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Impaired Cortisol Stress Response in Fish from Environments Polluted by PAHs, PCBs, and Mercury

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Abstract. The cortisol stress response to capture was investigated in two species of fish (*Perca flavescens* and *Esox lucius*) from sites polluted by high levels of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and mercury, and from reference sites in the St. Lawrence river system. Fish from the reference sites exhibited the normal elevation of serum cortisol in response to the acute stress of capture and had large pituitary corticotropes. In contrast, fish from the most polluted sites were unable to increase their serum cortisol in response to the acute stress of capture and their pituitary corticotropes were atrophied. These results suggest that a life-long exposure to chemical pollutants may lead to an exhaustion of the cortisol-producing endocrine system, possibly as a result of prolonged hyperactivity of the system.

Elevated blood cortisol is recognized as an indicator of general stress in fish (Adams 1990). The hypothalamo-pituitary-interrenal (HPI) axis in fish is activated by a wide range of stressors that stimulate the pituitary corticotropes to release corticotropin which in turn stimulates the synthesis and release of cortisol from the interrenal glands. Exposure to chemical pollutants such as some heavy metals, organic contaminants or acid water results in increased blood cortisol levels (Donaldson *et al.* 1984; Brown *et al.* 1989; Thomas 1990) as does capture and handling (Schreck 1981; Fryer 1975). Thus, cortisol, an energy mobilizing catabolic hormone promoting gluconeogenesis and lipolysis (Brown *et al.* 1984; Sheridan 1986), is part of a generalized stress response, in fish as well as in other vertebrates (Selye 1973). Although it is well established that acute exposure to chemical stressors increases cortisol levels, the effects of a chronic life-long exposure to pollutants are less well understood.

The sensitivity of the cortisol stress response to a variety of pollutants makes this hormone potentially useful as a bioindicator of general toxic stress since it reflects the homeostatic effort of fish in habitats polluted by a mixture of contaminants. Development and use of such bioindicators in the health assessment of fish from systems threatened by pollutants is a challenge facing environmental toxicologists (McCarthy and Shugart 1990; Huggett *et al.* 1992). Laboratory studies of the acute cortisol responses of fish to short-term (hours to weeks) exposures to single pollutants have yielded interpretable results since other stressors were carefully minimized. However, sensitivity of cortisol levels to capture and handling has complicated the interpretation of cortisol data from field studies. Thus, the role of the HPI axis and cortisol during life-long exposure to pollutants in the environment has not yet been clearly established. The objective of this study was to investigate the cortisol response to the acute stress of capture in fish chronically subjected to pollutants in their environment.

Materials and Methods

Sampling Sites and Capture of Animals

Yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) were captured in winter on hook and line by ice fishermen between 11^{00}h and 16^{00}h on each sampling day (water T = 3°C) to minimize variation possibly caused by daily cycles in endocrine activity (Peter *et al.* 1978). The fish were collected from sites differing in their contaminant load in the St. Lawrence river system. The pollution profile at the sites consisted of PAHs, PCBs and mercury (Table 1). The most contaminated site, Paix, in Lake St. Louis (part of the St. Lawrence river) receives effluents from several industries and its sediments have a higher toxicity ranking and contamination index than sediments from Perrot, the intermediate site (Champoux and Sloterdijk 1988; Desilets and Langlois 1989; Sloterdijk *et al.* 1989). Sites in Lake Magog (light industrial activity) and in Lake Massawippi (no industry) were the least contaminated sites (Paul and Laliberté 1989a, 1989b). Within 30 min of capture, fish (15–20 fish of each species from each site) were stunned and a blood sample was taken from the caudal vasculature with a 1 cc syringe. Blood was allowed to clot for 20 min, centrifuged, and serum was frozen on dry ice. Fork length, body weight, sex, and the stage of gonadal maturity were recorded.

