Ecotoxicology **6**, 1–12 (1997)

Structural and functional impairment of the hypothalamo-pituitary-interrenal axis in fish exposed to bleached kraft mill effluent in the St Maurice River, Québec

ALICE HONTELA[1], CLAUDE DANIEL[1] and JOSEPH B. RASMUSSEN[2]

[1]*Department of Biological Sciences, TOXEN Research Centre, University of Québec at Montréal, CP 8888, Succ. A, Montréal, Québec, Canada H3C 3P8*
[2]*Department of Biology, Limnology Center, McGill University, 1240 Avenue Docteur Penfield, Montréal, Québec, Canada H2P 6T8*

Received 25 April 1995; accepted 13 September 1995

The effects of bleached kraft mill effluent (BKME) on blood cortisol levels and the morphology of the pituitary-interrenal axis were investigated in two species of teleost fish, the northern pike, *Esox lucius*, and the yellow perch, *Perca flavescens*, sampled upstream and downstream from a pulp and paper mill on the St Maurice River, Québec. Fish were acutely stressed by a standardized capture and sampling protocol at both sites, and their ability to elevate blood cortisol levels in response to the capture stress was compared. Blood cortisol levels in fish from the upstream site (. 100 ng/ml plasma) were higher than the levels in fish from the BKME site, and the pituitary corticotropes and the interrenal steroidogenic cells of the upstream fish were larger and had larger nuclei compared with cells from the downstream fish. The low blood cortisol levels in fish exposed to BKME were correlated to cellular atrophy within the hypothalamo-pituitary-interrenal (HPI) axis. The reduced ability to elevate blood cortisol in response to an acute stress may be an endocrine dysfunction occuring in fish chronically exposed to chemical stressors in their environment.

Keywords: cortisol; BKME; fish; interrenal; pituitary; morphology; acute stress; endocrine dysfunction

Introduction

Despite increasing evidence in animal species for a causal relationship between pollutant-mediated disruptions of endocrine function and impaired health (Colborn and Clement, 1992), few field studies have unequivocally demonstrated endocrine dysfunctions in fish exposed to environmental doses of toxic chemicals. Reduced plasma levels of sex steroids and diminished reproductive performance has been observed in fish from areas in the Great Lakes impacted by bleached kraft mill effluent (BKME) (Munkittrick *et al*., 1991; McMaster *et al*., 1991; Van der Kraak *et al*., 1992). There is new evidence for an abnormal secretion of another steroid hormone, the glucocorticosteroid cortisol, in fish chronically exposed to pollutants in their environment. The endocrine impairment is characterized by a reduced ability to elevate blood cortisol in response to an acute stress; levels of plasma cortisol following a standardized capture and handling stress were lower in pike and perch from sites polluted by a mixture of heavy metals, PAHs and PCBs compared with fish from reference sites (Hontela *et al*., 1992, 1993, 1995). A similar syndrome, characterized by low blood cortisol and glucose levels after capture, was

earlier reported in pike contaminated by high levels of mercury (6.3–16 ìg^{-1} g in the muscle) accumulated from chronic environmental exposure (Lockhart *et al*., 1972). Some support for impaired cortisol secretion in fish chronically exposed to xenobiotics has been provided by the laboratory study of Kirubagaran and Joy (1991). A 90 day exposure to mercury at doses of 10% of the 96 hr LC50 in the laboratory induced structural damage in the pituitary and in the interrenal cells in the catfish, *Clarias batrachus*, and these morphological changes were associated with low blood cortisol.

The release of adrenocorticotropic hormone (ACTH) from pituitary corticotrope cells and the subsequent release of the glucocorticosteroid hormone cortisol from interrenal tissue are two steps in the sequence of neuro-endocrine and metabolic events activated in a teleost fish in response to a stress (Donaldson, 1990; Schreck, 1990). Acute exposures to a wide range of chemical contaminants elevate blood cortisol (Folmar, 1993), and physical stresses such as handling or confinement have a similar effect (Barton and Iwama, 1991). The time course of the cortisol response during short term exposures to a stress lasting minutes to hours has been investigated in some studies. Blood cortisol levels increase within minutes of the onset of the stress and while the stress is imposed, they may remain elevated or they may decrease after a period of time, depending on the type and the intensity of the stress (Barton and Iwama, 1991; Folmar, 1993). The effects of exposure to sublethal chemical stress lasting months or years on the secretion of cortisol are unknown, although those are likely to be the exposure regimes experienced by feral fish in contaminated environments, particularly by sedentary species such as perch and pike (Toner and Lawler, 1969; Aalto and Newsome, 1990). Since cortisol has a role in regulation of metabolism, reproduction, growth and the immune function (Sheridan, 1986; Maule *et al*., 1989; Vijayan and Moon, 1992), disruption of normal cortisol secretion may have adverse secondary effects on fish health.

