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Osmoregulatory and Ionoregulatory Changes and Associated Mortalities during the Transition of Maturing American Eels to a Marine Environment

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Abstract.—Serum Na^+, Cl^-, and K^+ concentrations, branchial chloride cells and Na^+,K^+-ATPase activity, and pollutant levels were examined in maturing American eels *Anguilla rostrata* migrating down the St. Lawrence River estuary. The purpose was to investigate physiological changes that normally occur during seaward migration and their reversibility, and to determine the cause(s) of heavy annual mortalities of eels that occur in the freshwater portion of the St. Lawrence. Serum ion and osmotic concentrations were slightly lower in healthy eels collected in fresh water than in salt water. Freshwater eels and saltwater eels kept 96 h in salt- and fresh water, respectively, had similar concentrations as controls. The number of chloride cells increased during the transition to the marine environment, within 96 h in eels collected in fresh water and transferred to salt water, but the number of chloride cells did not regress in eels collected in salt water and transferred to fresh water for 96 h. Gill Na^+,K^+-ATPase activity increased as the eels moved to salt water in the estuary. Freshwater eels kept in salt water 96 h also showed a marked increase, but saltwater eels maintained high though reduced gill Na^+,K^+-ATPase activity when kept 96 h in fresh water. Diseased eels had much lower serum ion and osmotic concentrations than healthy eels, but they recovered to near normal concentrations when kept 96 h in salt water. Branchial chloride cells and Na^+,K^+-ATPase activity followed the same trend as in freshwater eels. Diseased eels had similar heavy metal and pesticide loads as healthy eels. Pathological conditions of their gills, however, suggested that diseased eels migrated through highly polluted areas. The results indicate that maturing eels in fresh water possess significant hypoosmoregulatory ability. Increases in chloride cells and gill Na^+,K^+-ATPase activity occur upon exposure to salt water. Maturing American eels may rely on two different mechanisms to maintain relatively constant serum ion and osmotic concentrations as they migrate through the estuaries. Mortalities are associated with ion loss in fresh water, perhaps as a result of increased permeability of the branchial membrane due to damage caused by pollution in the St. Lawrence.

Physiological control of osmotic balance in teleosts has been the focus of much research particularly on those species migrating between fresh and salt water (reviewed by Evans 1979, 1980). Teleosts maintain the osmotic concentrations of their bodies within narrow limits as they migrate between fresh water and salt water, Na^+ and Cl^- contributing most to the limited changes in the ion content of the blood (Parry 1966). Members of the family Anguillidae are no exception (Boucher-Firly 1935; Sharratt et al. 1964; Munroe and Poluhowich 1974; Schmidt-Nielsen and Renfro 1975); migrating eels face the problems of penetration of salts and osmotic loss of water as they move into the marine environment. Transfer of eels from fresh water to salt water causes the eels to ingest salt water (Smith 1930, 1932; Keys 1933), causes a decrease in the production of urine (Sharratt et al. 1964; Schmidt-Nielsen and Renfro 1975), and causes an increase in the volume of water reabsorbed passively (preadaptive in maturing *Anguilla japonica*), following NaCl transport through the intestinal mucosa (Oidé and Utida 1967a, 1967b; Utida et al. 1967; Skadhauge 1969). Monovalent ions are not excreted renally (Evans

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1979), and an extrarenal structure is needed to maintain a constant osmotic concentration.

Gill chloride cells and Na^+,K^+-ATPase have been widely implicated in the branchial mechanisms of osmotic regulation. Chloride cells are located on the primary lamellae of the gills (reviewed by Laurent 1984). Transfer of eels to salt water results in the proliferation of gill chloride cells (Shirai and Utida 1970; Doyle and Epstein 1972), an increase in gill Na^+,K^+-ATPase activity (Epstein et al. 1967, 1971; Kamiya and Utida 1968, 1969; Bornancin and DeRenzis 1972; Butler and Carmichael 1972; Sargent and Thomson 1974; Sargent et al. 1975), and the formation of accessory cells (Dunel-Erb and Laurent 1980; Laurent and Dunel 1980) and leaky junctions (Sardet et al. 1979, 1980). Sodium ions (Mizuhira et al. 1970), Cl^- ions (Petrik 1968), and gill Na^+,K^+-ATPase (Mizuhira et al. 1970; Maetz and Bornancin 1975; Karnaky et al. 1976) are localized in association with the network of tubular smooth-surfaced endoplasmic reticulum of the chloride cells in saltwater-acclimated eels.

Increases in the number of branchial chloride cells and in activity of branchial Na^+,K^+-ATPase also occur in *Anguilla anguilla* and *A. japonica* in fresh water coincident with seaward migration but prior to saltwater entry (Utida et al. 1971; Thomson and Sargent 1977). Similar changes accompany the parr–smolt transformation in anadromous salmonids (see reviews by Hoar 1976; Wedemeyer et al. 1980; McCormick and Saunders 1987, this volume). During this transformation, osmoregulatory organs undergo structural and functional changes, while the fish are still in fresh water, that result in increased salinity tolerance. This process is under photoperiodic control (Wedemeyer et al. 1980) and can be triggered by migration (Zaugg et al. 1985) and water quality (Saunders et al. 1983). By analogy, environmental changes may influence the development of osmoregulatory organs prior to or during the downstream migration of eels.

Like most members of the genus *Anguilla*, American eels *A. rostrata* spend most of their life in lakes and rivers. They migrate out of fresh water at the onset of sexual maturation to reach the ocean, spawn, and die. This catadromous migration takes place throughout summer and autumn, and in the St. Lawrence River, Canada, results in heavy mortalities. Hundreds of dead eels are stranded on the shoreline. High mortalities date back to at least 1960. In the bad years of 1972 and 1973, losses were estimated to have been 100 metric tons. This rate has considerably decreased in recent years but losses still take place.

This study examines changes in serum Na^+, Cl^-, and K^+ concentrations, numbers of branchial chloride cells, and Na^+,K^+-ATPase activity that occur in healthy and diseased maturing American eels as they migrate down the St. Lawrence estuary and also in the laboratory following transfer from fresh water to salt water and from salt water to fresh water. The disease is shown to result from damage to the branchial membranes that cause disorders in the ionic and osmotic regulatory performance and in the rate of maturation, and is indicative of the bad conditions that maturing eels face as they migrate in the St. Lawrence.

Methods

The material collected in the period 1981–1985 is examined. Some of the 1981–1982 data have been published (Dutil 1984; Dutil and Lallier 1984). Some American eels were collected in 1983, 1984, and 1985 for histological examination of gills. Those eels collected in 1984 were also examined for heavy metals and pesticide contamination. Finally, Na^+, Cl^-, and K^+ contents and gill Na^+,K^+-ATPase activity were measured in eels collected in 1985.

Stations and collections.—Migrating eels were collected in commercial weirs in the St. Lawrence estuary near St. Nicolas (freshwater site, located 50 km above the highest point of penetration of salt water), and near Kamouraska (saltwater site, located 100 km downstream from the highest point of penetration of salt water) (Figure 1). Periods of sampling changed between locations, St. Nicolas eels being collected earlier than Kamouraska eels in general. The period extended from early August to late October. Conditions were homogeneous horizontally and vertically at St. Nicolas, but the circulation was very complex downstream where temperature and salinity varied horizontally and vertically. Temperature decreased from more than 20°C in mid-August to less than 10°C in mid-October. Salinity ranged between 0‰ at St. Nicolas to about 18–24‰ at Kamouraska. For information on the ion concentrations of the St. Lawrence River, refer to Cossa and Tremblay (1983).

