Responsiveness of the interrenal tissue of yellow perch (*Perca flavescens*) from contaminated sites to an ACTH challenge test in vivo

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Abstract: The effects of chronic toxic stress on the hypothalamo–pituitary–interrenal (HPI) axis were investigated in yellow perch (*Perca flavescens*) captured at a reference site (Lake Memphremagog) and two sites contaminated with heavy metals and organic contaminants (Ile Perrot and Iles de la Paix, Lake St. Louis) in spring, summer, and fall. Cortisol secretion of the fish was stimulated in situ by an acute capture stress or by an i.p. injection of 4 IU/100 g body mass of porcine corticotropin ($ACTH^{1–39}$). The response to both these challenges was lower in perch from the highly contaminated site than in perch from the reference site in the spring but not in summer. In fall, fish from the highly contaminated site had, as in spring, a lower response to ACTH than fish from the reference site. The reduced ability of perch to respond to capture stress or to ACTH indicates that the interrenal tissue in fish from contaminated sites is functionally impaired. Cortisol-impaired fish also had abnormal carbohydrate metabolism. The reduced ability of wild fish from contaminated sites to respond to a standardized ACTH challenge may be used as an early indicator of contamination-induced chronic stress.

Résumé : Les effets du stress toxique chronique sur l'axe hypothalamo–hypophyso–interrénal (axe HHI) ont été étudiés chez des perchaudes (*Perca flavescens*) capturées à un site référence (lac Memphrémagog) et à deux sites contaminés par des métaux lourds et des composés organiques (Île Perrot et Îles de la Paix au lac St-Louis) au printemps, en été et en automne. La sécrétion du cortisol par le tissu interrénal a été stimulée in situ de façon standardisée par un stress aigu de capture ou par l'injection intra-péritonéale de 4 UI/100 g (poids corporel) de corticotropine porcine ($ACTH^{1–39}$). La capacité de réponse des perchaudes du site fortement contaminé à ces épreuves physiologiques au printemps était plus faible qu'au site référence, mais pas en été. En automne, les perchaudes du site fortement contaminé stimulées par injection d'ACTH ont démontré une réponse cortisolique moindre que celle des poissons du site référence. La capacité réduite de répondre au stress peut être expliquée en partie par la défaillance fonctionnelle du tissu interrénal. Les mesures de glucose plasmatique et de glycogène hépatique ont révélé des irrégularités du métabolisme glucidique. La capacité des poissons sauvages à répondre à une stimulation hormonale standardisée peut être utilisée dans l'évaluation de la santé comme indicateur précoce de stress chronique dû aux substances toxiques.

Introduction

Physiologically disruptive effects of chronic and acute exposures to contaminants in fish have been investigated in the laboratory (Pratap and Wendelaar Bonga 1990; Johansen et al. 1994; Soimasuo et al. 1995; Bleau et al. 1996; Hontela et al. 1996) and in the field (Haux et al. 1985; Andersson et al. 1988; Munkittrick et al. 1992; Förlin et al. 1995). These mainly descriptive studies assessed the effects of pollutants on static physiological or endocrine endpoints. However, they provided limited information on the ability of the exposed fish to maintain homeostasis and cope with biotic and abiotic stressors, in contrast to more functional tests (Schreck 1990; Heath 1995; Hontela 1997).

Received October 22, 1996. Accepted June 26, 1997.
J13716

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Such tests have been used in some recent studies. Schreck and Lorz (1978) evaluated the ability of coho salmon (*Oncorhynchus kisutch*) to elevate plasma cortisol in response to confinement stress after an acute exposure to copper (Cu) or cadmium (Cd) in the laboratory; cortisol secretion could be activated even after a confinement period in Cu-exposed fish but not in Cd-exposed fish. Gill et al. (1993) exposed American eel (*Anguilla rostrata*) to environmentally realistic doses of Cd for up to 16 weeks in the laboratory; their ability to elevate plasma cortisol was unimpaired when a controlled standardized stressor (1-min exposure to CO_2 bubbles) was applied. Larsson et al. (1984) challenged perch (*Perca fluviatilis*) subjected in the laboratory to a heavy metal contaminated effluent by maintaining them for 3 min out of water; hyperglycemia and glycogenolysis following this acute stress were lower in exposed fish than in non-exposed controls, revealing a deficient carbohydrate metabolism in the fish exposed to heavy metals. In another series of experiments, yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) from reference and contaminated sites were subjected to capture stress and their ability to elevate blood cortisol was evaluated in situ. Fish from sites contaminated with heavy metals, PCBs, PAHs (Hontela et al. 1992, 1995), or bleached kraft mill effluents

Table 1. Contamination profile of the study sites, limnological characteristics, and water temperature at time of sampling.

	Lake Memphremagog[a] (reference)	Ile Perrot[b] (intermediate)	Iles de la Paix[b] (highly contaminated)
Concentration of contaminants (mg/kg)			
Hg			
Sediments	0.04	0.20 (55)	2.17 (56)
Perch flesh	0.09 (3)	0.14 (21)	0.42 (24)
Cd			
Sediments	na	0.83 (17)	2.20 (16)
Perch liver	na	0.018 (5)	0.18 (7)
PCB total			
Sediments	0.005	0.078 (32)	0.088 (31)
Perch flesh	0.02 (24)	0.06 (7)	0.02 (9)
PAH total			
Sediments	na	1.09 (10)	3.75 (9)
Perch whole	nd	0.0045 (7)	0.015 (9)
Limnological characteristics			
Temperature (°C)			
Spring	14	na	12
Summer	24±1	27±2	25
Fall	13	na	16±2
pH	6.4–8.4	8.1	8.3
Conductivity (μS; 25°C)	130–158	301–350	226–300
Alkalinity (mg/L $CaCO_3$)	48–56	74–83	74–83
Total P (μg/L)	16.2	23	20
Dissolved N (mg/L)	0.31	0.08	0.07
Total N (mg/L)	0.22	0.31	0.28

Note: Values in parentheses are the number of replicates. Water temperature was taken at the time of sampling (see text for details on the dates).

[a] Data were obtained from Paul and Laliberté (1989*a*, 1989*b*), D. Laliberté (Ministère de l'environnement et faune du Québec, personal communication), and Janus and Vollenweider (1981). na, not available. nd, non-detectable.

[b] Data were obtained from Hontela et al. (1995), Dumont (1996), D. Laliberté (Ministère de l'environnement et faune du Québec, personal communication).

(Hontela et al. 1997) had an impaired stress response, characterized by lower levels of plasma cortisol following an acute capture challenge. These latter studies suggested that the hormonal processes of the stress response are affected by chronic field exposure to contaminants.

Cortisol, the main glucocorticosteroid secreted by the interrenal tissue of teleosts in response to stimulation by adrenocorticotropic hormone (ACTH), rises along with catecholamines when the organism is subjected to noxious stressors (Donaldson 1981; Mazeaud and Mazeaud 1981). Acute stressors, such as handling and confinement (Barton and Iwama 1991), exposure to low pH (Brown et al. 1984) or to toxic chemicals (Folmar 1993) such as heavy metals (Gill et al. 1993; Bleau et al. 1996; Hontela et al. 1996), induce a strong elevation of plasma cortisol in several fish species. The activation of the hypothalamo–pituitary–interrenal axis (HPI axis) and the increased plasma cortisol enable the fish, in part through cortisol's lipolytic and gluconeogenic properties, to mobilize energy substrates and maintain homeostasis (Pickering 1993). Acute exposures to stressors activate carbohydrate metabolism, through a significant rise in plasma glucose and a depletion in liver glycogen stores (Vijayan and Moon 1992; Bleau et al. 1996; Hontela et al. 1996). Because cortisol plays a significant role in homeostatic mechanisms that maintain the normal physiological status of fish (Barton et al. 1987; Pickering 1993), the impairment of the cortisol stress response diagnosed in fish from polluted sites may adversely affect fish health.

