The use of selected physiological parameters as indicators of polychlorinated biphenyl exposure of rainbow trout, *Oncorhynchus mykiss*.

Tim Cano
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Abstract

Hatchery reared rainbow trout (*Oncorhynchus mykiss*) were caged at two locations in the St. Lawrence river near Cornwall, Ontario. One cage was anchored in an area of high polychlorinated biphenyl (PCB) contamination, within an iron enclosure near the General Motors Foundry at Massena, N.Y. during a dredging operation designed to remove PCB contaminated sediments from the river. A second control cage was placed in the Snye channel, known to have low PCB concentrations. Hatchery fish were sampled to provide a time 0 control, and fish from each site were collected following 21 and 41 day exposure. Plasma, muscle, bone, and liver were harvested and assayed to determine muscle PCB levels, plasma cortisol, electrolyte, lactate and glucose concentrations, bone calcium, muscle water and the activity of a variety of liver enzymes. Total PCB levels were significantly higher in fish muscle from the General Motors site but few differences were found in any of the physiological parameters. Levels of bone calcium decreased in fish exposed to high PCB levels, suggesting an effect of PCBs on either the thyroid or ultimobranchial bodies. Pyruvate kinase activity in the liver of PCB exposed fish suggests a shift in energy metabolism to a greater reliance on glycogenolysis. Both parameters require further study to determine the feasibility of their use as bioindicators of PCB stress.

Introduction

In 1986, in response to growing public concern about the state of the St. Lawrence River, government actions on both the Canadian and United States' sides of the border resulted in the inauguration of the St. Lawrence (Cornwall) and St. Lawrence River at Massena Remedial Action Programs (RAPs). The goal of the two cooperative RAPs was to remedy and to address pollution of the river by sources located on the watershed, including Lake Ontario and in particular those on the St. Lawrence River itself. The RAPs were designed in three stages: (1) a detailed assessment of problems and their causes (2) examination and recommendation of the best options to rectify the problems identified in stage 1, and (3) implementation of the recommendations from stage 2 and monitoring of subsequent environmental changes to determine the efficacy of the actions. The problems addressed by the RAPs are numerous and include the assessment of contamination by mercury, polychlorinated biphenyls (PCBs) and other pollutants in sediments, within the water column itself and in the biota including fish.

Because of human health concerns associated with the ingestion of contaminated fish, mercury contamination of the St. Lawrence River between Cornwall, Ontario and Valleyfield, Quebec was identified as a problem as early as the late 1960s. Mercury contamination results largely as a byproduct of industry which discharges this toxic substance directly into the water system. The mercury can then either disperse within the water column or be associated with particles and settle into the sediment. Bacteria living within the sediment layer can mobilise and recycle mercury back into the water column as methyl mercury which, in this form, can move into the food chain by being incorporated

into plant life. Ultimately, as a result of biomagnification, mercury accumulates in the body tissues of the top carnivores. The resulting high concentration of mercury in fish has resulted in the restriction of the consumption of both sportfish species and some commercially important fish species such as the American eel (*Anguilla rostrata*), carp (*Cyprinus* sp.) and white sucker (*Catastomus commersoni*). In the Cornwall area, the main sources of mercury pollution are ICI Forest Products, Domtar Fine Papers, Cornwall Chemicals, and the Cornwall sewage treatment plant (St. Lawrence RAP report, 1994). Though the persistent historic mercury pollution of the river sediment and water column must be addressed, measures to reduce or eliminate present mercury emissions by industry must also be undertaken.

Similarly, PCB contamination was identified in the river, but in this instance the primary polluters are in the United States. In particular, the General Motors Foundry, ALCOA and Reynolds Metals discharge PCB contaminated effluent directly into the river (St. Lawrence RAP report, 1994). Additionally, PCBs are suspected of leaching from waste dumps on various industrial properties. For one, high sediment contamination was identified near the General Motors plant in the United States, where levels as high as 13.8 µg PCB/ g sediment were measured. In comparison, the highest Canadian concentration, 1.0 µg PCB/ g sediment, was found at the Courtauld Fibres site near Cornwall (St. Lawrence RAP report, 1994). Fortunately, the U.S. Environmental Protection Agency (EPA) allocated money to facilitate the clean up of contamination at these sites. One such project is the dredging near the General Motors plant at Massena, New York, to remove PCB contaminated sediments.

PCBs belong to a chemical family containing more than 200 similar compounds. They do not degrade easily, are resistant to heat and are miscible in water but not in oil. Partially for these reasons, PCBs are a valuable industrial product and are used in a variety of ways, including as coolants in transformers, in inks, paints and fire retardants (Government of Canada, 1991). In the 1970s, however, it was determined that PCBs persisted and accumulated in the environment and became very toxic at high concentrations. Because of this toxicity, their use was greatly curtailed.

Biomagnification occurs throughout the food chain, and results in high and even toxic concentrations of PCBs in fish. In the St. Lawrence River, the Cornwall-Massena area is known to have PCB levels greater than those proposed as safe by the Ontario government (<1 ng/L) (St. Lawrence RAP report, 1994). Bottom sediments can provide a sink for PCBs but these can be remobilised by the general scouring action of the river current and are resuspended during maintenance dredging of the navigational channel. Similar to mercury, high PCB levels resulted in the restriction, and occasionally the closure, of commercial fishing for various species including both the eel and the carp. Clearly, action is required to remove the PCBs and, of equal importance, to prevent further contamination.

Remedial action to reduce mercury and PCB contamination and pollution are not the sole aim of the St. Lawrence RAPs. There are a number of other contaminants of concern that require attention to improve water quality and habitat. Organic pollutants, including phenolic compounds, oils, greases, dioxins, furans, polyaromatic hydrocarbons and pesticides also result in a variety of detrimental effects on animal populations in the

St. Lawrence River (St. Lawrence RAP report, 1994). But compared to the effects of mercury and PCBs, the impact of these pollutants is minimal. In the Cornwall-Massena area, contamination of sediments by metals is of greater concern. For example, in 1985, levels of zinc, lead, copper, chromium, nickel, arsenic and cadmium were judged to be toxic. Fortunately, Courtauld Fibres, the source of most of this metal pollution, ceased operations. However, the problem of how to deal with the severely contaminated sediments still remains.

