HYDROELECTRIC POWER, MERCURY, ENERGETICS, AND STRESS: BIOLOGICAL AND TOXICOLOGICAL IMPLICATIONS FOR FISH AND FISHERIES

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ABSTRACT

Hydroelectric power is a critical component of the global energy budget and its capacity is projected to increase by 73% over the next 20 years, with much of this new capacity installed in the developing world. It is therefore important that we understand the impacts of these developments and how they might affect human and ecological health. Mercury accumulation in fish in reservoirs after dam construction is a well-researched and established phenomenon. While increased mercury concentrations have also been observed in fish downstream of dams, less is known about the dynamics of mercury in downstream sites, meaning there is unassessed historical risk to downstream wildlife, fisheries, and consumers. Further, hydropeaking that leads to fish strandings may represent a contemporary environmental stressor to downstream fishes. This could exacerbate mercury exposure in downstream fish by reallocating their energy from growth to addressing the stressor, resulting in higher mercury concentrations due to decreased growth dilution. This thesis explores the potential relationships between mercury and energy stores in fish up and downstream of a hydroelectric dam to determine historical relationships of fish mercury between these locations, and whether ongoing dam operations may exacerbate mercury concentrations in downstream fish. It also introduces a novel stress challenge protocol using a common minnow species in order to assess potential chronic environmental stress.

Using historical records of commercial fishes from a reservoir and downstream fishery, I found rates of mercury decline were similar in fish populations within both sites since the 1970s. Yet where differences were noted, mercury consistently took longer to decline from downstream populations; mercury concentrations were also greater in fish immediately downstream of the dam relative to those from farther downstream despite minimal mercury concentrations (1-5 ng/L) in the water column.

Higher mercury concentrations were also found downstream of the dam in a common minnow species (spottail shiner; “shiner”: *Notropis hudsonius*), and the same populations showed reduced energy stores relative to upstream fish in both August and September of 2014. Despite this connection, I noted minimal effects on fish condition, and there were no direct predictive relationships between fish mercury concentrations, energy stores, and condition.

A newly developed acute stress challenge protocol also provided mixed evidence for the effects of hydropeaking on shiner stress responses. Glycogen concentrations were slightly higher

in downstream fish from a site of concern in October compared with September, though minimal differences were found across time points, months or sites. Where patterns were observed, concentrations were highest within the first five minutes of capture and ultimately reached basal levels after 15 minutes. Minimal differences were noted in triglyceride concentrations across site, month, or time point. Cortisol secretion was successfully induced by the stress challenge as measured by whole-body cortisol concentrations, and the two upstream sites showed nearly identical patterns of cortisol concentration increase over time across months, with concentrations peaking ≈45-minutes post-challenge at both sites in both months. Minimal differences were noted across time points and sites within months, though cortisol concentrations in fish at a downstream site of concern were slightly elevated compared to upstream sites in September, and dropped significantly from September to October of 2015.

While it is not possible to draw definitive conclusions about hydropeaking as a stressor based solely on these data, these results suggest that mercury concentrations may take longer to decline in fish populations downstream of dams, and that ongoing dam activities may be imparting direct or indirect effects on downstream fish that exacerbate long-term mercury concentrations. Finally, these results suggest that cortisol concentrations in shiner in response to an acute stressor may be successfully developed as a biomarker of chronic environmental stress.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

[Hg]	Mercury concentration
‰	Per mille
^{o}C	Degrees celsius
^{137}Cs	Cesium-137
^{210}Pb	Lead-210
CL	Cumberland Lake
CL-MR	Cumberland Lake-Mossy River Junction
CL-SR	Cumberland Lake-Saskatchewan River Junction
CRM	Certified reference material
DMA 80	Direct Mercury Analyzer 80
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DS1	Downstream site 1
DS2	Downstream site 2
EBC	Immediately downstream of the E. B. Campbell Dam
EIA	Enzyme immunoassay
g	Gram
Hg	Mercury
HPI axis	Hypothalamic-pituitary-interrenal axis
HSC axis	Hypothalamic-pituitary-chromaffin cell axis
IAEA	International Atomic Energy Agency
ICPMS	Inductively coupled plasma mass spectrometry
IUCN	International Union for Conservation of Nature
m^3/s	Metres cubed per second
MeHg	Methylmercury
mg/g	Milligrams per gram
mg/L	Milligrams per litre
mm	Millimetres
ng	nanograms
ng/g	nanograms per gram
SRD	Saskatchewan River Delta
TL	Tobin Lake
TMF	Trophic magnification factor
TMS	Trophic magnification slope
TSS	Total suspended solids
UNDP	United Nations Development Programme
US EIA	United States Energy Information Administration
US EPA	United States Environmental Protection Agency
w.w.	wet weight

YOY	young-of-the-year
$\delta^{15}N$	ratio of heavy nitrogen (^{15}N) to light nitrogen (^{14}N)
µg/g	micrograms per gram
µg/L	micrograms per litre

NOTE TO READERS

This thesis was prepared in a manuscript style, and will therefore have some redundancies across sections of research chapters. To reduce these redundancies, specific descriptions of methods and statistics are found in their respective chapters. Chapter 1 is a general introduction, and chapters 2-4 are written in the style of publishable manuscripts. Chapter 5 serves as a summary and conclusion to the overall thesis. Chapter 2 of this thesis was published in Archives of Environmental Contamination and Toxicology in June of 2016. The third chapter is being prepared for submission to the Canadian Journal of Fisheries and Aquatic Sciences, and the fourth is being prepared for the Journal of Comparative Physiology A. To avoid redundancies in citation lists, all citations have been provided in a combined section at the end of this thesis.

CHAPTER 1:
INTRODUCTION

1.1 Hydroelectric power, mercury, and human health

The latter half of the twentieth century saw increased public awareness of detrimental anthropogenic influences on the environment. Well-established ties between fossil fuels and adverse effects on environmental and human health have encouraged transitions to presumably more benign alternatives such as wind, solar, and hydroelectric power (United Nations Development Program 2000; Treyer et al. 2014).

As of 2012, hydroelectric facilities were responsible for approximately 6.8% of global power generation and expanding at a rate of 3-9% per year, with 73% expansion predicted over the next 20 years, largely in the developing world (Turkenburg et al. 2012; United States Energy Information Administration [US EIA] 2014; Zarfl et al. 2015). While hydroelectric power is an important part of green energy budgets and provides renewable energy in place of using unsustainable fossil fuels, it is increasingly implicated in damages to human and environmental health. The potential detriments include, but are not limited to, the production of greenhouse gases, significant alteration of downstream habitats and communities, and the bioaccumulation of potently neurotoxic methylmercury (MeHg) in local foodwebs (Rosenberg et al. 1995; McKinney et al. 2001; Graf 2006; Hertwich 2013; Schetagne and Therrian 2013).

Abundant literature relates reservoir construction to the biomethylation and subsequent bioaccumulation of mercury (Hg) within fish affected in artificial reservoirs and downstream (Schetagne et al. 2000; Bodaly et al. 2007; Yu et al. 2011). Biomethylation of Hg to MeHg may then expose all subsequent consumers to toxic concentrations of a potent neurotoxin, teratogen, and probable cardiovascular toxin (Health Canada 2007; Mergler et al. 2007; Karagas et al. 2012). The potential for human exposure is exacerbated by the tendency of MeHg to bioaccumulate within foodwebs and peak in popular high trophic level sportfish (Hammerschmidt and Fitzgerald 2006; Depew et al. 2013)

Though Hg can contaminate fish after reservoir construction, dams do not add this Hg to the system. Rather, Hg accumulates in reservoirs from the biomethylation of a background level of Hg (II) that contaminates the environment and comes from a mixture of natural and anthropogenic sources (Gustin et al. 2000). Human activities have significantly increased environmental Hg contamination through atmospheric fossil fuel combustion, artisanal gold mining and other industrial activities (Goodarzi et al. 2008; Pirrone et al. 2010). Mercury in the atmosphere subsequently propagates the globe via wind and water cycles (Keating et al. 1997). As a consequence, Hg has become a ubiquitous contaminant both near and far from industrial sources (Mierle 1990; Lucotte et al. 1995; Engstrom and Swain 1997).

As an environmental contaminant, aquatic Hg is predominantly found in the oxidized Hg(II) state (Morel et al. 1998), but changing a system from a river to a reservoir environment can facilitate its microbe-mediated conversion to toxic and bioaccumulative MeHg. The percent surface area flooded to make a reservoir has been shown to predict the magnitude of mercury accumulation in fish (Bodaly et al. 2007). This relationship exists because the rising waters cause flooding of riparian vegetation, which causes an inundation of dissolved organic carbon (DOC), and greater propensity for anoxic waters and low pH (Thornton et al. 1990; Porvari and Verta 1995; Paterson et al. 1998; Kelly et al. 2003). When these conditions combine with warmer seasonal temperatures the metabolism of iron- and sulfate-reducing bacteria is stimulated and they produce MeHg from the Hg(II) available in the environment (Wright and Hamilton 1982; St. Louis et al. 1994; Yu et al. 2011; Graham et al. 2012).

The contribution of these high carbon, low oxygen conditions to the methylation of Hg is corroborated by the amelioration of MeHg bioaccumulation as they decline (Hrabik and Watras 2002). Once produced, MeHg takes upwards of 10 years to reach peak concentrations in predatory fish and takes 2-3 decades thereafter to return to background concentrations (Bodaly et al. 2007). The factors commonly predictive of MeHg accumulated include the size of the catchment flooded (Bodaly et al. 2007), the extent of Hg inundation to the system (Harris et al. 2007), the length of the food web (Lavoie et al. 2013), and the age and body size of affected fish (Simoneau et al. 2005; Jardine et al. 2012).

While these trends have been well-established for [Hg] in fish from constructed reservoirs, much less is known about the potential for contamination and amelioration downstream (Rosenberg et al. 1997; Schetagne et al. 2000; Kasper et al. 2012). While elevated [Hg] have been

found downstream of artificial reservoirs, the factors controlling this accumulation have only recently begun to be defined (Anderson 2011). Taken together, the potential for contamination of Hg within biota in reservoirs and downstream warrants further investigation, to characterize the potential for wildlife and human consumers to face toxic exposure both on-site and downstream. These assessments are particularly important at higher latitudes where longer food chains of northern ecosystems increase the potential for Hg biomagnification (Lavoie et al. 2013).

The dynamics of these effects and their potential interactions with environmental stressors can be observed in the Saskatchewan River in a reservoir (Tobin Lake) and a downstream fishery (Cumberland Lake) near the community of Cumberland House, Saskatchewan.

1.2 Hydroelectric power and the Saskatchewan River Delta

The western Canadian provinces of Alberta, Saskatchewan, and Manitoba are tied through their centres by the waters of the Saskatchewan River. The headwaters begin in the Rocky Mountains of western Alberta and terminate within the Saskatchewan River Delta (SRD), which spans the border between Saskatchewan and Manitoba. As North America's largest inland delta, the SRD faces increasing stress from climate change-induced reductions in source waters (Schindler and Donahue 2006; Partners for the Saskatchewan River Basin 2009; Maran et al. 2014; Sagin et al. 2015), as well as hydroelectric development. The most immediate upstream structure is the E. B. Campbell hydroelectric dam.

Tobin Lake was formed as an artificial reservoir for the E. B. Campbell dam upon its commissioning in 1963. Shortly thereafter, concerns over Hg contamination in fish were raised for both Tobin Lake and 97 km downstream in the fishery of the SRD's largest lake, Cumberland Lake (Waldram 1988). Anecdotal reports also indicate potential degradation of fish populations downstream of the dam, and a commercial sturgeon fishery was closed due to declining catches in 1996. Literature exists to support both Hg (Schetagne et al. 2000; Bodaly et al. 2007) and population level concerns (MacPhee and Brusven 1976; Parasiewicz et al. 1998; Smith et al. 2007) in fish downstream of hydro dams, as stressors can reduce the fitness of downstream populations through reduced energy and growth. In this particular case, hydropeaking (the manipulation of water flows downstream of dams to efficiently tailor energy production to cyclic demand; Moog 1993; Young et al. 2011) may act as a chronic stressor, and fish strandings observed downstream of the E. B. Campbell dam after downramping (water flow restriction) suggest that this stressor

can be quite severe. While some Hg may have been contributed to this system from a now-defunct chlor-alkali plant ≈400km upstream on the South Saskatchewan River (Turner and Lindberg 1978; Hildebrand et al. 1980), the Hg bioaccumulation in the Tobin-Cumberland Lake system provides a unique opportunity to assess the breadth of Hg and physiological concerns in fish up and downstream of dams. It also allows assessment of the relationships between these measures between up and downstream sites, and the interactions between these measures that might arise from both the *construction* and *operation* of hydroelectric dams. It also represents an opportunity to address the persistent concerns about [Hg] in fish held by members of the Cumberland House community.

1.3 Dam imposed stressors

Hydroelectric facilities exist in several varieties, each with unique effects on up and downstream fluvial morphology and aquatic chemistry (Thornton et al. 1990). As a hydropeaking facility, the E. B. Campbell dam relies on the Tobin Lake reservoir to satisfy predicted and irregular demands on power production (Young et al. 2011). The construction of the Tobin Lake reservoir effectively transformed the affected portion of the Saskatchewan River from a riverine to a lacustrine system with mixing characteristics unique to reservoirs (Thornton et al. 1990). The physical influences and changes imposed on aquatic chemistry by dams depends upon the depth of the dam, the height of its release point, and the degree of hydropeaking exerted downstream (Thornton et al. 1990; Karr 1991; Cooke et al. 2005; Young et al. 2011). Depending upon these factors, stressors imparted on downstream fish can include rapid fluctuations in temperature, and decreases in dissolved oxygen (DO) (Thornton et al. et al. 1990), both of which can induce or exacerbate a stress response in fish (Donaldson et al. 2008; Cook and Herbert 2012). Additional stressors can include changes in water osmolarity, turbidity, and total suspended solids (TSS) (Thornton et al. 1990; Wendelaar Bonga 1997). Perhaps the most ubiquitous potential stressor inherent to hydropeaking facilities is the sequestration of water and alteration of downstream flow regimes. Dams on a similar order of magnitude as the E. B. Campbell exert an average 67% flow reduction to downstream ecosystems (Graf 2006). Further, the restricted flows downstream of the E. B. Campbell dam are also quite dynamic, and water depths can change more than 1 metre per day (Fig. 1.1).

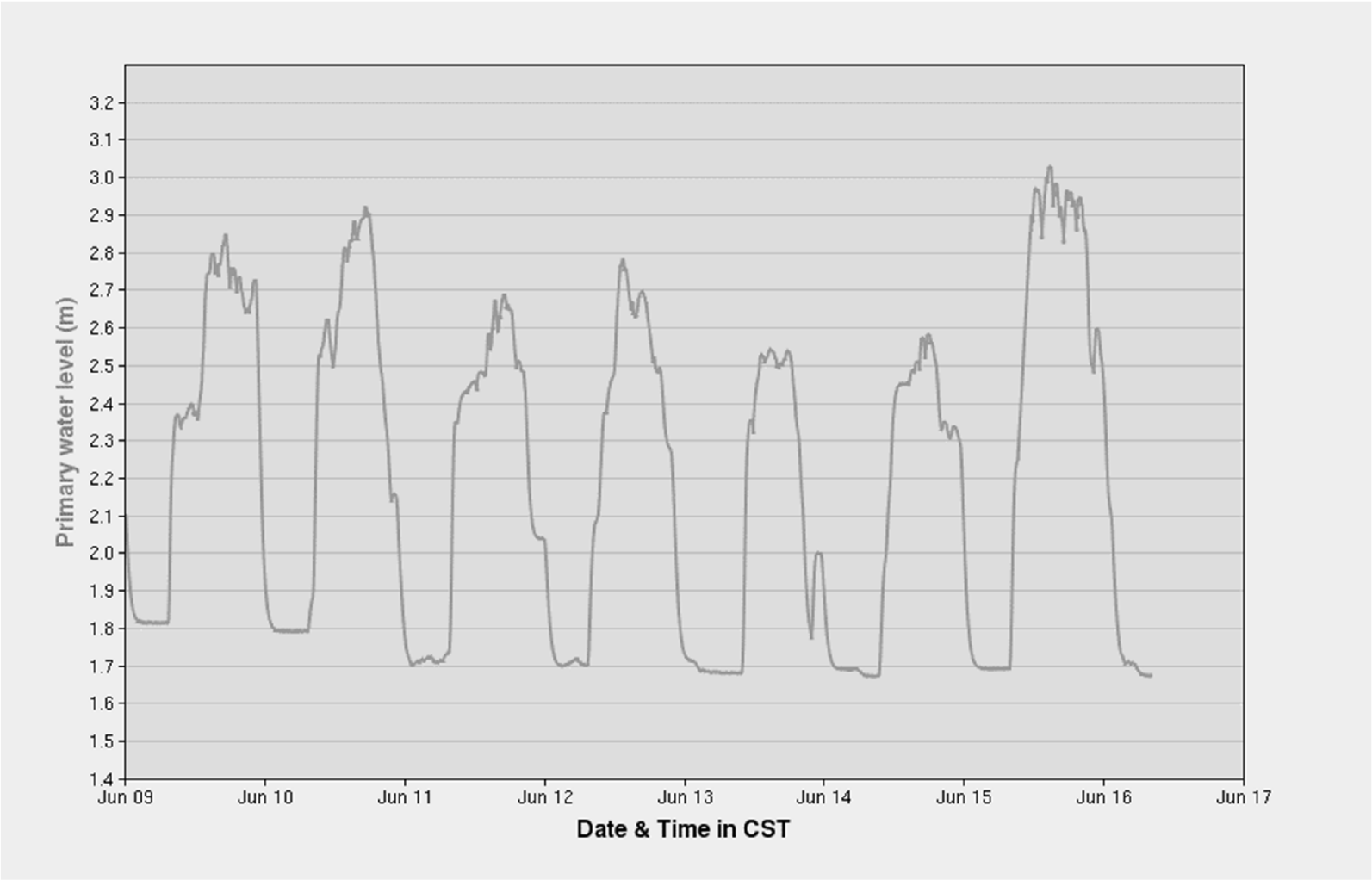

Fig. 1.1 Real time water levels (depth: m) immediately downstream of the E. B. Campbell dam, June 2015. Wateroffice, Government of Canada.

The irregular pulses of water that accompany hydopeaking facilities can have complex effects on the downstream macroinvertebrate prey community (Ogbeibu and Oribhabor 2002; Bednarek and Hart 2005; Armanini et al. 2014), and can impart negative influence on fish populations (McKinney et al. 2001; Young et al. 2011). Some studies suggest that flow alterations have a more direct effect on fish condition (measures of mass-at-length relationships) rather than an indirect effect through their macroinvertebrate prey (Shaw and Richardson 2001). Flow alterations may also act as a direct physical stressor in fish (Flodmark et al. 2002; Young et al. 2011) and limit the reproductive success of species who spawn along the shoreline (Young et al. 2011)

Some of the stressors imparted downstream are imparted by the lateral change in shoreline that accompanies flow manipulation. The shallow banks of certain stretches of the Saskatchewan River downstream of the dam are areas of particular concern for fish strandings (Young et al. 2011), a term which includes both beached fish and fish trapped in small refugia (pools and backwaters). Late season (summer/fall) fish strandings have been observed downstream of the E. B. Campbell dam (Fig. 1.2) which, in addition to direct mortality, may also pose sublethal risks to the downstream fish populations (Saltveit et al. 2001; Nagrodski et al. 2012).

Fig. 1.2 Stranded fish observed downstream of the E. B. Campbell Dam in summer of 2014. Photo Credits: Derek Green

Fish that are able to avoid or survive strandings may expend energy in the process that can reduce the critical lipid stores required for over-winter survival, an effect particularly salient for young fish (Cunjak and Power 1987; Cunjak 1988; Adams 1999; McKinney et al. 2001). The lateral recession of flow during down-ramping may also be harder to overcome for chronically stressed fish and being overwhelmed by downramping could be a sign of the concomitant effects of the above stressors (Sigismondi and Weber 1988). The stress imparted by sudden alterations in the lateral flow of the river may be particularly impactful on smaller fish (Hoffarth 2004).

The cyclic, non-overlapping nature of these imposed stressors (i.e. downramping: lateral habitat decrease; upramping: increased turbidity, longitudinal flow and TSS, changes in osmolarity, and decreased DO concentration and temperature), means that habitats downstream of hydroelectric facilities potentially face a combination of cyclic and acyclic stressors. While the stress response of fish has been well-studied (Wendelaar Bonga 1997; Mommsen et al. 1999; Barton 2002), the resounding conclusion is that species differences, experimental protocols and methods, and age can all obfuscate extrapolations to environmentally meaningful endpoints. These endpoints can include primary (e.g. neuroendocrine hormone release (Wendelaar Bonga 1997)), secondary (e.g. energy dysregulation; Barton et al. 1987; Cunjak and Power 1987), and tertiary or whole-body (e.g. growth; De Boeck et al. 2001; Wendelaar Bonga 1997) stress responses. When combined with Hg exposure these effects may increase [Hg] by minimizing growth dilution within fish tissues (Wendelaar Bonga 1997; Trudel and Rasmussen 2006; Sousa et al. 2010; Sandheinrich and Drevnick 2016). Given the potential for environmental stress downstream of a hydropeaking dam, and the intimate ties that stress could have on the performance and [Hg] of downstream fish populations, a better understanding of both energetics and Hg in fish downstream of dams is required.

1.3.1 Chronic stress and the primary stress response

The functional definition of stress is a departure from physiological homeostasis severe enough to elicit physiological and behavioural patterns that attempt to restore homeostasis to the organism (Chrousos and Gold 1992). Within teleost fish, the initial response to stressors is the rapid release of catecholamines (epinephrine and norepinephrine) from the head kidneys resulting from sympathetic innervation within the hypothalamic-sympathetic-chromaffin cell (HSC) axis (Mazeaud et al. 1977; Wendelaar Bonga 1997: Reid et al. 1998). Catecholamines subsequently

increase the activity of the cardiovascular system, improve blood oxygen transport capacity, and stimulate hyperglycemia via glycogenolysis (Wendelaar Bonga 1997). The HSC stress response is subsequently enhanced by the slower-acting, hormonally-activated (Barton 2002) influence of the hypothalamic-pituitary-interrenal (HPI) axis which ultimately stimulates the release of cortisol through a corticotropin-releasing hormone initiated adrenocorticotropic hormone releasing cascade (Wendelaar Bonga 1997). Recent research suggests that the ordered nature of these responses occurs not only due to differences in stimulation, but also because hyperglycemia from the HSC axis may stimulate responses from the HPI (Conde-Sierra et al. 2013).

During the stress response, cortisol generally plays a minor role in hepatic glycogenolysis and primarily encourages a shift to gluconeogenesis and subsequent downregulation of the stress response (Mommsen et al. 1999). Cortisol secretion is regulated by negative feedback exerted by the hormone on the interrenal cells, pituitary, and hypothalamus, which prevents excessive chronic elevation of cortisol in the stress response that can exert detrimental influences on reproduction, growth, and resistance to pathogens (Barton and Iwama 1991; Pickering 1993; Wendelaar Bonga 1997; De Boeck et al. 2001). Under conditions of acute stress the sequential responses of the HSC and HPI axes enhance the physiological capacity of fish to deal with sudden stressors.

Both the HSC and HPI axis can have their effects altered in response to chronic stress. Chromaffin cells in rainbow trout facing chronic physical stress showed decreased catecholamine release in response to cholinergic stimulation compared to controls, despite having similar or increased levels of catecholamine stores (Reid et al. 1994). In corroboration, fish exposed to daily acute stressors show attenuated, and perhaps in some cases habituated, cortisol responses to acute stressors (Barton et al. 1987; Reid et al. 1994; McCormick et al. 1998; Jentoft et al. 2005). Notably, in fish experiencing chronically stressful conditions, peak cortisol levels can be suppressed when acutely stressed (Barton et al. 1987; Jentoft et al. 2005). In these fish the primary stress response may ultimately be truncated by diminished HSC activity, as would agree with the top down stress response inhibition suggested by Conde-Sierra et al. (2013). Conversely, other studies have failed to show diminished acute stress responses in chronically stressed fish (Barcellos et al. 2006), and have suggested increased cortisol metabolism is responsible for decreased concentrations in chronically stressed fish facing an acute stressor (Wiseman et al. 2011), highlighting possible differences in methodology and/or species and a possibility of multiple simultaneous influences.

In cases where the stress response has become blunted, there is the risk of falsely concluding that the stressor has been appropriately habituated if other endpoints in the secondary and tertiary stress responses are not considered in tandem with cortisol concentrations in response to an acute stressor (Jentoft et al. 2005).

1.3.2 Chronic stress and the secondary and tertiary stress response

The potential for chronic stress to downregulate the stress response holds serious implications for affected fish as stress-induced breakdown of glycogen is a critical step in inducing the hyperglycemic response required to address immediate stressors (Wendelaar Bonga 1997). Plasma glucose concentrations are also reduced in chronically stressed fish facing acute stressors, suggesting that these fish are less able to mobilize glucose from glycogen stores in order to respond to an immediate stressor. It is therefore critical to measure glycogen concentrations in concert with cortisol concentrations, as neglecting the effects of chronic stress may otherwise lead to the erroneous conclusion that affected fish are exceptionally well primed to handle acute stressors.

The interactions between cortisol, glucose, and glycogen concentrations are still contentious; previous instances showing no relationship between these endpoints were possibly due to the effects of stocking density or food aversion (Pickering and Stewart 1984; Vijayan et al. 1990; Ellis et al. 2002). As catecholamine release causes glycogenolysis in the immediate stages of the acute stress response, it was anticipated that rapid collection of samples may yield observable glycogen decline in acutely stressed fish, and that an inhibition of glycogen decline would be noted in acutely stressed fish from chronically stressful environments due to concomitant inhibition of the catecholamine response (Reid et al. 1994; Wendelaar Bonga 1997).

In addition to alterations in glycogen metabolism, chronic stress/cortisol exposure is implicated in an overall decrease in growth and/or body condition of fish due to increased taxation of available resources and downregulation of triiodothyronine (T3) (Vijayan and Leatherland 1989; Wendelaar Bonga 1997; McCormick et al. 1998) or through inhibition of insulin-like growth factor I by acting through the somatotropic axis (Shepherd et al. 2011; Faught and Vijayan 2016). The influence of this effect is increasingly severe at northerly latitudes where overwintering periods are long, causing increased taxation on the lipids required for reproduction and population maintenance of small fishes (Shuter and Post 1990; Adams 1999; Post and Parkinson 2001; Biro et al. 2004). Smaller prey fishes are also among those most susceptible to energy deficiencies

owing to their relatively low lipid to metabolism ratios (Schultz and Conover 1997; Biro et al. 2004), and the weight of these secondary stress effects may manifest in tertiary level changes that reduce the viability individual fish and fish populations.

1.4 Thesis objectives, hypotheses, and purposes by chapter

1.4.1 Thesis objectives

By analyzing historical and contemporary drivers of Hg contamination within a river-reservoir system, as well as energetics and stress responses between fish from up and downstream of the dam, I intended to assess the ramifications of hydroelectric development for locally affected fish and fisheries. The purpose of my thesis was therefore threefold:

1. To determine the long-term trends and contemporary patterns of mercury concentrations in a reservoir and downstream fishery.
2. To determine if environmental stress (in the form of hydropeaking) imparted downstream of a hydropeaking dam is reflected by reduced energy stores and condition, and increased mercury concentrations in fish.
3. To determine the utility of spottail shiner ("shiner"; *Notropis hudsonius*) as a sentinel fish species in the study of environmental stressors including hydropeaking dams.

In order to address Hg and physiological concerns associated with hydroelectric dams, it was deemed necessary to contrast physiological markers, [Hg], and the stress response of fish throughout the system affected by the impoundment. The potential interaction between [Hg] and the secondary stress response means studying these endpoints together can provide the best resolution on long-term trends and ongoing effects on and elevated [Hg] in fish downstream of dams. To this end, my thesis research has been prepared in the following three parts.

1.4.2 Research chapter 1, thesis chapter 2: Historical and contemporary patterns of mercury in a hydroelectric reservoir and downstream fishery: Concentration decline in water and fishes

The purpose of this chapter was to assess and compare the historical and contemporary trends of mercury in fish from a hydroelectric reservoir (Tobin Lake) and downstream fishery (Cumberland Lake). The null hypotheses tested were as follows:

$H_0$1: There will be no pattern in [Hg] in fish from either site over time.

$H_0$2: Mercury concentrations in fish will not suggest relationships in contamination across sites.

$H_0$3: Analyses of contemporary [Hg] predictors (e.g. walleye age and length and trophic level) will not suggest continual mercury export to the downstream system.

1.4.3 Research chapter 2, thesis chapter 3: Energy stores and mercury concentrations in a common minnow up and downstream of a hydropeaking dam

The purpose of this chapter was to assess condition, energetics, and [Hg] in fish downstream of the dam affected by hydropeaking to compare these endpoints across sites, and to assess the possible predictive relationships between these endpoints. Null hypotheses tested included:

$H_0$1: Fish condition, triglyceride concentrations, and [Hg] will not be significantly different across environments experiencing different degrees of hydropeaking.

$H_0$2: Statistical models will show no predictive relationships between fish condition, triglyceride concentration, and [Hg].

1.4.4 Research chapter 3, thesis chapter 4: Cortisol as an early environmental biomarker of direct chronic stress in spottail shiner (*Notropis hudsonius*): Assessment of a novel protocol using a potential hydropeaking stressor

The purpose of this chapter was to refine the results of research chapter 3 using another year of data, and to determine the potential utility of an acute stress challenge protocol standardized to a single year-class of common, wild-caught fish. Null hypotheses tested included:

$H_0$1: Whole-body fish glycogen and triglyceride concentrations and fish condition will not be significantly different in fish collected from environments experiencing different degrees of hydropeaking.

$H_0$2: Whole-body fish glycogen, triglyceride, and cortisol concentrations will not change over time in response to an acute stress challenge, and concentration patterns observed over time will not differ across site or month in fish collected from environments experiencing different degrees of hydropeaking.

$H_0$3: Statistical models will show no predictive relationship between condition, energetic endpoints, and cortisol concentrations in response to an acute stressor in fish collected from sites experiencing different degrees of hydropeaking.

CHAPTER 2:

HISTORICAL AND CONTEMPORARY PATTERNS OF MERCURY IN A HYDROELECTRIC RESERVOIR AND DOWNSTREAM FISHERY: CONCENTRATION DECLINE IN WATER AND FISHES

Preface

The research in this chapter was designed to address the decline of mercury from water and fish contaminated with Hg in a hydroelectric reservoir and a downstream fishery in North-Eastern Saskatchewan. Rates of [Hg] decline were measured from commercially relevant fish populations from both sites where [Hg] were above the consumption guideline when monitoring began in the 1970s. It took approximately 5-20 years from the start of the study period (or 10-25 years post-reservoir construction) for [Hg] to return below the guideline, and one species had yet to return to background concentrations by the time the study was conducted in 2014-2015. Delayed Hg decline was observed in downstream fish, and trends in [Hg] indicated similar patterns between the Hg in the reservoir and fishery. Contemporary predictors of [Hg] in fish suggested Hg retention in downstream fish populations despite no evidence for ongoing aquatic contamination or persistent bioaccumulation.