The purpose of the present study was to develop a field-adapted, standardized method using ACTH to test in vivo the capability of the interrenal tissue of fish chronically exposed to pollutants in the wild to secrete cortisol. Yellow perch was selected for this study because its demic behaviour predisposes this species to lifelong exposure to site-specific contamination (Aalto and Newsome 1990) and because it is still abundant in the St. Lawrence river system (Dumont 1996). The specific objectives of the present study were (*i*) to determine the extent of cortisol impairment of yellow perch from reference and contaminated sites, using the activation of the HPI axis by capture stress and by the ACTH challenge test; (*ii*) to identify the site of the impairment within the HPI axis; (*iii*) to field validate this endocrine dysfunction as a potential new bioindicator of toxic stress by analyzing the effects of season, sex, and sexual maturity on the cortisol response. To further characterize the physiological status of the fish, carbohydrate metabolism was assessed by measures of plasma glucose and liver glycogen. The interrenal tissue was investigated with histomorphometric analyses to identify cellular alterations that could be related to chronic exposure to contaminants.

Materials and methods

Study sites and fish

Yellow perch (*Perca flavescens*) were sampled at sites representing a gradient of contamination (Table 1). The reference site, Lake Memphremagog, Quebec, at approximately 125 km east from Montréal, was chosen by consulting data previously published by the Ministère de l'environnement et faune, Quebec (Paul and Laliberté 1989*a*, 1989*b*; D. Laliberté, Ministère de l'environmement et faune, Quebec, personal communication). The lake is free of industrial activity, and its contamination is low. The heavily polluted site at Iles de la Paix is situated on the south shore of Lake St. Louis, an enlargement of the St. Lawrence River, near Montréal. The sediments, as well as flesh and liver of yellow perch from this site, are highly contaminated by mercury, Cd, PCBs, and PAHs, and the contaminant load is higher at this site than at the intermediate site, Ile Perrot, situated on the north shore of Lake St. Louis (Table 1). The highly contaminated site and the intermediate site were also studied by Hontela et al. (1995) and Dumont (1996).

Capture of the fish

Perch ranging from 20 to 250 g were captured using a 30 m long seine (2 m high at the extremities, 4 m high at the center, 1.5 cm mesh), between 09:00 and 12:00. Fish were transferred from the seine to a lid-covered tank and transported by boat to shore (5 min maximum).

The mean daily catch was approximately 70 fish, and the experiments were completed on two consecutive days at each site every season. Sampling was done at the same time of day to eliminate interference from the daily variations of plasma cortisol levels (Peter et al. 1978). The spring experiments were carried out from mid-May to the end of the month shortly after spawning, the summer sampling

extended from mid-July to mid-August, and fall experiments were done during the last 2 weeks of October. Only the fish sampled in summer at the intermediate site (Ile Perrot) were included in this study, because the number of fish caught at this site in spring and fall was too small.

Sampling procedure: capture stress test

Immediately after capture and transport to shore, fish were taken out of the holding tank by groups of four or five, kept out of the water for 5 s, and rapidly anaesthesized in a solution of 150 mg/L of tricaine methanesulphonate (MS-222) until they reached stage III of anaesthesia, as described by Iwama et al. (1989). This group is referred to as the "post-capture" group. After measuring body mass (±0.1 g) and total length, fish were bled by caudal puncture with a heparinized 1-mL syringe. Blood was centrifuged, plasma was separated in aliquots and instantly frozen in liquid nitrogen. Fish were killed by spinal transection, sex was determined, the liver and gonads were weighed, and pieces of the liver were instantly frozen in separate aliquots (<1 g) in liquid nitrogen. Fish with threadlike gonads were classified as immature. All samples were kept at –80°C until biochemical analyses. The interrenal and pituitary tissues were prepared for histology as described in Hontela et al. (1997). The sampling of all the fish was completed within 40 min.

ACTH challenge test

After capture and transport to shore, yellow perch were randomly distributed between four and six experimental enclosures (12 fish/0.5 m^3) made out of nylon nets (1 m length, 1 m depth, 0.5 m width, 0.5 cm mesh), attached to a floating (1 × 1 m) wooden frame. The frames were secured on a dock near shore, in approximately 1.5 m water depth. The enclosures were covered to reduce direct sun exposure and to ensure water temperatures similar to the lake (Table 1). Fish were allowed to recover from the capture stress for 24 h in the enclosures, with water flowing through and without feeding. They were then subjected to the ACTH challenge test.

Prior to field experiments, ACTH aliquots were prepared, using porcine $ACTH^{1–39}$ (Sigma Corp., St. Louis, Mo.) dissolved in deoxygenized and acidified (pH 6) double-distilled water, and lyophilized. The aliquots were transported to the field in liquid nitrogen. Immediately before taking the fish out of the enclosures, ACTH was dissolved in saline (0.7% NaCl), transferred into a 1-mL syringe, and kept on ice.

After the 24-h rest period, the net with the fish from each enclosure was submerged in a shallow container with the anaesthetic. This procedure lasted 2 min maximum. A group of fish ("ACTH" group) received an i.p. injection of 4 IU ACTH/100 g body mass (BM) per 100 µL of saline and the control group ("saline" group) received an i.p. injection of 100 µL saline/100 g BM. After injection, fish were put back in the enclosures, where they recovered from anaesthesia within a few minutes. Two hours later, the saline and the ACTH-treated groups were sampled using the same protocol as used with the post-capture group (see above). An uninjected group was also sampled after the 24-h rest period ("24-h" group), to monitor the levels of cortisol after capture.

The ACTH dose and the length of time before sampling (2 h) were selected in a pilot study and by consulting data of Ilan and Yaron (1976) and Nichols and Weisbart (1984). The pilot study was conducted in the field at the reference site during the same season, where yellow perch were injected with saline or with three different doses of ACTH (1, 2, or 4 IU). The dose of 4 IU ACTH elicited a significantly higher cortisol response (341.98 ± 29.56 ng/mL; mean ± SE) than the dose of 2 IU (231.38 ± 110.91 ng/mL) and the dose of 1 IU (163.81 ± 68.76 ng/mL), and cortisol in fish injected with 4 and 2 IU was higher than in fish injected with saline.

Biochemical analyses

Plasma cortisol was assayed with a radioimmunoassay (ICN Biomedicals, No. 07-221102). The plasma glucose levels were measured in deproteinized plasma, using a colorimetric method from Sigma Corp. (GOD–PAP reagent). Liver glycogen was analyzed using an enzymatic and colorimetric method described by Bleau et al. (1996).

Statistical analysis

The mean of the variables from the different experimental groups were compared using Student's *t*-test, one-way and two-way analysis of variance (ANOVA I and ANOVA II), or the Kruskall–Wallis test, on transformed or untransformed data, investigating the effects of treatment, sampling site, season, and sex on cortisol variations at a significance level of $\alpha = 0.05$. ANOVA II was specifically used for the comparisons of the cortisol levels in fish subjected to the ACTH challenge test, to take into account the effect of the "treatment" variable on the "site" variable. Fisher's LSD test or a modified nonparametric multiple-comparison test (Scherrer 1984) was used to discriminate between the different means. A nested one-way ANOVA was used to analyse the variations of the nuclear diameter of the interrenal cells in yellow perch sampled in spring, investigating the differences among fish from the same site and the differences among sites.

