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MERCURY CONTENT AND ULTRASTRUCTURE OF GILLS AND SCALES OF FISH FROM LAKES IN NORTH AND NORTHWESTERN RUSSIA THAT ARE POLLUTED BY ATMOSPHERIC DEPOSITION

Terry Haines[a], Victor Komov[b], Charles Jagoe[c], and Victoria Matey[b]

ABSTRACT

Field studies were conducted of lakes in Darwin National Reserve in 1989, and in Karelia in 1991. Darwin National Reserve is 300 km north of Moscow at the west end of Rybinsk Reservoir. The terrain is generally flat and consists of thick sandy till covered with hardwood forest. Karelia is north of St. Petersburg and consists of hilly terrain underlain with precambrian bedrock. In each case, remote lakes were visited and sampled for water and fish, primarily perch *Perca fluviatilis*. Water samples were analyzed for pH, color, specific conductance, and major cations and anions. Fish were weighed and measured, dorsal muscle tissue was collected and analyzed for mercury, and gills and scales were sampled for examination by light and electron microscopy. In both locations acidic lakes (acid neutralizing capacity <0) were common. Acidic lakes were both clear and colored and the dominant anion in both types was sulfate, indicating that the lakes were acidic because of atmospheric deposition of strong acids and not because of organic acids. Mercury content of fish was increased in acidic and in colored lakes. Mercury appears to enter the lakes by atmospheric deposition, as there are no local sources. Organic acids are believed to increase mercury bioavailability in lakes by transporting mercury from terrestrial regions and possibly contributing to methylation. Also, mercury methylation is probably enhanced in acidic lakes, increasing bioavailability. Gills of perch from acidic lakes had thicker lamellar epithelia and more ion-transporting cells than those collected in circumneutral lakes. The density of microridges on gill surfaces was reduced in perch from acidic lakes. These differences in gill structure serve to decrease gill surface area, increase diffusion distances, increase active ionic influx, and represent responses that allow perch to inhabit acidic waters. Scales of perch from acidic lakes had little or none of the pattern of ridges at the foci observed in scales of perch from higher pH lakes. This probably results from development and growth in low pH, low Ca water, and may be a useful indicator of stressful acidic conditions during early life stages.

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INTRODUCTION

Atmospheric transport and deposition of contaminants is a world-wide problem. The importance of atmospheric transport in contamination of aquatic ecosystems was first documented for emission of acidic gases such as sulfur oxides and nitrogen oxides and the deposition of strong acids, which resulted in water acidification and loss of fish populations (Schindler 1988). This process is also important in the mercury contamination of fish in waters of low acid neutralizing capacity (ANC) in northern Europe and North America (Lindqvist *et al.* 1991; Spry and Wiener 1991). Further, lake acidification increases mercury concentration in fish (Grieb *et al.* 1990; Häkanson *et al.* 1990; McMurtry *et al.* 1989).

Atmospheric deposition of sulfur compounds in Europe has been estimated using a model developed by the Cooperative Programme for Monitoring and Evaluation of the Long Range Transmission of Air Pollutants in Europe (EMEP). The results of this model give a deposition rate of 800-1,640 eq S/ha per year for northern Russia, which is comparable to or exceeds deposition rates for regions in Scandinavia where lake acidification and fish mercury accumulation has occurred (Chadwick and Kuylenstierna 1991). In undertaking this study, our objectives were to confirm the previously reported presence of clear-water and brown-water acidic lakes in two areas of Russia to determine whether fish inhabiting these lakes contained elevated levels of mercury, and to determine whether these fish exhibited symptoms of damage from acidity.

METHODS

This study was conducted in two phases. In May and June 1989 we sampled lakes in Darwin National Reserve, Yaroslavl Region, and in June 1991 we sampled lakes in the Karelia Autonomous Republic (Figure 1). Darwin National Reserve is a protected natural area created in 1943, located at the western end of Rybinsk Reservoir. It is characterized by thick tills and sandy soils (Ager 1980), and the lakes are primarily precipitation-dominated seepage lakes. In Karelia we visited lakes in the vicinity of Suoyarvi, which were primarily highly colored lakes, and lakes in the vicinity of Kondopoga, which were primarily clear-water lakes. Both areas were underlain by granitic bedrock covered with thin soil (Ager 1980), and the lakes were primarily drainage lakes. Most lakes were in forested catchments with no dwellings or roads. An exception was Suoyarvi Lake, which has a settlement at the southern end.

At each lake, a water sample was collected by immersion of cleaned polyethylene bottles just below the surface near the point of maximum depth. Bottles were tightly capped, held in the dark, and within a few hours analyzed for pH (Orion model SA210 meter equipped with a Ross combination electrode), ANC (acid neutralizing capacity, measured by inflection point titration), and true color (visual comparison with platinum-cobalt standards). The remaining water sample from each lake was refrigerated and later analyzed for major cations and anions. Calcium, magnesium, and sodium were determined by flame atomic absorption spectrophotometry, chloride, fluoride, sulfate, and nitrate by ion chromatography, and total aluminum by graphite furnace atomic absorption spectrophotometry.

Perch (*Perca fluviatilis*) were collected by angling from various points around the shoreline of each lake, except for Suoyarvi Lake, where fish were captured in a gill net set overnight. The second and third gill arches were dissected from each fish and fixed in either 0.1 M cacodylate buffer, pH 7.3, containing 2.5% glutaraldehyde, or in 0.1 M HEPES buffer, pH 7.4, containing 1% glutaraldehyde, 4% formaldehyde, and 5% sucrose. Fish were then placed in plastic bags and frozen (-5°C) within a few hours, and remained frozen until they were analyzed for mercury. Fish and fixed gill tissue were transported to the Institute for Biology of Inland Waters, Borok, in 1989, and the Institute for Research on Northern Fishes, Petrozavodsk, in 1991.

At the laboratories, the fish were thawed, weighed to the nearest gram, and measured (total length) to the nearest millimeter. Scales were removed from the left side of the fish adjacent to the dorsal fin and placed in paper envelopes for future ultrastructure determination. Fish were

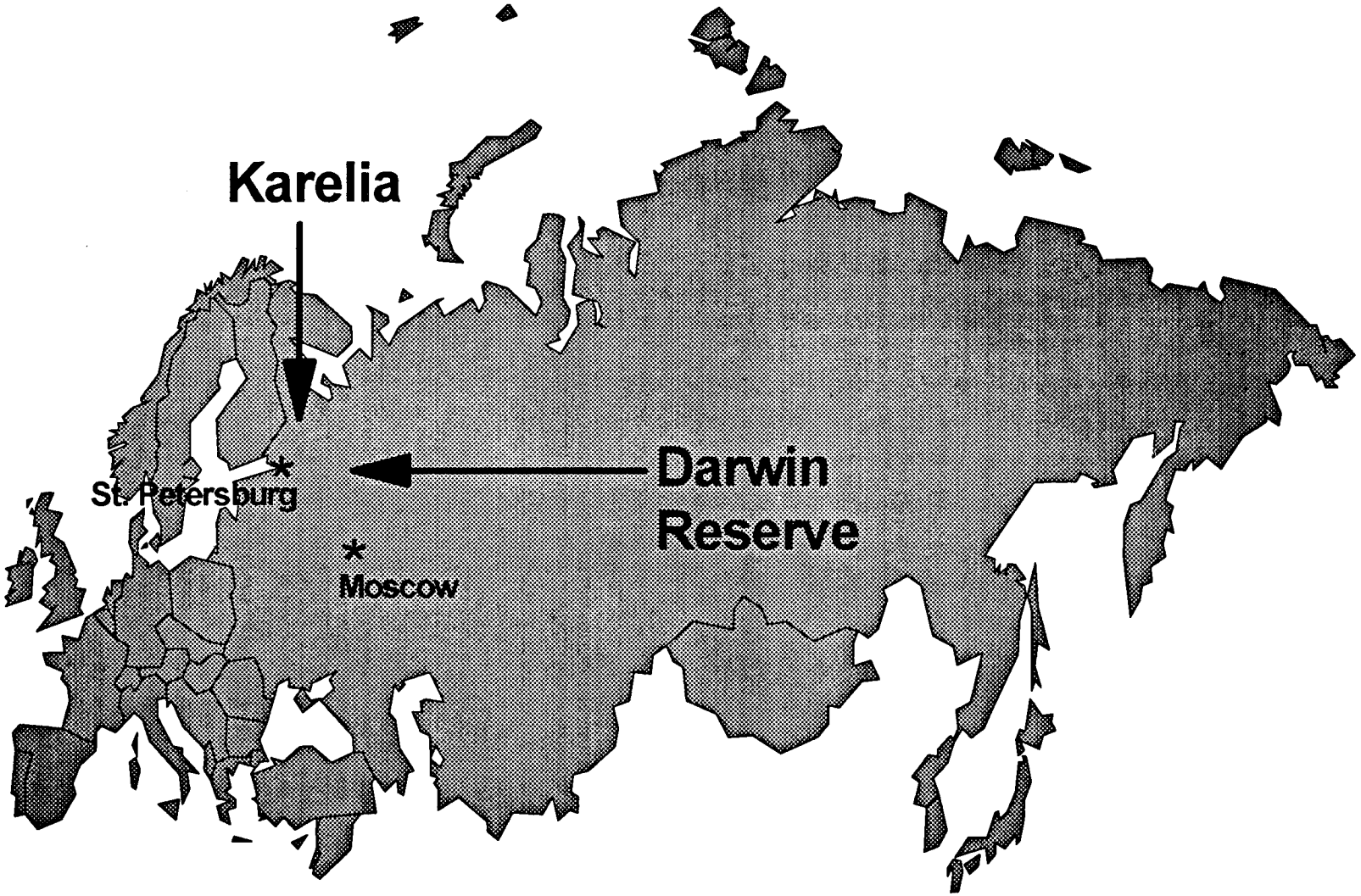


Figure 1. Map of Europe showing the location of the two study sites.

placed on an acid-washed plexiglass plate and a 2- to 3-g sample of skeletal muscle was dissected from the left side of the fish, adjacent to the dorsal fin and extending from the rib cage to the center of the dorsal surface. All dissecting instruments were stainless steel, and all instruments and glassware were cleaned with 10% nitric acid and rinsed with distilled water.

