

Supporting Information

Increasing mercury in yellow perch at a hotspot in Atlantic Canada, Kejimikujik National Park

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Methods

Sample collection and preparation

In 1996/97, all fish were lethally sampled. In 2006/07, perch < 18 cm were lethally sampled and perch > 18 cm were sampled using a dermal punch under protocols approved by the University of New Brunswick Animal Care Committee. A subset of fish >18 cm were also sampled lethally to compare results across tissues.

To calibrate the total Hg (THg) and isotope concentrations between the non-lethal [dermal punch;(I)] and lethal (fillet or whole body) samples, four to nine large (15-20 cm) yellow perch were collected in each of three lakes (Kejimikujik, Cobrielle, and Big Dam West) and a dermal punch, fillet, and whole body sample was processed for each fish. Fish were anaesthetized with clove oil, a dermal punch (4 mm diameter) was used to remove three samples of dorsal muscle tissue (34 – 164 mg wet weight each), and then the fish were sacrificed to obtain the fillet (30 – 300 mg wet weight each) and whole body samples. The epidermis was removed from the biopsy sample then all tissues were sealed in a plastic bag and frozen for later analyses. In all other lakes, fish > 18 cm were sampled with the dermal punch, the wound was covered with Vetbond, and then the fish was allowed to recover before it was returned to the lake.

In 2006 and 2007, littoral invertebrates were collected from each lake during the fish collections. Fish in these lakes feed mainly on littoral carbon sources (2); therefore, littoral primary consumers (Trichoptera or Lepidoptera) were collected to standardize the isotopic signatures of the fish for the baseline signature of each lake (3). Invertebrates were live-sorted to major taxa in the field, sealed in Whirl-Paks®, and frozen within 24 hr of collection.

In the laboratory, invertebrates and fish were sorted and prepared for isotope and THg analyses. All tools for handling invertebrates were rinsed in a 5% HCl acid bath between samples, and tools to homogenize fish were washed in soapy water and then rinsed with excess volumes of distilled water. Cases were removed from the invertebrates, and the trichopterans and lepidopterans were identified to family according to Merritt and Cummins (4). A skinless dorsal muscle sample was removed from each

fish for isotope analysis and then the whole bodies were homogenized. Fish muscle (dermal punch or fillet) and whole body homogenates, and whole body composites ($n \geq 2$) of invertebrates were freeze dried and homogenized for THg and stable isotope analyses. Fish samples were weighed before and after lyophilization to determine the percent moisture.

Mercury analyses, 1996/97

Whole bodies of 677 yellow perch were homogenized, then homogenates of one to three similar-length fish were pooled within lakes to create a total of 242 composite samples (5). The composite samples (maximum 9 per lake except for Kejimikujik and Peskawa, which had 9×3 sites/lake) were analysed for THg as described in Drysdale et al.(5). Briefly, fish samples were digested in acid, then reduced to elemental Hg, and THg was measured by cold vapour atomic absorption spectrometry. Runs had a mean reference material (DORM-2; dogfish muscle; National Research Council of Canada) recovery of $103.3 \pm 4.5\%$ ($93.33 - 107.96\%$; $n = 10$) and precision within 3.7% ($n = 40$). A subset of 23 composite tissues analysed for MeHg using an Advanced Mercury Analyser (AMA-254) had $95.5 \pm 6.7\%$ of THg as MeHg (5).

Quality assurance, 2006/07

Twenty yellow perch archived from the 1996/97 study were re-analyzed at the University of New Brunswick (UNB) and thirty yellow perch captured in 2006 were re-analysed by Environment Canada at the National Wildlife Research Centre (NWRC) in Ottawa. Recoveries of reference materials analysed at UNB were described in the main text. The 1996/97 perch re-analyzed at UNB had a mean of $0.34 \pm 0.23 \mu\text{g}\cdot\text{g}^{-1}$ ww in 2006 (original mean THg of $0.30 \pm 0.19 \mu\text{g}\cdot\text{g}^{-1}$ ww). Once the UNB data for the 1996/97 fish were corrected for moisture lost while in storage (average of 3.8%), the mean difference \pm SD between duplicate samples was $0.04 \pm 0.09 \mu\text{g}\cdot\text{g}^{-1}$ and there were no significant differences between labs (paired t-test, $p=0.556$). Results were not corrected for this difference. Samples analysed at NWRC in 2006 were processed using an AMA-254 and had a mean reference material recovery of $111.3 \pm 7.0\%$ (DOLT-3, dogfish liver tissue, National Research Council of Canada; OT-1566b, oyster tissue, National Institute of Standards and Technology; $n = 8$). Precision of duplicate samples at NWRC was

2.9 ± 2.0 ‰ (n = 10). Analyses at NWRC were an average of 21.3 ± 17.4% higher than at UNB, and the difference was reduced to non-significance (5.4%; paired t-test p = 0.87) after correcting for the recovery of reference materials.

Stable isotope analyses

Stable nitrogen isotope ratios of the fish were obtained from individual dermal punch or fillet samples; invertebrates were analysed as composites (n ≥ 2) of whole bodies. Analyses were performed as per Wyn et al.(2). Atmospheric nitrogen is the internationally recognized standard, and ammonium sulfate ($\delta^{15}\text{N} = 20.3\text{‰}$) was the lab standard. Accuracy of the standard was 0.24‰ (n = 10). Precision of duplicate samples had one standard deviation of 0.12‰ (n = 51).

Trophic position (TP) of each perch captured in 2006/07 was calculated by subtracting the mean $\delta^{15}\text{N}$ of primary consumers within the respective food webs (i.e., as Trichoptera or Lepidoptera) from the $\delta^{15}\text{N}$ of the fish. This adjusted value ($\delta^{15}\text{N}_{\text{adj}}$) was used then to calculate TP: $(\delta^{15}\text{N}_{\text{adj}}/ 3.4) + 2$. This equation assumes that the average N enrichment observed between subsequent trophic levels is 3.4‰ and that the primary consumer has a trophic level of 2 (3,6,7). Although $\delta^{15}\text{N}$ of the primary consumers (Trichoptera or Lepidoptera) was similar among lakes (ANOVA, p = 0.38; Table S5), there was a broad range in values (-0.25 to 5.75 ‰) suggesting that the comparisons of TP would be more accurate than comparisons of the raw isotopic data.

Statistical analyses

For >18 cm fish collected in 2006/07, linear regression analysis (general linear model, GLM) was used to convert the THg concentrations in dermal punch samples to whole body concentrations (smaller fish were lethally sampled).

Temporal analyses in Big Red and Cobrielle Lakes were limited to 10-20 cm sized fish because of low sample sizes of smaller fish.

Among-lake differences in mean log-lengths, log-weights, ages, and trophic position (2006/07 only) of yellow perch were evaluated within each period using ANOVA, followed by Tukey's multiple comparison tests.

Temporal changes in perch THg concentrations were analysed using polynomial regression within each lake (8). The polynomial regression model included a dummy variable for year (1996/97 = 0, 2006/07 = 1), and required that the log-length of each fish be centred to reduce correlation between terms [length-centred, $LC = \text{individual log-length} - \text{lake's mean log-length of both years combined}$; (8,9)]. The model was:

$$\text{Log-THg} = LC + LC^2 + \text{year} + \text{year} * LC + \text{year} * LC^2.$$

When removal of an outlier from the above polynomial regressions resulted in a non-significant result for the year variable, standardized THg concentration used in the % change analysis was calculated from the non-significant regression equation.