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Table 1. Concentrations of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and mercury in sediments at the four study sites[a]

Parameter	Sites: Paix	Perrot	Magog	Massawippi
Chemical pollutants				
mg/kg sediment				
Phenanthrene	0.1	0.09	<0.05	<0.05
Benzo(a)fluoranthene	0.3	0.08	<0.05	<0.05
Dibenzo(a,h)anthracene	0.8	0.31	<0.05	<0.05
Indeno(1,2,3,c,d)pyrene	0.5	0.08	<0.05	<0.05
Benzo(g,h,i)perylene	0.7	0.13	<0.05	<0.05
Benzo(a)pyrene	0.2	<0.05	<0.05	<0.05
Benzo(k)fluoranthene	0.08	<0.05	<0.05	<0.05
Total PAHs	3.7	0.69	<0.1	<0.1
Total PCBs	0.39	0.19	0.17	<0.05
Hg	1.4	0.39	0.52	0.03
Limnological characteristics				
Color (mg Pt/L)	15	15	20	15
pH	7.9	7.9	7.8	7.9
Phosphorus (μg/L)	14	14	20	15
Hardness (mg Ca/L)	34	34	16	28

[a] Data obtained from Champoux and Sloterdijk (1988), Desilets and Langlois (1989), and Paul and Laliberté (1989a,b)

Determinations of Serum Cortisol

The serum cortisol concentrations were measured with a radioimmunoassay kit (Kallestad diagnostics No. 825) with the detection limit calculated as 0.3 ng/ml. There was a parallelism between the cortisol standards and dilutions of fish serum. The arithmetic means were compared by a Tukey test at $p \leq 0.05$ following ANOVA (Wilkinson 1989).

Histomorphometry of Pituitary Corticotropes

The pituitary glands were dissected out immediately after the blood sampling. The glands were exposed by lifting a rectangular flap of the occipital bone and dissecting away the brain. The clearly visible pituitary was then fixed *in situ* with Bouin's fluid, dissected out of the cranium and preserved in Bouin's fluid until return to the laboratory (2–3 days). The glands (10 to 12/species per site) were then paraffin-embedded, cut at 5μ and stained by Cleveland & Wolf differential stain. Pituitary corticotropes of the rostral pars distalis (30 to 40 cells per pituitary) were observed and measured through an optic microscope (1000×) coupled to a computerized image analyser (Coreco Inc.). The means were compared with a Tukey multiple range test at $p \leq 0.05$.

Results

The length and the weight of the fish were similar at the different sites. The groups of perch contained similar proportions of sexually mature and sexually immature individuals at each sampling site; all the pike were sexually mature. Since a preliminary analysis of the cortisol data did not reveal a difference between cortisol levels of sexually mature and immature perch, nor differences between males and females, fish of different sexes or maturity from a particular site were grouped together in the analysis.

Northern pike at the most contaminated site (Paix) had the lowest ($p \leq 0.05$) concentrations of serum cortisol and did not display elevated cortisol levels characteristic of the normal healthy response to the acute stress of capture (Figure 1). Intermediate cortisol levels were detected at the intermediate site while high cortisol levels were evident in pike from the least contaminated site (Magog). Pike could not be obtained at Massawippi, the second reference site. A similar pattern was observed in the perch; the highest cortisol levels being detected in fish captured at the least contaminated site (Massawippi). The inability to elevate serum cortisol in response to capture was, however, evident already at the intermediate site (Perrot).

A histomorphometric analysis of the pituitary corticotropes revealed smaller ones in fish from the contaminated sites compared to fish from the reference sites. The corticotropes situated in the rostral pars distalis of the pituitary gland (Kerr 1942; Olivereau and Olivereau 1983) form a border along the neurohypophysis in both pike and perch. The corticotropes of the perch are elongated, have a rectangular shape, and their cellular membranes were clearly visible under the microscope (Figure 2). The length of the cells was significantly smaller ($p \leq 0.05$) in perch from the most contaminated site (Paix), fish that also had the lowest serum cortisol. The cell length of the corticotropes could not be measured in the pike because the cells in pituitaries from fish captured at the reference sites were too tightly packed together (Figure 2). However, spaces between the cells were observed in fish from the contaminated sites (Figure 2) and they were quantified using an image analyser program designed to quantify areas. The smallest intercellular spaces ($p \leq 0.05$) were observed between corticotropes in pike from the most contaminated site, fish which were also unable to elevate their serum cortisol in response to capture.