Moisture content of muscle tissue was determined by placing a portion of the tissue in a dried, tared glass dish and weighing before and after drying at 105°C. The remaining tissue, typically 1 to 2 g, was placed in a tared glass beaker, weighed, and digested in a 1:1 mixture of nitric acid and hydrogen peroxide (FAO/SIDA 1983).

Fish were processed in batches of 5 to 10. Two fish from each batch were randomly selected for use in determination of accuracy and precision of mercury determination. For these fish, the tissue was divided into two nearly equal portions. A known amount of mercury standard solution was added to one portion of tissue, and all portions were digested and analyzed. A sample of National Institute of Science and Technology (NIST) tuna reference material and a reagent blank was included with each batch of samples. After digestion, solutions were cooled and diluted to 25 ml with distilled water, and 1- to 2-ml portions were analyzed for total mercury with a gold film analyzer (Arizona Instrument Co., Jerome, Arizona USA). Each digestate was analyzed in duplicate. Mercury in all samples exceeded our calculated detection limit of 5 ng. The recovery of mercury from spiked samples averaged 95.4% (range 90-102%). The percent difference between replicate samples averaged 2.9% (range 1.7-5.2%). The measured mercury content of NIST tuna averaged 0.93 $\mu g/g$ (range 0.88-0.96 $\mu g/g$), and all analyses were within the certified range for this material.

Gill tissue was postfixed in buffered OsO_4 for two hours and dehydrated through a graded ethanol series into absolute acetone. Whole gill arches were critical point dried under liquid CO_2, sputter-coated with gold, and examined using a JEOL JSM-25S electron microscope operated at 15 KV. Scales were first placed in glass vials with 5% NaOH for 6-12 h, then hydrolyzed in a saturated solution of $K_2Cr_2O_7$ in 10% NaOH for 1-7 d. The scales were then washed in distilled water until all yellow color was removed, placed in 3:1 mixture of absolute ethanol and acetone and then in absolute acetone for 10-15 min each, air dried, mounted on stubs, and coated with gold and examined in the same manner as were gills.

RESULTS

The lakes surveyed varied in pH from 4.6 to 8.1 and in color from 3 to 188 Hazen (Figure 2, Table 1). Drainage and seepage lakes were relatively uniformly distributed over the range of color values, but all seepage lakes surveyed were acidic (pH $\leq$5.0). The lakes in Darwin Reserve were predominately seepage lakes whereas the lakes in Karelia were predominately drainage lakes.

The fish collected ranged in weight from 8 to 515 g, and in mercury content from 0.06 to 3.04 $\mu g/g$ wet weight (0.76-17.9 $\mu g/g$ dry weight) (Table 2). Omitting two unusually large fish from Tyomnoye Lake reduces the maximum weight to 196 g and the maximum mercury content to 1.03 $\mu g/g$ wet weight (6.87 $\mu g/g$ dry weight). Fish mercury content was correlated with fish size, especially weight (R^2=0.32, p=0.0001); therefore the least square mean mercury concentration, which represents fish mercury concentration normalized by fish weight, was computed by analysis of covariance using weight as the covariate.

Plotting fish mercury concentration against lake pH and color (Figure 3) indicates that fish from high pH lakes have relatively low mercury content regardless of lake color but in low pH lakes fish mercury content increases with lake color. Accordingly, stepwise regression analysis was performed using the non-intercorrelated variables pH, color, and sulfate, with the data stratified by lake drainage type. The results (Table 3) indicate that color is more important in the regression than is pH for seepage lakes, and that the relation between fish mercury and lake pH is not significant in the absence of color data for drainage lakes. The inclusion of lake sulfate did not significantly improve the regression for seepage lakes.

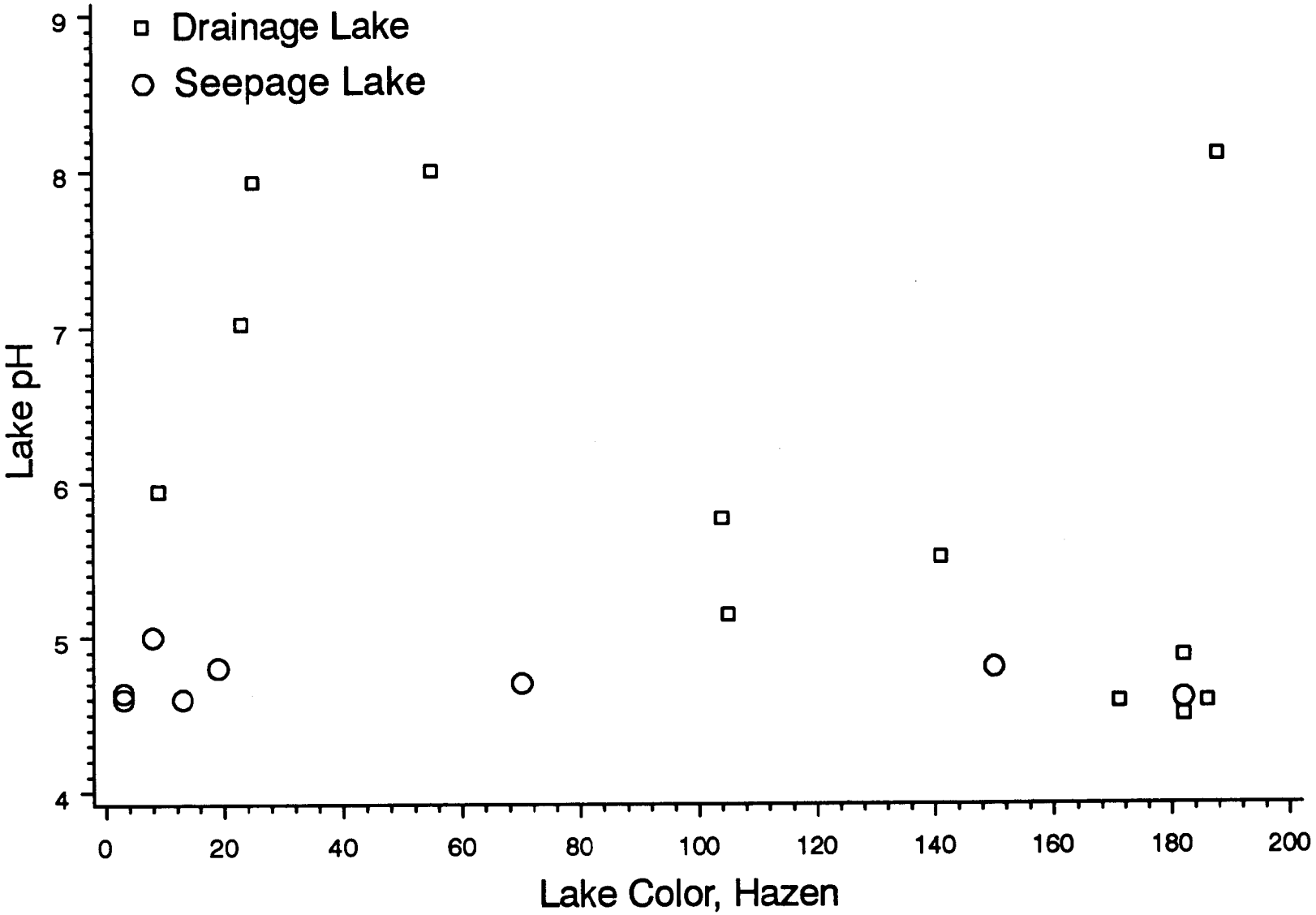


Figure 2. Plot of lake pH versus lake color for all lakes, by drainage type.

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE STUDY LAKES.

