

Highlighted article

Bioenergetics and growth of young-of-the-year northern pike (*Esox lucius*) and burbot (*Lota lota*) exposed to metal mining effluent [☆], [☆] [☆]

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Abstract

We hypothesized that exposure to metal mining effluent would reduce the ability of young-of-the-year fishes to accumulate energy reserves to survive the overwinter period (known as “winter stress syndrome”) in a Canadian boreal forest watershed. Northern pike (*Esox lucius*) and burbot (*Lota lota*) were collected immediately before and after winter from a reference lake and two lakes receiving effluent. Unexpectedly, total body lipid and triglyceride, and liver triglyceride levels were greater in effluent-exposed pike and burbot in both fall and spring. However, there were no lake or season differences in growth indices of length, weight, muscle RNA/DNA ratio, or muscle protein levels in pike. In addition, total lipids and triglycerides in burbot were greater in spring compared to fall, while no seasonal differences were observed in pike, suggesting that burbot continued to feed during winter. Findings do not support the winter stress syndrome hypothesis and suggest possible direct and indirect effects of metal mining effluent on lipid dynamics of juvenile fishes. © 2007 Elsevier Inc. All rights reserved.

Keywords: Overwinter survival; Winter stress syndrome; Uranium milling effluent; Northern pike; Burbot; Young-of-the-year; Triglycerides; Lipids; RNA/DNA ratio

1. Introduction

At higher latitudes, fish are subject to seasonally short periods of growth potential followed by a long overwintering period. This seasonal environment puts constraints on the ability of young-of-the-year (YOY) fish to acquire sufficient growth and energy reserves to survive

winter (Cunjak, 1988; Berg and Bremset, 1998; Post and Parkinson, 2001). A major source of winter mortality in underyearling fish is depletion of lipid reserves (Sogard and Olla, 2000; Pratt and Fox, 2002; Biro et al., 2004). Triglycerides (triacylglycerols) are the major energy storage form in fish and have important ecophysiological relevance as indicators of growth potential and survival (Lochmann et al., 1995; Jobling et al., 1998; MacFarlane and Norton, 2002; Heintz et al., 2004).

Environmental stressors can alter both the quality and quantity of energetic lipids in fish (Adams, 1999), which directly influences the ability of YOY fishes to survive the overwinter period. In addition, metabolic costs may be increased during exposure to toxicants such as metals (Sherwood et al., 2000; Levesque et al., 2002; Rajotte and Couture, 2002; Couture and Rajotte, 2003), thus decreasing production processes such as growth and lipid storage (Calow, 1991; Lemly, 1993; Adams, 1999; Congdon et al.,

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2001). Lemly (1996) used the term “winter stress syndrome” to describe the significant overwinter lipid depletion and reduced survival that can occur in fish exposed to toxicants. Fish with reduced activity and foraging during cold winter temperatures may develop winter stress syndrome if a metabolic stressor is present (Lemly, 1993; Lemly, 1996). Thus, in wild fish populations inhabiting north temperate aquatic systems that are impacted by metal mining activities, recruitment of individuals into the population could be impaired (Calow, 1991; Lemly, 1996). However, to our knowledge the winter stress syndrome hypothesis has not been investigated in field research involving indigenous coldwater fish species.

To assess possible impacts of metal mining effluent on juvenile fish condition and overwinter survival potential, the present study determined traditional morphometric measures of growth (length, weight, condition factor), and biochemical measures of energy storage (total body lipids, total body triglycerides, liver triglycerides) and growth (muscle protein, muscle RNA/DNA ratio) in juvenile fishes sampled immediately before and after the overwinter period. YOY northern pike (*Esox lucius*) and burbot (*Lota lota*) were collected from two lakes receiving effluent from a uranium milling operation and an uncontaminated reference lake in northern Saskatchewan, Canada. We hypothesized there would be decreased growth and lipid stores in juvenile fish exposed to effluent in both fall and spring compared to fish collected from the reference lake. We also hypothesized there would be decreased lipid and triglyceride content in fish collected in spring from all sites compared to fish collected in the fall, due to energy utilization during the overwinter period.

2. Materials and methods

2.1. Site description and fish collection

Fish were collected from two lakes downstream of uranium milling effluent discharge: Unknown Lake (high exposure), located approximately 2 km from effluent release and Delta Lake (low exposure), located approximately 10 km from effluent release (Fig. 1). The reference lake, David Lake, is approximately 5 km upstream of all milling activities in the same watershed as the two exposure lakes. The study site is located at Key Lake uranium operation in the boreal forest region of north-central Saskatchewan, Canada (57°13'N, 105°38'W). At this latitude, long, cold winters are typical and lakes are usually covered with ice for >200 days. Treated mill effluent is discharged at a rate of approximately 6000 m³/d into the local water drainage system (Fig. 1).

Juvenile northern pike and burbot were collected in early October 2003 and early June 2004 using boat and backpack electrofishing units. No burbot were collected from the reference lake in fall 2003. Spottail shiners (*Notropis hudsonius*) were collected in June 2004 at the low exposure and reference lakes. Fishes were captured, held for less than 1.5 h and over-anesthetized with MS-222 (3-aminobenzoic acid). Total lengths (to the nearest 0.1 cm) and wet weights (to the nearest 0.01 g) were recorded for all fish and ageing structures (cleithra and scales) were removed from pike. Fish were immediately stored in air tight bags on dry ice until transport back to a –80 °C freezer at the University of Saskatchewan.

2.2. Laboratory analyses

2.2.1. Fish dissection

Tails and a portion of caudal muscle were cut from each fish and immediately returned to the –80 °C freezer for subsequent RNA/DNA and protein determinations. Each carcass was dissected as follows. A small piece (< 270 mg) of liver was removed for triglyceride measurement and otoliths were removed from burbot. Stomach contents were removed, weighed, and identified as either invertebrate or vertebrate (fish) prey items. The wet weight of the stomach contents was subtracted from the

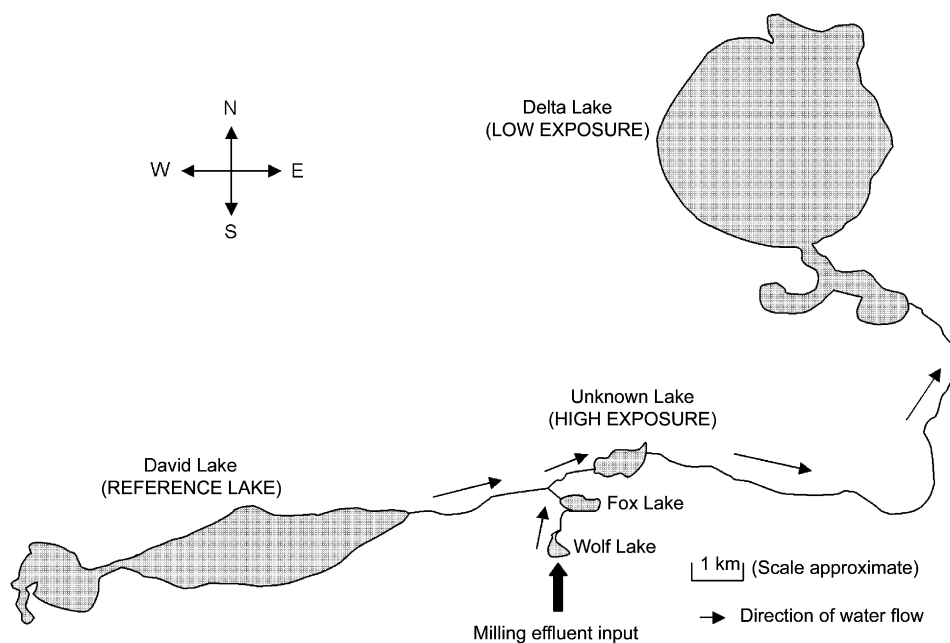


Fig. 1. Map of David Creek drainage containing treated uranium milling effluent at Key Lake uranium operation, Saskatchewan, Canada (57°13'N, 105°38'W). The direction of water flow is shown with arrows and lakes are shown in grey.

initial fish wet weight and the resulting value was used for subsequent calculations involving fish weight.