The St. Lawrence RAPs must be concerned not only with direct human inputs into the environment, but also with indirect effects of human activities that can reduce and even destroy available wildlife habitat. Of primary concern is the maintenance and protection of the wetland habitats, which are extremely important for use by animals. Additionally, viable wetlands improve water quality, reduce erosion and control flood events. Human activities such as dredging, filling to provide land for use in agriculture and housing, and human recreational activities all contribute to the destruction of these valuable wetland areas and reduce their value to the ecosystem. Alteration of the hydrological cycle of a wetland, such as by damming, also results in its degradation. The creation of a navigation channel by dredging results in changes in water flow, reduces the flow in nearshore areas and adversely affects species richness. Though considerable damage already has been done, the situation is not beyond redemption. But it is important to act quickly in order to preserve the remaining wetlands along the St. Lawrence and this will prevent further development that may have deleterious effects on the river system.

To address this problem, a multidisciplinary study was undertaken under the auspices of the Institute for Research on Environment and Economics (IREE, University of Ottawa). The study draws upon the skills of various researchers, including geologists, chemists and biologists, to provide a detailed picture of the present state of the St. Lawrence River. Exhaustive examination of ecosystem variables is being performed, including both sediments and water for chemical pollution and species richness of invertebrates. Studies on population dynamics and health are also underway. The principle aim of this study is ultimately to provide an accurate picture upon which to base recommendations for the remediation of the St. Lawrence River.

The St. Lawrence River has been, and continues to be, a site of intense industry and recreation and it is now clear that each of these activities have had detrimental effects on the overall quality of the system. But the continual discharge of effluent from industrial plants into the river has probably had the greatest negative impact as this activity produces high pollutant loads and some extremely toxic point sources have produced general deleterious effects on the resident flora and fauna. Indeed, the increased pollutant burden has resulted in numerous effects throughout the ecosystem. Perhaps the most dramatic effect is tumours found in populations of St. Lawrence Beluga whales (De Guise *et al.*, 1994), possibly occurring as a result of ingestion of eels highly contaminated with PCBs (Hodson *et al.*, 1994). Remedial action is required to restore the St. Lawrence River to a productive and healthy state. To undertake such action, it is first necessary to quantify the degree of strain being imposed on the system. However, complete quantification is impractical. Instead, one must look for easily measurable and reliable bioindicators. The

present study was initiated to determine if the measure of various parameters in fish might provide such indicators.

In determining the health of fish populations, changes in various biological, including such physiological parameters as histological changes in the pituitary of pollutant exposed fish (Hontela *et al*, 1992), can be used as indicators of the health of the community in general. This quantification is of primary importance to the Mohawk people at Awkwesasne, near Cornwall, Ontario. Historically, the river was an important source of food to the Mohawk people, and the degradation of water quality has resulted in a loss of this resource. Further, recent interest in aquaculture makes it necessary to ascertain whether or not the river can support this type of industry, and especially if farmed fish will be suitable for human consumption.

As part of a larger research program being conducted by the IREE, this particular study was designed to explore the physiological status of fish exposed to conditions in the St. Lawrence at a site which is highly polluted with PCBs (Kadlec, 1994). The rainbow trout, *Oncorhynchus mykiss*, because of its wide availability, well understood physiology and commercial importance, was chosen as a model animal. A second goal of this study was to evaluate the feasibility of aquaculture by measuring and comparing a variety of physiological indicators including liver enzymes, hormones, plasma variables and histological parameters of the gill among fish held in highly polluted areas, fish held in significantly less polluted areas, and fish which had not been so exposed.

Polychlorinated biphenyls, manufactured until the 1970s primarily for industrial uses, persist in the environment and are known to be detrimental throughout the life cycle

of teleost fish. This is most noticeable at highly contaminated sites, since PCB concentrations and their associated effects both correlate negatively with the distance from the pollution source (Stow, 1995). In particular, chronically exposed Northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) exhibited atrophy of the corticotropes which release the adrenocorticotropic hormone which in turn stimulates release of the stress hormone cortisol. This atrophy, which may be the result of chronic hyperactivity, certainly correlated with their inability to raise cortisol levels in response to the acute stress of either capture or handling. In the wild, such a deterioration of the stress response would likely render the fish less able to cope with the natural physical rigors of their environment (Hontela *et al*, 1992). Further, this atrophy may result as a response to PCB accumulation, because it is known that lipophilic chemicals tend to remain in the body for longer periods than those which are lipophobic (McKim and Erickson, 1991). As one example of this, lake trout, *Oncorhynchus namaycush*, displayed an increased body PCB burden which was dependent on the time of exposure to PCB polluted waters (Madenjian *et al*, 1994).

Diet is also known to have a profound effect on the concentration of PCBs in body tissues. For instance, Stow (1995) observed that PCB levels measured in Lake Michigan salmonids correlated well with populations of alewife, a forage fish upon which they feed and which mobilises PCBs through the food chain. Conversely, rainbow trout, which consume a more diverse diet than do lake trout, exhibit lower PCB levels (Stow, 1995). Though PCBs at levels normally found in the environment are insufficiently toxic to increase acute mortality in adult fish (Williams *et al*, 1992), the toxic effect of PCBs on

immature fish is well documented. For instance, Harris *et al* (1994b) injected Japanese medaka (*Oryzias latipes*) eggs with extracts derived from Lake Ontario rainbow trout and observed a reduction in swim bladder inflation and an increase in mortality at the swim up stage when yolk absorption occurred. At this time, lipophilic compounds are rapidly incorporated into the body. Injection of the PCB fraction resulted in tissue lesions, a reduction of blood flow in the caudal vein and internal hemorrhaging. Additionally, exposure of either red mullet, *Mullus barbatus* or scorpion fish, *Scorpaena porcis* L. to PCB contaminated water increased the PCB levels in both their muscle and liver tissue, and the degree of contamination correlated positively with an increase in the activity of various antioxidant enzymes such as peroxidase, glutathione, carotenoid and vitamin A, E and K levels (Rudneva-Titova & Zherko, 1994).