 The proper citation for this article is

Green, D. J., Duffy, M., Janz, D. M., McCullum, K., Carrière, G., and Jardine, T. D. **2016**. Historical and contemporary patterns of mercury in a hydroelectric reservoir and downstream fishery: concentration decline in water and fishes. *Archives of Environmental Contamination and Toxicology*. 71: 157-170.

The author contributions to chapter 2 of this thesis were as follows:

Derek Green (University of Saskatchewan) collected, processed, and analyzed contemporary field samples, performed all statistical analyses, and drafted the manuscript.

Mark Duffy (Saskatchewan Ministry of Environment) provided historical provincial mercury records for statistical analyses; reviewed and revised the manuscript, providing comments and corrections.

David Janz (University of Saskatchewan) provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Kevin McCullum (Saskatchewan Ministry of Environment) provided historical provincial mercury records; reviewed and revised the manuscript, providing comments and corrections.

Gary Carrière (Cumberland House Fisherman's Cooperative) provided contemporary walleye and pike samples, and insight and guidance to the influences of the E. B. Campbell dam on Cumberland Lake and the surrounding environment.

Tim Jardine (University of Saskatchewan) designed study; provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

2.1 Abstract

Mercury (Hg) contamination can pose risks to both human and animal health and commercial fisheries. Reservoir construction in riverine systems produces flooded conditions amenable to Hg (II) methylating bacteria that can transform this relatively benign environmental contaminant into the bioaccumulative, environmentally relevant, and neurotoxic MeHg. [Hg] in fishes from reservoirs can take decades to decline to pre-dam levels but less is known about Hg exported downstream and its dynamics within downstream fish populations. We examined and compared the multi-decadal rates of biotic [Hg] decline and contemporary factors affecting [Hg] in fish collected from a hydroelectric reservoir (Tobin Lake) and a related downstream fishery (Cumberland Lake) along the Saskatchewan River, Canada. Rates of [Hg] decline were considered in four species, northern pike (*Esox lucius*), sauger (*Sander canadensis*), goldeye (*Hiodon alosoides*), and walleye (*Sander vitreus*), all of which showed significant decline over time ($P < 0.001$) and are now below Health Canada consumption guidelines (0.5 μg/g). Rates of decline ranged from 0.5 to 3.9% per year and were similar between sites in the cases of northern pike and sauger. Contemporary factors affecting [Hg] in walleye collected downstream include fish length ($P<0.001$), age ($P<0.001$), and trophic magnification through the food web ($P<0.001$), and relationships between [Hg] and trophic level in predatory and prey fish are now similar to those found in non Hg inundated systems at similar latitude. Together, these results suggest connected contamination between the two sites, and delineate the timeline upon which [Hg] in a variety of fish species declined to non-toxic levels in both locations.

2.2 Introduction

Inorganic trace Hg is a common and fairly benign metallic environmental contaminant that can be methylated into the potent and more environmentally relevant neurotoxin, teratogen, and potential chronic cardiovascular toxin, MeHg (Karagas et al. 2012). Natural processes are responsible for a background signature of Hg in the environment; however, human industrial activities and the burning of fossil fuels has substantially increased the global pool of Hg that is transported across the globe via wind and water cycles (Keating et al. 1997; Goodarzi et al. 2008; Pirrone et al. 2010). The result is aquatic Hg pollution both near and far from industrial sources (Mierle 1990; Lucotte et al. 1995; Engstrom and Swain 1997) and concentrations in fish that may affect the health of fish-eating consumers (Mergler et al. 2007). Data from large scale sampling, when compared to consumption guidelines for total [Hg] in fish (e.g. 0.5 μg/g wet weight, Health Canada 2007), has indicated that many higher trophic level fish species regularly approach or exceed this limit (Depew et al. 2013), suggesting toxicological risk to consumers. Exacerbating this exposure, those fish which are most heavily contaminated are among those commonly consumed (Hammerschmidt and Fitzgerald 2006; Depew et al. 2013).

Mercury accumulation in aquatic food webs depends largely on local conditions, specifically those conducive to the activity of methylating bacteria. They include high dissolved organic carbon (DOC) in anoxic conditions (Graham et al. 2012), relatively warm temperatures (Wright and Hamilton 1982), reducible sulfate and/or iron (II) (Yu et al. 2011), and large amounts of decaying flooded vegetation and soil organic matter, which are the primary contributors of DOC (St. Louis et al. 1994; Kelly et al. 1997). Reservoirs can be contaminated by Hg from atmospheric deposition, run-off from the surrounding land, and upstream point sources (Mason et al. 1994; Selin 2009), and reservoir construction produces the aforementioned conditions conducive to Hg methylation, and consequently results in Hg contamination in fish (Jackson 1988; Louchouarn et al. 1993; Bodaly et al. 2007).

The influxes of inorganic Hg and DOC, followed by subsequent increases in methylation from flooding or point sources can contaminate areas within a short timeframe, though [Hg] in biota are known to decrease once the influx ceases (Hrabik and Watras 2002). Time-series analyses of [Hg] in fish from within newly flooded reservoirs show that concentrations may continue to rise for approximately one decade, followed by slow declines over approximately 20-30 years post-

impoundment (Bodaly et al. 2007) with larger, older organisms feeding at the top of long food chains showing the greatest degree of contamination (Cabana et al. 1994; Kidd et al. 1995).

Far less is known about downstream transport of MeHg produced in reservoirs and its long-term fate in downstream food webs (Rosenberg et al. 1997; Schetagne et al. 2000; Kasper et al. 2012), though point source reservoirs of MeHg may lead to elevated [Hg] as far as 320 km downstream that can accumulate within and subsequently decline from the food web (Anderson 2011). Given the proliferation of dams throughout the developing world (Finer and Jenkins 2012; Turkenburg et al. 2012), information on the consequences of reservoir formation for downstream fisheries is needed for responsible hydroelectric implementation and management.

The Saskatchewan River Delta (SRD) is a large wetland complex located in the boreal plain of north-central Canada. The SRD and its largest lake, Cumberland Lake (CL), currently supports a commercial fishery, largely focused on walleye (*Sander vitreus*) and northern pike (*Esox lucius*) (Royer et al. 1968; Wallace 1999) which are also highly represented in the local diet (Waldram 1988). A now-defunct fishery for lake sturgeon (*Acipenser fulvescens*) was closed briefly in the early 1970s because of high [Hg] (Waldram 1988), re-opened in 1973, and fished until 1997 when it was closed permanently due to population declines. The Upper Delta of the SRD is approximately 50 km downstream from the E.B. Campbell hydroelectric facility that was constructed in 1963, creating the large, shallow Tobin Lake reservoir (TL). This study site thus represents an opportunity to examine long term trends in fish [Hg] in an area downstream of an impoundment. While the source of the Hg to the reservoir itself is unknown, it is possible that some Hg may have been contributed by an upstream chlor-alkali plant that operated between 1964 and 1978 and led to high [Hg] in fishes in the South Saskatchewan River (Wobeser et al. 1970). However, the large distance between the plant and TL (approximately 400 km), additional dilution from the North Saskatchewan River, and the high deposition of sediment-bound Hg expected at the transition zone between the Saskatchewan River and TL suggest that MeHg within TL is comprised of atmospheric Hg deposition with minor contributions from upstream runoff, which was subsequently methylated within the reservoir (Thornton et al. 1990; Schetagne et al. 2000).

The objective of this study was to evaluate historical trends in and contemporary drivers of [Hg] in fish communities in three areas influenced by a hydroelectric dam: the reservoir, the river immediately downstream of the dam, and the downstream delta. We sought to estimate the annual percent change of [Hg] in commercial species since monitoring began in the 1970s. We predicted

that concentrations would show declines consistent with those observed within reservoirs (Bodaly et al. 2007) and that known factors that dictate fish [Hg] (i.e. age, size and trophic level), and relationships within and among contemporary fish populations would suggest that Hg methylation within the TL reservoir was a common source of contamination for both TL and CL fish populations. Further, we predicted that these data would suggest that downstream transport of Hg to CL has ceased, and that Hg has largely returned to background levels (Simoneau et al. 2005; Jardine et al. 2012). We conducted these analyses to evaluate the potential for adverse health effects of fish consumers in local communities, and to better understand the likely consequences of reservoir creation on active commercial and subsistence fisheries elsewhere in the developing world.

2.3 Methods

2.3.1 Study area

The E.B. Campbell Dam (53°41’19”N 103°20’50”W) was commissioned upstream of the SRD in 1963 forming TL (Fig. 2.1).

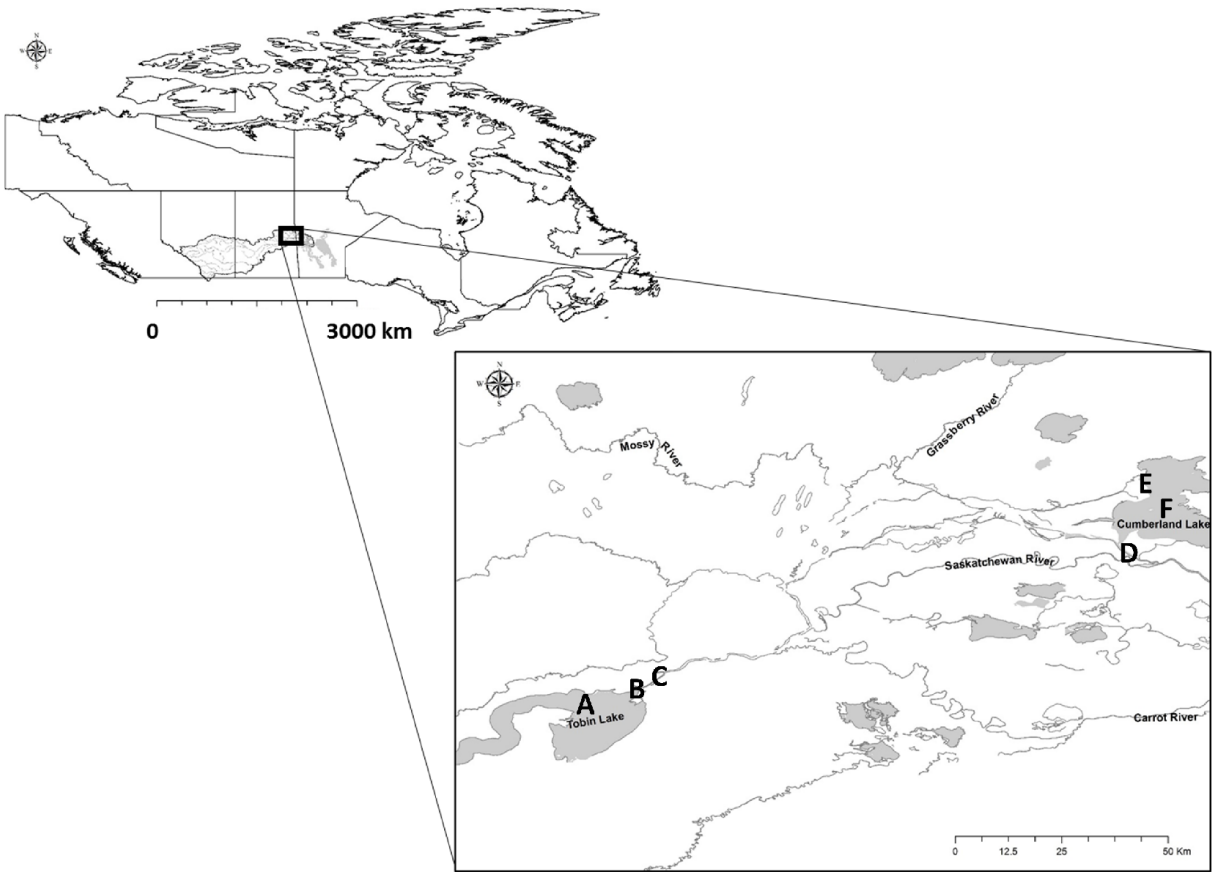

Fig. 2.1 Study sites: A) Tobin Lake (TL), B) E. B. Campbell Dam (formerly "Squaw Rapids Dam"), C) Immediately downstream of the E. B. Campbell Dam (EBC), D) Saskatchewan River junction with Cumberland Lake (CL-SR), E) Mossy River junction with Cumberland Lake (CL-MR), F) Cumberland Lake (CL).

The SRD is located at the border of Saskatchewan and Manitoba, encompasses 10,000 km^2 (Smith et al. 2014), and is divided into the Upper Delta and Lower Delta with the lower portion in Manitoba and the upper located primarily in Saskatchewan. The Upper Delta contains the affected fishery site at CL (54°3'0"N 102°18'2"W). The majority of flow to the lake is from the Saskatchewan River flowing through the E.B. Campbell Dam, with minor contributions from three tributaries, the Torch, Mossy-Grassberry and Sturgeon-Weir Rivers (Smith et al. 2014). The areas surrounding Cumberland Lake, including the Cumberland Marshes, are characterized by numerous wetlands and abandoned and active river channels. While upstream water resource development has reduced the magnitude of the annual summer flood, the delta still experiences significant flood-drought cycles (Sagin et al. 2015). A lack of industrial activity in the SRD means contributions from local point sources of Hg are minimal, and additional description of the quality and quantity of water in the Saskatchewan River can be found in Wheater and Gober (2013).

2.3.2 Water sampling and analysis

Historical water samples were collected by the provincial government of Saskatchewan between 1975 and 1990, and a total of 70 water samples were collected from immediately downstream of the E. B. Campbell Dam (EBC; 53°42'59"N 103°17'11"W) and analyzed for Hg. We collected contemporary water samples (2013-2014) in 250 ml narrow-mouth FPE bottles, and preserved at pH 2 with trace metal analysis grade nitric acid using clean hands techniques (United States Environmental Protection Agency [US EPA] 1996). Collection vessels were prepared, and samples were analyzed by SRC Analytical Laboratories, Saskatoon, Saskatchewan. Additional water chemistry parameters of contemporary samples were provided by SRC Analytical Laboratories.

Throughout the collection period [Hg] detection limits in water have varied substantially and were therefore inconsistent between dates, ranging from 0.1 µg/L in 1973 to 0.01 µg/L in 2014. Horizontally linear clusters of concentrations were presumed to represent censored values below detection within the relevant timeframe, and we substituted half the detection limit for data display purposes. Contemporary concentrations were initially analyzed using ICPMS which revealed concentrations to be below the detection limit of this method (0.01 µg/L). A subset of water samples were then analyzed for low level Hg by SRC Analytical Laboratories, in accordance with

EPA 1631 methodology and using MERX Automated Total Analytical System which allows for detection of concentrations as low as 1ng/L (US EPA 2002).

2.3.3 Fish sampling, processing, and analysis

Two sets of fish data were considered in this study. Historical records of fish muscle [Hg] (μg/g wet weight) were obtained from the provincial government of Saskatchewan and span from 1970 to 2013. Datasets derived from these data included muscle [Hg] concentrations, fork length, and date of capture for several commercially and recreationally important fish species, including northern pike, walleye, sauger (*Sander canadensis*), and goldeye (*Hiodon alosoides*). Field collected historical samples were filleted on site, and muscle samples were frozen immediately and subsequently processed and analyzed in accordance with Environment Canada guidelines using cold vapour atomic absorption techniques (Environment Canada 1977). Historical fish concentrations were used to derive rates of [Hg] decline over time. Datasets were derived for TL (53°37'11"N 103°31'43"W) and CL (54°04'48"N 102°21'3"W), as well as from several lakes near, but not connected to the Saskatchewan River to serve as reference sites. These unconnected reference lakes included Candle (53°48'59"N 105°18'00"W), Montreal (54°15'36"N 105°44'37"W), and Big Sandy (54°25'26"N 104°5'30"W) Lakes and were chosen for their proximity to the SRD and their robust datasets, which included adequate samples spanning multiple years in the cases of northern pike and walleye.

Contemporary samples came from TL, EBC, and from two sites near CL, the mouth of the Mossy River where it empties into CL (CL-MR; 54°04'48"N 102°21'3"W) and along the Saskatchewan River south of CL (CL-SR; 53°57'16"N 102°23'9"W) (Fig. 2.1). Contemporary fish samples were provided by the Cumberland House Fisherman's Co-op in the spring and summer of 2013 and 2014. Additional samples of small-bodied, low trophic level species (various shiners, perch, minnows) were collected via bag seine. As the Fisherman's Co-op operates as a commercial fishery, often only heads were available for analysis. In these cases the total length of the walleye was extrapolated using the following formula:

Total length = 1.526+(3.777 x head length) 2.1

Where head length is the distance between the tip of the snout and the edge of the operculum. This isometric relationship was derived during preliminary analysis of fish of known length (n=113, r^2=0.87). In order to standardize fish lengths in our contemporary dataset, all contemporary fish lengths were extrapolated using this formula. All contemporary pike and walleye [Hg] was derived from muscle samples that were excised from the filet remaining at the posterior of the heads supplied by the Co-op. Mercury concentrations in muscle taken from this area correlate strongly with those from fillet samples (Bank et al. 2007). Whole-body Hg analysis, with head and tail removed, was used to determine [Hg] in shiner, minnow, and perch species.

Where available, data collected on contemporary fish samples included total length, fork length, and mass. To better understand contemporary trophic magnification of Hg at these sites, we measured [Hg] and trophic level of the northern pike and/or walleye provided by Cumberland House fishermen, as well as four of their common prey species: spottail shiner, blackchin shiner (*Notropis heterodon*), brassy minnow (*Hybognathus hakinsoni)* and yellow perch (*Perca flavescens*). Muscle [Hg] in pike and walleye of a given size within a given geographic location is relatively robust to seasonal variation and short term annual cycles (≈1-2% change per year; Rasmussen et al. 2007; Neff et al. 2012); therefore, samples were pooled from summer collections in 2013 and 2014 for this analysis. Only prey species collected in the summer of 2013 were considered for analysis in an attempt to minimize seasonal influences over whole-body [Hg] in these fish (Korthals and Winfrey 1987; Greenfield et al. 2013).

All contemporary samples were desiccated for approximately 48 hours in a drying oven set to 50ºC to maximize Hg recovery (Ortiz et al. 2002) and ground to a fine powder using an acid-washed mortar and pestle.

Fifty mg of each contemporary sample was subsequently analyzed in a Direct Mercury Analyzer (DMA 80, Milestone Inc., Shelton, Connecticut, USA). Mercury recovered from the collected samples was referenced against Certified Reference Materials (CRMs; dogfish muscle DORM and lobster hepatopancreas TORT from National Research Council [Ottawa, Ontario, Canada], human hair IAEA-85 from International Atomic Energy Agency [Vienna, Austria]). Recovery of these CRMs was 100 +/- 14% (n = 20), 93 +/- 7% (n = 8) and 97 +/- 1% (n = 4) respectively. Blank sample boats contained less than half the detection limit of Hg (0.04 ng) and therefore the test samples were not blank corrected. In order to remain analytically consistent between historical and contemporary samples, wet weight concentrations of muscle were

subsequently derived from contemporary dry weight concentrations by applying a 0.25 multiplier, assuming 75% moisture (May et al., 2009: Lavoie et al., 2010). For the purposes of this study [Hg] is assumed as a proxy measure for [MeHg], as analyses of numerous fish species from different habitats confirm that virtually all Hg in fish tissues is in the methylated form and bound to thiol groups in proteins (Bloom 1992; Lemes and Wang 2009; Greenfield and Jahn 2010).

Heavy nitrogen isotopes (^{15}N) bioaccumulate at a rate of approximately 3.4‰ per trophic level over their lighter counterpart, ^{14}N (Post 2002). To assess trophic magnification of Hg (Lavoie et al. 2013), nitrogen stable isotope ratios ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) were determined by combusting 1.0 (±0.2) mg of dried, powdered samples of predators (walleye, pike) and prey fish (minnows) in a PDZ Europa ANCA-GSL elemental analyzer followed by delivery of N_2 gases to a PDZ Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility. Samples analyzed in duplicate differed by an average of 0.2‰.

To determine the effects of age on [Hg] in a subset of fish, otoliths were collected from contemporary walleye sampled from CL-MR. Otoliths were extracted, processed, and aged using the procedures developed by the Fisheries Management Branch of Alberta Environment and Sustainable Resource Development (Watkins and Spencer 2009) and analyzed by North Shore Environmental Services, Thunder Bay, Canada.

2.3.4 Statistics

Much of the historical water sampling was conducted without the use of clean hands techniques, and there was appreciable ambiguity in the detection limits over time. For these reasons trends in water [Hg] over time were not analyzed statistically, and were instead only included for display purposes.

To compare spatial and temporal changes in fish [Hg], we used historical data for four species (northern pike, sauger, walleye, and goldeye) from both TL and CL. Log transformed [Hg] was compared against time (year) for each species across each location, using an ANCOVA with location as the factor and year as the covariate, after testing for parallel slopes. All ANCOVAs were considered significant when $P<0.01$. Rates of [Hg] decline from each species were determined from regression formulae derived from Log[Hg] in fish muscle over time (year), with the slope of the line taken as a measure of percent decline per year. Because larger fish within species are known to have higher [Hg] in some cases, but not all (Scott and Armstrong 1972;

Simoneau et al. 2005; Fowlie et al. 2008), and preliminary analyses revealed significant increases in fish length sampled over time in some populations, we accounted for this potential confound by standardizing to [Hg] of a uniform size. Log[Hg] per cm of fish length was calculated as Log[Hg]/Log Length(cm) for both TL and CL data on all species, and ANCOVAs were re-run to compare these adjusted rates of [Hg] decline over time with location as the factor and time (year) as the covariate. Reference lake data were analyzed using linear regression to assess whether their trends over time were comparable to our study sites. As these analyses were run on pike and walleye, and the length of these species is known to correlate positively with muscle [Hg] in Saskatchewan (Saskatchewan Ministry of Environment 2015), these data were length adjusted as previously described before being analyzed against year of capture. Reference lake mean fish [Hg] is presented as either Log[Hg], or length adjusted Log[Hg] as indicated.

To assess spatial and biological factors driving [Hg] in our contemporary data set (2013 and 2014), we first ran an ANCOVA to compare the influence of location (factor) and length (covariate) on Log[Hg] in a single species, walleye, across three locations (EBC, CL-MR, and CL-SR). Next, we compared the relative influence of length, age and $\delta^{15}N$ on [Hg] within CL-MR walleye where we had a range of body sizes and age data. We used a forward stepwise linear regression to compare the fit and significance of the three variables.

Finally, we determined if trophic level, as estimated from $\delta^{15}N$, provided additional explanatory power for [Hg] across fish species. We examined Hg transfer through the fish food web by calculating Trophic Magnification Factors (TMFs) for three locations (TL, EBC and CL, with the latter including both CL-MR and CL-SR) by regressing log-transformed [Hg] against $\delta^{15}N$ values for commercial fishes and prey fishes (Kidd et al. 2003; Jardine et al. 2006; Jardine et al. 2012; Lavoie et al. 2013). These trophic magnification slopes (TMSs) can be converted to TMFs using $TMF = 10^{m}$, where m is the TMS multiplied by the average increase in $\delta^{15}N$ across trophic levels (3.4‰; Post 2002). Differences in TMSs were tested among the three locations using ANCOVA, with $\delta^{15}N$ as the covariate and location as a factor.

2.4 Results

2.4.1 Water [Hg] and chemistry

Concentrations of Hg in water were occasionally above detection in the historical period, reaching as high as 2 to 4 μg/L in the 1970s, though data collected before the advent of clean hands techniques are to be interpreted cautiously (Fig. 2.2**)**.

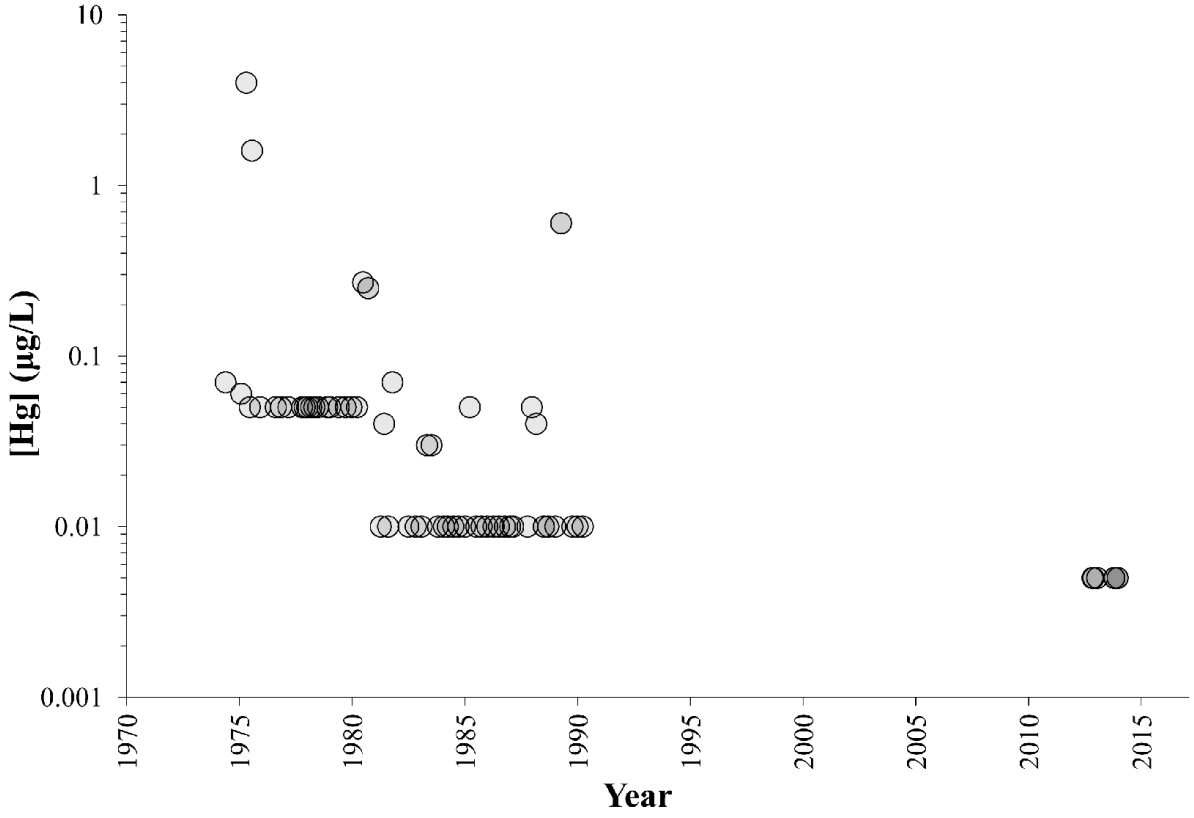

Fig. 2.2 Water [Hg] from historical (1975-2013) and contemporary (2013-2014) samples collected immediately downstream of the E.B. Campbell Dam (n=70) versus sampling year. Data is presented using a log-scaled y-axis.

While the frequency of detection appears to have declined over the study period, ambiguities in the detection limits over time precludes any statistical analyses. All contemporary water samples contained [Hg] below 0.01 µg/L, and subsequent low-level analyses showed that current concentrations ranged from 1 to 5 ng/L (data not shown). As water concentration data were only presented for illustrative purposes and were not analyzed statistically, our historical and contemporary concentrations are shown on a single plot in Fig. 2.2.

Since the Saskatchewan River is the common water source for both the reservoir and the downstream delta, there is limited variability in water chemistry among locations sampled in this study. Contemporary water samples are neutral to basic, with moderate solute and DOC concentrations (Appendix, Table C2.S1). Nutrient levels from each location can be classified as eutrophic, with the exception of mesotrophic levels of chlorophyll α observed across sites (Nürnberg 1996).

2.4.2 Historical trends in fish [Hg]

Initial sampling in 1970 revealed mean [Hg] above or nearing modern consumption guidelines (0.5 µg/g wet weight; Health Canada 2007) in fillets of all species at both TL and CL, with values ranging from 0.3-1.8 µg/g in goldeye, <0.1-2.0 µg/g in northern pike, 0.5-3.5 µg/g in walleye, and 0.7-2.6 µg/g in sauger. Comparing rates of Log[Hg] decline within species across TL and CL fishes revealed significant rates of [Hg] decline in both sites for all species (Fig. 2.3; Table 2.1), though the strength of the regression varied (r^2 range from 0.03 to 0.47; $P < 0.001$).

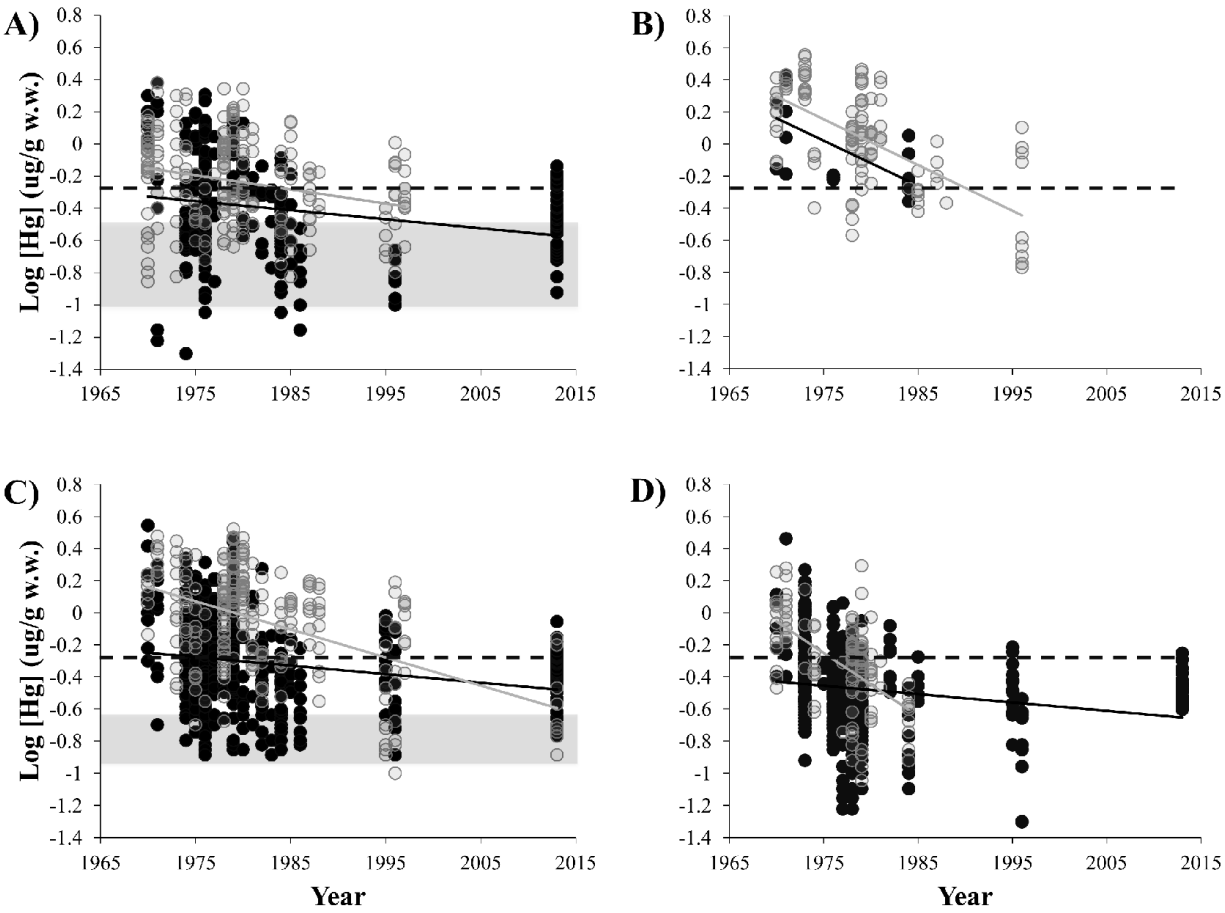

Fig. 2.3 Historical Log[Hg] in fish muscle vs. sampling year (1970-2013) for species sampled in Tobin Lake (TL; grey) and Cumberland Lake (CL; black): a) northern pike (TL, n = 215; CL, n = 275), b) sauger (TL, n = 114; CL, n = 21), c) walleye (TL, n = 232; CL, n = 483), and d) goldeye (TL, n = 118; CL, n = 396). The horizontal dashed black line represents the current consumption guideline, 0.5 µg/g (Health Canada, 2007), shaded areas represent reference lake mean Log[Hg] (± SD) of filets from northern pike (Fig. 2.3a; collection dates 1971-2002; n = 89) and walleye (Fig. 2.3c; collection dates 1970-2002; n = 96). See Table 2.1 for regression analyses and Table 2.2 for ANCOVA results.