Backward stepwise regression was used to identify terms significantly related to log-THg in each lake, and only these terms were retained in the temporal trend analysis.

Results

Dermal punch analyses, 2006

Twenty-one yellow perch were sampled in 2006 for THg concentrations in dermal punch, fillet, and whole body. A highly significant relationship was observed between log-THg in dermal punches and whole bodies:

Whole-body equivalent [THg ww] = $0.60 \times \text{dermal punch [THg ww]}$; $r^2 = 0.942$; GLM, $p < 0.001$

Whenever only dermal punches were collected, THg concentrations were adjusted to whole-body equivalents using the above equation. Hg in dermal punches and fillet samples were also significantly related (fillet [THg ww] = $0.998 * \text{punch [THg ww]}$, $r^2 = 0.992$; GLM, $p < 0.001$) such that fillet concentrations did not require transformation.

Dermal punch $\delta^{15}\text{N}$ of the 21 yellow perch from the three lakes were significantly related to fillet $\delta^{15}\text{N}$ values. The resulting equation was used to convert all dermal punch isotope ratios to fillet equivalents:

$$\text{Fillet-equivalent } \delta^{15}\text{N} = 0.94 \times \text{Dermal punch } \delta^{15}\text{N}; r^2 = 0.895; \text{GLM, } p < 0.0001.$$

Yellow perch 1996/97 [data from (5)]

Lengths, weights, and ages of yellow perch collected in 1996/97 did not vary significantly (ANOVA, $p > 0.30$) among the 16 lakes in KNPNS; within-lake means ranged from 10.7 ± 3.5 to 14.6 ± 2.5 cm for length, 17.52 ± 16.93 to 39.32 ± 20.08 g for weight (data not shown), and 3.7 ± 1.5 to 6.8 ± 2.1 y for age (Table 1). In contrast, growth rates (ANCOVA interaction, $p < 0.004$) and condition (ANCOVA intercept, $p < 0.05$) varied significantly among the lakes, with within-lake means ranging from 0.02 to $0.11 \text{ cm}\cdot\text{y}^{-1}$ and 1.08 and $1.31 \text{ g}\cdot\text{cm}^{-3}$, respectively (Tables 1 and S4).

Log-THg concentrations were significantly different among the lakes where all sizes (5-20 cm) of fish were captured (ANOVA, $p < 0.02$; Table 1). Log-THg was positively (GLM, $p < 0.05$, $r^2 > 0.43$) related to log-length, log-weight, or age in most lakes (Table S6). Log-THg*age accumulation rates were also significantly (ANCOVA interaction, $p < 0.001$) different among the lakes (Table S6). No differences in THg accumulation with log-length or log-weight were observed among lakes (ANCOVA interaction, $p > 0.26$).

Across lakes, length-standardized THg concentrations were positively related to mean age (GLM, $p = 0.03$, $r^2 = 0.29$), aqueous THg ($p = 0.05$, $r^2 = 0.25$), specific conductivity ($p = 0.01$, $r^2 = 0.36$), TOC ($p = 0.02$, $r^2 = 0.37$; Figure S2a), TN ($p = 0.003$, $r^2 = 0.49$), and percent wetlands in the watershed ($p = 0.12$, $r^2 = 0.16$ reduced to $p = 0.02$, $r^2 = 0.35$ when one outlier was removed). Standardized THg concentrations were also lowest in the lakes with the highest pH ($p = 0.01$, $r^2 = 0.39$; Figure S2b).

Yellow perch 2006/07

No significant differences in size were observed among lakes for yellow perch captured in 2006/07 (ANOVA, $p > 0.18$), and within-lake mean (un-pooled) lengths and weights ranged from 10.9 ± 2.7 to 14.4 ± 1.8 cm and 16.33 ± 11.97 to 34.76 ± 14.75 g, respectively (Table S7). In contrast, fish from two lakes (Upper Silver and Mountain) were approximately 50 % younger ($p < 0.04$) than those captured at eight other sites (Cobrielle, Peskowsk, Frozen Ocean, Peskawa, Kejimikujik, North Cranberry, Puzzle, and Big Red; Table S7). Perch growth also varied significantly (ANCOVA interaction, $p < 0.001$) among the 16 study lakes, ranging from 0.01 to $0.09 \text{ cm}\cdot\text{y}^{-1}$ (Table S4). Among-lake differences in condition could not be assessed for fish collected in 2006/07 because of a significant interaction

between length and weight (ANCOVA, $p < 0.008$), although means ranged from $1.04 \text{ g}\cdot\text{cm}^{-3}$ to $1.24 \text{ g}\cdot\text{cm}^{-3}$ (Table S7).

Mean trophic positions of yellow perch were significantly (Tukey test, $p < 0.001$) higher in three lakes (Beaverskin, North Cranberry, and Loon) than for the perch in the other lakes, while the perch from one lake (Mountain) had a significantly ($p < 0.001$) lower mean TP than the fish in all other lakes (Table S5). Trophic position of perch varied by up to 1.06 levels within a given lake, and increased significantly (GLM, $p < 0.03$, $r^2 > 0.18$) with length of perch in most lakes (all except Back, Beaverskin, Big Dam West, Big Red, Pebbleloggitch, Peskowsk, and Upper Silver).

Log-THg was significantly (GLM, $p < 0.03$) positively related to log-length, log-weight, or age for the yellow perch within most lakes (Figure S4, Table S6). Relationships between concentrations of THg and age varied significantly among lakes (ANCOVA interaction of log-THg*age, $p = 0.006$; Table S6), although bioaccumulation rates based on log-THg and log-length or log-weight were not significantly different ($p > 0.26$) across lakes.

The variability in THg concentrations in perch from the lakes sampled in 2006/07 was explained more by biological than by physical or chemical factors. Standardized (12-cm) THg concentrations ranged from $0.12 \mu\text{g}\cdot\text{g}^{-1}$ in the lake with the youngest perch to a high of $0.36 \mu\text{g}\cdot\text{g}^{-1}$ in lakes with some of the oldest perch (Table S7). Although there were significant differences in fish age, growth, and trophic positions across the 16 study lakes, standardized THg concentrations were only related to the mean age of perch (positive GLM, $p < 0.001$, $r^2 = 0.69$); among lakes these THg concentrations were not related to log-weight ($p = 0.09$, reduced to $p = 0.17$ after removal of one outlier), condition ($p = 0.64$) or trophic position ($p = 0.31$). In contrast to data from 1996/97, standardized THg concentrations of perch collected in 2006/07 were significantly (simple GLM, $p = 0.05$, $r^2 = 0.25$) negatively related to aqueous alkalinity and marginally to pH ($p = 0.08$, $r^2 = 0.20$) but not to any other physical or chemical parameters (e.g., TOC, Figure S2; $p > 0.15$).

Temporal trends

It is worth noting that the polynomial regression indicated that the relationship between THg and length (i.e., as determined by variables included in the regression) changed between 1996/97 and 2006/07 for four lakes (Table S3; Figure S4).

Within some lakes, relationships between THg concentrations and length, weight or age of yellow perch changed from 1996/97 to 2006/07. Rates of THg accumulation with log-length, log-weight, or age decreased (ANCOVA interaction, $p < 0.03$) in four lakes but accumulation with length increased in another lake ($p < 0.05$; Figure S4; Table S6). These relationships did not change significantly in the remaining lakes. The % change of THg in perch was not significantly predicted by the % change of bioaccumulation with age (the only bioaccumulation metric that changed significantly through time; $p = 0.72$) or with bioaccumulation with age, as measured in 2006/07 ($p = 0.78$).