2.2.2. Total body lipids

The remaining carcass was weighed, added to $\times 2$ volume of distilled deionized (nanopure) water, finely minced with scissors, homogenized (3×30 s) using a Tissue Tearor (BioSpec Products Inc., Bartlesville, OK, USA) and a 4 ml aliquot of the resulting homogenate was used for Bligh and Dyer solvent lipid extraction (Bligh and Dyer, 1959). The chloroform–methanol lipid extraction was conducted on all fish using a final ratio of 1.8:2:2 for water to chloroform to methanol as per Bligh and Dyer, with the following modifications: chloroform contained 50 mg/L butylated hydroxytoluene (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada) to reduce oxidation of fatty acids (Weber et al., 2003), samples were centrifuged instead of filtered, and chloroform fractions were dried at room temperature under a gentle stream of nitrogen. Lipid weight was determined in the fish sample (mg lipid per gram of fish carcass) using a gravimetric technique where triplicate aliquots of the chloroform fraction were evaporated in pre-weighed glass vials under a nitrogen atmosphere to prevent lipid oxidation.

2.2.3. Triglyceride assay

Whole body triglycerides were determined in the unextracted fraction of the same fish carcass homogenized in $\times 2$ volume of water using a modified clinical serum assay based on the McGowan et al. (1983) method (Weber et al., 2003). An aliquot of the fish homogenate was added to an equal volume of 0.2 M sodium citrate. The sample was homogenized on ice, placed in a heating block at 100 °C for 5 min and immediately returned to ice. All samples were centrifuged for 5 min at 500g prior to assaying in order to remove insoluble materials such as connective tissue from the liquid fraction. The frozen piece of fish liver was weighed, added to $\times 3$ volume of ice-cold 0.2 M sodium citrate, minced with scissors and homogenized on ice in a glass mortar with a motorized Teflon pestle. The sample was capped, placed in a heating block at 100 °C for 5 min and immediately returned to ice. Triglycerides were then determined in the liver tissue as per the triglyceride assay described earlier.

2.2.4. RNA/DNA assay

Tail muscle was weighed (0.010–0.020 g wet weight) and added to 450 μ l ice-cold TE (Tris–EDTA) buffer (10 mM Tris, 1 mM EDTA, pH 8.0) plus 0.2 M NaCl. Tissue was finely minced with scissors, homogenized with a Tissue Tearor (2×10 s), 5 μ l of 1% sodium dodecyl sulphate was added, and the sample was shaken and incubated at 65 °C for two hours. Samples were centrifuged at 5000g for 10 min at 4 °C, the supernatant was removed and an aliquot was taken for subsequent protein assay. Ice-cold

isopropanol and 3 M sodium acetate were added to the remainder of supernatant and the sample was stored overnight at -20 °C to allow precipitation of nucleic acids. After centrifugation at 15,000g for 30 min, the supernatant was removed, TE buffer was added to the pellet, the sample was incubated at 65 °C for 1 h and stored overnight at 4 °C to ensure complete resuspension of nucleic acids. The following day, RNA and DNA concentrations and resulting RNA/DNA ratio were determined using a modified dual fluorescent dye method (Clemmesen, 1988; Weber et al., 2003). Calf thymus DNA and yeast RNA were used as standards.

2.2.5. Protein assay

An aliquot of the tail muscle homogenate prepared for nucleic acid determination was used to determine muscle protein concentration. A modification of the Lowry et al. (1951) protein assay (BioRad, Hercules, CA, USA) was used with bovine serum albumin as the standard. Duplicate 5 μ l samples were read on a 96-well microplate at 750 nm absorbance.

2.3. Statistical analyses

Two-way analysis of variance (ANOVA) was used to analyze data, with lake and season as factors. Data were transformed (log 10 or square root) if parametric assumptions were not met. When data were missing (i.e. fall 2003 burbot), one-way ANOVA was performed to compare lakes between fall and spring, and *t*-tests were used to compare mean values for a lake in fall versus spring. Mann–Whitney rank sum test was used if data failed parametric assumptions for *t*-tests. Pair-wise multiple comparisons were performed using Tukey's post-test following significant one- or two-way ANOVA. Results were considered significant if $P < 0.05$. Data are reported as mean \pm standard error of the mean (SEM).

Intra-assay variability was calculated for biochemical assays by determining the coefficient of variation (%CV = standard deviation/mean*100) for six replicates of a pooled sample measured in the same assay. Inter-assay variability was determined by calculating the %CV among six replicates of the same pooled sample performed in two separate assays.

3. Results

3.1. Abiotic environment

Water chemistry data collected at study lakes are presented in Table 1. Conductivity, total hardness,

Table 1
Water chemistry variables for lakes at Key Lake uranium operation

Variable	Lake		
	Reference	Low exposure	High exposure
Dissolved oxygen (mg/L)	10.0 \pm 0.2	10.0 \pm 0.1	9.5 \pm 0.2
Temperature (°C)	15.6 \pm 1.0	14.9 \pm 1.0	14.9 \pm 0.8
pH	6.7 \pm 0.1	5.8 \pm 0.6	5.3 \pm 0.1
Conductivity (μ S/cm)	15.2 \pm 2.3	582.0 \pm 73.8	719.3 \pm 126.0
Total hardness (mg/L)	4	221	317
Ammonia (as N) (mg/L)	0.03	0.19	2.1
Nitrate (mg/L)	<0.04	1.4	4.1
Arsenic (μ g/L)	0.1	0.9	3.8
Molybdenum (μ g/L)	<0.1	126	108
Nickel (μ g/L)	<0.1	2.2	6.6
Selenium (μ g/L)	0.1	1.0	3.0
Uranium (μ g/L)	<0.1	<0.1	0.4

Data are means \pm standard error of the mean for combined fall and spring values. Hardness, ammonia, nitrate and trace metal values are from Golder (2005) collected in fall 2004.

ammonia, and nitrate values were higher in the low and high exposure lakes compared to reference (high exposure > low exposure). Lake pH was lower in both exposure lakes compared to reference lake. Previous studies have found that the exposure lakes are elevated in various trace metals including arsenic (As), molybdenum (Mo), nickel (Ni), selenium (Se), and uranium (U) compared to the reference sites (Pyle et al., 2001; Golder, 2005; Muscatello et al., 2006). Values for these metals from Golder (2005) are presented in Table 1.

3.2. Sample size, age, and stomach contents

In the fall collection, a total of 38 northern pike were collected from the three study lakes and 12 burbot were collected from the two exposure lakes, approximately 2 weeks before ice-on. No burbot were collected from the reference lake in the fall due to a sampling error. During the spring sampling period, approximately 2 weeks after ice-off, a total of 39 northern pike and 33 burbot were collected from the reference and exposure lakes. Fish were aged and verified to be YOY (i.e. age-0 in fall 2003 and age-1 in spring 2004), using otoliths for burbot, and cleithra and scales for pike. In the spring sampling period, 18 spottail shiners were collected from the reference lake and 16 shiners were collected from the low exposure lake. Shiners were immediately frozen and transported as described above. The spottail shiners were not aged.

Invertebrates were the only type of food present in burbot stomachs; 69% of all burbot had prey items in their stomachs and of these, 100% were invertebrates. The northern pike fed on both invertebrates and spottail shiners; 71% of all pike stomachs contained food, and of these, 38% had recently consumed shiners and 62% had recently consumed invertebrates. There were no shiners present in the high exposure lake, as evidenced by stomach content analysis and extensive electrofishing effort during fish collection. In the spring, 54% of burbot collected from the reference site had retrievable stomach content, whereas

burbot from the low and high exposure lakes had prey items in 91% and 89% of all individuals. The average wet weight of stomach contents from burbot collected in the spring also varied for fish from the reference (0.07 ± 0.02 g), low (0.34 ± 0.08 g), and high exposure (0.26 ± 0.09 g) lakes.