Lakes	Area (ha)	Depth (m)	Drainage Type	Color (Hazen)	pH	ANC (μeq/l)	SO_4 (μeq/l)
Darwin Lakes							
Dorojiv	200	3	Seepage	13	4.6	-41	71
Dubrovskoye	20	2	Seepage	182	4.6	-50	64
Hotavets	160	3	Drainage	188	8.1	227	32
Motykino	2	4	Seepage	19	4.8	-38	49
Rybinsk	395,000	30	Impoundment	55	8.0	1,369	420
Tyomnoye	20	2	Seepage	70	4.7	-53	52
Uteshkovo	5	3	Seepage	150	4.8	-33	29
Karelia Lakes							
Blue Lamba	307	4	Seepage	3	4.6	-17	67
Chuchyarvi	112	5	Seepage	8	5.0	-9	48
Grushna Lamba	3	5	Seepage	3	4.6	-19	48
Ilyakalkenyarvi	104	6	Drainage	171	4.6	-25	54
Kabozero	210	3	Drainage	141	5.5	13	60
Lamba Vegarous	7	4	Drainage	182	4.5	-39	75
Leukunyarvi	-	-	Drainage	182	4.9	-9	68
Sargozero	200	4	Drainage	25	7.9	252	29
Suoyarvi	6,070	5	Drainage	104	5.8	23	83
Uros	426	3	Drainage	9	5.9	16	72
Vegarousyarvi	1,880	5	Drainage	105	5.1	-2	49
Venderskoye	998	6	Drainage	23	7.0	167	44
Vuontelenyarvi	394	3	Drainage	186	4.6	-24	68
Mean Values							
Drainage Lakes[a]	1,045	4.2	Drainage	120	5.8	56	58
Seepage Lakes	84	3.5	Seepage	56	4.7	-33	53

[a]Rybinsk omitted.

TABLE 2. MEAN AND RANGE OF FISH WEIGHT, MOISTURE CONTENT, AND MERCURY CONCENTRATION IN FISH, AND LEAST SQUARE MEAN MERCURY CONCENTRATION IN FISH FROM THE STUDY LAKES.

Lake	Weight, g Mean (Range)	Moisture, % Mean (Range)	Mercury, μg/g wet weight Arithmetic Mean (Range)	Mercury, μg/g wet weight Least Square Mean
Blue Lamba	22 (18-28)	85.6 (81.1-89.7)	0.34 (0.26-0.44)	0.41
Chuchyarvi	59 (18-133)	90.6 (83.2-93.9)	0.10 (0.08-0.13)	0.08
Dorojiv	62 (31-94)	80.9 (78.0-86.2)	0.50 (0.36-0.71)	0.47
Dubrovskoye	27 (23-30)	83.6 (77.8-88.1)	0.64 (0.56-0.68)	0.71
Grushna Lamba	38 (28-48)	81.5 (76.9-86.0)	0.30 (0.20-0.43)	0.33
Hotavets	62 (37-150)	88.6 (80.4-93.8)	0.11 (0.08-0.16)	0.09
Ilyakalkenyarvi	35 (20-78)	82.4 (72.0-94.2)	0.28 (0.19-0.36)	0.31
Kabozero	35 (24-47)	81.5 (61.7-87.2)	0.31 (0.22-0.39)	0.34
Lamba Vegarous	55 (28-80)	80.5 (75.9-85.1)	0.40 (0.30-0.52)	0.39
Leukunyarvi	30 (8-54)	88.2 (82.8-96.3)	0.21 (0.17-0.24)	0.25
Motykino	60 (43-75)	81.4 (79.3-87.9)	0.57 (0.43-0.97)	0.54
Rybinsk	86 (59-163)	80.8 (78.1-82.6)	0.19 (0.08-0.45)	0.11
Sargozero	18 (8-27)	85.2 (75.0-89.6)	0.12 (0.09-0.18)	0.19
Suoyarvi	98 (53-131)	81.5 (79.6-83.6)	0.29 (0.19-0.38)	0.18
Tyomnoye	155 (50-515)	79.6 (74.6-83.4)	1.06 (0.45-3.04)	0.56

Uros	19 (14-21)	83.7 (76.1-90.3)	0.12 (0.06-0.18)	0.19
Uteshkovo	45 (18-98)	80.4 (75.7-85.8)	0.78 (0.54-0.97)	0.80
Vegarousyarvi	26 (17-45)	79.5 (76.9-82.8)	0.34 (0.20-0.44)	0.41
Venderskoye	41 (12-107)	79.8 (72.9-81.0)	0.15 (0.10-0.24)	0.17
Vuontelenyarvi	43 (16-196)	84.5 (76.9-93.0)	0.53 (0.32-1.03)	0.54

TABLE 3. STATISTICALLY SIGNIFICANT ($P \leq 0.05$) REGRESSION EQUATIONS, CORRELATION COEFFICIENTS, AND PROBABILITY FOR STEPWISE REGRESSION OF LAKE PHYSICAL AND CHEMICAL VARIABLES ON FISH MERCURY CONTENT. ANALYSES ARE PRESENTED FOR ALL LAKES, AND STRATIFIED BY DRAINAGE TYPE.

Lake Type	Number of Variables	Equation	R^2	P
All	1	-0.0347 pH + 0.317	0.46	0.001
	2	-0.0336 pH + 0.000144 color + 0.298	0.49	0.0035
	3	-0.0331 pH + 0.000143 color -0.000019 sulfate + 0.297	0.50	0.0119
Seepage	1	0.000711 color + 0.128	0.56	0.0332
	2	0.000671 color - 0.173 pH + 0.946	0.69	0.05
Drainage	2	-0.0260 pH + 0.000030 color + 0.252	0.68	0.0058
	3	-0.0256 pH + 0.0000284 color -0.0000167 sulfate + 0.251	0.68	0.0216

The structure of the gills of perch has been previously described by Matey (1984). Primary lamellae or filaments project in two parallel rows from the gill arch, and each primary lamella supports two rows of secondary lamellae (Figure 4a). The epithelium covering the gill consists primarily of thin, squamous respiratory cells, along with mucous cells and chloride (ion transporting) cells, underlain by undifferentiated cells. Most chloride cells occur in the primary lamellar epithelium, especially in the spaces between secondary lamellae. Normally, the surfaces

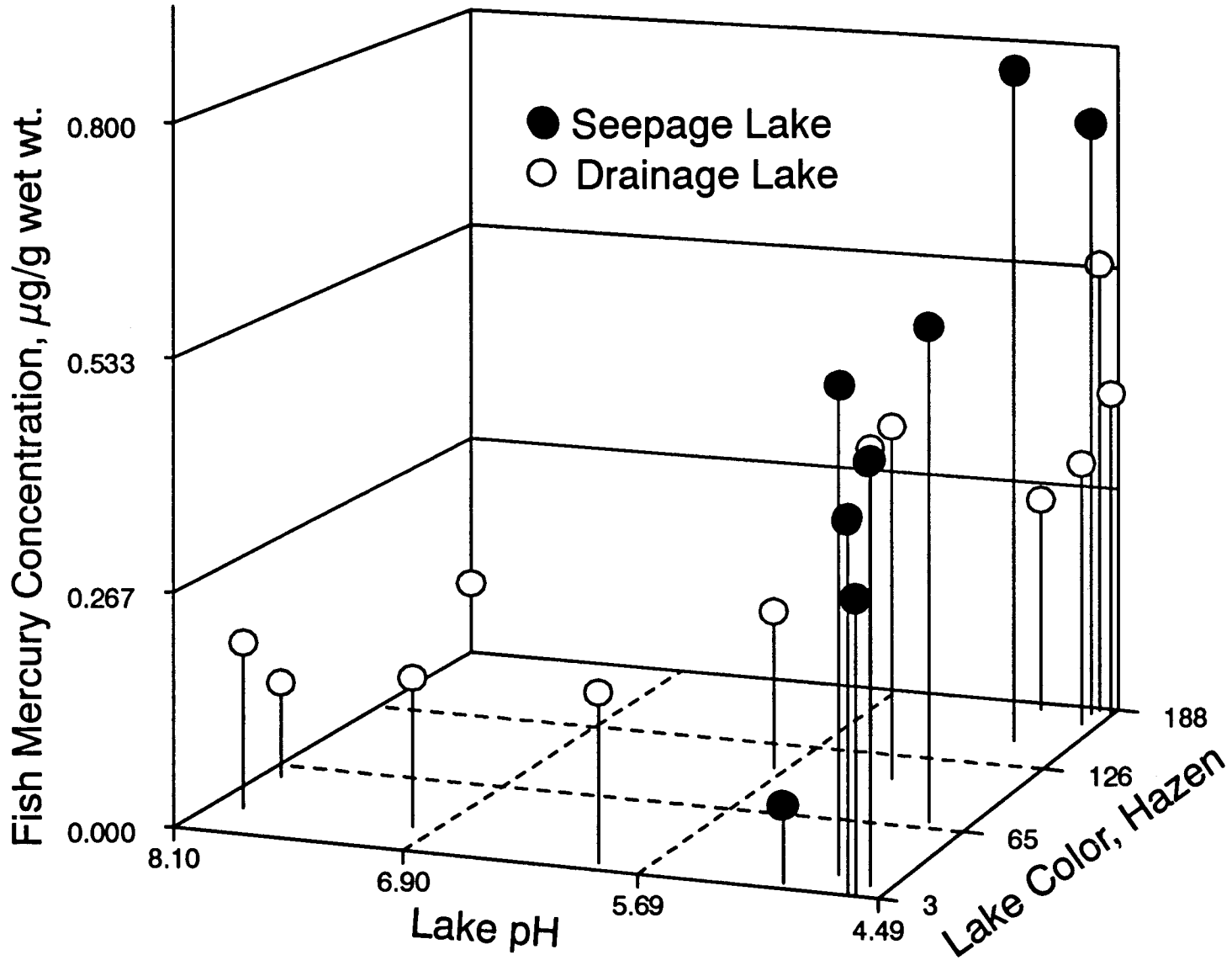


Figure 3. Three-dimensional plot of least square mean fish mercury content versus lake pH and color, by lake drainage type.