Discussion

Yellow perch and northern pike are relatively sedentary species (Toner and Lawler 1969; Fortin and Magnin 1972) and therefore the pollution profile at the capture sites should

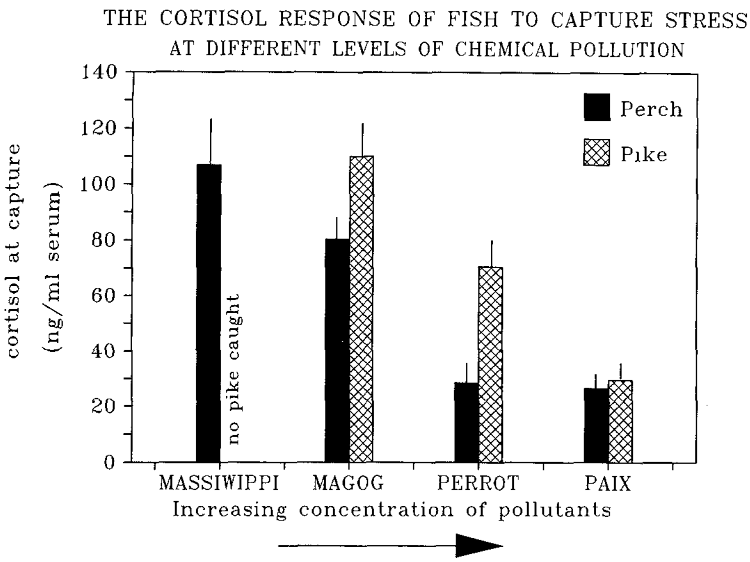

Fig. 1. Northern pike at the most contaminated site (Paix) had the lowest ($p \leq 0.05$) serum cortisol levels (mean ± SE) and did not display the normal elevation of serum cortisol in response to the acute stress of capture. The highest cortisol levels at capture were evident in pike from the least contaminated site (Magog). A similar pattern was observed in the perch, the highest cortisol levels being detected in fish captured at the least contaminated site (Massawippi). The inability to elevate serum cortisol in response to capture was evident already at the intermediate site (Perrot)

reflect the conditions to which the fish were exposed throughout their life. Blood cortisol levels in acutely stressed fish usually exceed 100 ng/ml but may be below 10 ng/ml in unstressed fish (Pickering *et al.* 1982; Pickering 1989). The levels detected at capture in pike and perch from the reference sites (Magog, Massawippi) reached 114 ng/ml and these concentrations indicate that capture by hook and line constitutes an acute stress. This is in agreement with numerous reports of elevated serum cortisol concentrations in fish experiencing acute stress caused by handling (Fryer 1975; Donaldson *et al.* 1984).

Our study is first to report that fish chronically subjected to contaminants in their environment are unable to respond normally to the acute stress of capture. The physiological perturbation of the stress response was quantified at two different levels in the HPI axis, in the pituitary by histomorphometry of the corticotropes, and in the blood by assessing the levels of cortisol. Serum cortisol levels at capture were low in both species at the most contaminated site (Paix, Figure 1) and in addition, their corticotropes appeared atrophied (Figures 3 and 4). Cortisol concentrations were also lower in perch from Magog, a site subjected to light industrial activity, than in perch from Massawippi where there is no industry.

