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Physiological stress response of Mountain Whitefish (*Prosopium williamsoni*) and White Sucker (*Catostomus commersoni)* sampled along a gradient of temperature and agrichemicals in the Oldman River, Alberta

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Abstract Species differences in tolerance to environmental stressors can contribute to differences in species distribution and abundance along river gradients. Climate change and intensive agriculture are likely to have major effects on fish populations in temperate zones, yet understanding of the interactions between temperature and chemical stressors on fish physiology is limited. The objective of this study was to compare the stress responses of the Mountain Whitefish, (*Prosopium williamsoni*, a cold-water fish) and White Sucker (*Catostomus commerson*i, a cool-water fish), along a temperature and pesticide gradient in the Oldman River, Southern Alberta in spring and summer. Fish were seined, placed into an enclosure, and plasma cortisol, glucose, liver glycogen, and condition factor were measured. Plasma acetylcholinesterase (AChE) activity was used as an indicator of exposure to organophosphate and carbamates pesticides. Whitefish had lower plasma AChE activity and lower liver glycogen reserves compared to suckers at all sites and all sampling times but the differences in plasma cortisol were not species-specific and there were no differences in plasma glucose levels, except at one site. Plasma cortisol increased, and plasma glucose decreased along a downstream river gradient in whitefish in both spring and summer; in sucker only plasma cortisol fluctuated and only in the summer. Liver glycogen decreased along the river gradient in both species at both seasons. Our study detected important species-specific differences in AChE activities and responses of the physiological stress axis, suggesting that whitefish are more sensitive to temperature and pesticide stress than suckers.

Keywords Fish · Pesticides · Temperature · Stress · Whitefish · Suckers · AChE · Cortisol · Glycogen

Introduction

Rivers are complex systems where many fish species coexist along elevational gradients, and species differences in tolerance to environmental stressors can contribute to differences in distribution and abundance. Fish in rivers draining agricultural areas may be exposed to a variety of concurrent stressors (Eder et al. 2007; Couillard et al. 2008a, b), including agrichemicals, increasing water temperatures, and habitat alterations resulting from flow regulation (dams, weirs, and water withdrawals). It is difficult to assess the impact of these multiple stressors on the physiological integrity of fish, especially in species for which the physiological tolerance thresholds have not been characterized.

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Species-specific responses of fish have been documented for many endpoints, including vulnerability to toxicants, acetylcholinesterase (AChE) inhibition, metal uptake and metallothionein concentration (Van Dolah et al. 1997; Linde-Arias et al. 2008; Miller et al. 2009a), cortisol secretion (Lacroix and Hontela 2004; Jentoft et al. 2005), and vulnerability to oxidative stress (Miller et al. 2009b). Differences in tolerance to thermal stress also exist; fish are classified as warm-, cool- or cold-water species according to their thermal preference (Eaton et al. 1995). Cool- and cold-water fish species often coexist, particularly when their movements are restricted by dams and weirs. As climate changes and water temperature increases, cold-water fish species in rivers draining agricultural areas in temperate zones may be particularly vulnerable since they may be exposed concurrently to agrichemicals and high temperatures (Couillard et al. 2008a, b).

In agricultural drainages fish may be exposed to short-term spikes of pesticides during spraying and rain events, or chronic exposures in areas where streams and irrigation canals drain sprayed areas (Morrison and Wells 1981). Exposures to organophosphate (OP) and carbamate (CB) pesticides lower the activity of the enzyme AChE, and cause changes in behaviour, metabolism, feeding (Pavlov et al. 1992; de Aguiar et al. 2004; Kavitha and Venkateswara Rao 2008) and reproduction (Bhattacharya 1993). Pesticides also influence the physiological stress response and cortisol secretion in fish (Bisson and Hontela 2002; Dorval et al. 2005; Teles et al. 2007), and decrease the upper temperature tolerance limits (Patra et al. 2007). There is also evidence that toxicity of pesticides to fish increases with temperature (Altinok et al. 2006).

Fish have specific thermal requirements and select habitats near their optimal temperature (Eaton et al. 1995; Eaton and Scheller 1996; Dill 1987). However, they will expand their temperature range in response to food availability, predators, competition, or social dominance (Beitinger and Magnuson 1975; Dill 1987). Increasing water temperature is already considered a widespread and problematic stressor in salmonids on the West Coast and displacement of cold-water, northern, high latitude and high altitude species of fish is predicted in many global warming models (Daufresne et al. 2003; Couillard et al. 2008a). Water temperatures outside the thermal preference range, specifically warmer water temperatures, can cause thermal stress which depletes energy reserves (Viant et al. 2003), decreases growth rates (Meeuwig et al. 2004), impairs reproduction (Daufresne et al. 2003; Ito et al. 2008) and changes behaviour (Quigley and Hinch 2006).

A combination of climate change and intensive agriculture is likely to have major effects on fish populations in temperate zones, yet the understanding of the interactions between temperature and chemical stressors on the physiological status of fish remains limited. Moreover, basic physiological data for many wild fish species are lacking. The objective of this study was to investigate physiological species-specific responses to multiple concurrent stressors (confinement, pesticides and warm temperature) in Mountain Whitefish (*Prosopium williamsoni*, a cold-water fish) and White Sucker (*Catostomus commersoni*, a cool-water fish) in a river draining an area of intensive agriculture in Southern Alberta. Plasma cortisol, glucose, liver glycogen, and condition factor were measured as the physiological stress response endpoints, and plasma AChE activity was used as an indicator of exposure to OP and CB pesticides. We hypothesized that species-specific differences in responses to multiple stressors exist and that at temperature-impacted sites, the Mountain Whitefish will exhibit an increased stress response compared to the White Sucker.