For the purpose of this thesis, stress will be defined as a condition of disturbed homeostasis under the influence of an environmental factor, the "stressor" (Chrousos and Gold, 1992). An animal's response to stress can be described by the General Adaptation Syndrome (GAS) (for review, see Barton and Iwama, 1991). This model states that the stress response can be divided into three distinct phases: the alarm reaction, adaptation and exhaustion. During the alarm reaction an animal undergoes a rapid alteration of the physiological state which leads to a higher state of resistance to the stress. For example, and often typically, cortisol is released in response to an acute stress such as handling (Pankhurst and Dedual, 1994) and is the primary hormone associated with this portion of the GAS. The second stage of the GAS is adaptation to the stressor by the organism, and a return to the homeostatic condition. This second stage was demonstrated by Laidley

and Leatherland (1988) who observed a return of cortisol in rainbow trout to control levels after an initial increase in response to crowding stress. The final stage is exhaustion, the point at which the organism can no longer cope with the stressor and is unable to maintain homeostasis. This stage has been associated with both severe and chronic stresses to which the animal is unable to respond further. Animals at this stage of the GAS may demonstrate increased mortality either as a result of the exhaustion of the stress-response mechanisms or even as a direct result of the continued action of this system. For example, prolonged elevation of cortisol in the brown trout, *Salmo trutta*, can result in immunosuppression and increased mortality as a result of infection (Pickering and Duston, 1983).

Cortisol, a corticosteroid hormone, is released in fish from the interrenal tissue (homologous to the adrenal cortex in mammals) in response to stress. The ability to raise cortisol levels was observed in juvenile rainbow trout, as early as 5 weeks post hatching at a point known as the 'critical period' during which high mortality occurs and the success of the age class is determined (Pottinger and Mosuwe, 1994). The release of cortisol in teleost fish, as in other vertebrates, is mediated by the hypothalamo-pituitary-interrenal (HPI) axis. Corticotropin releasing factor (CRF) from the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the corticotropes in the anterior pituitary gland and ACTH in turn stimulates the release of cortisol from interrenal cells. Basal levels of circulating cortisol in salmonids reported in the literature range from 0-5 ng ml^{-1} (Pickering and Pottinger, 1989) to 16.4 ng ml^{-1} (Nielsen *et al*, 1994), though the latter value may be artefactually high due to sampling stress. Under normal circumstances,

the secretion of cortisol is controlled by a negative feedback loop of the hormone on the HPI axis (reviewed by Barton and Iwama, 1991). Cortisol in the blood acts upon the pituitary gland to prevent the further release of ACTH, which in turn, reduces circulating cortisol.

Teleost fish release cortisol in response to a wide variety of physical stressors including confinement (Pankhurst and Dedual, 1994), ionoregulatory disturbance (Greco *et al*, 1995) and capture (Hontela *et al*, 1992). Indeed, this response has been used as an indicator of stress in a wide variety of teleost fish, including the snapper, *Pagrus auratus* (Bollard *et al*., 1993), striped bass, *Morone saxatilis* (Hopkins and Cech, 1992), channel catfish, *Ictalurus punctatus* (Tomasso *et al*, 1981) and many salmonids [chinook salmon, *Oncorhynchus tshawytscha* (Gadomski *et al*, 1994) and rainbow trout (Brown *et al*, 1986; for review, see Pickering and Pottinger, 1989)]. However, these data must be carefully assessed as the cortisol response may be induced by the stress of blood sampling itself (Nielsen *et al*, 1994). But this does indicate the sensitivity of this parameter.

Stress, and the associated increase in cortisol levels, also affects the reproductive success of teleost fish. Campbell *et al* (1992) observed multiple effects of emersion (denoting draining and refilling of water in experimental tanks) stress on the gamete quality of the rainbow trout. Stressed females, whose cortisol levels were raised to 30 ng ml^{-1}, demonstrated delayed ovulation, ovulated for shorter periods of time, and produced eggs of a reduced size and weight than nonstressed control fish. Males subjected to similar stress had reduced sperm count and the progeny of stressed fish demonstrated a decreased survival rate, primarily at the time of hatching.

The present study was designed to test a variety of physiological parameters in order to find which of them might function as useful bioindicators of PCB toxicity in the rainbow trout. To be designated as an effective bioindicator, several criteria were set: (1) it must be easily measured, (2) it must be reflective of the degree of stress, and (3) it must be consistent. Because there was evidence that cortisol, glucose, lactate and various blood electrolytes seemed to meet the criteria, they were selected. Similarly, gill tissue is easily obtained and can be examined microscopically to detect variations in histology. Both bone calcium and muscle water content may be affected by pollution stress and were also quantified. It was hypothesized that one or more of these parameters would prove to be useful bioindicators in spite of the fact that each had specific deficiencies.

In long term studies of stress, cortisol levels may not be fully representative of the state of stress due to the possibility of changes in the metabolic clearance rate of the hormone or the downregulation of its receptors. This is evident in Northern pike, because when they were exposed to high levels of polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), or mercury, Hontela *et al* (1992) found that cortisol levels did not rise in response to capture by angling. This result is consistent with the hypothesis that the constant exposure to pollutants resulted in the observed atrophy of the corticotropes, possibly as a result of exhaustion due to prolonged hyperactivity. Long term stress may also result in an increase in the metabolic clearance rate (MCR) of cortisol from the body. Rainbow trout, showed an increase in MCR after prolonged periods of exercise (Nielsen *et al*, 1994) and the Atlantic salmon, *Salmo salar*, demonstrated increased MCR during seawater acclimation, although the physiological basis of this response is not known

(Nichols and Weisbart, 1985). A reduction in the number of receptors for cortisol was observed in stressed chinook salmon (Shrimpton and Randall, 1994), possibly resulting in a mediation of the effects of increased cortisol levels. Thus, the use of levels of plasma cortisol may be misleading and an increase in MCR (Brown *et al*, 1986) or a down regulation of cortisol receptors (Shrimpton and Randall, 1994) must be considered as a possible explanation for low cortisol levels in fish known to be stressed.