Cortisol impairment characterized by a reduced ability to elevate blood cortisol following an acute stress has thus far only been detected in fish chronically subjected to either heavy metals or mixtures of heavy metals, PAHs and PCBs (Lockhart *et al*., 1972; Kirubagaran and Joy, 1991; Hontela *et al*., 1992, 1995). It is not known how chemical-specific such an endocrine dysfunction is or indeed whether chronic exposure to any chemical stress will eventually impair cortisol secretion. In the present study, the effects of chronic exposure to BKME on blood cortisol were investigated. BKME is a complex mixture of chlorinated chemicals, plant resins and acids (Suntio *et al*., 1988). Effects of BKME on fish include disturbances of numerous biochemical parameters in the blood and liver, reduced levels of plasma sex steroid hormones, effects on growth and fecundity, and increased mortality rates (Andersson *et al*., 1988; Sandström and Thoresson, 1988; Karas *et al*., 1991; McMaster *et al*., 1991; Munkittrick *et al*., 1991; Hodson *et al*., 1992; van der Kraak *et al*., 1992; Gagnon *et al*., 1994). The effects of BKME on cortisol secretion and the hypothalamo-pituitary-interrenal (HPI) axis have not yet been investigated.

The objectives of the present study were i) to compare blood cortisol levels in fish from a site contaminated by BKME and from a reference site to assess whether chronic exposure to BKME impairs cortisol secretion, and ii) to examine the pituitary corticotropes and the interrenal cells and quantify, with a histomorphometric analysis, the morphological changes within the HPI axis in fish chronically exposed to BKME.

Methods

Test species and study sites

Northern pike, *Esox lucius*, and yellow perch, *Perca flavescens*, were selected as test species because these fish are relatively sedentary (Toner and Lawler, 1969; Aalto and Newsome, 1990), they are present in many ecosystems in the temperate zone and both species exhibited cortisol impairment in our previous studies at sites contaminated by a mixture of heavy metals and organics. Fish were sampled upstream (site 1) and downstream (site 2) from a bleached kraft pulp and paper mill located at La Tuque, Québec (47°22′N, 72°26′W) on the St Maurice river (Fig. 1) in May. Site 1 was situated 10 km upstream from the mill in a reservoir created by a hydroelectric dam located at La Tuque. The site was free of industrial and agricultural activity except for logs floating towards the mill during periods of log release. The sediment was mud with much wood debris and emergent vegetation; the current was slow. Site 2 was situated below the dam 2 km downstream from the mill. Water at both sites was highly coloured, water temperature was 15 °C. Fish movement from site 2 to site 1 was limited by the hydroelectric dam.

The water quality at sites 1 and 2 was described in several studies carried out within the same year as the present study (Hodson *et al*., 1992; Gagnon *et al*., 1994) and characteristics such as turbidity, colour and temperature were similar at the two sites. Mean daily production of the mill was 1427 t of air-dried pulp with a daily wastewater discharge of 240 000 m^3 and an effluent dilution ratio in the river of 94:1 (Hodson *et al*., 1992). Chlorophenols and chloroguaiacols were detected downstream from the mill (Hodson *et al*., 1992), the daily emissions of absorbable organic chlorine (AOX) were

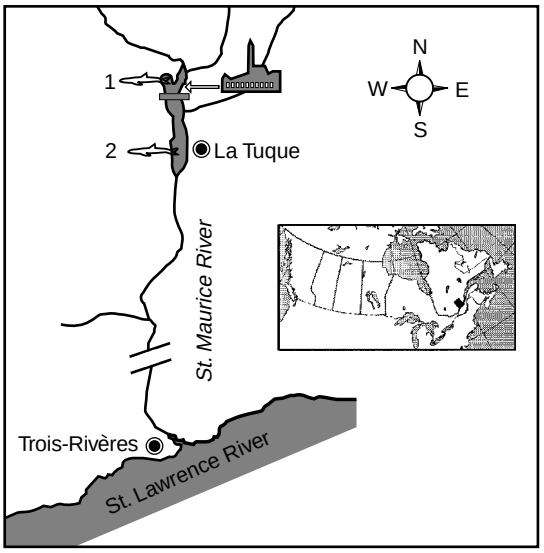

Fig. 1. Location of the sampling sites on the St Maurice River 10 km upstream (site 1) and 5 km downstream (site 2) from the bleached kraft pulp and paper mill at La Tuque, Québec. A dam 1 km below the mill separates the two sites and prevents upstream movement of fish from site 2 to site 1.