Study sites and methods

Description of sampling sites and fish species

Fish were sampled at five sites along the Oldman River (Fig. 1), in southern Alberta, Canada during spring and summer 2005 (Table 1). The Oldman River originates in the Rocky Mountains and flows east, draining an area of intensive grain and livestock production. Runoff from these operations contains agricultural chemicals including pesticides that have been detected in the Oldman River (Koning et al. 2006; Alberta Environment Pesticide Monitoring Program, Table 2). Moreover, the river exhibits a west to east temperature gradient, with higher temperatures in the lower reaches (Fig. 2). Whitefish and suckers were sampled for this study because both species are present in the Oldman River and they have different species-specific temperature optima. Whitefish represent a cold-water fish species with a maximum temperature tolerance of 23.1°C (Eaton

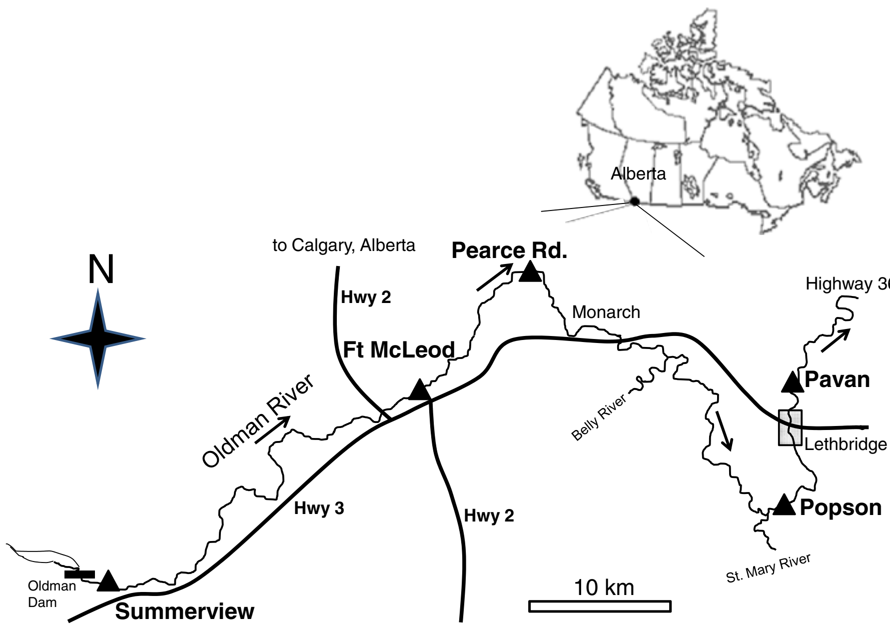

Fig. 1 Location of sampling sites (Summerview, Ft McLeod, Pearce Rd, Popson and Pavan) in the Oldman River Basin, Southern Alberta. Black triangles represent site locations, the heavy bar **I** is the location of the Oldman Dam and arrows indicate flow direction, with Summerview the most upstream site and Pavan the most downstream site. Highway 36, a site where water temperature and pesticides were monitored by Alberta Environment is also included

and Scheller 1996) and a thermal preference range between 12.8°C to 17.7°C [upper temperature preference (UTP)] (Ihnat and Bulkley 1984). Suckers represent a cool-water species with a maximum temperature tolerance of 27.4°C (Eaton and Scheller 1996) and a thermal preference range of 18.3°C to 24°C (UTP) (Eaton et al. 1995). The UTPs for sucker and whitefish are indicated in Fig. 2.

Capture and sampling of the fish

Fish were captured by a beach seine (30×1.8 m, 0.4 cm mesh) between 10:00 and 13:30 each day. The seine was deployed from a boat, and was pulled in and closed in about 20 min. Fish were transferred from the seine to floating enclosures (0.68×1×0.47 m) and were kept in the enclosure until 14:00 when they were removed in groups of 3 to 5. They were deeply anaesthetized in a solution of 0.15 gL^{-1} of tricaine methanesulphonate (MS-222) in about 1 min; blood sampled from the caudal vasculature (~30 s), and plasma was collected and frozen in liquid nitrogen. The fish were euthanized by spinal transection, weights and lengths recorded, and the liver was dissected and frozen in liquid nitrogen. Plasma and liver samples were kept frozen at -80°C until analysis.

Table 1 Characteristics of sites sampled in the Oldman River, Alberta

Site	Distance from Oldman dam (km)	Latitude and longitude	Date	Depth[a] (m)	Velocity[a] ($m\cdot s^{-1}$)	Flow[a] ($m^3\cdot s^{-1}$)
Summerview	7	49°33'N,113°49'W	Summer 2005	0.6	0.4	3.6-6
Fort McLeod	70	49°44'N, 113°13'W	Spring 2005	0.4	0.3	2.0
			Summer 2005	0.3-0.7	0.1-0.6	0.9-5.2
Pearce Road	105	49°48'N, 113°13'W	Spring 2005	0.4-0.7	0-0.5	
			Summer 2005	0.4-0.5	0.1-0.7	0.5-5.5
Popson Park	160	49°44'N, 112°51'W	Spring 2005	0.3-1.1	0.1-0.5	0.5-5.0
			Summer 2005	0.7	-	-
Pavan Park	175	49°38'N, 112°51'W	Spring 2005	0.4-0.6	0.1-0.4	0.6-3.0
			Summer 2005	0.4-0.8	1.4-1.6	9.6-31.5