The effects of a chronic stress, that which poses a continuous challenge to the fish and may result in illness or death (Pickering *et al*, 1982), are numerous. In teleost fish, these effects can include an increase in muscle water content probably as a result of cortisol mediated catabolism of skeletal muscle via gluconeogenesis (Pickering and Duston, 1983; Redding *et al*, 1991; Van der Boon *et al*, 1991). This loss of muscle mass could result in a decrease in predator avoidance ability as seen in chinook salmon (Mesa, 1994) or feeding capacity as described in brown trout (Pickering *et al*, 1982). Both the brown trout (Pickering and Duston, 1983) and the rainbow trout (Pickering *et* al, 1991) exhibited increased mortality in response to increased cortisol levels. Brook charr, *Salvelinus fontinalis*, stressed by soft acidic water demonstrated increased mortality resulting from ionoregulatory and respiratory dysfunctions (Conklin *et al*, 1992).

Increased plasma cortisol may cause an increase in the lactate level of the blood, though observations are inconsistent. Gadomski *et al* (1994) observed no correlation between lactate and cortisol levels in rainbow trout whereas cortisol injections in the snapper resulted in increased lactate levels (Bollard *et al*, 1993). These conflicting results may occur due to the rapid removal of lactate from the tissues by oxidation, conversion to

glucose or excretion (Pickering *et al*, 1982). Nevertheless, lactate may be a useful indicator of physical disturbances. For example, in the brown trout, emersion resulted in an increased level of lactate, likely due to an extreme exercise level (Pickering *et al*, 1982). A similar observation was made in the rainbow trout, which raised blood lactate in response to as little as a 5 minute handling stress (Pankhurst and Dedual, 1994). Though lactate may not be a reliable indicator of stress, it could prove to be a useful bioindicator of physical disturbance.

The use of blood glucose levels as indicators of stress poses similar problems. Glucose levels increased for periods of up to 72 hours in brown trout (Pickering *et al*, 1982) and to 300 minutes post stress (Casillas and Smith, 1977) and as a secondary effect of stress (Laidley and Leatherland, 1988) in rainbow trout. Barton *et al* (1987), however, observed that cortisol fed juvenile rainbow trout showed no change in glucose levels, and suggested that catecholamines may be involved in mediation of the hyperglycemic response. These results agree with work by Nielsen *et al* (1994), who found glucose levels to be extremely variable in rainbow trout. Stressed chinook salmon also exhibited no difference in glucose levels as compared to control fish (Gadomski *et al*, 1994). It has been suggested that cortisol secretion mediates gluconeogenesis from proteins in either the short or long term (for review, see Van der Boon *et al*, 1991), which may cause difficulties in the use of glucose as a bioindicator.

Because of the difficulties associated with the use of both lactate and glucose levels as indicators of stress, examination of the factors affecting cortisol induced gluconeogenesis was deemed to be more promising. Gluconeogenic liver enzymes, for

example, may be more stable in the short term and thus have greater predictive value. This is supported by the observations of Vijayan *et al* (1991), who described a cortisol induced increase in gluconeogenesis in the brook charr by demonstrating an increased activity of fructose bis-phosphatase (FBPase). Instead, it was proposed that cortisol may have a role in the mediation of glycerol utilisation in glucogenesis. This is further supported by the absence of changes in levels of pyruvate kinase (PK), glutamate dehydrogenase (GDH), glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) in stressed brook charr. Increased phosphofructokinase (PFK) activity indicated that liver glycogen was mobilised (Vijayan *et al*, 1990). However, Foster and Moon (1986), using hepatocyte preparations derived from cortisol injected American eels, observed an increase in both PEPCK and GOT activity, suggesting that gluconeogenesis shifted to use of lactate as a preferential substrate as opposed to amino acids. Metabolic depression was also observed. Similar conflicting findings are reported elsewhere. Cortisol treatment is known to induce GOT, but reports on its effect on GPT activity and liver glycogen are inconsistent (for review, see Van der Boon *et al*, 1991). Activity of lactate dehydrogenase (LDH) was also shown to decrease after cortisol injection in American eels (Foster and Moon, 1986). Moreover, PCB exposure in the Japanese medaka resulted in a reduction of both LDH and carbonic anhydrase activity, causing increased mortality as a result of failure of swim bladder inflation (Harris *et al*, 1994a). These enzymes, then, may be more reliable bioindicators than the products of the metabolic pathway in which they operate.

Stress also reduced growth in fish and this is characteristically associated with increased plasma cortisol; in the teleost fish, the stress response results in muscle catabolism (Van der Boon *et al*, 1991). Rainbow trout subjected to stress showed higher activity of the HPI axis concomitant with a reduction in circulating levels of growth hormone in the plasma (Pickering *et al*, 1991). Peritoneal cortisol implants in the brook charr resulted in a reduction of feeding; this may be a maladaptative response whereby body reserves are utilised for maintenance of function, even when food is available (Vijayan *et al*, 1990). Pickering and Duston (1983) demonstrated that, in brown trout, cortisol in the diet resulted in a reduction of growth as a result of increased catabolism. Food absorption processes are less efficient in the rainbow trout as a result of increased cortisol levels, possibly as a result of morphological changes in the cardiac stomach. Increased fecal matter was also seen in experimental tanks, perhaps indicating a reduction in food digestibility or conversion (Barton *et al*, 1987).