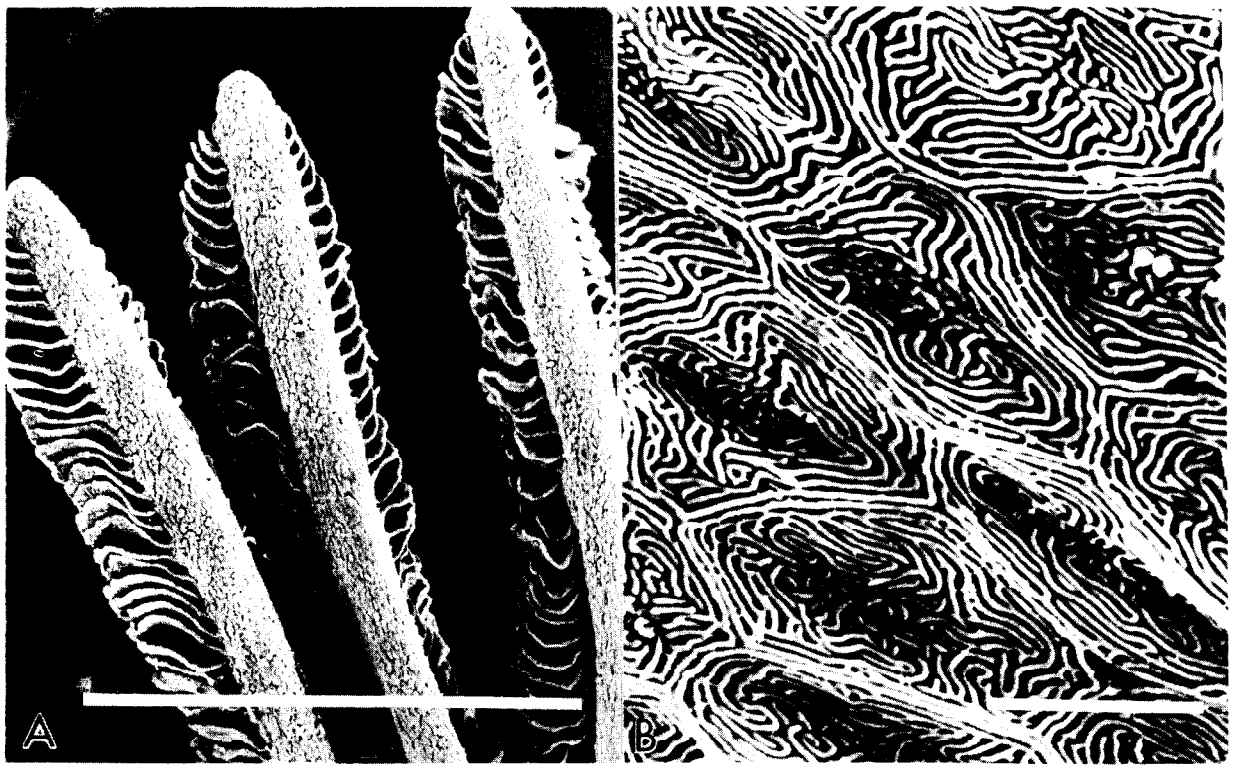


Figure 4. Scanning electron micrographs of normal gill structure. A. Gill filaments with secondary lamellae. Bar= 1 mm. B. Epithelial surface. Bar= 10 μm.

of the respiratory cells are covered by a well-developed system of microridges, which may serve to anchor mucus, increase surface area, or create micro-turbulence (Figure 4b). This structural arrangement is found in most teleost fishes (Hughes 1984; Laurent 1984).

For the Darwin Reserve lakes, all fish examined from Hotavets Lake (pH 8.1) had normal, well-formed gills. The primary lamellar epithelia contained pavement cells with well-formed microridges and few chloride cells (Figure 5a), and secondary lamellae were thin and regular (Figure 5b). Perch from the more acidic lakes (Dubrovskoye, Dorojiv, Motykino, and Uteshkovo lakes) all had alterations in gill structure. Chloride cell numbers were greatly increased along the primary lamellae (Figure 5c). Some chloride cells had apical crypts (Figure 5d), which were not observed in fish from the higher pH lake. Chloride cells were also present on many secondary lamellae (Figure 5e). Mucus secretion was elevated, as evidenced by increased numbers of mucus droplets and secretory pores associated with mucous cell activity (Figure 5f). The density of microridges on the gill epithelia was reduced in fish from the acid lakes compared with fish from the higher pH lake (Figure 5g; compare with Figure 4b).

For the highly colored lakes in Karelia, fish from lakes of pH <5.5 had thickened and swollen secondary lamellae (Figure 6a). This phenomenon was especially severe in fish from Leukunyarve, Vegarousyarvi, and Lamba near Vegarous lakes, where epithelial hyperplasia produced regions of fused secondary lamellae (Figure 6b). These fish also often had locally swollen or evaginated regions on the primary lamellae (Figure 6c), although these abnormalities were not observed in fish from Ilyakalkenyarvi Lake, which had a pH of 4.6. Chloride cell number on primary lamellae appeared to increase with decreasing pH, and was greatly elevated in fish from Vuontelenyarvi, Ilyakalkenyarvi, and Lamba near Vegarous lakes (Figure 6d). In perch from lakes with pH below 5.1, chloride cells were also abundant on secondary lamellar epithelia (Figure 6e).

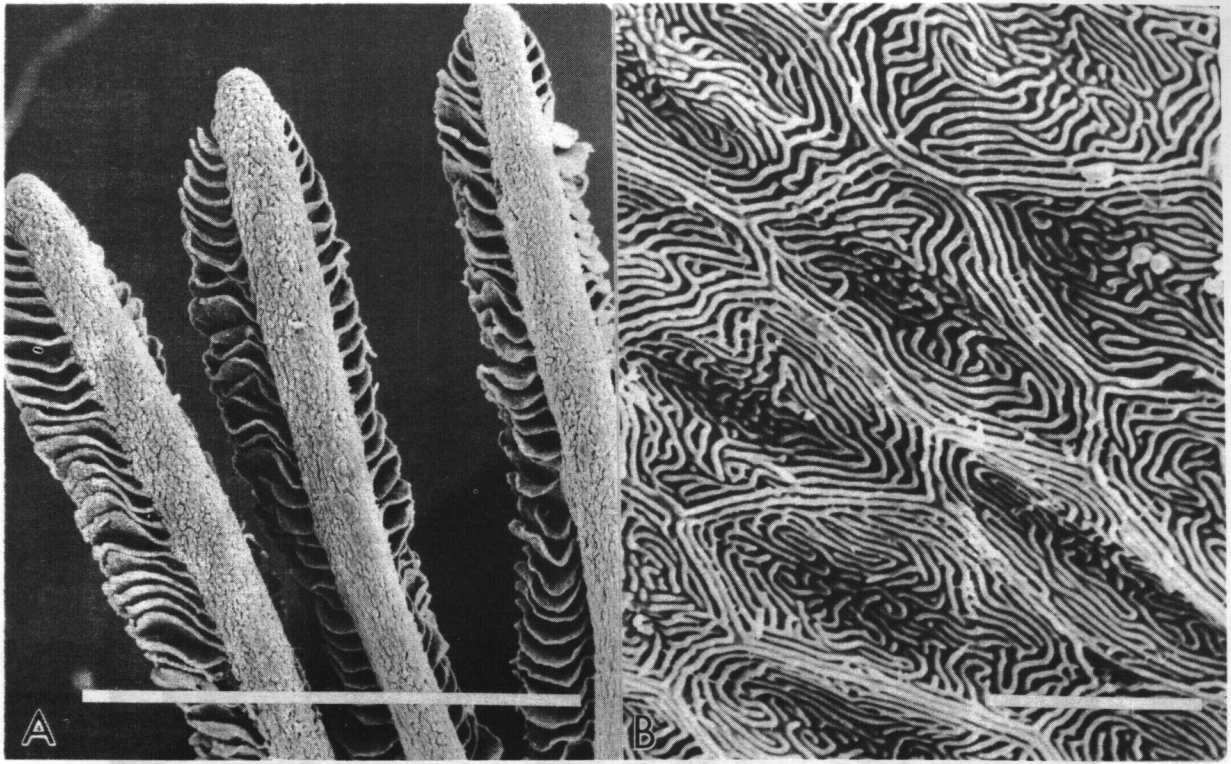


Figure 4. Scanning electron micrographs of normal gill structure. A. Gill filaments with secondary lamellae. Bar= 1 mm. B. Epithelial surface. Bar= 10 μm.