Why fish that are chronically subjected to pollutants in their environment were unable to elevate their serum cortisol in response to the acute stress of capture is not clear. The dynamics of the stress response have been extensively documented in mammals. Selye (1973), a leader in stress studies in man and experimental animals described an initial alarm stage, the ensuing stage of resistance and the final stage of exhaustion. The alarm reaction, characterized by a sharp increase in blood cortisol levels, has been reported in fish (Schreck 1981; Donaldson *et al.* 1984). The stage of resistance is less well understood, with some authors reporting a return to normal cortisol levels after 1 to 2 weeks despite the continued presence of the stressor, and others showing continued cortisol elevation for at least 25 days (Schreck 1981; Pickering and Stewart 1984). The stage of exhaustion may be reached if the stressful conditions are severe and sufficiently prolonged (Selye 1973). In mammals, this stage is characterized by decreasing levels of cortisol, depletion of liver glycogen, immunosuppression, and other changes reducing the survivorship of the organisms. The inability of fish from our most polluted sites to elevate blood cortisol in response to the acute stress of capture indicates that the fish may have reached this stage. The atrophied appearance of the pituitary corticotropes (shorter cells in the perch, and greater intercellular spaces in the pike) is compatible with this hypothesis. Few studies in fish have investigated the exhaustion stage of the stress response (Ram and Singh 1988). Exposure of a teleost fish, *Channa punctatus,* to a sublethal dose of a nitrosulfate fertilizer for a period of 6 months or 1 year resulted in degenerative changes in the pituitary corticotropes and in the adrenal cortisol-producing cells. Almost complete liver glycogen depletion and a decrease in blood glucose by 35% was detected in trout subjected to components of pulpmill effluent for 11 days (Oikari and Nakari 1982). However, blood cortisol levels were not measured in these studies.

The HPI axis of the perch may be more sensitive than that of the pike since the maximum decrease in the cortisol response and in corticotrope size was attained at a lower level of contamination in perch (Figure 5). Thus, while the pike from Perrot (intermediate level of contamination) exhibited intermediate serum cortisol levels and corticotrope condition, perch from Perrot were not distinguishable from those of the most contaminated site (Paix) in either of these traits. The perch HPI axis might therefore be useful as an indicator of general toxic stress at lower levels of contamination than the HPI axis of pike. Species differences in responses to pollutants have been reported for other early warning indicators of toxicity such as metallothioneins in fish (Brown *et al.* 1987). Differences in maturity may also influence the response to toxic substances (Cleveland *et al.* 1986). Our groups of perch contained similar numbers of immature and mature fish at each site while all the pike were sexually mature.

The data indicate that under chronic life-long exposure to chemical pollution (specifically PAHs, PCBs, and mercury), the normal responsiveness of the HPI axis to acute stress is

