Impaired cortisol secretion in yellow perch (*Perca flavescens*) from lakes contaminated by heavy metals: in vivo and in vitro assessment

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Abstract: The characteristic elevation of plasma cortisol levels in response to an acute stress of capture was impaired in both male and female yellow perch (*Perca flavescens*) from lakes contaminated by heavy metals. The impairment of the cortisol stress response was observed in fish 4+ years and older whereas the capacity to elevate plasma cortisol levels of fish younger than 4+ was not significantly different at contaminated and reference sites. The responsiveness to ACTH of the interrenal tissue of 4+ yellow perch was evalutated in vitro to determine whether the impairment of the cortisol stress response is caused by a dysfunction of the interrenal tissue or if the dysfunction is located elsewhere in the hypothalamo–pituitary–interrenal axis controlling the secretion of cortisol. The amount of cortisol secreted by the interrenal tissue of yellow perch from a contaminated site in response to a 10-min stimulation with 10^{-7} M ACTH was significantly lower compared with fish from the reference site. These results indicate that the impairment of the cortisol stress response observed in fish from polluted sites is caused, at least in part, by a dysfunction of the interrenal tissue.

Résumé : L'augmentation caractéristique des concentrations plasmatiques de cortisol en réponse à un stress de capture aigu était significativement réduite chez les perchaudes (*Perca flavescens*) males et femelles provenant de sites contaminés par les métaux lourds. Cette altération de la sécrétion de cortisol en réponse au stress a été observée chez les perchaudes de 4 ans et plus, alors que la capacité des perchaudes de moins de 4 ans à augmenter leurs niveaux de cortisol plasmatique n'était pas significativement différente entre les sites contaminés et les sites références. La sensibilité à l'ACTH du tissu interrénal des perchaudes de 4 ans a été évaluée in vitro de façon à déterminer si l'altération de la sécrétion de cortisol en réponse au stress est due à un dysfonctionnement du tissu interrénal ou si le dysfonctionnement se situe ailleurs dans l'axe hypothalamo–hypophyso–interrénal responsable de la sécrétion de cortisol. La quantité de cortisol sécrétée in vitro par le tissu interrénal de perchaudes provenant d'un site contaminé par les métaux en réponse à une stimulation de 10 min avec 10^{-7} M ACTH était significativement réduite comparativement au groupe contrôle. Ces résultats indiquent que l'altération de la sécrétion de cortisol en réponse au stress observée chez les poissons des sites contaminés est causée, en partie du moins, par un dysfonctionnement du tissu interrénal.

Introduction

Many of the human-made chemicals released into the environment have the potential to disrupt the endocrine system of animals (Colborn and Clement 1992). The consequences of such a perturbation can be important because of the crucial role played by the endocrine system in the coordination of physiological processes and the maintenance of homeostasis (Brouwer et al. 1990).

Disruption of interrenal function has been reported in several fish species from sites contaminated by various types of pollutants (Lockhart et al. 1972; Hontela et al. 1992, 1995, 1997; McMaster et al. 1994). This dysfunction is characterized by an impairment of the typical elevation of plasma cortisol levels in response to an acute stress of capture. In fish, the adrenocorticotropic hormone (ACTH) released from the pituitary gland under the control of the hypothalamus is the main stimulus for cortisol secretion by the interrenal tissue diffusely distributed within the head kidneys (Butler 1973; Donaldson 1981). The stimulation of cortisol secretion is a nonspecific response to the alteration of fish homeostasis by a variety of stressors (Thomas 1990). The gluconeogenic actions of cortisol enable the organism to meet the increased energy demands from various homeostatic mechanisms and cope with the stressors (Thomas 1990). Fish incapable of mounting a normal cortisol stress response are likely to have a reduced ability to cope with the continous challenges imposed on their homeostatic systems by the normal demands of the aquatic environment. An impairment of cortisol secretion may also compromise the health of the fish because cortisol has a regulatory role in osmoregulation (Flick and Perry 1989; Madsen 1990), metabolism (Leach and Taylor 1982; Janssens and Waterman 1988), immune function (Pickering 1984; Maule et al. 1989), and reproduction (Wingfield and Grimm 1977; Bry 1985).