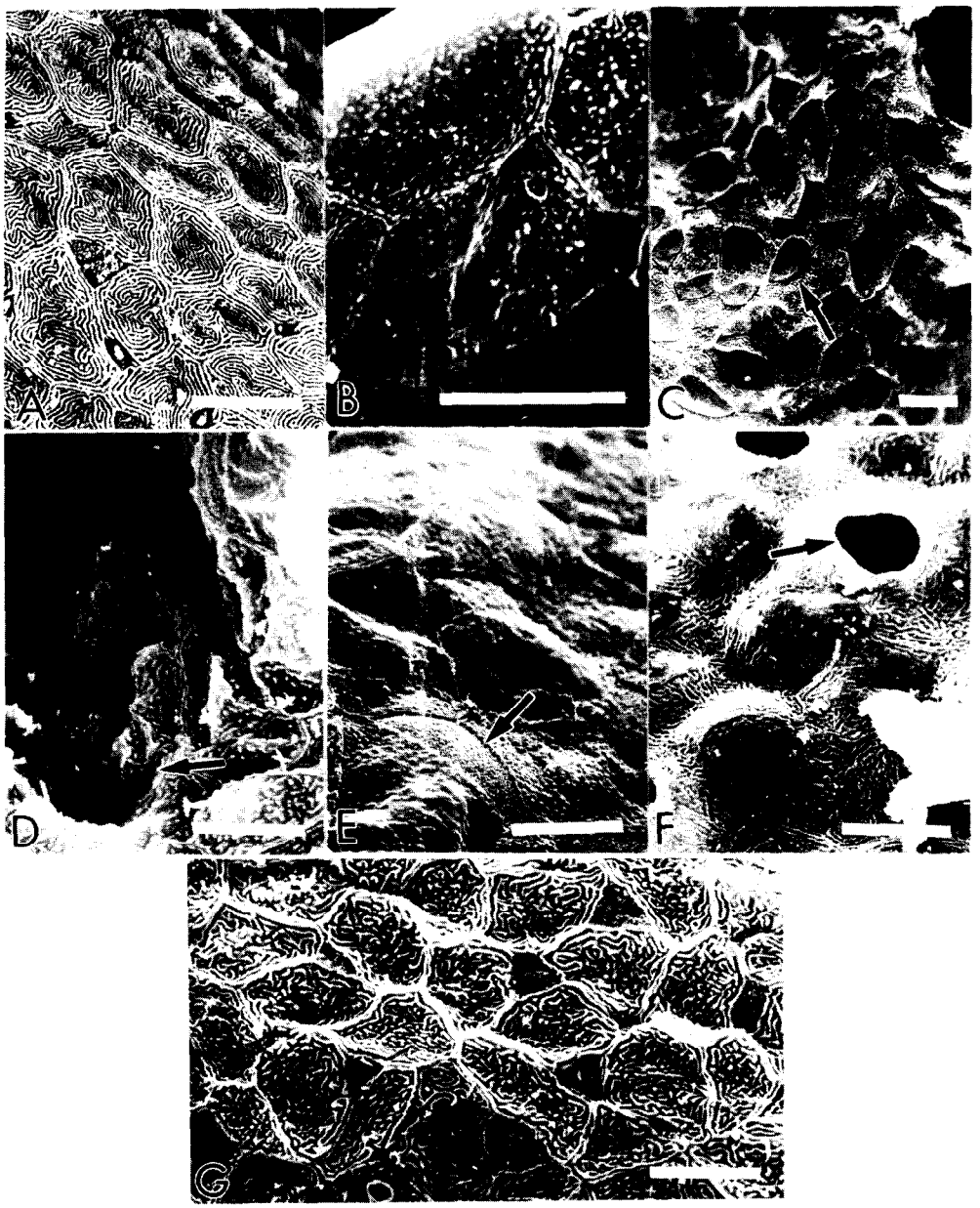


Figure 5. Scanning electron micrographs of gills from Darwin Reserve fish. Bar=10 μm in all cases. A. Primary lamellar epithelium of a fish from a high pH lake, showing respiratory pavement cells and a lack of chloride cells. B. Secondary lamellar epithelium of a fish from the same lake as A. C. Primary lamellar epithelium of a fish from an acidic lake showing numerous chloride cells (arrow). D. Chloride cell with an apical crypt (arrow). E. Secondary lamellar epithelium of a fish from an acidic lake showing numerous chloride cells (arow). F. Secondary lamellar epithelium of a fish from an acidic lake showing numerous secretory pores (arrow). G. Primary lamellar epithelium of a fish from an acidic lake showing reduced microridge density.

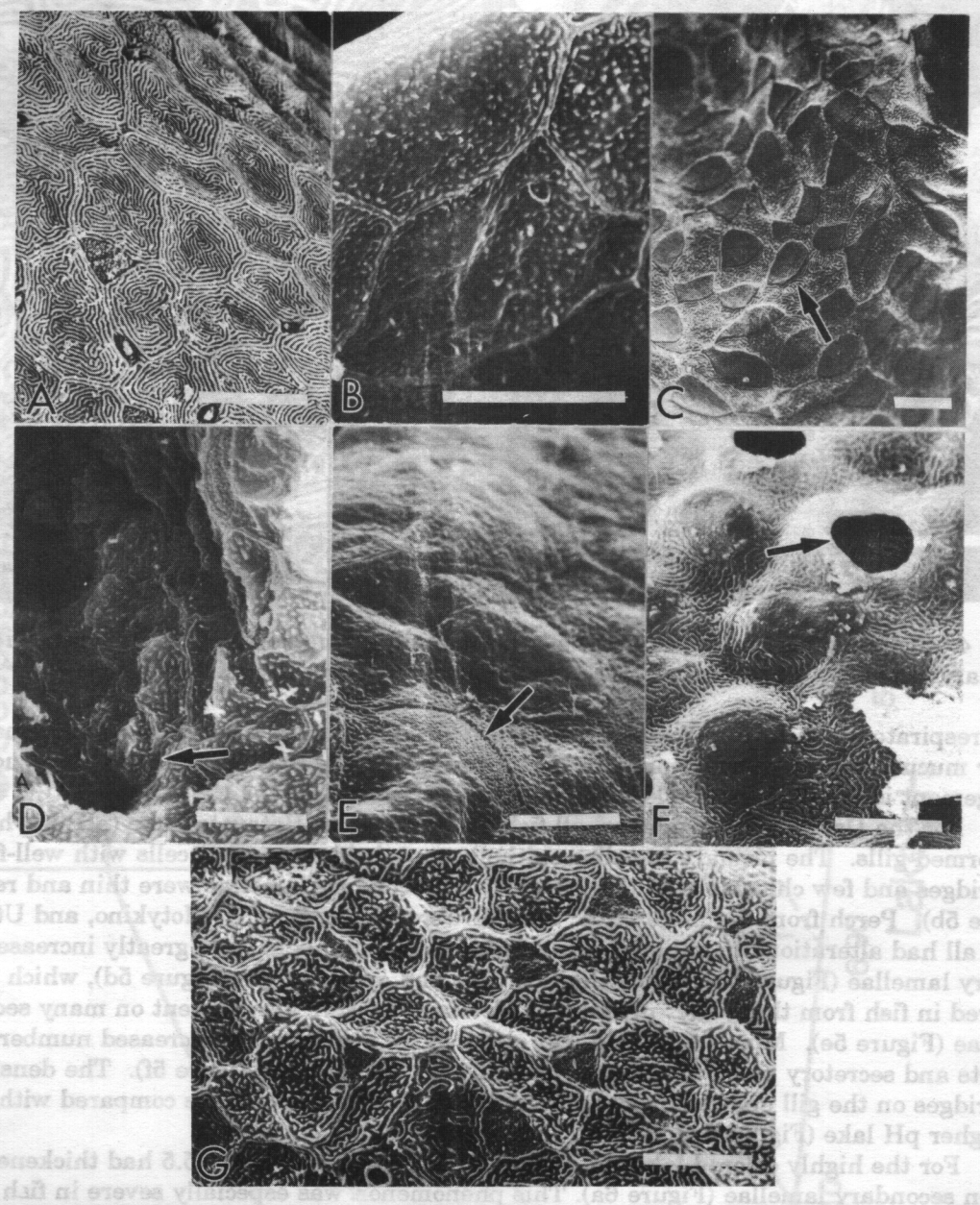


Figure 5. Scanning electron micrographs of gills from Darwin Reserve fish. Bar=10 μm in all cases. A. Primary lamellar epithelium of a fish from a high pH lake, showing respiratory pavement cells and a lack of chloride cells. B. Secondary lamellar epithelium of a fish from the same lake as A. C. Primary lamellar epithelium of a fish from an acidic lake showing numerous chloride cells (arrow). D. Chloride cell with an apical crypt (arrow). E. Secondary lamellar epithelium of a fish from an acidic lake showing numerous chloride cells (arow). F. Secondary lamellar epithelium of a fish from an acidic lake showing numerous secretory pores (arrow). G. Primary lamellar epithelium of a fish from an acidic lake showing reduced microridge density.