3.3. Morphometric results

3.3.1. Northern pike

Northern pike were of similar length, weight, and condition factor in reference and exposure lakes in both fall and spring (Table 2). There were no significant differences in length, weight, or condition factor when comparing pike from high or low exposure lakes to the reference lake for each season. There was an overall significant ($P < 0.01$) seasonal difference in length. In the low exposure lake, the pike were significantly longer in the spring compared to the previous fall. There were no significant differences in weight or condition factor of pike within the reference and high exposure lake between fall and spring.

3.3.2. Burbot

Overall, the morphometric endpoints for YOY burbot were different between reference and exposure lakes and from fall to spring within a lake (Table 3). Body weights of burbot were greater in the spring compared to the fall in both low and high exposure lakes ($P < 0.05$ for both lakes). Within the spring, burbot collected from both the low and high exposure lakes had significantly greater body weights than fish from the reference lake ($P < 0.05$ and $P < 0.01$, respectively). Compared to fall values, burbot collected in the spring were significantly longer in the low exposure lake ($P < 0.01$) and high exposure lake ($P < 0.05$). Comparing burbot collected from all lakes in the spring, fish from both the low and high exposure lakes were significantly longer than reference fish ($P < 0.001$ and $P < 0.05$, respectively). In the spring, burbot collected from the high exposure lake had a significantly greater condition factor compared to

Table 2
Morphometric and biochemical measures of growth and condition in young-of-the-year northern pike (*Esox lucius*) collected from one reference lake and two lakes downstream of uranium milling effluent discharge (low and high exposure)

	Reference		Low exposure		High exposure	
	Fall 2003	Spring 2004	Fall 2003	Spring 2004	Fall 2003	Spring 2004
<i>n</i>	12	10	15	15	11	14
Wet weight (g)	17.30 ± 1.58	21.53 ± 1.87	22.69 ± 2.58	31.02 ± 4.15	19.50 ± 2.02	22.69 ± 1.94
Total length (cm)	14.3 ± 0.5	15.4 ± 0.4	15.6 ± 0.6	$17.4 \pm 0.7^\dagger$	14.8 ± 0.5	15.8 ± 0.4
Condition factor	0.58 ± 0.014	0.57 ± 0.015	0.57 ± 0.012	0.53 ± 0.014	0.59 ± 0.0091	0.56 ± 0.013
Total body lipids (%)	0.74 ± 0.042	$1.15 \pm 0.079^{\dagger\dagger}$	$1.57 \pm 0.16^{***}$	$1.80 \pm 0.10^{**}$	$1.14 \pm 0.16^*$	$1.64 \pm 0.12^{*\dagger\dagger}$
Muscle RNA/DNA ratio	2.59 ± 0.53	2.17 ± 0.49	2.52 ± 0.34	2.74 ± 0.46	2.41 ± 0.32	2.07 ± 0.24
Muscle protein (mg/g)	28.83 ± 2.68	32.61 ± 2.90	26.35 ± 1.77	27.30 ± 1.49	26.27 ± 1.57	29.18 ± 1.69

Fish were collected in fall 2003 and spring 2004 at the Key Lake uranium operation, SK, Canada. Results are means \pm standard error of the mean. Condition factor = (body weight/total length³)100.

Significantly different from reference lake in the same season (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Significantly different within a lake comparing fall and spring values ($^\dagger P < 0.05$, $^{\dagger\dagger} P < 0.01$).

Table 3

Morphometric and biochemical measures of growth and condition in young-of-the-year burbot (*Lota lota*) collected from one reference lake and two lakes downstream of uranium milling effluent discharge (low and high exposure)

	Reference		Low exposure		High exposure	
	Fall 2003	Spring 2004	Fall 2003	Spring 2004	Fall 2003	Spring 2004
<i>n</i>	0	13	6	11	6	9
Wet weight (g)	n/a	5.01±0.66	5.80±0.54	9.40±0.98*†	4.65±0.61	10.04±1.81**†
Total length (cm)	n/a	9.2±0.4	9.6±0.3	11.8±0.4***††	8.7±0.5	11.1±0.6*†
Condition factor	n/a	0.60±0.022	0.64±0.042	0.56±0.013†††	0.70±0.062	0.67±0.019*
Total body lipids (%)	n/a	2.16±0.13	1.13±0.057	3.02±0.35††	1.14±0.039	3.89±0.28***††
Muscle RNA/DNA ratio	n/a	8.24±0.48	6.64±0.34	5.94±0.59*	7.61±0.65	9.96±0.54*
Muscle protein (mg/g)	n/a	24.97±1.58	27.29±1.78	20.85±1.13††	25.99±2.50	21.94±0.87

Fish were collected in fall 2003 and spring 2004 at the Key Lake uranium operation, SK, Canada. Results are means±standard error of the mean. Condition factor = (body weight/total length³)100.

Significantly different from reference lake in the same season (* $P<0.05$, ** $P<0.01$, *** $P<0.001$)

Significantly different within a lake comparing fall and spring values († $P<0.05$, †† $P<0.01$, ††† $P<0.001$).

n/a = data not available.

fish collected from the reference lake ($P<0.05$) and low exposure lake ($P<0.001$). There was a seasonal difference in condition factor for burbot collected from the low exposure lake: condition factor was lower in the spring compared to the fall ($P<0.001$; Table 3).

3.3.3. Spottail shiners

There were no differences in wet weight, length, and condition factor for spottail shiners between the reference and low exposure sites (data not shown).

3.4. Biochemical results

3.4.1. Northern pike

Two-way ANOVA indicated significant differences in total body lipids among lakes and seasons ($P<0.001$). Post-tests revealed that in both fall and spring, YOY pike collected from the high and low exposure lakes had significantly greater total body lipids compared to pike collected from the reference lake (Table 2). Pike collected from the high exposure lake had greater total body lipids than reference pike in fall ($P<0.05$) and spring ($P<0.05$). Compared to pike from the reference lake, fish collected from the low exposure lake had greater total body lipids in the fall ($P<0.001$) and spring ($P<0.01$). Among seasons, pike collected from the reference and high exposure lakes had greater total body lipids in the spring compared to the fall ($P<0.01$). When comparing results between the two exposure lakes, total body lipids were significantly lower ($P<0.05$) in the fall in pike collected from the high exposure lake compared to fish from the low exposure lake (Table 2).

There were significant differences in northern pike total body triglycerides among lakes ($P<0.001$) but not seasons (Fig. 2A). Total body triglycerides were significantly greater in pike collected from the low exposure lake compared to fish from the reference lake in both fall ($P<0.01$) and spring ($P<0.001$). Total body triglycerides

were significantly greater in pike collected from the high exposure lake compared to the reference lake in spring only ($P<0.001$). Similar to total body triglycerides, liver triglycerides in northern pike were significantly different among lakes ($P<0.01$) but not seasons (Fig. 3A). Liver triglycerides were significantly greater in pike collected from the low exposure lake in the fall ($P<0.01$) and significantly greater in pike from the high exposure lake in the spring compared to reference pike ($P<0.05$).

Muscle RNA/DNA ratio in northern pike was not significantly different between lakes within a season or within lakes over the two seasons (Table 2). Protein content was not different in tail muscle from northern pike among lakes or seasons (Table 2).

3.4.2. Burbot

In the spring, YOY burbot collected from the high exposure lake had significantly greater total body lipids compared to burbot collected from the reference lake ($P<0.001$; Table 3). In addition, burbot collected from both low and high exposure lakes had significantly greater total body lipids in the spring compared to the fall ($P<0.01$). Total body triglycerides in burbot followed a similar trend as total body lipids. In the spring, burbot from the high exposure lake had significantly greater total body triglycerides compared to reference burbot ($P<0.01$). Burbot had greater total body triglycerides in both the low ($P<0.001$) and high ($P<0.01$) exposure lakes in the spring compared to the fall (Fig. 2B). Although there was a trend for higher liver triglycerides in burbot collected from the exposure lakes compared to the reference lake (Fig. 3B), there was no statistically significant difference in this biochemical endpoint between lakes in the spring ($P=0.062$). However, liver triglycerides were significantly higher in the spring compared to the fall for burbot collected from both low ($P<0.001$) and high ($P<0.01$) exposure lakes.