Table 2.1 Annual rates and regression results of Log[Hg] decline over year from the muscle of northern pike, sauger, walleye, and goldeye populations sampled from Tobin Lake (TL) and Cumberland Lake (CL) between 1970 and 2013.

Species	**N**	**% Annual [Hg] Decline**	**r^2**	**F**	**P**
Northern Pike$_{TL}$	215	0.9	0.06	14.70	< 0.001
Northern Pike$_{CL}$	275	0.6	0.05	14.56	< 0.001
Sauger$_{TL}$	114	2.9	0.41	76.97	< 0.001
Sauger$_{CL}$	21	2.8	0.47	16.62	< 0.001
Walleye$_{TL}$	232	1.8	0.25	78.08	< 0.001
Walleye$_{CL}$	483	0.5	0.04	21.63	< 0.001
Goldeye$_{TL}$	118	3.9	0.33	55.80	< 0.001
Goldeye$_{CL}$	396	0.5	0.03	11.63	< 0.001

Location explained significant variation in [Hg] in walleye and goldeye (Table 2.2), but the interaction term was significant for these two species, suggesting a more rapid decline in TL and thus limiting our ability to conclude that either site had higher concentrations for a given year.

Table 2.2 Analysis of covariance table comparing effects of location and year on Log[Hg] in the filets of northern pike (n = 490), sauger (n = 135), walleye (n = 715), and goldeye (n = 514) collected from Tobin Lake and Cumberland Lake between 1970 and 2013.

Species	d.f.	MS	F	P
Northern Pike:				
Location	1	0.262	3.2	0.076
Year	1	2.123	25.7	<0.001
Location*Year	1	0.110	1.3	0.248
Sauger:				
Location	1	0.003	0.1	0.804
Year	1	2.242	41.35	<0.001
Location*Year	1	0.000	0.0	0.956
Walleye:				
Location	1	3.391	45.7	<0.001
Year	1	7.592	102.2	<0.001
Location*Year	1	2.161	29.1	<0.001
Goldeye:				
Location	1	2.440	34.7	<0.001
Year	1	3.760	53.5	<0.001
Location*Year	1	2.204	31.3	<0.001

For pike and sauger, [Hg] decline was similar between locations (Table 2.1, Table 2.2, Fig. 2.3). Interaction terms were removed from both the pike and sauger analyses due to non-significance. Subsequent equal slopes analyses of the simplified model indicated effects of location and year for both species (pike location: $F = 29.77$, $p < 0.001$; year: $F = 27.46$, $p < 0.001$; sauger location: $F = 5.66$, $p = 0.019$; year: $F=93.23$, $P<0.001$). Holm-Sidak post hoc tests revealed significant differences in mean [Hg] between locations in both pike ($t = 5.456$, $p < 0.001$) and sauger ($t = 2.379$, $p = 0.019$), with higher concentrations in TL fishes for both species.

Length-adjusted [Hg] in all fish showed similar trends to those observed in non-adjusted data sets, showing significant declines in all species in both locations (Appendix: Tables C2.S2, C2.S3; Fig. C2.S1). Significant rates of decline were observed across sites and species ($p \leq 0.001$). Interaction terms were removed from both the pike and sauger analyses due to non-significance. Subsequent equal slopes analyses indicated effects of location and year for both species (pike

location: $F = 24.48$, $p < 0.001$; year: $F = 21.68$, $p < 0.001$; sauger location: $F = 4.75$, $p = 0.031$; year: $F = 89.97$, $p < 0.001$). Holm-Sidak post-hoc analysis revealed significant differences remained between locations when considering length adjusted Log[Hg] against year in pike ($t = 4.948$, $p < 0.001$) and sauger ($t = 2.179$, $p = 0.03$), with higher concentrations in TL fishes for both species.

While periods of data availability for the reference lakes were typically shorter than those for TL and CL, these former lakes exhibited limited change in fish [Hg] over time. There were no significant changes over time in Candle Lake pike (1992-2002, $n = 30$, $p = 0.131$), Montreal Lake pike (1970-1988, $n = 27$, $p = 0.308$), Montreal Lake walleye (1983-1988, $n = 15$, $p = 0.350$), Big Sandy Lake pike (1978-1999, $n = 32$, $p = 0.083$), or Big Sandy Lake walleye (1978-1999, $n = 39$, $p = 0.883$). Candle Lake walleye (1970-2002, $n = 42$, $p < 0.001$) had a slight positive increase in [Hg] over time.

2.4.3 Contemporary predictors of fish [Hg]

In the contemporary walleye samples collected near (EBC) and far (CL-MR and CL-SR) from the dam, the interaction term was not significant, suggesting similar [Hg] vs. length relationships among sites ($F = 2.016$, $p = 0.136$, Fig. 2.4).

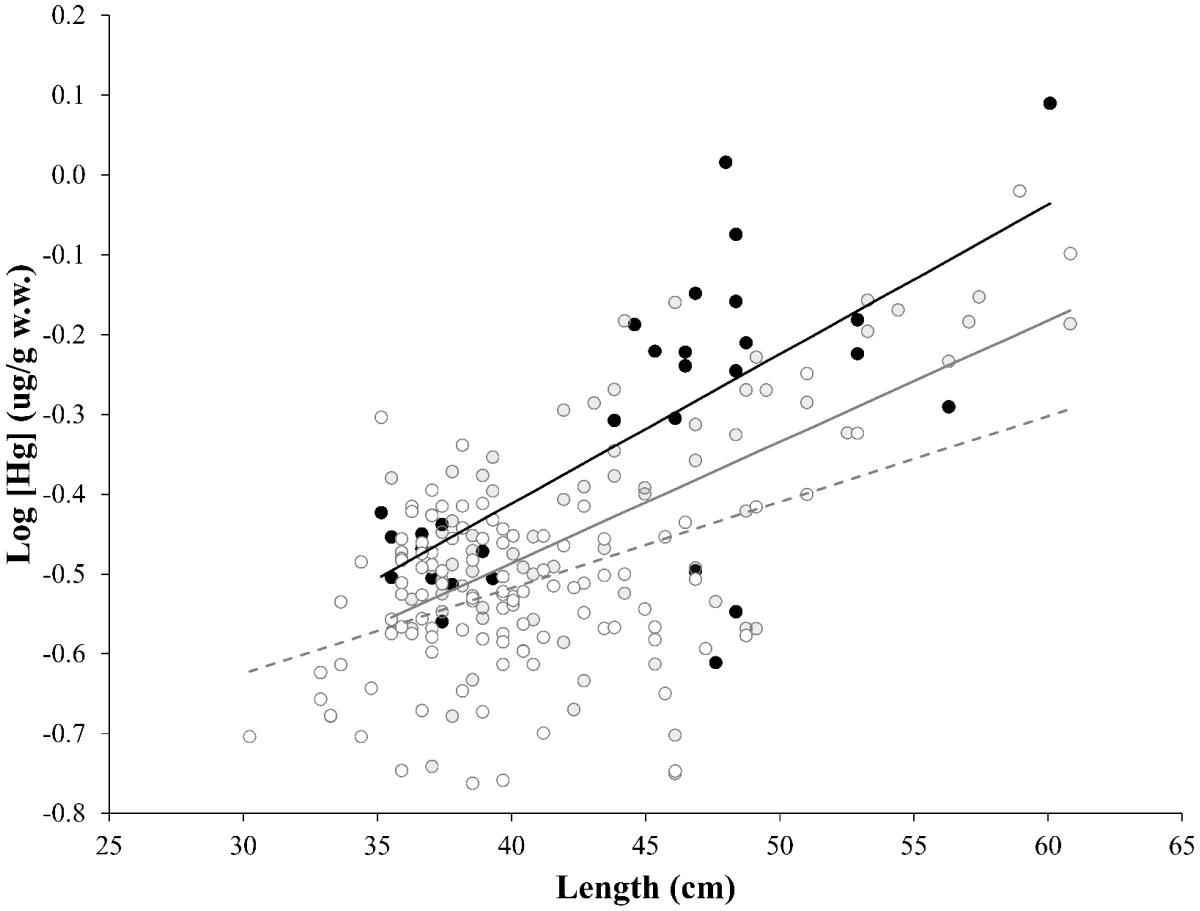

Fig. 2.4 Log[Hg] in contemporary muscle samples versus length (cm) for walleye collected from immediately downstream of the E.B. Campbell Dam (Solid circles, black best-fit line; n = 30), Cumberland Lake juncture with Mossy River (Shaded circles, grey best-fit line; n = 66), and Cumberland Lake juncture with the Saskatchewan River (Open circles, dashed best-fit line; n = 112).

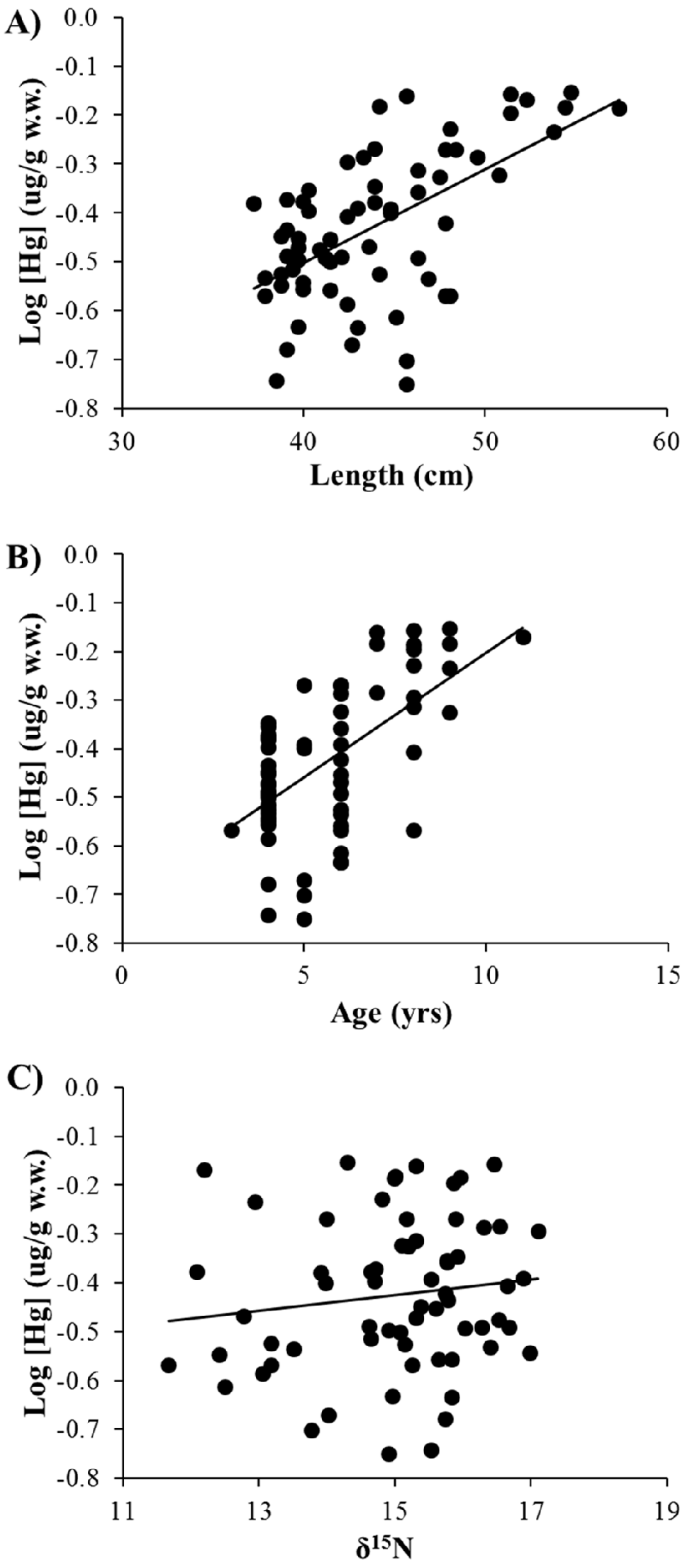

Fig. 2.5 Log[Hg] of muscle versus A) Length (cm) B) Age (years); and C) $\delta^{15}N$ (‰) in walleye sampled from the juncture of Cumberland Lake with Mossy River (2014; n = 65).

With the interaction term removed, length and location were both significant predictors of [Hg] ($F = 91.046$, $p < 0.001$; $F = 13.648$, $p < 0.001$). Holm-Sidak post-hoc analysis revealed that fish from immediately downstream of the dam have higher [Hg] than both CL-MR and CL-SR ($t = 3.665$, $p < 0.001$; $t = 5.224$, $p < 0.001$), while [Hg] from the two downstream locations were not significantly different from each other ($t = 1.876$, $p = 0.062$) (Fig. 2.4).

Within walleye from CL-MR, forward stepwise regression analysis revealed both age and length positively predicted [Hg] ($F = 33.777$, $p < 0.001$; $F = 33.011$, $p < 0.001$), and derivation of our strongest simplified model included only age, which proved to be the single best predictor of [Hg] ($p < 0.001$, $r^2 = 0.35$). Within this species, $\delta^{15}N$ proved an ineffective predictor of [Hg] ($F = 1.17$, $p = 0.284$) (Fig. 2.5).

The strength of the Log[Hg] vs. $\delta^{15}N$ regressions for food webs with multiple fish species varied among sites. There was a strong and significant relationship at TL ($r^2 = 0.85$, $F = 71.969$, $p < 0.001$), a weak but significant relationship at CL ($r^2 = 0.34$, $F = 50.212$, $p < 0.001$), and a non-significant relationship at EBC ($r^2 = 0.11$, $F = 3.093$, $p = 0.09$) (Fig. 2.6). Across sites both $\delta^{15}N$ and location were significant predictors of [Hg] ($F = 55.273$, $p < 0.001$; $F = 8.135$, $p < 0.001$). However, the differences in slopes resulted in a significant interaction term ($F = 5.998$, $p = 0.003$), indicating different Hg biomagnification among sites, possible differences in the structure of the food webs or insufficient characterization of the breadth of $\delta^{15}N$ at the EBC site. Estimated slopes at the three sites correspond to average increases in [Hg] per trophic level of 9.0 (TL), 2.9 (CL) and 2.6 (EBC) (Table 2.3).

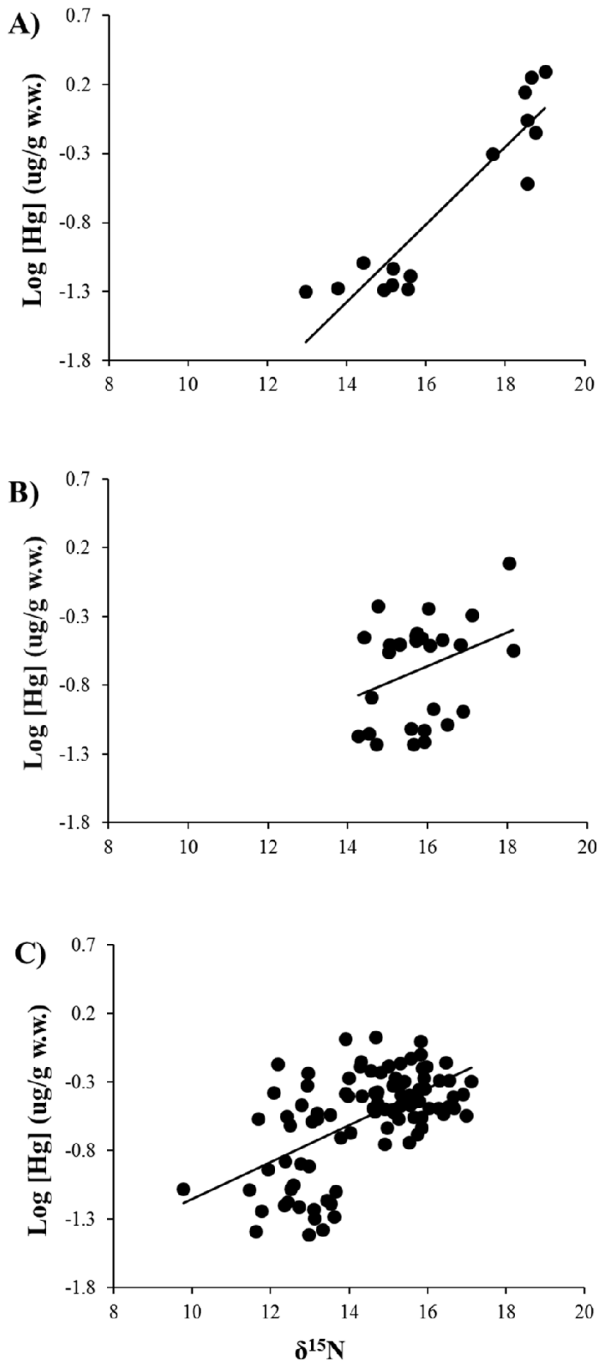

Fig. 2.6 Log [Hg] of muscle (pike, walleye) and whole-body homogenate (all others) versus $\delta^{15}N$ in A) Tobin Lake (walleye, n = 7, yellow perch, n = 3; spottail shiner, n = 5), B) Immediately downstream of the E.B. Campbell Dam (walleye, n = 17, yellow perch, n = 8; spottail shiner, n = 3), C) Cumberland Lake (including samples from the Cumberland Lake and Mossy River junctures with the Saskatchewan River; northern pike, n = 18, walleye, n = 65, spottail shiner, n = 6; blackchin shiner, n = 2; brassy minnow, n = 3; yellow perch, n = 6).

Table 2.3 Trophic Magnification Slopes (TMS), calculated from regressions of Log[Hg] v. $\delta^{15}N$ in fish muscle (northern pike, walleye) and whole-body homogenates (spottail and blackchin shiner, yellow perch, brassy minnow), and corresponding Trophic Magnification Factors (TMF) for Tobin Lake (TL), immediately downstream of the E.B. Campbell Dam (EBC), and Cumberland Lake (CL).

Site	n	TMS±SE	r^2	P	TMF±SE
TL	15	0.28±0.03	0.85	< 0.001	9.0±1.3
EBC	28	0.12±0.07	0.11	0.090	2.6±1.7
CL	100	0.14±0.02	0.34	< 0.001	2.9±1.2

2.5 Discussion

Analyses of a 40-year historical record revealed significant [Hg] decline within the muscle of multiple fish species in the TL reservoir and a downstream fishery at CL, providing new information on rates of [Hg] decline post-impoundment in an array of commercially relevant species (Bodaly et al. 2007; Anderson 2011). Further analyses of contemporary samples found length and age to be strong significant predictors of [Hg] in walleye, which will allow consumers to use either parameter to avoid consuming walleye that may yet retain high [Hg], or to guide more efficient walleye sample selection in the ongoing monitoring of [Hg] recovery in this system. $\delta^{15}N$ analyses proved useful in predicting [Hg] across species within sites, but not within species or across sites and suggested current [Hg] are at or approaching a new equilibrium in affected fish. Taken together these data can help to delineate the effective timeline of [Hg] decline from impounded and downstream fish contaminated as a result of hydroelectric reservoir construction, as well as the factors controlling rates of Hg uptake and clearance. These data can inform expected consequences of reservoir-initiated Hg inundation as the world develops an increasing reliance upon hydroelectric power generation (Finer and Jenkins 2012; Turkenburg et al. 2012).

Although we lack pre-dam data for these systems, the patterns of [Hg] decline beginning in 1970 agrees with the approximate seven-to-ten-year post-impoundment peak [Hg] observed for pike and walleye in similar systems (Bodaly et al. 2007; Schetagne and Therrien 2013). The higher [Hg] in walleye immediately downstream of the reservoir agrees with the persistent but attenuated export of Hg from reservoirs (Schetagne et al. 2000) despite the very low concentrations in contemporary water samples. The connection between TL and CL is strengthened by the consistent

patterns of [Hg] decline observed in the muscle of the four fish species studied, an occurrence unlikely in unrelated sites, and by the lack of chronological trends in fish [Hg] in reference lakes where concentrations were always below those in TL and CL (Lathrop et al. 1991; Harris and Bodaly 1998; Simoneau et al. 2005). As a result of declines in [Hg] from TL and CL fish over the last 40 years, the species studied are now, on average, below consumption guidelines (Fig. 2.3). The exception is CL sauger, though this disparity is likely an artifact of the truncated data set for this species as the existing trend suggests current [Hg] may be below guidelines. Notably, rates of [Hg] decline from pike in our data set (0.6% yr^{-1} or ~0.0016 d^{-1}) were on the same order of magnitude as those observed in pike in laboratory depurations (K = 0.00072-0.0011 d^{-1}) as summarized by Trudel and Rasmussen (1997). Disparities between the sizes of walleye between Trudel and Rasmussen's (1997) data and the present study preclude similar comparisons for that species.

Within our three piscivorous species, sauger exhibited relatively rapid declines in [Hg] compared to northern pike and walleye, which is consistent with its relatively short lifespan (Scott and Crossman 1973) rapidly turning over cohorts exposed to cleaner waters after peak methylation. As dietary uptake is the primary route of Hg exposure (Harris and Bodaly 1998; Pickhardt et al. 2006), the similarly rapid decline of [Hg] in TL goldeye support the conclusions from previous researchers that small-bodied, non-piscivorous species should show the most rapid [Hg] declines due the relatively short length of their food chain (Scott and Crossman 1973; Cabana et al. 1994; Moon et al. 1998; Bodaly et al. 2007). Significantly slower [Hg] declines were observed in CL goldeye and walleye, and converging trends of pike [Hg] suggest slower declines in the downstream population, though the interaction between the rates was not significant for this species. These patterns may represent an attenuated and more persistent MeHg exposure as the MeHg was transported between the sites (Schetagne et al. 2000). Though sauger data do not adhere to these trends, we suspect that a more robust sauger dataset would have unveiled similar patterns. It is also possible that benthic versus pelagic feeding habits of these species may account for differences in downstream exposure post-inundation (Eagles-Smith et al. 2008). Unfortunately, there is considerable overlap in the dietary habits of our predatory species, and differences in feeding habits were not possible to confirm as we did not conduct detailed stomach content analyses. Where rates of [Hg] decline were similar enough to be compared across sites within species (pike and sauger), TL [Hg] was consistently higher than CL.

As is the case with much of Northern Canada, the SRD is a critical foundation for the subsistence economies of Cree, Métis, and non-status First Nations peoples. Elevated [Hg] in fish became a concern for Cumberland House fisheries in the 1970s, though no cause could be ascribed (Waldram 1988). The parallel declines in [Hg] in TL and CL fish and higher [Hg] in walleye nearest the dam observed in this study coupled with similar observations in other reservoir influenced systems (Bodaly et al. 1984; Bodaly et al. 2007; Anderson 2011), suggests that the implementation of the TL reservoir upstream may be responsible for the biotic contamination. The fishery downstream was then susceptible to MeHg inundations from this source through aqueous and to a lesser extent biotic transport (Schetagne et al. 2000; Baker et al. 2009). At present, muscle [Hg] concentrations have fallen below consumption guidelines in three of four species, with trends in sauger data suggesting contemporary sauger [Hg] is below guideline [Hg] as well (Health Canada 2007). The human population of Cumberland House faced elevated [Hg] for decades following the implementation of the dam, and though maximum concentrations in the 1970s were lower than those in the well-documented Grassy Narrows incident in Northwestern Ontario (e.g. 15 µg/g in walleye muscle from Clay Lake in 1973; Kinghorn et al. 2007), further studies may be required to determine if any subclinical symptoms are evident in the human population (Takoaka et al. 2014).

In agreement with previous research, age and length were strong predictors of muscle [Hg] (Simoneau et al. 2005; Rasmussen et al. 2007) in walleye from the downstream sites. In contrast to the strong effects of length and age, $\delta^{15}N$ failed to predict Log[Hg] concentrations within this species (Jardine et al. 2012), likely reflecting the limited trophic variation in walleye over 15cm in length inhabiting similar habitats (Galarowicz et al. 2006). Taken together, these results support the standard use of walleye body length by various jurisdictions as a suitable proxy for [Hg] in fish consumption advisories (Ontario Ministry of the Environment 2015; Saskatchewan Ministry of Environment 2015).

With the exception of the EBC site, the observed significant TMSs were consistent with those predicted for northern latitudes (Lavoie et al. 2013), rather than being substantially lower, suggesting that current [Hg] in fish tissues has either reached or is nearing equilibrium. Average TMSs in freshwater are 0.16 ± 0.1 S.D, meaning values obtained from CL are well within expected ranges, while the TMS from TL slightly exceeds the upper range of the scale (Lavoie et al. 2013). The high value observed in TL may reflect that the predators from TL available for this analysis

were relatively large compared to those from EBC and CL, as by convention [Hg] were not standardized against length for trophic analyses (Lavoie et al. 2013). The divergence in these trends may also stem from sampling success, as prey species sampled from TL were only successfully captured in June of 2013 while those from EBC and CL were captured in August, which may have artificially inflated the [Hg] of prey species at the latter two sites relative to the former (Korthals and Winfrey 1987; Greenfield et al. 2013).

While previous studies have suggested <30 years are required for [Hg] to return to baseline in impounded fish (Bodaly et al. 2007) and in those downstream of impoundments (Anderson 2011), present data suggests a less optimistic timeline of upwards of 40 years. Further study will be required to fully describe the ultimate timeline of Hg inundation and decline, particularly because we lack pre-impoundment data. Future studies may corroborate the influence of reservoir liberated Hg on downstream fisheries by constructing ^{137}Cs and/or ^{210}Pb [Hg] timelines from affected and reference sediment profiles (Van Metre et al. 2004; Muir et al. 2009). These data are necessary to ensure that the increasing reliance on hydroelectric power generation does not come at the cost of human and environmental health. A more robust understanding of the dynamics of Hg within and downstream of an affected reservoir and its biota hold promise to ensure more ecologically sound implementation of future reservoirs.

2.6 Disclosure of conflict of interest

Funding for this study was partially provided by SaskPower, the crown corporation which operates the E. B. Campbell dam. SaskPower had no part in the collection, analysis, interpretation, or writing of this report. The final copy was submitted for their review and approval before submission for publication. Additionally, two authors are employees of Saskatchewan Ministry of Environment, and one works in the commercial fishery at Cumberland House.

CHAPTER 3:
ENERGY STORES AND MERCURY CONCENTRATIONS IN A COMMON MINNOW (SPOTTAIL SHINER; NOTROPIS HUDSONIUS) UP AND DOWNSTREAM OF A HYDROPEAKING DAM

Preface

This chapter was intended to compare the energy storage, body condition, and mercury concentrations across sites and month in shiner up and downstream of a hydropeaking dam. Hydropeaking is the alteration of water flow rates through turbines to efficiently tailor energy production to daily demand, and results in substantial changes in water flow rates downstream of the facility. Hydropeaking is known to elicit the stress response in fish, and the results of this chapter show that this stress may tax the energy stores of shiner, leaving them with less energy available for growth, and relatively higher [Hg] through reduced growth dilution. However, more information is required before concrete conclusions can be drawn, as there were no predictive relationships between these endpoints among populations studied.

Chapter 3 of this thesis is currently being prepared for publication in the Canadian Journal of Fisheries and Aquatic Sciences. The author contributions to Chapter 3 of this thesis were as follows:

Derek Green (University of Saskatchewan) co-designed study; collected, processed, and analyzed all field samples, performed all statistical analyses, and drafted the manuscript.

David Janz (University of Saskatchewan) provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Lynn Weber (University of Saskatchewan) provided scientific guidance; reviewed and revised the manuscript, providing comments and corrections.

Tim Jardine (University of Saskatchewan) co-designed study; aided in sample collection; provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

3.1 Abstract

Hydropeaking hydroelectric facilities release water from dams to increase or decrease energy production and match demand, often on a daily cycle. These fluctuating flows downstream can exert several potential stressors on organisms and may inhibit their growth, indirectly causing higher contaminant concentrations through reduced growth dilution. We collected shiner at two sites upstream and two sites downstream of a hydropeaking hydroelectric dam in east-central Saskatchewan and compared their body condition, triglyceride concentrations, and mercury concentrations. Condition declined from August to September in downstream fish and triglyceride concentrations were consistently lower downstream of the dam, with lowest concentrations at the site where hydropeaking is most severe ($p < 0.01$). Mercury concentrations were significantly greater at both downstream sites relative to upstream ($p < 0.01$). Despite these results, inconsistencies in physiological responses limited our ability to isolate the effects of hydropeaking as a direct cause of observed results, and suggest indirect effects such as shifts in algal and macroinvertebrate communities may be responsible. These findings highlight both direct and indirect effects of hydropeaking on downstream resident fishes.

3.2 Introduction

Hydroelectric generation is an integral source of renewable energy that accounts for 6% of annual global energy production, with production capacities expected to rise 73% by 2034, with 3100 large facilities either planned or under construction in the developing world (Turkenburg et al. 2012; Zarfl et al. 2015). While hydroelectric power is a critical component of renewable energy, the construction and operation of dams and reservoirs can cause mercury (Hg) bioaccumulation in, and impose environmental stress on, downstream fish and other organisms (Mailman et al. 2006; Poff and Zimmerman 2010).