Figure 6. Scanning electron micrographs of gills of fish from colored, acidic Karelian lakes. A. Swollen and thickened secondary lamellae (arrow). Bar=100 μm. B. Epithelial hyperplasia (arrow). Bar=1 mm. C. Primary lamelar epithelial swelling (arrow). Bar=100 μm. D. Primary lamellar epithelium with numerous chloride cells (arrow). Bar=100 μm. E. Secondary lamellar epithelium with numerous chloride cells (arrow). Bar=10 μ.

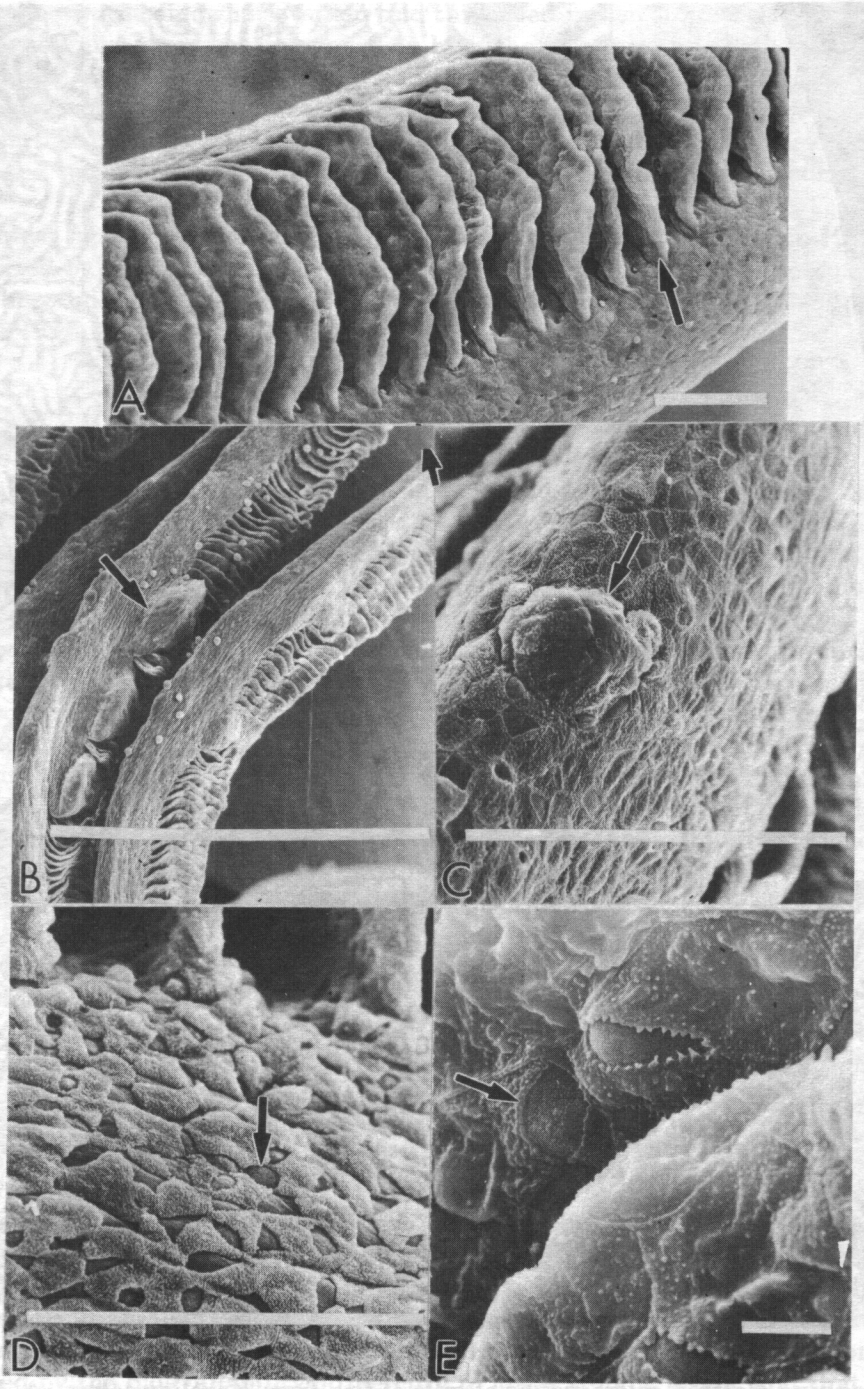


Figure 6. Scanning electron micrographs of gills of fish from colored, acidic Karelian lakes. A. Swollen and thickened secondary lamellae (arrow). Bar=100 μm. B. Epithelial hyperplasia (arrow). Bar=1 mm. C. Primary lamelar epithelial swelling (arrow). Bar=100 μm. D. Primary lamellar epithelium with numerous chloride cells (arrow). Bar=100 μm. E. Secondary lamellar epithelium with numerous chloride cells (arrow). Bar=10 μ.

Figure 7. Scanning electron micrographs of gills of fish from Karelian lakes. A. Epithelial hyperplasia leading to secondary lamellar fusion (arrow) in a fish from a colored, acidic lake. Bar=100 μm. B. Cell surface microridge density in a fish from a high pH lake. Bar=10 μm. C. Cell surface microridge density in a fish from a low pH, clear lake. Bar=10 μm. D. Cell surface microridge density in a fish from a low pH, colored lake. Bar=10 μ.