Because the impairment of cortisol secretion seems to be one of the early physiological alterations caused by pollutants and because it may precede more significant health problems, this variable could be used in the detection and assessment of

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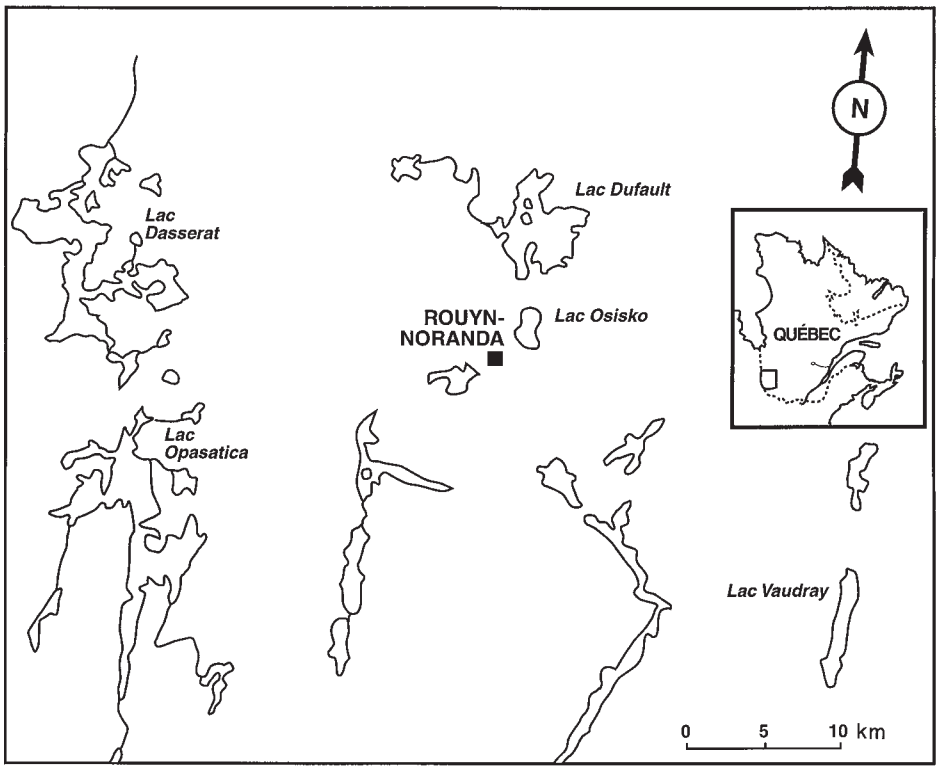
Fig. 1. Location of the lakes sampled around Rouyn-Noranda, Abitibi, Quebec.



sublethal toxic effects of chemical pollutants in fish (Hontela et al. 1993). A better understanding of the mechanisms underlying the disruption of the interrenal function and of the effects of factors such as sex, age, and season is, however, needed before this phenomenon can be used in diagnosis of pollutant-induced physiological anomalies (Huggett et al. 1992).

In the present study, the plasma cortisol levels in response to a standardized capture stress were measured in yellow perch (*Perca flavescens*) from reference lakes and lakes contaminated by heavy metals to determine if chronic exposures to heavy metals lead to an impairment of the cortisol stress response, similar to the dysfunction previously diagnosed in fish from sites contaminated by mercury (Hg), bleached kraft mill effluents, and mixtures of pollutants (Lockhart et al. 1972; Hontela et al. 1992, 1995, 1997; McMaster et al. 1994). Males and females of a wide range of ages were sampled in each lake to determine the effects of sex and age on the cortisol stress response. The functional integrity of the interrenal tissue of yellow perch from contaminated and reference lakes was also evaluated in vitro to determine whether the impairment of the cortisol stress response is associated with a dysfunction of the interrenal tissue or if the dysfunction is located elsewhere in the hypothalamo–pituitary–interrenal axis (HPI axis) controlling the secretion of cortisol.

Methods

Study area

The sampling was conducted in the region of Rouyn-Noranda (Abitibi, northwestern Quebec) where a large smelter has been operating since 1927 and many surrounding lakes are contaminated with heavy metals (Couillard et al. 1993). Of the four lakes sampled (Fig. 1), Lakes Opasatica and Dasserat, situated upwind from the smelter and protected from atmospheric fallout, were used as reference sites. The two other lakes sampled (Dufault and Osisko) both receive atmospheric fallout from the smelter, and one of them, Lake Osisko, also received liquid effluents from the smelting operations during many years in the past.