Diseased eels referred to in this study were found exclusively in fresh water, mainly downstream from Lake St. Pierre, and were collected in St. Nicolas. There was no means of recognizing early stages in the disease; diseased eels collected

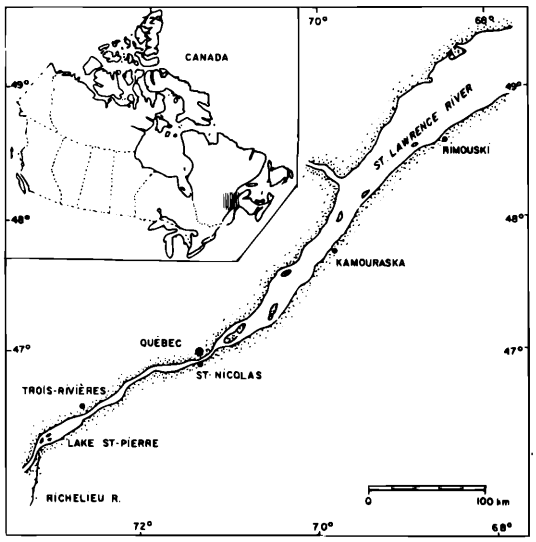

FIGURE 1.—Location of the collecting sites for American eels in the St. Lawrence River estuary.

in this study were sometimes moribund. They were distinguished from normal eels by their mottled pattern of skin pigmentation, their lack of any reaction to manipulations, their tendency to stay near the surface, and sometimes their incapacity to maintain vertical position as they moved. For details on the history of the disease, see Dutil and Lallier (1984).

Treatments.—Transportation to our laboratory took 15 min from the freshwater site and 2 h from the saltwater site. The eels were transported in river water from St. Nicolas and in moist air from Kamouraska. They were either killed immediately upon their arrival at our laboratory (no treatment) or assigned to one of two treatments: either 4 d in fresh water or 4 d in saltwater (20‰ chloride). The ion content of water in freshwater tanks fluctuated around these values: Na^+, 580 $\mu eq \cdot L^{-1}$; Cl^-, 800 $\mu eq \cdot L^{-1}$; K^+, 32 $\mu eq \cdot L^{-1}$; Ca^{++}, 950 $\mu eq \cdot L^{-1}$; Mg^{++}, 200 $\mu eq \cdot L^{-1}$. Mean annual concentrations in the St. Lawrence in St. Nicolas were: Na^+, 460 $\mu eq \cdot L^{-1}$; Cl^-, 590 $\mu eq \cdot L^{-1}$; K^+, 30 $\mu eq \cdot L^{-1}$; Ca^{++}, 1.4 $meq \cdot L^{-1}$; Mg^{++}, 600 $\mu eq \cdot L^{-1}$ (Cossa and Tremblay 1983). Saltwater was made by adding to tap water: NaCl, 16.1 $g \cdot L^{-1}$; $CaCl_2$, 1.0 $g \cdot L^{-1}$; KCl, 0.5 $g \cdot L^{-1}$; $NaHCO_3$, 0.1 $g \cdot L^{-1}$; $MgCl_2$, 3.1 $g \cdot L^{-1}$; $MgSO_4$, 3.8 $g \cdot L^{-1}$. Ion concentrations in the saltwater tanks were: Na^+, 260 $meq \cdot L^{-1}$; Cl^-, 300 $meq \cdot L^{-1}$; K^+, 6.8 $meq \cdot L^{-1}$; Ca^{++}, 14 $meq \cdot L^{-1}$; Mg^{++}, 64 $meq \cdot L^{-1}$. These are similar to concentrations in brackish water at Kamouraska, measured on occasion: Na^+, 230–310 $meq \cdot L^{-1}$; Cl^-, 280–360 $meq \cdot L^{-1}$; K^+, 4.8–6.6 $meq \cdot L^{-1}$ (Ca^{++} and Mg^{++} were not measured). The eels were kept at 15.0°C in 500-L tanks and received no food. They were killed by a blow on the head, decerebrated, measured (±1 mm), and weighed (±1 g). Though the length-distribution for the pooled samples (1981–1985) looks homogeneous (Figure 2), there was a difference in mean length and mean weight between healthy eels collected in fresh water (hereafter referred to as FW eels) and healthy eels collected in salt water (hereafter referred to as SW eels) ($P < 0.05$) (Figure 2). The distribution for the diseased eels was also different from those of the FW and the SW eels ($P < 0.05$). However, based on histological examination of the gonads, the samples were homogeneous in that they were exclusively maturing females. This is not an unusual situation in the St. Lawrence (Dutil et al. 1985).

Histology.—Part of the gonad was dissected out, fixed in Bouin's solution 2 d, and dehydrated in alcohol (Miles Scientific Tissue Tek V.I.P. 1000). Tissues were embedded in paraffin (Paraplast +) and 8-μm sections were cut and mounted, stained in hematoxylin, and counterstained in eosine (Shandon Varistain 24-3). The slides were examined microscopically for sex and maturity based on the mean value of the maximum diameter of 10 randomly selected oocytes showing a nucleus.

Branchial filaments, taken from any gill arch, were cut longitudinally. Sections (6 μm) made through the afferent side were stained as above. The cytoplasm of chloride cells shows a strong reaction to eosine (Keys and Willmer 1932). Some of the tissues fixed in Bouin's solution were stained with PAS (periodic acid–Shiff) and PIC (picro–indigo–carmine) methods (Gabe 1968) to make sure that mucous cells had not been mistaken for chloride cells. Finally, the identification of chloride cells was corroborated by the use of osmium tetroxide. This chemical blackens chloride cells on a pale yellow background (Gabe 1968; Garcia-Romeu and Masoni 1970).

Branchial sections were examined for the relative abundance of chloride cells on primary and secondary lamellae. Relative abundance was scaled into four classes based on the examination of numerous interlamellar spaces on four or five primary lamellae: (1) sections showing less than one chloride cell per interlamellar space (i.e., between two secondary lamellae on the primary lamellae); (2) sections showing regularly one or two chloride cells per space and scattered cells