The % change in sulfate concentration in each lake between 1995-1997 and 2005-2007 was significantly and positively related to % changes in specific conductance, Al, Ca, Cl, K, Na, and Mg concentrations (GLM, $p < 0.01$). Percent change of TN and sulfate were marginally positively related to drainage basin area and latitude, respectively ($p < 0.07$); the % change in pH was not related to any physical characteristics of the lakes.

Discussion

Several studies have shown that Hg concentrations are higher in fish that occupy a higher trophic position [determined using $\delta^{15}\text{N}$; (10) and references therein], thus an increase in $\delta^{15}\text{N}$ or a change in food web structure through time could help explain the temporal increases in fish THg. $\delta^{15}\text{N}$ was measured in a subset of perch collected in 1996/97 and the limited results suggest that lakes where THg concentrations increased through time also had increases in $\delta^{15}\text{N}$ of these fish (Table S5). Changes in food web structure may have contributed to the temporal trends observed in the lakes at KNPNHs, but increases in $\delta^{15}\text{N}$ values of lower-trophic-level organisms over time may also explain this trend and cannot currently be ruled out.

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Table S1. Physical characteristics of selected lakes in Kejimikujik National Park and National Historic Site¹ (11,12).

Lake	Longitude	Latitude	Surface Area	Maximum Depth	Mean Depth	Volume	Drainage Basin Area	Wetland	Flushing Rate
	(° W)	(° N)	(Ha)	(m)	(m)	(1000 m ³)	(km ²)	(%)	(year ⁻¹)
Back	65.27	44.29	79.9	5.8	2.2	1706	9.3	1.0	2.0
Beaverskin	65.33	44.31	41.8	6.3	2.2	864	4.8	0.0	1.0
Big Dam East	65.27	44.45	45.5	4.2	2.3	1055	131	0.0	1.6
Big Dam West	65.29	44.46	105	9.5	2.5	2593	131	5.4	13.1
Big Red	65.38	44.35	77.9	2.2	1.0	704	120	3.2	9.6
Cobrielle	65.23	44.32	136	6.3	2.0	2595	98	14.9	3.8
Frozen Ocean	65.35	44.45	228	7.6	1.9	4241	131	6.7	23.2
Kejimikujik	65.22	44.37	2632	19.2	4.4	106,017	842	6.9	5.5
Loon	65.18	44.32	76.5	8.5	2.0	1,471	842	10.8	418.0
Mountain	65.26	44.33	137.2	14.3	4.3	5,790	98	14.2	1.2
N. Cranberry	65.23	44.33	34.4	5.0	1.4	498	4.6	21.3	6.1
Pebbleloggitch	65.35	44.30	33.4	2.5	1.4	474	2	17.6	2.9
Peskawa	65.36	44.32	390	9.0	3.2	12,249	98	4.1	4.6

Table S1. Continued.

Lake	Longitude	Latitude	Surface Area	Maximum Depth	Mean Depth	Volume	Drainage Basin Area	Wetland	Flushing Rate
	(° W)	(° N)	(Ha)	(m)	(m)	(1000 m ³)	(km ²)	(%)	(year ⁻¹)
Peskowesk	65.28	44.32	737	13.0	3.8	26,356	98	4.6	2.7
Puzzle	65.23	44.33	33.7	6.1	2.7	911	4.6	35.3	2.0
Upper Silver	65.25	44.28	24.3	5.8	2.3	566		1.3	1.4

¹ Sites are polymictic, with stratification and oxygen depletion occurring only in the deepest locations that represent < 1% of lake volume [Kerekes 1975 #318].

Table S2. Mean chemical characteristics of selected lakes in Kejimikujik sampled in spring and fall of 1995-1997 and 2005-2007; significant changes are marked with an asterisk {Drysdale, Burgess, et al. 2005 #76}.

Lake	Year	n	pH	THg ¹	TOC ²	Alk. ³	Sp. Cond. ⁴	TN ⁵	SO ₄	Al	Ca	Na
				(ng•L ⁻¹)	(mg•L ⁻¹)	(ng•L ⁻¹)	(µS•cm ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)
Back	1996	6	5.4	1.5	3.8	0.3	21.5	0.07	1.9	0.079	0.45	2.5
	2006	6	5.5*	2.0	4.1	0.3	21.4	0.16*	1.6*	0.080	0.42	2.4
Beaverskin	1996	6	5.3	1.5	2.6	-0.1	21.8	0.06	1.9	0.039	0.30	2.6
	2006	6	5.5*	0.8	2.6	0.2	20.6	0.13*	1.7*	0.035	0.31	2.5
Big Dam East	1996 ⁶	1	5.9	2.8	3.7	0.9	23.8	0.07	1.8	0.070	0.61	2.9
	2006	6	6.0	1.5	3.9	1.1	24.3	0.12	1.7	0.072	0.58	2.9
Big Dam West	1996	6	5.0	2.8	9.3	0.0	29.8	0.10	1.8	0.198	0.60	3.5
	2006	6	5.1	4.2	8.8	0.2	33.3	0.19	1.7	0.185	0.62	3.6
Big Red	1996	6	4.3	5.0	14.8	-2.4	37.6	0.12	1.9	0.175	0.25	2.7
	2006	6	4.3	7.0	15.4	0.0	40.8	0.27	1.7	0.234	0.27	2.6
Cobrielle	1996	6	5.3	2.6	4.3	0.1	22.3	0.07	1.8	0.101	0.37	2.6
	2006 ⁷	5	5.5	1.3	3.4	0.3	20.1	0.13*	1.5*	0.082	0.31	2.4

Table S2. Continued

Lake	Year	n	pH	THg ¹	TOC ²	Alk. ³	Sp. Cond. ⁴	TN ⁵	SO ₄	Al	Ca	Na
				(ng•L ⁻¹)	(mg•L ⁻¹)	(ng•L ⁻¹)	(µS•cm ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)	(mg•L ⁻¹)
Frozen Ocean	1996	6	4.9	4.8	9.4	-0.3	28.5	0.10	1.8	0.195	0.52	3.1
	2006	6	4.9	4.4	9.2	0.1	30.8	0.21*	1.7	0.212	0.53	3.2
Kejimkujik	1996	6	5.0	3.5	7.4	-0.1	27.9	0.08	2.0	0.164	0.58	3.1
	2006	6	5.0	4.3	8.3	0.2	30.0	0.19*	1.8	0.182	0.54	3.1
Loon	1996 ⁶	1	5.1	3.9	6.9	0.0	27.9	0.08	2.1	0.150	0.62	3.2
	2006	6	5.0	4.1	7.8	0.2	29.6	0.19	1.8	0.171	0.58	3.1
Mountain	1996	6	5.4	1.8	3.7	0.2	21.4	0.07	1.8*	0.117	0.43	2.5
	2006	6	5.2	2.0	4.6	0.1	21.0	0.12*	1.5	0.124	0.31	2.4
N. Cranberry	1996 ⁶	1	5.1	1.9	4.5	-0.1	21.1	0.10	1.7	0.080	0.42	2.3
	2006 ⁸	6	5.1	2.7	5.2	0.2	26.3	0.22	1.8	0.141	0.53	2.8
Pebblelogitch	1996	6	4.5	5.3	10.7	-1.3	30.4	0.11	1.9	0.190	0.29	2.6
	2006	6	4.5	5.3	10.1	0.0	33.4	0.23	1.6	0.236	0.29	2.4
Peskawa	1996	6	4.7	3.8	6.4	-0.8	26.8	0.09	2.0	0.200	0.27	2.7
	2006	6	4.7	4.6	8.8	0.0	28.9	0.19*	1.8	0.260	0.32	2.6

Table S2. Continued.