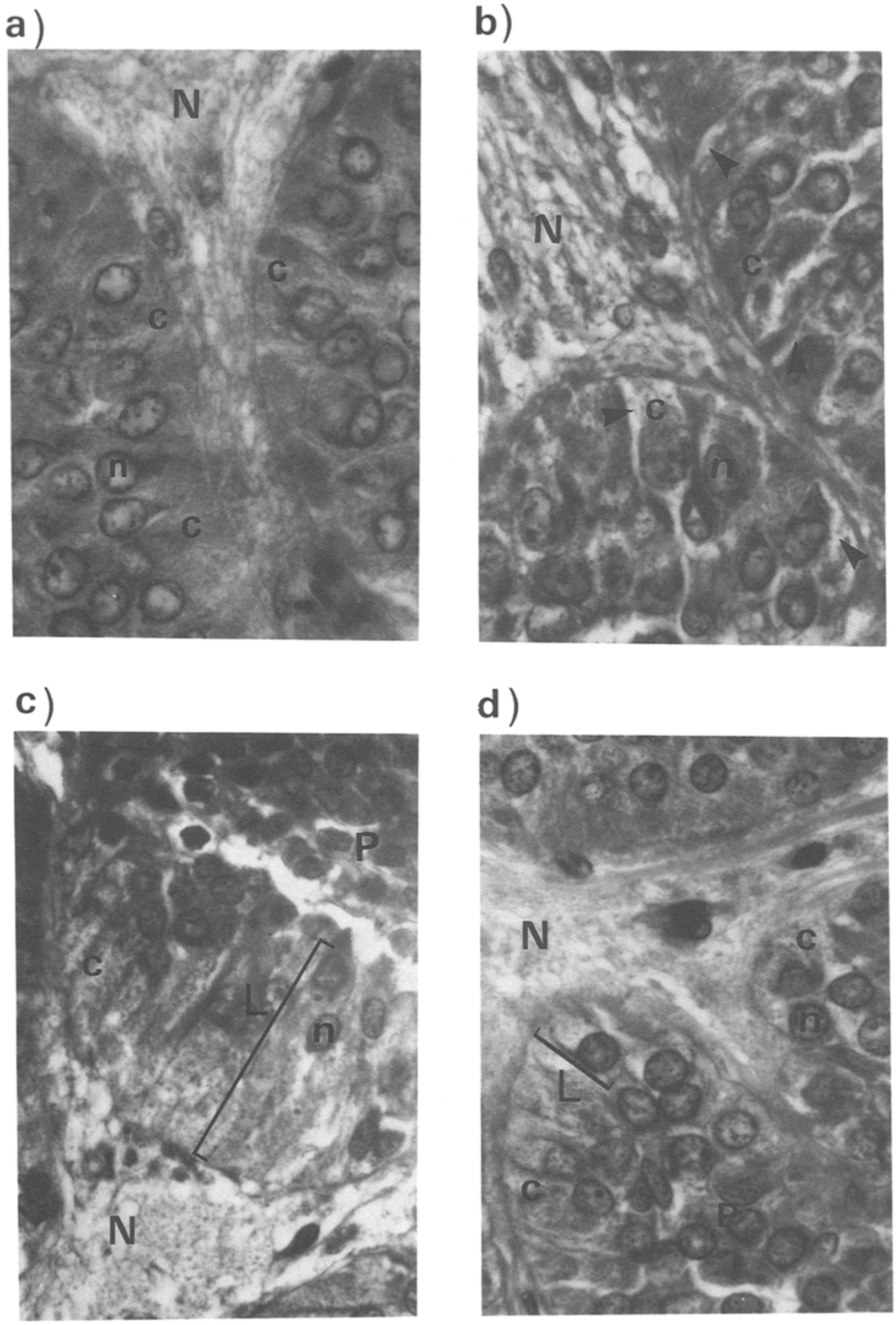

Fig. 2. Pituitary corticotropes of the rostral pars distalis stained by Cleveland & Wolf differential stain. a) Normal cells characteristic of pike from the reference site (Magog), b) atrophic corticotropes in pike from the most polluted site (Paix), c) normal corticotropes of perch from a reference site (Massawippi), and d) atrophic corticotropes of perch from Paix. N, neurohypophysis; n, nucleus; C, corticotropes; P, prolactin; L, cell length; intercellular spaces indicated by arrows (1000×)

impaired, possibly as a result of prolonged hyperactivity of the axis. Prolonged cortisol elevation leads to muscle wasting and lower growth rate in fish as well as to disturbances of the reproductive axis (Freeman and Idler 1973; Billard *et al.* 1981). A government study (Dumont 1990) in which fish were tagged and recaptured over several years, found lower growth rate and fecundity in fish from our most polluted site (Paix) compared to the intermediate site (Perrot). The absence of the cortisol response to capture also implies that fish from the polluted sites lack the gluconeogenic and lipolytic energy-mobilizing capacity. Fish in such a physiological state may fail to display the normal behavioral responses to conspecifics, prey and predators, thus further jeopardizing their own survivorship.

Mechanisms other than prolonged activity of the HPI axis might also contribute to the low cortisol concentrations in