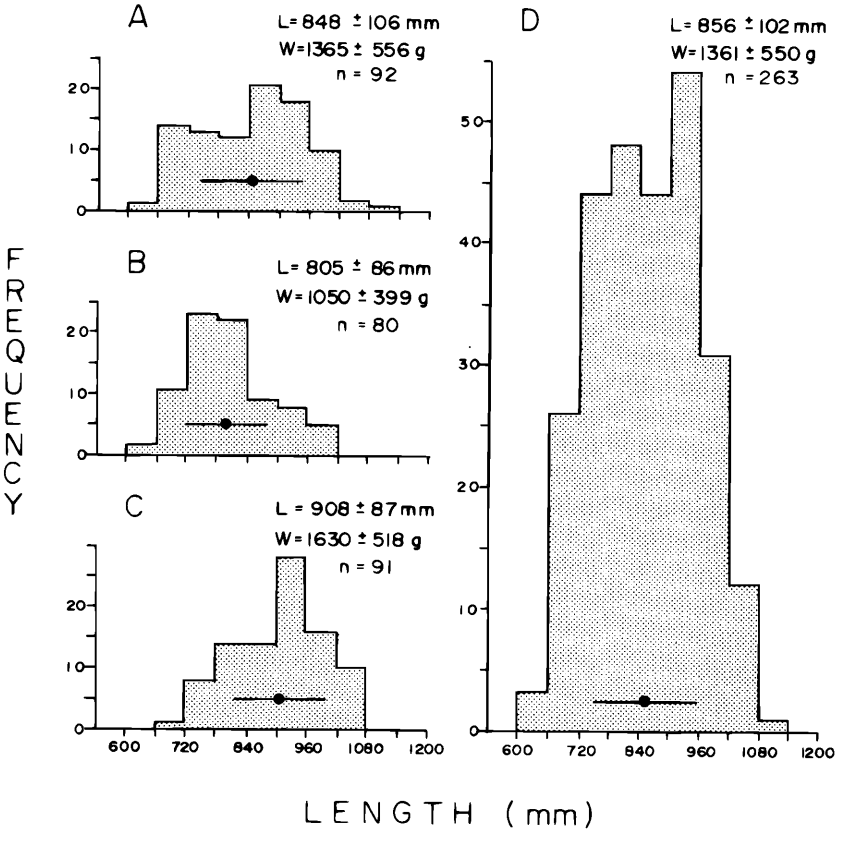

FIGURE 2.—Length-frequency distributions of diseased American eels (A), freshwater eels (B), saltwater eels (C), and pooled distribution (D). Mean lengths (L and solid circles), mean weights (W), standard deviations (± and horizontal lines), and numbers of fish (n) are shown.

near the arch; (3) sections showing regularly three to five chloride cells per space and numerous cells near the arch; (4) sections showing regularly three to five chloride cells per space plus a few cells located on the lower third of secondary lamellae, and numerous cells on secondary lamellae near the arch. The presence or absence of isolated chloride cells on secondary lamellae of individuals in classes 1 and 2 was also noted as were the pathological conditions.

Blood analysis.—Blood samples were collected from the posterior cardinal vein, allowed to clot for 5–10 min, and centrifuged at 4,000 revolutions/min for 5 min. The serum was stored at 4°C for less than 5 d prior to determinations of Na^+, Cl^-, and K^+ concentrations by flame spectrophotometry and of osmolality by freezing point depression measurement. The results are expressed in meq·L^{-1} and mOsmol·kg^{-1}.

Gill Na^+,K^+-ATPase.—The Na^+,K^+-ATPase activity of branchial tissue samples (150–200 mg) stored at −80°C for less than 40 d was determined following Zaugg (1982). Modifications were made to Zaugg's method, based on characteristics of the enzyme in eels, following Butler and Carmichael (1972) and Thomson and Sargent (1977). Solution A was modified to 100 mM NaCl, 20 mM KCl, 6 mM $MgCl_2 \cdot 6H_2O$, and 40 mM imidazole. The pH of solution A and that of ATP was adjusted to 7.2. Centrifugation times were prolonged to 10 min.

Heavy metals and pesticides.—Carcasses stored at −20°C for 6 months were deheaded, eviscerated, and homogenized and their heavy metal (9 variables), pesticide (16 variables), and PCB contents were measured following the methods outlined in the Chemical Methods Manual of the Inspection Branch of the Department of Fisheries and Oceans.

Statistical tests.—Analysis of variance was used to test for significant differences in mean lengths and mean weights (one fixed factor), serum ion concentrations, serum osmolality, and

branchial Na^+,K^+-ATPase activity (two fixed factors, unbalanced design). Chloride cell counts were tested by median tests (Siegel 1956). Pairwise tests were made a posteriori following the Student–Newman–Keuls procedure (Sokal and Rohlf 1969) when the analysis of variance detected significant differences among means. Those tests were run only on distributions having homogeneous variances and at the same probability level as tests of homogeneity of variances (Underwood 1981). Homogeneity of variances was checked using Cochran's *C*-test (Winer 1971). Homogeneity of regression lines was tested by analysis of covariance (Snedecor and Cochran 1967).

Results

Na^+, Cl^-, and K^+ Concentrations

Sodium ion concentrations (Tables 1, 2) in untreated FW eels (147.4 meq·L^{-1}) were lower by 20 meq·L^{-1} than in untreated SW eels (167.7 ± 5.3). They were also lower in eels kept 4 d in fresh water than in eels kept 4 d in salt water, both for FW eels (144.8 meq·L^{-1} in fresh water versus 154.8 in salt water, NS) and SW eels (149.6 meq·L^{-1} in fresh water versus 157.9 in salt water). Saltwater eels tended to maintain slightly higher concentrations than FW eels in both treatments (NS). Freshwater eels and SW eels kept in fresh water also maintained a similar sodium concentration (144.8 and 149.6 meq·L^{-1}, respectively) to the untreated FW eels (147.4) but the concentration was lower in FW eels and in SW eels kept 4 d in salt water (154.8 and 157.9 meq·L^{-1}) than in the untreated SW eels (167.7).

The diseased eels had consistently lower Na^+ concentrations (117.4 meq·L^{-1}) than either FW or SW eels in the St. Lawrence (147.4 and 167.7 meq·L^{-1}). They behaved similarly to FW eels when transferred to fresh water for 4 d: their Na^+ concentration did not change. Transfer to salt water, however, resulted in a greater increase in Na^+ concentrations in diseased eels (117.4–136.0 meq·L^{-1}) than in FW eels (147.4–154.8 meq·L^{-1}, NS). Though the mean concentration was still lower than in FW eels, some individuals had recovered to near-normal levels as indicated by the range in concentrations.

The results for Cl^- (Tables 1, 2) were very similar to those for Na^+: the concentrations were much lower in untreated FW eels (110.0 meq·L^{-1}) than in untreated SW eels (122.2), and in fresh water (118.8 meq·L^{-1} for FW eels and 121.7 for SW eels) than in saltwater (132.2 meq·L^{-1} for FW eels and 133.4 for SW eels). There were also some dissimilarities; concentrations were higher in both FW and SW eels kept 4 d in fresh water than in untreated FW eels. Freshwater and SW eels kept 4 d in salt water maintained higher Cl^- concentrations than untreated SW eels.

Serum Cl^- concentrations in diseased eels followed the same trend as those for Na^+, but the gap between healthy eels and diseased eels was even wider and ranged from 30 to 50 meq·L^{-1}. Most interesting was the recovery of some individuals in salt water as indicated by an overlap in the range between the diseased eels in salt water (61–118 meq·L^{-1}) and the untreated SW eels (109–131 meq·L^{-1}), and the variability in the data for the diseased eels in salt water.

TABLE 1.—Mean Na^+, Cl^-, and K^+ concentrations and their standard deviations (meq·L^{-1}), in the sera of freshwater, saltwater, and diseased American eels subjected to 4-d treatments in fresh water or salt water or to no treatment. The range is shown in parentheses.