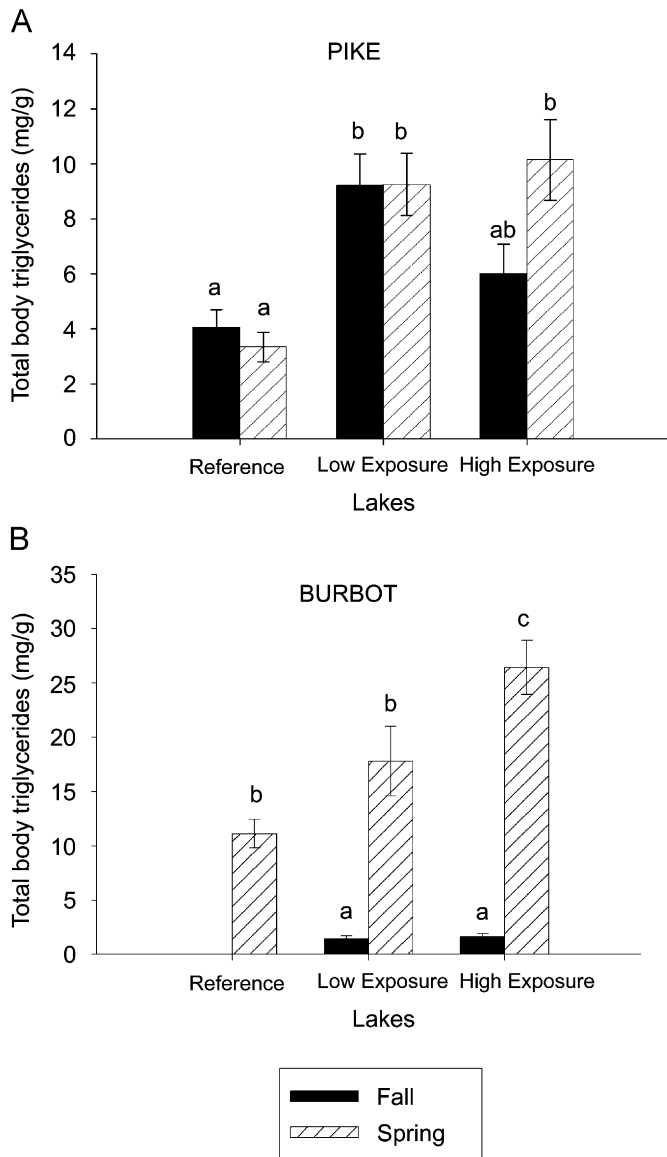


Fig. 2. Total body triglycerides (mg triglyceride per gram fish tissue) in young-of-the-year northern pike (*Esox lucius*) (A) and burbot (*Lota lota*) (B) collected in fall and spring from a reference lake and two lakes receiving uranium milling effluent. Bars without letters in common are significantly different ($P < 0.05$) between lakes within a season and within a lake over two seasons.

Muscle RNA/DNA ratio measured in burbot collected from the low exposure lake was significantly lower ($P < 0.05$) than both reference and high exposure burbot in the spring (Table 3). Burbot from the high exposure lake had a significantly higher ($P < 0.05$) RNA/DNA ratio compared to reference fish (Table 3). Muscle protein measured in burbot from the low exposure lake was lower ($P < 0.01$) in the spring compared to the fall (Table 3).

Except where noted (northern pike total body lipids; burbot condition factor and RNA/DNA ratio), there were no statistically significant differences in any morphological or biochemical endpoint between the low and high exposure lakes in either fall or spring.

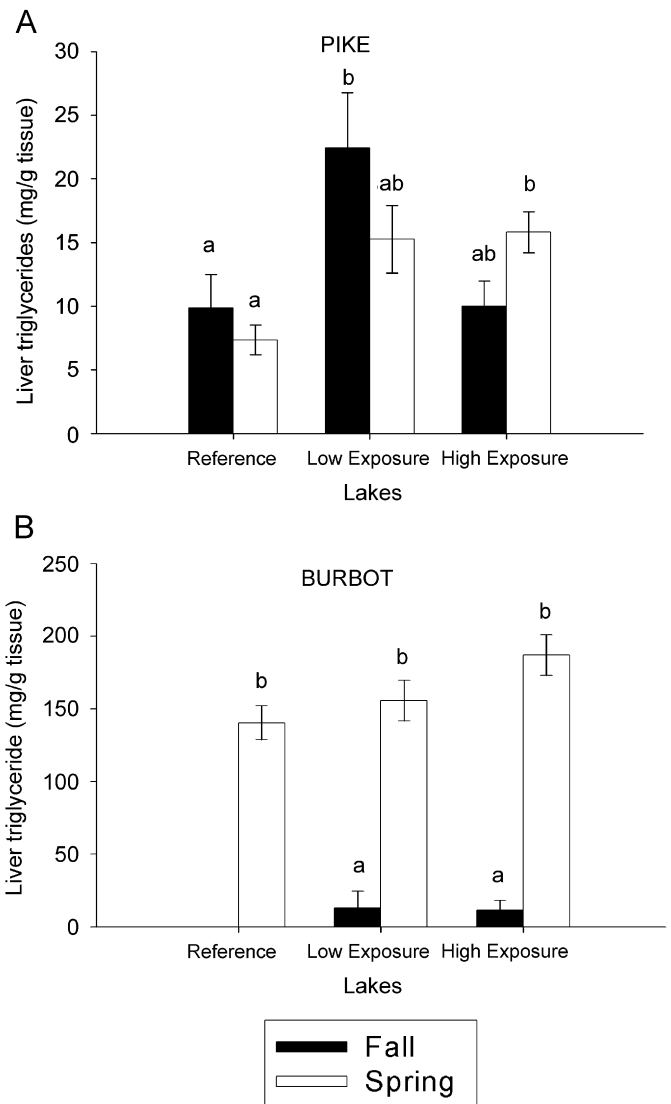


Fig. 3. Liver triglycerides (mg triglyceride per gram liver) in young-of-the-year northern pike (*Esox lucius*) (A) and burbot (*Lota lota*) (B) collected in fall and spring from a reference lake and two lakes receiving uranium milling effluent. Bars without letters in common are significantly different ($P < 0.05$) between lakes within a season and within a lake over two seasons.

3.4.3. Spottail shiners

Total body triglycerides were significantly greater in spottail shiners collected from the low exposure lake compared to reference lake in the spring ($P < 0.001$; Fig. 4).

3.5. Assay performance

Both intra- and inter-assay coefficients of variation were below 10% for all assays. Intra-assay variability was 4.6%, 3.5%, 3.7%, 3.0%, and 4.1% for the RNA/DNA ratio, protein, total body lipids, total body triglycerides, and liver triglyceride assays, respectively. The inter-assay variability was 8.7%, 5.0%, 5.6%, 4.1%, and 4.5% for the RNA/DNA ratio, protein, total body lipids, total body

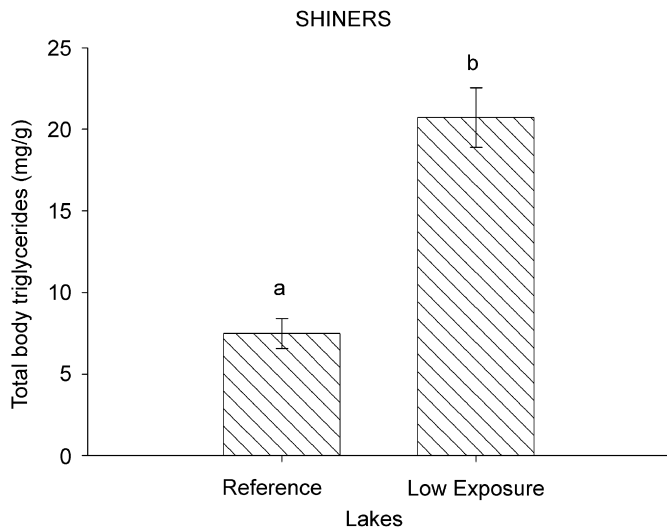


Fig. 4. Total body triglycerides (mg triglyceride/g fish tissue) in spottail shiners (*Notropis hudsonius*) collected in the spring. Bars without letters in common are significantly different ($P < 0.05$).

triglycerides, and liver triglyceride assays, respectively (data not shown).