Stress also changes various other blood parameters. In rainbow trout, stress increased hematocrit and decreased blood clotting time (Casillas and Smith, 1977). Changes in blood electrolyte levels are also associated with stress. Both Gadomski *et al* (1994) and Nielsen *et al* (1994) demonstrated an increase in plasma potassium levels in rainbow trout after stress, but this may have resulted from excessive leakage from the active muscle tissue. Changes in plasma sodium levels were also noted in stressed rainbow trout (Gadomski *et al*, 1994; Laidley and Leatherland, 1988). These results are indicative of temporary ionic disturbances associated with an increase in muscle activity, alteration of ion flux, or both (Laidley and Leatherland, 1988).

Teleost fish exhibiting either induced or artificially raised cortisol levels also show a marked suppression of the immune system and salmonid fish become more susceptible to disease causing pathogens when the fish are exposed to environmental stress (Pickering, 1993). Most significant to the present study, when rainbow trout are exposed to sublethal PCB levels they demonstrate a reduced capacity to produce antibodies against the bacteria *V. anguillarum* (Thuvander and Carlstein, 1991). Further, cortisol implants in brown trout, even though producing only slightly elevated cortisol levels (~10 ng ml^{-1}), caused an increase in mortality from furunculosis, *saprolegnia* infection and bacterial fin rot (Pickering and Duston, 1983; Pickering and Pottinger, 1989). Similarly, wild blue mao mao (*Scorpis violaceus*), captured and confined for a period of four months, became more susceptible to parasitic infection as a result of the stress of confinement and concomitant increase in plasma cortisol levels (Pankhurst *et al*, 1992). The demonstrated immunosuppression may be a result of stress mediated reductions in the number of circulating lymphocytes post stress, as seen in stressed brown trout (Pickering, 1984).

Similar maladaptive changes occur in the gill, mainly affecting the population and surface area of chloride cells, as a response to a wide variety of stresses and related increases in cortisol titers. Intramuscular injections of cortisol in rainbow trout increased the fractional surface area of chloride cells on the gill filament epithelium by increasing both the number and the size of the cells (Laurent and Perry, 1990). Chloride cell population is also increased by toxicants which cause an increased ion loss or compromised ion uptake across the gill epithelium (Laurent and Perry, 1991). Juvenile brook trout exposed to soft, acidic water exhibited an increase followed by rapid

degeneration of chloride cell population. In addition, decrease in length of both the filaments and lamellae and an increase in filamental fusions was observed (Conklin *et al*, 1992).

Such changes in the populations of chloride cells can result in respiratory dysfunction and an inability to adapt to reductions in water oxygen content. Bindon *et al* (1994) showed that rainbow trout injected with growth hormone and cortisol demonstrated an increase in chloride cell number which was associated with acidosis, probably as a result of an increased partial pressure of carbon dioxide resulting from impeded interlamellar water flow across the gill surface. A similar decrease in water flow across the gills as a result of increased lamellar size and thickness was reported by Greco *et al* (1995b). Softwater acclimated rainbow trout increased ventilation by 36% during normoxic conditions (Greco *et al*, 1995a), likely as blood to water diffusion distance was increased by approximately 2 times, impairing gas transfer (Greco *et al*, 1995b). Such an observed proliferation in chloride cell number and fractional area, which can be induced by cortisol, may be maladaptive, causing impaired gas transfer and reducing fitness of fish. The importance of these morphological changes is that they occur slowly enough that they are not affected by the immediate stress of collection.

It is known that exposure to PCBs results in a variety of stresses on the physiology of teleost fish and that many responses to stress are mediated by the release of cortisol. Thus, it is reasonable to hypothesize that, in response to the presumed stress of PCB exposure, the rainbow trout will release cortisol, which in turn will affect blood glucose, lactate, electrolytes, muscle water, bone calcium and gill histology. Though changes in

cortisol release are extremely sensitive to stress, occurring very quickly and rendering the hormone itself of little use as a bioindicator in general, and in wild fish in particular, secondary affects associated with its release may therefore be more reliable and prove to be valuable bioindicators of PCB stresses in the wild. This would make them very useful tools in remedial action programs. The primary purpose of this study was to explore this possibility.

Materials and Methods

Experimental Animals

Sexually immature federal strain rainbow trout, *Oncorhynchus mykiss*, weighing 150-200g, were obtained from the Hinchenbrooke Trout Farm (Chateauquay, N.Y.) and caged at two locations in the St. Lawrence River, in either the Snye channel near Yellow Island (low PCB contamination) or near the General Motors Foundry (high PCB contamination) at Massena, New York (Fig.1). At the latter site, fish were caged within an iron enclosure designed to contain PCB contaminated sediments suspended in the water column as a result of dredging operations.

The fish were enclosed in 1m x 1m x 2m cages constructed of 1 cm x 1 cm plastic mesh. The cages were kept afloat by a framework of PVC pipe to which the mesh was attached. A flap was cut in the top of the cage to facilitate fish removal and was wired shut except during sampling. The cage at the General Motors site was attached directly to the metal enclosure wall whereas the cage at the Snye site was anchored to the river bottom with cinder blocks. Fish were fed daily with a commercial pellet diet from day 0 to day 31. Because access to the cage at the General Motors site was restricted after day 31, fish were not fed at either site after this time.

Fish were sampled at 0, 21 and 41 days. Animals were removed from the cages and immediately killed by a sharp blow to the head. Approximately 1 ml of blood was removed via caudal puncture with a heparinised 23 gauge needle into a heparinised syringe, immediately transferred to heparinised 1.5 ml centrifuge tubes which were placed on ice for approximately 30 minutes until centrifugation to facilitate plasma separation.