Lake	Year	n	pH	THg ¹ (ng•L ⁻¹)	TOC ² (mg•L ⁻¹)	Alk. ³ (ng•L ⁻¹)	Sp. Cond. ⁴ (μS•cm ⁻¹)	TN ⁵ (mg•L ⁻¹)	SO ₄ (mg•L ⁻¹)	Al (mg•L ⁻¹)	Ca (mg•L ⁻¹)	Na (mg•L ⁻¹)
Peskowesk	1996	6	4.8	2.7	5.8	-0.6	24.9	0.09	2.0	0.235	0.31	2.6
	2006	6	4.8	3.5	7.1	0.0	26.0	0.19*	1.7*	0.238	0.32	2.6
Puzzle	1996 ⁶	1	5.3	0.9	3.3	0.0	20.5	0.09	1.6	0.050	0.35	2.3
	2006 ⁹	3	5.5	1.5	3.3	0.3	21.6	0.18	1.6	0.067	0.42	2.4
Upper Silver	1996	6	5.9	1.8	3.2	0.9	21.6	0.07	2.0	0.056	0.61	2.6
	2006	6	6.1*	1.2	3.4	1.1	21.7	0.13*	1.7*	0.060	0.58	2.5

¹Total Mercury (n=1-2); ²Total Organic Carbon; ³Alkalinity; ⁴Specific Conductivity; ⁵Total Nitrogen; ⁶Means of the 1995–1997 results were obtained from (5), i.e., n = 1 for this study while original results were collected in spring and/or fall 1995, 1996, 1997; ⁷Cobrielle was sampled in spring 2005, spring and fall of 2006, 2007 (n = 5); ⁸North Cranberry was sampled in fall 2006, spring 2007 (n = 2); ⁹Puzzle was sampled in fall 2006, spring and fall 2007 (n = 3).

Table S3. Results of polynomial regressions calculated to determine differences in yellow perch THg concentrations between 1996/97 and 2006/07 (mathematically pooled data; FLC = fork length, centred). Presence of coefficients for the variable “Year, Year*L₁₀FLC,” or “Year*L₁₀FLC²” shows that yellow perch had significantly different THg concentrations between the two periods. Inclusion of a coefficient for the Year*L₁₀FLC² also indicates a changes in the shape of the slope of the relationship between years.

Lake	Intercept	L ₁₀ FLC	L ₁₀ FLC*L ₁₀ FLC	Year	Year*L ₁₀ FLC	Year*L ₁₀ FLC ²	p	r ²
Back	-0.912	0.816		0.201			<0.001	0.70
Beaverskin	-0.746	1.355		0.198	-1.368		<0.001	0.72
Big Dam East	-0.798	1.403		0.150		-8.236	<0.001	0.90
Big Dam West	-0.643	1.238	-3.085			-5.140	<0.001	0.91
Big Red	-0.321	1.084					0.04	0.34
Cobrielle	-0.603	1.213		0.103			0.004	0.71
Frozen Ocean	-0.652	1.434					<0.001	0.83
Kejimkujik	-0.559	1.036					<0.001	0.68
Loon	-0.624	1.147		0.087		-3.974	<0.001	0.87
Mountain	-0.741	1.372			0.530		<0.001	0.93
Mountain ¹	-0.756	1.740					<0.001	0.96

Table S3. Continued.

Lake	Intercept	L ₁₀ FLC	L ₁₀ FLC*L ₁₀ FLC	Year	Year*L ₁₀ FLC	Year*L ₁₀ FLC ²	p	r ²
N. Cranberry	-0.502	1.122	3.709	0.075			<0.001	0.89
Pebbleloggitch	-0.734	0.445	-3.797	0.073			<0.001	0.74
Pebbleloggitch ¹	-0.718	0.490	-3.433				<0.001	0.76
Peskawa	-0.645	1.345			-0.582		<0.001	0.68
Peskowesk	-0.696	1.061	4.708	0.119			<0.001	0.80
Puzzle	-0.657	1.095		0.116			<0.001	0.91
Upper Silver	-0.755	0.854	-6.717	-0.195		8.234	<0.001	0.82

¹ One outlier removed from analysis.

Table S4. Regression coefficients for log length-age relationships of yellow perch captured in Kejimikujik in 1996/97 and 2006/07. ANCOVA p values represent among-period differences in the slopes (growth rates) of the pooled data, while letters represent statistical differences among lakes within the appropriate period.

Lake	Year	Intercept	Slope	p	r ²	ANCOVA
						Interaction p
Back	1996	0.747	0.067b	<0.001	0.85	0.04
	2006 ¹	0.715	0.103	<0.001	0.96	
	2006 ²	0.760	0.090c	<0.001	0.85	
Beaverskin	1996	0.719	0.090c	<0.001	0.81	0.29
	2006 ¹	0.772	0.073	<0.001	0.98	
	2006 ²	0.784	0.069b	<0.001	0.95	
Big Dam East	1996	0.726	0.066b	<0.001	0.93	0.09
	2006 ¹	0.610	0.107	0.004	0.78	
	2006 ²	0.750	0.070b	<0.001	0.52	
Big Dam West	1996	0.697	0.059b	<0.001	0.95	0.49
	2006 ¹	0.800	0.054	<0.001	0.98	
	2006 ²	0.814	0.051b	<0.001	0.89	
Big Red	1996	1.009	0.019a	0.30	0.26	0.42
	2006 ¹	0.926	0.037	0.003	0.85	
	2006 ²	1.058	0.013a	0.02	0.30	
Cobrielle	1996	0.858	0.059b	0.01	0.86	0.88
	2006 ¹	0.905	0.054	0.12	0.49	
	2006 ²	0.964	0.040b	0.004	0.44	

Table S4. Continued

Lake	Year	Intercept	Slope	p	r ²	ANCOVA
						Interaction p
Frozen Ocean	1996	0.632	0.070b	<0.001	0.92	0.01 ³
	2006 ¹	0.843	0.051	<0.001	0.96	
	2006 ²	0.849	0.050b	<0.001	0.92	
Kejimkujik	1996 ⁴	0.781	0.050b	<0.001	0.84	0.002
	2006 ²	0.702	0.068b	<0.001	0.95	
Loon	1996	0.768	0.053b	<0.001	0.91	0.02 ³
	2006 ¹	0.790	0.075	<0.001	0.97	
	2006 ²	0.805	0.071b	<0.001	0.91	
Mountain	1996	0.745	0.061b	<0.001	0.94	0.19
	2006 ¹	0.834	0.078	<0.001	0.90	
	2006 ²	0.830	0.079b	<0.001	0.87	
North	1996	0.807	0.051b	<0.001	0.94	0.54
Cranberry	2006 ¹	0.791	0.054	<0.001	0.98	
	2006 ²	0.811	0.050b	<0.001	0.95	
Pebbleloggitch	1996	0.709	0.068b	<0.001	0.88	0.18
	2006 ¹	0.649	0.100	0.002	0.78	
	2006 ²	0.746	0.076b	<0.001	0.59	
Peskawa	1996 ⁴	0.714	0.068b	<0.001	0.92	0.68
	2006 ²	0.725	0.066b	<0.001	0.95	
Peskowesk	1996	0.735	0.065b	<0.001	0.87	0.33
	2006 ¹	0.807	0.053	<0.001	0.89	
	2006 ²	0.815	0.050b	<0.001	0.85	

Table S4. Continued

Lake	Year	Intercept	Slope	p	r ²	ANCOVA
						Interaction p
Puzzle	1996	0.738	0.061b	<0.001	0.88	0.78
	2006 ¹	0.761	0.058	<0.001	0.95	
	2006 ²	0.768	0.057b	<0.001	0.93	
Upper Silver	1996	0.600	0.112c	<0.001	0.97	0.004³
	2006 ¹	0.888	0.067	<0.001	0.92	
	2006 ²	0.890	0.064b	<0.001	0.84	

¹Mathematically pooled data; see Methods for details.