4. Discussion

4.1. Abiotic environment

YOY northern pike and burbot inhabiting the low and high exposure lakes experienced a much different environment, in terms of water chemistry, compared to fish inhabiting the reference lake. A direct result of uranium milling effluent input is that exposure lakes were characterized by higher conductivity and hardness, and lower pH compared to the reference site. Various trace metals, including As, Mo, Ni, Se, and U are reported to be consistently elevated in the exposure lakes compared to unimpacted lakes near the uranium operation (Pyle et al., 2001; Golder, 2005; Muscatello et al., 2006). Aside from possible metal and ion effects, there also existed a potential nutrient effect, with increased nitrogen (in the form of ammonia and nitrate) resulting from effluent input into the uranium milling effluent impacted lakes.

4.2. Site differences in lipids and triglycerides

We hypothesized that fishes inhabiting exposure lakes would have lower total body lipids and triglycerides compared to fish from the reference lake in both seasons, due to impacts of environmental contaminants on fish energy storage and metabolism (Calow, 1991; Adams, 1999; Congdon et al., 2001; Levesque et al., 2002). However, YOY northern pike and burbot collected from exposure lakes generally exhibited greater total body lipids and triglycerides compared to reference fish in either season. The observed effects were consistent in both fish

species over two seasons. The higher total lipids and triglycerides in exposed pike and burbot suggests that these fish may be in better overall condition than reference fish. Northern pike from exposure sites generally had higher liver triglyceride content compared to reference fish, with no difference between seasons. In contrast, burbot from exposure lakes exhibited clear seasonal differences in liver triglyceride content, with greater liver triglycerides in the spring compared to fall. However, there were no differences in liver triglyceride content in burbot from exposure and reference lakes in the spring. Overall, liver triglyceride values followed similar trends to total body triglycerides, indicating that total body triglyceride levels are closely related to liver triglyceride concentrations for both species. Trace metal exposure has varying effects on lipid dynamics (Adams, 1999). Katti and Sathyanesan (1984) found that cadmium exposure increased liver lipid levels in catfish (*Clarias batrachus*). In contrast, Levesque et al. (2002) reported significantly lower liver triglyceride content in yellow perch (*Perca flavescens*) from a Cd-, Zn-, and Cu-contaminated lake compared to reference fish during two sampling periods (fall and summer).

Many northern freshwater systems are nitrogen and phosphorus limited, and elevated levels of these nutrients may increase phytoplankton biomass in a lake, which may influence the biomass and productivity of higher trophic levels such as fish (Dillon et al., 2004). In the present study, nitrogen (ammonia and nitrate) was higher in the exposure lakes compared to the reference lake. However, elevated phosphorus has not been reported downstream of effluent release, with recent water chemistry data indicating that total phosphorus levels are $< 10 \mu\text{g/L}$ in both the low exposure and reference lakes (K.T. Himbeault, 2006, pers. comm.). Although it is possible that nitrogen alone could increase productivity to a point at which phosphorus would become the primary limiting nutrient, further work is needed to assess effects of nutrient input on the productivity of these systems.

Boreal lakes experiencing nutrient enrichment as a result of fire regimes have higher macroinvertebrate biomass compared to reference lakes (Scrimgeour et al., 2001). Fish energy stores depend on the food supply available during the summer growing season, which is ultimately linked to lake productivity (Eckmann, 2004). If uranium milling effluent is increasing the productivity of exposure lakes, this may explain the higher triglyceride levels in YOY fish collected in the fall and spring sampling periods compared to the reference fish. A concurrent study observed higher total macroinvertebrate density in the high exposure lake, but not the low exposure lake, compared to the reference lake in fall 2003 (Robertson, 2006). Higher macroinvertebrate densities in exposure lakes was supported by burbot stomach content analysis in the spring, where approximately half of the burbot from the reference lake had empty stomachs while the majority of individuals from the low and high exposure lakes had recently ingested invertebrates. In addition, the average mass of YOY

burbot stomach contents was much lower in fish from the reference site compared to fish from both exposure sites. However, macroinvertebrate density varied among study lakes and it is important to note that density varied between the high and low exposure sites. More specifically, the low exposure site had similar macroinvertebrate density to the reference site (high exposure > low exposure = reference; Robertson, 2006). This suggests that the observed site differences in lipids and triglycerides in YOY fish do not entirely result from differences in prey quantity in the study lakes. Trends in fish lipids and triglycerides showed differences between the reference and low exposure sites; however, macroinvertebrate density did not follow a similar pattern.

Differences in prey quality between the low exposure and reference lakes were also observed. Total body triglycerides in spottail shiners followed a trend similar to that for the northern pike, with higher total body triglycerides in shiners collected from the low exposure lake compared to shiners from the reference lake. This suggests that uranium milling effluent may have an indirect, food web-based impact on burbot and northern pike energy reserves: if available prey possess higher energy content (quality), then predatory YOY fishes should gain more lipids and triglycerides than reference fish. However, shiners were not present in the high exposure lake and YOY burbot were not preying on shiners in any lake, as evidenced by stomach content analysis. Thus, changes in fish triglycerides may not be solely related to milling effluent, as increases in these energy storage endpoints were not necessarily exposure-dependent when comparing low and high exposure sites. Other studies have concluded that differences in fish condition have been the result of indirect contaminant effects through an aquatic food web (Iles and Rasmussen, 2005). Improved survival of age-0 fish in nutrient-enriched systems is generally attributed to decreased starvation due to increased food availability and decreased predation due to increased growth (Grant and Tonn, 2002). Overall, a combination of increased prey quantity and quality in the present study may explain the observed higher lipid and triglyceride content in burbot and pike collected from exposure lakes.

4.3. Seasonal differences in lipids and triglycerides

Not only can overwinter mortality of young fish vary with species, population density, prey abundance, and predator density (Pratt and Fox, 2002), evidence here suggests that lipid depletion following a long, northern winter may not always occur and may also vary with these ecological variables. Contrary to logic and various papers on overwintering (e.g. Cunjak, 1988; Lemly, 1996; Berg and Bremset, 1998; Post and Parkinson, 2001; Biro et al., 2004), effluent exposed burbot consistently had higher total body lipids, total body triglycerides and liver triglycerides in the spring compared to the previous fall. The concept that fishes must rely on stored energy to survive winter

months is related to low food availability (Johnson and Evans, 1991; Foy and Paul, 1999) as well as decreased food digestion associated with low temperatures (Toneys and Coble, 1980). However, Hurst and Conover (1998) noted that many details related to winter feeding ecology of the majority of fish species remain unstudied. Indeed, various reports have challenged the idea that fish are unable to feed or assimilate energy from food during winter (Sogard and Olla, 2000; Biro et al., 2004; Parrish et al., 2004). Bauer and Schlott (2004) reported that common carp (*Cyprinus carpio*) were relatively active in the winter, despite previous beliefs that the fish were inactive. McCollum et al. (2003) found that winter food availability regulated the energetic condition of age-0 white crappies (*Pomoxis annularis*) entering the spring. Biro et al. (2004) reported that fed YOY rainbow trout (*Oncorhynchus mykiss*) in a laboratory setting under simulated winter conditions were capable of doubling their lipid content over a 100 day period. Similarly, juvenile Atlantic salmon (*Salmo salar*) provided with shelter from predators in a setting with high food availability experienced increased growth and survival over the winter, as there was an advantage for fish to expend energy to feed for growth and weight maintenance (Parrish et al., 2004). Clearly, lipid depletion following winter does not occur with all fish species and may vary depending on various ecological variables including foraging strategy, predator density, prey abundance, and competition.