Ion and treatment	Freshwater eels	Saltwater eels	Diseased eels
Na^+			
Fresh water-4 d	144.8±8.8 (132–154) *N*=5	149.6±2.8 (144–152) *N*=7	114.8±8.3 (109–129) *N*=5
Salt water-4 d	154.8±3.2 (150–157) *N*=5	157.9±3.4 (151–160) *N*=7	136.0±12.5 (126–150) *N*=3
None	147.4±8.6 (135–156) *N*=5	167.7±5.3 (158–172) *N*=6	117.4±6.3 (106–125) *N*=8
Cl^-			
Fresh water-4 d	118.8±10.5 (107–129) *N*=5	121.7±7.7 (106–128) *N*=7	66.4±17.1 (53–96) *N*=5
Salt water-4 d	132.2±3.5 (128–136) *N*=5	133.4±4.2 (129–139) *N*=7	97.0±31.3 (61–118) *N*=3
None	110.0±14.3 (98–134) *N*=5	122.2±7.4 (109–131) *N*=6	72.9±11.8 (56–89) *N*=8
K^+			
Fresh water-4 d	4.23±1.96 (2.15–6.34) *N*=5	4.81±1.36 (3.30–6.70) *N*=7	7.49±2.97 (4.21–12.16) *N*=5
Salt water-4 d	3.71±0.96 (2.60–4.74) *N*=5	4.56±0.99 (3.11–5.96) *N*=7	4.20±0.23 (3.94–4.34) *N*=3
None	6.03±1.12 (4.33–7.05) *N*=5	4.67±1.37 (2.80–5.88) *N*=6	6.41±2.00 (4.09–10.44) *N*=8

TABLE 2.—Results of pairwise tests on mean serum Na^+ concentrations, serum osmolality, and gill Na^+,K^+-ATPase activity in healthy American eels taken from freshwater (FW eels) or saltwater (SW eels) portions of the St. Lawrence River estuary and subjected to 4 d in fresh or salt water, or to no treatment, and in diseased eels. Asterisks denote significant differences.

Comparison by treatment				Comparison by status			
Comparison	Diseased	FW eels	SW eels	Comparison	Fresh water 4 d	Salt water 4 d	No treatment
Na (statistical threshold: $P = 0.01$)							
Fresh water-4 d × no treatment			*	Diseased eels × FW eels	*	*	*
Saltwater-4 d × no treatment	*		*	Diseased eels × SW eels	*	*	*
Fresh water-4 d × saltwater-4 d	*		*	FW eels × SW eels			*
Osmolality (statistical threshold: $P = 0.05$)							
Fresh water-4 d × no treatment			*	Diseased eels × FW eels	*	*	*
Saltwater-4 d × no treatment			*	Diseased eels × SW eels	*	*	*
Fresh water-4 d × saltwater-4 d				FW eels × SW eels		*	*
Na^+,K^+-ATPase (statistical threshold: $P = 0.05$)							
Fresh water-4 d × no treatment		*[a]	*	Diseased eels × FW eels			
Saltwater-4 d × no treatment				Diseased eels × SW eels	*	*	*
Fresh water-4 d × saltwater-4 d		*[a]	*	FW eels × SW eels	*	*	*

[a] Diseased eels and FW eels combined.

Results for serum K^+ (Tables 1, 2) are not so conclusive but they tend to confirm results on Na^+ and Cl^- for diseased eels kept 4 d in salt water. Plots of mean Na^+ and Cl^- concentrations against mean K^+ concentrations (Figure 3) both indicate extreme values for diseased eels kept in fresh water and for diseased eels that received no treatment, whereas the mean K^+ concentration of diseased eels kept in salt water does not show such an extreme and has by far the smallest coefficient of variation (6%).

Heterogeneity in the data was revealing in that it was associated with variability in the results for the diseased eels (Table 1). Variances in the Na^+ data were heterogeneous at the 5% level but not at the 1% level; those of Cl^- and K^+ proved heterogeneous at both the 1% and 5% levels. This suggests there was a biological basis for heterogeneity and may explain that transformations failed to restore the homogeneity of variances. The analysis of variance was run on Na^+ concentrations and not on Cl^- and K^+ concentrations. The interaction between the two factors (categories and treatments) turned out to be significant ($P = 0.002$) but much smaller than the main effects, particularly the categories of eels (mean square, categories = 6,320; mean square, treatments = 546; mean square, interaction = 298).

Serum Osmolality

Serum osmolality (Tables 2, 3) was lower in untreated FW eels (323.6 mOsmols·kg^{-1}) than in untreated SW eels (392.8). Freshwater eels in fresh water had a lower serum osmolality than FW eels in salt water (303.0 versus 319.0 mOsmol·kg^{-1}, NS). There was a similar and significant increase between SW eels kept in fresh water and SW eels kept in salt water (323.9 to 335.0 mOsmol·kg^{-1}), but transfer of SW eels to either fresh water or salt water resulted in a sharp decline in osmolality, not unlike the decrease in Na^+ (not Cl^-) concentration. These results, on the other hand, differ from those on Na^+ and Cl^- in that mean osmolality in SW eels kept 4 d in fresh water (323.9) was still higher than the mean osmolality of FW eels kept 4 d in salt water (319.0); the ranges overlap substantially.

TABLE 3.—Mean osmolality and standard deviations (mOsmol·kg^{-1}), in the sera of freshwater, saltwater, and diseased American eels subjected to 4-d treatments in fresh water or salt water or to no treatment. The range is shown in parentheses.

Treatment	Freshwater eels	Saltwater eels	Diseased eels
Fresh water-4 d	303.0±17.9 (272–317) $N=5$	323.9±4.0 (320–329) $N=7$	270.2±29.6 (235–300) $N=5$
Salt water-4 d	319.0±4.5 (313–324) $N=5$	335.0±3.6 (330–339) $N=7$	280.0±27.4 (259–311) $N=3$
None	323.6±22.5 (297–349) $N=5$	392.8±30.0 (355–422) $N=6$	257.1±16.4 (236–281) $N=8$

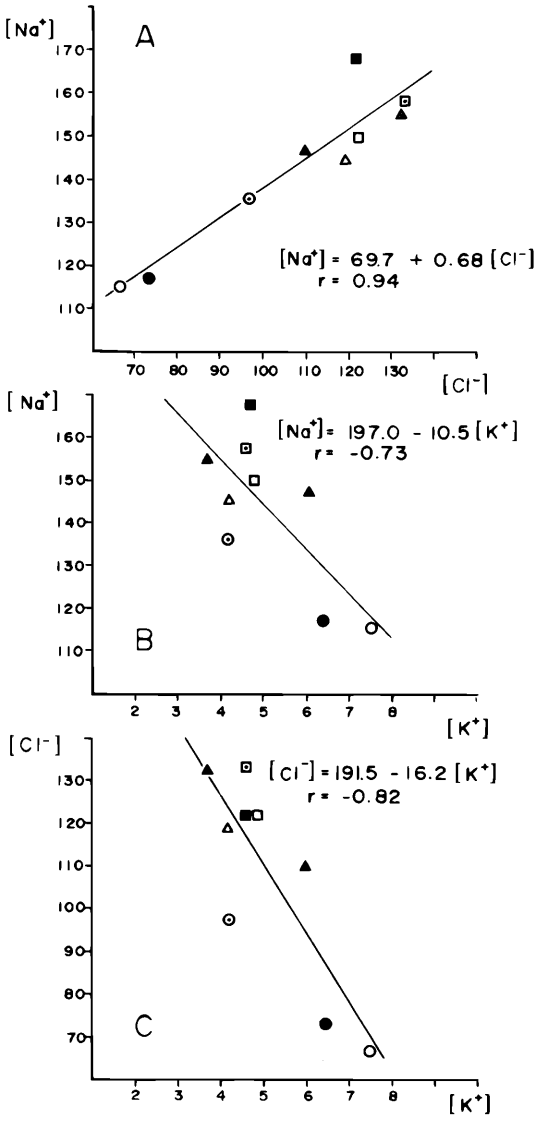

FIGURE 3.—Linear regressions of mean concentrations (meq · L^{-1}) of Na^+ on Cl^- (A), Na^+ on K^+ (B), and Cl^- on K^+(C) for American eels. ● Diseased eels with no treatment; ○ diseased eels in freshwater tanks; ⊙ diseased eels in saltwater tanks. ▲ Freshwater eels with no treatment; △ freshwater eels in freshwater tanks; ◬ freshwater eels in saltwater tanks. ■ Saltwater eels with no treatment; □ saltwater eels in freshwater tanks; ⊡ saltwater eels in saltwater tanks.