Results

Response pattern

At the reference site (Lake Memphremagog) in spring 1995, the capture procedure induced relatively high plasma cortisol levels (post-capture group: 374.41 ± 32.32 ng/mL), whereas the 24 h-rest period allowed the fish to return to lower cortisol levels (24-h group: 89.09 ± 23.30 ng/mL) ($p < 0.001$, *t*-test) (Fig. 1). The 24-h groups had significantly lower plasma cortisol levels than their respective post-capture groups at every site and season (Table 2). Injection of the vehicle (saline group) slightly increased plasma cortisol (155.09 ± 15.23 ng/mL), but the injection of ACTH induced a strong rise in plasma cortisol (461.48 ± 30.73 ng/mL) ($p < 0.001$, *t*-test), compared with the saline (Fig. 1).

Spring experiments

The capture stress test in spring 1995 (Fig. 2A) revealed that perch from the highly contaminated site (Iles de la Paix) had a lower cortisol response ($p = 0.001$, *t*-test) than fish from the reference site. Moreover, fish from the highly contaminated site also had a lower response to the ACTH challenge ($p < 0.05$, ANOVA II) (Fig. 2B).

Females in spring at the reference site had a higher cortisol response to the capture stress test than males ($p < 0.01$, *t*-test) (Fig. 3), but no differences between sexes were observed at the highly contaminated site. There was no difference between the plasma cortisol levels of males and females injected with ACTH at the reference site and at the highly contaminated site (Table 3).

Summer experiments

No significant differences were detected between sites sampled in summer using the capture stress test (Fig. 4A). The ACTH challenge did not reveal differences between the sites in the cortisol response to ACTH injection (Fig. 4B). However, fish from the highly contaminated site had higher levels of cortisol when injected with saline than fish from the reference site ($p < 0.001$, ANOVA II). Fish from the highly

Fig. 1. Response pattern of plasma cortisol (mean ± SE) in yellow perch (*Perca flavescens*) to the experimental treatments. Fish were sampled at the reference site (Lake Memphremagog, Quebec) in spring 1995. *, Significant difference between post-capture and 24-h groups ($p < 0.05$, *t*-test); †, significant difference between saline- and ACTH-injected groups ($p < 0.05$, *t*-test).

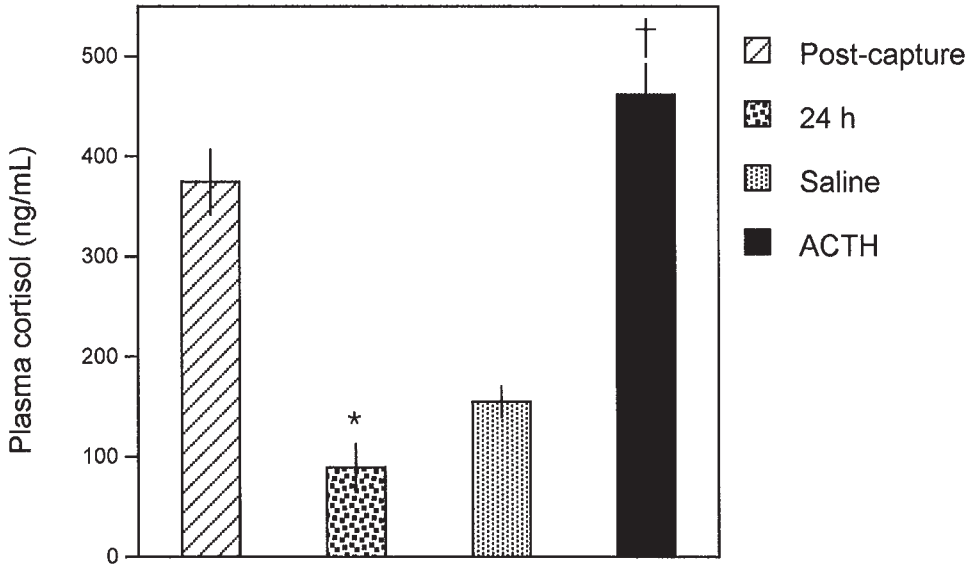


Table 2. Plasma cortisol (mean ± SE (*n*)) in yellow perch from the reference site (Lake Memphremagog), intermediate site (Ile Perrot), and highly contaminated site (Iles de la Paix) immediately after capture by seine or after a 24-h rest period in spring, summer, and fall.

		Cortisol (ng/mL)	
Season	Site	Post-capture group	24-h group
Spring	Lake Memphremagog	374.41±32.32 (20)	98.07±23.30 (11)*
	Iles de la Paix	236.53±22.77 (31)‡	135.38±16.17 (18)*
Summer	Lake Memphremagog	241.00±28.02 (16)†	54.22±9.43 (11)*
	Ile Perrot	289.00±24.07 (18)	43.21±11.64 (8) *
	Iles de la Paix	225.04±26.91 (20)	101.55±22.23 (17)*†‡
Fall	Lake Memphremagog	163.74±15.65 (20)†	na[a]
	Iles de la Paix	148.26±13.90 (21)†	64.01±13.31 (10)*†

[a]Not available.
*Significantly different from the post-capture group ($p < 0.05$, *t*-test).
†Significant difference among seasons ($p < 0.05$, ANOVA I).
‡Significantly different from the less contaminated site ($p < 0.05$, *t*-test).

contaminated site also had higher ($p < 0.05$, ANOVA I) plasma cortisol levels 24 h after capture (24-h group) (Table 2) compared with the less contaminated sites.

There was no significant differences between the cortisol response of male, female, and immature fish to the experimental treatments in the summer (data not shown for the capture stress test and shown in Table 3 for the ACTH challenge test).

