 0+-age and yearling, reared in freshwater. Aquaculture 14: 365-382.

McCarthy, L. S. and A. H. Houston. 1977. Na^+:K^+- and HCO^{3-}-stimulated ATPase activities in the gills and kidneys of thermally acclimated rainbow trout, Salmo gairdneri. Can. J. Zool. 55: 704-712.

McGlade J. and H. MacCrimmon. 1979. Taxonomic congruence of three populations of Quebec brook trout, Salvelinus fontinalis (Mitchill). Can. J. Zool. 57: 1998-2009.

McInerny, J. E. 1964. Salinity preference: an orientation mechanism in salmon migration. J. Fish. Res. Bd. Can. 21: 995-1018.

Montgomery, W. L. 1980. Tides and water movements in the Moisie River estuary. In: Matamek Annual Rept. for 1979. (ed.: Naiman, R. J.). Woods Hole Oceanographic Institution Tech. Rept. 80-31.

Montgomery, W. L., S. D. McCormick, R. J. Naiman, F. G. Whoriskey and G. A. Black. 1983. Spring migratory synchrony of salmonid, catostomid and cyprinid fishes in Rivière à la Truite, Québec. Can. J. Zool. in press.

Mullan, J. W. 1958. The sea-run or "salter" brook trout (Salvelinus fontinalis) fishery of the coastal streams of Cape Cod, Massachusetts. Mass. Div, Fish and Game. Bull. No. 17,

Phillips, A. M. 1969. Nutrition, digestion and energy utilization. In: Fish Physiology, Vol I. (ed.: Hoar, W. S. and D. J. Randall). pp. 599-675. Academic Press, New York

Reimers, T. J., J. P. McCann, R. G. Cowan and P. W. Concannon. 1982. Effects of storage, hemolysis, and freezing and thawing on concentrations of thyroxine, cortisol and insulin in blood samples. Proc. Soc. Exp. Biol. Med. 170: 509-516.

Rousnefell, G. A. 1958. Anadromy in North American Salmonidae. U.S. Fish Wildl. Serv. Fish. Bull. 58: 171-185.

Saunders, R. L. and E. B. Henderson. 1978. Changes in gill ATPase activity and smolt status of Atlantic salmon (Salmo salar). J. Fish. Res. Bd. Can. 35: 1542-1546.

Scott, W. B. and E. J. Crossman. 1973. Freshwater Fishes of Canada. Fish. Res. Bd. Can. Bull. 184.

Smith, M. W. and J. W. Saunders. 1958. Movements of brook trout, Salvelinus fontinalis (Mitchill) between and within fresh and salt water. J. Fish. Res. Bd. Can. 15: 1403-1449.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Co. San Fransisco. 776 p.

Wedemeyer, G. A., R. L. Saunders and W. C. Clarke. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42: 1-14.

Weisbart, M. 1968. Osmotic and ionic regulation in embryos, alevins, and fry of the five species of Pacific salmon. Can. J. Zool. 46: 385-397.

White, H. C. 1940. Life-history of sea-running brook trout (Salvelinus fontinalis) of Moser river, Nova Scotia. J. Fish. Res. Bd. Can. 5: 176-186.

White, B. A. & N. E. Henderson. (1977). Annual variation in the circulating levels of thyroid hormones in the brook trout, Salvelinus fontinalis, as measured by radioimmunosassay. Can. J. Zool. 55: 475-481.

Whoriskey, F. G., R. J. Naiman, and W. L. Montgomery. 1981. Experimental sea ranching of brook trout, Salvelinus fontinalis, Mitchill. J. Fish Biol. 19: 637-651.

Wilder, D. G. 1952. A comparative study of anadromous and freshwater populations of brook trout (Salvelinus fontinalis (Mitchill)). J. Fish. Res. Bd. Can. 9: 169-203.

Zaugg, W. S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aq. Sci. 39: 215-217.

Zaugg, W. S. and L. R. McLain. 1972. Changes in adenosine triphosphatase activity associated with parr-smolt transformation in steelhead trout, coho and spring chinook salmon. J. Fish. Res. Bd. Can. 29: 167-171.

SUMMARY

The effects of size, age and photoperiod on salinity tolerance and osmoregulatory ability of brook trout were investigated using laboratory and field experiments. Physiological changes associated with salmonid smoltification were examined to determine whether this process occurred in brook trout. The overall objective was to establish the factors which limit natural anadromy of brook trout and their introduction into salt water for sea ranching, farming and natural enhancement.