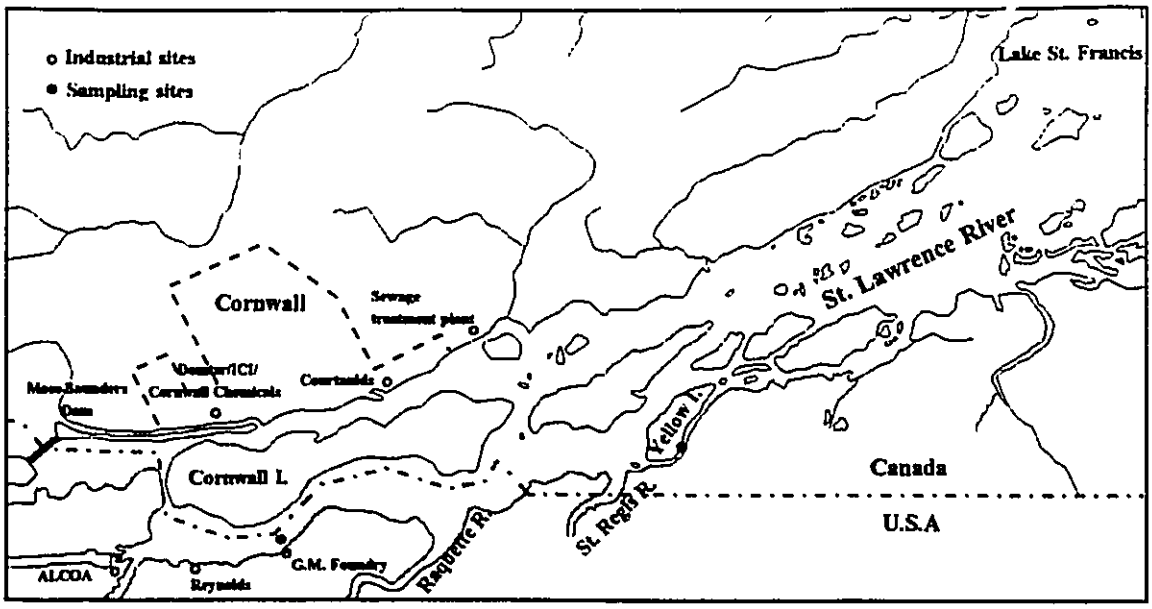


Fig. 1. Location of sampling sites

Livers were then removed and placed in liquid nitrogen until they could be stored in an ultra-low deep freezer at -80° C.

Preliminary processing of gills for scanning electron microscopy was performed as described by Greco *et al* (1995b). The second gill arch on the left side of each fish was removed, rinsed in ice cold 0.9% saline solution to remove excess blood and mucus and fixed for 12 hours in 5% glutaraldehyde (w:v) in 0.15 M sodium cacodylate buffer solution. Carcasses less livers, part of the gills, and about 1 ml of blood were weighed, placed in numbered bags on ice and returned to the laboratory in Ottawa, Ontario. Fish were weighed last in order to minimise any undue effects on physiological parameters.

Sample Analysis

Bone Calcium

Bone calcium was measured following the procedure of Fenwick *et al* (1994). The left side of the jaw was removed from the carcass and attached skin and tissue was scraped from the bone. The bone which remained was dried in an oven at 70° C for 24 h, then weighed and digested overnight in 1 ml of concentrated nitric acid (BDH, Toronto, Ontario). After digestion was complete, 10 ml of 65 mM lanthanum chloride was added as a quenching agent and the calcium concentration of the resulting solution was determined by atomic absorption spectrophotometry using a Varian SpectrAA 250 plus. Bone calcium in mg/g dry bone was calculated using the formula:

$$\frac{[Ca^{2+}] \text{ x dilution factor x molecular weight of calcium}}{\text{dry weight of bone}}$$

Muscle Water Content and PCB analysis

Muscle samples weighing about 3g were taken from the epaxial white muscle on the left side of the fish near the dorsal fin, weighed and dried in an oven at 60° C for 72 hours. Dry muscle weights were recorded and percent muscle water content was determined. A second sample of epaxial white muscle from the right side of the fish near the dorsal fin was removed, wrapped in aluminum foil, packed in dry ice, and sent to the Wadsworth Center, Albany, N.Y to determine PCB levels. PCB analysis for 70 congeners was performed by B. Bush and A.C. Casey using high resolution glass capillary gas chromatography as described by Bush *et al* (1989). PCB levels were determined in parts per billion (PPB) wet weight of tissue.

Plasma Parameters

Plasma was thawed and cortisol was determined using a commercially available radioimmunoassay (Immunocorp Sciences, Montreal, Canada) modified to facilitate measurement of cortisol in rainbow trout (Andersen *et al*, 1991). Fifty microliters of both plasma and cortisol standards were deproteinised in ice cold 8% perchloric acid (PCA; 2:1 w:v). Tubes were vortexed for 30 seconds, neutralised with 15 µl of potassium carbonate, placed on ice for 5 minutes and then centrifuged at 12000g for 5 minutes. The remainder of the assay was performed using the resultant supernatant and measured on a Packard Cobra autogamma counter.

Plasma for lactate determination was deproteinised following the same method as used for cortisol and measured using a colorimetric assay (Sigma, Cat. No. 826-UV, St. Louis, Missouri) based on the following reaction:

$$\text{lactate} + \text{NAD} \xrightarrow{\text{lactate dehydrogenase}} \text{pyruvate} + \text{NADH}$$

and calculating concentration based on changes in the extinction coefficient of NADH at 340 nm.

Glucose was measured using a similar colorimetric commercial assay (Sigma, Cat. No. 16-UV, St. Louis, Missouri) based on the reactions:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate (G-6-P)} + \text{ADP}$$

and

$$\text{G-6-P} + \text{NAD} \xrightarrow{\text{G-6-P dehydrogenase}} \text{6-PG} + \text{NADH}$$

Glucose concentration was determined based on changes in the extinction coefficient at 340 nm. Both assays were performed using a Beckman DU 640 spectrophotometer.

Plasma was diluted 150 times in deionised water and plasma calcium was measured using flame atomic absorption (Varian SpectrAA 250 plus). To measure potassium and sodium, plasma was diluted by a factor of one thousand and measured using flame emission photospectrometry. Plasma chloride was measured using a colorimetric assay on a Milton Roy Spectronic 1001 plus spectrophotometer as described by Zall *et al* (1956).