Total body triglycerides, a more ecologically relevant measure of energy storage than total body lipids, exhibited a seasonal (fall to spring) increase in YOY burbot, but not in northern pike. Eckmann (2004) found that YOY perch (*Perca fluviatilis*) had decreased lipid levels after the winter, whereas ruffe (*Gymnocephalus cernuus*) were actually higher in lipids following winter. This was attributed to differences in predation: ruffe use sensory ability to forage whereas perch are a visually oriented predator (Eckmann, 2004). Although both species in the present study are carnivorous, pike are pelagic carnivores and burbot tend to forage in the benthic area they inhabit (Scott and Crossman, 1973). This difference in feeding niche was supported by stomach content analysis as only the northern pike had consumed shiners, a pelagic prey item. Burbot locate prey with olfactory and tactile cues by touching the substrate with their pelvic fins and barbels (Hinkens and Cochran, 1988), whereas pike are visual predators (Scott and Crossman, 1973). Since winter feeding conditions are characterized by low light due to ice cover and photoperiod, a species that relies primarily on vision for feeding will experience a reduction in foraging success compared to a sensory and olfactory predator, as seen in the present study and in Eckmann (2004). Hofmann and Fischer (2003) reported that juvenile burbot exhibited a two-fold increase in food conversion efficiency when moving from 20 to 5–6 °C and therefore, the need for food is lower at lower temperatures. If food is available and burbot are foraging well during the dark winter months using their sensory system, any food obtained will thus be converted

efficiently to energy storage or growth during winter and may explain the seasonal differences in lipids and triglycerides observed in burbot in the present study.

Evidence generated in the present study suggests that fishes in these freshwater systems may not be relying completely on stored energy to survive the winter. It appears that the key element of Lemly's (1996) winter stress syndrome hypothesis, that fish respond to low water temperatures and short photoperiod with reduced feeding, may not be occurring with YOY northern pike and burbot inhabiting north temperate aquatic systems. Thus, YOY northern pike and burbot may not be vulnerable to winter stress syndrome, as their feeding ecology and life history characteristics suggest that these fish can feed and consequently maintain or increase energy stores during winter. In addition, Lemly's (1996) idea that winter stress syndrome results as the combination of cold temperatures and a metabolic stressor would increase an individual's susceptibility to overwinter mortality. However, the effects of winter temperatures on metabolism must be considered. Since fish are poikilothermic, their metabolic rate and many of their physiological functions are fundamentally influenced by temperature (Fry, 1971). Perhaps during winter, when temperatures are low, any impacts of a metabolic stressor in exposure sites would be less than expected in the summer. For instance, elevated ammonia and low pH were not found to impact liver and gill protein turnover in rainbow trout during winter (Morgan et al., 1998), although similar studies at warmer temperatures found alteration in tissue protein synthesis (Wilson et al., 1996; Linton et al., 1997). These differences in effects of sublethal exposures dependent on temperature may thus be related to low metabolic rates associated with low temperatures (Morgan et al., 1998). Thus, the results of the present field study do not provide support for Lemly's (1996) winter stress syndrome hypothesis.

4.4. Size-dependent overwinter mortality

Although it is possible that burbot were gaining lipids and triglycerides during winter, the possibility of size-dependent overwinter mortality must be considered. Since smaller fish have a higher mass-specific metabolic rate and lower energy density than larger fish, size-dependent overwinter mortality may occur (Peters, 1983; Post and Parkinson, 2001; McCollum et al., 2003). Numerous laboratory and field studies with various fish species have established that smaller YOY fish indeed suffer higher mortality over the winter than larger individuals (Toneys and Coble, 1980; Johnson and Evans, 1991; Post and Evans, 1989; Griffiths and Kirkwood, 1995; Foy and Paul, 1999; Gotceitas et al., 1999; Kristiansen et al., 2000; Sogard and Olla, 2000; Grant and Tonn, 2002; Biro et al., 2004). In addition, size-dependent predation can regulate winter mortality, as smaller fish are more vulnerable to gape-limited predators (Werner and Gilliam, 1984; Kristiansen et al., 2000).

In the present study, mortality of smaller burbot with reduced energy stores during winter could have potentially contributed to the observed seasonal increase in total body lipids and triglycerides. In addition, burbot collected in spring from exposure lakes had greater lengths compared to the previous fall, further suggesting that size-dependent overwinter mortality may have occurred. Several studies have reported that size-dependent overwinter mortality was responsible for an increase in average length or mass of individuals in spring compared to the previous fall (Post and Evans, 1989; Griffiths and Kirkwood, 1995; Grant and Tonn, 2002). However, size-dependent mortality is not a universal phenomenon for all fish species. For instance, size-dependent overwinter mortality may not occur in natural walleye (*Sander vitreus vitreus*) populations (Pratt and Fox, 2002). Immature Atlantic salmon parr exhibited growth in length during winter, which was not a result of size-dependent mortality since overall cohort survival was estimated to be 98% (Parrish et al., 2004). If burbot in this study were not succumbing to size-dependent overwinter mortality, then their seasonal increase in total length may be a result of winter growth. Unfortunately, neither temperature growth thresholds nor minimum lipid requirements for overwinter survival in YOY northern pike and burbot are known. However, some evidence suggests that fish are capable of growth during winter. Age-0 cod (*Gadus morhua*) achieved some positive growth under winter temperatures (Gotceitas et al., 1999), juvenile burbot were reported to have a growth rate of approximately 0.005 cm/day under fed conditions at 7.8 °C (Hofmann and Fischer, 2003) and perch were shown to grow at temperatures < 10 °C (Karås, 1990).

As an indication of size- or energy-dependent overwinter mortality, we compared the variability in our data in the fall and spring fish collections. Calculation of the coefficient of variation (standard deviation/mean) for weight, length, and total body lipids indicated there was higher variability in all of these endpoints in spring compared to fall for both northern pike and burbot. If only individuals in the best condition preferentially survived the overwinter period, we would expect much lower variability in spring, since phenotypic variability in the population would have been reduced. Therefore, it is unclear if size-selective overwinter mortality or overwinter growth was responsible for increased length in YOY burbot in the exposure lakes in spring compared to the previous fall.

4.5. Site and season differences in biochemical measures of growth

In fishes, growth is related to various environmental factors including food availability, toxicant exposure, and temperature. Muscle RNA to DNA ratio provides an estimate of short-term (days to weeks) growth (Clemmesen, 1988). While DNA per cell remains relatively constant, the amount of RNA is proportional to the amount of cellular

protein synthesis, providing an estimate of growth. Total protein content provides a measure of longer-term growth (weeks to months) and to a lesser extent, energy storage. Protein synthesis in an active tissue such as caudal muscle reflects recent growth and therefore nutritional status of the individual fish. We hypothesized that YOY fish would grow faster in the reference lake compared to exposure lakes, since increased metabolic activity associated with inhabiting a contaminated environment may channel a greater proportion of available energy into metabolism and away from growth. However, we found that the YOY pike and burbot did not exhibit any exposure-dependent site differences in muscle RNA/DNA ratio or muscle protein in either season. It is interesting to note that although YOY pike in the exposure sites had higher lipids and triglycerides, an increase in growth measured with muscle RNA/DNA ratio or muscle protein was not observed in this species. Further investigation is required to determine if this combination of factors could indicate metabolic disruption, where although energy was available, it did not appear to be allocated to growth. Overall, fish biochemical estimates of growth in this study did not appear to be sensitive indicators of uranium milling effluent exposure.

4.6. Other energetic considerations

Possible food web changes are only one of numerous environmental and biological variables that can influence energy allocation and growth in wild fish. A discussion of bioenergetics must consider energy expenditures related to predation. Through personal observation during fish collection, we observed fewer large, adult northern pike in the relatively small high exposure lake compared to the larger low exposure and reference lakes. Fish with low predation risk can employ an active feeding regime and forage optimally (Post and Parkinson, 2001), which may further explain the higher lipids found in fish from the high exposure lake. Field-based experiments have shown that the presence of a predator will reduce growth and survival of YOY fish (Landry et al., 1999; Biro et al., 2003). Larger fish are more likely to survive than smaller fish due to size-dependent predation (Kristiansen et al., 2000). As mentioned previously, this is due to the fact that small fish are more vulnerable to gape-limited predators (Werner and Gilliam, 1984) and larger fish have relatively more energy and therefore, would be in better condition to actively escape predators (McCollum et al., 2003).