3.4 kg^{-1} t of dried pulp per day in 1991 (Personal communication, W.M. Vrooman, Canadian Pacific Forest Products). Although the fish sampled in the present study were not analysed for chlorinated chemicals, dioxines and furans were detected in the flesh of white suckers collected at site 2 while fish from site 1 were not contaminated (Hodson *et al*., 1992; Gagnon *et al*., 1994).

Sampling procedure

The nets were set in shallow water at depth of 1–3 m at both sites, in slow current. Fish were captured from 1100–1800 h on four consecutive days with a seine or a gill net set for periods of 30 min. The fish were removed from the nets and placed in a holding container for a maximum of 10 min prior to sampling. They were stunned with a sharp blow to the head, and body length and weight were recorded. Blood was withdrawn from the caudal vasculature with a 1 cc non-heparinized syringe; serum was separated and immediately frozen on dry ice. The pituitary gland and the head kidney containing the interrenal tissue were rapidly dissected and fixed in Bouin's Hollande solution (pituitary gland) or Bouin's solution (interrenal tissue). To eliminate the effects of sampling methodology on cortisol levels, identical sampling procedures were used at the two sampling sites as far as time for setting the nets, number of fish placed in the holding container, the time required for blood sampling and dissections, and number of fish sampled per site on each day. No significant trends with time were detected in blood cortisol levels, fish sampled first at a site were not significantly different from fish sampled last.

Radioimmunoassay for cortisol

Plasma samples were assayed in duplicates (20 ìl per tube) for cortisol with a diagnostic kit (No. 825 Kallestaad Diagnostics, Montréal). Characteristics of the assay were published previously (Hontela *et al*., 1995.

Histomorphometric analysis of pituitary corticotropes and interrenal cells

The interrenal tissue and the pituitary gland were processed for histology and stained with differential stains (Cleveland & Wolff stain for pituitary, Trichrome de Masson stain for interrenal). Morphological characteristics of the corticotropes from the rostral pars distalis region of the pituitary and of the steroidogenic interrenal cells were compared on slides using an Image Analyser system (Quadra 840AV MacIntosh computer linked to a colour camera). Two programmes were used for the histomorphometric analysis. The NIH Image programme was used to measure the diameter of the nuclei in the interrenal cells and in the pituitary corticotropes. The Stereology Tool Box^{TM} was used to quantify the cellular area and the intercellular spaces within the interrenal tissue as an A/a ratio which corresponds to the ratio of the total surface occupied by a specific cell type in relation to the total sampling surface. Data and photographs of the histological slides were stored on laser disks.

Results

Gross morphology of the fish

No significant differences in body weight, length or gonadosomatic index between the upstream and the downstream fish were detected (Table 1).

Table 1. Morphological characteristics of fish sampled upstream and downstream from a pulp and paper mill on the St Maurice River, Québec

Site and species	GSI (%)	Body weight (g)	Body length (cm)	Condition[a]
Upstream perch (*n*=9)	, 1	101 6 19	13 6 0.5	0.41
Downstream perch (*n* = 10)	, 1	110 6 21	14 6 0.9	0.42
Upstream pike (*n* = 18)	3.4 6 0.8	922 6 125	51 6 2.0	0.53
Downstream pike (*n* = 12)	2.6 6 0.6	980 6 147	51 6 1.7	0.54

[a]condition = (Body weight (total length)^3) 3 10^5

Plasma cortisol

Blood cortisol levels in pike and perch from the upstream reference site were greater than 100 ng^{-1} ml of plasma and were significantly higher (p , 0.05) than the cortisol levels in fish from the downstream site (Fig. 2). Values from males and females were grouped together at each site since sex differences in blood cortisol were not detected.

Morphology and morphometric analysis of the pituitary gland and the interrenal tissue

Pituitary corticotropes and the steroidogenic interrenal cells were examined in histological preparations. The pink staining corticotropes with dark nuclei were located between the orange staining prolactin cells and the neurohypophysis (Fig. 3A, B). The islets of interrenal steroidogenic cells were found in the proximity of the cardinal vein sinuses within the head kidney (Fig. 3C, D). The cells were large and tightly packed in the upstream fish, without any intercellular spaces between cells (Fig. 4A). A significantly smaller proportion of the total area within interrenal tissue was occupied by interrenal cells; large spaces between individual cells being apparent in the