To characterize the contamination level of the four lakes sampled, the concentrations of cadmium (Cd), copper (Cu), iron (Fe), and zinc (Zn) in the liver of 3- to 5-year-old yellow perch were determined by atomic absorption. The procedure described by Manca et al. (1992) was used to measure Cd. The other metals were measured using a similar procedure, described in Ricard et al. (1997), and the recovery determined from standards (National Bureau of Standards; bovine liver No. 1577b, 0.50 μg Cd/g, 160 μg Cu/g, 184 μg Fe/g, 127 μg Zn/g) was more than 90% for all metals measured. The values are reported on a wet mass basis (correction factor for dry mass: 3.40) and are not corrected for recovery.

Test species and capture of the fish

Yellow perch was selected for this study because it is a sedentary fish (Aalto and Newsome 1990) that reflects the contamination of its milieu, it is abundant in many aquatic systems of the temperate zone, and it exhibited an impaired cortisol stress response in previous studies conducted at sites contaminated by bleached kraft mill effluents or by mixtures of pollutants (Hontela et al. 1992, 1995, 1997). Fish were captured by seine or gill net in September when water temperature was 16.9 ± 1.5°C. The fishing was always done in the morning and the gill nets were set for periods not exceeding 60 min. Previous experiments demonstrated that yellow perch show a similar cortisol stress response when they are captured either by seine or by gill net (Brodeur et al. 1997).

Evaluation of the cortisol stress response

Following capture, fish were held in a large container onboard the boat until fishing was completed. Fish spent less than 2.5 h in the

container and the water was partially replaced every 30 min to insure that it was sufficiently oxygenated. After the fish were subjected to this capture and holding stress, they were anesthetized by immersion in a solution of tricaine methanesulfonate (MS 222, 0.15 g/L) (Sigma, St. Louis, Mo.) and their body length was recorded. A blood sample was taken from the caudal vasculature with a heparinized syringe and fish were sacrificed by cutting the neural cord behind the brain. The plasma was separated by centrifugation (5 min, 13 000 rpm, ambient temperature) and immediately frozen on dry ice. Fish were also frozen and measurements of body characteristics were completed in the laboratory.

To ensure that the last fish sampled were as stressed as the first ones, the water of the holding container was agitated frequently while the boat was at the dock and fish were removed by groups of seven or eight to be sampled. The sampling procedure was completed in about 40 min, and it was always executed in the afternoon to insure that the daily cycles of cortisol secretion did not interfere with the stress response (Pickering and Pottinger 1983; Audet and Claireaux 1992). Previous studies using this protocol demonstrated that the plasma cortisol of fish sampled last did not differ significantly from that of fish sampled first (Hontela et al. 1995). Fish of a wide range of body sizes were sampled to investigate the effects of age on the cortisol stress response.

In the laboratory, fish were weighed and the condition factor was calculated as [mass (g)]/[length (cm)]^3 × 100. The gonads were dissected and weighed to calculate the gonadosomatic index (GSI) of the fish as [gonad mass (g)]/[body mass (g)] × 100, and an operculum was removed to determine the age of the fish.

In vitro determination of the functional integrity of the interrenal tissue by perifusion

After capture, fish were placed for 24 h in floating enclosures (0.5 m width × 1 m long × 1 m deep) made of net (11 fish/enclosure) to allow a partial recovery from the effects of the capture stress on cortisol secretion. Following the rest period, fish were anesthetized by immersion in a solution of 0.15 mg MS 222/L before they were exsanguinated from the caudal vasculature and sacrificed by section of the spinal cord. They were then transported to the powered installations where the perifusion system was set up. The in vitro perifusion procedure used to determine the functional integrity of the interrenal tissue has been described in detail elsewhere (Brodeur et al. 1997). It involves dissecting the head kidneys where the interrenal tissue is diffusely distributed and placing them in an incubation chamber that is connected to a peristaltic pump to continuously supply the tissues with fresh incubation medium. After perifusion for 110 min to reach the basal rate of cortisol secretion, the interrenal tissue is stimulated by adding 10^{-7} M porcine ACTH$^{1–39}$ to the incubation medium for 10 min. The response of the interrenal tissue is monitored by collecting the incubation medium continously evacuated from the chamber in 10-min fractions for 120 min and measuring the cortisol concentrations in the fractions by radioimmunoassay. The cortisol released, when summed over all the fractions, was used to compare the response to ACTH of fish from different lakes. The in vitro experiments have been carried out in one reference lake (Dasserat) and one contaminated lake (Osisko). Yellow perch measuring from about 18.5 to 20 cm (4+ year-class) were selected for the perifusion experiments to decrease variability in the age of fish used.