[a] Measurements taken at points in the river where fish were seined; -, data not available

Table 2 Concentrations of chemicals detected in water at sites situated along the Oldman River, Alberta

Compound		Summerview	Lethbridge	Popson Park	Hwy 36
Herbicide ($\mu g \cdot L^{-1}$)[b]	2,4-D	<0.005(04)[a]	0.005-0.026(04)		0.006-0.082(04)
	Dichlorprop (2,4-DP)	<0.005(04)	<0.005-0.007(04)		<0.005(04)
	MCPA	<0.005(04)	<0.005-0.01(04)		0.003-0.022(04)
	MCPP (Mecoprop)	<0.005(04)	<0.005(04)		0.004-0.018(04)
	Dicamba (Banvel)	<0.005(04)	<0.005(04)		<0.005-0.006
Insecticide ($\mu g \cdot L^{-1}$)[b]	Dimethoate (Cygon)	<0.05(04)	<0.05(04)		<0.05(04)
Other Chemicals[c] ($ng \cdot L^{-1}$)	Cholesterol	74.70(05)		108.15(05)	217.98(05)
	Desmosterol	28.46(05)		36.34(05)	51.58(05)
	Cholestanol	3.61(05)		12.26(05)	36.27(05)
	7-Ketocholesterol	1.65(05)		7.27(05)	9.90(05)
	β-Sitosterol	40.56(05)		48.62(05)	118.74(05)
	Campesterol	9.43(05)		10.12(05)	16.75(05)
	Stigmastanol	2.47(05)		10.64(05)	33.30(05)
	Stigmasterol	19.80(05)		42.01(05)	118.20(05)
	Fucosterol	65.18(05)		111.63(05)	208.14(05)

[a] Year of sampling indicated in bracket; 2004 as (04) and 2005 as (05)

[b] data from Alberta Environment Monitoring Program (http://www3.gov.ab.ca/env/)

[c] Data from Jeffries et al. 2008

Biochemical analyses

Acetylcholinesterase (AChE)

The AChE activity was measured with an assay modified from Chuiko (2000). Plasma (2 μL) was pipetted into a microplate and 120μL of ISO-OMPA (Sigma Tetraisopropyl pyrophosphoramide, T1505) was added. The samples were incubated at room temperature for 10 min and 10 μL of DTNB (Sigma 5,5' Dithiobis [2-nitro-benzoic acid], D8130) and 10 μL of Acethylthiochlorine Iodine (Sigma minimum 98% TLC, A5751) were added, followed by a 10 min incubation at room temperature. The microplate was read at 405 nm in a microplate reader every 2 min for 10 min. Concentration of AChE for each sample was measured from the slope; internal standards (Normal Serum Control, TC-TROL[N], Teco Diagnostics) were used to ensure the accuracy of the assay was maintained.

Physiological stress response

Plasma cortisol was determined with diagnostic kits (MP Biomedicals Diagnostics Division 07-221102); assay characteristics and accuracy were verified, as described previously (Hontela et al. 1995). Plasma glucose was determined with a colorimetric assay using the GOD-PAP reagent (Roche 1929526) at 512 nm. Liver glycogen was determined using a method modified from Levesque et al. (2002).

Statistical analysis

The statistical program JMP IN version 5.1 was used to perform statistical tests at $p<0.05$. One way ANOVA was used for single factor comparisons, a *t*-test was used to compare species at each site. Significant differences between means were determined using Tukey-Kramer HSD post hoc test and log-normal data were normalized using a log transformation.

Results

Temperature regimes

Water temperatures recorded by Alberta Environment in 2004 and 2005 show that the sampling sites used in this study are situated along a temperature gradient, with Summerview as the coldest site and Pavane the warmest site (Fig. 2). Temperatures measured on sampling days by our team confirmed the gradient.