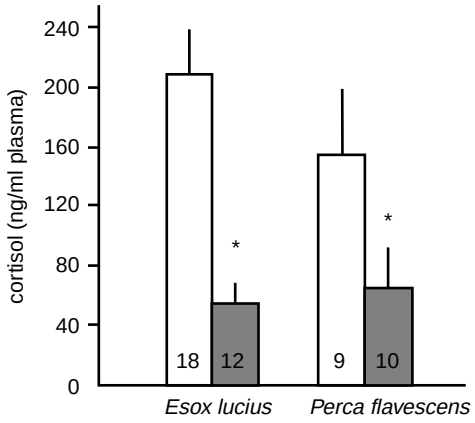

Fig. 2. Plasma cortisol stress response (mean 6 SE) in northern pike, *Esox lucius*, and yellow perch, *Perca flavescens* exposed to BKME sampled upstream u and downstream j from the mill at La Tuque. Numbers of fish sampled are indicated in the bars. *significantly different from the upstream fish at p , 0.05 (One way ANOVA).

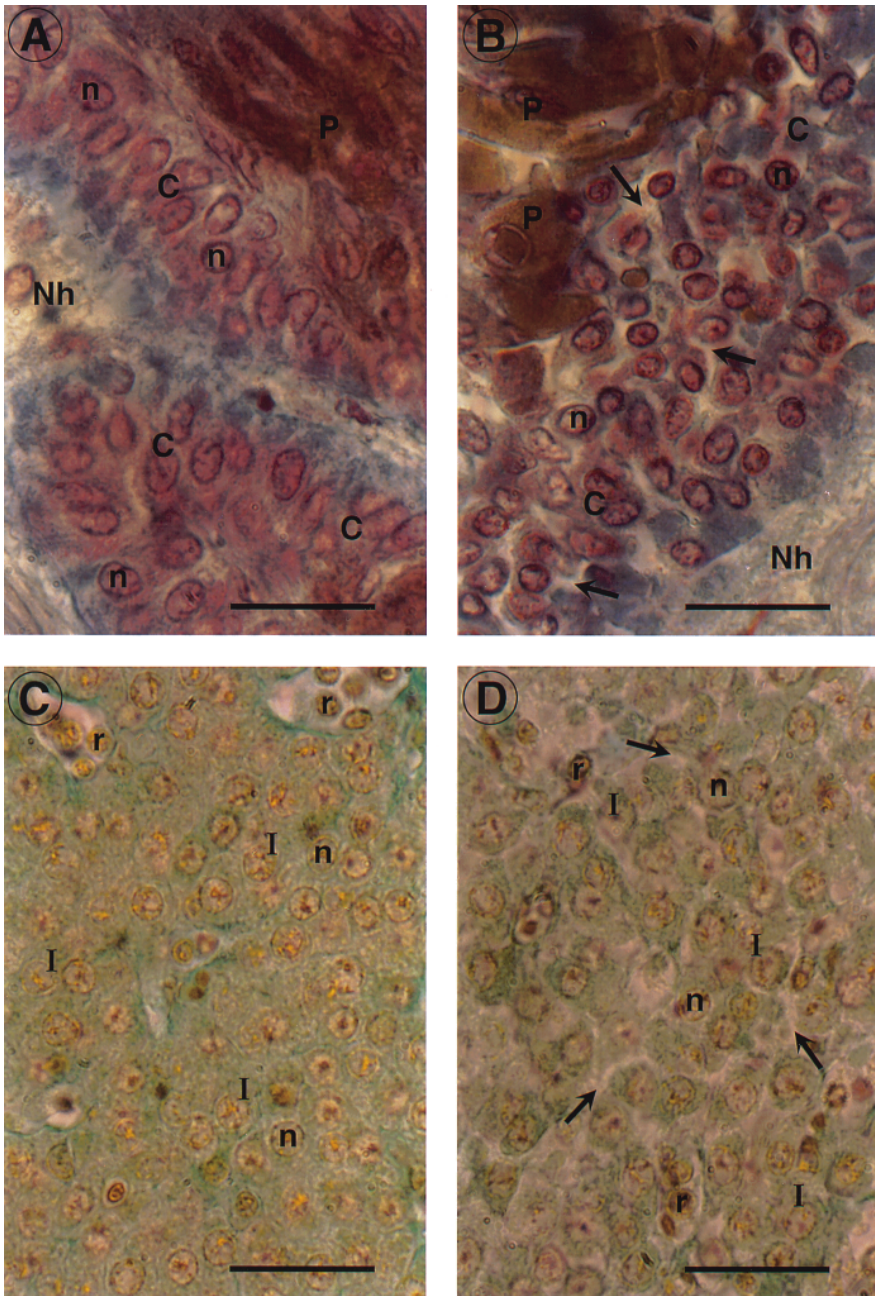

Fig. 3. Pituitary corticotropes in the pars rostralis of pike sampled at (A) the reference upstream site and (B) the downstream site receiving BKME. Stained by Cleveland & Wolff differential stain. C: corticotrope; n: nucleus; P: prolactin cells; Nh: neurohypophysis; Arrows indicate intercellular spaces. Magnification 3 1000. Black bar measures 20 ìm. Interrenal steroidogenic cells within the pronephros of pike sampled at (C) the upstream site and (D) the downstream site receiving BKME. Stained by Trichrome de Masson. I: interrenal cells; n: nucleus; r: red blood cells within blood vessels. Arrows indicate intercellular spaces. Magnification 3 1000. Black bar measures 20 ìm.