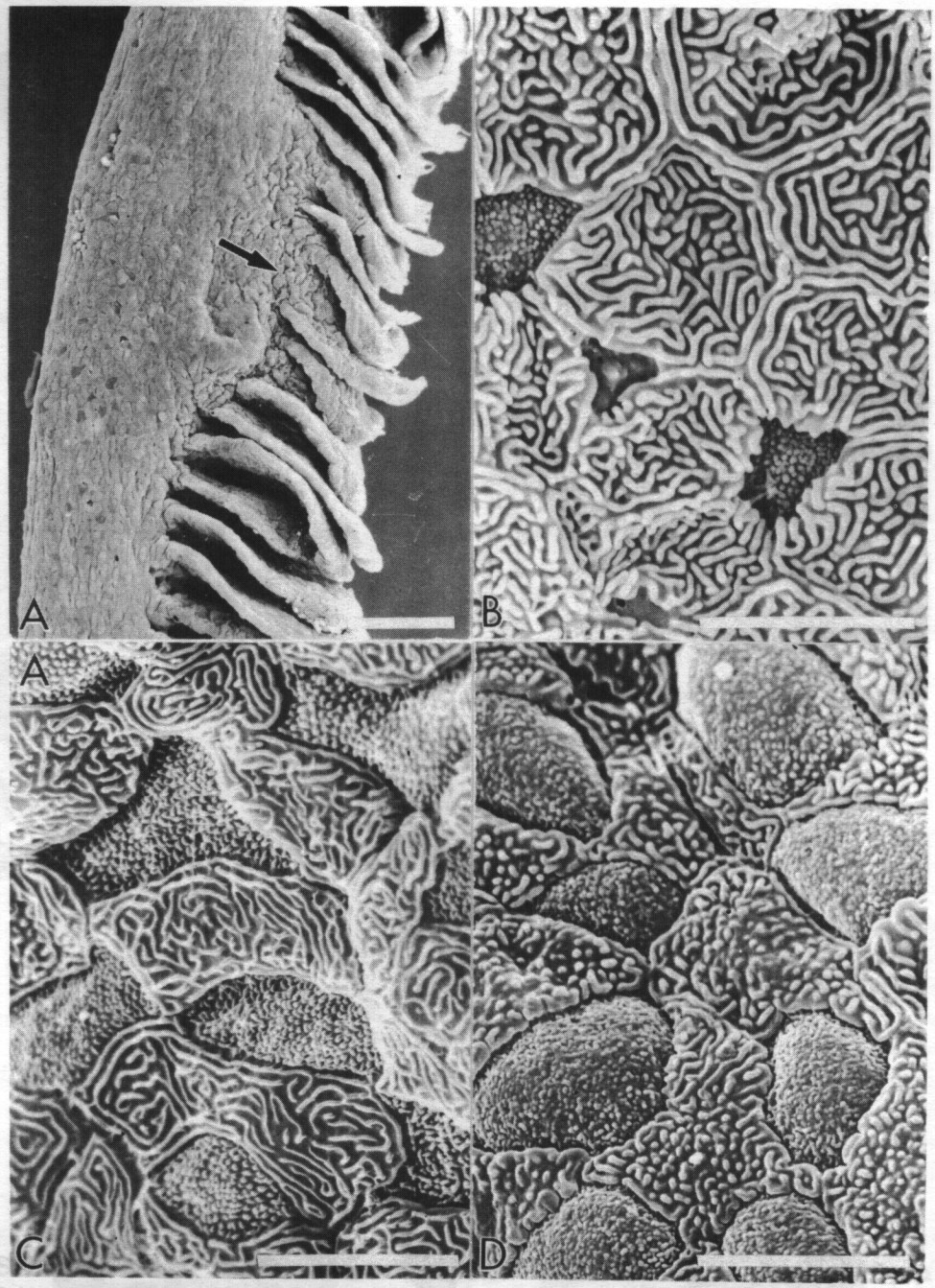


Figure 7. Scanning electron micrographs of gills of fish from Karelian lakes. A. Epithelial hyperplasia leading to secondary lamellar fusion (arrow) in a fish from a colored, acidic lake. Bar=100 μm. B. Cell surface microridge density in a fish from a high pH lake. Bar=10 μm. C. Cell surface microridge density in a fish from a low pH, clear lake. Bar=10 μm. D. Cell surface microridge density in a fish from a low pH, colored lake. Bar=10 μ.

Alterations in gill structure associated with low pH appeared less severe in fish from clear-water lakes in Karelia than in those from colored-water lakes. Epithelial hyperplasia leading to secondary lamellar fusions was observed only in fish from Chuchyarvi, pH 5.0 (Figure 7a). Secondary lamellae were somewhat swollen and thickened in fish from the lower pH clear-water lakes. Chloride cell numbers were increased on both the primary and secondary lamellae in fish from lakes with pH <5.9. Compared with perch from lakes of higher pH (Figure 7b), cell surface microridge density was slightly decreased in fish from the most acidic clear-water lakes (Figure 7c). In contrast, fish from low pH, colored-water lakes had substantially reduced microridge patterns, with the typical labyrinth appearance replaced by shorter, smaller surface ridges (Figure 7d).

Perch have typical ctenoid scales (Figure 8a). The scales of perch from Hotavets (pH 8.1) were characterized by a focus consisting of a number of microridges creating a complex pattern (Figure 8b). These microridges were lacking in the scales of the fish from lakes in Darwin Reserve with pH 4.4-4.8 (Figures 8c, 8d).

DISCUSSION

The areas of Russia where we surveyed lakes receive relatively high levels of sulfur deposition. Karelia receives 420-800 eq/ha/yr, comparable to southern Finland, Sweden, and Norway, and Yaroslavl region receives 800-1640 eq/ha/yr, comparable to northern Germany (Chadwick and Kuylenstierna 1991). The critical loading of acidity is estimated at 200 eq/ha/yr or less for Karelia and 200-500 eq/ha/yr for Yaroslavl region, and deposition is estimated to exceed critical loading by 500-1000 eq/ha per year in Karelia, but only 200 eq/ha per year or less in Yaroslavl region (Hettelingh *et al.* 1991). Consequently, we found no acidic drainage lakes in Darwin Reserve. The seepage lakes were precipitation-dominated as reflected in the low ion content, and were not influenced by the chemistry of the soils or surficial material. The acidity of the seepage lakes in Darwin Reserve reflects the acidity of the precipitation they receive. In Karelia, both drainage and seepage lakes were acidic. The situation here is similar to that in Finland, Sweden, and southern Norway, where acidic precipitation falling on a landscape consisting of resistant bedrock and thin, nutrient-poor soils has resulted in the acidification of thousands of lakes, with resultant biological effects (Rosseland *et al.* 1986).

The mercury content of perch from undisturbed lakes in Russia is comparable to that reported for this species from similar lakes in Sweden and Finland, disregarding the two large fish from Tyomnoye lake, which had unusually high mercury concentrations. Mercury concentrations were reported to be 0.2 to 2.0 μg/g dry weight for "small" perch and 0.6 to 6.0 μg/g dry weight for "large" perch in Swedish forest lakes (Lindqvist *et al.* 1991). Mercury content of perch muscle tissue in 11 Swedish lakes of pH 5.2-6.2 was between 0.04 and 0.29 μg/g wet weight (Paulsson and Lundbergh 1991). In Finland, mercury concentrations between 0.03-0.53 μg/g in perch from circumneutral lakes and 0.15-0.63 in fish from acidic lakes have been noted (Verta *et al.* 1990). The larger fish from the most acidic lakes in our study approached or exceeded mercury concentrations believed harmful to fish consumers (Wiener 1987; Grant 1991).

The association of elevated mercury content in fish with low water pH has been widely reported (Lindqvist *et al.* 1991; Spry and Wiener 1991). Our results confirm these findings and extend them to two regions of Russia not previously investigated. However, the phenomenon is much more complex than a simple water acidity-fish mercury relation. An extensive study of mercury accumulation by fish in Swedish forest lakes led to the conclusion that concentrations were determined by the bioavailability of mercury to lower trophic levels, and that lake humicity, productivity, and acidity controlled bioavailability (Lindqvist *et al.* 1991). Our results suggest that acidity and humicity are important factors in affecting fish mercury content in Russian lakes (we did not investigate productivity). We found low concentrations of mercury in fish from high pH lakes regardless of color, which suggests that acidity is a necessary but not sufficient factor in mercury accumulation by fish in these lakes.