4.7. Morphometric versus biochemical endpoints

Previous studies have reported that fish exposed to metals have decreased condition factor relative to unexposed fish (Laflamme et al., 2000; Sherwood et al., 2000; Levesque et al., 2002; Rajotte and Couture, 2002). However, lower condition factor in fishes inhabiting uranium milling effluent impacted lakes was not observed

in the present study. In fact, burbot from the high exposure lake had higher condition factor compared to reference fish in the spring. Overall, the northern pike were similar in morphometric indices among lakes, while the burbot had greater lengths and weights in the exposure lakes compared to burbot collected from the reference lake. Although there were no site differences observed in spring burbot using biochemical measures of growth (muscle RNA/DNA ratio or muscle protein), there were site differences in total length, suggesting that body length is a more reliable indicator of growth than the biochemical endpoints of growth used in this study.

4.8. Reference data

Although the results of this study revealed certain growth and energy storage endpoints were different for fish inhabiting reference versus exposure sites, the use of only one reference site may cause some concern about the validity of the conclusions. Only one reference lake was sampled in the current study due to time and budget constraints. The possibility that the results may have been different if compared to additional (i.e. replicate) reference sites must be acknowledged. However, in a similar study, pike and burbot were sampled in fall and spring at reference lakes in the boreal forest approximately 100 km from Key Lake operation (Bennett, 2006). Results for biochemical and morphometric endpoints in YOY pike and burbot were similar among reference sites at the different locations (Bennett, 2006), supporting the assumption that the reference site sampled in the present study was indeed indicative of the baseline condition for pike and burbot from this region.

5. Conclusions

To evaluate changes in fish physiology due to environmental stress, employment of a suite of biochemical measures is recommended. Results of this study highlight the value of using certain biochemical measures of condition. For instance, in northern pike there were no differences in the standard morphometric measures of condition such as weight, length or condition factor among study lakes. However, we found significant differences in lipids and triglycerides in pike among lakes and seasons. Thus, exposure to uranium milling effluent had an influence on YOY fish that may not be detected with traditional morphological measurements in all species. Since lipids, including triglycerides, are important for estimating fish condition they continue to be valuable assessment tools in fisheries research. However, we found that muscle RNA/DNA ratio and muscle protein concentration did not detect relevant site or season differences for either species. Perhaps growth measured morphometrically with total length may be more relevant than variations in relatively short term growth rates measured with

RNA/DNA ratio or protein, particularly in fish that have survived beyond the fry stage (Weber et al., 2003).

Increased lipids and triglycerides in both species, as well as weight and length in burbot, following exposure to uranium milling effluent may be an indirect effect via food web enrichment, characterized by higher prey quantity and quality in combination with overwinter mortality of individuals with lower energy stores. In a study in metal-contaminated lakes, Iles and Rasmussen (2005) suggested that indirect, food web-mediated effects may have a relatively higher impact on yellow perch than direct, physiological effects. In addition, the energetic costs associated with predation risk may also have contributed to the observed site differences in energy storage. However, direct effects of metal exposure on fish physiology can have energetic costs (Campbell et al., 2003) and thus cannot be ruled out. Nevertheless, results of the present study in northern populations of pike and burbot do not support the winter stress syndrome hypothesis of Lemly (1996) and illustrate the complexities involved in extrapolating laboratory-based results to field investigations in native fish species. Our continuing research in this area is focusing on the relative importance of direct versus indirect causes for the observed differences in bioenergetics of juvenile fishes exposed to metal mining discharges.

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References

- Adams, S.M., 1999. Ecological role of lipids in the health and success of fish populations. In: Arts, M.T., Wainman, B.C. (Eds.), *Lipids in Freshwater Ecosystems*. Springer, New York, pp. 132–160.
- Bauer, C., Schlott, G., 2004. Overwintering of farmed common carp (*Cyprinus carpio* L.) in the ponds of a central European aquaculture facility—measurement of activity by radio telemetry. *Aquaculture* 241, 301–317.
- Bennett, P.M., 2006. Effects of uranium mining and milling effluents on juvenile fish bioenergetics, growth and overwinter survival. M.Sc. Thesis, University of Saskatchewan, Saskatoon, Canada.
- Berg, O.K., Bremset, G., 1998. Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *J. Fish Biol.* 52, 1272–1288.
- Biro, P.A., Post, J.R., Parkinson, E.A., 2003. Population consequences of a predator-induced habitat shift by trout in whole-lake experiments. *Ecology* 84, 691–700.
- Biro, P.A., Morton, A.E., Post, J.R., Parkinson, E.A., 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 61, 1513–1519.
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917.
- Calow, P., 1991. Physiological costs of combating chemical toxicants: ecological implications. *Comp. Biochem. Physiol. C* 100, 3–6.
- Campbell, P.G.C., Hontela, A., Rasmussen, J.B., Giguère, A., Gravel, A., Kraemer, L., Kovescs, J., Lacroix, A., Levesque, H., Sherwood, G., 2003. Differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal-impacted lakes. *Human Ecol. Risk Asses.* 9, 847–866.
- Clemmesen, C.M., 1988. A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. *Meeresforsch* 32, 134–143.
- Congdon, J.D., Dunham, A.E., Hopkins, W.A., Rowe, C.L., Hinton, T.G., 2001. Resource allocation-based life histories: a conceptual basis for studies of ecological toxicology. *Environ. Toxicol. Chem.* 20, 1698–1703.
- Couture, P., Rajotte, J.W., 2003. Morphometric and metabolic indicators of metal stress in wild yellow perch (*Perca flavescens*) from Sudbury, Ontario: a review. *J. Environ. Monit.* 5, 216–221.
- Cunjak, R.A., 1988. Physiological consequences of overwintering in streams: the cost of acclimatization. *Can. J. Fish. Aquat. Sci.* 45, 443–452.
- Dillon, P.J., Clark, B.J., Evans, H.E., 2004. The effects of phosphorus and nitrogen on lake trout (*Salvelinus namaycush*) production and habitat. In: Gunn, J.M., Steedman, R.J., Ryder, R.A. (Eds.), *Boreal Shield Watersheds: Lake Trout Ecosystems in a Changing Environment*. Lewis Publishers, Boca Raton, pp. 119–131.
- Eckmann, R., 2004. Overwinter changes in mass and lipid content of *Perca fluviatilis* and *Gymnocephalus cernuus*. *J. Fish Biol.* 65, 1498–1511.
- Foy, R.J., Paul, A.J., 1999. Winter feeding and changes in somatic energy content of age-0 Pacific herring in Prince William Sound, Alaska. *Trans. Am. Fish. Soc.* 128, 1193–1200.
- Fry, J.E.J., 1971. The effects of environmental factors on the physiology of fish. In: Hoar, W.S., Randall, D.J. (Eds.), *Fish Physiology*, Vol. 6. Academic Press, New York, pp. 1–98.
- Golder, 2005. Key Lake Operation Comprehensive Environmental Effects Monitoring Program Interpretive Report. Prepared for Cameco Corporation, Saskatoon, SK by Golder Associates Ltd., Saskatoon, SK, Canada.
- Gotceitas, V., Methven, D.A., Fraser, S., Brown, J.A., 1999. Effects of body size and food ration on over-winter survival and growth of age-0 Atlantic cod, *Gadus morhua*. *Environ. Biol. Fish.* 54, 413–420.
- Grant, S.C.H., Tonn, W.M., 2002. Effects of nutrient enrichment on recruitment of age-0 fathead minnows (*Pimephales promelas*): potential impacts of environmental change on the Boreal Plains. *Can. J. Fish. Aquat. Sci.* 59, 759–767.
- Griffiths, D., Kirkwood, R.C., 1995. Seasonal variation in growth, mortality and fat stores of roach and perch in Lough Neagh, Northern Ireland. *J. Fish Biol.* 47, 537–554.
- Heintz, R.A., Nelson, B.D., Hudson, J., Larsen, M., Holland, L., 2004. Marine subsidies in freshwater: effects of salmon carcasses on lipid class and fatty acid composition of juvenile coho salmon. *Trans. Am. Fish. Soc.* 133, 559–567.
- Hinkens, E., Cochran, P.A., 1988. Taste buds on pelvic fin rays of the burbot, *Lota lota* (L.). *J. Fish Biol.* 32, 975–976.
- Hofmann, N., Fischer, P., 2003. Impacts of temperature on food intake and growth in juvenile burbot. *J. Fish Biol.* 63, 1295–1305.
- Hurst, T.P., Conover, D.O., 1998. Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. *Can. J. Fish. Aquat. Sci.* 55, 1122–1130.
- Iles, A.C., Rasmussen, J.B., 2005. Indirect effects of metal contamination on energetics of yellow perch (*Perca flavescens*) resulting from food web simplification. *Freshwater Biol.* 50, 976–992.
- Jobling, M., Johansen, S.J.S., Foshaug, H., Burkow, I.C., Jørgensen, E.H., 1998. Lipid dynamics in anadromous Arctic charr, *Salvelinus alpinus* (L.): seasonal variations in lipid storage depots and lipid class composition. *Fish Physiol. Biochem.* 18, 225–240.