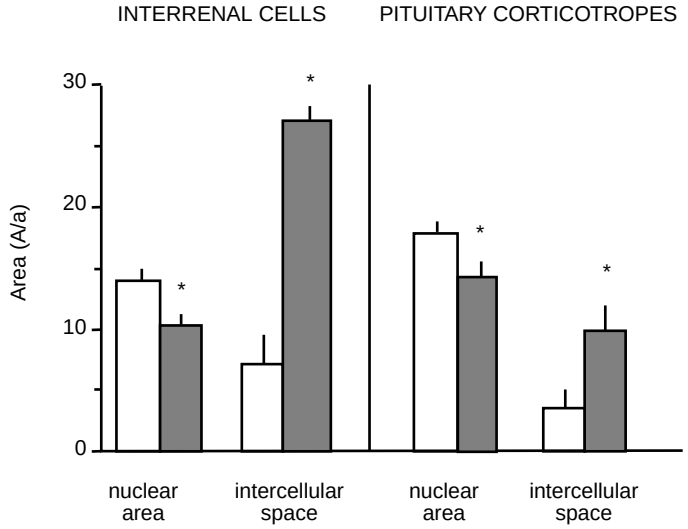

Fig. 4. Morphometric analysis of the interrenal cells and of the pituitary corticotropes in the pike, *Esox lucius*. The total area occupied by nuclei, relative to the total area measured (A/a ratio) was smaller in cells of fish exposed to BKME (downstream fish; j). The A/a ratio for intercellular spaces within the interrenal tissue was larger in the downstream fish. *significantly different from the upstream (u) group. Number of fish examined in each group is indicated in Fig. 2.

downstream fish, compared with interrenal tissue of upstream fish. The length (data not shown) and the area (expressed as an A/a ratio) of the nuclei profile were smaller in the interrenal and in the corticotropic cells of the downstream pike than in the upstream fish (Fig. 4). Vacuolization was however not evident in the pituitary-interrenal axis of BKME exposed pike. A similar trend indicative of cellular atrophy in the BKME exposed fish was also evident in perch; pituitary corticotropes and interrenal cells were smaller and intercellular spaces were larger in the exposed fish. A significant correlation was detected between plasma cortisol levels and the total steroidogenic cell area within the interrenal tissue in pike and in perch (Fig. 5). Only fish with small intercellular spaces and large cell area in the interrenal tissue were able to produce the maximal plasma cortisol in response to the acute stress of capture, although some of these fish did not have plasma cortisol levels . 100 ng ml^{-1}. However, none of the fish with atrophic interrenal cells exhibited the maximal cortisol response.

Discussion

This study provided evidence that the HPI axis of fish chronically exposed to BKME is structurally and functionally impaired. Cortisol levels greater than 100 ng ml^{-1} of plasma following an acute stress such as capture by a seine and handling indicate that the fish are able to activate the normal neuro-endocrine response to an acute stress (Barton and Iwama, 1991). The normal cortisol stress response was detected in pike and perch sampled upstream from the pulp and paper mill. The reduced ability of the downstream

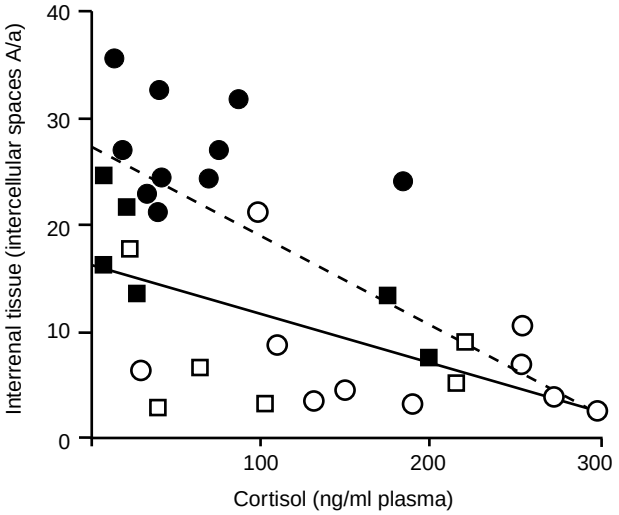

Fig. 5. Correlation between plasma cortisol levels and the total area occupied by intercellular spaces, relative to the total area measured (A/a ratio) within the interrenal tissue of yellow perch (squares) and of northern pike (circles) sampled upstream (hollow) and downstream (solid) from the pulp and paper mill at La Tuque, Québec. $r = 0.52$, $y = 16.1657\ 2\ 0.0453\ x$ for perch (solid line); $r = 0.69$, $y = 27.28\ 2\ 0.0838\ x$ for pike (broken line).