The survival of brook trout after exposure to seawater was found to be primarily size dependent. Age affected seawater survival only through its covariance with size. The effect of size on seawater survival slowed after fish reached a fork length of 14.0 cm. Regulation of plasma ions and osmolarity after seawater exposure was also size dependent. Gill Na^+,K^+-ATPase activity after 20 d in seawater decreased with increasing size, possibly reflecting a lower demand for active transport in larger fish due to a more favorable surface area to volume ratio. These results establish size as a basic physiological constraint to salmonid euryhalinity. A conceptual model was developed which depicts the phylogenic relationship of size dependent ion transport ability and size dependent seawater survival among salmonids.

Photoperiod influenced salinity tolerance of brook trout through its control of the maturation cycle. Seawater survival of mature males was lower than mature females during spring, summer and fall photoperiods. Lowered salinity tolerance of adult males becomes acute

during autumn photoperiod when normal spawning occurs. This did not occur in immature males. Ion transport ability after seawater exposure was poorer in mature males than in mature females. No other photoperiod-related pattern in seawater survival was found.

Field and laboratory studies indicate that preparatory adaptations for seawater entry, such as those that occur in smolting salmonids, are undeveloped or non-existent in brook trout. Size, age and photoperiod period had no effect on gill Na^+,K^+-ATPase activities. Plamsa thyroxine was found to be associated with differences in somatic growth rates. Although an annual cycle of plasma thyroxine existed, it did not influence osmoregulatory functions. Similar results were obtained when natural anadromous and non-anadromous populations of brook trout were compared; spring increases in plasma thyroxine were found, but there were no differences in plasma thyroxine, gill Na^+,K^+-ATPase activity or hypoosmoregulatory ability.

Gradual seawater acclimation of brook trout (as opposed to direct seawater transfer) increased survival. Gradual acclimation was also important under natural conditions; acclimation of brook trout in estuaries was found to increase hypoosmoregulatory ability. In contrast, Atlantic salmon smolts possessed preparatory adaptations for seawater entry, and spent only a few days in the estuary. This ability to directly enter seawater without gradual estuarine acclimation may represent a fundamental advancement of smolting salmonids, an adaptation which appears to be absent in brook trout.

These results indicate three major factors will limit anadromy of brook trout under natural conditions: 1) Size dependent osmoregulatory

ability, 2) necessity of gradual acclimation and estuarine residence and 3) decreased mature male seawater survival which becomes acute during spawning. These physiological limitations account for several of the prominent features of brook trout seaward migration, which include size dependent migration, estuarine residence, return to freshwater prior to spawning, and sex ratios of anadromous brook trout populations which are skewed toward a greater number of females.

These results are particularly illuminating when placed in the context of salmonid evolution. Though it is generally accepted that the genus Salmo is intermediate between Oncorhynchus and Salvelinus (Jordan and McGregor, 1925), there has been some debate over which of the latter genera is more primitive. Those who argue for a seawater origin of the subfamily Salmoninae place Oncorhynchus as the forerunner, while those in favor of a freshwater origin place Salvelinus as the primitive group (a short review of these arguments can be found in Hoar, 1976 and Thorpe, 1982). Hoar (1976, pg. 1235) summarized the arguments of Tchernavin (1939) for a freshwater origin of salmonids: " 1) the broad distribution of salmonids in fresh water; 2) the existence in many species of parallel migratory and strictly freshwater forms; 3) the universal habit of breeding and incubating eggs in freshwater; 4) the tendency of migratory species to return to a "home" stream; 5) the presence of many features in the life of freshwater forms that are found only during the young stafe of the migrant individuals before they reach the sea; and 6) the fact that there are numerous species of salmon that complete their life cycle in freshwater but none that is strictly marine ...".

Arguments for a seawater origin of Salmoninae rest primarily on the fossil record of Smilodonicthyhys rastrosus, which has been hypothesized to be an anadromous, planktonic feeder in Pliocene oceans (Cavender and Miller, 1972). Based on this interpretation, Balon (1980, pp. 552-2) states that "An ancient predecessor of salmonins, therefore, would be a pelagophil with an associated sequence of relatively slow differentiation in the embryonic period, followed by a long larval period, with metamorphosis preceding the juvenile period ... Consequently, all recent salmonins may, in various degrees, be considered as juvenilized or secondarily altricial, though Pacific salmons of the genus Oncorhynchus least so." In other words, the genus Salvelinus is the more 'advanced' form which has lengthened its residence in freshwater (i.e. decreased anadromy) through neoteny.