²Raw, unpooled data; see Methods for details.

³One outlier removed from analysis.

⁴Sample size was similar in 1996/97 and 2006/07; therefore, raw, unpooled data were compared. See Methods for details.

Table S5. Mean (\pm SD) $\delta^{15}\text{N}$ (‰) and trophic position of primary consumers (PC) and yellow perch (YP) caught in 1996/97 and 2006/07 from 16 lakes in Kejimikujik. See text for details on trophic position calculations; letters represent statistical differences (ANOVA) among lakes within the 2006/07 period.

Lake	Year	n	PC $\delta^{15}\text{N}$ (n) (‰)	YP $\delta^{15}\text{N}$ (n) (‰)	Trophic Position
Back	1996	9	-	7.18 (1)	-
	2006 ¹	10	-	7.49 \pm 0.26	3.69 \pm 0.08
	2006 ²	27	1.54 / 1.97 (2)	7.50 \pm 0.43	3.69 \pm 0.13b
Beaverskin	1996	10	-	7.45 (1)	-
	2006 ¹	8	-	7.98 \pm 0.17	4.05 \pm 0.05
	2006 ²	24	1.00 \pm 0.35 (3)	7.98 \pm 0.36	4.05 \pm 0.11c
Big Dam East	1996	8	-	7.17 (1)	-
	2006 ¹	8	-	7.41 \pm 0.41	3.56 \pm 0.12
	2006 ²	21	2.13 \pm 0.78 (3)	7.39 \pm 0.47	3.55 \pm 0.14b
Big Dam West	1996	7	-	7.40 (1)	-
	2006 ¹	8	-	6.80 \pm 0.40	3.71 \pm 0.12
	2006 ²	22	0.99 (1)	6.78 \pm 0.53	3.70 \pm 0.16b
Big Red	1996	6	-	8.03 (1)	-
	2006 ¹	7	-	7.04 \pm 0.20	3.64 \pm 0.06
	2006 ²	18	0.97 / 1.94 (2)	7.02 \pm 0.31	3.64 \pm 0.09b
Cobrielle	1996	6	-	7.35 (1)	-
	2006 ¹	6	-	7.75 \pm 0.22	3.54 \pm 0.06
	2006 ²	17	2.51 (1)	7.72 \pm 0.22	3.53 \pm 0.07b

Table S5. Continued

Lake	Year	n	PC $\delta^{15}\text{N}$ (n)	YP $\delta^{15}\text{N}$ (n)	Trophic Position
			(‰)	(‰)	
Frozen Ocean	1996	9	-	7.46 (1)	-
	2006 ¹	9	-	6.91 ± 0.37	3.82 ± 0.11
	2006 ²	27	0.73 ± 1.56 (3)	6.91 ± 0.51	3.92 ± 0.15b
Kejimkujik	1996	23	-	7.63 ± 0.46 (4)	-
	2006 ²	26	1.66 (1)	7.37 ± 0.62	3.68 ± 0.18b
Loon	1996	9	-	7.68 (1)	-
	2006 ¹	9	-	7.35 ± 0.32	3.96 ± 0.09
	2006 ²	27	0.68 ± 0.43 (5)	7.35 ± 0.40	3.96 ± 0.12c
Mountain	1996	8	-	7.90 (1)	-
	2006 ¹	9	-	6.59 ± 0.71	3.21 ± 0.21
	2006 ²	23	0.54 / 4.40 (2)	6.51 ± 0.79	3.19 ± 0.23a
N. Cranberry	1996	9	-	6.00 / 9.53 (2)	-
	2006 ¹	9	-	8.40 ± 0.52	4.05 ± 0.15
	2006 ²	25	1.45 ± 0.43 (3)	8.30 ± 0.53	4.02 ± 0.16c
Pebbleloggitch	1996	9	-	5.40 / 6.96 (2)	-
	2006 ¹	9	-	6.81 ± 0.20	3.76 ± 0.06
	2006 ²	27	0.82 ± 0.11 (3)	6.81 ± 0.55	3.76 ± 0.16b
Peskawa	1996	20	-	-	-
	2006 ²	27	-0.04 (1)	6.25 ± 0.40	3.85 ± 0.12b
Peskowesk	1996	8	-	5.72 (1)	-
	2006 ¹	10	-	6.09 ± 0.37	3.57 ± 0.11
	2006 ²	27	0.74 (1)	6.04 ± 0.47	3.56 ± 0.14b

Table S5. Continued

Lake	Year	n	PC $\delta^{15}\text{N}$ (n) (‰)	YP $\delta^{15}\text{N}$ (‰)	Trophic Position
Puzzle	1996	8	-	7.60 / 8.12 (2)	
	2006 ¹	9	-	7.62 ± 0.41	3.57 ± 0.12
	2006 ²	26	2.27 ± 0.20 (3)	7.60 ± 0.51	3.57 ± 0.15b
Upper Silver	1996	7	-	-	-
	2006 ¹	10	-	7.18 ± 0.26	3.76 ± 0.08
	2006 ²	24	1.14 / 1.26 (2)	7.16 ± 0.48	3.75 ± 0.14b

¹Mathematically pooled data; see text for details.

²Raw, unpooled data; see text for details.

Table S6. Regression coefficients for log THg-log length, -log weight, or -age relationships of yellow perch captured in Kejimikujik in 1996/97 and 2006/07. ANCOVA interaction p represents the among-period difference in the bioaccumulation rates (i.e., slopes) of the pooled data; letters represent among-lake differences in the rates within each period.

Lake	Year	Log THg - Log Length			Log THg - Log Weight			Log THg - Age		
		Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction
Back	1996	1.085	0.01	0.07	0.349	0.004	0.06	0.063b	0.052	0.68
	2006 ¹	0.401	0.08		0.129	0.07		0.045	0.055	
	2006 ²	0.336	0.18		0.106	0.18		0.044b	0.03³	
Beaverskin	1996	1.355	0.01	0.01	0.465	0.01	0.004	0.138b	0.01	0.002
	2006 ¹	-0.013	0.92		-0.005	0.89		0.000	0.98	
	2006 ²	-0.041	0.73		-0.010	0.79		-0.004a	0.67	
Big Dam East	1996	1.501	<0.001	0.34	0.486	0.002	0.40	0.104b	<0.001	0.52
	2006 ¹	1.137	0.01		0.369	0.01		0.130	0.02	
	2006 ²	1.253	<0.001		0.400	<0.001		0.107b	0.003	
Big Dam West	1996	1.564	<0.001	0.08	0.502	<0.001	0.08	0.097b	<0.001	0.08 ³
	2006 ¹	0.787	0.07		0.245	0.07		0.041	0.08 ³	
	2006 ²	0.885	0.01		0.265	0.01		0.047b	0.01	

Table S6. Continued.