fish to elevate blood cortisol in response to the acute stress of capture suggest that the neuroendocrine components of the HPI axis were adversely affected by the chronic exposure to BKME. The morphological alterations consistent with cellular atrophy observed in pituitary corticotropes and interrenal steroidogenic cells in fish from the BKME site provided additional evidence for an impairment of the HPI axis. Both species of fish investigated in the present study exhibited an impaired cortisol stress response at the site receiving the BKME since their plasma levels of cortisol following the standardized capture and handling stress were much below 100 $ng\,ml^{-1}$ while the fish upstream had the expected high blood cortisol levels (Fig. 2). The ability to increase blood levels of cortisol in response to the acute stress of capture and handling in a field situation is a potential indicator of physiological competence of the fish.

We have previously reported atrophic changes of the pituitary corticotropes in cortisol-impaired fish from sites contaminated by heavy metals, PAHs and PCBs (Hontela *et al*., 1992). The present study is the first quantitative report of morphological changes in the pituitary as well as in the interrenal component of the HPI axis in fish exposed to BKME in their environment. The reduced size of the nuclei, the reduced cell area and the presence of large intercellular spaces between cells of the corticotropic zone of the pituitary gland and between the steroidogenic cells of the interrenal tissue suggest that these cells are atrophic and that the interrenal tissue is structurally impaired. The significant negative correlation between plasma cortisol levels following an acute capture stress and the degree of cellular atrophy within the HPI axis (Fig. 5) provides further evidence that environmental exposure to BKME causes a functional and structural impairment of the HPI axis in fish. There were no fish with a high degree of cellular atrophy in the HPI axis that were able to increase plasma cortisol to

levels . 100 ng ml^{-1}; levels characteristic of the normal endocrine response to an acute stress. Although fish with normal interrenal tissue had a significant tendency to exhibit high blood cortisol, some individuals with normal interrenal cells and low plasma cortisol (, 100 ng ml^{-1}) were obtained. This variability of the hormonal response to stress is compatible with the present knowledge of the physiological mechanisms underlying the acute stress response in fish (Haux *et al*., 1985; Schreck, 1990). A physiologically competent fish (HPI axis unimpaired) may elevate its plasma cortisol in response to an acute stress or it may maintain low plasma cortisol, if the stress threshold of the individual has not been reached (Schreck, 1990; Barton and Iwama, 1991). Alternatively, the exposure threshold (concentration and/or duration) to induce structural impairment of the interrenal tissue may be higher than the threshold for the functional impairment of the cortisol stress response.

The mechanisms by which chronic exposures to environmental contaminants impair the ability to elevate blood cortisol in fish have not yet been established. Selye (1973), in his studies with laboratory rodents, provided some experimental evidence that long term exposures to stressful conditions may eventually cause an exhaustion of the neuro-endocrine axis that mediates the physiological stress response and reduce the survivorship of the organism. Laboratory studies designed to investigate the effects of long term exposures to stress in fish are difficult to carry out and only a few such studies have been reported. Kirubagaran and Joy (1991) exposed a catfish to sublethal doses of mercury (10% of the 96-hr LC50) for three months and reported lower blood cortisol and atrophic changes in the pituitary corticotropes and the interrenal cells. Although their study did not provide data concerning the temporal dynamics of the cortisol stress response to the xenobiotic (blood cortisol was not assayed in the early phase of the exposure), it provided some support to the hypothesis that long term exposure to contaminants leads to an exhaustion of the cortisol secreting system.