Diseased eels had very low osmolalities compared with FW eels and SW eels for all treatments, reaching a low value of 235 mOsmol·kg^{-1}. Transfer of the diseased eels to either fresh water or salt water resulted in an increase in mean osmolality. Though the normal condition was restored in some individuals following transfer to fresh water and more particularly to salt water, where values as high as 310 mOsmol·kg^{-1} were reached, treatments did not restore normal concentrations in all individuals.

The analysis of variance showed that interaction was highly significant between the two factors (Figure 4) ($P < 0.0001$; mean square = 3,490) and that mean osmotic concentrations differed between categories ($P < 0.0001$; mean square = 32,096) and between treatments ($P = 0.001$; mean square = 2,915). Variances were not heterogeneous ($P > 0.05$).

Branchial Na^+,K^+-ATPase

Branchial Na^+,K^+-ATPase activity was similar in the diseased eels to that in the FW eels (Tables 2, 4), indicating that the low serum NaCl and osmotic concentrations observed in the diseased eels could not be associated with increased branchial enzymatic activity. Diseased eels and FW eels were pooled to test treatments (Table 2). The

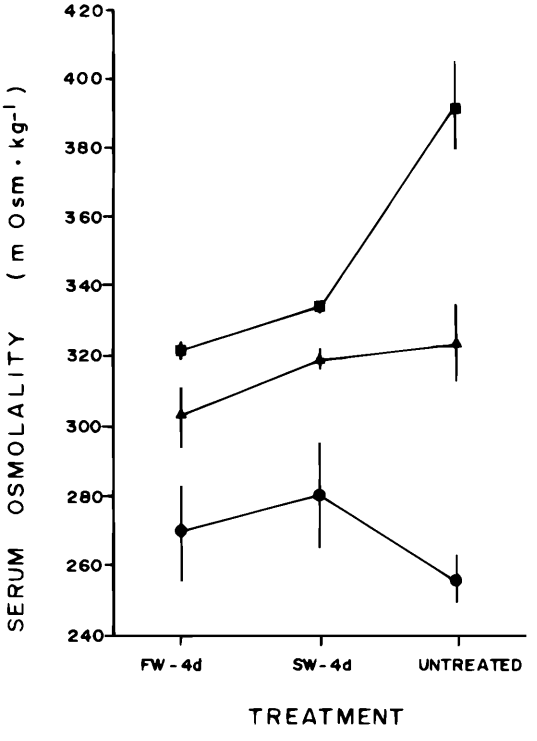

FIGURE 4.—Interaction between categories (freshwater or saltwater) and treatments (4 d in fresh or salt water, or no treatment) on mean serum osmolality in diseased American eels (●), freshwater eels (▲), and saltwater eels (■). Standard errors are shown as vertical bars.

TABLE 4.—Mean branchial Na^+,K^+-ATPase activity and standard deviations (μmol P_i·mg protein^{-1}·h^{-1}) of freshwater, saltwater, and diseased American eels subjected to 4-d treatments (fresh water or salt water or to no treatment). The range is shown in parentheses.

Treatment	Freshwater eels	Saltwater eels	Diseased eels
Fresh water-4 d	5.56±2.62 (3.3–9.6) N=5	29.06±9.99 (12.0–41.5) N=7	10.28±6.14 (4.3–19.6) N=5
Salt water-4 d	20.98±8.31 (12.9–33.9) N=5	45.00±9.32 (28.0–55.3) N=7	20.98±9.63 (10.3–32.6) N=4
None	19.24±13.61 (4.6–34.8) N=5	46.53±9.54 (37.7–61.8) N=6	14.98±5.40 (6.7–26.3) N=10

variances were not heterogeneous ($P > 0.05$) and the means differed between categories and treatments ($P < 0.0001$), but there was no interaction ($P = 0.4266$). The gill Na^+,K^+-ATPase activity (μmol P_i·mg protein^{-1}·h^{-1}) of untreated FW eels (including the diseased individuals) decreased upon transfer to fresh water (16.40 to 7.92) and increased (NS) upon transfer to salt water (20.98). Similarly, untreated SW eels transferred to fresh water showed a sharp decline in enzymatic activity (46.53 to 29.06), but no change when transferred to salt water (45.00). Gill Na^+,K^+-ATPase activity was also much higher in untreated SW eels transferred to fresh water than in untreated FW eels transferred to salt water. Finally, the level of activity in SW eels in any treatment was much higher than in the FW eels.

TABLE 5.—Frequency of occurrence of chloride cells, expressed as classes of relative abundance, observed in the gill sections of 94 American eels collected in 1983, 1984, and 1985 from the St. Lawrence River.

Category, treatment	Class of relative abundance[a] 1	2	3	4
Diseased eels + freshwater eels				
Fresh water-4 d	5	4	1	0
Salt water-4 d	1	3	5	2
Saltwater eels				
Fresh water-4 d	0	1	2	2
Salt water-4 d	0	0	5	2
No treatment				
Diseased eels	2	5	9	4
Freshwater eels	5	5	5	5
Saltwater eels	0	2	9	10

[a] Relative abundance of chloride cells increases with class number.

Gill Histology and Contamination

The number of chloride cells (Table 5) in the diseased eels was similar to that in FW eels ($\chi^2 = 0.41$). This observation is consistent with the results obtained for the branchial Na^+,K^+-ATPase activity. Diseased eels and FW eels also had fewer chloride cells than SW eels ($\chi^2 = 8.83$). Transfer of FW eels to salt water brought about proliferation of the chloride cells ($P < 0.02$), but the number of chloride cells was not reduced by transferring SW eels to fresh water for 4 d ($P = 0.42$) (Figures 5, 6). Median tests were performed on chloride cell abundance data after pooling of classes 1 and 2 and of classes 3 and 4.

The incidence of pathological conditions in the branchial tissues, however, differed markedly between diseased and healthy eels. Diseased eels had the highest incidence (86%), but some of the FW (28%) and SW eels (18%) also had similar damage (Table 6). Pathological conditions of the gills were so common when sections were examined for the relative abundance of chloride cells that the number of slides showing gill damage was assessed. Damage was not local and could sometimes be detected on macroscopic examination, particularly in 1985. Most common conditions included hypertrophic and hyperplastic cells, necrotic tissues, and some cases of aneurisms (Figure 6c). Interestingly, many diseased eels also had isolated chloride cells on the secondary lamellae (Table 6).