Lake	Year	Log THg - Log Length			Log THg - Log Weight			Log THg - Age		
		Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction
Big Red	1996	0.702	0.30	0.22	0.228	0.28	0.26	-0.019a	0.45	0.11
	2006 ¹	1.975	0.04		0.599	0.053		0.060	0.17	
	2006 ²	1.901	0.01		0.552	0.01		0.029b	0.10	
Cobrielle	1996	1.413	0.01	0.46	0.430	0.02	0.51	0.090b	0.01	0.25
	2006 ¹	0.908	0.20		0.294	0.15		0.021	0.73	
	2006 ²	0.741	0.22		0.270	0.11		0.043b	0.24	
Frozen Ocean	1996	1.658	<0.001	0.08	0.518	<0.001	0.09	0.112b	0.003	0.06
	2006 ¹	1.033	0.01		0.319	0.01		0.054	0.01	
	2006 ²	1.039	<0.001		0.310	<0.001		0.056b	<0.001	
Kejimkujik	1996	1.242	<0.001	0.14	0.384	<0.001	0.18	0.061b	<0.001	0.79
	2006 ²	0.921	<0.001		0.287	<0.001		0.065b	<0.001	
Loon	1996	1.377	<0.001	0.21	0.456	<0.001	0.16	0.081b	<0.001	0.71
	2006 ¹	1.000	0.004		0.317	0.01		0.073	0.01	
	2006 ²	1.011	<0.001		0.319	<0.001		0.076b	<0.001	

Table S6. Continued.

Lake	Year	Log THg-Log Length			Log THg - Log Weight			Log THg - Age		
		Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction
Mountain	1996	1.369	<0.001	0.045	0.443	<0.001	0.07	0.08b	<0.001	0.051
	2006 ¹	1.898	<0.001		0.607	<0.001		0.144	<0.001	
	2006 ²	1.939	<0.001		0.616	<0.001		0.152c	<0.001	
N. Cranberry	1996	1.260	<0.001	0.03³	0.398	<0.001	0.03³	0.070b	<0.001	0.01³
	2006 ¹	0.643	0.01³		0.194	0.01		0.033	0.02³	
	2006 ²	0.649	0.001		0.196	<0.001		0.036b	<0.001	
Pebbleloggitch	1996	0.654	0.01	0.61	0.206	0.01	0.63	0.039a	0.054	0.27
	2006 ¹	0.473	0.16		0.150	0.16		0.078	0.02	
	2006 ²	0.472	0.06		0.146	0.08		0.056b	0.02	
Peskawa	1996	1.346	<0.001	0.01	0.431	<0.001	0.01	0.097b	<0.001	0.01
	2006 ²	0.763	<0.001		0.234	<0.001		0.052b	<0.001	
Peskowesk	1996	1.099	0.02	0.66	0.349	0.02	0.62	0.063b	0.08	0.48
	2006 ¹	0.903	0.01		0.277	0.02		0.038	0.09	
	2006 ²	0.843	0.001		0.263	0.001		0.023b	0.08 ³	

Table S6. Continued.

Lake	Year	Log THg-Log Length			Log THg - Log Weight			Log THg - Age		
		Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction	Slope	p	ANCOVA Interaction
Puzzle	1996	1.130	0.001	0.77	0.360	<0.001	0.64	0.069b	0.01	0.65
	2006 ¹	1.063	<0.001		0.327	<0.001		0.061	<0.001	
	2006 ²	1.052	<0.001		0.319	<0.001		0.057b	<0.001	
Upper Silver	1996	1.438	0.01	0.10	0.449	0.02	0.14	0.131b	0.03	0.047
	2006 ¹	0.762	0.001		0.252	0.001		0.054	<0.001	
	2006 ²	0.756	0.001		0.244	<0.001		0.049b	<0.001	

¹Mathematically pooled data; see text for details.

²Raw, unpooled data; see text for details.

³ One outlier removed from analysis

Table S7. Mean (\pm SD) length, weight, condition, age, and THg (raw and standardized) of yellow perch caught in 2006/07 from 16 lakes in Kejimikujik (raw, unpooled data); letters represent among-lake differences in perch age ($p < 0.05$).

Lake	n	Length (cm)	Weight (g)	Condition ($\text{g}\cdot\text{cm}^{-3}$)	Age (y)	THg ($\mu\text{g}\cdot\text{g}^{-1}$ ww)	12-cm THg ($\mu\text{g}\cdot\text{g}^{-1}$ ww)
Back	27	12.1 \pm 3.1	22.39 \pm 18.39	1.04 \pm 0.14	3.4 \pm 1.1ab	0.20 \pm 0.07	0.20
Beaverskin	24	12.4 \pm 3.8	27.79 \pm 25.52	1.11 \pm 0.07	4.2 \pm 1.8ab	0.29 \pm 0.05	0.28
Big Dam East	21	10.9 \pm 2.7	16.33 \pm 11.97	1.06 \pm 0.07	4.0 \pm 1.1ab	0.19 \pm 0.08	0.25
Big Dam West	22	11.4 \pm 2.7	20.00 \pm 16.90	1.24 \pm 0.09	4.5 \pm 1.9ab	0.20 \pm 0.08	0.23
Big Red	18	13.6 \pm 1.4	30.87 \pm 9.87	1.19 \pm 0.09	5.6 \pm 1.9b	0.46 \pm 0.15	0.36
Cobrielle	17	14.4 \pm 1.8	34.76 \pm 14.75	1.10 \pm 0.14	4.7 \pm 0.9b	0.32 \pm 0.10	0.25
Frozen Ocean	27	12.7 \pm 3.4	30.86 \pm 23.31	1.20 \pm 0.11	4.8 \pm 2.4b	0.26 \pm 0.14	0.22
Kejimikujik	26	12.3 \pm 4.3	31.52 \pm 27.83	1.21 \pm 0.14	5.3 \pm 2.3b	0.32 \pm 0.14	0.29
Loon	27	12.3 \pm 3.8	28.52 \pm 26.34	1.16 \pm 0.10	3.7 \pm 1.8ab	0.26 \pm 0.13	0.24
Mountain	23	11.2 \pm 3.3	19.53 \pm 17.18	1.08 \pm 0.09	2.5 \pm 1.6a	0.19 \pm 0.12	0.20
N. Cranberry	25	12.4 \pm 3.4	27.37 \pm 22.38	1.10 \pm 0.12	5.3 \pm 2.4b	0.42 \pm 0.12	0.36
Pebbleloggitch	27	12.3 \pm 3.4	26.24 \pm 18.94	1.14 \pm 0.08	4.3 \pm 1.3ab	0.20 \pm 0.08	0.22
Peskawa	27	12.3 \pm 4.5	32.23 \pm 28.34	1.20 \pm 0.13	5.2 \pm 2.6b	0.25 \pm 0.11	0.24

Table S7. Continued.