Elevated [Hg] in fish have been observed in hydroelectric reservoirs across Canada, posing risks to fisheries and consumers (Bodaly et al. 2007; Anderson 2011; Chapter 2). Mercury concentrations in fish rise when chemical and physical changes associated with reservoir flooding induce iron and sulfate reducing bacteria to biomethylate inorganic environmental Hg (II) to MeHg, a bioaccumulative neurotoxin and teratogen (Kelly et al. 2003; Yu et al. 2011; Graham et al. 2012). [Hg] then peak in commercial sportfish (e.g. northern pike and walleye in 2-8 years post-impoundment (Bodaly et al. 2007; Willacker et al. 2016) with maximum concentrations increasing in proportion to the area flooded (Bodaly et al. 2007). After inundation, [Hg] return to baseline over the following decades with the ultimate time course depending upon the trophic levels and feeding habits of contaminated fish (Bodaly et al. 2007; Anderson 2011; Chapter 2).

Elevated [Hg] have also been observed in fish downstream of reservoirs as reservoir-discharged MeHg is advected to downstream environments (Schetagne et al. 2000; Anderson 2011; Chapter 2). Concentrations in river-dwelling fish downstream of dams can take significantly longer to return to baseline than those in reservoirs (Chapter 2). These persistently high concentrations in fish occur despite minimal [Hg] in the water column, suggesting that physiological factors such as growth rates may play a role (Trudel and Rasmussen 2006; Sousa et al. 2010; Sandheinrich and Drevnick 2016), and impinged growth rates have been observed in Atlantic salmon (*Salmo salar*) relative to controls in simulated hydropeaking conditions (Puffer et al. 2015). It is possible, therefore, that a delayed decline in [Hg] may be due to the concurrent effects of stressors imparted by hydropeaking on the downstream ecosystem.

Stressful events disturb the homeostasis of organisms, eliciting physiological and/or behavioral compensatory responses (Wendelaar Bonga 1997). These responses necessarily require energy that comes at the expense of energy required for other purposes, including growth, which

can increase [Hg] in fish by minimizing growth dilution, the reduction in contaminant concentrations within fish due to more efficient tissue production and growth (Ward et al. 2010; Sousa et al. 2010). Direct stressors on fish downstream of dams can be chemical or physical depending on the depth of the reservoir, release point of the dam, and dam type, and often include distinct differences between upstream and downstream water flows (Thornton et al. 1990; Bednarek 2001; Cooke et al. 2005). Hydropeaking facilities exert physical disturbances on downstream environments as they alter rates of water flow depending on daily energy demand (Young et al. 2011; Puffer et al. 2015). This hydrological stress can increase the risk of fish stranding, reduce spawning success, and reduce juvenile survival (Young et al. 2011), by affecting the growth and fat accumulation of fish due to trade-offs between growth and locomotion and energetic taxation of the stress response (Wendelaar Bonga 1997; Sousa et al. 2010; Puffer et al. 2015).

Based on these observations, we tested whether a small-bodied fish (spottail shiner; "shiner": *Notropis hudsonius*) living downstream of a hydropeaking dam with a history of mercury contamination (Chapter 2) on the Saskatchewan River, SK, showed evidence of reallocation of energy from growth to stressor responses. We predicted that fish downstream of the dam would have reduced energy stores, evidenced in the form of whole-body triglycerides and condition, compared with fish captured upstream. Further, we predicted that these effects would be accompanied by greater [Hg] due to reduced effects of growth dilution (Fig. 3.1). Finally, we predicted that these results would be most apparent at downstream sites with low-pitched shorelines that experience a greater degree of shoreline fluctuation in response to altered flow regimes.

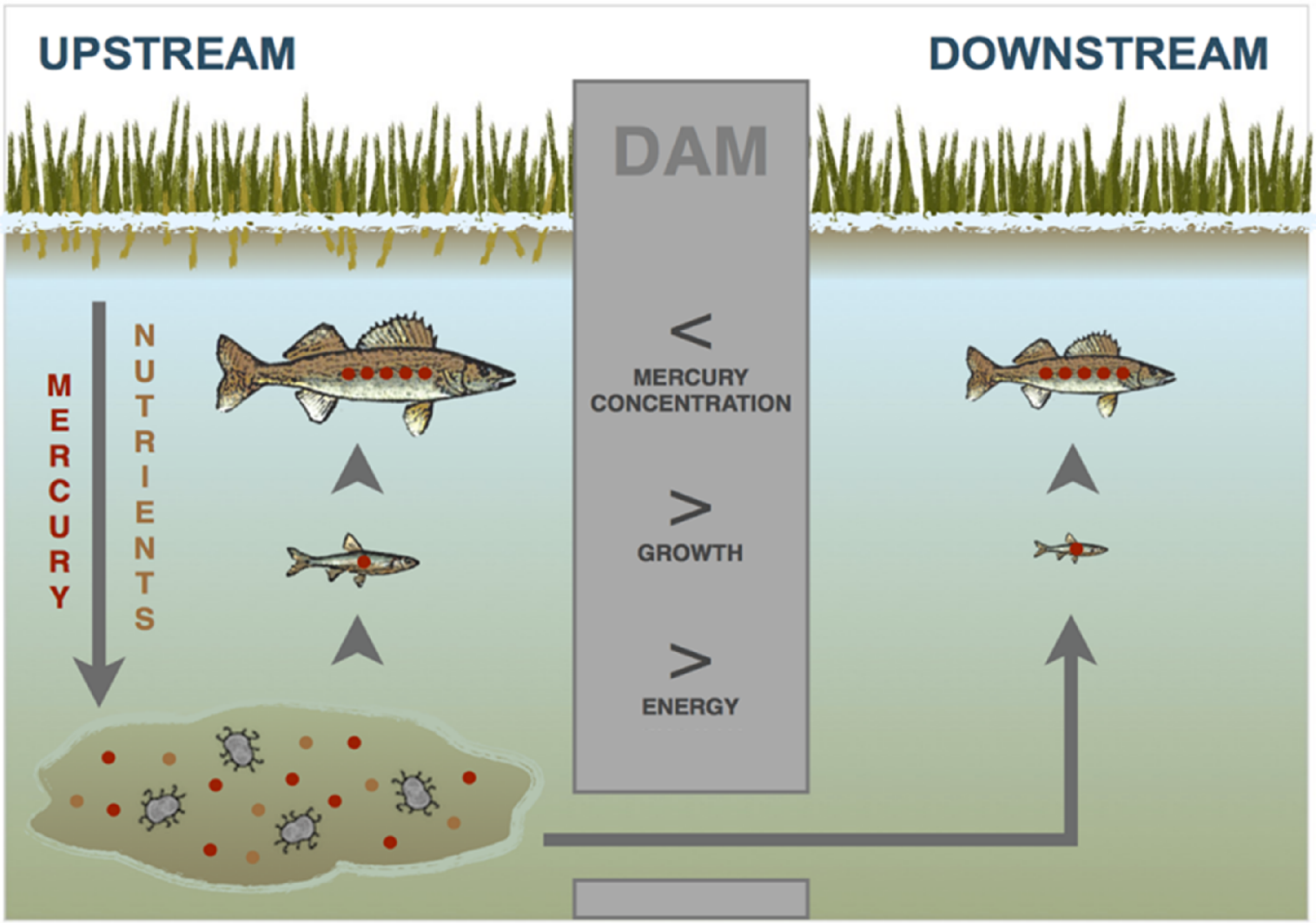

Fig. 3.1 Conceptual model depicting the predicted relationships across sites if there is a chronic stressor in the downstream environment, including relatively lower energy concentrations and growth, and elevated Hg concentrations in downstream fish through the effects of minimized growth dilution.

3.3 Methods

3.3.1 Study area

During August and September 2014, shiner were collected up- and down-stream of the E. B. Campbell hydroelectric dam (53°41’19”N 103 °20’50”W), which is found on the terminal arm of the Saskatchewan River before it empties into the Saskatchewan River Delta (Fig. 3.2). The E. B. Campbell dam is a hydropeaking dam that produces marked change in downstream hourly flows relative to those measured upstream (Fig. 3.3). Daily changes in flow cause between 0.2 and 1.4 meter changes in depth in downstream waters, depending on channel morphology. Shoreline elevation gradients temper or exacerbate the influence of hydropeaking, with steep shorelines seeing minimal lateral depth fluctuations relative to sites with low pitched shores (Fig. 3.4).

Fig. 3.2 The Saskatchewan River and study sites surrounding the E. B. Campbell Dam in 2014, including A) Weldon Ferry, B) Water level gauge 05KD007, C) Wapiti, D) Codette Lake, E) Tobin Lake, F) E. B. Campbell Dam, G) Water level gauge 0KD5003, H) Downstream site 1 (DS1), and I) Downstream site 2 (DS2). Figure modified from Mihalicz et al., unpublished.

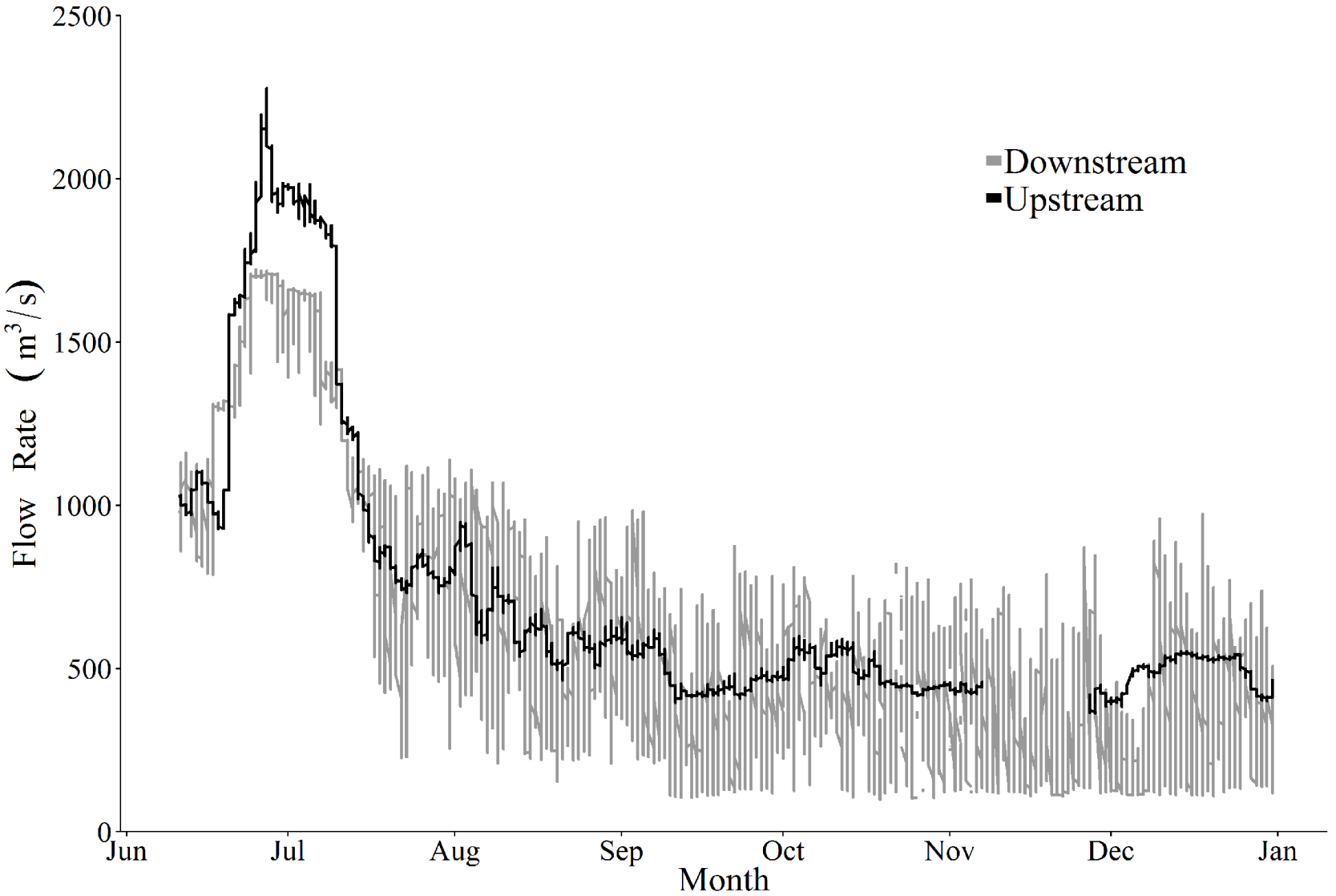

Fig. 3.3 Comparison of flow rates up and downstream of the E. B. Campbell hydroelectric dam in 2014. Hydrographic data obtained from the Water Survey of Canada gauges 05KD007 and 05KD003.

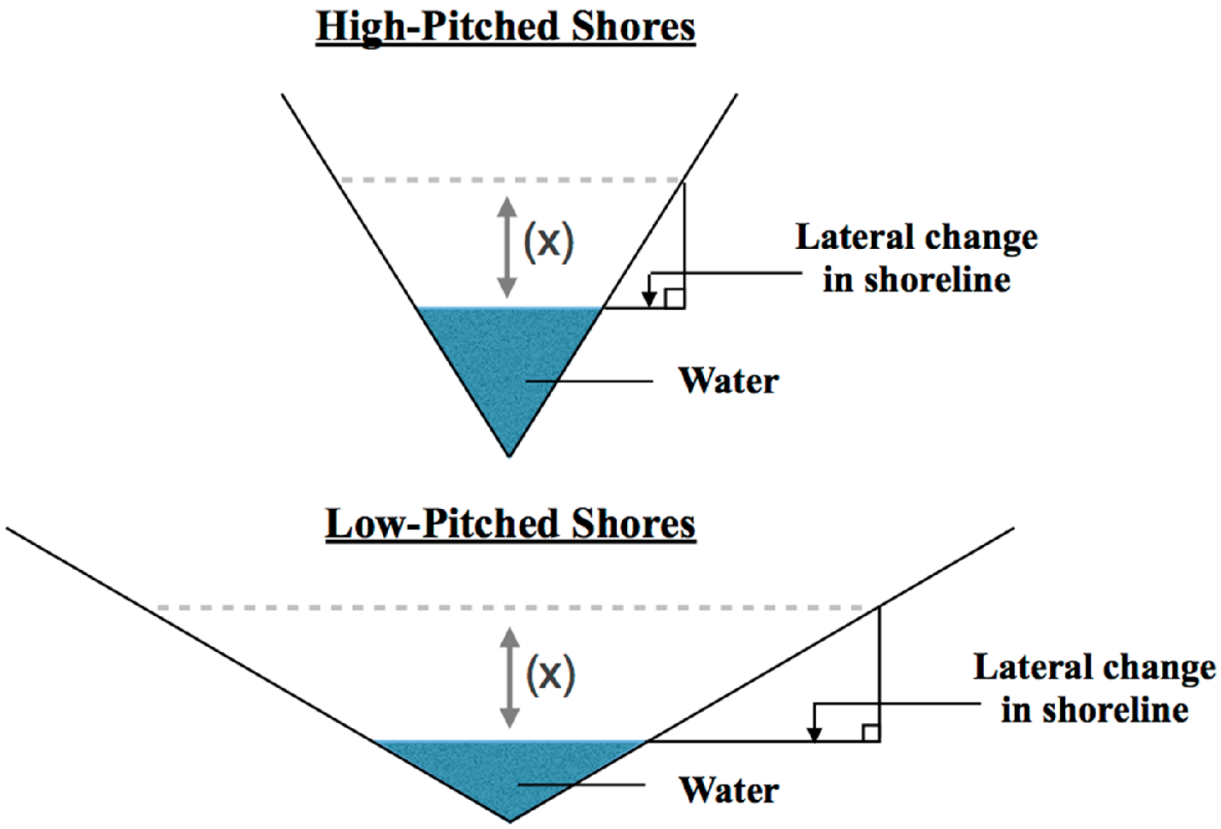

Fig. 3.4 Conceptual diagram of the relationship between shoreline pitch and lateral shoreline fluctuation for a given change in water level (X). Diagram not to scale of sites under study.

Upstream sites were selected on both the South Saskatchewan River at Weldon Ferry (53°10’58”N 105°9’49”W) and on the Saskatchewan River at Wapiti (53°13’14”N 104°41’11”W, Fig. 3.1). This second location was selected to represent the river reach that was downstream of the confluence of the North and South Saskatchewan rivers while remaining upstream of the transition zone into Codette Lake, the reservoir of the Francois Finlay Hydro Station (Thornton et al. 1990; Cooke et al. 2005). Downstream sites 1 (DS1; 53°42’53”N 103°17’18”W) and 2 (DS2; 53°43’45”N 103°7’57”W) were selected to represent sites with different pitched shoreline, which translates to substantial differences in lateral shoreline change (Appendix C3.S1). DS1 has a deeper main channel, while stranded fish have been observed at DS2 after downramping.

3.3.2 Species selection and sampling

Shiner are recommended as a sentinel species for studying environmental effects of hydroelectric dams by Wells and House (1974) and are ideal for this purpose for several reasons. The range of North American shiner has high overlap with areas of hydroelectric development (Page et al. 1991; US EIA 2014). The species typically spawns in June through July (Peer 1966; Wells and House 1974), leaving small, young-of- the-year (YOY) shiner with minimal time to acquire the size and energy stores needed to maximize the probability of over-winter survival (Shuter and Post 1990; Biro et al. 2004; Perez and Munch 2010), particularly in a northern environment where shiner are generally smaller, and colder winter climates increase stranding risks (Peer 1966; Saltveit et al. 2001). Young of the year shiner preferentially inhabit shallower waters making them susceptible to the influence of high hydropeaking magnitudes (Wells and House 1974) but also allow their capture via bag seine during sampling. Age classes of shiner can be approximated by their length (Wells and House 1974) with mean and modal fork lengths of YOY reaching 30-77 mm by the end of year one at Canadian latitudes, with length generally inversely related to latitude (Peer 1966; Wells and House 1974; Bond and Berry 1980). Finally, shiner are abundant, indicating they can be studied using lethal sampling without depleting the population (Page et al. 1991; International Union for Conservation of Nature 2013).

Each site was sampled in each month for a total of eight sample sets. Monthly collections took place within a seven-day span to minimize the influence of within-month change on across-month analyses of physiological endpoints. Fish were captured using a bag seine, with each netting taking 10-15 minutes, and were immediately euthanized with a sharp blow to the head and stored

in pre-labeled 50 ml falcon tubes (Corning Science; Reynosa, Mexico). Samples from Weldon Ferry, DS1, and DS2 were immediately snap frozen in liquid nitrogen, while samples from the Wapiti site were held on ice for upwards of two hours before being snap frozen due to the remoteness of the site. Samples were then stored in the lab at -80^{o}C before further processing.

At each month we took spot measurements of temperature, pH, turbidity and conductivity up and downstream of the dam. Aqueous [Hg] are already known to be minimal (low ng/L) in this system (Chapter 2), but low-level mercury analyses were conducted regardless in July and September.

3.3.3 Sample preparation and analysis

Analyses were restricted to juvenile YOY shiner to minimize the confounding influence of ontogeny on physiological endpoints, and to address effects on shiner at a critical life stage.

Mass-at-length relationships were used as a measure of condition, and triglycerides were selected as our energetic biomarker as they are the primary metabolic swimming fuel in fish under non-fasting conditions (Moyes and West 1995). Triglyceride analyses were conducted on whole-body homogenates in citrate buffer using the colourimetric assay protocol developed and validated by Weber et al. (2003; 2008). Sex ratios were presumed to be approximately equal in sampled YOY populations as per the observations of Wells and House (1974), and sex effects were therefore presumed homogeneous if sampled fish were YOY, All homogenates were subject to a 5-minute heat block treatment (100^{o}C) immediately after homogenization to inactivate amylase, as this homogenization protocol is also used in glycogen analyses . Concentrations were quantified using a glycerol standard curve and plated in duplicate, with values accepted at CV<15%. Glycerol standard (G7793), free glycerol (F6428) and lipase reagents (T2449) were all purchased from Sigma-Aldrich, Oakville, Ontario, Canada. Citrate buffer was made using sodium citrate dihydrate (S279-500; Fair Lawn, New Jersey, USA) and nano-pure water and adjusted to pH 5 with hydrochloric acid. Samples were homogenized for biochemical analysis using techniques developed by Weber et al. (2003; 2008) and described by Thomas and Janz (2011). Samples were analyzed in duplicate using a SpectraMax 190 spectrophotometer (Molecular Devices Corp, Sunnyvale, California, USA).

[Hg] was measured by desiccating homogenates in a drying oven at 50^{o}C in order to maximize Hg recovery (Ortiz et al. 2002). Homogenized samples were crushed to a fine powder

using an acid-washed glass stirring rod and analyzed for total Hg in a Direct Mercury Analyzer (DMA 80, Milestone, Shelton, Connecticut, USA). Total Hg measures were considered a suitable proximal measure of MeHg, as >90% of Hg in fish tissues is methylated (Bloom 1992; Jackson 1991; Greenfield and Jahn 2010). Sample masses analyzed ranged from 10 to 30 mg depending upon homogenate volume. Recoveries of Hg were estimated from the dogfish muscle (DORM-4) certified reference material analyzed concurrently (National Research Council, Ottawa, Ontario, Canada). Recovery of this CRM was 94 ± 4% (n = 58). Blank sample boats contained less than half the detection limit of Hg (0.04 ng), and samples were not blank corrected. Dried [Hg] were converted to wet concentrations assuming 75% moisture (May et al. 2009; Lavoie et al. 2013).

3.3.4 Statistics

All data were initially assessed for normality and equality of variance using visual inspection (Zuur et al. 2010). Mass, length, and triglyceride concentration were $\log_{10}$ transformed to improve normality and homogeneity of variance. Before testing for effects across sites, statistical models were derived from maximal models to test for the influence of physiological covariates on mass, triglycerides, and [Hg] (Johnson and Omland 2004; Lenth 2016). Covariates for mass included length and triglyceride concentration, while maximal triglyceride models included length and condition factor ([K]; $K = 100\,000*W(g)/L(mm)^3$; Fulton 1904), and maximal Hg models included length, triglyceride concentrations, and K as covariates. Month and site were included as main effects in all maximal models. Maximal models included three-way interactions between site, month, and individual covariates, as well as all two way interactions between each pair of these variables. Analyses also included two-way interactions between covariates and main effects of all predictors. Minimally adequate models were selected on the basis of the AIC criterion (Johnson and Omland 2004; Aho et al. 2014). Terms were removed starting with highest order interactions based on three criteria: a) their removal provided less than a +2 change to AIC, b) term removal did not cause significant change in model deviance, and c) terms were not a component of a higher order interaction. All term removals were considered before individual terms were removed. Distribution, normality, and leverage of residuals were then visually inspected using residuals versus fitted values, standardized residuals versus normal Q-Q, scale-location, and residuals versus leverage plots before conducting hypothesis testing on minimally adequate models.

Main effects and covariates of minimally adequate models were analyzed using the Anova function from the 'car' package for R using type III sums of squares to account for imbalanced sample design (Fox and Weisberg 2011). Post-hoc analyses were conducted using least-squares trends and least-squares means analyses to account for unequal sample sizes and the presence of covariates (Lenth 2016). To account for multiple comparisons, all post-hoc results were considered significant at $p < 0.01$ to minimize the chances of type I error without unduly losing power. All analyses were conducted using R (RStudio, V. 0.99.893; RStudio Team 2015; R Core Team 2015).

3.4 Results

3.4.1 Water [Hg] and chemistry

Water chemistry parameters were not statistically analyzed as only spot-measures were taken during sampling, but minimal differences were observed in parameters between up and downstream samples barring turbidity, which was generally lower downstream (Table 3.1).

Table 3.1 Water chemistry parameters for sites combined upstream (measures taken on North and South Saskatchewan River forks pre-confluence and Wapiti; n = 3) and downstream (DS1 and 2; n = 2) in August and September of 2014.

Month	Sample Location	Temperature (°C)	pH	Turbidity(NTU)	Conductivity (µS/cm)
August	Upstream	22.4±2.2	8.57±0.28	8.84±5.14	450±14
August	Downstream	22.5±0.2	8.45±0.47	3.44±0.44	453±16
September	Upstream	15.9±2.1	8.63±0.10	6.78±1.66	NA
September	Downstream	13.9±0.1	8.62±0.06	3.1±0.52	NA

Low level Hg analyses confirmed that concentrations both up and downstream of the dam were low (July, downstream = 1.2 ± 0.2 ng/L, n = 2; September, upstream = 1.3 ng/L, n = 1; downstream = 0.9 ± 0.1 ng/L, n = 2) (Sorenson et al. 1990; Kelly et al. 1995; Chapter 2).

3.4.2 Condition analyses

Mean shiner lengths ranged from 32 to 47 mm across months and sites, and shiner were presumed to be YOY based on length-age relationships derived in prior work that used scale annulus counts and length distributions for shiner at similar latitudes (Peer 1966; Wells and House 1974; Suns and Reese 1978). Fish were generally smallest at Weldon Ferry (Table 3.2).

Table 3.2 Descriptive statistics (ranges and mean ± standard deviation) for length, mass, triglycerides concentration, condition (K), and mercury concentration of spottail shiner sampled in August and September of 2014 at Weldon Ferry, Wapiti, Downstream Site 1 (DS1), and Downstream Site 2 (DS2).

			Total Length (mm)		Mass (g)		Triglycerides (mg/g)		Condition (K)		Mercury (ng/g)	
Month	Site	n	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
August	Weldon Ferry	31	22 - 41	32 ± 4	0.09 - 0.58	0.28 ± 0.11	0.53 - 3.16	1.43 ± 0.54	0.43 - 1.00	0.82 ± 0.09	21.5 - 73.3	44.9 ± 13.9
	Wapiti	21	31 - 47	41 ± 4	0.26 - 0.89	0.59 ± 0.13	0.50 - 2.13	1.19 ± 0.46	0.73 - 0.97	0.84 ± 0.06	31.1 - 84.1	46.1 ± 12.0
	DS1	21	33 - 51	40 ± 4	0.31 - 1.02	0.56 ± 0.18	0.78 - 2.80	1.45 ± 0.64	0.69 - 0.97	0.82 ± 0.07	34.4 - 78.6	52.9 ± 10.4
	DS2	37	32 - 47	39 ± 4	0.26 - 0.77	0.51 ± 0.11	0.39 - 3.68	0.84 ± 0.52	0.66 - 0.94	0.82 ± 0.07	24.7 - 62.6	46.5 ± 10.3
September	Weldon Ferry	10	26 - 42	32 ± 5	0.19 - 0.57	0.29 ± 0.13	1.04 - 2.42	1.75 ± 0.44	0.77 - 1.18	0.88 ± 0.13	20.8 - 51.1	41.9 ± 12.5
	Wapiti	18	33 - 43	38 ± 2	0.29 - 0.65	0.46 ± 0.08	1.41 - 3.74	2.61 ± 0.76	0.75 - 0.93	0.82 ± 0.05	25.2 - 98.8	42.0 ± 17.1
	DS1	31	37 - 57	47 ± 5	0.37 - 1.46	0.80 ± 0.28	0.68 - 4.01	1.94 ± 0.78	0.67 - 0.84	0.74 ± 0.04	35.0 - 76.3	53.6 ± 9.2
	DS2	34	31 - 52	42 ± 5	0.17 - 1.09	0.61 ± 0.19	0.56 - 2.53	1.28 ± 0.51	0.58 - 0.91	0.78 ± 0.07	35.0 - 89.3	56.0 ± 12.6

The maximal model predicting fish mass was statistically significant ($F = 212.7$, degrees of freedom (df) = 24 and 161, $p < 0.001$, adjusted $r^2 = 0.97$) with an AIC of -1187.38. The minimally adequate model was also statistically significant ($F = 350.4$, df = 15 and 183, $p < 0.001$, adjusted $r^2 = 0.96$) with an AIC of -1282.90, and resulted in removal of the triglyceride term. The fitted model displayed acceptable homogeneity of variance, normality, and leverage of residuals. Two residuals caused a left skew, but neither was exerting excessive leverage so both points were left in the model. Analysis of the main effects and covariates of the minimally adequate model revealed a significant relationship between mass and the covariate length ($F = 1467.7$, df = 1, $p < 0.001$), a significant two-way interaction between month and site ($F = 4.0$, df = 3, $p = 0.009$), and a significant three-way interaction between month, site, and length ($F = 3.7$, df = 3, $p = 0.013$; Fig. 3.5). Pairwise least-squares trends analyses of mass-at-length slopes revealed no interactions between the slopes of mass at length across sites or month barring DS2 August and DS2 September ($p < 0.01$), precluding least-squares means comparisons across months at this site. No significant differences were found between mass-at-length relationships across sites within either August or September. Only DS1 showed a significant change in mass-at-length relationships across months, with a lower mass for a given length in September compared to August ($p < 0.01$).

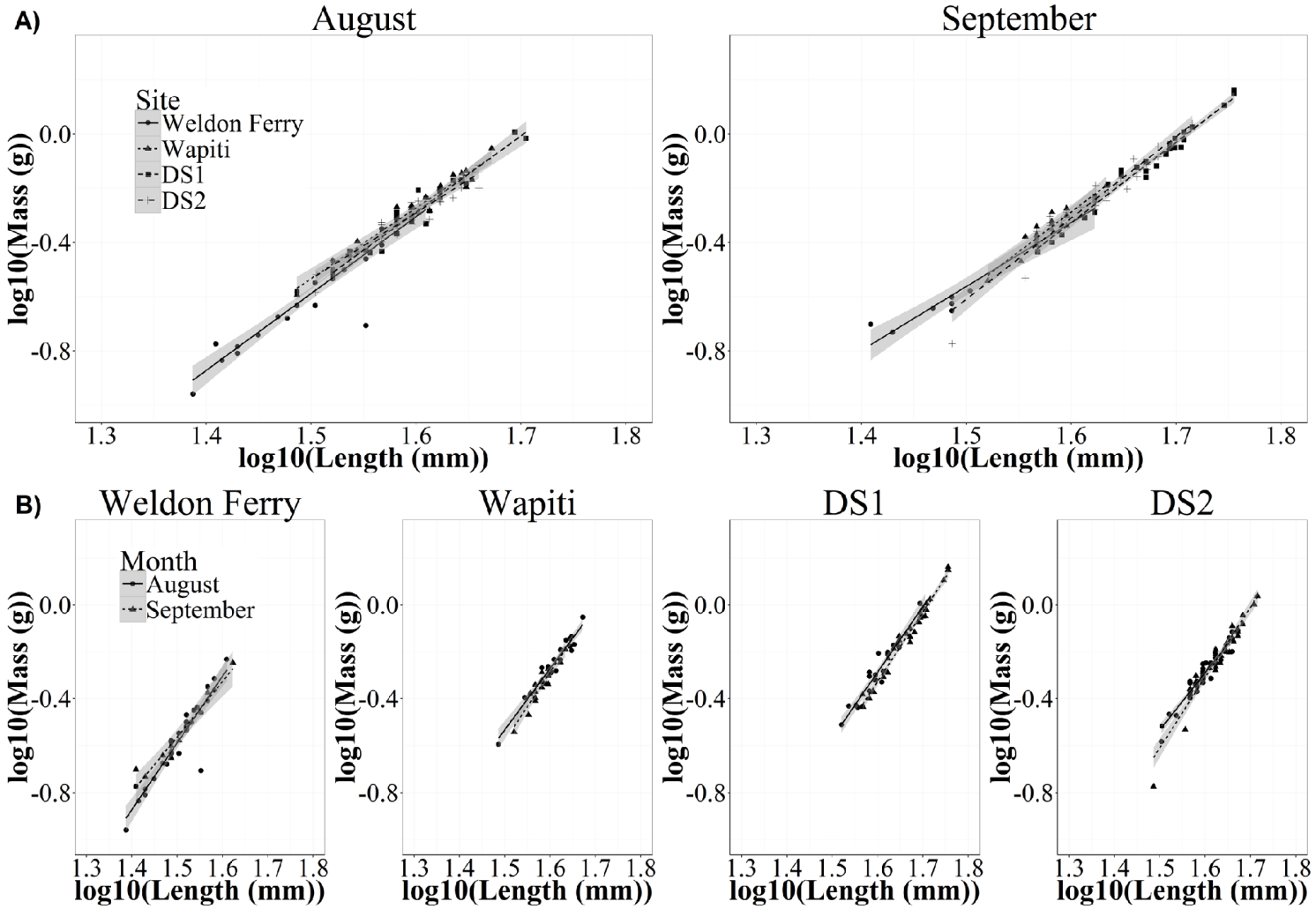

Fig. 3.5 Log transformed mass-length relationships with 95% confidence intervals (shaded) compared A) within months across sites, and B) within sites across months for spottail shiner captured in August and September of 2014 (Weldon Ferry: August [n = 31], September [n = 9]; Wapiti: August [n = 20], September [n = 18]; DS1: August [n = 21], September [n = 29]; DS2: August [n = 37], September [n = 34]).