- Johnson, T.B., Evans, D.O., 1991. Behaviour, energetics, and associated mortality of young-of-the-year white perch (*Morone americana*) and yellow perch (*Perca flavescens*) under simulated winter conditions. *Can. J. Fish. Aquat. Sci.* 48, 672–680.
- Karås, P., 1990. Seasonal changes in growth and standard metabolic rate of juvenile perch, *Perca fluviatilis* L. *J. Fish Biol.* 37, 913–920.
- Katti, S.R., Sathyanesan, A.G., 1984. Changes in tissue lipid and cholesterol content in the catfish *Clarias batrachus* (L.) exposed to cadmium chloride. *Bull. Environ. Contam. Toxicol.* 32, 486–490.
- Kristiansen, T.S., Otterå, H., Svåsand, T., 2000. Size-dependent mortality of juvenile reared Atlantic cod released in a small fjord. *J. Fish Biol.* 56, 792–801.
- Laflamme, J.-S., Couillard, Y., Campbell, P.G.C., Hontela, A., 2000. Interrenal metallothionein and cortisol secretion in relation to Cd, Cu, and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. *Can. J. Fish. Aquat. Sci.* 57, 1692–1700.
- Landry, F., Post, J.R., Parkinson, E.A., 1999. Spatial ontogeny of lentic age-0 rainbow trout, *Oncorhynchus mykiss*: whole-lake manipulations of population size structure. *Can. J. Fish. Aquat. Sci.* 56, 1916–1928.
- Lemly, A.D., 1993. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat. Toxicol.* 27, 133–158.
- Lemly, A.D., 1996. Winter stress syndrome: an important consideration for hazard assessment of aquatic pollutants. *Ecotoxicol. Environ. Saf.* 34, 223–227.
- Levesque, H.M., Moon, T.W., Campbell, P.G.C., Hontela, A., 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquat. Toxicol.* 60, 257–267.
- Linton, T.K., Reid, S.D., Wood, C.M., 1997. The metabolic costs and physiological consequences to juvenile rainbow trout of a simulated summer warming scenario in the presence and absence of sublethal ammonia. *Trans. Am. Fish. Soc.* 126, 259–272.
- Lochmann, S.E., Maillet, G.L., Frank, K.T., Taggart, C.T., 1995. Lipid class composition as a measure of nutritional condition in individual larval Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 52, 1294–1306.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- MacFarlane, R.B., Norton, E.C., 2002. Physiological ecology of juvenile chinook salmon (*Oncorhynchus tshawytscha*) at the southern end of their distribution, the San Francisco Estuary and the Gulf of the Farallones, California. *Fish. Bull.* 100, 244–257.
- McCullum, A.B., Bunnell, D.B., Stein, R.A., 2003. Cold, northern winters: the importance of temperature to overwinter mortality of age-0 white crappies. *Trans. Am. Fish. Soc.* 132, 977–987.
- McGowan, M.W., Artiss, J.D., Strandbergh, D.R., Zak, B., 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* 29, 538–542.
- Morgan, I.J., D'Cruz, L.M., Dockray, J.J., Linton, T.K., McDonald, D.G., Wood, C.M., 1998. The effects of elevated winter temperature and sub-lethal pollutants (low pH, elevated ammonia) on protein turnover in the gill and liver of rainbow trout (*Oncorhynchus mykiss*). *Fish. Physiol. Biochem.* 19, 377–389.
- Muscattello, J.R., Bennett, P.M., Himbeault, K.T., Belknap, A.M., Janz, D.M., 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. *Environ. Sci. Technol.* 40, 6506–6512.
- Parrish, D.L., Hawes, E.J., Whalen, K.G., 2004. Winter growth and survival of juvenile Atlantic salmon (*Salmo salar*) in experimental raceways. *Can. J. Fish. Aquat. Sci.* 61, 2350–2357.
- Peters, R.H., 1983. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.
- Post, J.R., Evans, D.O., 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosures, and field experiments. *Can. J. Fish. Aquat. Sci.* 46, 1958–1968.
- Post, J.R., Parkinson, E.A., 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology* 82, 1040–1051.
- Pratt, T.C., Fox, M.G., 2002. Influence of predation risk on the overwinter mortality and energetic relationships of young-of-year walleyes. *Trans. Am. Fish. Soc.* 131, 885–898.
- Pyle, G.G., Swanson, S.M., Lemkuhl, D.M., 2001. Toxicity of uranium mine-receiving waters to caged fathead minnows, *Pimephales promelas*. *Ecotoxicol. Environ. Saf.* 48, 202–214.
- Rajotte, J.W., Couture, P., 2002. Effects of environmental metal contamination on the condition, swimming performance, and tissue metabolic capacities of wild yellow perch (*Perca flavescens*). *Can. J. Fish. Aquat. Sci.* 59, 1296–1304.
- Robertson, E.L., 2006. Investigating the cause(s) of benthic macroinvertebrate community impairment downstream of two Saskatchewan uranium operations. M.Sc. Thesis, University of Saskatchewan, Saskatoon, Canada.
- Scott W.B., Crossman, E.J., 1973. *Freshwater fishes of Canada*. *Bull. Fish. Res. Board Can.* 184.
- Scrimgeour, G.J., Tonn, W.M., Paszkowski, C.A., Goater, C., 2001. Benthic macroinvertebrate biomass and wildfires: evidence for enrichment of boreal subarctic lakes. *Freshwater Biol.* 46, 367–378.
- Sherwood, G.D., Rasmussen, J.B., Rowan, D.J., Brodeur, J., Hontela, A., 2000. Bioenergetic costs of heavy metal exposure in yellow perch (*Perca flavescens*): in situ estimates with a radiotracer (¹³⁷Cs) technique. *Can. J. Fish. Aquat. Sci.* 57, 441–450.
- Sogard, S.M., Olla, B.L., 2000. Endurance of simulated winter conditions by age-0 walleye pollock: effects of body size, water temperature and energy stores. *J. Fish Biol.* 56, 1–21.
- Toneys, M.L., Coble, D.W., 1980. Mortality, hematocrit, osmolality, electrolyte regulation, and fat depletion of young-of-the-year freshwater fishes under simulated winter conditions. *Can. J. Fish. Aquat. Sci.* 37, 225–232.
- Weber, L.P., Higgins, P.S., Carlson, R.I., Janz, D.M., 2003. Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. *J. Fish Biol.* 63, 637–658.
- Werner, E.E., Gilliam, J.F., 1984. The ontogenetic niche and species interactions in size-structured populations. *Ann. Rev. Ecol. Syst.* 15, 393–425.
- Wilson, R.W., Wood, C.M., Houlihan, D.F., 1996. Growth and protein turnover during acclimation to acid and aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* 53, 802–811.