Fall experiments

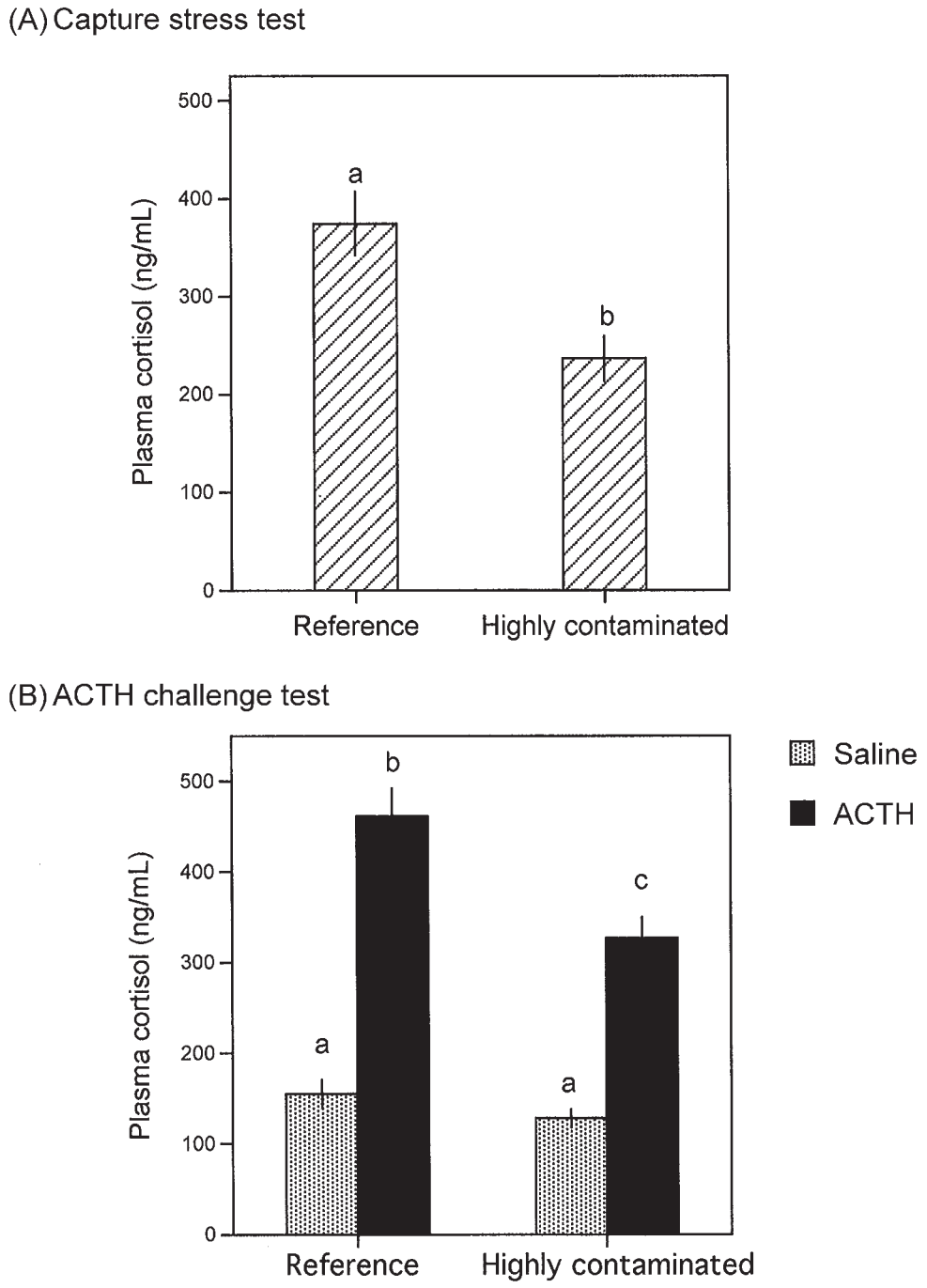
There were no significant differences in the responsiveness to capture stress of yellow perch from the reference and the highly contaminated site (Fig. 5A), but the response pattern to the ACTH challenge test resembled that of the spring experiment (Fig. 5B). Indeed, the response to the ACTH challenge at the highly contaminated site was lower than at the reference site ($p = 0.001$, ANOVA II).

The capture stress induced a similar cortisol response in males and females from the reference site and the highly contaminated site (Fig. 3). Females from the reference site had a higher cortisol response to ACTH than males (Table 3) ($p < 0.001$, ANOVA II), but no difference between males and females was observed at the contaminated site. The ANOVA II revealed that males from the contaminated site had lower levels of plasma cortisol in response to ACTH compared with males from the reference site (Table 3). This is also true for females from the contaminated site injected with ACTH, which have significantly lower levels of plasma cortisol than females from the reference site (Table 3).

Carbohydrate metabolism

Statistical analyses for plasma glucose and liver glycogen content were made on total groups, because subgroups based on sex were generally too small ($n < 8$). In spring, fish from the reference site and the highly contaminated sites had similar plasma glucose levels following either the capture stress test or the ACTH challenge test (Table 4). However, fish subjected to

Fig. 2. Spring experiments. Plasma cortisol (mean ± SE) in yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog) and from the highly contaminated site (Iles de la Paix) subjected to (A) the capture stress test and (B) the ACTH challenge test. Means with different letters are significantly different ($p < 0.05$, ANOVA I).



the capture stress test in summer and fall had higher levels of glucose at the highly contaminated site compared with the reference site ($p < 0.05$, ANOVA I or *t*-test). The injection of saline or ACTH tended to increase plasma glucose, compared with levels measured immediately post-capture, but site or seasonal differences were not consistent.

Liver glycogen was measured in the 24-h group to compare animals that recovered, at least partially, from the capture stress. In spring, fish from the highly contaminated site had a higher level of liver glycogen than fish from the reference site (Fig. 6), although the difference was not significant ($p = 0.053$, *t*-test). In summer, on the other hand, fish from the highly contaminated site had a lower level of liver glycogen than fish from the reference site ($p < 0.01$, *t*-test). No differences between the two sites were observed in fall.

Condition factor, LSI, and GSI

The condition factor (CF) of perch from the intermediate and the highly contaminated sites was in general higher compared to the reference site ($p < 0.01$, *t*-test and ANOVA I and II)

Fig. 3. Seasonal variations of plasma cortisol levels (mean ± SE) in sex subgroups of yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog) and the highly contaminated site (Iles de la Paix) subjected to the capture stress test in (A) spring and (B) fall. *, Significant difference between sexes ($p < 0.05$, ANOVA I); **, significantly different from the reference site ($p < 0.05$, ANOVA I); †, significantly different from spring ($p < 0.05$, ANOVA I).

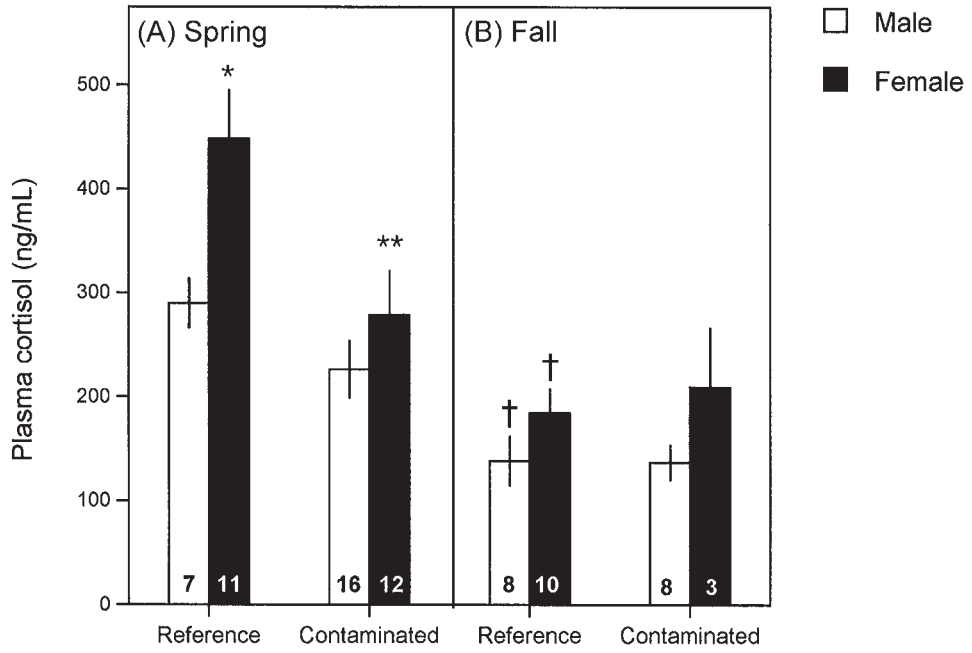


Table 3. Plasma cortisol levels (mean ± SE (*n*)) in male, female, and immature yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog), intermediate site (Ile Perrot), and highly contaminated site (Iles de la Paix) sampled in spring, summer, and fall and subjected to the ACTH challenge test.