3.4.3 Triglyceride analyses

The maximal model for predicting triglyceride concentration was statistically significant ($F = 8.3$, $df = 24$ and 161, $p < 0.001$, adjusted $r^2 = 0.49$) with an AIC of -675.4. The minimally adequate model was also significant ($F = 16.2$, $df = 11$ and 177, $p < 0.001$, adjusted $r^2 = 0.47$) with an AIC of -692.2. Condition and interactions involving condition were completely removed from the minimally adequate model. Normality, and fit and leverage of residuals all indicated good model fit. One point caused observable right-skew, but the point was left in the model as it did not exert excessive leverage. Analysis of the main effects of the minimally adequate model showed significant effects for month ($F = 53.9$, $df = 1$, $p < 0.001$) and site ($F = 3.4$, $df = 3$, $p = 0.018$), and a significant relationship with the covariate, length ($F = 12.6$, $df = 1$, $p < 0.001$). There were also significant two-way interactions between month and site ($F = 6.97$, $df = 3$, $p < 0.001$) and site and length ($F = 4.09$, $df = 3$, $p = 0.008$). Pairwise least-squares trends comparisons of the slopes of triglyceride concentration-at-length revealed parallel slopes within all sites across months, and significant interactions within month across site ($p < 0.01$). Within month, equal slopes were found between DS2 and DS1, and DS1 and all other sites, allowing for least-squares means comparisons within those groupings as well as within sites across month. Overall, DS2 stood out for its significantly low triglyceride concentration-at-length compared to other sites. Within August, significantly lower triglyceride concentration-at-length were found in DS2 compared to DS1, though DS1 was not significantly different from any other sites (Fig. 3.6). Significantly greater triglyceride concentrations were also found at Weldon Ferry compared to Wapiti ($p < 0.01$). No direct comparisons could be made between DS2 and Weldon Ferry and Wapiti within August due to the interactions between their slopes. Within September, triglyceride concentration-at-length were similar between DS2 and DS1, and DS1 triglyceride concentration-at-length were significantly lesser compared to Wapiti but not compared to Weldon Ferry, and Weldon Ferry and Wapiti were not significantly different from each other ($p > 0.01$). Interactions between triglyceride-at-length relationships precluded direct comparisons between DS2 and Weldon Ferry and Wapiti sites. Within sites, both DS2 and Wapiti had significantly greater triglyceride concentration-at-length in September compared to August ($p < 0.01$).

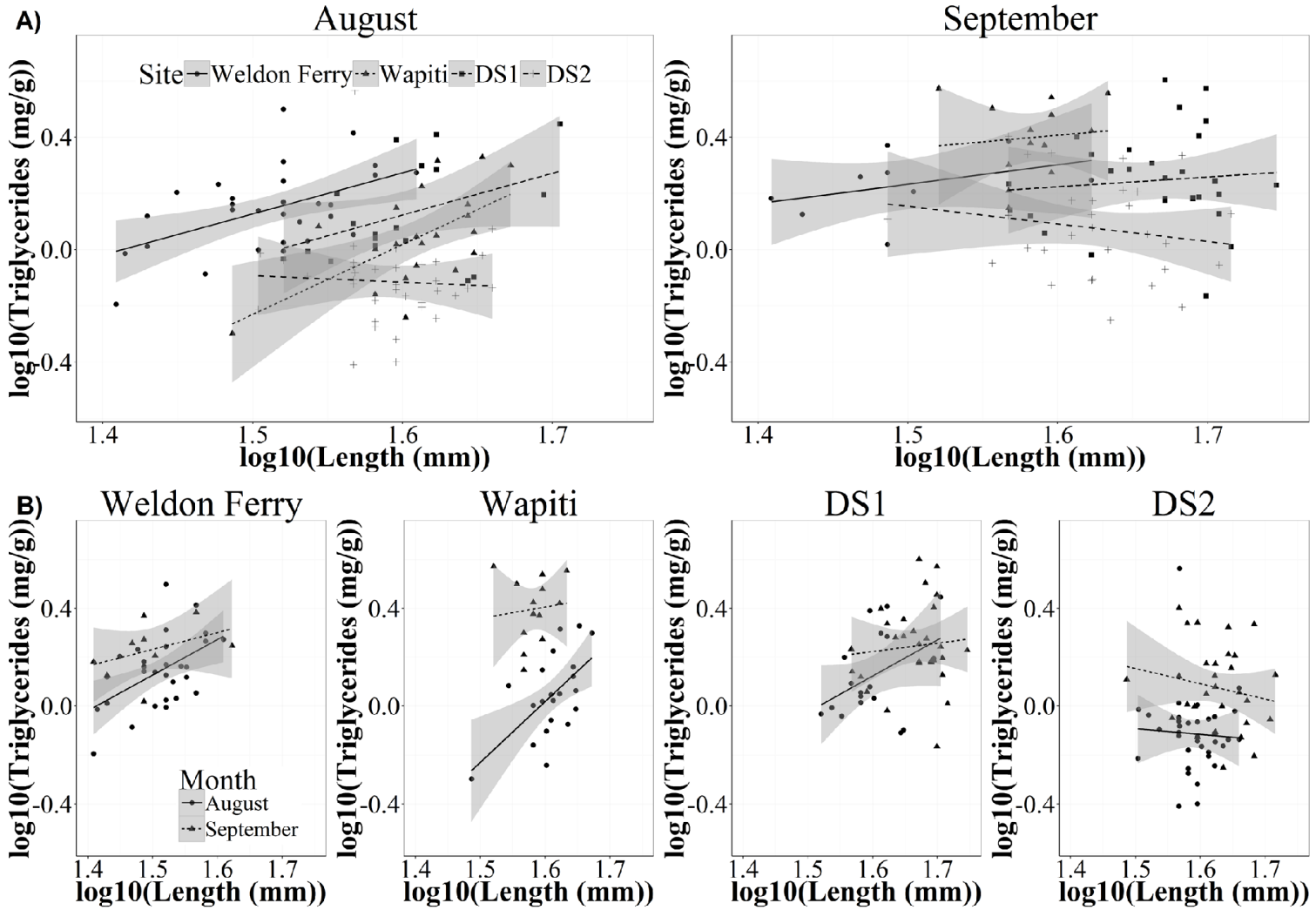

Fig. 3.6 Log transformed triglyceride-length relationships with 95% confidence intervals (shaded) compared A) within months across sites and B) within sites across months for spottail shiner captured in August and September of 2014 (Weldon Ferry: August [n = 31], September [n = 9]; Wapiti: August [n = 21], September [n = 13]; DS1: August [n = 18], September [n = 31]; DS2: August [n = 35], September [n = 31].

3.4.4 Hg analyses

The maximal model predicting [Hg] was statistically significant, but model fit was lower than models for mass-at-length (condition) and triglycerides ($F = 1.7$, $df = 34$ and 125, $p < 0.015$, adjusted $r^2 = 0.14$; AIC = -674.78). The minimally adequate model excluded all covariates but length, and was also statistically significant ($F = 4.1$, $df = 11$ and 161, $p < 0.001$, adjusted $r^2 = 0.167$; AIC = -759.36). Assumption of residual normality, homogeneity of variance, and leverage of residuals were satisfied upon visual inspection. The model residuals showed slight departures from normality, with one particular point causing a right-skew. Despite minor deviations from normality, no residuals were causing undue leverage in the model, and no points were removed. Main effects were significant for site ($F = 2.8$, $df = 3$, $p = 0.041$), but not month ($F = 0.07$, $df = 1$, $p = 0.79$). Significant interactions were found between month and site ($F = 3.7$, $df = 3$, $p = 0.014$) and site and length ($F = 2.9$, $df = 3$, $p = 0.037$), though length showed no significance as a covariate ($F = 1.31$, $df = 1$, $p = 0.25$). Least-squares trends analysis revealed no significant interactions between slopes ($p < 0.01$), and Hg data were normalized against length for least-squares means analyses. In general, least-squares means comparisons revealed greater [Hg] in shiners collected downstream compared to upstream. Within August, there were significantly greater [Hg]-at-length at DS1 compared to Weldon Ferry, and no significant differences between any other pairings ($p < 0.01$; Fig. 3.7). Within September, DS2 [Hg] were equal to DS1, and significantly greater than Wapiti and Weldon Ferry concentrations. DS1 concentrations were also greater than Weldon Ferry, but not significantly greater than Wapiti, and concentrations were similar between the upstream sites Weldon Ferry and Wapiti ($p > 0.01$). The only significant difference within sites and across months was an increase in [Hg] at DS2 between August and September ($p < 0.01$).

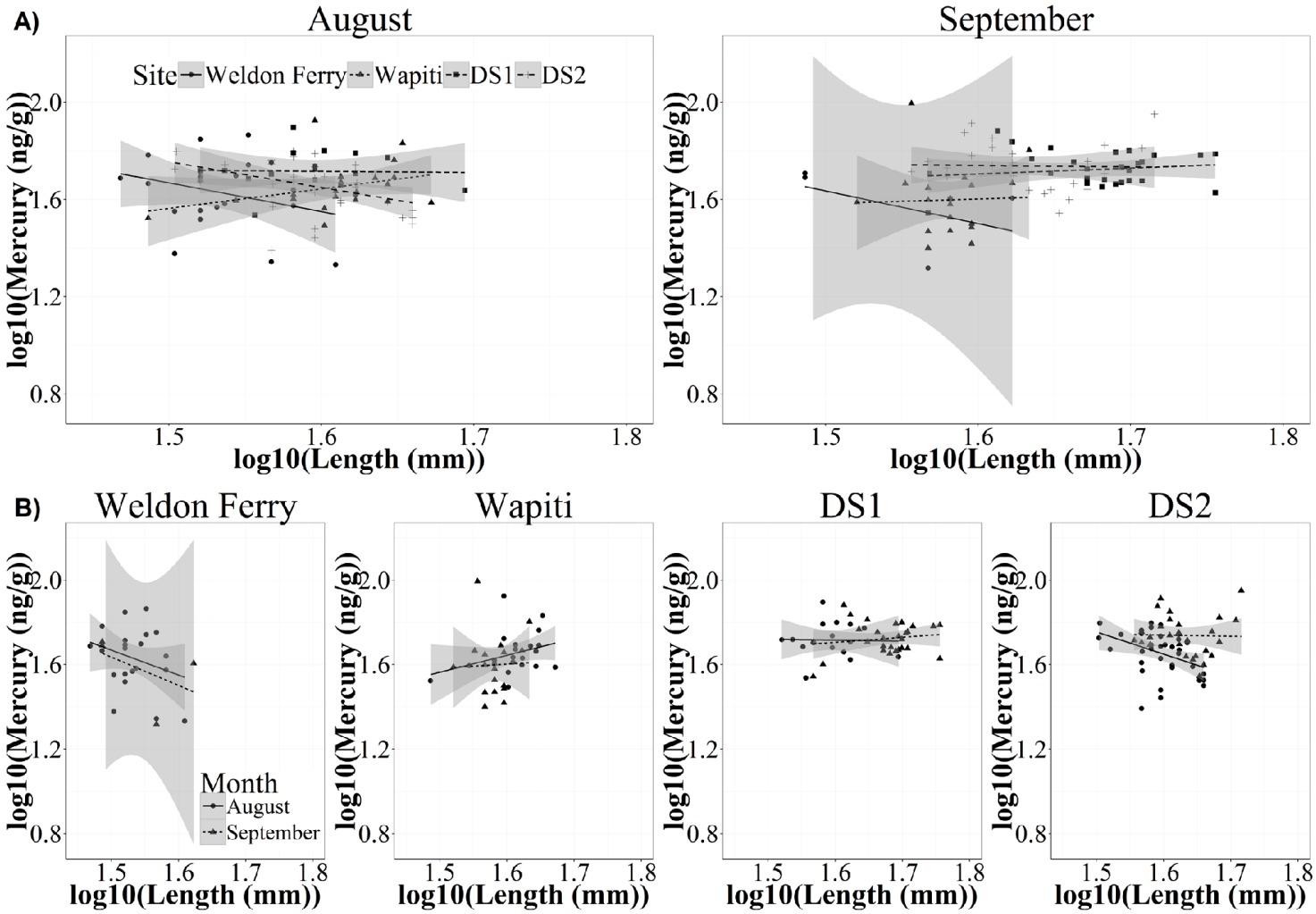

Fig. 3.7 Log transformed mercury-length relationships with 95% confidence intervals (shaded) compared A) within months across sites and B) within sites across months for spottail shiner captured in August and September of 2014 (Weldon Ferry: August [n = 22], September [n = 4]; Wapiti: August [n = 21], September [n = 18]; DS1: August [n = 16], September [n = 28]; DS2: August [n = 36], September [n = 28]).

3.5 Discussion

Shiner captured downstream of the dam had lower triglyceride concentrations and elevated [Hg] relative to upstream sites. While these results provide evidence that modifications to the flow regime affect both energy balance and [Hg] in fish, we cannot conclude that they are directly linked through the effects of hydropeaking on fish growth. No minimally adequate models included covariates with significant links between condition, triglycerides, and Hg. Furthermore, there were no consistent patterns of greater effects at DS2 compared with DS1. This suggests that hydropeaking may affect both fish physiology and [Hg], but perhaps at least partially through an indirect route or influences of other factors. Despite a lack of clear links between response variables, our observation of effects in downstream fish populations support the conclusions of other researchers that water flow manipulations downstream of dams should be carefully considered against energy production efficiency to maximize the health of downstream ecosystems and organisms (Young et al. 2011; Armanini et al. 2014).

Fish condition (K) and mass-at-length relationships are commonly used as a crude measure of overall fish health and can be cause for concern when K at an effect site is as little as 10% lower than at reference sites (Munkittrick et al. 2009). Shiner mass was strongly correlated with length, and comparisons of mass-at-length across months revealed significantly decreased condition at DS1, as might be expected in fish from a chronically stressful environment (Pickering 1993; McCormick et al. 1998). Condition declined by 10% between September and October at DS1, indicating a critical change in this measure over time by some metrics (Munkittrick et al. 2009; Environment Canada 2012). While a greater effect size was expected at DS2 commensurate with the greater degree of hydropeaking, an interaction in the mass-at-length slopes between months precluded comparisons of DS2 across months. Regardless, downstream sites showed condition deficits that may indicate energetic differences between up and downstream shiner populations.

The lowest triglyceride concentrations were found at DS2. Previous studies have shown a predictive relationship between lipid concentrations and length in perch and trout (Biro et al. 2004; Bocherding et al. 2007); the slope of the relationship between triglyceride concentrations and length was lower in DS2 shiner compared to those from Weldon Ferry and Wapiti sites, indicating that DS2 fish were not accumulating triglycerides as they aged and grew relative to fish from upstream of the dam. This is consistent with hydropeaking acting as a direct stressor at DS2 where we had once observed stranded fish (Pickering 1993; McCormick et al. 1998). In August and

September, triglyceride vs length regression slopes at DS1 were similar to the two sites upstream, but DS1 also displayed significantly lower covariate adjusted concentrations compared with upstream sites in August and mean adjusted triglyceride concentrations at DS2 were 43% lower than at DS1, indicating a large effect size in this metric in August across sites (Cohen 1988). Triglyceride concentrations increased in Wapiti and DS2 sites from August to September, yet concentrations remained lowest at DS2, indicating that shiner from this location were not accumulating the same critical triglyceride stores as their upstream counterparts in the lead up to winter. The decreasing triglyceride slopes across the three sites upstream of DS2 indicate that these increases across months were likely preceding the loss of lipid reserves observed in other species in winter (Biro et al. 2004; Kooka et al. 2009), or that there is positive selection for fish with greater energy stores leading into winter. The overall trend of lower triglyceride concentrations at DS2 relative to sites receiving no or lesser hydropeaking effects is perhaps the strongest evidence from this study that hydropeaking may act as a chronic stressor.

Water chemistry values were similar between up and downstream samples barring turbidity, which was lower at downstream sites, indicating little reason to assume that stresses are being imparted by changes in physical and chemical parameters measured in this study (Wendelaar Bonga 1997; Sutherland and Meyer 2007; Kemp et al. 2011). Aquatic Hg concentrations in this system were confirmed to be among the lowest observed in freshwater systems in both up and downstream samples (Sorensen et al. 1990; Kelly et al. 1995; Chapter 2).

Mercury-at-length concentrations were greatest in fish from downstream sites, particularly in September. DS2 was also the only site where fish experienced a significant increase in [Hg] between months. Patterns in [Hg] in fishes downstream of hydropeaking dams have been minimally explored in other literature. Relatively low [Hg] were observed in shiner sampled downstream of the Moses-Saunders hydropeaking dam in Ontario, Canada, compared with samples from upstream (Choy et al. 2008), but hydropeaking effects on the shores downstream of that dam are minimal (Federal Energy Regulatory Commission 2003). Our results suggest that hydropeaking may influence [Hg] in fish downstream, but perhaps not through effects on growth, as neither K nor triglyceride concentrations were predictive of [Hg].

Statistical models provided valuable insight into covariates that should be considered when studying physiological endpoints in shiner. As expected, length significantly predicted body mass (Wells and House 1974), and the significant relationship between length and triglyceride

concentration indicates length should be included as a covariate when making comparisons of shiner triglycerides, as has been suggested for other species in previous studies (Biro et al. 2004; Bocherding et al. 2007). While length also significantly predicted [Hg] and was accounted for in statistical analyses, there were no consistent patterns in direction of the effect. This suggests that Hg-length interactions observed in this study may be spurious. Though many fishes show a positive relationship between length and [Hg] owing to Hg accumulation with age and dietary shifts, our sampled fish were a single age class with limited variation in diet that otherwise would have allowed the relationship between length and mercury to develop as has been observed in other species (Fowlie et al. 2008; Gewurtz et al. 2011).

Given the lack of a predictive relationship between indicators of growth (condition, triglycerides) and [Hg], the effects we observed in downstream fish may have been driven by other indirect influences of hydropeaking. Hg in fish is derived from the diet, so concentrations in prey items may simply differ up and downstream of the dam (Painter et al. 2015). Shiner consume benthic macroinvertebrates and zooplankton alike (Crowder et al. 1981; Hartman et al. 1992; Happel et al. 2015), with selection following seasonal changes (Hartman et al. 1992; Happel et al. 2015). Macroinvertebrate assemblages are affected by hydroelectric dams and the presence of reservoirs (Ogbeibu and Oribhabor 2002; Xiaocheng et al. 2008; Armanini et al. 2014) which could shift community composition towards prey species that more readily accumulate Hg (Karimi et al. 2016). There are significant differences in macroinvertebrate assemblages among sites in the current study, with those downstream dominated by reservoir-derived taxa such as amphipods and chironomids (T.D. Jardine, unpublished data). Future studies should examine stomach contents to ascertain both their contribution to whole body energy concentrations and the possible influence of diet composition on fish [Hg] and morphometric and energetic endpoints (Jauncey 1982; Salhi et al. 2004). Further, reservoirs and hydropeaking can indirectly affect invertebrates and fish through effects on primary producers (Finger et al. 2007; McCartney 2009); algal quality is capable of affecting growth dilution in invertebrates (Karimi et al. 2007), and changes in prey Hg may have subsequent effects on [Hg] in fish (Karimi et al. 2016). Lower turbidity that leads to large mats of filamentous algae (D. Green, pers. obs.) downstream of the dam suggest these variables may have influenced our results. While it was beyond the scope of this study to included detailed analyses of primary producers and macroinvertebrate communities, these findings highlight the complexity of interactions between hydrology, benthic communities, fish physiology, and mercury

bioaccumulation associated with hydropeaking dams and suggests that data on all of these parameters may be necessary to understand Hg dynamics downstream of dams.

3.6 Disclosure of conflict of interest

Funding for this study was provided in part by Saskpower, the crown corporation that owns and operates the E. B. Campbell dam. Saskpower had no part in the collection, analysis, or interpretation of the samples in this study, and they did not partake in writing the preceding manuscript, though they will be presented with a copy for review before the article is submitted for publication.

CHAPTER 4:
CORTISOL AS AN ENVIRONMENTAL BIOMARKER OF CHRONIC STRESS IN SPOTTAIL SHINER (NOTROPIS HUDSONIUS): ASSESSMENT OF A NOVEL PROTOCOL USING A POTENTIAL HYDROPEAKING STRESSOR

Preface

This study was conducted to expand the assessment of hydropeaking as a chronic stressor exacerbating mercury concentrations in spottail shiner ("shiner") downstream of a hydropeaking hydroelectric facility. As was observed in 2014, energetic and condition endpoints provided mixed results of hydropeaking as a stressor. Here, cortisol responses of shiner were analyzed in response to an acute stress challenge and revealed signs of response inhibition in fish from the downstream site of concern, though this inhibition was still within the range of natural variation. Cortisol concentrations also revealed two distinct patterns of cortisol secretion in response to stress challenge as measured by cortisol concentration across time points across sites and months. Concentrations reliably increased over time and plateaued or nearly plateaued within 90-minutes post-stress challenge. The increases in cortisol concentrations observed over time after an acute stress challenge in shiner in this study were similar to patterns observed in other species, and suggested that the cortisol response may be developed into a reliable biomarker of chronic environmental stress in this species.

Chapter 4 of this thesis is currently being prepared for publication in the Journal of Comparative Physiology A. The author contributions to Chapter 4 of this thesis were as follows:

Derek Green (University of Saskatchewan) co-designed study; collected, processed, and analyzed all field samples, performed all statistical analyses, and drafted the manuscript.

David Janz (University of Saskatchewan) provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

Lynn Weber (University of Saskatchewan) provided scientific guidance; reviewed and revised the manuscript, providing comments and corrections.

Tim Jardine (University of Saskatchewan) co-designed study; aided in sample collection; provided scientific input and guidance; reviewed and revised the manuscript, providing comments and corrections; procured and provided funding required to conduct the research.

4.1 Abstract

Spottail shiner downstream of a hydropeaking dam have been shown to exhibit secondary signs of stress (e.g. decreased condition and triglyceride concentrations) and increased mercury concentrations that may be indicative of a chronically stressful environment. Here we develop a novel, rapid, field-deployable protocol for measuring the cortisol response as a biomarker to further assess this potentially stressful environment. Shiner were chosen as a sentinel species due to their North American ubiquity, and this standardized species may be used in broad-spectrum tests to identify less-habitable environments. Analyses of body condition and energetics (whole-body triglyceride and glycogen concentrations) showed mixed support for chronic stress from hydropeaking. Whole-body cortisol concentrations increased over time in wild-caught shiner after an acute stress challenge as would be expected from studies on other fish species, and there was some evidence for inhibition in downstream fish at the site most affected by hydropeaking. While a direct relationship between hydropeaking, stress, and the secondary stress responses cannot be confirmed from these data, they do suggest that the standardized stress-challenge protocol may have substantial utility in assessing chemical and physical stressors that can blunt the stress response.

4.2 Introduction

Cortisol is the primary glucucorticoid hormone in the physiological stress response of teleost fish and primary hormone of interest in the study of fish stress (Wendelaar Bonga 1997; Barton 2002; Ellis et al. 2012). It plays an integral role in the normal physiology of fish growth (Brown et al. 2014), maturation (Björnsson et al. 2011), osmoregulation (Ellis et al. 2012), and behavior (Barton 2002; McConnachie et al. 2012a). Cortisol also plays a significant metabolic role in the stress response by promoting gluconeogenesis and oxygen uptake, and inhibiting net glycogen synthesis in response to stress, actions which require energy investment, causing a reduction in energy available for other biological functions (Wendelaar Bonga 1997; Milligan 2003; Sousa et al. 2010; Ellis et al. 2012). Events that cause chronic stress or dysregulation of the stress or cortisol response can subsequently cause significant reductions in fish fitness, causing deficiencies in reproduction, immune function, energy stores, and growth, which can manifest as population level reductions in performance (Pankhurst 2011; Young et al. 2011; Ellis et al. 2012). While cortisol is well-studied in teleost fish under controlled conditions (Pankhurst 2011), it is poorly developed as a biomarker of stress in wild fish populations because there are numerous factors that can complicate its assessment.

Unlike the catecholamines (adrenaline and noradrenaline; Reid et al. 1998), cortisol is a steroid hormone, meaning it cannot be stored and must be produced *de novo* in response to each stressor (Ellis et al. 2012). Its production is controlled by a hormone cascade along the hypothalamus-pituitary-interrenal (HPI) axis in fish, meaning there is a response lag in cortisol production compared with the catecholamines released by sympathetic innervation (Wendelaar Bonga 1997; Barton 2002). After an acute stressor, cortisol concentrations rise in plasma and ultimately peak within one hour in most species before declining through a combination of the negative feedback effect of cortisol on each of the primary components of the HPI axis, and through depletion of corticotrophs (Wendelaar Bonga 1997; Barton 2002; Hontela and Vijayan 2008). Disruptions at any point in this feedback loop can dysregulate cortisol, and disruption of the cortisol response is known to be a sensitive and broad-spectrum biomarker to acute environmental stressors, with inhibited responses shown in fish from environments contaminated with metals and chemical pollutants (Hontela et al. 1992; Brodeur et al. 1997), and in laboratory tests of physical and chemical stressors (Barton et al. 1987; McCormick et al. 1998; Jentoft et al. 2005 Wiseman et al. 2011; Koakoski et al. 2014).

Despite its potential utility as a broad-spectrum biomarker of environmental stress, field-based assessment of the stress response is difficult because it can also be modified by genetic, developmental, and environmental factors. Basal cortisol concentrations fluctuate in diel cycles in fish (Cousineau et al. 2014), and the magnitude, timing, and duration of the stress response can all vary depending upon many factors, including stress exposure history (Barton et al. 1987; Barcellos et al. 2007; Auperin and Geslin 2008), fish species (Barton et al. 1987; Jentoft et al. 2005 Barcellos et al. 2006), and age (Barcellos et al. 2012; Koakoski et al. 2012). While there is much variability in the stress response, the effects of acute or chronic stressors can be relatively consistent within related species (Pickering and Pottinger 1989; Barton et al. 1987; Jentoft et al. 2005). Despite its potential usefulness as an assessment tool, more research is needed before the stress response in fishes can be useful in regulatory decision making or environmental risk assessment (Pankhurst 2011).

This study was conducted to test a novel, field deployable stress challenge protocol to refine the use of cortisol as a biomarker of environmental stress, and to study the role of hydropeaking as a potential environmental stressor for fish (Chapter 3). Hydropeaking is the manipulation of water flow rates through hydroelectric stations in response to local fluvial conditions and/or changes in electricity demand, often on daily and/or weekly cycles, and has been shown to elicit the stress response in fish and cause deleterious effects on fish and fish populations (Flodmark et al. 2002; Young et al, 2011). Aquatic ecosystems downstream of dams face a variety of direct and indirect stressors as a result of hydropeaking depending upon the characteristics of the dam, reservoir, and downstream environment, which can have deleterious effects on downstream organisms and ecology (Thornton et al. 1990; Young et al. 2011; Armanini et al. 2014). Secondary stress response endpoints known to be affected by hydropeaking in fish include reduced growth and energy accumulation (Young et al. 2011; Puffer et al. 2015), while tertiary effects include stranding risk (Saltveit et al. 2001; Young et al. 2011), survivorship, reproduction, and reduced rearing survival (Barton 2002; Young et al. 2011; Ellis et al. 2012). While hydropeaking was determined to be a stress that fish could rapidly habituate by Flodmark et al. (2002), that study focused purely on flow rates, rather than lateral shoreline displacement proposed as an exacerbating agent in our study sites (Chapter 1). Where the effects of hydropeaking on a low-pitched shoreline have been examined, strandings were noted even when species preferentially inhabit deeper river channels (Cocherell et al. 2012).

In order to account for species differences in the timing and magnitude of the stress response, as well as other confounds that can affect the stress response in wild caught fish, a protocol must be derived for a standardized species or set of species. Species selected must have a broad geographical range and high abundance to ensure they can be reliably sampled within potential sites of concern and appropriate reference environments. Spottail shiner ("shiner") were selected for this study as they are a ubiquitous and abundant North American minnow (Page et al. 1991; International Union for Conservation of Nature 2013). They are common in many aquatic environments, including rivers, lakes, wetlands, and reservoirs (Brazner 1997; Choy et al. 2008; Chapter 2). They are used for site-specific environmental impact assessments due to their small YOY home range, facultative schooling, and the propensity of YOY to inhabit shoreline environments (Wells 1968; Suns and Reese 1978; Choy et al. 2008). These characteristics mean sensitive YOY populations can be sampled conveniently using a bag seine and taken to represent influences of the immediate environment (Suns and Rees 1978; Choy et al. 2008). Young-of-the-year are hatched after the summer spawn and grow rapidly in order to achieve the size and energy stores required to maximize odds of over-winter survival, a challenge exacerbated at northern latitudes where shiner spawn later and are typically smaller by the onset of winter (Peer 1966; Wells and House 1974; Shuter and Post 1990; Biro et al. 2004; Perez and Munch 2010), and there is evidence that shiner reach spring with lower caloric content relative to what they accrued prior to winter (Bryan et al. 1996). They also show particular risk of being affected by a hydropeaking stressor, as the stressor is relatively greater at low-pitched shores (Chapter 3), and these fish preferentially seek out shallower waters to spawn (Wells and House 1974). These features make YOY shiner ideal candidates to study hydropeaking and other environmental stressors that might affect growth or energy acquisition of fish.