Liver Enzyme Analysis

Frozen liver pieces weighing approximately 0.75 g were placed in a mortar, covered with liquid nitrogen and then ground frozen to a fine powder. A stopping buffer

[50 mM imidazole, 15 mM β-mercaptoethanol, 100 mM KF, 5mM EDTA, 5mM EGTA, 0.1 mM PMSF (dissolved in EtOH), 20% glycerol and 10 mM P_i (as K_2HPO_4)] was added (3:1, v:w) to prevent enzyme alteration. The tissue was then homogenised using a Brinkman polytron tissue homogeniser, centrifuged for 5 minutes at 22,500 x g in a Sorval RC5C centifuge at 4° C and the supernatant was frozen in 300 µl aliquots at -80° C.

Liver homogenates were analysed for the activity of lactate dehydrogenase (LDH), pyruvate kinase (PK), phosphoenolpyruvate carboxykinase (PEPCK) and glutamate-pyruvate transaminase (GPT) as well as for glycogen content. Prior to analysis for PK, homogenate samples were placed on a 1 ml column of Sephadex G-25 and centrifuged at speed 4 in a clinical centrifuge for 2 minutes to remove any small molecules which may act to inhibit or activate the enzymes.

All enzyme activities were analysed for kinetic properties using a Ceres UV 900 HDi plate spectrophotometer at room temperature (21° ± 1° C) and a 340 nm filter every 10 seconds for a period of 10 minutes. Final well volumes in all cases were 200 µl + 20 µl homogenate. Assay conditions were optimised for substrate to achieve maximum activity of each enzyme and concentrations were as follows:

LDH - 7.33 mM pyruvate and 0.15 mM NADH.

PEPCK - 3.95 mM $MnCl_2$, 0.8 mM deoxyguanosine diphosphate, 7.9 mM $NaHCO_3$, 4 units/ml glycerol free malate dehydrogenase (MDH), and 20 mM phospho(enol) pyruvate (PEP).

GPT - 22.6 mM α - ketoglutarate (α-KG), 35.2 units/ml lactate dehydrogenase (LDH), 440 mM L-alanine, and 0.15 mM NADH.

PK - 97.5 mM KCl, 9.75 mM $MgCl_2$, 2.5 mM ADP, 20 units/ml dialysed LDH, 5 mM PEP, and 0.15 mM NADH.

Protein content of liver homogenates was determined using the colorimetric bicinchonic acid protein assay described by Smith *et al* (1985). All samples were compared against a standard curve based on bovine serum albumin (Sigma, St. Louis, Missouri) and measured on a Ceres 900 HDi plate reader. Maximum activity (µmol/min/mg tissue) of each enzyme was calculated for each treatment group.

A 200 µl sample of liver homogenate for glycogen analysis was taken and frozen before centrifugation. Later, the sample was thawed, and a 100 µl aliquot was incubated at 40° C for 2 hours in a Acme shaking waterbath set at 380 speed with 1 ml of amyloglucosidase (1 mg/ml). Liver glycogen was calculated based on the difference in glucose concentration between homogenate and supernatant samples.

Gill Histology

Gill tissue was prepared for SEM examination of chloride cell fractional area as described by Greco *et al* (1995b). After 12 h, gills were transferred to a 0.15 M sodium cacodylate buffer solution until post fixation in unbuffered 1% osmium tetroxide for 1 h. Following post fixation, cartilaginous material was removed from the gill arch using a scalpel. Pairs of gill filaments were removed from the arch, dehydrated in a series of ethanols, bathed in 1,1,1,3,3,3-hexamethyldisilizane (Aldrich, Milwaukee, Wisconsin) and air dried for 24 h. Filament pairs were attached to stubs compatible with a Phillips 500 scanning electron microscope using silver paste. Filaments were examined using scanning

electron microscopy at approximately 1000 times magnification and four photographs from random filaments were taken per fish at the point where the lamellae and filament meet. Chloride cell area was measured by using a digitising tablet and tracing the perimeter of both the individual chloride cells and the entire micrograph. Chloride cell fractional area (CCFA) was determined using the following formula:

$$CCFA = \frac{\text{Area of entire and partial chloride cells}}{\text{picture area}} \times 100$$

Statistical Analysis

One way Analyses of Variance (ANOVA) were used to discern any differences between parameters at a single site over time. A two-tailed T-Test was used to compare control and experimental sites at a particular time. In cases where data was not normally distributed, a nonparametric ANOVA on ranks was performed followed by one of a Dunn's pairwise, Student-Newman-Keuls or Mann-Whitney test. The fiducial limit was set at $p < 0.05$.

Results

PCB Analysis

Analysis of water samples taken at the General Motors site and at the mouth of the Raquette River on June 22, 1995 (Time 0) using mass spectrophotometry showed that PCB levels at the GM site were 0.34 µg/L while PCB levels at the mouth of the Raquette River, which was assumed to be comparable to the Snye site were below the detection limit of 0.062 µg/L. Water samples taken on day 37 of the study (July 29, 1995) showed levels of 1.32 µg/L while Raquette River values were again below the detectable limit.

Examination of white muscle PCB levels showed that the PCB levels at the highly polluted General Motors site rose from 51.2 $\pm$ 11.5 parts per billion (PPB) at time 0 to 2080.0 $\pm$ 345.3 PPB at 21 days. At 41 days the value was not significantly different from the value at 21 days. Control site values were not significantly different from time 0 after 21 days but increased significantly from 21 day levels of 22.6 $\pm$ 3.93 PPB to 59.5 $\pm$ 9.8 PPB at 41 days. PCB levels were significantly different between control and experimental sites at both 21 and 41 days (Fig.2)

Plasma Parameters

Comparison of plasma cortisol levels showed no differences, either over the 41 day experimental period or between the Snye site and the PCB contaminated General Motors site (Fig. 3). Time 0 samples were not analysed due to transfer stress. Plasma glucose levels were not different after 21 days, but were lower after 41 days relative to fish

Fig. 2. Concentration of PCBs (parts per billion) ± SEM in white muscle tissue obtained from *Oncorhynchus mykiss* after 0, 21, and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Time 0 denotes no exposure to polluted waters. Differences were accepted at $p<0.05$ and are denoted as follows: * = different from Time 0, + = different from previous sampling at same site and ! = different from control (Snye) site. Sample sizes are indicated in parentheses at the bottom of each column.