			Cortisol (ng/mL)	
Season	Sex	Site	Saline group	ACTH group
Spring	Male	Lake Memphremagog	161.61±35.17 (12)	419.70±60.31 (8)
		Iles de la Paix	141.82±22.01 (12)	334.80±31.42 (13)
	Female	Lake Memphremagog	173.23±18.38 (15)	494.76±34.71 (22)
		Iles de la Paix	135.10±15.67 (5)	202.68±90.20 (3)
Summer	Female	Lake Memphremagog	73.57±22.36 (12)	358.66±35.42 (11)
		Ile Perrot	203.93±54.35 (7)*	375.90±67.52 (11)
		Iles de la Paix	370.70±58.47 (11)*	364.71±38.39 (17)
	Immature	Lake Memphremagog	132.64±28.49 (13)	331.08±37.28 (15)
		Ile Perrot	211.14±48.57 (8)	339.29±61.85 (4)
		Iles de la Paix	219.97±21.32 (23)*	345.38±51.79 (13)
Fall	Male	Lake Memphremagog	153.06±18.13 (8)	244.26±26.81 (7)
		Iles de la Paix	113.38±9.97 (16)	181.07±21.69 (12)*
	Female	Lake Memphremagog	240.18±26.55 (11)	400.22±43.94 (13)†
		Iles de la Paix	132.22±17.74 (5)	181.00±27.36 (6)*

Note: In all the cases, cortisol levels of the ACTH group are significantly higher than the saline group ($p < 0.05$, ANOVA II). The results of few ($n < 3$) immature fish captured in spring and fall, and males captured in summer are not shown.

*Significantly different from the reference site ($p < 0.05$, ANOVA II).

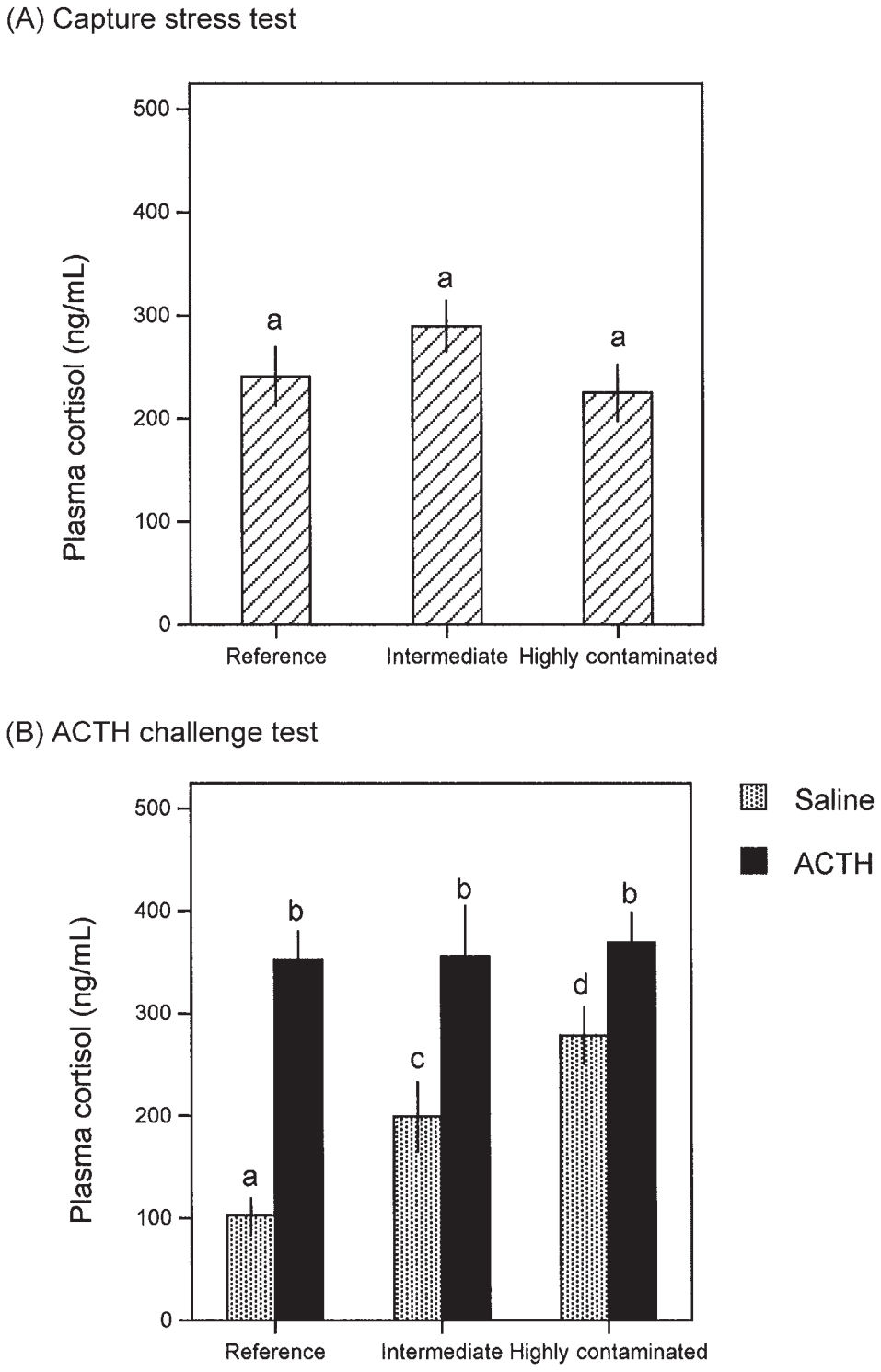
†Significantly different from the males ($p < 0.001$, ANOVA II).

(Table 5). The liver somatic index (LSI) was similar in the total groups, although some inconsistent differences among sites were observed in some subgroups based on sex (Table 5). The gonadosomatic index (GSI) was generally not different between the highly contaminated site and the reference site (Table 6) but in spring, females from the highly contaminated site had a lower GSI ($p < 0.001$, *t*-test) than females from the reference site and the opposite was true in fall.

Seasonal variations

The responsiveness of yellow perch to capture stress varied among seasons (Fig. 3). Indeed, the plasma cortisol levels of