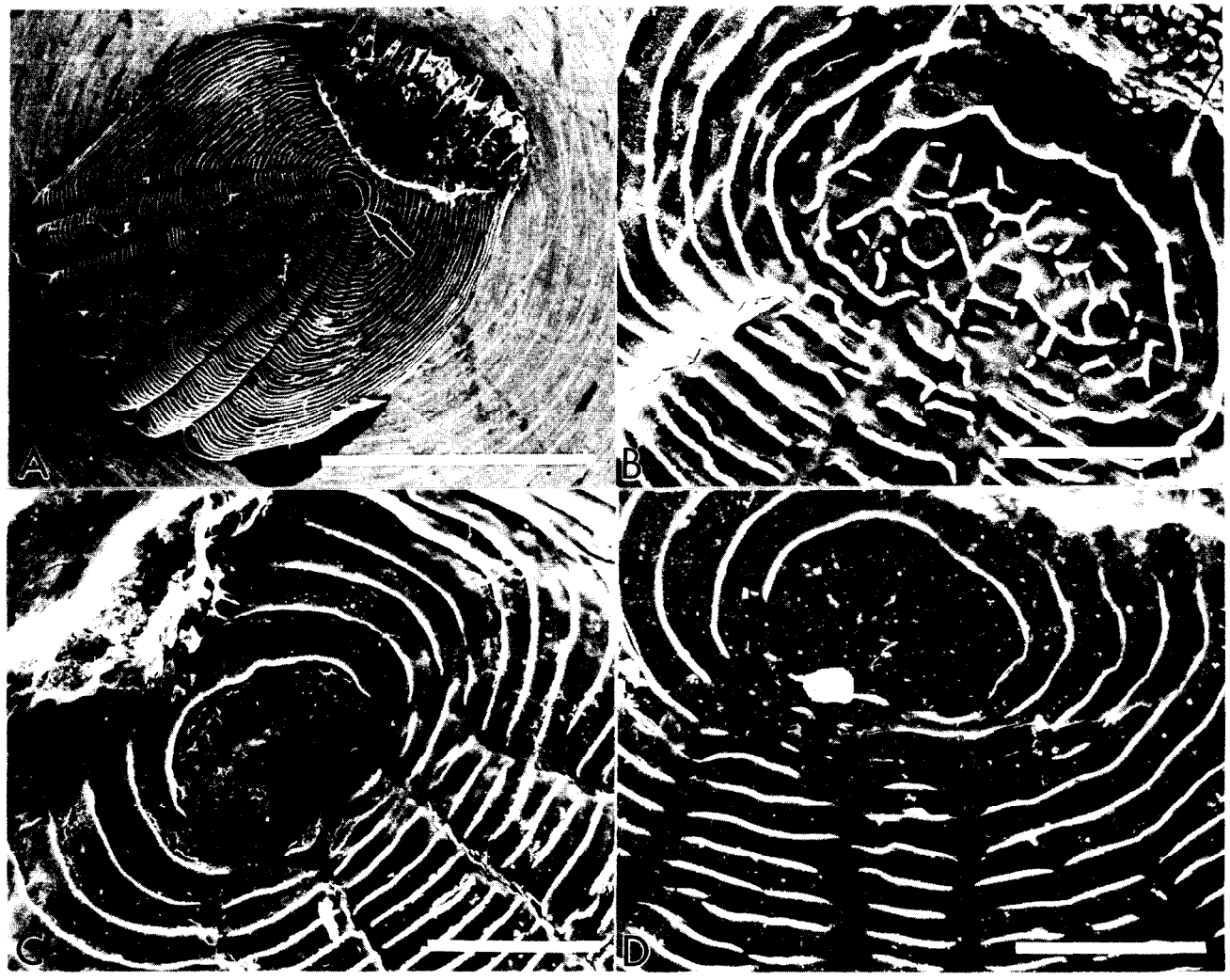
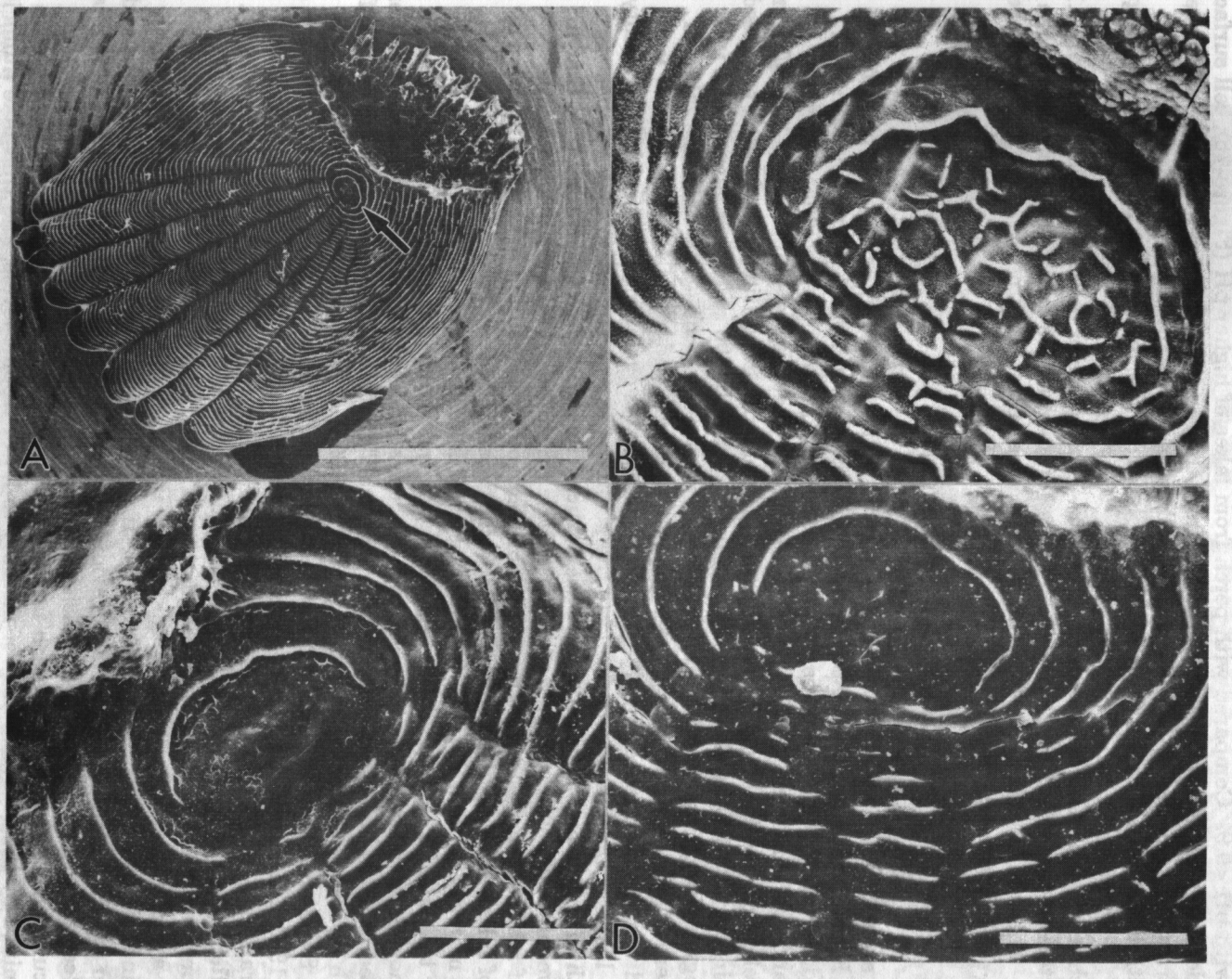


Figure 8. Scanning electron micrographs of scales from Darwin Reserve fish. A. View of entire scale. Bar=1 mm. B. Focus of scale from Hotavets Lake (pH8.1) fish showing normal ridge pattern. Bar=100 μm. C. Focus of scale from Motykino Lake (pH 4.8) fish showing lack of normal microridges. Bar=100 μm. D. Focus of scale from Tyomnoye Lake (pH 4.4) fish showing lack of normal microridges. Bar=100 μm.



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Humic matter was found to control the solubility and watershed export of mercury deposited in precipitation in Sweden and Canada (Iverfeldt and Johansson 1988; Mierle and Ingram 1991). Further, water concentrations of mercury were highly correlated with water color (Meili *et al.* 1991). Most of the mercury in clearwater lakes was deposited in the sediment whereas most of the mercury in humic lakes was retained in the water column (Meili 1991). Thus humic matter may affect fish mercury content by affecting the mercury loading to a lake and by retaining mercury in the water column where it is available for uptake in biota.

Alterations in gill morphology have been reported in a number of fish species in response to water acidification (Jagoe and Haines 1983; Matey 1984; Evans *et al.* 1988; Tietge *et al.* 1988). Most studies have examined effects after laboratory exposure; relatively few studies have been conducted on wild fish from chronically acidic environments. Chevalier *et al.* (1985) reported gill abnormalities associated with acidification in brook trout (*Salvelinus fontinalis*) collected from acidic lakes, and Leino *et al.* (1987) found changes in the gill epithelium of two species of cyprinids collected from an experimentally acidified lake. Our results expand these observations to perch inhabiting acidified environments in Russia, and allow comparisons from colored and clear acidic waters.

Exposure to low pH water causes osmoregulatory and ionoregulatory disturbances in fish (McDonald 1983), primarily because of increased passive efflux of sodium. The changes in gill morphology we observed may represent adaptation of acclimation to minimize this effect. The thickening of the epithelium would lengthen the diffusion pathway by which ion losses occur, and increasing numbers of chloride cells would allow increased active uptake of ions from the environment. Perch are known to be relatively acid tolerant and are frequently the only species of fish inhabiting acidic lakes (Rask and Tuunainen 1990), including the ones we studied. Perhaps these changes in gill morphology allow this species to survive at low pH.

Ctenoid fish scales consist of two layers: an upper, ridged osseous layer with cteni and a lower fibrillary plate (Fouda 1979). The focus is formed early in the life of the individual (Sire 1986) and does not change appreciably after formation. Thus the morphology of this structure reflects environmental conditions early in the life of the fish. The loss of microridges from the scale focus in fish from acidic lakes may reflect disruption of calcium metabolism in these fish. Steingraeber and Gingerich (1991) found that brook trout exposed to pH 5 water had reduced body calcium, and Reader *et al.* (1989) found a similar effect for brown trout (*Salmo trutta*) at pH 4.5. Majewski *et al.* (1990) found reduced bone calcium concentration in adult Atlantic salmon (*Salmo salar*) held in a river of pH 4.7-5.2. Therefore, scale structure may be a sensitive measure of physiological stress from water acidity in fish. Because scale structure remains unchanged after formation, examination of scales of older fish could be used as an indicator of water chemistry at the time the scale was formed.

SUMMARY AND CONCLUSIONS

We surveyed 20 lakes in two regions of Russia to assess lake acidity, and to determine the effects of water acidity on fish mercury content and gill and scale ultrastructure. The lakes surveyed ranged in pH from 4.5 to 8.1 and in color from 3 to 188 Hazen. The mercury content of fish ranged from 0.06 to 3.04 μg/g wet weight, which was comparable to that reported for this species from forest lakes in Sweden and Finland. Inasmuch as these were all remote lakes with no local sources of pollution, atmospheric deposition is presumed to be the source of both acidity and mercury. Lake acidity and color, or humic content, were the major lake characteristics related to fish mercury content. Fish from high pH lakes were low in mercury regardless of other lake characteristics. Fish from low pH lakes varied widely in mercury content, with fish from colored lakes having higher concentrations than those from clear lakes. Regressions including lake pH and color, separated by drainage type, explained about 70% of the variance in fish mercury content. Acidity and color may affect fish mercury content by regulating loading and bioavailability of mercury to lower trophic levels in these lakes.

Gills and scales of fish from acidic lakes were morphologically different from those of fish from circumneutral lakes. In acidic lakes, fish gills had thickened secondary lamellae, increased numbers of chloride cells, apical crypts in some chloride cells, increased mucous production, and decreased microridge density. These changes were more severe in highly colored acidic lakes than in clear-water lakes. Scales of fish from acidic lakes lacked microridges in the focus. The gill abnormalities are all changes that would tend to reduce the effects of ion loss, which is the major physiological effect of water acidity to fish. The scale abnormality may result from disrupted calcium metabolism in acidic lakes.

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