In order to assess whether hydropeaking acts as a chronic physical stressor, we measured whole-body cortisol, glycogen, and triglyceride concentrations in fish following challenge by an acute stressor (seine netting and aerial exposure), and fish condition in late summer at sites that differed in water level fluctuations. Hydropeaking was implicated as a stressor at these sites as previous research revealed elevated [Hg] in downstream shiner through possible deficiencies in energy and growth (Chapter 3). There were also observed fish strandings at the site most heavily affected by hydropeaking (DS2, Chapter 1), suggesting flow in these environments may be acting as a chronic physical stressor. However, it was not possible to determine whether influences on

these endpoints were due to direct physical effects of hydropeaking or indirect effects through other organisms. Based on this previous work and literature, we predicted significantly reduced body condition and energy storage in shiner from sites that experience chronic hydropeaking. Further, we predicted these energy stores would decrease following an acute stressor and that whole-body cortisol concentrations would show signs of inhibition at sites maximally affected by hydropeaking.

4.3 Methods

4.3.1 Study area

Protocol development was conducted in 2015 at three sites upstream and two sites downstream of the E. B. Campbell Dam (EBC; 53º41’19”N, 103 º20’50”W), a hydropeaking dam in the Saskatchewan River in east-central Saskatchewan, Canada (Fig. 4.1).

Fig. 4.1 The Saskatchewan River and study sites surrounding the E. B. Campbell Dam in 2015, including A) Cecil Ferry, B) Weldon Ferry, C) Wapiti, D) Codette Lake, E) Tobin Lake, F) E. B. Campbell Dam, G) Downstream site 1 (DS1), and H) Downstream site 2 (DS2). Figure modified from Mihalicz et al., unpublished.

The three upstream locations were in the North Saskatchewan River (Cecil Ferry; 53°14'43"N 105°26'10"W), South Saskatchewan River (Weldon Ferry; 53°10'58"N 105°9'49"W), and the Saskatchewan River immediately upstream of Codette Lake reservoir (Wapiti; 53°13'14"N 104°41'11"W). Downstream sites included a location that experiences relatively minimal effects of hydropeaking (DS1; 53°42'53"N 103°17'18"W) because of a deep, narrow channel, and a site that experiences hydropeaking to a greater degree (DS2; 53°43'45"N 103°7'57"W) with a wider, shallower channel (Chapter 1; Chapter 3). Shiner YOY were of catchable size only by September, suggesting that shiner spawned later in 2015 than in a previous study in 2014 (Chapter 3). As the cortisol response is a heritable trait (Pottinger and Carrick 1999; Barton 2002), samples were collected across sites and months to look for population level differences in response (across site) and differences in a population's stress response over time (across months), which may indicate a chronically stressful environment (Barton et al. 1987; Jentoft et al. 2005). Changes over time were also considered, as seasonal differences in lipid concentrations and basal and maximal cortisol concentrations have been observed in other fish (Vollenweider et al. 2011; Belanger et al. 2016). Further, a minimum of one-month was used for within site cross-time comparisons, as a minimum four-week chronic stress exposure seems to demarcate studies that observe an inhibition of the cortisol response from those that do not (Pickering and Stewart 1984; Barton et al. 1987; Rotllant and Tort 1997; Barcellos et al. 2006)

Shiner were easily captured at all sites and times with the exception of DS1 in September and Cecil Ferry in October.

4.3.2 Stress challenge protocol

The stress challenge protocol was designed to expose field-sampled shiner to a standardized stressor that could be used to gauge the subsequent rate and magnitude of their stress response. Fish were captured using a bag seine, with each attempt taking 5-10 minutes. Captured shiner were subjected to a five second aerial exposure by lifting the bag of the seine from the water, and then left in crowded, minimally watered conditions in the bag of the seine for approximately 2 minutes as ≈10 (depending up on capture success) shiner were then randomly distributed to one of four watered and identical plastic tanks (12.4"x7.2"x5.5"). A subset of samples was collected immediately for the assessment of basal concentrations of whole-body cortisol and energy stores

(time 0). Tanks with fish were left undisturbed and then collected at 5, 15, 45, and 90-minute time points.

Though the protocol employed a relatively short aerial stressor (Barton et al. 1987; Cousineau et al. 2014), this length was deemed appropriate as shiner began to succumb relatively quickly when this combination of stressors included longer aerial exposures in preliminary trials.

During September sampling at Cecil Ferry there were too few shiner to distribute among time 0 through 90 minute groups. The decision was made to exclude the 5 minute timepoint from consideration at this site and time in order to prioritize the basal levels observed at time 0.

4.3.3 Fish processing

Total length and mass were used to determine weight-at-length relationships as a measure of condition. The study also employed the condition factor ($K = 10\ 000*W/L^3$) as developed by Fulton (1904) in order to assess condition as a predictor of other endpoints. Samples were homogenized and processed using methods developed by Weber et al. (2003; 2008) and described in Chapter 3 and by Thomas and Janz (2011). Fish homogenates were vortexed and distributed among three snap-cap vials when they were of sufficient volume (≈ 150-200 µl or more) to maximize the number of fish analyzed by all three (cortisol, glycogen, and triglyceride) assays and re-frozen at -80ºC to await further processing

4.3.3.1 Cortisol extraction and analysis

Cortisol was extracted from homogenates using diethyl ether as described previously by members of our lab (Thomas and Janz 2011). Homogenate volumes (20-470 µl) were added to a borosilicate glass vial and adjusted to 1 ml using an enzyme immunoassay (EIA) phosphate buffer and combined with 5 ml of ether and vortexed for 40 seconds. Samples were then left undisturbed for 5-10 minutes until the aqueous and organic fractions had separated. The aqueous layer was then snap frozen in liquid nitrogen for 20 seconds, hand warmed for 10 seconds, and then subjected to a second 20 second freezing in liquid nitrogen. The organic layer was then carefully decanted into a second borosilicate vial. Ether vials were then transferred to a heating block set to 50ºC and the ether was evaporated under an N_2 stream and the cortisol was reconstituted in a known volume (200-250 µl) of EIA buffer. Homogenate mixtures were then allowed to thaw, and the process was

repeated two additional times for each sample with each ether phase being decanted into the same receiving vial for a given sample to ensure all extracted cortisol was combined for a given sample.

Extracted cortisol was reconstituted overnight in EIA buffer at 4°C with intermittent vortexing. Once collected the reconstituted samples were either returned to -80°C for later analysis, or assayed immediately using an enzyme-linked immunosorbent assay (ELISA) kit from Oxford Biomedical Sciences (Oxford, Michigan, USA). Cortisol extraction and analysis had not been previously validated for shiner homogenates in our lab, and the assay was validated using spike-recovery, inter and intra-assay variation, and parallelism assessments. All spike solutions were comprised of 32 µl of 0.5 µg/mL kit-provided cortisol stock solution diluted to 0.0125 µg/mL using EIA buffer. Spike solutions were added to and extracted from homogenates that had previously undergone cortisol extraction. Inter and intra-assay validations were performed using pooled high concentration shiner samples, and parallelism was testing using serial dilutions of pooled high cortisol shiner samples. All cortisol plates were read in a SpectraMax 190 spectrophotometer (Molecular Devices Corp., Sunnyvale, California, USA).

4.3.3.1.1 Cortisol assay validation

Spike-recovery averaged 103% (n = 9). The intra and inter-assay coefficients of variation were 4.9% (n = 6) and 6.6% (n = 13), respectively, and tests of parallelism confirmed parallel slopes between standards and serially diluted sample extracts ($F = 0.003$, $df1 = 3$, $df2 = 32$, $p = 1$).

4.3.3.2 Glycogen relevance and analysis

Glycogen is a critical energy store in fish and liver glycogen is rapidly depolymerized to glucose in the presence of an acute stressor, though whether this signal is detectable through the muscle glycogen remaining in a whole-body homogenate remains to be determined (Wendelaar Bonga 1997; Kelly and Janz 2008; Goertzen et al. 2012. Catecholamine release causes glycogenolysis in the initial stages of the acute stress response, and catecholamine release can also be inhibited in chronically stressed fish (Reid et al. 1994 Wendelaar Bonga 1007). It was therefore predicted that rapid collection of samples would reveal a significant glycogen decline in acutely stressed fish, and that fish from chronically stressful environments would show reduced glycogen depolymerization. Glycogen concentrations were determined using an amylase treatment against an amylase-treated glycogen standard (Weber et al. 2008).

4.3.3.3 Triglyceride relevance and analysis

Triglycerides are a common measure of fish energetics as they are the primary aerobic swimming fuel; activity and stress can both tax available energy, decreasing energy available for growth and other endpoints (Moyes and West 1995; Wendelaar Bonga 1997; Sousa et al. 2010; Ellis et al. 2012). Triglycerides were analyzed using a glycerol standard curve in a lipase-treated colourimetric assay developed by Weber et al. (2003; 2008), and as described in Chapter 3.

4.3.4 Statistics

Data were initially inspected in accordance with Zuur et al. (2010). All dependent variables and covariates were log-transformed as needed to improve the homogeneity and normality of residuals. Response data were then fit with maximal models that include all potential physiological covariates. The predictive value of site, month, and covariates on the response variables mass, and glycogen, triglyceride, and cortisol concentrations were determined using linear models. In order to account for the missing time point "5" data from Cecil Ferry in September, all variables to be contrasted over time points (cortisol, glycogen, and triglycerides) were collectively compared within response variables at the 0 and all 5-minute time points using Welch's two-sample t-tests. The difference between the time points was non-significant in the case of glycogen ($p = 0.47$) so the two time points were combined to a collective group called "2". Cortisol and triglyceride concentrations were significantly different between the 0 and 5-minute time points ($p = 0.001$ and $p < 0.001$, respectively). Thus, the 5-minute time point was dropped from triglyceride and cortisol analyses as the inclusion of both time points was originally an arbitrary selection and basal levels were of greater importance.

In the cases of cortisol, glycogen, and triglyceride analyses, all linear covariate interactions were considered to a maximum of a three-way interaction with all paired combinations of the factors month, site, and time point. These latter three factors were also considered against one another using a three-way interaction. Two-way interactions and main effects were also considered between all covariate and factor combinations. Mass-at-length (condition) models were constructed using similar formulas barring the inclusion of time point as a factor. Cortisol was modeled using length, K, triglycerides, and glycogen as covariates. Glycogen concentrations were assessed using length, K, and triglyceride concentrations as covariates. Triglyceride concentrations

were assessed using length, K, and glycogen concentration as covariates. Mass-at-length (condition) was assessed using mass and length relationships, with triglyceride and glycogen concentrations included as additional covariates.

A fully comprehensive model including all sites, months, and time points was not constructed because of the missing data for DS1 in September and Cecil Ferry in September and October. Consequently, three models were run for each dependent variable in order to produce the most robust across-month (within-site) and across-site (within-month) models for hypothesis testing. **Model 1** compared across months (within-site) using data from Weldon Ferry, Wapiti, and DS2. **Model 2** compared across sites in September using data from Weldon Ferry, Cecil Ferry, Wapiti, and DS2. **Model 3** compared across sites in October using data from Weldon Ferry, Wapiti, DS1, and DS2.

Model selection was conducted by removing the highest order interactions whose removal failed to raise the AIC value of the model by >2 and provided the lowest non-significant difference in the deviance of the model as determined using χ^2. Non-significant terms were not removed if they were a component of a higher order interaction. Models were visually inspected for homogeneity and normality of residuals and while occasional, minor departures from normality were noted, no data exerted undue leverage within the models, and no outliers were removed. Data were log transformed as required, and hypotheses were tested using the Anova function in the 'car' package (Fox and Weisberg 2011). Analyses were conducted using type-III sums of squares, and all post-hoc tests were conducted with least-squares means and trends (lsmeans; lstrends) as provided by the "lsmeans" package for R in order to account for imbalanced data (Fox and Weisberg 2011; Lenth 2016). Post-hoc tests were conducted after adjusting for all significant covariates provided they did not significantly interact over levels of a factor. In order to adjust for multiple comparisons all post-hoc tests, including interactions, were considered significant at $p < 0.01$ to minimize the risk of type I error without unduly decreasing the power of analyses. All modeling and statistical analysis was conducted in RStudio (RStudio, V. 0.99.893; RStudio Team 2015; R Core Team 2015).

4.4 Results

4.4.1 Shiner descriptive data

Mean lengths of shiner within groups and months were between 35 and 52 mm, consistent with sizes of YOY at similar latitudes as determined using scale annulus counts and length distributions (Peer 1966; Suns and Reese 1978), and with the sizes of shiner captured in chapter 3. Descriptive statistics for fish length, mass, and condition can be found in Table 4.1, and for triglyceride, glycogen, and cortisol concentrations in Table 4.2.

Table 4.1 Descriptive statistics (range and mean ± standard deviation) for total length, total mass, and condition of spottail shiner sampled in September and October of 2015 at Weldon Ferry, Cecil Ferry, Wapiti, Downstream Site 1 (DS1) and Downstream Site 2 (DS2).

			Total Length (mm)		Total Mass (g)		Condition (K)	
Month	**Site**	**n**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
September	Weldon Ferry	52	32-63	45±7	0.26-1.91	0.75±0.32	0.48-0.98	0.77±0.09
	Cecil Ferry	33	42-64	52±6	0.57-1.97	1.17±0.36	0.54-0.90	0.78±0.07
	Wapiti	43	37-63	48±6	0.38-2.14	0.96±0.4	0.55-1.01	0.80±0.07
	DS1	0	NA	NA	NA	NA	NA	NA
	DS2	50	29-48	40±3	0.23-0.79	0.51±0.13	0.59-0.96	0.81±0.07
October	Weldon Ferry	62	29-56	44±6	0.19-1.23	0.66±0.25	0.58-0.90	0.74±0.05
	Cecil Ferry	0	NA	NA	NA	NA	NA	NA
	Wapiti	40	24-56	41±7	0.13-1.35	0.57±0.28	0.65-0.91	0.78±0.06
	DS1	41	34-59	45±7	0.29-1.40	0.70±0.28	0.47-0.88	0.74±0.08
	DS2	50	26-53	35±5	0.13-1.13	0.35±0.17	0.63-0.86	0.75±0.05

Table 4.2 Descriptive statistics (range and mean ± standard deviation) for triglyceride, glycogen, averaged across time point, and basal (Time 0) and maximal (Time 90) cortisol concentrations of spottail shiner sampled in September and October of 2015 at Weldon Ferry, Cecil Ferry, Wapiti, Downstream Site 1 Site (DS1) and Downstream Site 2 (DS2).

			Triglycerides (mg/g)		**Glycogen (mg/g)**		**Basal Cortisol (ng/g)**		**Maximal Cortisol (ng/g)**	
Month	**Site**	**n**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
September	Weldon Ferry	52	0.84-5.13	2.23±0.85	0.30-5.79	2.48±1.69	0.9-4.5	2.8±1.3	13.2-102.8	41.0±26.0
	Cecil Ferry	33	1.02-4.05	2.56±0.86	0.38-5.95	2.02±1.47	1.9-10.5	6.1±3.3	27.4-68.2	48.4±13.4
	Wapiti	43	1.10-5.31	2.53±0.78	0.29-4.60	1.48±0.80	2.6-19.1	7.7±6.0	28.3-75.7	46.9±15.5
	DS1	0	NA	NA	NA	NA	NA	NA	NA	NA
	DS2	50	1.41-5.13	2.84±0.78	0.34-4.19	1.73±0.93	4.1-55.3	20.5±14.3	63.4-123.2	85.6±21.8
October	Weldon Ferry	62	1.34-8.07	3.22±1.36	0.44-6.08	2.61±1.59	2.8-19.0	10.4±4.7	20.9-80.0	41.0±18.0
	Cecil Ferry	0	NA	NA	NA	NA	NA	NA	NA	NA
	Wapiti	40	1.41-4.95	3.07±0.84	0.44-6.37	2.54±1.66	2.4-19.0	8.9±5.1	26.7-66.4	46.6±14.0
	DS1	41	1.23-4.53	2.66±0.81	0.06-5.55	1.95±1.29	18.3-38.3	26.8±9.2	39.9-83.4	58.6±14.8
	DS2	50	1.61-6.09	3.32±1.01	0.36-9.36	3.59±1.70	2.6-20.6	8.8±5.6	43.3-58.6	49.4±5.1

4.4.2 Cortisol analyses

Cortisol concentrations showed a distinct pattern of concentration increase over time within all models, and generally peaked around 45-minutes post-stress challenge (Fig. 4.2).

Maximal and minimal models predicting cortisol concentration (ng/g) were both statistically significant using **Model 1** ($F = 15.28$, $df = 53$ and 145, $p < 0.001$; adjusted $r^2 = 0.79$; AIC = - 629.78; $F = 30.95$, $df = 29$ and 192, $p < 0.001$: adjusted $r^2 = 0.80$; AIC = -739.11; minimal model overview: Table 4.3).

Table 4.3 Significant terms of the minimal model predicting cortisol concentration (ng/g) of spottail shiner captured in September and October of 2015 using **Model 1**.

	SS	DF	F	P	
Intercept	0.026	1	0.821	0.366	
Time Point	19.793	3	208.750	0.000	***
Month	0.267	1	8.461	0.004	**
Site	0.159	2	2.509	0.084	.
Triglycerides	0.267	1	8.437	0.004	**
K Factor	0.212	1	6.715	0.010	*
Month:Site	1.809	2	28.616	0.000	***
Month:K Factor	0.241	1	7.624	0.006	**
Site:K Factor	0.264	2	4.178	0.017	*
Triglycerides:K Factor	0.244	1	7.705	0.006	**
Time Point:Month	0.261	3	2.756	0.044	*
Time Point:Month:Site	1.261	12	3.326	0.000	***
Residuals	6.068	192			

Interactions precluded comparisons across the main effects of month, site, and time point. Pairwise comparisons of time point across month within site showed general trends of increased cortisol over time, with peak cortisol concentrations reached as early as 45 minutes after the acute stressor (Fig. 4.2). Cortisol concentration patterns were nearly identical over time points across months at Weldon Ferry and Wapiti, but they were significantly reduced at DS2 in October compared with September.

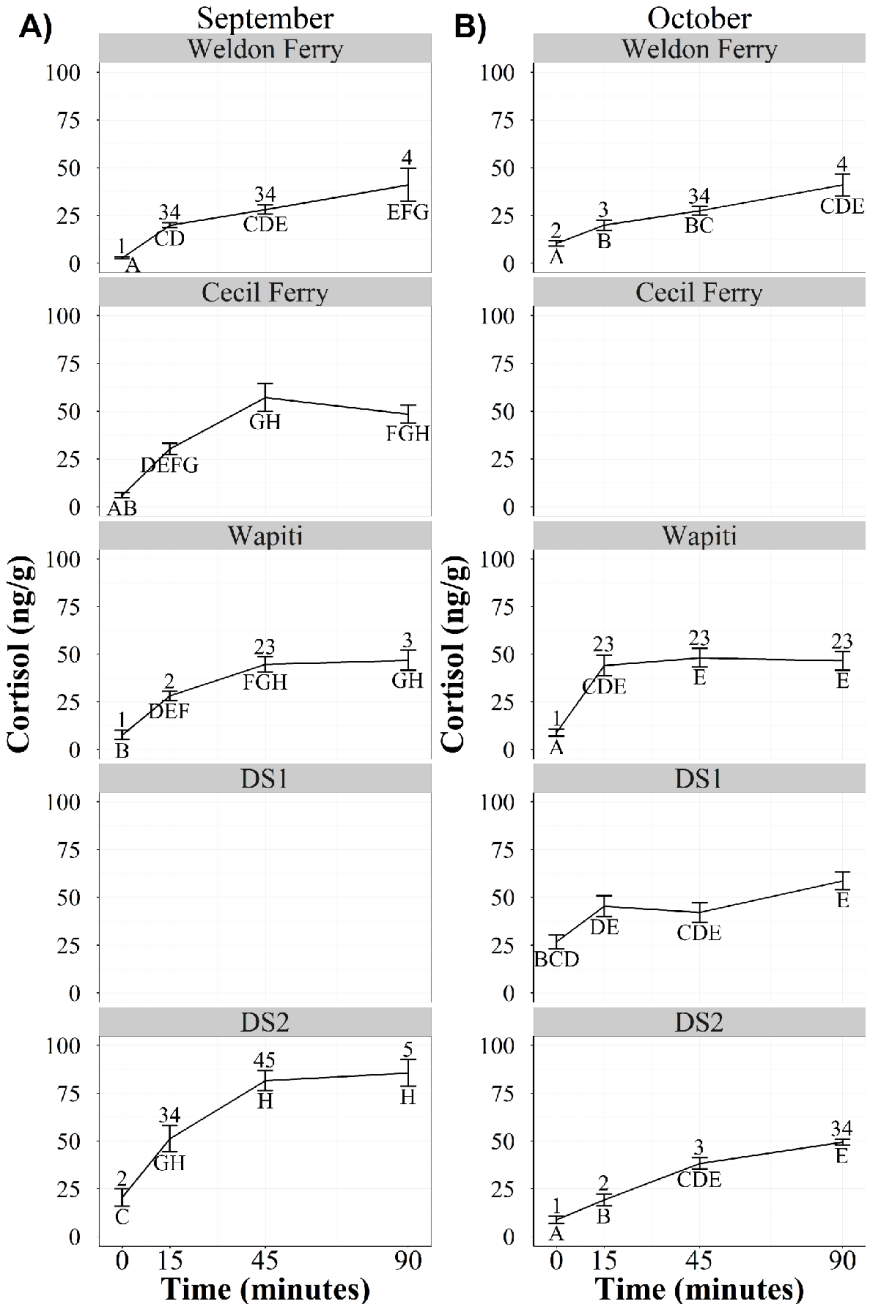

Fig. 4.2 Mean (±SE) cortisol concentrations in spottail shiner subjected to a stress challenge. Numbers correspond to comparisons made with **Model 1** (within and across months for sites with data for both months); time points sharing numbers are not significantly different within or across sites [$p \geq 0.01$]). Letters correspond to comparisons made with **Model 2** (panel A, September only) and **Model 3** (panel B, October only); time points sharing letters were not significantly different across time point when no interaction between time point and site was present, and across all time points when an interaction is present [$p \geq 0.01$]) (n = 4-14 per time point). Analyses conducted after adjusting for covariates.

Both maximal and minimal models predicting cortisol concentrations were statistically significant using **Model 2** ($F = 20.69$, $df = 37$ and 92, $p < 0.001$; adjusted $r^2 = 0.85$; AIC = -426.36; $F = 32.30$, $df = 22$ and 107, $p < 0.001$; adjusted $r^2 = 0.84$; AIC = -430.53; minimal model overview: Table 4.4).

Table 4.4 Significant terms of the minimal model predicting cortisol concentrations (ng/g) of spottail shiner captured in September of 2015 using **Model 2**.

	SS	DF	F	P	
Intercept	2.389	1	76.853	0.000	***
Time Point	14.058	3	150.727	0.000	***
Site	0.131	3	1.404	0.246	
Triglycerides	0.067	1	2.151	0.145	
Glycogen	0.207	1	6.665	0.011	*
K Factor	0.136	1	4.370	0.039	*
Time Point:Site	0.571	9	2.042	0.041	*
Site:K Factor	0.214	3	2.293	0.082	.
Triglycerides:Glycogen	0.211	1	6.784	0.011	*
Residuals	3.326	107			

Interactions prevented the direct comparisons of cortisol concentrations across site or time point, and pairwise comparisons across site and time point revealed few significant differences, though there was a trend of elevated cortisol concentrations at DS2 across all time points (Fig. 4.2A).

Maximal and minimal models predicting cortisol concentration were significant using **Model 3** ($F = 8.104$, $df = 37$ and 84, $p < 0.001$; adjusted $r^2 = 0.68$; AIC = -382.42; $F = 26.29$, $df = 15$ and 129, $p < 0.001$; adjusted $r^2 = 0.72$; AIC = -496.84; minimal model overview: Table 4.5).

Table 4.5 Significant terms of the minimal model predicting cortisol concentrations (ng/g) of spottail shiner captured in October of 2015 using **Model 3**.

	SS	DF	F	P
Intercept	273.308	1	9328.374	0.000
Time Point	7.738	3	88.039	0.000
Site	1.845	3	20.995	0.000
Time Point:Site	1.317	9	4.994	0.000
Residuals	3.780	129		

Interactions precluded the direct comparison of the main effects across sites and time points, and pairwise comparisons of time point across sites revealed relatively high cortisol concentrations at DS1 for the 0 and 15-minute time points (Fig. 4.2B).

4.4.3 Glycogen analyses

Maximal and minimal models predicting glycogen concentrations were both significant using **Model 1** ($F = 3.01$, $df = 44$ and 210, $p < 0.001$; adjusted $r^2 = 0.26$; AIC = -614.3; $F = 4.42$, $df = 24$ and 231, $p < 0.001$; adjusted $r^2 = 0.24$; AIC= -625.7; minimal model overview: Table 4.6).

Table 4.6 Significant terms of the minimal model predicting glycogen concentrations (mg/g) of spottail shiner captured in September and October of 2015 using **Model 1**.

	SS	DF	F	P	
Intercept	0.990	1	12.511	0.000	***
Time Point	1.517	3	6.392	0.000	***
Month	2.957	1	37.365	0.000	***
Site	1.025	2	6.479	0.002	**
K Factor	1.770	1	22.369	0.000	***
Month:Site	0.822	2	5.196	0.006	**
Time Point:Month	0.170	3	0.717	0.543	
Time Point:Site	0.357	6	0.752	0.609	
Time Point:Month:Site	1.106	6	2.329	0.033	*
Residuals	18.279	231			

The three way interactions precluded direct comparisons of adjusted glycogen concentrations across the main effects of time point, month, and site. Statistical relationships within month across site and time point are summarized in Fig. 4.3, and while no patterns were clearly discernable across time point and site, there were elevated concentrations found at time point 45 at Wapiti in October compared with September. There were also significantly higher glycogen concentrations at time points 2 and 90 at DS2 in October as compared with September.

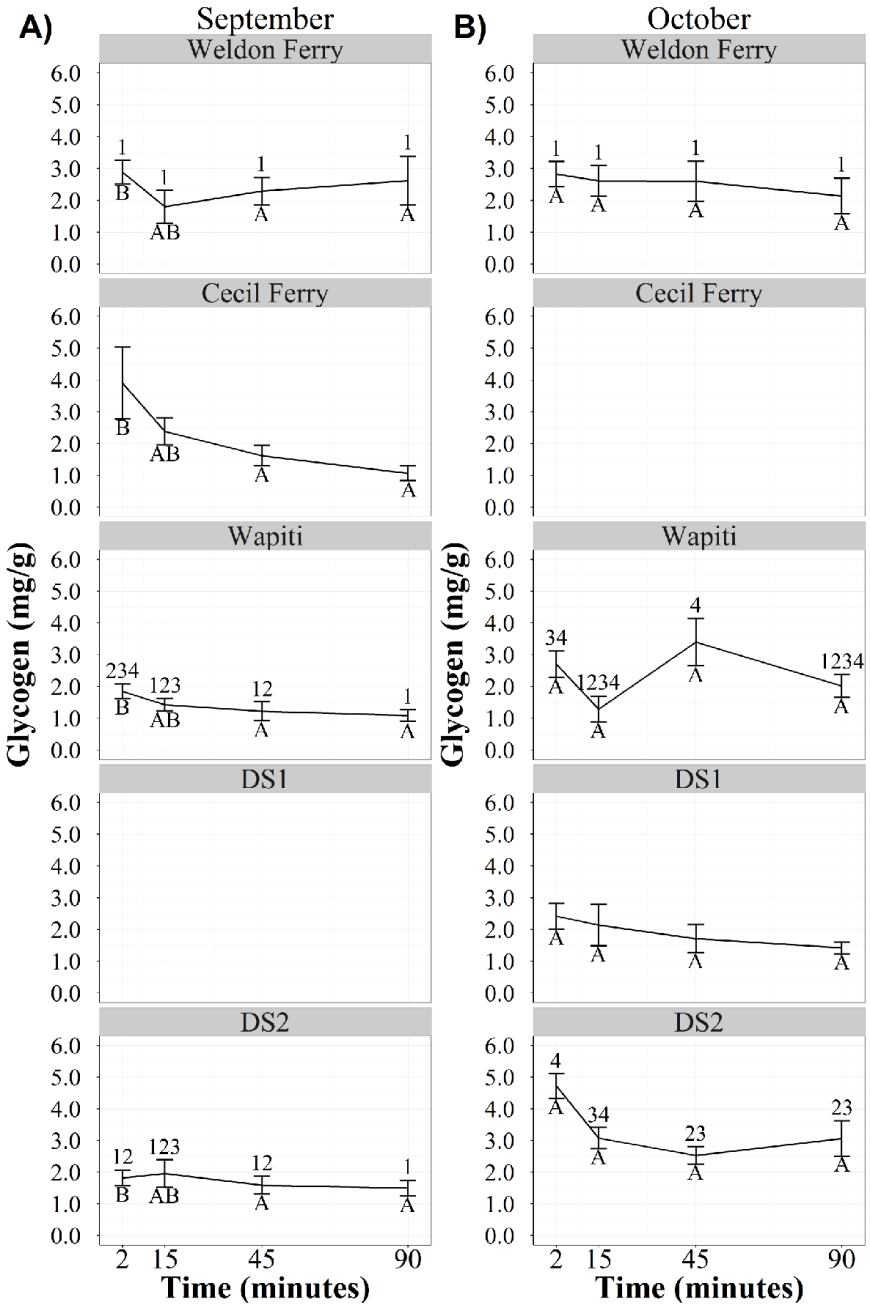

Fig. 4.3 Mean (±SE) glycogen concentrations in spottail shiner subjected to a stress challenge. Numbers correspond to comparisons made with **Model 1** (within and across months for sites with data for both months); time points sharing numbers were not significantly different within or across sites [$p \geq 0.01$]). Letters correspond to comparisons made with **Model 2** (panel A, September only) and **Model 3** (panel B, October only); time points sharing letters were not significantly different across time point when no interaction between time point and site was present, and across all time points when an interaction was present [$p \geq 0.01$]) (n = 4-19 per time point). Analyses conducted after adjusting for covariates.

Maximal and minimal models predicting glycogen concentrations were also significant using **Model 2** ($F = 1.56$, $df = 30$ and 126, $p = 0.049$; adjusted $r^2 = 0.10$; AIC = -351.36; $F = 3.76$, $df = 7$ and 149, $p < 0.001$; adjusted $r^2 = 0.11$; AIC = -373.4; minimal model overview: Table 4.7).