However, diseased eels did not have higher concentrations of contaminants than healthy eels collected in fresh water or in salt water (Table 7).

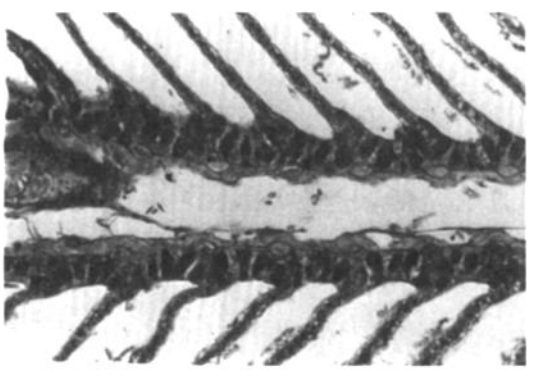

FIGURE 5.—Chloride cells, stained black by osmium tetroxide, in the branchial lamellae of an American eel. This eel was collected at the saltwater site in the St. Lawrence River and shows a moderately high number of chloride cells.

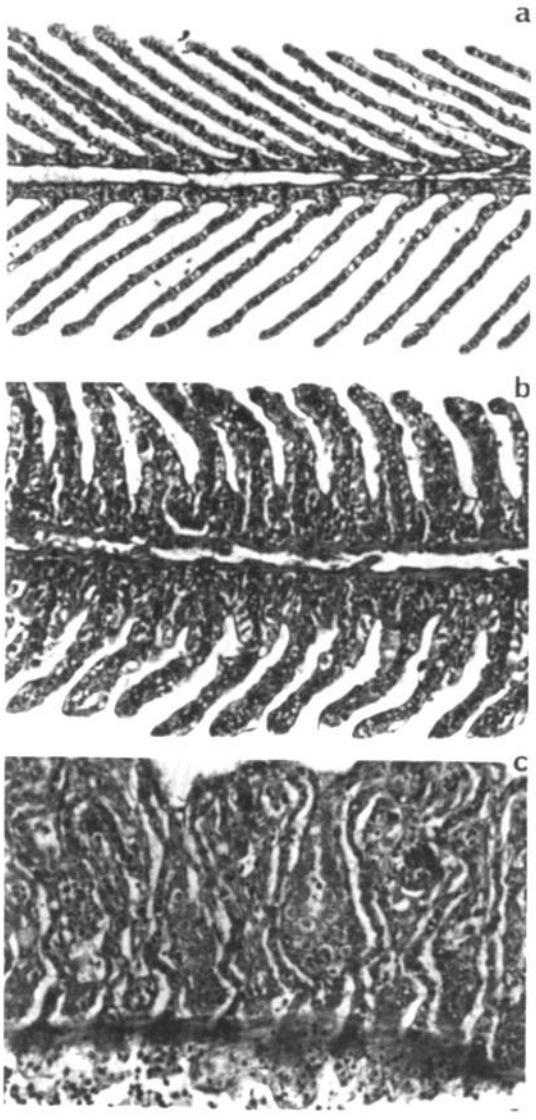

FIGURE 6.—Normal and pathological conditions of gill lamellae in American eels from the St. Lawrence River. **A:** Normal condition of primary and secondary lamellae. **B:** Reduction of the interlamellar space due to swelling of the gill lamellae. **C:** Thickening of membranes and fusion of secondary lamellae; the interlamellar space becomes nonexistent in such an extreme condition.

Generally, heavy metal, PCB, and pesticide concentrations were actually higher in SW eels than in FW eels and diseased eels. Mean heavy metal concentrations were slightly lower in diseased than in FW eels, and those of PCB and pesticides higher in diseased than in FW eels, but in those instances where they were higher than in FW eels,they were still lower than in SW eels. This rules out the possibility that the disease is related to contamination. The data indicate that the eels had spent part of their freshwater residence in polluted areas. Dutil et al. (1985) have shown that 70% of the eels caught in the St. Lawrence estuary came from Lake Ontario.

TABLE 6.—Frequency of occurrence of pathological conditions observed in the gill sections of 94 American eels collected in 1983, 1984, and 1985 from the St. Lawrence River, and occurrence of lamellar chloride cells.

Feature	Diseased eels $N=29$	Freshwater eels $N=32$	Saltwater eels $N=33$
Pathological conditions	25	9	6
Isolated chloride cells in secondary lamellae	17	2	1

Maturation

Maturation rate, measured as change in the

TABLE 7.—Mean concentrations and standard deviations ($\mu g \cdot g^{-1}$, wet-weight basis) of heavy metals, PCBs, and pesticides in American eels collected in 1984 from the St. Lawrence River.

Chemical	Diseased eels, $N=16$	Freshwater eels, $N=15$	Saltwater eels, $N=20$
Hg	0.34±0.21	0.36±0.15	0.39±0.21
Cd	0.03±0.01	0.04±0.04	0.02±0.02
Co	<0.05	<0.05	<0.05
Cr	0.05±0.04	0.06±0.06	0.06±0.02
Cu	0.77±0.19	0.90±0.28	0.93±0.22
Mn	1.06±0.28	1.14±0.35	0.82±0.24
Ni	<0.10	<0.10	<0.10
Pb	0.33±0.12	0.36±0.08	0.31±0.05
Zn	34.78±7.42	37.97±7.36	34.34±5.73
α-hexachlorocyclohexane	0.017±0.004	0.016±0.004	0.029±0.041
γ-hexachlorocyclohexane	0.003±0.001	0.003±0.001	0.004±0.001
Heptachlor	0.022±0.020	0.013±0.018	0.031±0.025
Heptachlor epoxide	0.017±0.013	0.012±0.004	0.023±0.013
Oxychlordane	0.016±0.014	0.010±0.006	0.022±0.013
α-chlordane	0.058±0.064	0.032±0.028	0.062±0.040
γ-chlordane	0.020±0.019	0.015±0.012	0.021±0.011
Aldrin	0.007±0.004	0.006±0.002	0.008±0.002
Dieldrin	0.061±0.062	0.034±0.020	0.103±0.068
Endrin	0.011±0.008	0.007±0.004	0.015±0.011
P-P'-DDD	0.144±0.102	0.103±0.082	0.196±0.111
P-P'-DDE	0.261±0.230	0.155±0.156	0.412±0.270
P-P'-DDT	0.061±0.054	0.037±0.027	0.126±0.085
DDD + DDE + DDT	0.460±0.346	0.296±0.196	0.734±0.450
Hexachlorobenzene	0.054±0.047	0.026±0.016	0.099±0.055
PCB	5.323±4.415	3.030±2.156	6.688±3.673
Mirex	0.189±0.185	0.056±0.090	0.301±0.222

natural logarithm of the mean diameter (μm) of 10 oocytes (Y) versus date (X), was slower in diseased than healthy eels.

$$\text{Healthy: } Y = 3.6936 + 0.0058X; \quad N = 524; r^2 = 0.72. \tag{1}$$

$$\text{Diseased: } Y = 4.4274 + 0.0026X; \quad N = 125; r^2 = 0.16. \tag{2}$$

In a 2-month period (mid-August to mid-October), the diameter of oocytes increased from 149 to 174 μm in diseased eels and from 151 to 214 μm in healthy eels. The sections of gonadal tissues were as reported by Wenner and Musick (1974) for migrating American eels caught in Chesapeake Bay in late November; the oocytes were smaller but the mean diameter in some individuals caught late in October reached 250 μm. There was a great deal of variability in the data for diseased eels, but the variances were not significantly heterogeneous ($P > 0.05$). The slopes were significant ($P < 0.05$) and differed ($F = 20.05$; $P < 0.05$). The relationship between the diameter of oocytes and time in maturing American eels has not been reported in the literature. Data in an earlier study showed that immature eels had a mean oocyte diameter of 65 ± 7 μm that did not increase in the summer period ($P = 0.60$; Dutil et al. 1985). Maturing eels in equation (1) were collected between 1981 and 1985 from May to mid-October. Diseased eels in equation (2) were also collected between 1981 and 1985 but over a shorter period, mid-August to mid-October. However, the conclusions remained unchanged when eels collected before mid-August were excluded from the analysis.