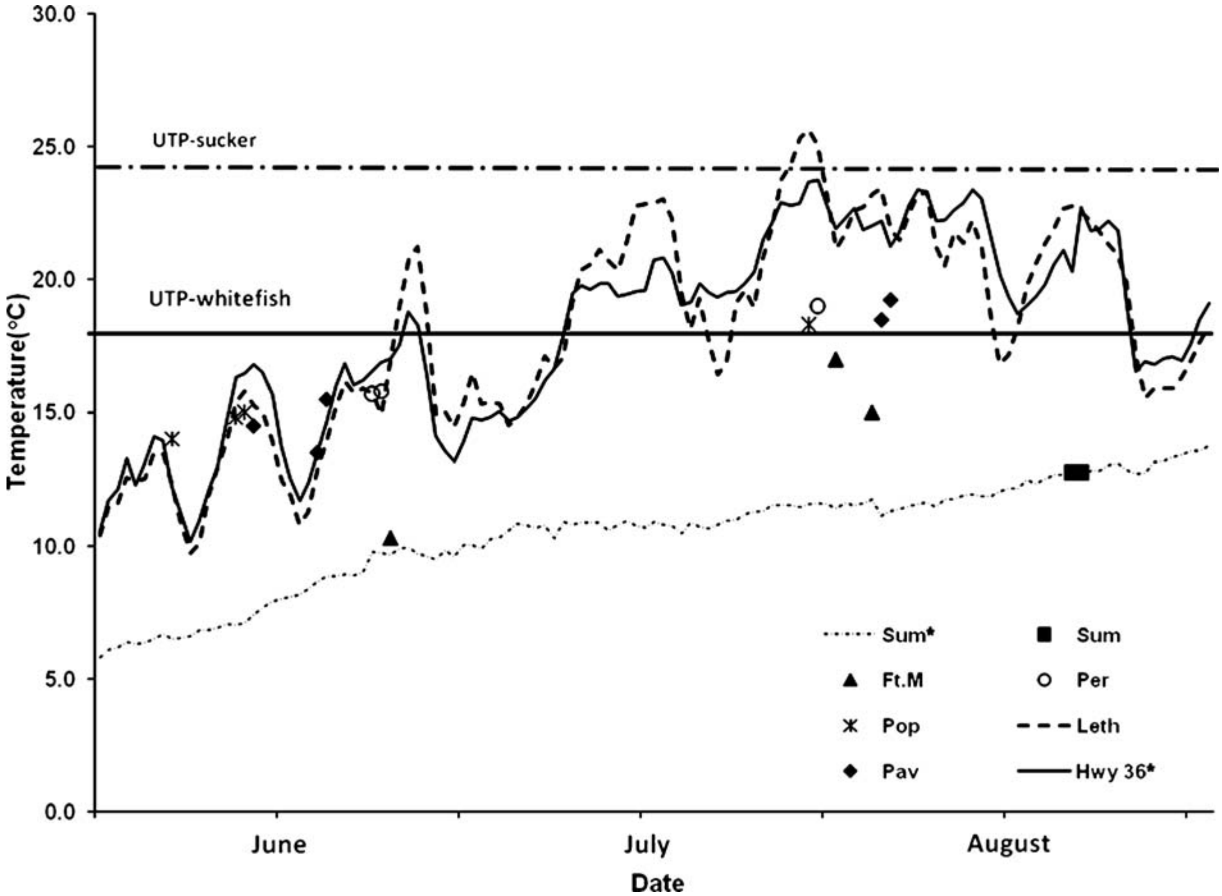

Fig. 2 Average water temperatures for each site during sampling in summer 2004 and 2005. Data for Summerview, Lethbridge and Highway 36 received from Alberta Environment. Upper thermal preference (UTP) for sucker and for whitefish are indicated by the lines on the graph for sucker (▬ • ▬ •) and whitefish (▬▬▬)

Water temperatures fluctuated throughout the sampling period and were always below the maximum temperature tolerance for whitefish and suckers, 23.1°C and 27.4°C respectively (Eaton and Scheller 1996). However water temperatures did exceed the UTP (17.7°C) for whitefish during summer sampling at Pearce Rd. site (Fig. 2). Water temperatures consistently exceeded UTP for whitefish at the most downstream sites Popson and Pavan where whitefish were not caught in summer. Suckers were not caught at the Summerview site (<15°C) in any of the sampling periods.

Pesticide exposure

Water concentrations of herbicides and other chemicals in the Oldman river increased along a west to east gradient (Table 2), however several of the OP and CB pesticides monitored by Alberta Environment (Koning et al. 2006; http://environment.alberta.ca/) were below the detection limits at all sites: Chlorpyrifos-ethyl, Diazinon and Phorate <0.005 $\mu g \cdot l^{-1}$, Parathion< 0.01 $\mu g \cdot l^{-1}$, Terbufos<0.03 $\mu g \cdot l^{-1}$, Malathion and Triallate<0.05 $\mu g \cdot l^{-1}$, Ethiion<0.1 $\mu g \cdot l^{-1}$, Disulfoton and Gluthion <0.2 $\mu g \cdot l^{-1}$. Plasma AChE activities, used as indicators of exposure to OP and CB pesticides, are presented in Fig. 3. At all sites and all sampling times, whitefish had significantly lower plasma AChE activity ($p<0.05$, *t*-test) compared to suckers. In spring, plasma AChE activities in whitefish were higher at upstream sites while plasma AChE in suckers were not significantly different among sites ($p>0.05$, ANOVA) (Fig. 3a). Plasma AChE activity in summer was lower at upstream sites in both suckers and whitefish (Fig. 3b).