Table 4.7 Significant terms of the minimal model predicting glycogen concentrations (mg/g) of spottail shiner in September of 2015 using **Model 2**.

	SS	DF	F	P	
Intercept	0.310	1	3.514	0.063	
Time Point	1.333	3	5.038	0.002	**
Site	0.867	3	3.278	0.023	*
K Factor	0.575	1	6.521	0.012	*
Residuals	13.141	149			

Post-hoc tests revealed that across sites glycogen concentrations were significantly higher in Weldon Ferry fish as compared to Wapiti ($p < 0.01$) though no other differences were significant across sites. Comparisons of the main effects of time point revealed that the highest glycogen concentrations were found immediately after capture and reached minimal levels around the 15-minute time point in September as shown in Fig. 4.3A.

Maximal and minimal models predicting glycogen concentration were also significant using **Model 3** ($F = 4.10$, $df = 30$ and 126, $p < 0.001$; adjusted $r^2 = 0.37$; AIC = -397.08); $F = 5.26$, $df = 24$ and 134, $p < 0.001$; adjusted $r^2 = 0.39$; AIC = -408.56; Table 4.8).

Table 4.8 Significant terms of the minimal model predicting glycogen concentrations (mg/g) of spottail shiner captured in October of 2015 using **Model 3**.

	SS	DF	F	P	
Intercept	0.902	1	13.595	0.000	***
Time Point	0.330	3	1.659	0.179	
Site	1.331	3	6.686	0.000	***
Length	0.946	1	14.266	0.000	***
K Factor	0.753	1	11.349	0.001	**
Time Point:Site	1.064	9	1.783	0.077	
Site:Length	0.877	3	4.406	0.005	**
Site:K Factor	1.389	3	6.979	0.000	***
Length:K Factor	0.809	1	12.192	0.001	**
Residuals	8.889	134			

The interaction between site and length did not reveal significantly different slopes upon pairwise comparison, though the negative slope between glycogen concentration and condition at DS1 was significantly different from the two upstream sites, precluding direct comparisons of effects across these sites. Despite this complication, there were no significant differences within the lsmeans compared across sites with similar slopes, meaning no site was significantly different than the others ($p > 0.01$). Unlike in September, there were no significant trends across time point in October (Fig. 4.3B).

4.4.4 Triglyceride analyses

Maximal and minimal models predicting triglyceride concentrations were both statistically significant using **Model 1** ($F = 3.94$, $df = 44$ and 157, $p < 0.001$; adjusted $r^2 = 0.39$; AIC = -795.8; $F = 7.74$, $df = 20$ and 181, $p < 0.001$; adjusted $r^2 = 0.40$; AIC = -818.3: minimal model overview: Table 4.9).

Table 4.9 Significant terms of the minimal model predicting triglyceride concentrations (mg/g) of spottail shiner captured in September and October of 2015 using **Model 1**.

	SS	DF	F	P	
Intercept	0.162	1	10.275	0.002	**
Time Point	0.302	3	6.378	0.000	***
Month	0.217	1	13.759	0.000	***
Site	0.136	2	4.300	0.015	**
Length	0.153	1	9.716	0.002	**
Glycogen	0.076	1	4.789	0.030	*
K Factor	0.139	1	8.796	0.003	**
Month:Site	0.153	2	4.842	0.009	**
Site:Length	0.131	2	4.141	0.017	*
Length:K Factor	0.148	1	9.397	0.003	**
Time Point:Site	0.532	6	5.617	0.000	***
Residuals	2.856	181			

The site-length interaction was not significant under the more stringent post-hoc criteria ($p < 0.01$), and all further analyses were adjusted for length, glycogen concentration, and K. Comparisons of standardized triglyceride concentrations compared across site and month revealed

that the interaction observed between month and site was due to significant increases in triglyceride concentrations across months in Weldon Ferry fish ($p < 0.01$) compared with non-significant increases observed in both Wapiti and DS2 fish ($p > 0.01$). No site showed significantly different triglyceride concentrations within month. No consistent patterns appeared to exist across time point for triglycerides, and a comprehensive summary of the pairwise concentration comparisons responsible for the interaction between site and time point can be found in Fig. 4.4.

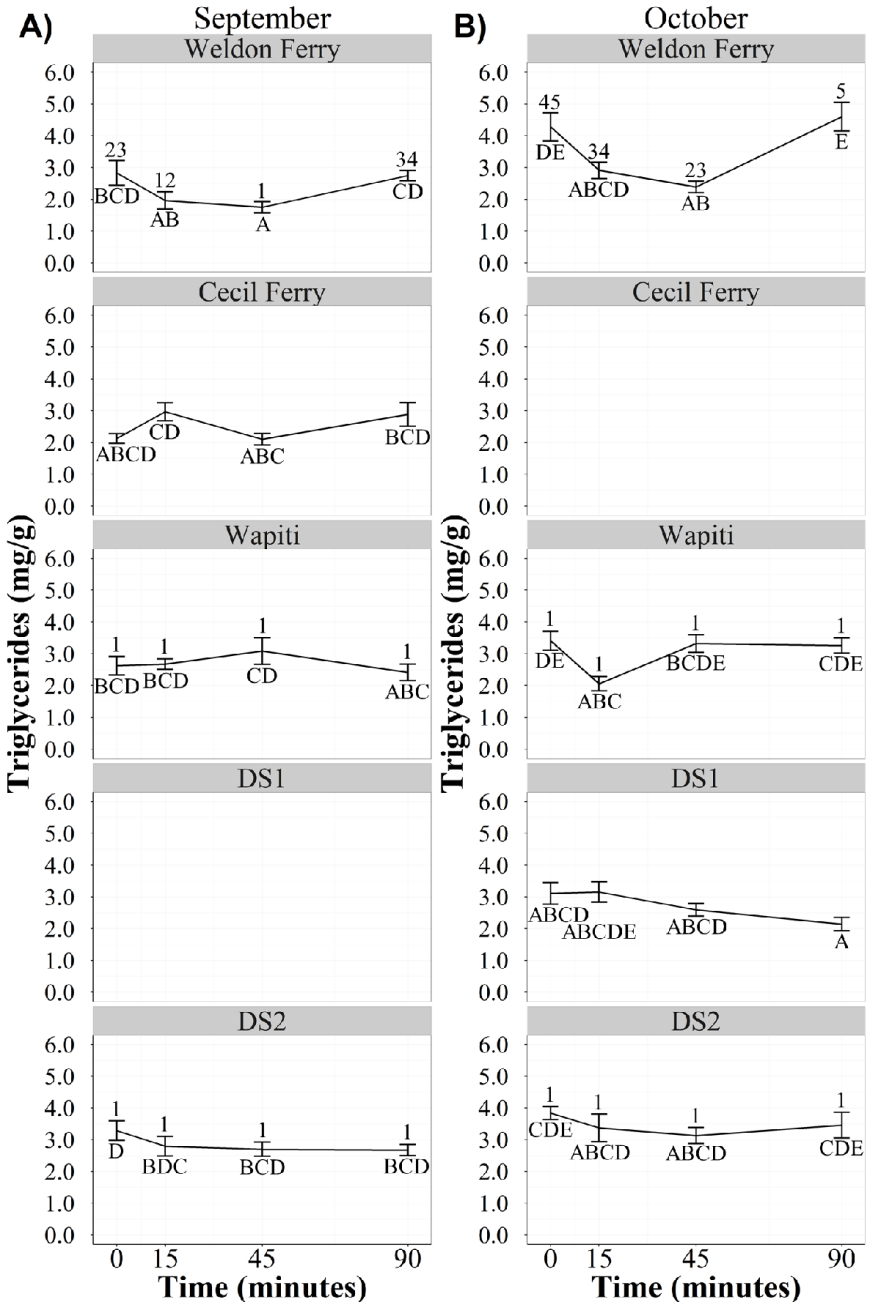

Fig. 4.4 Mean (±SE) triglyceride concentrations in spottail shiner subjected to a stress challenge. Numbers correspond to comparisons made with **Model 1** (within and across months for sites with data for both months); time points sharing numbers were not significantly different within or across sites [$p \geq 0.01$]). Letters correspond to comparisons made with **Model 2** (panel A, September only) and **Model 3** (panel B, October only); time points sharing letters were not significantly different across time point when no interaction between time point and site was present, and across all time points when an interaction was present [$p \geq 0.01$]) (n = 4-14 per time point). Analyses conducted after adjusting for covariates.

Maximal and minimal models predicting triglyceride concentrations were also significant using **Model 2** ($F = 2.93$, $df = 30$ and 99, $p < 0.001$; adjusted $r^2 = 0.31$; AIC = -514.20; $F = 3.63$, $df = 16$ and 129, $p < 0.001$; adjusted $r^2 = 0.22$; AIC = -566.02; Table 4.10).

Table 4.10 Significant terms of the minimal model predicting triglyceride concentrations (mg/g) of spottail shiner captured in September of 2015 using **Model 2**.

	SS	DF	F	P	
Intercept	0.000	1	0.000	0.999	
Time Point	0.104	3	1.875	0.137	
Site	0.171	3	3.074	0.030	*
K Factor	0.173	1	9.333	0.003	**
Time Point:Site	0.486	9	2.906	0.004	**
Residuals	2.396	129			

Interactions between site and time point precluded direct comparisons of triglyceride concentration across time point and site, and the statistical relationships between time point and site in September are summarized in Fig. 4.4A. Dependent variable groupings show minimal difference in triglyceride concentration across site.

Maximal and minimal models predicting triglyceride concentrations were also significant using **Model 3** ($F = 2.86$, $df = 30$ and 94, $p < 0.001$; adjusted $r^2 = 0.31$; AIC = -495.33; $F = 5.00$, $df = 16$ and 113, $p < 0.001$; adjusted $r^2 = 0.33$; AIC = -528.61; minimal model overview: Table 4.11).

Table 4.11 Significant terms of the minimal model predicting triglyceride concentrations (mg/g) of spottail shiner captured in October of 2015 using **Model 3**.

	SS	DF	F	P	
Intercept	11.210	1	738.404	0.000	***
Time Point	0.214	3	4.705	0.004	**
Site	0.094	3	2.065	0.109	
Glycogen	0.133	1	8.791	0.004	**
Time Point:Site	0.551	9	4.034	0.000	***
Residuals	1.716	113			

The interaction between time point and site precluded direct comparison of triglyceride concentration across the main factor levels of site and time point, and the statistical relationships across site and time point in October are summarized in Fig. 4.4B.

4.4.5 Mass-at-length (condition) analyses

Maximal and minimal models significantly predicted mass using **Model 1** ($F = 342.60$, degrees of freedom [df] = 26 and 228, $p < 0.001$; adjusted $r^2 = 0.97$; AIC = -1660.8; $F = 1113.00$, df = 8 and 246, $p < 0.001$; adjusted $r^2 = 0.97$; AIC: -1677.9; minimal model overview: Table 4.12).

Table 4.12 Significant terms of the minimal model predicting mass-at-length (condition, g/mm) of spottail shiner captured in September and October of 2015 using **Model 1**.

	SS	DF	F	P	
Intercept	1.341	1	1000.169	0.000	***
Month	0.000	1	0.035	0.852	.
Site	0.018	2	6.825	0.001	**
Length	1.237	1	922.863	0.000	***
Triglycerides	0.009	1	6.716	0.010	*
Glycogen	0.007	1	4.997	0.026	*
Length:Triglycerides	0.010	1	7.257	0.008	**
Month:Triglycerides	0.007	1	5.394	0.021	*
Residuals	0.330	246			

Interactions across month were non-significant at the more stringent post-hoc criteria ($p < 0.01$), indicating minimal interaction, and no site showed significant change in mass at length condition across month (Fig. 4.5). Adjusted comparisons averaged across month revealed the lowest mass-at-length at Weldon Ferry ($p < 0.01$), which was significantly lower than at Wapiti ($p < 0.01$) but not DS2 ($p > 0.01$). Mass-at-length at DS2 was not different from Wapiti ($p > 0.01$).

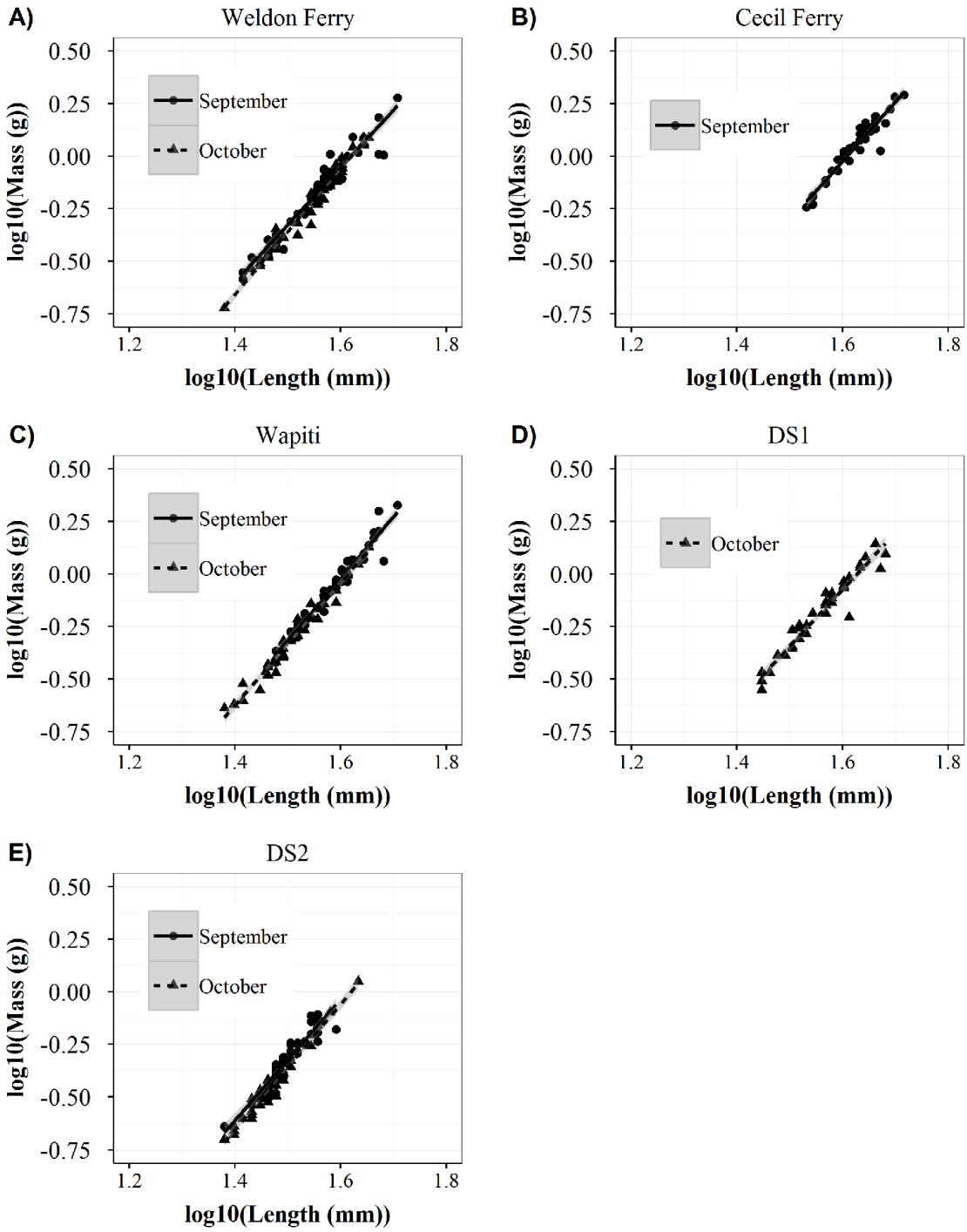

Fig. 4.5 Log-transformed mass-at-length relationships with 95% confidence intervals (shaded) compared within site across month (**Model 1**) for spottail shiner captured in 2015 from A) Weldon Ferry (September: n = 51; October: n = 57), B) Cecil Ferry (September: n = 33; October: n = NA), C) Wapiti (September: n = 41; October: n = 40), D) Downstream Site 1 (DS1; September: n = NA; October: n = 38), and E) Downstream Site 2 (DS2; September: n = 49; October: n = 51).

Maximal and minimal models also significantly predicted mass using **Model 2** ($F = 193.00$, $df = 18$ and 138, $p < 0.001$; adjusted $r^2 = 0.96$; AIC = -980.56; $F = 422.60$, $df = 9$ and 164, $p < 0.001$; adjusted $r^2 = 0.96$; AIC = -1090.24; minimal model overview: Table 4.13).

Table 4.13 Significant terms of the minimal model predicting mass-at-length (condition, g/mm) of spottail shiner captured in September of 2015 using **Model 2**.

	SS	**DF**	**F**	**P**	
Intercept	0.589	1	327.570	0.000	***
Site	0.021	3	3.938	0.010	**
Length	0.567	1	315.351	0.000	***
Triglycerides	0.007	1	3.715	0.056	.
Site:Triglycerides	0.014	3	2.560	0.057	.
Length:Triglycerides	0.007	1	4.038	0.046	*
Residuals	0.295	164			

Adjusted cross-site comparisons revealed no significant differences across site within September ($p > 0.01$) (Fig. 4.6A).

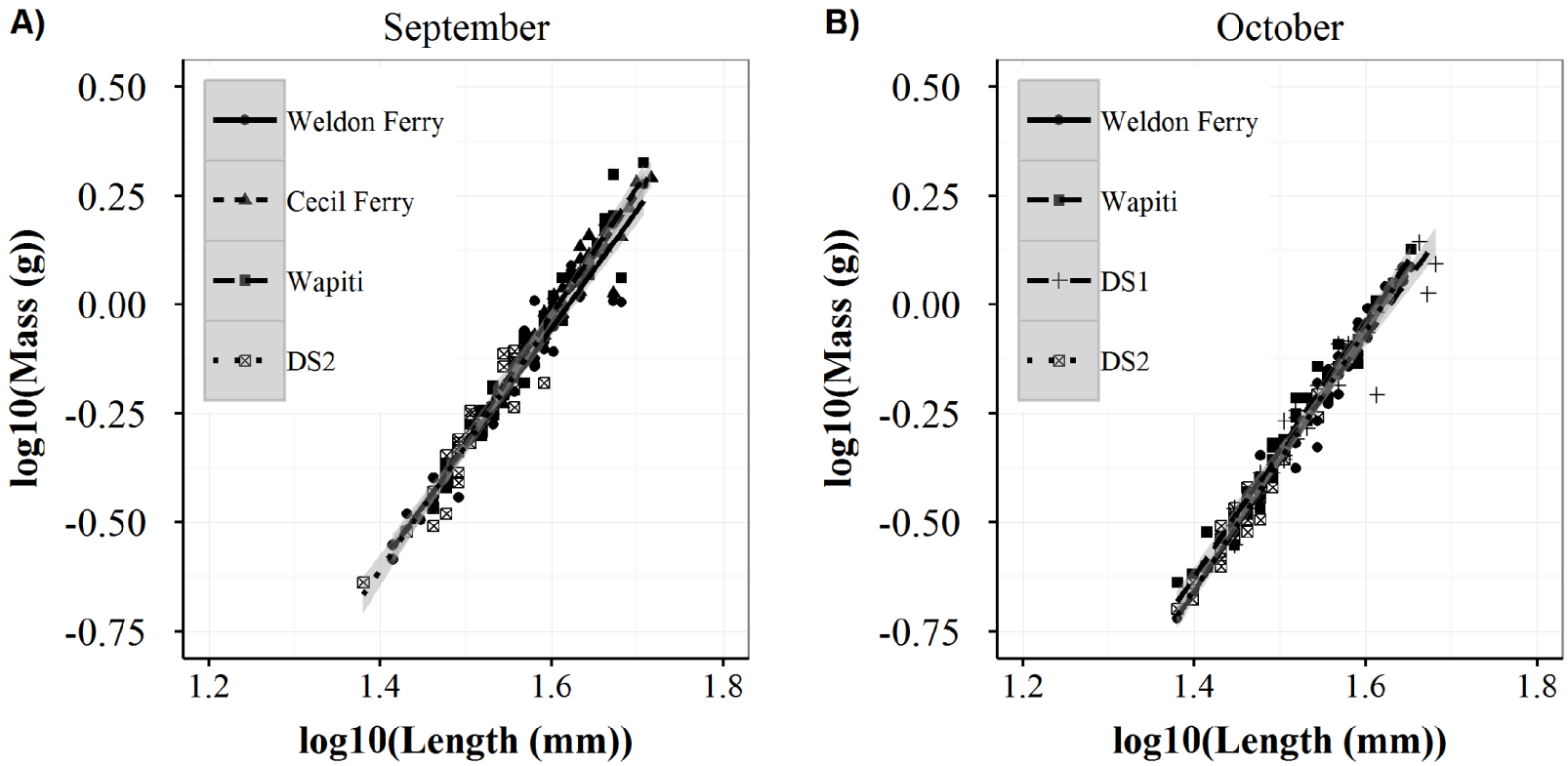

Fig. 4.6 Log-transformed mass-at-length relationships with 95% confidence intervals (shaded) compared within month across site for spottail shiner captured in 2015 in A) September (**Model 2**; Weldon Ferry: n = 51; Cecil Ferry: n = 33; Wapiti: n = 41; Downstream Site 1 [DS1]: n = NA; Downstream Site 2 [DS2]: n = 49) and B) October (**Model 3**; Weldon Ferry: n = 57; Cecil Ferry: n = NA; Wapiti: n = 40; Downstream Site 1 [DS1]: n = 38; Downstream Site 2 [DS2]: n = 51).

Maximal and minimal models predicting mass were both statistically significant using **Model 3** ($F = 326.00$, $df = 18$ and 138, $p < 0.001$; adjusted $r^2 = 0.97$; AIC = -1032.5; $F = 1117$, $df = 5$ and 153, $p < 0.001$; adjusted $r^2 = 0.97$; AIC = -1050.4; minimal model overview: Table 4.14).

Table 4.14 Significant terms of the minimal model predicting mass-at-length (condition) (g/mm) of spottail shiner in October of 2015 using **Model 3**.

	SS	DF	F	P	
Intercept	4.588	1	3521.458	0.000	***
Site	0.011	3	2.727	0.046	*
Length	4.184	1	3211.756	0.000	***
Glycogen	0.010	1	7.694	0.006	**
Residuals	0.199	153			

Post-hoc lsmeans comparisons revealed DS2 to have significantly lower mass-at-length compared with Wapiti ($p < 0.01$), though no significant differences were observed among any other sites ($p > 0.01$) (Fig. 4.6B).

4.5 Discussion

The results of this study provide further mixed evidence for hydropeaking as a chronic environmental stressor, but suggest whole-body cortisol concentration may be developed as a biomarker of chronic stress using shiner as a sentinel species. Shiner proved to be an excellent sentinel species as they were common and abundant, and showed comparable patterns of increased cortisol concentrations, peaking about 45-minutes post-stress challenge, within most samplings.

In this study the strongest predictor of mass was length, though mass was also predicted by triglyceride and glycogen concentrations in **Models 1 and 3** respectively, showing a predictive relationship between growth and energy stores, as observed in previous studies (Chellappa et al. 1995; Pangle and Sutton 2005). Mass-at-length (condition) of shiner at DS2 was significantly low compared with Wapiti shiner in October but not September, which could represent the effects of a chronically stressful environment (Wendelaar Bonga 1997; Ellis et al. 2012). However, these observations may simply represent random variation among sites, as evidenced by significantly lower condition of Weldon Ferry compared to Wapiti shiner when compared across site and averaged across month. Significantly lower condition at Weldon Ferry and DS2 fish may therefore

indicate energy constraints at these sites or may simply be due to Type I errors, since differences in K at either site compared with Wapiti yielded minimal effect sizes by any metric (<5%, Munkittrick et al. 2009), which is less than half what was observed in 2014 (Chapter 3).

In agreement with results from 2014 (Chapter 3), whole-body triglycerides increased across months within sites. This suggests shiner can still accumulate energy stores in the autumn. Triglyceride concentrations decline in early winter in other forage species (Chellappa et al. 1995; Guijarro et al. 2003; Vollenweider et al. 2011), and later sampling may be needed to observe decreased triglyceride concentrations observed in other fish. Whole-body triglyceride concentrations observed in this study were approximately one-third of those observed in shiner collected from a reference location in northern Saskatchewan (Bennett and Janz 2007). Triglyceride concentration was predicted by length, as was observed in YOY perch (*Perca fluvialitis*) (Bocherding et al. 2007). While direct effects of site were not considered due to interactions between site and time point, analyses within month do not suggest downstream energy deficiencies as was observed in 2014 (Chapter 3), which suggests that if hydropeaking is acting as a stressor it had yet to yield appreciable secondary effects in fish by October of 2015 (Barton 2002; Ellis et al. 2012). The lack of a pattern in triglyceride concentrations across time point suggests either minimal direct links between cortisol and triglyceride concentrations in shiner (Wendelaar Bonga 1997), or that the effects on triglyceride concentrations cannot be captured within 90 minutes following acute stress.

Lipids and tissue energy content (joules/gram) have all been predicted by condition factor in other fish species (Chellappa et al. 1995, Jonas et al. 1996, Pangle and Sutton 2005). In this study K predicted triglyceride concentrations in shiner within sites and within September, but not October. This result suggests a variable relationship between condition and triglyceride concentrations as observed in muskellunge (*Esox masquinongy*; Jonas et al. 1996) and three-spined stickleback (Chellappa et al. 1995). Further, condition remained the same across months while losing predictive power over the higher triglyceride concentrations in October, suggesting that these shiner were beginning to prioritize energy storage over somatic growth in preparation for the winter (Shuter and Post 1990; Post and Parkinson 2001). This may maximize winter survivorship of this and other fast-growing species (Wells and House 1974; Post and Parkinson 2001). While it remains to be confirmed, potential detection of this pattern would have been missed by condition

assessments alone, and further supports calls to include more biochemical assessments in condition analyses (Simpkins et al. 2003; Weber et al. 2003).

Acute stress primarily mobilizes liver glycogen (Wendelaar Bonga 1997), and whole-body glycogen concentrations reached minima after 15 minutes in fish captured in September. However, this trend was not observed in October despite similar stress responses across months, suggesting that changes in liver glycogen concentrations may have a narrow window of detection as it is catabolized, particularly in whole-body homogenates. Difficulty detecting this change is likely exacerbated by using a whole-body homogenate in which high liver glycogen concentrations have been combined with the lesser glycogen concentrations found in the relatively large volume of the muscle and skeletal mass (Kelly and Janz 2008; Goertzen et al. 2012). Overall, mean whole-body glycogen concentrations were lower in shiner from this study compared with liver concentrations of shiner captured from a reference site at a similar latitude in Saskatchewan (Goertzen et al. 2012) but higher than the concentrations observed in the muscle, and similar to concentrations observed in zebrafish (*Danio rerio*; Thomas and Janz 2011). High glycogen concentrations at DS2 in October compared with September may reflect glycogen retention, which would agree with the diminished cortisol response observed in that month and may suggest a chronically stressful environment. Decreased plasma glucose concentrations are also observed following an acute challenge of chronically stressed trout, suggesting retained glycogen (Barton et al. 1987; Jentoft et al. 2005). However, we were not able to capture a pattern of glycogen decline across time points in all sites and months, preventing a conclusive assessment of this endpoint.

Whole-body cortisol concentrations increased following acute stress in all fish captured in all month and site combinations, and generally reached peak concentrations 45-90 minutes after stress challenge. The 5-fold increase from basal to peak cortisol concentrations observed in this study are of the same magnitude as other minnow species, including whitetail shiner (*Cyprinella galactura*), spotfin chubs (*Erimonax monachus*), and zebrafish (Sutherland et al. 2008; Ramsay et al. 2009). The acute stress challenge elicited a similar cortisol response in shiner as has been observed in juvenile rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), and sparid red porgy (*Pagrus pagrus*) (Barton et al. 1987; Schreck et al. 1995; Rotllant and Tort 1997). Time point was consistently the strongest predictor of cortisol concentrations across models, followed closely by site and the interaction between these two variables. Other significant predictors of cortisol concentration included K and triglyceride and

glycogen concentrations, suggesting a direct relationship between cortisol, condition, and energy stores in these fish as would be expected from previous literature (Wendelaar Bonga 1997; Sousa et al. 2010; Cook et al. 2012; Ellis et al. 2012).

Cortisol concentrations in DS2 fish in September were relatively high post-stress challenge compared with other sites, but were statistically within the natural variation observed across reference sites. In October, this pattern was reversed, with DS2 fish showing slightly lower concentrations across earlier time points relative to both DS2 in September and Wapiti and DS1 in October. High basal concentrations in DS1 captured in October may have been caused by the stress of predation (Wendelaar Bonga 1997; Barcellos et al. 2007), as a northern pike was captured in the seine net along with these samples (D. Green, personal observation). While previous studies have shown additivity of stress challenges reflected in the cortisol response (Barton 2002), the lack of a subsequent and additional rise in cortisol concentrations in October DS1 fish after capture suggests that the stress challenge as employed was sufficient to elicit the maximum physiological cortisol response from these fish. This result supports that of Cousineau et al. (2012) who showed that diel differences in basal cortisol concentrations upon capture do not affect the maximum cortisol amplitude induced by an acute stressor using wild-caught bluegill (*Lepomis macrochirus*), though supraphysiological doses of cortisol did elicit an opposite effect in the same species (McConnachie et al. 2012b).

Cortisol responses showed remarkable similarities within Weldon Ferry and Wapiti populations across months suggesting a conserved genetic or environmental signature within these populations over time (Pottinger and Carrick 1999; Barton 2002). Across months, only DS2 showed a significant decrease in the magnitude of the cortisol response. This pattern, similar to that observed in chronically stressed trout exposed to an acute stressor (Barton et al. 1987; Jentoft et al. 2005), suggests fish at DS2 may be facing a chronically stressful environment. Curiously, this shift made the DS2 response in October nearly identical to that in both months at Weldon Ferry, possibly signaling a chronic stressor at this upstream reference site. It should also be noted that the cortisol response patterns in Weldon Ferry fish across months and DS2 fish in October compared to Wapiti in either month or Cecil Ferry in September are also very similar to the pattern observed in older as compared to younger silver catfish (*Rhamdia quelen*; Barcellos et al. 2012).

In sum, this study provides mixed results implicating hydropeaking as an environmental stressor. Other studies on hydropeaking found rapid habituation of the cortisol response in

response to peaking (Flodmark et al. 2002), but this study focused purely on flow rates. We expected lateral shoreline displacement as the major agent of stress in our study sites (Chapter 1; Chapter 3). Where the effects of hydropeaking on a low-pitched shoreline have been examined, stranding and behavioral impacts were noted even among species that preferentially inhabit deeper river channels (Cocherell et al. 2012). Reduced triglyceride concentrations were not observed in fish downstream from the dam in this study, unlike 2014 (Chapter 3), and no consistent effects were noted in glycogen analyses. An inhibited stress response and glycogen retention were observed in shiner from DS2 in October, but similarities with one of the reference sites (Weldon Ferry) complicated the interpretation of this difference. While no stressor is currently proposed for this latter site, recent research indicates hypolimnetic water releases from a large reservoir upstream of Weldon Ferry (Phillips et al. 2015) may cause cooler temperatures at this site. This could produce changes in the stress response, and changes in secondary and tertiary stress indicators that reflect chronic stress (Davis and Parker 1990; Wendelaar Bonga 1997; Donaldson et al. 2008;), particularly in the case of shiner, a species which preferentially inhabits warmer waters (Wells and House 1974).