Radioimmunoassay of cortisol

Concentrations of cortisol in plasma and in the incubation medium collected from perifusion were determined with a radioimmunoassay kit (No. 07-221102, ICN Biomedicals Canada Ltd., Montreal, Que.). A standard curve prepared by adding synthetic cortisol (Sigma) to incubation medium at concentrations used in the kit's standard curve was used to measure cortisol in samples collected from perifusion. The characteristics of assays done with this standard curve have been described previously (Brodeur et al. 1997).

Statistical analysis

Differences among groups were tested with *t*-tests, one-way and two-way ANOVA, or Kruskall–Wallis tests when normality or homoscedasticity could not be obtained. Student–Newman–Keuls (SNK) or Dunn tests were used for multiple comparisons when a significant difference was detected.

Results

Exposure of the fish was assessed by measuring concentrations of metals in the liver. Liver concentrations of Cd, Cu, Zn, and Fe were significantly higher in yellow perch from Lakes Dufault and Osisko than in yellow perch from Lakes Opasatica and Dasserat (Table 1).

The cortisol stress response to capture of yellow perch younger than 4+ from reference and contaminated sites was not significantly different (Fig. 2*a*). However, yellow perch 4+ and older from both contaminated sites exhibited significantly lower levels of plasma cortisol compared with fish of the same age in the two reference sites (Fig. 2*b*); this reduction was observed in both sexes (Fig. 2*b*). Whereas capture stress elicited a similar increase of plasma cortisol levels in females 4+ and older from both reference sites, males 4+ and older from the reference Lake Dasserat had significantly lower levels of plasma cortisol compared with the males of the same age from the other reference site, Lake Opasatica (Fig. 2*b*). The plasma cortisol levels of yellow perch younger than 4+ from the reference Lake Opasatica were significantly lower than the levels measured in yellow perch 4+ and older (Fig. 2).

Plasma cortisol levels after 24 h in the enclosure were significantly lower than levels immediately postcapture in both reference and contaminated sites, and the difference that was observed between the sites immediately after capture was no longer detected (Fig. 3). The interrenal tissue of both the males and females from the contaminated lake (Osisko) secreted a significantly lower amount of cortisol in response to a 10-min stimulation with 10^{-7} M ACTH compared with the interrenal tissue of yellow perch from the reference lake (Dasserat) (Fig. 4).

Females from both contaminated lakes (Dufault and Osisko) had a lower condition factor compared with females from reference lakes (Table 2). The condition factor of males from the contaminated Lake Dufault was also significantly lower than that of males from both reference lakes whereas the condition factor of males from the contaminated Lake Osisko was significantly different only from reference Lake Dasserat (Table 2). Males from the reference Lake Dasserat had a higher GSI than males from the three other lakes whereas females from the reference Lake Dasserat had a higher GSI compared with only one group, females from the contaminated Lake Dufault (Table 2).

Discussion

Because the liver is the major site of metal accumulation in fish, exposure of wild fish to heavy metals can be assessed by measuring concentrations of metals in the liver (Sorensen 1991). The lower concentrations of metals measured in the liver of yellow perch from Lakes Opasatica and Dasserat,

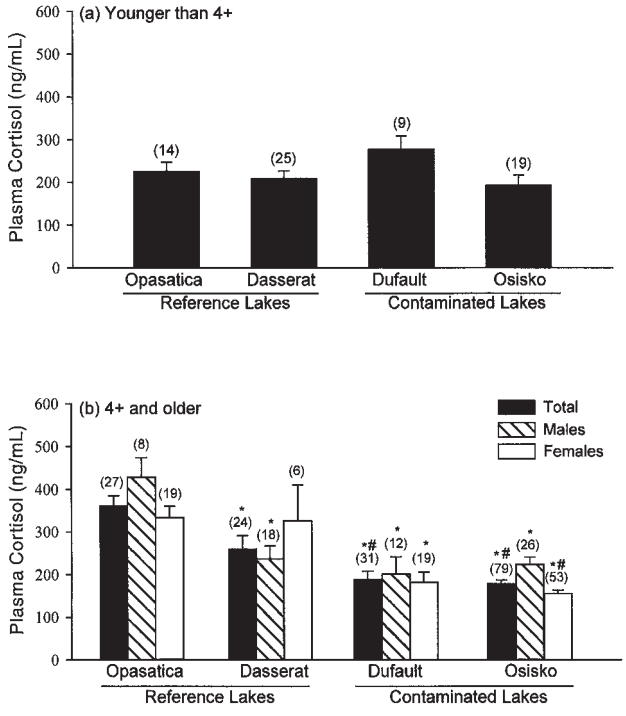
Table 1. Limnological characteristics and concentrations of metallothioneins and heavy metals (means ± SE) in the liver of yellow perch sampled in four lakes around Rouyn-Noranda, Quebec (numbers of samples in parentheses).