Resolution of these arguments is unlikely to occur without more evidence from the fossil record. For the purpose of making physiological comparisons, however, I shall assume a freshwater origin of salmonins. I find the arguments of Tchernavin (1939) to be more persuasive because of their greater explanatory power; in order to explain the present distribution and biology of salmonins this theory need only postulate increased exploitation of the oceans. Proponents of a seawater origin of salmonins must postulate increased exploitation of freshwater and the loss of wholly marine species (i.e. those that can reproduce in seawater) from the fossil record and present day biota.

Examination of a single physiological characteristic (such as osmoregulatory ability) does not allow one to delineate the path of evolution. Assuming a freshwater origin of salmonids, however, allows

one to make intructive phylogenetic comparisons in salmonid osmoreulatory physiology. Seaward migrations of brook trout can be visualized as opportunistic movements into areas of high food availability. Anadromy may also have facilitated colonization of new rivers during periods of glacial recession. Euryhalinity in brook trout lacks the specialized preparatory adaptations of smoltification which occurs in more advanced salmonids. Increased exploitation of the sea by salmonids can be viewed as a progressive decrease in the size at which survival in seawater is possible. A conceptual model is presented in Chapter 2 in which generic decreases in the size at which seawater survival occurs can be explained by changes in size dependent hypoosmoregulatory ability.

The osmoregulatory limitations outlined above will also affect the use of brook trout in sea ranching, farming and natural enhancement programs. Size dependent salinity tolerance of brook trout indicates that accelerated growth will allow earlier survival in seawater, an important economic consideration. However, maturation of brook trout is also size dependent, and has a negative affect on male seawater survival. In addition, the size at which brook trout can successfully adapt to seawater is larger than many other salmonids. Variability in the effect of size on both salinity tolerance and maturation of brook trout presents 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.

REFERENCES

Balon, E. K. 1980. Early ontogeny of the lake charr, Salvelinus (Cristivomer) namaycush. In: Charrs (edited by Balon, E. K.), pp. 485-562. Dr. W. Junk bv Publishers, The Hague.

Cavender, T. M. and R. E. Miller. 1972. Smilodonichthys rastrosus, a new pliocene salmonid fish from western United States. Bull. Mus. Nat. Hist. Univ. Oregon (Eugene) 18, 1-44.

Hoar, W. S. 1976. Smolt transformation: evolution, behavior and physiology. J. Fish. Res. Bd. Can. 33, 1234-1252.

Tchernavin, V. 1939. The origin of salmon. Salmon Trout Mag. 95, 1-21.

Thorpe, J. E. 1982. Migration in salmonids, with special reference to juvenile movements in freshwater. In: Proceedings of the Salmon and Trout Migratory Behavior Symposium (edited by Brannan, E. L. and E. O. Salo), pp. 86-97. Univ. of Washington, Seattle.

APPENDIX A

Measuring Gill Na^+,K^+-ATPase Activity

Measurement of gill Na^+,K^+-ATPase activity in brook trout was performed using the partial purification method of Zaugg (1982). This method yields higher specific activities than crude homogenates and circumvents several precautions necessary when using crude homogenates. The partial purification method gives specific activities which are similar in magnitude to purified microsomal fractions but avoids several time-consuming steps (Zaugg, 1982).

Dissection, storage and preparation of SEI (buffered storage) solution is reported in the text (Chapter 1).

Enzyme Preparation- Enzyme preparation is a two step procedure in which 1) soluble cellular material is removed, and 2) partially purified microsomes are prepared. Trimmed gill filaments are removed from the freezer and allowed to thaw on ice. After placing filaments (with SEI solution) in a conical glass homogenizer, soft tissue is separated from the cartilage using 8-10 strokes. The homogenate is poured into a conical centrifuge tube on ice. The homogenizer is rinsed with 1 ml SEI solution which is also added to the centrifuge tube, being careful to leave cartilagenous supports behind. 2 ml cold distilled water is also added to the centrifuge tube which is centrifuged at 3500 rpm for 8 min at room temperature. The supernatant is discarded and tubes inverted to thoroughly drain. Pellets are suspended in 0.5 to 1 ml (dependent on pellet size) SEI

with deoxycholate (0.1 g/100 ml, adjusted to pH 7.1), and homogenized (30 strokes). Homogenate is centrifuged as before and a 0.4 ml aliquot removed and placed on ice for enzyme activity and protein determinations.