Discussion

The changes in serum Na^+, Cl^-, K^+ and osmotic concentrations, and in branchial chloride cells and Na^+,K^+-ATPase activity measured in this study in maturing American eels migrating down the St. Lawrence estuary and in maturing eels transferred directly into fresh water or salt water may have been influenced by maturity. Some of the control mechanisms involved in the transition of eels to salt water are preparatory, i.e., they are set up when maturing eels are still in fresh water (Oidé and Utida 1967a, 1967b; Utida et al. 1967; Thomson and Sargent 1977). The eels in the present study varied in length and weight between locations but they were all females and their gonads were maturing. The changes may also have been influenced by variations in water quality, as they are in Atlantic salmon *Salmo salar* (Saunders et al. 1983), and there are some indications that conditions in the tanks did not reflect natural conditions precisely. Saltwater eels kept in saltwater tanks had lower Na^+ concentrations than SW eels in nature. Freshwater eels kept in freshwater tanks also had higher Cl^- concentrations than FW eels in nature. There were lower calcium and magnesium concentrations in the freshwater tanks than in the St. Lawrence at St. Nicolas, and no doubt many more divalent ions were present in the St. Lawrence that were not in freshwater and saltwater tanks. Stress, associated with handling, is most likely to have occurred in SW eels as they were transported in moist air for 2 h before they were processed. This may have resulted in increases in the concentrations of metabolic by-products such as NH_4^+ and HCO_3^-. Bradley and Rourke (1985) recently demonstrated that decreased NH_4^+ excretion, associated with low environmental Na^+ concentrations, could result in elevated branchial NH_4^+, which causes edema and hyperplasia in rainbow trout *Salmo gairdneri*. Finally, the experimental salinities, while similar to those of the saltwater site, were also significantly less than the 35‰ that migratory eels would eventually encounter.

Migrating anadromous and catadromous fishes rely on two strategies to survive changes in salinity. Motais (1967) made the observation that contradictions in findings concerning the euryhaline mummichog *Fundulus heteroclitus* and European eel *Anguilla anguilla* should make us aware that the same mechanisms may not necessarily be common to all teleosts. Folmar and Dickhoff (1980) concluded that species such as mummichog might not have the same mechanisms of gill Na^+,K^+-ATPase regulation as salmonids. The number of ouabain binding sites is a function of external salinity in mummichogs, suggesting that these animals can react instantaneously to variations in salinity (Towle et al. 1977). The mechanism of short-term control has been modeled by Towle et al. (1977) and Sardet et al. (1979). Salmonids require a longer time to react to changes in salinity. Turnover rates of DNA measured in gill filaments of coho salmon *Oncorhynchus kisutch* indicated that protein synthesis required 4–6 d (Conte and Lin 1967). This is the time required to increase branchial Na^+,K^+-ATPase activity in coho salmon transferred to salt water (Folmar and Dickhoff 1979). Numerous reviews have been published recently on the role

of chloride cells (Kirschner 1977; Payan et al. 1984), branchial Na^+,K^+-ATPase (Cantley 1981; Schuurmans Stekhoven and Bonting 1981; De-Renzis and Bornancin 1984), and branchial mechanisms of osmotic regulation (Evans 1979, 1980; Evans et al. 1982; Hoar and Randall 1984a, 1984b).

The results in this study indicate that maturing American eels migrating down the estuary might rely on a strategy based on both short-term and long-term mechanisms to maintain relatively constant serum ionic and osmotic concentrations in salt water. Maturing eels in nature exhibited the same mechanisms as used by salmonids (long-term control). The number of chloride cells increased markedly and gill Na^+,K^+-ATPase activity more than doubled in salt water. This resulted in relatively small increases in Na^+, Cl^-, and osmotic concentrations. The increases in serum Na^+ and Cl^- concentrations were much the same as observed in an earlier study (Dutil 1984). However, serum K^+ and osmolality were higher. These changes probably took place over a 4-week period because commercial landings peak 4 weeks earlier in St. Nicolas than in Kamouraska.

Transfer of FW eels to salt water demonstrated that maturing eels had the ability to maintain relatively constant ionic and osmotic concentrations without increasing their gill Na^+,K^+-ATPase activity (short-term control). Freshwater eels transferred to salt water maintained similar serum ionic and osmotic concentrations as SW eels in nature, and their serum osmotic concentration remained close to that of FW eels in nature, indicating that seaward-migrating eels in fresh water possess significant salinity tolerance and hypoosmoregulatory ability. The increase in ionic and osmotic concentrations reported in this study for FW eels (maturing) transferred to salt water are smaller than those reported for immature eels (Sharratt et al. 1964; Munroe and Polluhowich 1974) and similar to those reported for maturing eels (Sharratt et al. 1964; Butler 1966). Chloride cells proliferated over the 4-d period but there was no detectable change in gill Na^+,K^+-ATPase activity. The number of chloride cells had increased significantly in 4 d and peaked 10 d later in immature *A. japonica* transferred to salt water (Shirai and Utida 1970). Proliferation of chloride cells in immature European eels also preceded the change in gill Na^+,K^+-ATPase activity and a maximum was reached in less than 10 d but, in our study with American eels, there was no proliferation of chloride cells in fresh water such as reported in maturing European eels (Thomson and Sargent 1977).

Transfer of SW eels to fresh water also points to the existence of a short-term mechanism enabling maturing eels to maintain osmotic integrity as salinity conditions change over a short period. Na^+, Cl^-, and K^+ concentrations returned in 4 d to the same level as in FW eels in fresh water, but there was no reduction in the number of chloride cells. Gill Na^+,K^+-ATPase activity decreased 30% but remained higher than in FW eels kept 4 d in salt water. The slight reduction of osmotic concentration can be explained by the increased permeability of the gills of SW eels in fresh water, resulting in the rapid passage of water through the membrane (Isaia 1984). These results suggest the existence of a mechanism to react to short-term variations in salinity. This is vital in such a situation and may also be vital during migration through estuaries. However, the reversibility of these mechanisms is not total in that ionic and osmotic concentrations in SW eels, though they tended to get near those of FW eels in both treatments, always remained higher than in FW eels.