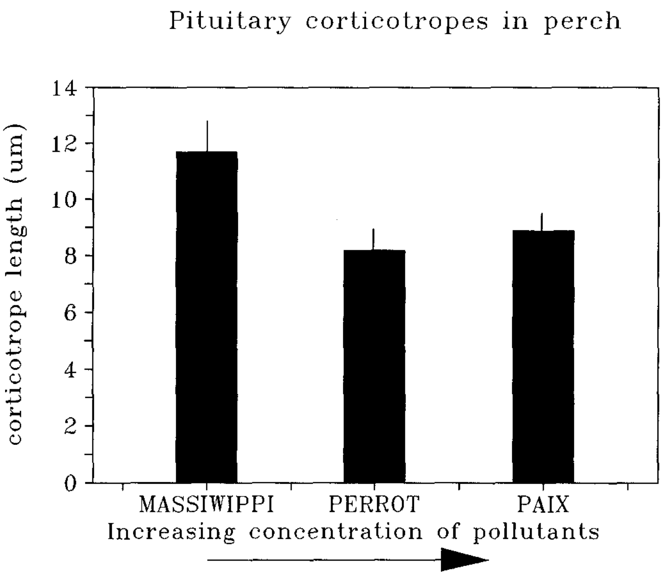

Fig. 3. The corticotropes were shortest (mean length ± SE; $p \leq 0.05$) in perch from the two more contaminated sites, fish which were also unable to elevate their serum cortisol in response to the acute stress of capture

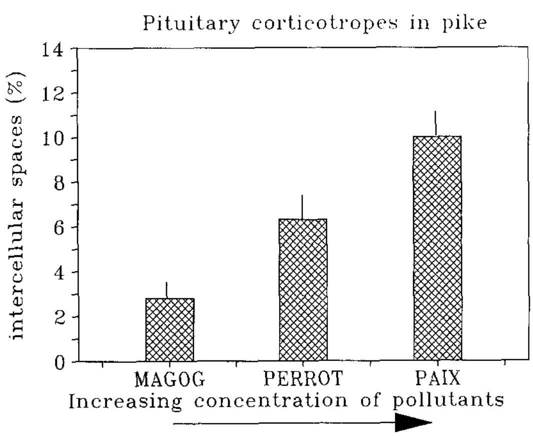

Fig. 4. Intercellular spaces between the pike corticotropes were quantified as % of the total corticotropic area. The largest intercellular spaces (mean ± SE) were observed between corticotropes of pike from the most contaminated site ($p \leq 0.05$), fish unable to elevate their serum cortisol in response to capture

fish from polluted sites. Specific hepatic enzymes, induced by some contaminants, alter metabolism and excretion of sex steroids (Hansson *et al.* 1980; Forlin and Haux 1985; Munkittrick *et al.* 1991). These enzymes, also referred to as the mixed function oxidase (MFO) system, may influence the metabolism of corticosteroids as well. The relationship between cortisol, the MFOs and pollutants is being presently investigated by our group. Endocrine parameters, through their integrative role in the maintenance of homeostasis, are emerging as useful diagnostic tools in the detection of early or low level responses to pollutants (Hontela *et al.* 1989, 1991); responses which may precede the onset of pathologies and mortality.

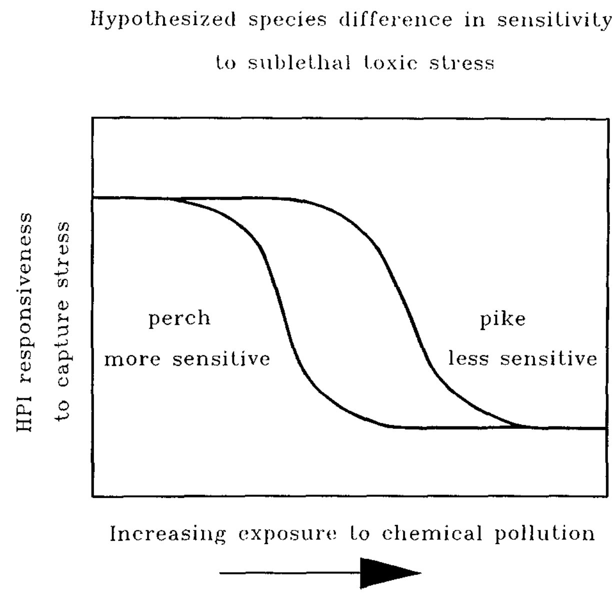

Fig. 5. Both species exhibited the greatest responsiveness of the HPI axis to the stress of capture (elevated serum cortisol, large corticotropes) at the least contaminated sites while reduced responsiveness was displayed at the most contaminated sites. The HPI axis of perch appears more sensitive than that of pike to the chronic effect of the pollutants since the maximum reduction of responsiveness was detected even at the intermediate site in the perch

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