Mammalian studies provide evidence that specific chemicals may exert direct toxic effects on cortisol producing endocrine tissue and impair cortisol secretion by specific cytotoxicity. Metabolites of DDT were cytotoxic in the *zona fasciculata* the cortisol producing layer of the mammalian adrenal gland, where they formed covalent bonds (Jönsson *et al*., 1993) and induced micronecrotic foci and hemorraghes (Lund, 1994). DDT also reduced cortisol secretion in a teleost fish (Ilan and Yaron, 1983). Whether low blood cortisol detected in fish exposed in the field to BKME is caused by an exhaustion of the HPI axis through chronic exposures or by cytotoxicity of BKME chemicals in the interrenal gland is not presently known. The evidence for an impairment of the synthesis of sex steroids in fish exposed to BKME in their environment (Munkittrick *et al*., 1991; Van der Kraak *et al*., 1992; McMaster *et al*., 1991; Gagnon *et al*., 1994) suggested that components of BKME may interfere with synthesis of steroid hormones; sex steroids as well as glucocorticosteroids such as cortisol. Masculinization, linked to abnormal sex steroid synthesis, has been also reported in fish from the family *Poecilidae* in streams receiving BKME (Davis and Bortone, 1992). Gagnon *et al*. (1994) reported reduced levels of 11-ketotestosterone and 17B estradiol in female white suckers *Catostomus commersoni* sampled at the downstream site (site 2) in the present study, compared with steroid levels in fish from the reference site (site 1).

Cortisol impairment has been shown in fish exposed to mixtures of heavy metals and organics (Lockhart *et al*., 1972; Hontela *et al*., 1992, 1995) and the present study

reports a similar phenomenon in fish exposed to BKME. Cortisol impairment, characterized by the reduced ability to elevate blood cortisol in response to an acute standardized stress, may be useful in diagnosis of sublethal effects of low level chronic exposures to environmental contaminants in fish. Development of such biomarkers is an active area of environmental toxicology (Thomas, 1990; Mayer *et al*., 1992). The non-destructive nature of this methodology (blood sampling) and the easy and sensitive method of the hormone assay, make this parameter potentially useful in health assessments of endangered species. Yellow perch is a sentinel fish species of interest for the temperate zone systems since this species is still relatively abundant in Eastern Canada, and yet is sufficiently sensitive to xenobiotics. Increased mortality rates and decreased recruitment in perch populations exposed to BKME have been documented in Scandinavia (Sandström and Thoresen 1988; Karas *et al*., 1991). Future studies should determine the mechanisms of action of the contaminants on the HPI axis and field validate the cortisol impairment in other teleost species.

Acknowledgements

We thank G. Downing, J. Waterman, J. Liepke and N. van Heuer for help with the field work and Dr. G. Chevalier for use of the laboratory facilities at the Université du Québec à Montréal. The study was funded by grants from NSERC and CNTC (Reproductive and Endocrine Toxicology Program).

References

Aalto, S.K. and Newsome, G.E. (1990) Additional evidence supporting demic behaviour of a yellow perch (*Perca flavescens*) population. *Can. J. Fish. Aquat. Sci.* **47**, 1959–62.

Andersson, T., Förlin, L., Härdig, J. and Larsson, A. (1988) Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. *Can. J. Fish. Aquat. Sci.* **45**, 1525–36.

Barton, B.A. and Iwama, G.K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Ann. Rev. Fish Dis.* **1**, 3–26.

Colborn, T. and Clement, C. (1992) *Chemically-Induced Alterations in Sexual and Functional Development: the Wildlife Human Connection, Volume XXI, Advances in Modern Environmental Toxicology,*: Princeton Scientific Publishing Co.

Davis, W.P. and Bortone, S.A. (1992) Effects of kraft mill effluent on the sexuality of fishes: an environmental early warning? In Colborn, T. and Clement, C. eds. *Chemically-induced alterations in sexual and functional development: the wildlife/human connection, volume XXI, Advances in Modern Environmental Toxicology,*: Princeton Scientific Publishing Co., Princeton, New Jersey. pp. 113–27.

Donaldson, E.M. (1990) Reproductive indices as measures of the effects of environmental stressors in fish. *Am. Fish. Soc. Symp.* **8**, 109–22.

Folmar, L.C. (1993) Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected papers. *Environ. Toxicol. Chem.* **12**, 337–75.

Gagnon, M., Dodson, J.J., Hodson, P.V., van der Kraak, G. and Carey, J.H. (1994) Seasonal effects of bleached kraft mill effluent on reproductive parameters of white sucker (*Catostomus commersoni*) populations of the St Maurice River, Québec, Canada. *Can. J. Fish. Aquat. Sci.* **51**, 337–47.

Haux, C., Sjöbeck, M. and Larsson, A. (1985) Physiological stress responses in a wild fish population of perch (*Perca fluviatilis*) after capture and during subsequent recovery. *Mar. Environ. Res.* **15**, 77–95.

Hodson, P.V., McWhirter, M., Ralph, K., Gray, B., Thivierge, D., Carey, J.H., Van Der Kraak, G., Whittle, D.M. and Levesque, M.-C. (1992) Effects of bleached kraft mill effluent on fish in the St Maurice River, Quebec. *Environ. Toxicol. Chem.* **11**, 1635–51.