	Lakes			
	Opasatica	Dasserat	Dufault	Osisko
Limnological characteristics				
Alkalinity (mg $CaCO_3$/L)	29	23.8	13.7	9.5
Conductivity (μS, 25°C)	87.6	72.2	173.4	300.4
Oxygen (ppm)	7.9	8.5	6	9
pH	5.84	7.39	6.52	5.9
Liver metallothioneins (μg/mg protein)	na	1.4±0.2 (5)	na	5.6±1.8* (7)
Liver concentrations of metals (μg/g)				
Cd	3.2±0.8 *c* (20)	1.9±0.2 *c* (28)	23±1.8 *a* (20)	14±0.8 *b* (49)
Cu	9.7±1.6 *b* (20)	7.7±1.2 *b* (28)	19±2.3 *a* (20)	27±2.8 *a* (49)
Fe	100±32 *b* (20)	100±12 *b* (28)	214±27 *b* (20)	860±52 *a* (48)
Zn	40±1.4 *b* (20)	31±2.3 *b* (25)	49±5.9 *a* (16)	50±2 *a* (48)

Note: Means followed by the same letter are not significantly different ($p < 0.05$, Krukall–Wallis and Dunn tests).

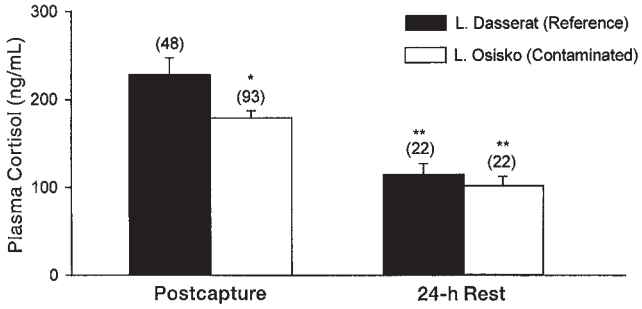
*Significantly different from Lake Dasserat ($p < 0.01$, *t*-test). na, not available. Data for limnological characteristics were obtained from F. Girard (Ministère Environnement du Québec, personal communication). Liver metallothionein data were obtained from Dr. P. Moffat (Department of Chemistry, Université du Québec à Montréal, personal communication).

Fig. 2. Plasma cortisol levels (mean ± SE) after a standardized capture stress in (*a*) younger than 4+ and (*b*) 4+ and older yellow perch from reference and contaminated lakes. Numbers of fish sampled are indicated on top of the error bars. Numbers of fish sampled were too small to separate fish younger than 4+ by sex. There was no significant difference between lakes in fish younger than 4+ (one-way ANOVA). *, Significantly different from the reference Lake Opasatica ($p < 0.05$, two-way ANOVA and SNK test); #, significantly different from the reference Lake Dasserat ($p < 0.05$, two-way ANOVA and SNK test).



compared with yellow perch from Lakes Dufault and Osisko, indicate that the exposure of these fish is low and justify the use of lakes Opasatica and Dasserat as reference sites. Lower metallothionein concentrations measured in the liver of yellow perch from Lake Dasserat compared with Lake Osisko provide further evidence that yellow perch from Lake Dasserat are exposed to lower levels of heavy metals.

Fig. 3. Plasma cortisol levels (mean ± SE) of yellow perch from reference and contaminated lakes immediately after capture or after spending 24 h in an enclosure. Numbers of fish sampled are indicated on top of the error bars. *, Significantly different from the reference lake ($p < 0.05$, two-way ANOVA and SNK test); **, significantly different from the postcapture group ($p < 0.05$, two-way ANOVA and SNK test).