The contradiction in the results, indicating that the number of chloride cells increased while gill Na^+,K^+-ATPase activity remained unchanged 4 d after transfer of maturing eels to salt water, may also have been due to the great variability in initial (untreated) ATPase levels. This variability was perhaps a reflection of variations in developmental stages of migratory eels. Both the variability and absolute levels of gill Na^+,K^+-ATPase activity were reduced following a 4-d exposure to fresh water. Levels of gill Na^+,K^+-ATPase activity of FW eels kept 4 d in fresh water were significantly lower than those of FW eels kept 4 d in salt water. If the low levels of gill Na^+,K^+-ATPase activity after 4 d in fresh water are considered "basal" levels, then higher and more variable levels in untreated FW eels may reflect a preparatory adaptation for their marine migration.

Since no mortality took place in eels that reached the brackish section of the estuary, the "disease" is likely to be linked to osmoregulatory failure. Bacterial and viral pathogens have been ruled out as potential causes of the mortality (Dutil and Lallier 1984; Robin, unpublished). The present study also rules out the possibility that mortalities are linked to higher concentrations of heavy metals and pesticides in the tissues of diseased eels through a delayed action of the chemicals on the changing eel physiology, such as

described by Kerswill (1967). Dutil (1984) indicated that diseased eels had unusually low Na^+ and, more particularly, low Cl^- concentrations in their serum. Their serum osmotic concentrations were among the lowest recorded in the literature (as low as 217 $mOsmol \cdot kg^{-1}$ in extreme cases). The present study corroborates the earlier observations, i.e., low Na^+, low Cl^-, and extremely low osmotic concentrations (mean, 257 ± 16 $mOsmol \cdot kg^{-1}$) and suggests that iono- and osmoregulatory failure could also have resulted in a decrease in the rate of maturation. This failure to maintain constant ionic and osmotic concentrations is similar to that reported in maturing eels by Callamand and Fontaine (1940a, 1940b). Fontaine and Koch (1950) explained this by the fact that eels, unlike most teleosts, cannot absorb Cl^- from fresh water (Krogh 1937; Kirsch 1972). Normally, the eels receive minerals from their food but they stop feeding at the onset of maturation. Starvation might also be the reason for low Cl^- values reported, for instance, by Schmidt-Nielsen and Renfro (1975).

Developmental changes that are part of the preparatory adaptations for life of eels in the sea may be subject to interference. Schreck (1982) has reviewed the increased susceptibility of salmonids to stress during the parr–smolt transformation. McCormick and Saunders (1987) have argued that ion losses sometimes observed in fresh water during transformation are not solely the result of differentiation of osmoregulatory organs, but are due to a synergy between differentiation and environmental change. Perhaps a similar condition may explain ion losses and mortality in diseased eels. This explanation, however, is not supported by the results of Utida et al. (1967) showing that maturing eels had lower branchial ion losses than immature eels.

The high incidence of pathological conditions in gill sections of diseased American eels suggests that iono- and osmoregulatory failure result from a change in permeability of branchial membranes that might have been caused by pollution in the St. Lawrence estuary. Low Na^+, Cl^-, and osmotic concentrations cannot be explained by the proliferation of chloride cells and enhanced Na^+,K^+-ATPase activity in diseased eels in fresh water. Chloride loss through intact branchial membranes of eels is very slow, particularly in maturing individuals (Kirsch 1972), and cannot explain the lower Cl^- concentrations in diseased eels. However, high K^+ concentrations in diseased eels indicate that cells were being damaged. Pathological conditions of the gills in this study included necrosis and a high incidence of hypertrophy and hyperplasia. Heavy metals injected into the blood of rainbow trout did not alter gill structure and function (Skidmore 1970), but pesticides and heavy metals in the environment do alter the gill structure of fishes (reviewed by Mallatt 1985). Alterations can be localized to any of the gill arches or some of the lamellae (Gardner and Yevitch 1970). Hypertrophy and hyperplasia are among the most common alterations (Gardner and Yevitch 1970; Temmink et al. 1983), and are considered initial reactions to membrane damage (Trump et al. 1981). In this study, there were also many instances of isolated chloride cells budding out in the secondary lamellae. Instances of increases in the number of chloride cells have been reported occasionally (Baker 1969; Matthiessen and Brafield 1973; Crespo et al. 1981; Tuurala and Soivio 1982). Laurent and Dunel (1980) concluded that skin damage and transfer of eels into deionized water induced the proliferation of chloride cells in the secondary lamellae and suggested that chloride cells in secondary lamellae may provide a mechanism for increasing net ion gain. Fishes experiencing such structural damage showed characteristic behaviors such as those observed in this study. They swam erratically, lost equilibrium, stayed close to the surface, and showed no reactions to manipulations (Lewis and Lewis 1971; Skidmore and Tovell 1972; Walsh and Ribelin 1975).

Structural alterations in gills have been shown to bring about respiratory distress (Skidmore 1970; Skidmore and Tovell 1972; Tuurala and Soivio 1982; Van Der Putte et al. 1982), and to perturb the acid–base balance resulting in iono- and osmoregulatory failure (McKim et al. 1970; Lewis and Lewis 1971; McCarty and Houston 1976; Lock et al. 1981; Tuurala and Soivio 1982; Van Der Putte et al. 1982; Spry and Wood 1985). The relative incidence of these two mechanisms is debatable, but results so far indicate that respiratory distress might occur in cases of acute toxicities and iono- and osmoregulatory problems might occur in cases of chronic sublethal toxicities (Spry and Wood 1985). Three factors may make eels more vulnerable to osmoregulatory problems than nonmigratory species in the St. Lawrence River. First, maturing eels cannot avoid moving through highly polluted, presumably fishless areas in order to reach the estuary and so are liable to damages resulting in increased permeability and loss of minerals through the branchial membranes, such

as described in this study (though the causative pollutant has not been detected). Second, maturing eels do not feed as they migrate in fresh water, so they cannot make up for loss of minerals by food intake. Third, eels, in contrast to most teleosts, cannot absorb Cl^- through the gills in a freshwater environment (Krogh 1937; Kirsch 1972). These factors may act in synergy to produce the observed ion losses and, unless fish reach the marine environment in time, mortality.

Transferring diseased eels to salt water did not prevent mortality in all instances but the condition of some individuals became less critical. Iono- and osmoregulatory problems can be prevented by adding NaCl to experimental tanks (Lewis and Lewis 1971; see also references in Lock et al. 1981). The increase in serum Na^+ concentration was greater in diseased eels than in healthy individuals upon transfer to salt water (8 meq·L^{-1} and 20 meq·L^{-1} for healthy and diseased eels, respectively). Serum Cl^- concentrations followed the same trend, the increase being 24 meq·L^{-1}. Serum K^+ was reduced and stabilized; the coefficient of variation decreased from 30 to 6%. Similarly, there was an increase in serum osmotic concentration. Some diseased individuals died during the experiment, so these results should also be considered in terms of their variability. The ranges indicate that some individuals recovered to normal ion concentrations, based on untreated FW eels. For instance, the range in Na^+ concentrations in untreated diseased eels was 106–125 meq·L^{-1}. This became 126–150 meq·L^{-1} in diseased eels kept 4 d in salt water compared with 135–156 meq·L^{-1} in untreated FW eels and 150–157 meq·L^{-1} in FW eels kept 4 d in salt water. Two of the diseased individuals survived to the end of the 4-d period in salt water. Serum Na^+, Cl^-, and osmotic concentrations of those individuals were 132 and 150 meq·L^{-1}, 112 and 118 meq·L^{-1}, and 270 and 311 mOsmol·kg^{-1}, respectively.

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