Future development of this protocol should incorporate aging structure analysis to assess if differences in age within year-classes can affect the shiner cortisol response (Barcellos et al. 2012), and measure free glucose or lactate concentrations as a secondary measures of the stress response (Vijayan and Moon 1992). Free glucose is a reliable blood parameter that changes with cortisol but also peaks later after stress, making changes in this parameter easier to detect than changes in glycogen depolymerization (Barton et al. 1987; Jentoft et al. 2005; O'Connor et al. 2011), though patterns in whole-body concentrations would need to be shown to correlate with plasma concentrations. Sex effects have been shown to influence the stress response in bluegill sunfish (Cook et al. 2012), and now that the viability of the shiner stress response as a biomarker has been demonstrated, future studies should assess whether sex differences in cortisol production exist in shiner as well. It would also be useful to analyze stomach contents for dietary differences that might affect the energetic endpoints under study (Jauncey 1982). Finally, in the interest of reducing animal usage, future studies could be refined by employing non-lethal methods of cortisol analysis that would allow the release of tested fish back to the wild (Martínez-Porchas et al. 2009; Zuberi et al. 2011), and collecting fish only past the 15-minute time point for glycogen and triglyceride analyses, as neither of these measures showed significant patterns of change between

15 and 90-minutes. Lab studies should be conducted to assess the shiner stress response in controlled settings, and to determine the contribution of receptor density, transcriptional changes, and cortisol metabolism on the cortisol concentrations elicited by acute and chronic stressors in this species (Wendelaar Bonga 1997; Aluru and Vijayan 2009; Wiseman et al. 2011), and lab or field studies should consider a longer post-stress window to determine the full time course of the shiner stress response. These additional refinements will optimize the utility of this stress challenge protocol, and increase its application in identifying stressful environments for fishes.

4.6 Disclosure of conflict of interest

Funding for this study was provided in part by Saskpower, the crown corporation that owns and operates the E. B. Campbell dam. Saskpower had no part in the collection, analysis, or interpretation of the samples in this study, and they did not partake in writing the preceding manuscript, though they will be presented with a copy for review.

CHAPTER 5:
DISCUSSION AND SUMMARY

5.1 Discussion of key findings

Hydroelectric power is a substantial and renewable component of global power production (Turkenberg et al. 2012). Largely due to expansion in the developing world, global hydroelectric output is projected to increase 73% in the next 20 years (Zarfl et al. 2015). It is therefore important that we understand the potential effects of dams and reservoirs, both on site and downstream, to minimize impacts of hydroelectric development on the environment and people (Rosenberg et al. 1995; Finer and Jenkins 2012).

The objectives of this thesis were to characterize historical and contemporary [Hg] in fish impounded in a reservoir and downstream fishery, to gauge the influence of hydropeaking on physiological markers of fish condition and [Hg], and to determine the viability of the cortisol response in a model species as a biomarker of chronic environmental stress. To these ends, I present the following key findings of my thesis, followed by a discussion of their relevance to this study and potential future research directions.

1. Reservoirs are unique and complex systems, and site specific studies are required to assess the physical, chemical, and biological impacts imposed by dam construction, activity, and decommissioning to the impounded and downstream environment.
2. [Hg] may be elevated longer in fish populations downstream of dams, exacerbating risks of chronic Hg exposure to human consumers.
3. Hydropeaking may exacerbate [Hg] in fish from some downstream environments by affecting fish physiology, though more data are required to complete this assessment.
4. The shiner stress response may be a useful biomarker in the detection of chronic physical and/or chemical environmental stressors, though more research is required.

1. Reservoirs are unique and complex systems, and site specific studies are required to assess the physical, chemical, and biological impacts imposed by dam construction, activity, and decommissioning to the impounded and downstream environment

Thornton et al. (1990) describes reservoirs as dynamic systems that cannot be appropriately compared with similar aquatic systems. Differences in water influx and efflux, geologic history, and morphology across reservoirs and lakes, and across reservoirs themselves, mean there are differences in the degree and magnitude of contaminants, nutrients, and suspended particles entering and leaving these waterbodies, and that their mixing and settling dynamics are inherently different (Cooke et al. 2005). Consequently, reservoirs also change the characteristics of downstream water bodies depending upon the settling and liberation dynamics of suspended particles, nutrients, and contaminants (Thornton et al. 1990; Cooke et al. 2005). Flow rates themselves are identified as an important source of variation for the downstream environment (Kingsford 2000; Guo et al. 2012), and the continued assessment of the reservoir-river-lake continuum will necessarily need to address the effects of physical and chemical changes in the waters released to the downstream ecosystem (Guo et al. 2012).

In the case of the Saskatchewan River, the most notable change in waters downstream of the E. B. Campbell dam was decreased turbidity, a common change downstream of dams worldwide (Kondolf 1997; Smith et al. 2016). In a physical sense, lower turbidity may benefit downstream fish by minimizing the negative effects of sediment deposition on eggs and juvenile growth (Sutherland and Meyer 2007; Kemp et al. 2011). Despite these possible benefits, the consequences of altered turbidity are complex (Wilber and Clark 2001), and may have mixed effects on downstream environments. Dissolved particles can be important vectors in the advection of both contaminants and nutrients (Warren et al. 2003; Vaze and Chiew 2004), and changes in turbidity can therefore change the deliveries of both sorbed constituents to downstream environments. In this instance, the reduced turbidity downstream of the E. B Campbell dam may have meant less MeHg transported to the downstream environment, as a major fraction of aquatic MeHg is sorbed to particulates (Schetagne et al. 2000). Conversely, this may not have altered the Hg transportation to a great degree as dissolved MeHg can comprise the major proportion of aquatic MeHg concentrations (Schetagne et al. 2000). In either case, a history of the unique Hg

transportation characteristics and how they may have influenced fish [Hg] within this system could possibly be informed using sediment cores.

Sediment cores can be used to reconstruct historical timelines of nutrient and contaminant deposition in aquatic environments (Schelske and Hodell 1995; Muir et al. 2009). During this study we attempted to uncover the historic trends of Hg deposition in Cumberland Lake using core analysis. Unfortunately, we found that continual sediment re-suspension and mixing in the lake precluded the construction of a meaningful timeline at the site selected for coring (L. Doig, unpublished data). The reservoir itself, Tobin Lake, is typically shallow, but deeper locations could be cored to allow for timeline construction which could be used to measure rates of nutrient and contaminant deposition in the reservoir (Schelske and Hodell 1995; Santschi et al. 2001) and determine which constituents carried by the water became less likely to make it downstream after reservoir construction. These data could also prove invaluable in constructing a historical timeline of Hg deposition that may have been contributed from the upstream chlor-alkali plant near Saskatoon (Wobeser et al. 1970). It is also worth noting that agriculture dominates the landscape along the river upstream of Tobin Lake, and sediment core information could also reveal how reservoir construction changed the delivery rates of pesticides and other contaminants that could affect downstream populations if sediments were ever remobilized (Bednarek 2001; Cooke et al. 2005).

In an ideal scenario, we would not lack the pre-dam data on fish from this system, and would be able to assess the potential effects of altered flow and turbidity in greater detail. By characterizing the transportation and remobilization risk of contaminants and nutrients between these sites, future studies will ensure we assess the historical, contemporary, and future risks associated with this hydroelectric development.

2. [Hg] may be elevated longer in fish populations downstream of dams, exacerbating risks of chronic Hg exposure to human consumers

Rates of Hg decline from Tobin Lake and Cumberland Lake fish were on the order of magnitude expected from research on reservoirs at similar latitudes (Bodaly et al. 2007), with slowest returns to baseline typically observed in pike and walleye at both locations. [Hg] were consistently lower in fish from Cumberland Lake relative to Tobin Lake at the start of the study

period (Chapter 2) and Cumberland Lake fish populations took less time to return below the consumption guideline of 0.5 mg/kg (25 vs 30 years, respectively; Health Canada 2007), despite significantly lower rates of Hg decline from walleye and goldeye populations downstream. Fish tissue concentrations were typically below consumption guidelines by 5-20 years after the point when records were kept (1970), and despite the reservoir being commissioned in 1963 [Hg] in some populations had yet to reach background concentrations by 2014-2015.

The observed delay in downstream fish [Hg] decline was corroborated by higher [Hg]-at-length in walleye immediately downstream of the dam (Chapter 2), and by elevated [Hg] observed in YOY shiner at sites downstream of the dam compared with upstream (Chapter 3). Collectively these data show elevated [Hg] have been retained in fish as far as 100 km downstream of the E. B. Campbell dam despite minimal [Hg] in the water column. This delay is of concern to downstream human consumers as it extends the duration of chronic Hg exposure. Mercury exposure is associated with neurological, reproductive, and cardiovascular effects (Kim et al. 2016) and exposures are of particular concern to sensitive populations such as pregnant women, children, and/or people who frequently consume fish (Health Canada 2007). The results of this thesis show that sites downstream of reservoirs should be considered when assessing Hg risk to human consumers (Schetagne et al. 2000; Anderson 2011), and suggests that regulatory decisions regarding [Hg] within fish need to be made to account for sensitive members of downstream human populations (Calder et al. in press).

3. Hydropeaking may exacerbate [Hg] in fish from some environments by affecting fish physiology, though more data are required to complete this assessment

Different dams will have different degrees and types of impacts on physical and chemical water parameters depending upon their geomorphology, the depth of the reservoir, the height of the release point of the dam, and whether they engage in hydropeaking (Thornton et al. 1990; Cooke et al. 2005; Young et al. 2011). Depending upon the degree of the effect on the downstream environment, changes in these parameters may be detrimental to downstream ecosystems. Early in my field work, observations of fish stranded after a rapid reduction in flow (Chapter 1) made it clear that on at least one occasion, the daily peaking cycle was creating an environment that was sufficiently stressful as to be physically overwhelming to small fish. Dynamic energy budget

theory and bioenergetic modeling explain how the finite energy available to meet a fish's homeostatic demands is taxed by chronic stress and can cause changes in fish that can lower growth efficiency, thereby reducing the effects of growth dilution on contaminant concentrations (Trudel and Rasmussen 2006; Sousa et al. 2010; Ellis et al. 2012; Sandheinrich and Drevnick 2016). For these reasons hydropeaking was investigated as an environmental stressor that may contribute to the delayed [Hg] decline from downstream fish population.

Hydropeaking has been implicated as a stressor as flow rate change has been shown to elicit the physiological stress response in fish, and chronic stress can potentially influence [Hg] in fish by minimizing growth dilution (Flodmark et al. 2002; Trudel and Rasmussen 2006; Sousa et al. 2010; Sandheinrich and Drevnick 2016). Flodmark et al. (2002) showed juvenile brown trout (*Salmo trutta*) secreted cortisol in response to changes in flow rate meant to simulate hydropeaking, but rapidly habituated to flow changes as evidenced by lowering of previously elevated cortisol and glucose concentrations after seven days of exposure to the hydropeaking stressor. While this study provided valuable information on the ability of fish to habituate to flow rate changes, they did not account for lateral shoreline fluctuations exacerbating chronic stress. Studies that have accounted for this change suggest this stress can be as overwhelming as observations downstream of E. B. Campbell would suggest, with 8% of fish susceptible to stranding after downramping (Cocherell et al. 2012), and increased mortality predicted for fish in simulated peaking streams (Puffer et al. 2015).

In this study, the potential effects of hydropeaking stress were first explored by analyzing the energy stores, condition, and [Hg] of shiner and how these measures predicted each other. Shiner analyses from both 2014 and 2015 revealed mixed support for increased [Hg] as a result of effects on secondary endpoints (condition, energy concentrations) in fish. Though relatively low condition and triglyceride concentrations, and relatively high [Hg] were found in downstream shiner (Chapter 3), inconsistent results across years and variation among upstream sites suggested these results may fall within natural variation (Chapter 3, 4). Therefore, in order to develop clearer conclusions about the role of hydropeaking stress on downstream fish it will be necessary to more fully characterize the natural variation in energetic and morphological endpoints of fish at additional upstream reference and downstream sites. These results could prove invaluable in future assessments of [Hg] in fish downstream of hydropeaking dams, as they may identify mechanisms

by which dam operators can minimize the influence of hydropeaking on delayed Hg clearance from, and other deleterious effects on, downstream fish populations (Young et al. 2011).

4. The shiner stress response may be a useful biomarker in the detection of chronic physical and/or chemical environmental stressors, though more research is required

As the results from 2014 were not conclusive, the following year's shiner collections were modified to include an acute stress challenge. While there is not an established protocol for testing environmental stress using the physiological stress response of fish, it was hoped that the results of this study would provide sufficient data to encourage the further development of a standardized protocol(s) for assessing environmental stress with shiner as a sentinel species.

Results again provided mixed support for hydropeaking as a stressor on downstream fish, though at a minimum the study revealed a great deal of similarity in the physiological relationships observed in shiner as compared to other wild and lab sampled fish (e.g. growth/condition and energy and cortisol concentrations; Pangle and Sutton 2005; Cook et al. 2012; Chapter 4). Notably, cortisol responses in shiner produced a reliable, time-delayed increase in cortisol concentrations in response to an acute stressor as observed in other species (Barton et al. 1987; Schreck et al. 1995; Rotllant and Tort 1997; Jentoft et al. 2005), with similar concentration patterns observed over time points across sites and months (Chapter 4). This finding provides the critical foundation necessary to begin further explorations of the shiner stress response as an indicator of environmental stress.

As cortisol concentrations in shiner increased over time in response to acute stress challenge as in other species, inhibition of the cortisol response in shiner may be a useful biomarker in making preliminary assessments of aquatic environments under potential chemical or physical chronic stressors (Barton et al. 1987; Jentoft et al. 2005; Wiseman et al. 2011; Koakoski et al. 2014). Within this study, the cortisol responses from the upstream site Weldon Ferry were low across months and nearly identical to those in DS2 in October, but only DS2 (where strandings had been observed) showed a decreased stress response over time as has been observed in chronically stressed fish (Barton et al. 1987; Jentoft et al. 2005). However, the concentrations observed at this site were still within the range of natural variation across sites within months (Chapter 4). Future research should sample additional sites to consider whether the patterns

observed in this study are a result of chronic stress, natural variation, or are confounded by other factors. For example, fish age has been shown to elicit distinct changes in cortisol response patterns in silver catfish (*Rhamdia quelen*) that were visibly similar to the different patterns observed in this study (Barcellos et al. 2012). The consistent ability to collect shiner of a single age class means that the effects of age should be minimal within our study, but greater detail is needed about the change in shiner stress response as they age in order to fully rule out potential confounding effects.

Interestingly, where physiological differences (e.g. condition) were noted across sites in 2015, they were most frequently observed to be low in Weldon Ferry or DS2 fish when compared with those from Wapiti. While no stressor was presumed for Weldon Ferry, the similarity between Weldon Ferry cortisol patterns in both months and the DS2 cortisol pattern in October suggests there may be two distinct cortisol response profiles in response to the acute stress challenge in shiner; a smooth upward arc peaking in 45-90-minutes post-stress (September: Wapiti, Cecil Ferry, DS2; October: Wapiti), and a response with significant attenuation observed within 15-minutes post-acute-stress and producing concentrations that may still be rising 90-minutes post-stress (September: Weldon Ferry; October: Weldon Ferry, DS2; Chapter 4). Further, while Weldon Ferry was originally selected as an upstream reference site, recent research indicates that this site is subject to cool water releases due to hypolimnetic water released from an upstream reservoir (Phillips et al. 2015). Temperature can modify the stress response of fish (Davis and Parker 1990), and cold shock stress may induce primary, secondary, and tertiary stress responses in fish (Donaldson et al. 2008). This could cause exaggerated effects on shiner due to their preference for warmer habitats (Wells and House 1974), and it is possible that this latter pattern represents a conserved cortisol secretion signature in shiner from chronically stressed environments. Future studies should not only explore whether these differences can be characterized at other control and affected sites, but also extend the window for fish collection post-stress challenge to fully characterize the stress response in fish from sites that were potentially increasing their cortisol concentrations past the 90-minute collection point in this study.

While glycogen was used as a secondary measure of stress in this study, glucose may have been a more appropriate choice (Barton et al. 2002). Wiseman et al. (2011) found a decreased cortisol response in trout exposed to selenomethionine that was accompanied by lower levels of plasma glucose, and decreased plasma glucose was also observed following a stress challenge of rainbow trout exposed to a chronic physical stressor (repeated handling and aerial exposures;

Barton et al.1987). A possible explanation for these trends is reduced glycogenolysis in response to the stressor, or increased glucose metabolism in chronically stressed or contaminant-exposed fish, suggesting the attenuation of the stress response reflects an explicit adaptive mechanism meant to preserve energy over time in stressful environments, as cortisol elevation is known to be energetically taxing in fish (Wendelaar Bonga 1997; De Boeck et al. 2001; Ellis et al. 2012). It is recommended that future studies measure glucose at the same time points selected for cortisol concentration assessments (Barton et al. 1987; Wiseman et al. 2011).

Future studies on these endpoints may be able to fill knowledge gaps while minimizing ecological effects by adapting minor modifications to the stress challenge protocol to allow for non-lethal sampling of collected shiner (Martínez-Porchas et al. 2009; Zuberi et al. 2011). This would leave a minimal requirement for lethal sampling to further address physiological comparisons between wild-caught shiner and other lab and field studied species. While analyses of triglycerides and glucose may yet require lethal sampling of small fish, efforts are currently underway to develop non-lethal methods for these endpoints as well (Olsen et al. 2013; Wu et al. 2015). For the time being, the number of fish collected for triglyceride and glucose analyses could be reduced by collecting samples from any time point past 15-minutes post-stress-challenge as there were no patterns of change in either parameter over time point past 15-minutes.

5.2 Summary and conclusions

The results of this thesis suggest that there are interrelationships between dam and reservoir construction, ongoing dam activities, fish physiology, and biochemical cycling of Hg in impounded and downstream foodwebs that may exacerbate risks of chronic Hg exposure to downstream human consumers. Delayed Hg decline was observed in downstream fish populations, and while definitive conclusions could not be drawn on the effects of ongoing dam activities and this delay, the results obtained suggest that Hg retention may be related to ongoing hydropeaking activities. Further, as elevated [Hg] were observed in both walleye *and* shiner immediately downstream of the dam, it is possible that the elevated [Hg] may be observed in prey items lower in the foodweb, and ongoing research within our lab is addressing the potential role of altered benthic macroinvertebrate community structure in Hg bioaccumulation dynamics downstream of the dam (Mihalicz et al. unpublished; Karimi et al. 2016).

The results of this thesis have provided invaluable data on the similarities and differences between [Hg] and physiological relationships in fish from reservoirs and the waters up and downstream. These data can now be used to inform future site-specific risk assessments using the suggestions described above. Finally, the results of this thesis suggest that with further refinement whole-body cortisol concentrations in small-bodied fishes may be a useful biomarker of chronic physical and chemical environmental stressors. Application and development of the proposed stress challenge protocol may therefore allow for a rapid preliminary measure of environmental stress and aid regulatory bodies in assessing the site specific risks imposed on aquatic life by industrial development. In turn, this will allow for more rapid and cost effective identification and remediation of chemically or physically disrupted environments.

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APPENDIX

This chapter includes supplementary information from the preceding research chapters. Tables and figures are identified using chapter number (ch) and table or figure number (sy) (ch.sy).

Table C2.S1 Mean water chemistry values (±SE), summer 2013, in Tobin Lake (TL), immediately downstream of the E.B. Campbell dam (EBC), and in Cumberland Lake (CL) (n=3-9 per location).

Site	pH	Total Dissolved Ions (mg/L)	Total Dissolved Solids (mg/L)	DOC (mg/L)	Total P (mg/L)	Total N (mg/L)	chl *a* (µg/L)
TL	7.89 ± 0.27	393±32	314 ± 33	6.44 ± 1.04	0.02±0.01	0.69±0.08	6.23±0.44
EBC	7.77 ± 0.42	391±14	314 ± 18	5.44 ± 0.77	0.03±0.00	0.63±0.08	5.77±0.94
CL	7.38 ± 0.50	337±45	254 ± 34	7.09 ± 0.81	0.09±0.07	0.68±0.24	6.71±1.03

Table C2.S2 Annual rates of length-adjusted Log[Hg] decline (Log[Hg]/Log Length(cm)) vs. year from the muscle of northern pike, sauger, walleye, and goldeye populations sampled from Tobin Lake (TL) and Cumberland Lake (CL) between 1970 and 2013.

Species	N	% Annual [Hg] Decline	r^2	F	P
$\text{Northern Pike}_{TL}$	214	0.4	0.05	10.67	0.001
$\text{Northern Pike}_{CL}$	265	0.3	0.04	11.93	< 0.001
Sauger_{TL}	114	1.8	0.40	74.54	< 0.001
Sauger_{CL}	21	1.8	0.45	15.33	< 0.001
Walleye_{TL}	231	1.1	0.25	74.36	< 0.001
Walleye_{CL}	476	0.3	0.05	22.42	< 0.001
Goldeye_{TL}	118	2.6	0.31	52.87	< 0.001
Goldeye_{CL}	384	0.4	0.03	11.41	< 0.001

Table C2.S3 Analysis of covariance table comparing effects of location and year on length-adjusted Log[Hg] (Log[Hg]/Log Length(cm)) vs. year in the filets of northern pike (n=479), sauger (n=135), walleye (n=707), and goldeye (n=502) collected from Tobin Lake and Cumberland Lake between 1970 and 2013.

Species	**d.f.**	**MS**	**F**	**P**
Northern Pike:				
Location	1	0.023	0.84	0.360
Year	1	0.553	19.95	<0.001
Location*Year	1	0.020	0.71	0.372
Sauger:				
Location	1	0.000	0.01	0.915
Year	1	0.915	41.08	<0.001
Location*Year	1	0.000	0.01	0.944
Walleye:				
Location	1	0.774	27.33	<0.001
Year	1	2.843	100.43	<0.001
Location*Year	1	0.757	26.75	<0.001
Goldeye:				
Location	1	1.061	31.05	<0.001
Year	1	1.712	50.13	<0.001
Location*Year	1	0.957	28.03	<0.001

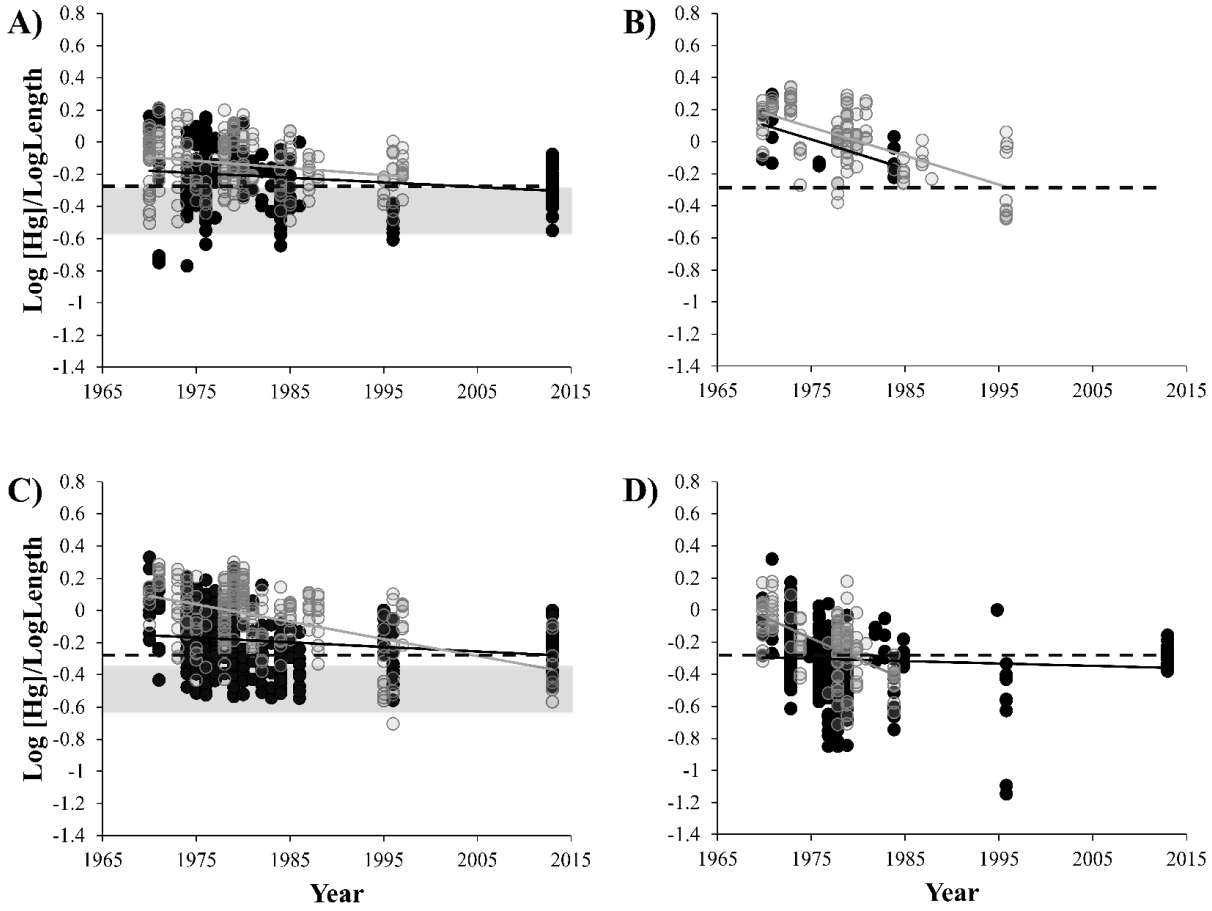

Fig. C2.S1 Historical Log[Hg] in fish muscle standardized by length (Log(cm)) vs. Year for Tobin Lake (TL; grey) and Cumberland Lake (CL; black) in a) northern pike (TL, n=214; CL, n=265), b) sauger (TL, n=114; CL, n=21), c) walleye (TL, n=231; CL, n=476), d) goldeye (TL, n=118; CL, n=384). The horizontal dashed black line represents the current consumption guideline, 0.5 µg/g (Health Canada, 2007), shaded areas represent reference lake mean Log[Hg]/LogLength(cm) (±SD) of filets from northern pike (figure 3a; collection dates 1971-2002; n=89) and walleye (figure 3c; collection dates 1970-2002; n=96). See appendix table C2.S2 for regression analyses and appendix table C2.S3 for ANCOVA results.

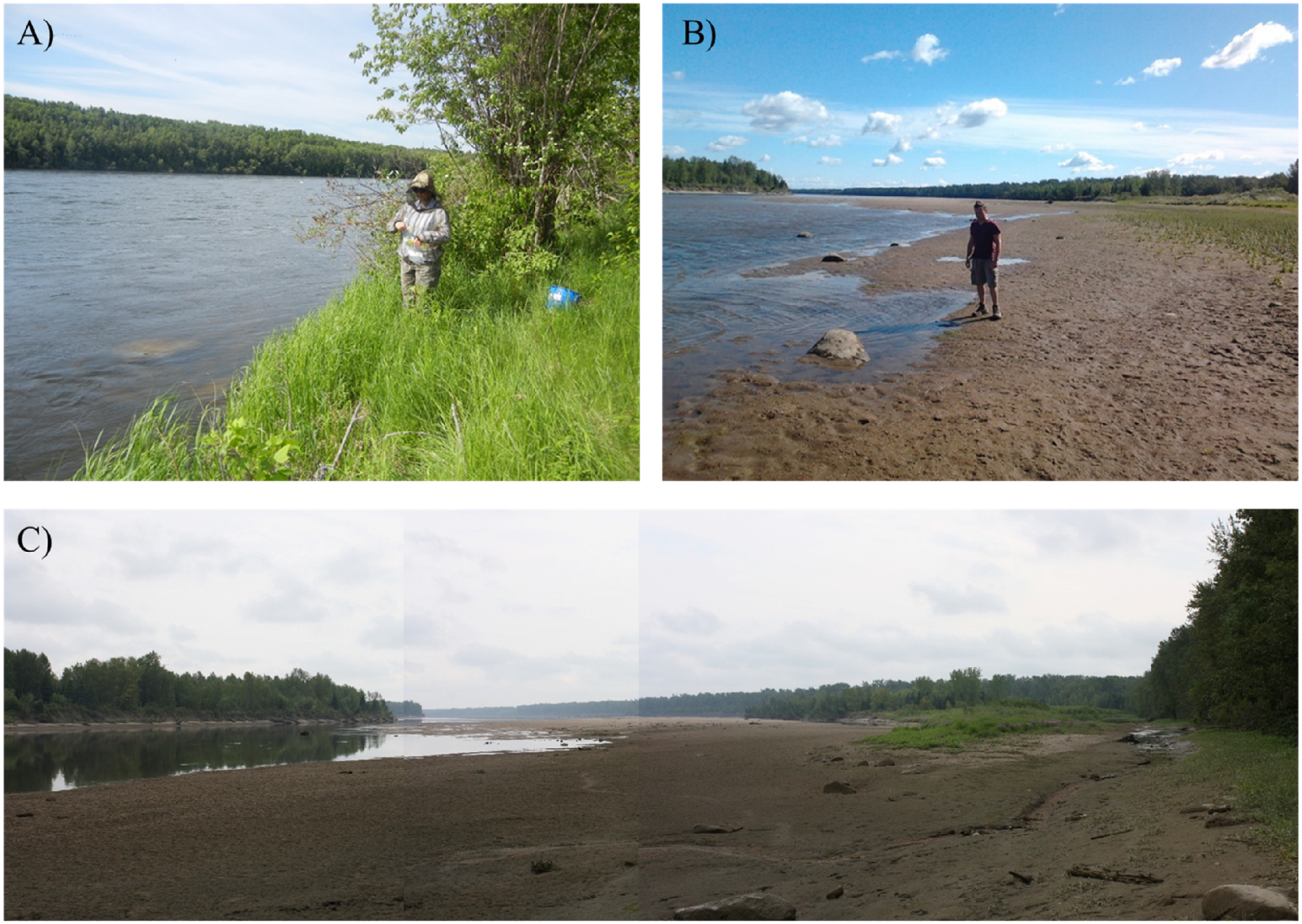

Fig. C3.S1 Topographies of A) Downstream site 1 (DS1; view looking upstream), B) Downstream site 2 (DS2; view looking downstream), and C) DS2 composite panorama of exposed shoreline at DS2 after water is restricted by downramping (view looking downstream). Photo Credit A) and B): Tim Jardine**,** C) Derek Green.