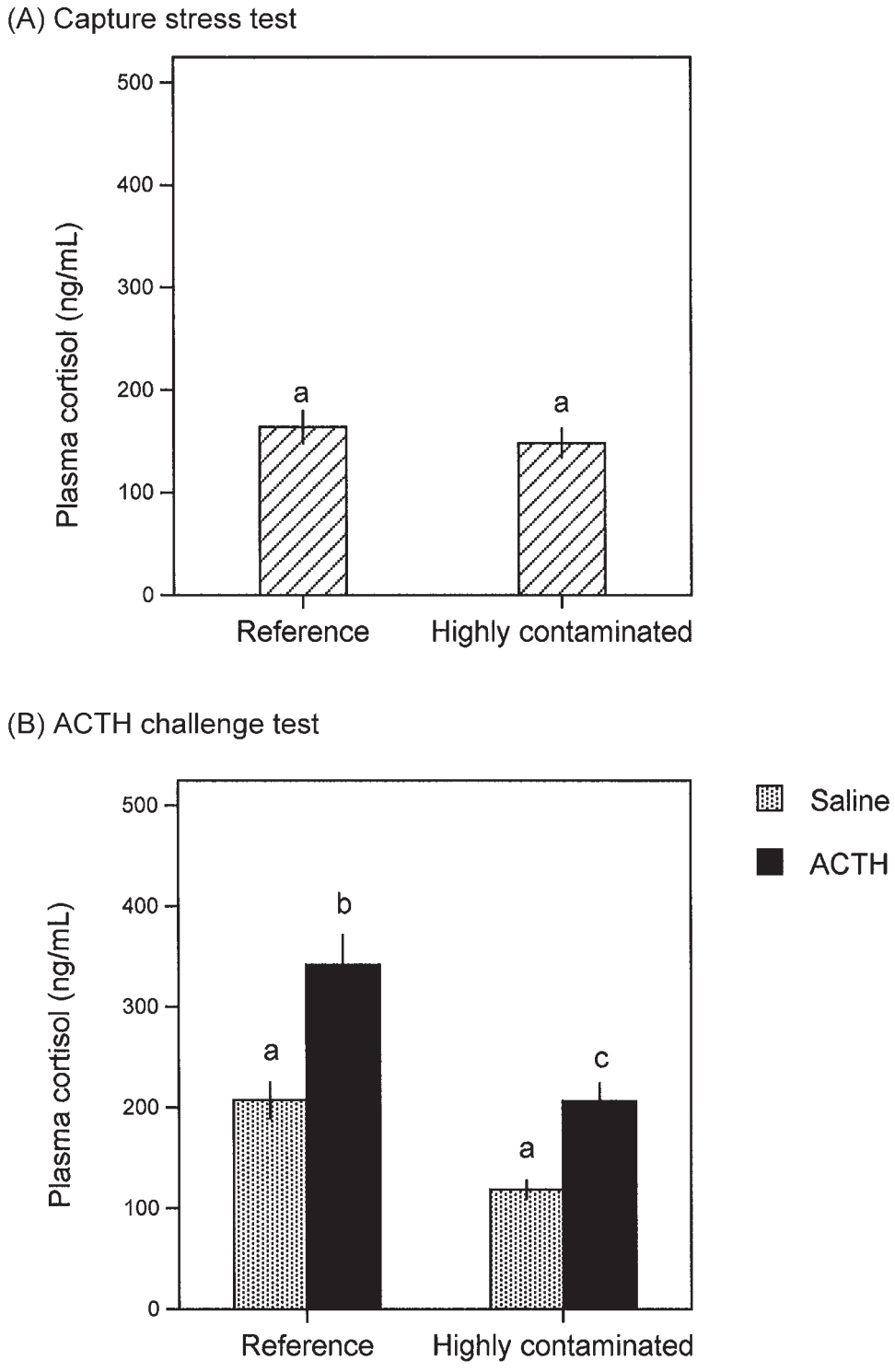
Fig. 4. Summer experiments. Plasma cortisol (mean ± SE) in yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog) and from the highly contaminated site (Iles de la Paix) subjected to (A) the capture stress test and (B) the ACTH challenge test. See Fig. 2 for details.



fish from the reference site were lower ($p < 0.05$, ANOVA I) in summer (241.00 ± 28.02 ng/mL) and fall (163.74 ± 15.65 ng/mL) than in spring (374.41 ± 32.32 ng/mL). Similarly, at the highly contaminated site, plasma cortisol levels in fall were lower than in spring ($p < 0.05$, ANOVA I). The responsiveness to the ACTH challenge test was also lower ($p < 0.01$, ANOVA II) in summer and fall compared with spring for yellow perch from the reference and the highly contaminated sites (Table 3).

Yellow perch from both the highly contaminated and the reference sites, subjected to the capture stress test or to the ACTH challenge test, had higher plasma glucose levels in

Fig. 5. Fall experiments. Plasma cortisol (mean ± SE) in yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog) and from the highly contaminated site (Iles de la Paix) subjected to (A) the capture stress test and (B) the ACTH challenge test. See Fig. 2 for details.



spring compared with summer ($p < 0.01$, *t*-test, ANOVA I or II) (Table 4). Seasonal variations of liver glycogen were also detected. At the reference site, fish in summer had higher levels of liver glycogen than fish sampled at the other seasons ($p < 0.05$, ANOVA I), but seasonal differences were not detected in fish from the highly contaminated site.

Histology

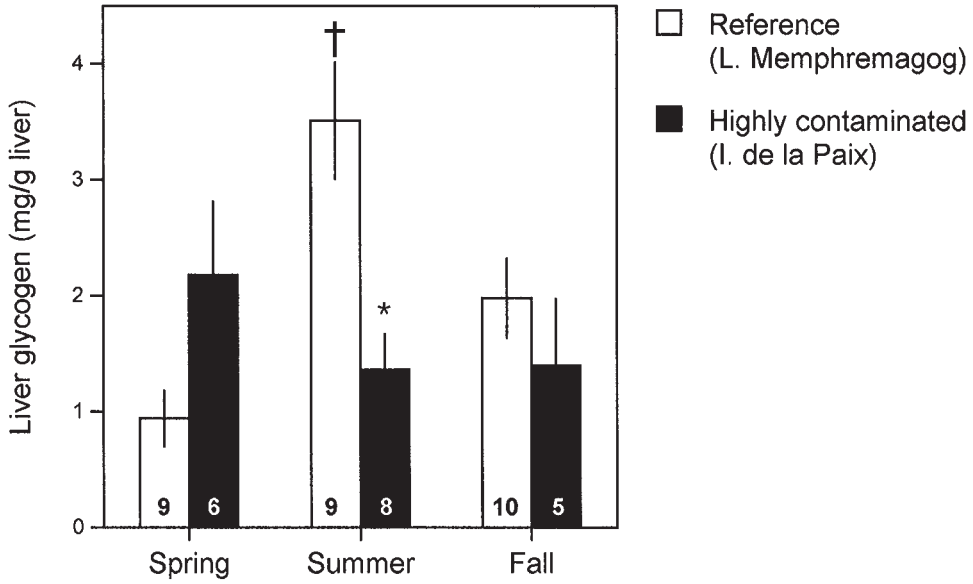
Histomorphometrical analyses of the interrenal tissue of fish from the reference site and the highly contaminated site did not reveal differences in the nuclear diameter (reference site: 4.369 ± 0.451 μm ($n = 11$); highly contaminated site: 4.253 ± 0.474 μm ($n = 11$)).

Table 4. Plasma glucose (mean ± SE (*n*)) in yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog), intermediate site (Ile Perrot), and highly contaminated site (Iles de la Paix) subjected to the four experimental treatments in spring, summer, and fall.

		Plasma glucose (mg/mL)			
Season	Site	Post-capture	24 h	Saline	ACTH
Spring	Reference	0.35±0.03 (20)	1.01±0.02 (10)	1.03±0.04 (27)	1.04±0.03 (23)†
	Highly contaminated	0.40±0.03 (26)	0.57±0.08 (5)	0.92±0.04 (22)	0.94±0.04 (23)
Summer	Reference	0.08±0.02 (11)†	0.24±0.02 (8)	0.41±0.03 (19)	0.50±0.03 (18)
	Intermediate	0.10±0.02 (12)	—	0.61±0.04 (12)*	0.56±0.04 (14)
	Highly contaminated	0.26±0.03 (14)*†	0.47±0.05 (8)	0.52±0.03 (19)*	0.50±0.03 (22)†
Fall	Reference	0.37±0.03 (16)	0.56±0.04 (8)	0.74±0.04 (20)	0.61±0.04 (19)
	Highly contaminated	0.48±0.04 (17)*	—	0.56±0.04 (21)*	0.62±0.04 (22)

*Significantly different from the reference site ($p < 0.05$, ANOVA I or II).
†Significantly different from the other seasons ($p < 0.05$, ANOVA I or II).

Fig. 6. Liver glycogen content (mg/g liver; mean ± SE) in yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog) and from the highly contaminated site (Iles de la Paix) after a 24-h rest period. *, Significantly different from the reference site ($p < 0.05$, *t*-test); †, significantly different from the other seasons ($p < 0.05$, ANOVA I).