The present study demonstrated that yellow perch submitted to a life-long exposure to sublethal levels of heavy metals exhibit an impairment of the cortisol stress response to capture, similar to the one observed previously in fish from sites polluted by Hg, bleached kraft mill effluents, or a mixture of chemicals (Lockhart et al. 1972; Hontela et al. 1992, 1995, 1997; McMaster et al. 1994). The analysis of plasma cortisol data in terms of age also demonstrates for the first time that the impairment of the cortisol stress response is only clearly evident in fish 4+ and older.

To determine whether the impairment of the cortisol stress response observed in fish 4+ and older is caused by a dysfunction of the interrenal tissue, the functional integrity of the interrenal tissue was evaluated in vitro by perifusion in yellow perch from the contaminated Lake Osisko and a reference lake. The interrenal tissue of both male and female yellow perch from the contaminated Lake Osisko secreted significantly less cortisol in vitro in response to the 10-min pulse of 10^{-7} M

Table 2. Characteristics (mean ± SE) of female and male yellow perch collected from reference (Opasatica and Dasserat) and contaminated (Dufault and Osisko) lakes.

Lake	*n*	Age (years)	Weight (g)	Length (cm)	Condition factor	GSI (%)
			Females			
Opasatica	27	4.26±0.38 *ab*	81.86±12.28 *a*	18.50±0.87 *b*	1.06±0.02 *a*	1.65±0.10 *ab*
Dasserat	14	3.79±0.48 *b*	88.29±20.12 *a*	18.33±1.27 *ab*	1.11±0.04 *a*	2.43±0.41 *a*
Dufault	22	5.00±0.30 *a*	33.95±2.09 *b*	15.37±0.31 *b*	0.91±0.01 *c*	1.48±0.09 *b*
Osisko	57	4.07±0.08 *b*	93.33±3.64 *a*	21.02±0.29 *a*	0.96±0.01 *b*	1.58±0.05 *ab*
			Males			
Opasatica	11	4.27±1.28 *a*	50.95±6.58 *a*	16.48±0.62 *a*	1.07±0.04 *ab*	2.06±0.40 *b*
Dasserat	32	4.00±0.22 *a*	56.52±5.40 *a*	16.68±0.48 *a*	1.11±0.01 *a*	4.80±0.35 *a*
Dufault	17	4.41±0.51 *a*	21.94±2.75 *b*	13.02±0.43 *b*	0.93±0.01 *c*	2.56±0.23 *b*
Osisko	33	3.67±0.15 *a*	52.88±4.19 *a*	17.03±0.52 *a*	0.98±0.01 *bc*	2.98±0.25 *b*

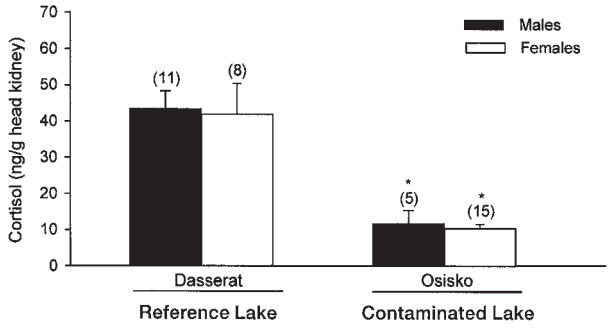
Note: Means followed by the same letter are not significantly different ($p < 0.05$, Krukall–Wallis and Dunn tests).

ACTH compared with the interrenal tissue of yellow perch from the reference Lake Dasserat. Because ACTH is the main stimulus of the interrenal tissue, this lower responsiveness to ACTH of the interrenal tissue of fish from the polluted site indicates that the life-long exposure to sublethal levels of metals has altered the normal functioning of the interrenal tissue. These results thus provide evidence that the decreased capacity to elevate plasma cortisol levels in response to capture stress of yellow perch from the contaminated Lake Osisko is caused by a dysfunction of the cortisol-secreting interrenal tissue, although they do not rule out the possibility that other levels of the HPI axis are also impaired.