Hontela, A., Rasmussen, J.B., Audet, C. and Chevalier, G. (1992) Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. *Arch. Environ. Contam. Toxicol.* **22**, 278–83.

Hontela, A., Rasmussen, J.B. and Chevalier, G. (1993) Endocrine responses as indicators of sublethal toxic stress in fish from polluted environments. *Water Poll. Res. J. Canada* **28(4)**, 767–80.

Hontela, A., Dumont, P., Duclos, D. and Fortin, R. (1995) Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St Lawrence river. *Environ. Toxicol. Chem.* **14**, 725–31.

Ilan, Z. and Yaron, Z. (1983) Interference of o,p'DDD with interrenal function and cortisol metabolism in *Sarotherodon aureus* (Steindachner). *J. Fish. Biol.* **22**, 657–69.

Jönsson, C.-J., Lund, B.-O. and Brandt, I. (1993) Adrenolytic DDT-metabolites: studies in mink, *Mustela vision* and otter, *Lutra lutra*. *Ecotoxicology* **2**, 41–53.

Karas, P., Neuman, E. and Sandström, D. (1991) Effects of a pulp mill effluent on the population dynamics of perch, *Perca fluviatilis*. *Can. J. Fish. Aquat. Sci*. **48**, 28–34.

Kirubagaran, R. and Joy, K.P. (1991) Changes in adrenocortical-pituitary activity in the Catfish, *Clarias batrachus* (L.), after mercury treatment. *Ecotoxicol. and Environ. Safety* **22**, 36–44.

Lockhart, W.L., Uthe, J.F., Kenney, A.R. and Mehrle, P.M. (1972) Methylmercury in northern pike (*Esox lucius*): distribution, elimination, and some biochemical characteristics of contaminated fish. *J. Fish. Res. Board Can* **29**, 1519–23.

Lund, B.-O. (1994) *In vitro* adrenal bioactivation and effects on steroid metabolism of DDT, PCBs and their metabolites in the Gray seal (*Halichoerus grypus*). *Environ. Toxicol. Chem.* **13**, 911–17.

Maule, A.G., Tripp, R.A., Kaattari, S.A. and Schreck, C.B. (1989) Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). *J. Endocrinol*. **120**, 135–42.

Mayer, F.L., Versteeg, D.J., McKee, M.J., Folmar, L.C., Graney, R.L., McCume, D.C. and Rattner, B.A. (1992) Physiological and non specific biomarkers. In: Huggett, R.J., Kimerle, R.A., Mehrle, P.M. and Bergman, H.L., eds, *Biomarkers- Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*, Boca Raton: Lewis Publishers, pp. 5–85.

McMaster, M.E., Van der Kraak, G.J., Portt, C.B., Munkittrick, K.R., Sibley, P.K., Smith, I.R. and Dixon, D.G. (1991) Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. *Aquat. Toxicol.* **21**, 199–218.

Munkittrick, K.R., Port, C.B., Van der Kraak, G.J., Smith, I.R. and Rokosh, D.A. (1991) Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. *Can. J. Fish. Aquat. Sci*. **48**, 1371–80.

Sandström, O. and Thoresson, G. (1988) Mortality in perch populations in a baltic pulp mill effluent area. *Mar. Pollut. Bull.* **19**, 564–7.

Selye, H. (1973) The evolution of the stress concept. *American Scientist* **61**, 692–9.

Sheridan, M. (1986) Effects of T4, cortisol, GH and prolactin on lipid metabolism of coho salmon during smoltification. *Gen. Comp. Endocrinol*. **64**, 220–38.

Schreck, C.B. (1990) Physiological, behavioural, and performance indicators of stress. *Am. Fish. Soc. Symp.* **8**, 29–37.

Suntio, L.R., Shiu, W.Y. and Mackay, D. (1988) A review of the nature and properties of chemicals present in pulp mill effluents. *Chemosphere* **17**, 1249–90.

Thomas, P. (1990) Molecular and biochemical responses of fish to stressors and their potential use in environmental monitoring. *Am. Fish. Soc. Symp.* **8**, 9–28.

Toner, E.D. and Lawler, G.H. (1969) synopsis of biological data on the pike *Esox lucius. FAO Fisheries Synopsis* No. 30, Rev. 1.

Van der Kraak, G.J., Munkittrick, K.R., McMaster, M.E., Port, C.B. and Chang, J.P. (1992) Exposure to bleached kraft pulp mill effluent disrupts the pituitary-gonadal axis of white sucker at multiple sites. *Toxicol. Appl. Pharmacol*. **115**, 224–33.

Vijayan, M.M. and Moon, T.W. (1992) Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **49**, 2260–6.