Discussion

The interrenal function was investigated in yellow perch captured at a reference site and at two sites contaminated with heavy metals, PCBs, and PAHs. Earlier investigations reported lower plasma cortisol in response to capture stress in yellow perch from contaminated sites (Hontela et al. 1992, 1995) and in the present study, the functional integrity of the cortisol-secreting interrenal tissue was evaluated using a standardized i.p. injection of porcine ACTH$^{1-39}$. The field-adapted ACTH challenge test revealed that the interrenal tissue of fish from the highly contaminated site had a significantly reduced ability to respond to ACTH, compared with fish from the reference site. This endocrine dysfunction may be partially or completely responsible for the impaired ability of fish from polluted sites to elevate plasma cortisol after an acute stimulation by capture stress. Our standardized protocol using exogenous ACTH enabled us to test, for the first time in a wild fish sampled in situ, the functional integrity of the HPI axis. The protocol has several key aspects crucial for this type of field investigation.

Seine-fishing, a non-destructive and relatively rapid capture technique, provided acutely stressed yet unharmed specimens. The method of anaesthesia was carefully selected since the procedure can influence plasma cortisol levels. An immobilizing dose of 100 mg MS-222/L was used because similar doses used in other fish did not induce a rise in plasma cortisol (Iwama et al. 1989). Fish were maintained post-capture in experimental enclosures during 24 h, which allowed the acutely stressed fish to significantly lower their plasma cortisol levels compared with fish from the post-capture groups, sampled immediately after capture (Table 2). The 24-h period has been used by others to let plasma cortisol levels settle to pre-confinement or pre-handling stress levels in fish kept in the laboratory (Pickering and Pottinger 1987) or in fish captured by angling (Pankhurst and Dedual 1994). The assessment of basal physiological status in wild fish in their habitat does remain a challenge, however, because of the sensitivity of physiological endpoints within the HPI axis to field-specific sampling procedures. To carry out the standardized functional testing of the HPI axis, it is important to reach cortisol levels that are not influenced by the stress of capture. The protocol described here provides an experimental setup in which such

Table 5. Gross morphology (mean ± SE (*n*)) of yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog), intermediate site (Ile Perrot), and highly contaminated site (Iles de la Paix) sampled in spring, summer, and fall.

Season	Site	CF[a]	LSI (%)[b]
Spring	Lake Memphremagog	1.07±0.1 (112)	1.31±0.03 (102)
	Iles de la Paix	1.12±0.02 (109)*	1.38±0.1 (94)†
Summer	Lake Memphremagog	1.03±0.01 (92)	1.27±0.04 (92)
	Ile Perrot	1.31±0.2 (55)*	0.96±0.03 (53)
	Iles de la Paix	1.15±0.01 (109)*	0.82±0.02 (109)
Fall	Lake Memphremagog	0.97±0.01 (80)	1.10±0.05 (58)
	Iles de la Paix	1.03±0.01 (56)*	0.99±0.01 (46)

[a]Condition factor (CF) = (body weight/(total length)^3) × 10^5.
[b]Liver somatic index (LSI) is the percentage of body weight constituted by the liver.
*Significantly different from the reference site ($p < 0.01$, *t*-test or ANOVA I).
†Significant difference among the seasons ($p < 0.05$, ANOVA I).

Table 6. Gonadosomatic index (GSI) of male, female, and immature yellow perch (*Perca flavescens*) from the reference site (Lake Memphremagog), intermediate site (Ile Perrot), and highly contaminated site (Iles de la Paix) sampled in spring, summer, and fall.

		GSI (%)[a]		
Season	Site	Male	Female	Immature
Spring	Lake Memphremagog	1.10±0.1 (33)	0.93±0.1 (54)†	1.26±0.7 (6)
	Iles de la Paix	1.28±0.2 (43)	0.38±0.1 (17)*	1.01±0.6 (4)
Summer	Lake Memphremagog	0.21±0.1 (10)	0.40±0.01 (36)	0.21±0.02 (23)
	Ile Perrot	—[b]	0.36±0.02 (31)	0.31±0.02 (19)
	Iles de la Paix	0.22±0.02 (14)	0.38±0.02 (43)	0.18±0.02 (41)
Fall	Lake Memphremagog	6.35±0.45 (16)†	0.49±0.1 (31)	0.07±0.004 (3)
	Iles de la Paix	6.04±0.3 (34)†	2.11±0.1 (15)*	0.57±0.1 (3)

[a]Gonadosomatic index is the percentage of body weight constituted by the gonads.
[b]Number of fish captured was too small (<3) to calculate the GSI.
*Significantly different from the reference site ($p < 0.01$, *t*-test or ANOVA I).
†Significant difference among the seasons ($p < 0.05$, ANOVA I).

levels can be attained, although they are not necessarily the basal cortisol levels. These levels are presently not known for yellow perch in the field, but a laboratory study showed that wild yellow perch maintained for 6 weeks in captivity had plasma cortisol levels of 55 ng/mL after injection of saline (C. Girard, unpublished). Basal levels of cortisol in this fish species are thus expected to be even lower.

The dose of 4 IU ACTH/100 g BM, used to illustrate the response pattern (Fig. 1), shows that a clear cortisol response induced by ACTH can be observed in the field situation using the enclosures and the 24-h rest period. Indeed, the injection of the vehicle (saline) does not itself induce a strong elevation in plasma cortisol compared with the non-injected 24-h group. Moreover, a significant difference in the plasma cortisol levels of saline-injected and ACTH-injected fish was seen in all the experiments at all the sites. Thus, the protocol described in the present study was appropriate for the assessment of the endocrine response to a standardized ACTH challenge. Haux et al. (1985) captured perch (*Perca fluviatilis*) and monitored the recovery of several stress variables of these wild fish maintained in floating wooden chests for up to 8 days. After approximately 3 days, blood glucose and lactate were significantly reduced compared with levels immediately after capture. Our data suggest that, at least for variables within the HPI axis, a 24-h period may be sufficient in yellow perch to detect the cortisol response specifically elicited by ACTH injection.

The spring experiments revealed that yellow perch from the highly contaminated site had a reduced ability to elevate plasma cortisol when subjected to an acute and standardized capture stress or to the ACTH challenge test, compared with fish from a reference site (Fig. 2A). In fall also, the responsiveness to the ACTH injection in fish from the highly contaminated site was lower than in fish of the reference site. In contrast to spring, however, no difference could be detected between sites in fall when the capture stress test was used to elevate plasma cortisol. A lower sensitivity of the HPI axis, possibly caused by the colder fall temperatures (see Seasonal variations in Results), may mask the impairment of the cortisol response that is revealed with the ACTH challenge test. The results from the spring and fall experiments confirm the results of earlier studies with perch (Hontela et al. 1992, 1995), which demonstrated a reduced capacity to elevate plasma cortisol in response to capture in fish from polluted waters. The ACTH challenge test in particular provided evidence that, in fish from the contaminated site, the interrenal tissue is a functionally impaired site within the HPI axis. An experiment in vitro showed that the temporal dynamics of the response of the interrenal tissue of yellow perch to exogenous ACTH was similar between fish from a contaminated site and from a reference

site (Brodeur et al. 1997*a*, 1997*b*). The amplitude of the response was, however, lower in fish from the contaminated site, also indicating a functionally impaired interrenal tissue. The specific cellular mechanisms responsible for this endocrine disruption are not yet understood.

Xenobiotics can directly alter the HPI axis by cytotoxic effects (Ilan and Yaron 1983), they may interfere with the bioactivity of ACTH (Bestervelt et al. 1993), or they may inhibit the steroidogenic pathways in adrenal cells (Mgbonyebi et al. 1993). Increased clearance rates of plasma cortisol could also explain the lower cortisol levels in chronically stressed fish (Schreck et al. 1985). The induction of biotransformation enzymes, possibly increasing clearance of hormones, have also been linked to changes in steroid levels in fish exposed to various contaminants (Munkittrick et al. 1992).