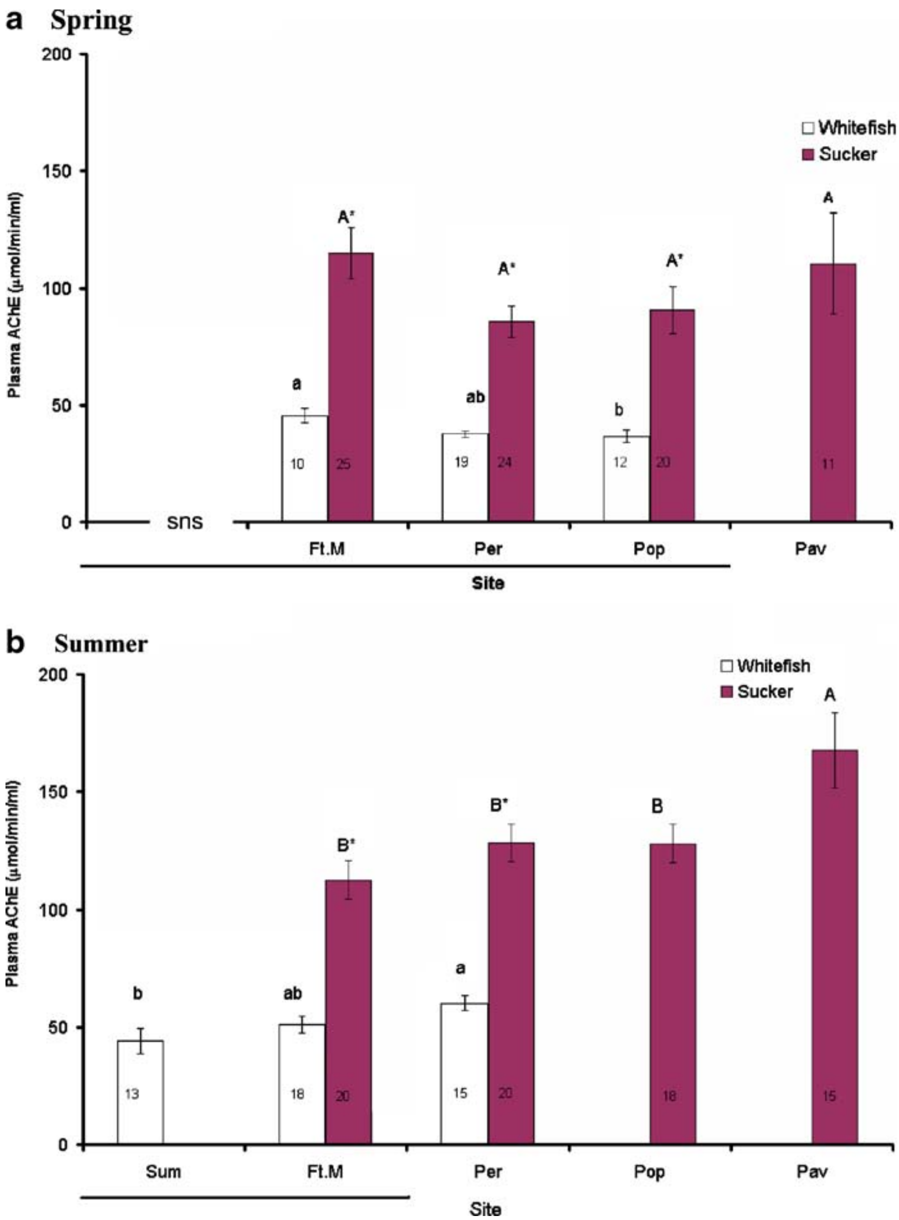

Fig. 3 Plasma AChE (mean±SE) in whitefish and suckers sampled at sites along the Oldman River in **a** Spring, and **b** Summer. Numbers in the bars indicate the sample size for each site. Lines under sites represent sites within the thermal preference of whitefish. Capital letters represent significant differences among sites for suckers and lower case letters represent significant differences among sites for whitefish (ANOVA and Tukey-Kramer HSD test α=0.05). Asterisks represent significant differences between species at a site (Student's *t*-test α=0.05). Sites with only one bar indicate that only one species was caught at that site; sns, site not sampled

Physiological stress response

Plasma cortisol levels are presented in Fig. 4. In contrast to AchE activities, differences in plasma cortisol were not species-specific. Cortisol levels in whitefish were higher ($p<0.05$) compared to suckers at Pearce Rd. in spring and also in the summer when water temperature exceeded UTP for whitefish (Fig. 2). In both spring and summer, plasma cortisol levels were higher in whitefish sampled at downstream sites compared to upstream sites. Whitefish were not captured at all at the most downstream sites (Pavan in spring, Popson and Pavan in summer). In suckers, a similar pattern with higher cortisol levels measured in fish sampled at more downstream sites was also observed in the summer, but not in the spring. It is of interest that plasma cortisol in suckers was higher ($p<0.05$) than in whitefish both in spring and summer at the Ft. McLeod site, the most upstream site where suckers were caught. (Summerview site could not be sampled in Spring 2005).

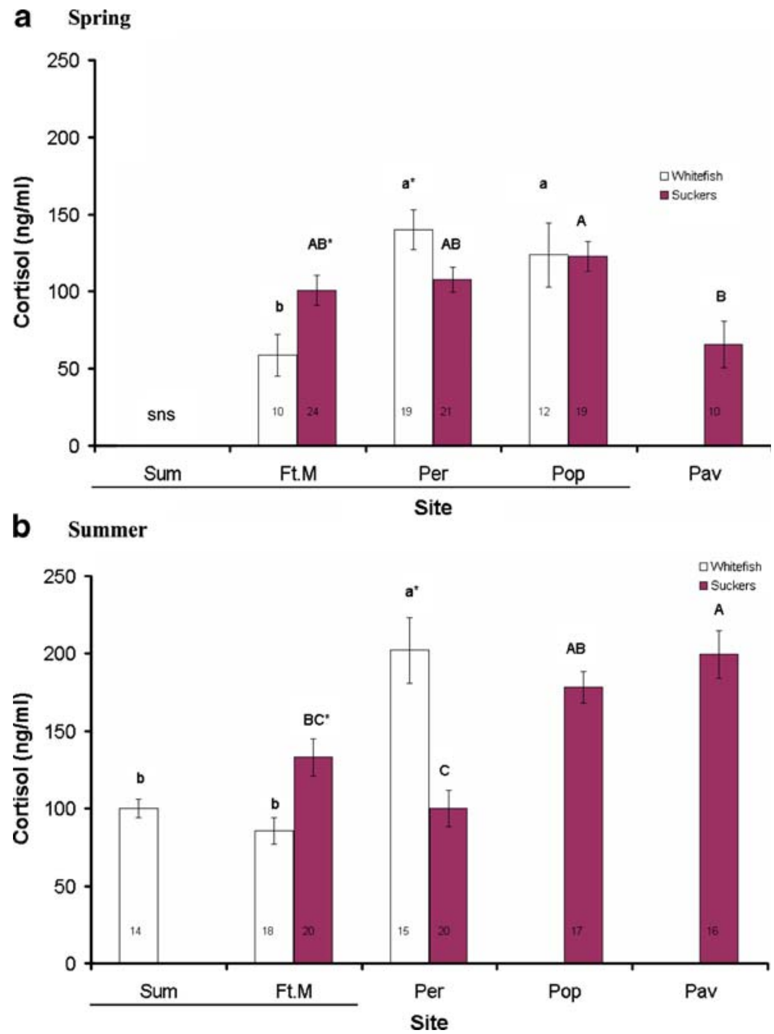

Fig. 4 Plasma cortisol (mean±SE) in whitefish and suckers sampled at sites along the Oldman River in **a** Spring, and **b** Summer. (See legend of Fig. 3 for details)