Lake	n	Length (cm)	Weight (g)	Condition (g•cm ⁻³)	Age (y)	THg (μg•g ⁻¹ ww)	12-cm THg (μg•g ⁻¹ ww)
Peskowesk	27	11.9 ± 3.4	24.57 ± 18.16	1.16 ± 0.10	4.7 ± 2.4b	0.33 ± 0.20	0.27
Puzzle	26	12.5 ± 3.9	28.42 ± 24.09	1.07 ± 0.11	5.3 ± 2.4b	0.32 ± 0.12	0.30
Upper Silver	24	11.5 ± 3.2	20.85 ± 20.82	1.08 ± 0.09	2.4 ± 1.6a	0.12 ± 0.04	0.12

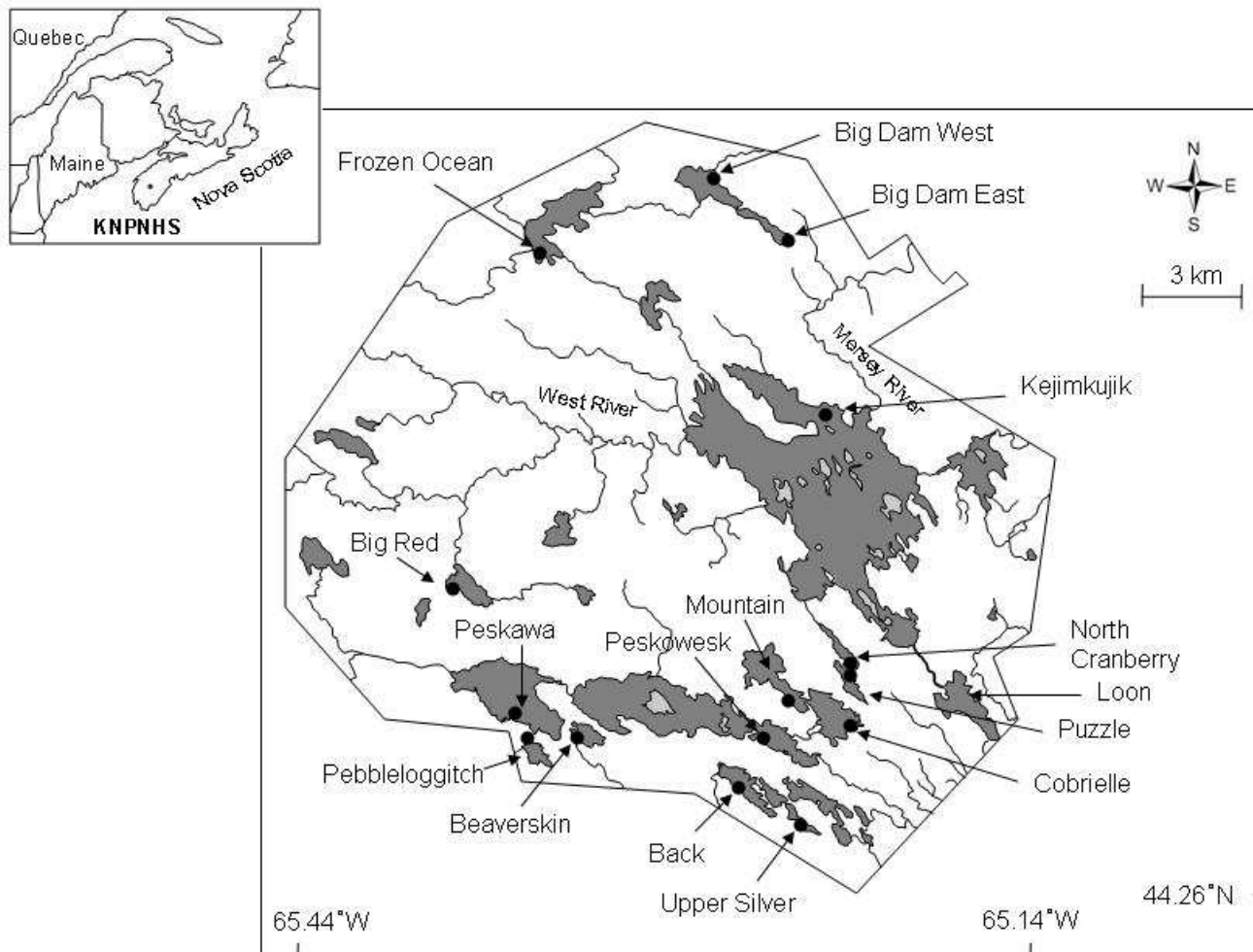


Figure S1. Sixteen study lakes in Kejimikujik National Park and National Historic Site, Nova Scotia, Canada.