Enzyme Activity- Na^+,K^+-ATPase activity is measured by calculating the difference in phosphate production (equivalent to ATP hydrolysis) in buffered salt solutions with and without ouabain, a specific inhibitor of Na^+,K^+-ATPase activity. Buffered salt reaction media contained 4.68 g $MgCl_2{\cdot}H_2O$, 9.07 g NaCl, 5.6 g KCl and 7.83 g imidazole in a final volume of 1.0 l including adjustment to pH 7.0 with HCl. Inhibitor solution was prepared by adding 0.43 g ouabain to 1.0 l of the above solution.

Duplicate 16x100 mm tubes containing 0.65 ml of each reaction solution are placed in an ice bath. 10 µl of each enzyme preparation, and 0.1 ml of 0.03 M Na_2ATP (1.84 g/100 ml adjusted to pH 7.0 with NaOH) is added to each tube. Duplicate reagent blanks contain 0.65 ml reaction solution, 0.1 ml Na_2ATP and 10 µl SEI with deoxycholate. After addition of ATP, racks with reaction tubes were shaken, placed in a 37 C water bath and shaken for 1 min. After 10 min at 37 C, the rack of tubes is removed and placed in ice water for 1 min. 1.85 ml of 0.95 $^o/o$ (v/v) $HClO_4$ is dispensed with moderate force down the side of each tube to cause mixing, followed immediately by addition of 3.0 ml 2-Octanol. Phosphate is extracted into the Octanol and measured spectrophotometrically (Zaugg, 1982). The protein content of enzyme preparations are measured by the method of Lowry et al. (1951) as modified by Millar (1959).

Protein and Wet Weight Reference- Enzyme activities are commonly referenced against the wet weight of tissue used in preparing the enzyme or the final amount of protein in the enzyme preparation. Since changes in cellular chemistry may occur under osmotic stress and thereby influence these references, we compared the validity of basing gill Na^+,K^+-ATPase activities on these two references in both freshwater and seawater.

Primary filaments of freshwater and seawater adapted brook trout were blotted dry on damp chamois cloth and weighed to the nearest 0.01 g prior to freezing and analysis. No significant difference ($p = 0.08$, student's t-test) in protein extractability occured as a result of environmental salinity (Table 1). Gill Na^+,K^+-ATPase activity expressed either on a per mg protein or per mg wet weight tissue basis are both significantly different ($p < 0.05$) for freshwater and seawater adapted animals (Table 1). Furthermore, gill Na^+,K^+-ATPase values of the same individual expressed as $\mu MP_i \cdot mg\ prot.^{-1} \cdot hr^{-1}$ or $\mu MP_i \cdot mg\ wet\ wt.^{-1} \cdot hr^{-1}$ show a strong and significant correlation ($r = 0.98$). Weight of extractable protein and wet weight of tissue are, therefore, equally acceptable references upon which to base activity measures of gill Na^+,K^+-ATPase in brook trout. We report gill Na^+,K^+-ATPase activities as $\mu MP_i \cdot mg\ prot.^{-1} \cdot hr^{-1}$.

Table 1. Protein concentration of final gill homogenates and gill Na^+,K^+-ATPase activities of freshwater and seawater adapted brook trout expressed on a per mg protein and per mg wet weight tissue basis. Values are mean $\pm$ 1 standard deviation. * and α indicate significant differences at $p < 0.001$ using student's t-test.

	Freshwater Adapted (N = 33)	Seawater Adapted (N = 23)
Homogenate Protein Content mg prot. · g wet tissue^{-1}	10.1 $\pm$ 1.6	9.6 $\pm$ 1.0
Gill Na^+,K^+-ATPase activity μMP_i · mg prot.$^{-1}$ · h^{-1}	8.1 $\pm$ 3.0 *	29.2 $\pm$ 9.4 *
μMP_i · mg tissue^{-1} · h^{-1}	.081 $\pm$ .031 α	.284 $\pm$ .109 α

REFERENCES

Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Miller, G. L. 1959. Protein determination for large numbers of samples. Anal. Chem. 31, 964.

Zaugg, W. S. 1982. A simplified preparation for adenosine triphosphatase determination in gill tissue. Can. J. Fish. Aq. Sci. 39, 215-217.

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16. Abstract (Limit: 200 words)

Brook trout (Salvelinus fontinalis) raised from eggs under two photoperiod and two feeding regimes were tested for physiological changes preparatory for transition from freshwater to seawater. Size, age, growth rate, photoperiod, and diel rhythms were examined for possible influences on plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$, $[Mg^{2+}]$, thyroxine concentration, hematocrit, and gill Na^+, K^+ - ATPase activity of brook trout in freshwater.

The results indicate that smoltification is relatively undeveloped in brook trout and that estuarine residence is important in salinity adaptation and eventual seaward migration.

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