Another mechanism that could be responsible for the lower plasma cortisol in fish from contaminated sites is a functional exhaustion of one level in the HPI axis. Indeed, acute exposures of fish to contaminants elevate plasma cortisol (Pickering 1993; Bleau et al. 1996; Hontela et al. 1996); however, the effects of lifelong exposure to one contaminant or to mixtures of toxic compounds have not been as well characterized. A reduced capacity to increase plasma cortisol in fish from contaminated sites has been documented (Lockhart et al. 1972; Hontela et al. 1992, 1995, 1997) as well as atrophy of the interrenal cells (Hontela et al. 1997). The ACTH challenge test performed here on yellow perch from reference and contaminated sites was designed to determine whether the impaired cortisol secretion previously observed in yellow perch from these contaminated sites could be counteracted by a standardized stimulation by ACTH. The lower response to ACTH observed in spring and fall in perch from the highly contaminated site (Figs. 2B and 5B), provides evidence that a partial functional failure of the interrenal tissue is implicated in the low cortisol stress response. However, the histomorphometry of the nucleus of the interrenal cells examined for fish sampled in spring did not reveal morphological differences among fish from the experimental sites, in contrast to a study with perch from sites contaminated by bleached kraft mill effluents (Hontela et al. 1997). Only a tendency for a slightly lower mean nuclear diameter in fish from the highly contaminated site compared with fish from the reference site was noted, indicating a slight atrophy of the interrenal cells.

In summer, fish from the three experimental sites responded similarly to both the capture stress test and the ACTH stimulation. Indeed, plasma cortisol was almost as high as in spring, yet no differences between sites were detected (Figs. 4A and 4B). The significant interaction between the site and the treatment variables ($p < 0.001$, ANOVA II) illustrates the fact that the relationship between cortisol levels of saline-injected and ACTH-injected fish varied from one site to the other. The higher levels of cortisol in the saline group of the highly contaminated site may be caused by the effects of seasonal factors, such as temperature or sexual condition, to which fish from this site may be more sensitive. Metabolic clearance rate of cortisol is influenced by temperature (Pickering and Pottinger 1983), and it is possible that the clearance rate of ACTH was enhanced in the summer. A higher dose of ACTH may have been necessary in summer to reveal differences between sites, since ACTH could be eliminated more rapidly and its full effect on secretion may not have been observed. This seasonal aspect of the response to the ACTH challenge test will be investigated in future studies.

Sexual maturity is another factor that could influence the function of the HPI axis (Pottinger et al. 1995). Analyses of the effects of sexual maturity on the responsiveness of the HPI axis revealed that females were more responsive to the capture stress and to the ACTH injection, since the differences between the contaminated sites and the reference site were more pronounced in females than in males (e.g., Fig. 1). A general seasonal trend in the responses to the capture stress test as well as to the ACTH challenge was observed; the levels of plasma cortisol in fall were significantly lower than in spring and, in certain cases, than in summer (Table 3, Fig. 4). The seasonal variations of sex-based subgroups were generally consistent with this trend, especially for capture-stressed fish (Fig. 1). Seasonal variations of plasma cortisol have been linked to variations in water temperature and sexual maturity (Planas et al. 1990; Audet and Claireaux 1992). In yellow perch, the ability to elevate plasma cortisol in response to capture stress and to ACTH stimulation was significantly lower in fall, a period of low temperature and high GSI (especially for males; Table 5).

The effects of the exposure to mixtures of contaminants on the endocrine and metabolic status were investigated in the present study by evaluating carbohydrate metabolism. Acute handling stress and exposure to xenobiotics are known to induce an elevation in plasma glucose in response to glucocorticosteroids and diminish liver glycogen stores (Haux et al. 1985; Thomas 1990; Vijayan and Moon 1992; Folmar 1993). Abnormal carbohydrate metabolism was observed in perch (*Perca fluviatilis*) exposed to bleached kraft pulp mill effluents. Fish from the contaminated site had significantly higher liver glycogen levels and their plasma glucose levels were variable compared with fish from a reference site (Andersson et al. 1988; Förlin et al. 1995). Hontela et al. (1995) also observed abnormally high levels of liver glycogen in yellow perch exposed to heavy metals and organic compounds, as well as markedly lower plasma glucose levels at the contaminated site than at the reference site in sexually immature fish. In the present study, no general trend in the site-related variations of plasma glucose levels could be detected (Table 4), and no difference of plasma glucose between the saline-injected and ACTH-injected fish was observed. The time course of the ACTH challenge (2 h) may have been too brief to stimulate gluconeogenesis and to attain the threshold level for the formation of new glucose (Vijayan and Leatherland 1989).

In spring, liver glycogen in fish from the highly contaminated site tended to be higher ($p = 0.053$, *t*-test) than at the reference site (Fig. 6), indicating in these fish, as seen in recent studies, an abnormal glycogen metabolism (Andersson et al. 1988; Förlin et al. 1995; Hontela et al. 1995). In summer, fish from the highly contaminated site had markedly lower liver glycogen stores. Vijayan and Leatherland (1989) proposed that cortisol has a direct effect on liver glycogen metabolism. Glycogen mobilization and hence a decrease in glycogen stores could be favored by the higher resting plasma cortisol levels (in the 24-h group) measured in summer at the contaminated site.

In the present study, the link between functional impairment of the HPI axis and deleterious effects on the physiological status of fish was investigated by measuring, in addition to

metabolic responses, endpoints indicative of growth and gonadal development. The condition factor (CF) is used to compare growth and has been interpreted as a depletion of the energy stores, such as liver glycogen or body fat (Goede and Barton 1990). Dumont (1996) sampled a large number of yellow perch in the St. Lawrence River and showed that fish from a contaminated site had a lower CF than fish from a reference site. On the other hand, perch (*Perca fluviatilis*) exposed to bleached kraft mill effluents had a higher CF than fish from a reference site (Sandström 1994), perhaps reflecting stimulated growth, as in our study, where the CF of yellow perch from the highly contaminated site was generally higher compared with fish from the reference site in spring, summer, and fall. Further studies are required to elucidate the effects of xenobiotics on growth in wild fish.

Our study provided evidence that the chronic toxic stress impairs the interrenal tissue and cortisol secretion, an endocrine system that plays an important role in maintenance of homeostasis. The ACTH challenge test, a new method to evaluate the functional integrity of the interrenal tissue of fish exposed to contaminants, revealed that yellow perch from a highly contaminated site had a lower ability to elevate plasma cortisol in response to an i.p. injection of exogenous ACTH, compared with fish from a reference site. The functional impairment of the HPI axis was most evident in spring and fall, and females were the most impaired. Although variations in plasma cortisol levels among fish from different sites can be associated with environmental variables other than site-specific contamination, there is increasingly strong evidence that a causal link between the impairment of cortisol secretion and chronic exposure to low levels of contaminants exists (Lockhart et al. 1972; Hontela et al. 1992, 1995, 1997; Brodeur et al. 1997*b*). The ability of wild fish to respond to a standardized hormonal challenge may be used to detect an abnormal neuroendocrine stress response in fish from polluted sites. The repercussions of this endocrine dysfunction on behavior, growth, and fitness need investigation.

Acknowledgements

The authors gratefully acknowledge Jérôme Allard for technical field assistance and Alain Blanchard, Anne C. Ricard, and Claude Daniel for technical support and advice. We thank Dr. Pierre Dumont, from the Ministère de l'environnement et faune, for critical review of the manuscript. We thank also the Limnology Center of McGill University for providing access to the Lake Memphremagog field station. This study was funded by the Canadian Network of Toxicology Centers (Reproductive and Endocrine Toxicology program) and Université du Québec à Montréal Research Funds.

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