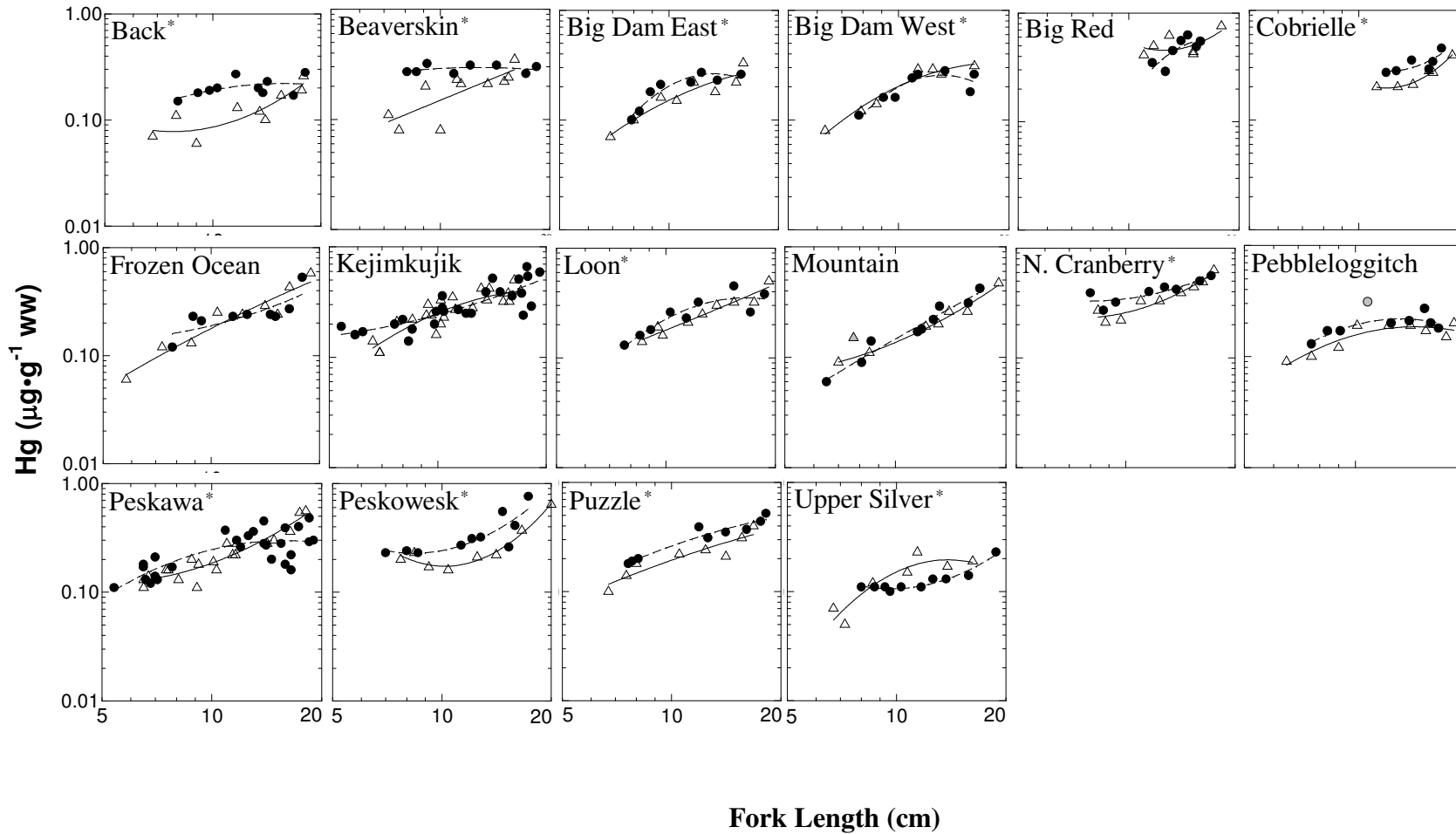


Figure S2. Log THg-log length relationships for yellow perch collected in 1996/97 and 2006/07 from 16 lakes in Kejimkujik. 1996/97 perch are represented by the open symbols and the solid line; 2006/07 perch as the closed symbols and dashed line; outliers are presented as the gray symbol for the respective sampling period; lakes with significant changes through time (excluding outliers) are indicated by an asterisk (changes in Mountain and Pebbleloggitch lakes were also significantly different before removal of one outlier each). See Table S3 for the polynomial regression equations.

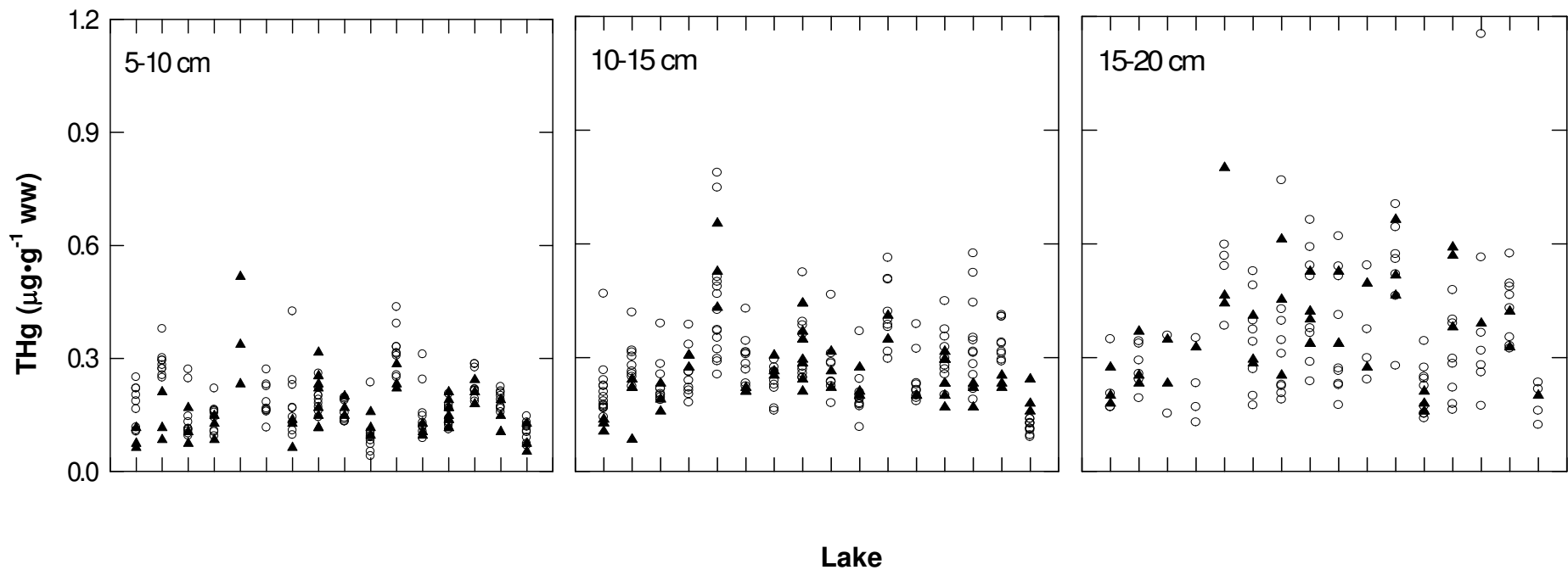


Figure S3. THg concentrations of individual yellow perch (separated by size class) collected from 16 lakes in Kejimikujik National Park in 1996/97 and 2006/07. 1996/97 perch are represented as the closed symbols; 2006/07 perch as the open symbols and typed values (note the symbol reversal from Figures S2 and S4). Lakes are presented in alphabetical order (left to right).

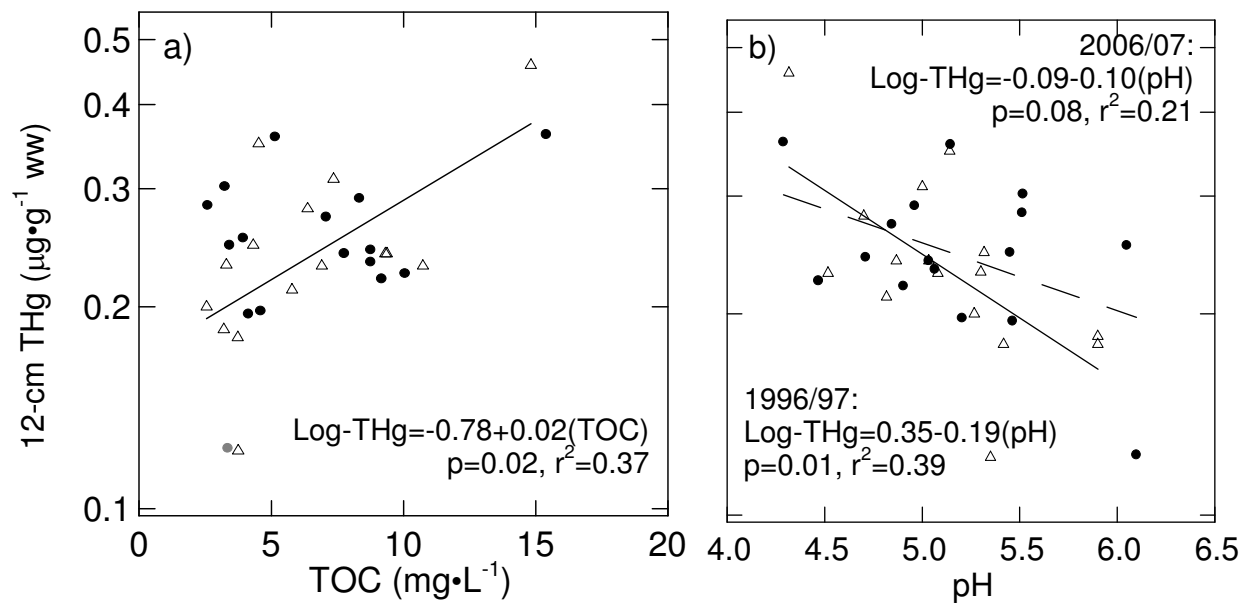


Figure S4. Relationships between log-standardized (12-cm) THg concentrations in yellow perch and a) TOC or b) pH. 1996/97 perch are represented by the open symbols and the solid line; 2006/07 perch as the closed symbols and dashed line (non-significant regression for TOC, $p = 0.45$); outlier presented as the gray symbol for the respective sampling period.