It is interesting to note that the in vitro responsiveness to ACTH of the interrenal tissue of males from the reference Lake Dasserat was significantly higher compared with the interrenal tissue of males from the contaminated Lake Osisko despite the fact that the plasma cortisol stress response of these males was not significantly different. These results indicate that the low cortisol stress response of the males from the reference Lake Dasserat is not the result of an impairment of cortisol secretion and suggest that the in vitro ACTH challenge test is a better diagnostic tool for assessing the functional integrity of the interrenal tissue than is the capacity to elevate plasma cortisol levels in response to capture.

A reduced responsiveness to ACTH of the interrenal tissue similar to the one observed in the present study has been previously demonstrated in yellow perch showing an impaired cortisol stress response at sites polluted by a mixture of heavy metals and organic pollutants (Brodeur et al. 1997; Girard et al. 1998). The present study is, however, the first one to link the dysfunction of the interrenal tissue to heavy metals. Metals could lead to the impairment of cortisol secretion by having direct toxic effects in the steroidogenic cells of the interrenal tissue, since Cd, Cu, and Hg have been found to exert toxic effects in the adrenal gland of mammals, the homologue of fish interrenal tissue (Veltman and Maines 1986*a*, 1986*b*; Mgbonyebi et al. 1994). Because the increase of cortisol secretion is part of the general adaptation response of the animal to stressors, it can also be expected that the interrenal tissue of fish living in contaminated environments chronically experiences periods of elevated metabolic activity that could eventually lead to cellular alterations and impaired cortisol secretion. This nonspecific mechanism of action would also be consistent with the various types of chemical pollution in which the impairment of cortisol secretion has now been observed.

Fig. 4. Cortisol secreted (mean ± SE) by female and male yellow perch head kidneys stimulated with 10^{-7} M ACTH during 10 min in perifusion. Fish from reference and contaminated lakes were tested. Numbers of fish sampled are indicated on top of the error bars. *, Significantly different from the reference lake ($p < 0.05$, two-way ANOVA and SNK test).



The present study and others conducted at sites contaminated by a mixture of organic pollutants and heavy metals (Brodeur et al. 1997; Girard et al. 1998) have taken an important first step in the understanding of the mechanisms underlying the impairment of the plasma cortisol stress response by identifying the interrenal tissue as a site of dysfunction within the three levels of the HPI axis that could theoretically be impaired. Heavy metals were present at all these sites, and it is not known if the dysfunction of the interrenal tissue is a phenomenon specific to metal-contaminated sites or if it can also explain the impairment of the cortisol stress response observed in sites polluted by other types of contaminants. The signs of atrophy in addition to impaired cortisol stress response observed previously in the interrenal cells of fish from a site contaminated by bleached kraft mill effluent (Hontela et al. 1997), however, suggest that the interrenal tissue of these fish may also be dysfunctional.

As more data become available, the impairment of the cortisol stress response appears to be a general phenomenon in fish from polluted environments, since, beside the present study with yellow perch from lakes contaminated by heavy metals, it has also been observed in northern pike (*Esox lucius*) from sites polluted by Hg (Lockhart et al. 1972), white sucker

(*Catostomus commersoni*), yellow perch, and northern pike from sites polluted by bleached kraft mill effluents (McMaster et al. 1994; Hontela et al. 1997), and yellow perch and northern pike from sites polluted by a mixture of organic pollutants and heavy metals (Hontela et al. 1992, 1995; Girard et al. 1998). The mudpuppy, an amphibian, also has a reduced capacity to respond to ACTH and to secrete corticosterone in response to acute capture stress at sites contaminated by chlorinated hydrocarbons (Gendron et al. 1997).

The impairment of cortisol secretion observed in fish from polluted environments could be used as an early warning biomarker of toxic effect, since this endocrine dysfunction will most likely result in more important health problems because of the major role played by cortisol in the maintenance of homeostasis. The low condition factor of yellow perch from both contaminated lakes indeed suggests that fish exhibiting an impairment of cortisol secretion have more difficulty in coping with their environment, since it indicates that they have decreased energy reserves (Goede and Barton 1990). However, before a biochemical parameter can be used appropriately in health assessment, it is necessary to characterize it in a field situation and determine how it is affected by biotic (age, sex, interindividual variation, etc.) and abiotic (temperature, season, pH, etc.) factors (van Gestel and van Brummelen 1996). The effects of season on the cortisol stress response are reported in Girard et al. (1998). The present study provided some insights on the influence of sex and age on the impairment of cortisol secretion by showing that males and females are similarly affected and that the older fish of the population seem to be more seriously impaired.

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