The concentrations of liver glycogen (Fig. 5) were significantly lower ($p<0.05$) in whitefish compared to suckers at all sites and seasons, except Ft. McLeod in spring where there were no significant differences between the two species. Liver glycogen reserves in both whitefish and suckers decreased along the river gradient in spring and also in summer.

There were no significant differences in plasma glucose (Fig. 6) between whitefish and suckers, in both spring and summer, except at Popson in spring. Plasma glucose levels in whitefish sampled in the spring and also the summer were lower at downstream sites along the river gradient, while in the sucker they were not significantly different among sites.

Condition factor did not follow a gradient pattern in either whitefish or suckers (Table 3). The sizes of the fish sampled (fork lengths and body weights, Table 3) were similar among sites in summer in both whitefish and suckers; there were some site differences in body weights of suckers sampled in spring.

Discussion

The Oldman River exhibits a pronounced elevational gradient in temperature, with the downstream warmer reaches impacted by agriculture and flow regulation. The gradient was used in this study as a model system for examining the effects of physico-chemical gradients on fish physiology. The distribution of fish along the river reflects the temperature gradient, as mainly cold-water species, such as Bull Trout

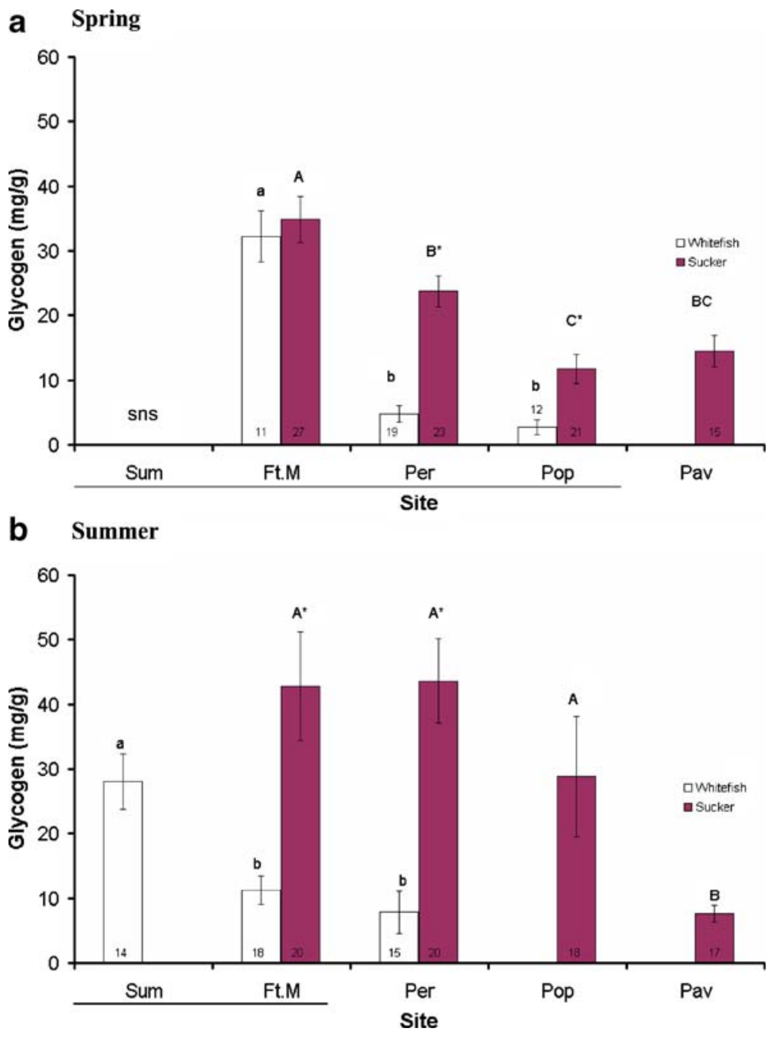

Fig. 5 Liver glycogen (mean±SE) in whitefish and suckers sampled at sites along the Oldman River in **a** Spring, and **b** Summer. (See legend of Fig. 3 for details)

(*Salvelinus confluentus*), Westslope Cutthroat Trout (*O. clarki lewisi*) and Mountain Whitefish are present in the upper reaches while cool-water fish such as suckers, Mooneye (*Hidon tergisus* Lesueur), Goldeye (*Hiodon alosoides*), and Walleye (*Stizostedion vitreum*) dominate the lower reaches. With the increasing intensity of agriculture downstream, concentrations of herbicides (2,4-D, Mecoprop) and other compounds, such as cholesterol or desmosterol (Koning et al. 2006; Jeffries et al. 2008) increase. Although OPs and CBs, pesticides with a short half-life, were not detected in the Oldman River in the present study, their effects, including AChE inhibition, may be detected even after a recovery period (Beauvais et al. 2000) and trace amounts of different OPs and CB can have an effect on organisms because they act through a similar mechanism (Scholz et al. 2006).

Differences in AChE were detected, with AChE activities in whitefish always lower compared to suckers, at all sites and seasons. Further studies are needed to determine whether these differences are related to exposure, through different use of habitat (Thompson and Davies 1976) or diets (Saint-Jacques et al. 2000), or a fundamental physiological difference. Species differences in the magnitude of AChE inhibition caused by exposure to the same pesticide have been reported for other fish species (Van Dolah et al. 1997). Along with differences in AChE activities related to fish species, small site-related differences were also observed. The lower AChE activities in whitefish in spring at downstream sites may be linked to an increase in pesticide exposure during a period of intensive pesticide application in Southern Alberta. Sucker AChE activities, higher than

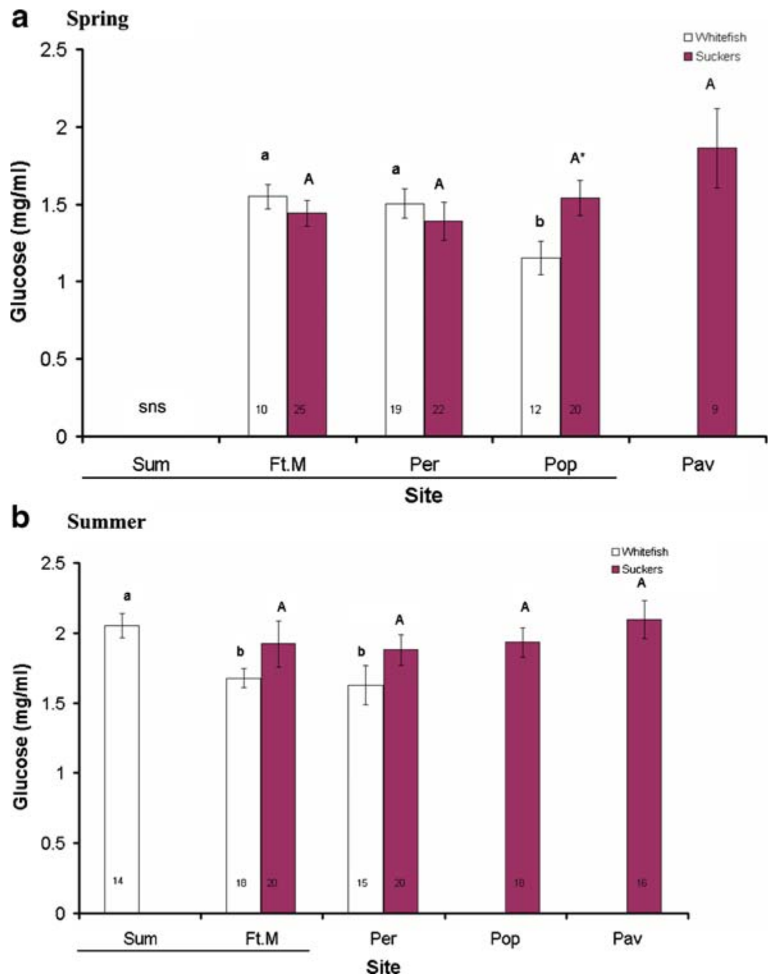

Fig. 6 Plasma glucose (mean±SE) in whitefish and suckers sampled at sites along the Oldman River in **a** Spring, and **b** Summer. (See legend of Fig. 3 for details)

whitefish, were not affected. In the summer, AChE in both species was however lower at more upstream sites which were cooler than the downstream sites. Hogan (1970) described a linear relationship between AChE activity and temperature in Bluegill, however we did not detect a significant relationship between AChE activity and temperature in our study. The relationship between temperature and pesticide exposure on AChE inhibition needs to be explored in greater depth, so that this marker of exposure to pesticides can be validated for use in a wide range of native fish species and temperature regimes. Ours is a first set of data for AChE in whitefish and first seasonal data for sucker.

The present study also provided a first set of data on confinement-induced plasma cortisol in Mountain Whitefish. Temperature is a known stressor and can increase plasma cortisol in fish at water temperatures outside of the thermal preference (Davis 2004). Whitefish were not caught at the most downstream (warmest) sites, and their plasma cortisol levels were higher at the downstream sites where they were still present, both in spring and summer. These results suggest that elevated plasma cortisol may indicate limits of the distribution of this species. A similar pattern of plasma cortisol was also detected in the sucker in summer, with higher levels at the warmest downstream sites, but not in the spring. Interestingly, suckers had higher plasma cortisol than whitefish at Ft. McLeod, the most western and also coldest site where suckers were caught in the Oldman River. Temperature below the optimal temperature range is known to increase plasma cortisol in some fish species (Davis and Peterson 2006), as temperatures above this range do. Several studies have shown that plasma cortisol levels are species-dependent; two

Table 3 Condition factor (mean±SE) in whitefish and suckers sampled at sites along the Oldman River in spring and summer

Site							
Sampling period		Species	Sum	Ft.M	Per	Pop	Pav
Spring		Whitefish	sns	1.11±0.02(11)B	1.19±0.03(18)A	1.09±0.01(12)B	-
Summer	Condition		1.13±0.0(18)A	0.99±0.02(15)B	1.06±0.03(12)AB	-	-
Spring	Factor	Sucker	sns	1.10±0.01(27)AB	1.15±0.14(22)A	1.07±0.2(20)B	0.99±0.08(14)AB
Summer			-	1.11±0.02(19)A	1.06±0.02(20)A	1.08±0.02(18)A	1.08±0.02(16)A
Spring		Whitefish	sns	14.05±1.1(11)B	22.59±0.9(18)A	17.38±1.4(12)B	-
Summer	Length		20.23±1.6(14)A	19.09±0.9(18)A	21.76±0.9(15)A	-	-
Spring		Sucker	sns	11.67±0.5(26)B	14.37±0.4(23)A	14.90±0.7(21)A	9.53±0.5(15)C
Summer			-	14.09±0.8(20)A	14.15±0.8(20)A	15.94±0.5(18)A	13.51±0.6(17)A
Spring		Whitefish	sns	38.30±14.6(11)B	146.92±15.0(18)A	70.65±17.1(12)B	-
Summer	Weight		95.67±16.4(14)A	78.89±11.3(18)A	119.22±13.7(15)A	-	-
Spring		Sucker	sns	21.00±4.0(26)BC	36.09±2.8(23)A	35.33±5.7(21)AB	10.98±3.0(15)C
Summer			-	38.5±6.3(20)A	35.18±3.5(20)A	46.63±4.9(18)A	27.19±4.3(17)A

Letters represent significant differences among sites (ANOVA and Tukey-Kramer HSD test $\alpha=0.05$); sns, site not sampled; – species not caught during sampling period; Condition factor=weight*100/length3

species may have different levels of plasma cortisol when exposed to the same stressor (Lacroix and Hontela 2004; Jentoft et al. 2005). The largest species-specific difference in plasma cortisol in this study was detected at the Pearce Rd. site in the summer when water temperatures were above the UPT for whitefish. Even though plasma cortisol values have not been published for Mountain Whitefish and ours is the first report for confined field sampled fish, the cortisol and AChE data suggest that whitefish may be highly vulnerable to the combined stress effects of pesticides and increased temperature. Future studies need to characterize the interactive effects of multiple concurrent stressors such as temperature, agrichemicals or sampling methods (seining, confinement) on physiological status, including plasma cortisol or AChE activity, in fish species from systems likely to be impacted by climate change.

To further characterize the physiological stress response in whitefish and the sucker, liver glycogen and plasma glucose were measured. Whitefish had lower levels of liver glycogen compared to suckers at all sites and sampling periods, however whether this differences between species is related to a difference in sensitivity to temperature, pesticides and/or confinement, or a physiological species difference and a lower capacity for storage of glycogen in the liver of whitefish is not known. Species-specific differences in liver glycogen levels were reported in other fish species (Krogdahl et al. 2004). In both whitefish and suckers, liver glycogen reserves decreased along a west-east gradient, suggesting that the increase of temperature and/or pesticides may lead to a depletion of glycogen reserves. Liver glycogen levels have been shown to decrease when fish are exposed to increased temperature (Viant et al. 2003) or pesticides (de Aguiar et al. 2004), however the interactions between temperature and pesticides have not been characterized thus far.

One of the characteristic responses associated with an increase in plasma cortisol and a decrease of liver glycogen, is a rise in plasma glucose, the energy source used to maintain homeostasis (Mommsen et al. 1999). Despite large differences in liver glycogen between the two species, their plasma glucose concentrations were different at only one site (Popson in spring). Plasma glucose concentrations in whitefish were lower in fish sampled at the downstream sites in both spring and summer, while site-related differences were not detected in the sucker. These results provide additional physiological evidence that whitefish may be more vulnerable than suckers to multiple stressors.

Condition factor, often used as an indicator of overall health, was not affected in whitefish or sucker sampled in this study. It is difficult to conclude whether the condition factors were within the normal range, especially for whitefish (CF=0.99 – 1.19), because there are limited physiological data for this species. Swanson et al. (1994) reported a condition factor of 1.14±0.22 for whitefish at a reference site while whitefish at two sites exposed to bleached kraft mill effluent had a condition factor of 1.20±0.18 and 1.07±0.17. Numerous studies reported the condition factor in suckers, ranging from 1.10 - 1.82 at reference sites and 1.23 - 1.72 at contaminated sites (Munkittrick and Dixon, 1988; Swanson et al. 1994). Although condition factor did not vary along the river gradient in this study, others have shown that as water temperatures rise, foraging frequency and durations decrease (Neill and Magnuson 1974), leading over time to a decreased condition factor.

Our study reported important species-specific differences in AChE activities and responses of the physiological stress axis between whitefish and suckers, suggesting that whitefish may be at greater risk if water temperatures and pesticides inputs continue to increase. The environmental scenario for which evidence is rapidly accumulating is an increase in water temperatures, in water withdrawals in agricultural areas and in pesticide loading of aquatic systems (Bloomfield et al. 2006). Global climate change models suggest as water temperatures continue to increase there may also be a shift in the distribution of fish with decreases in cold-water species and the invasion of cool- or warm-water species (Eaton and Scheller 1996; Daufresne et al. 2003). Use of species sensitivity distributions for temperature effects, potentially in combination with other stressors, has been proposed for environmental risk assessment in Europe and water quality objectives (de Vries et al. 2008). Physiological data provided by our study could be used, along with data validated under controlled exposures to specific stressors in the laboratory or in mesocosms, for development and application of location- and species – specific risk assessments. Further monitoring of the Oldman River and a greater understanding of Mountain Whitefish physiology are needed to determine how elevated temperature and agrichemicals influence the range and stability of whitefish populations, particularly at downstream river reaches where these stressors co-occur and interact. These studies are becoming important as climate changes, and agricultural activities expand and are modified in response to the climate change.

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