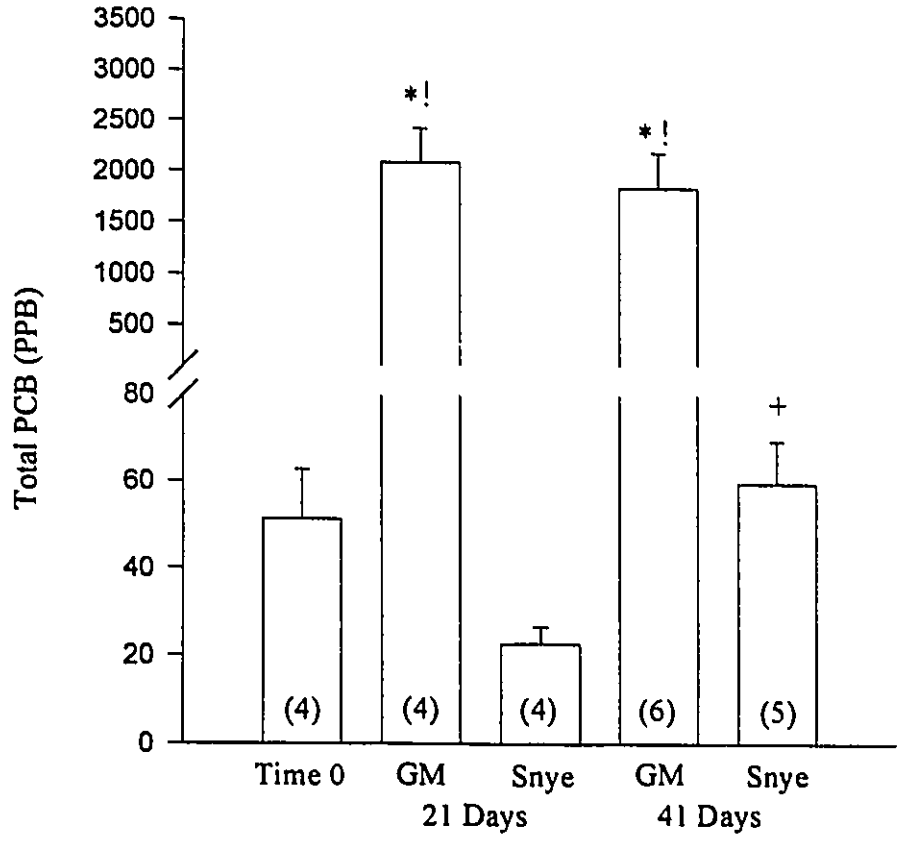
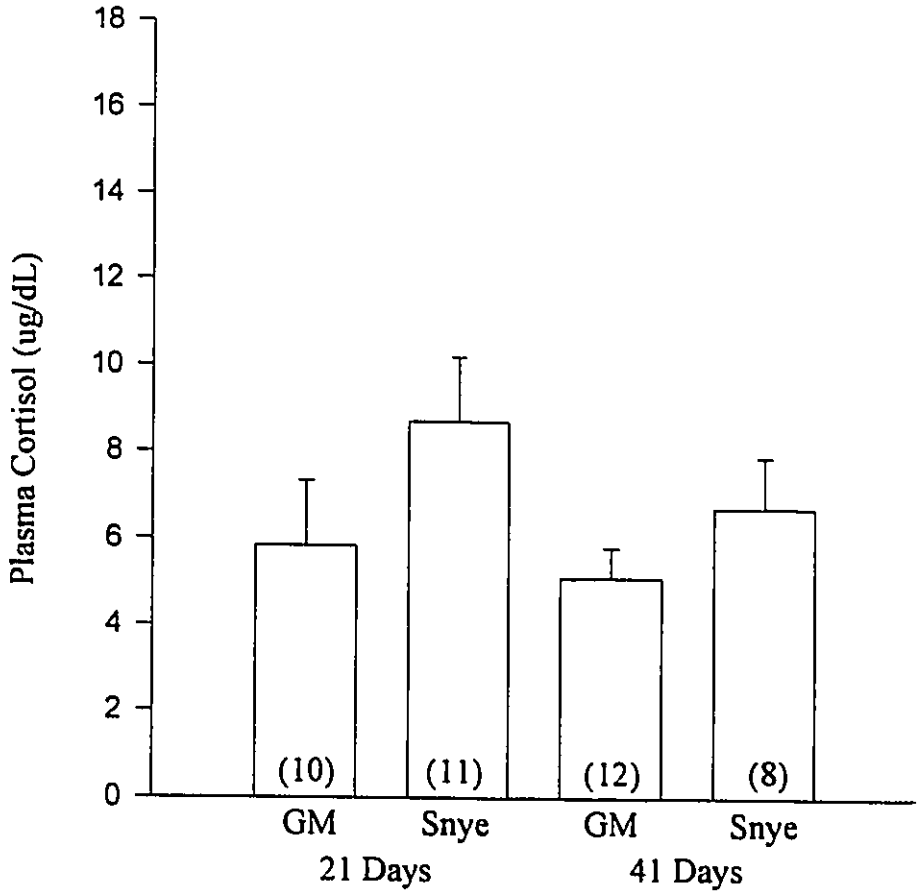

Total PCB (PPB)
3500
3000
2500
2000
1500
1000
500
80
60
40
20
0
*!
*!
+
(4)
(4)
(4)
(6)
(5)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 3. Plasma cortisol concentrations (± SEM) of *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Time 0 samples were not examined. No significant differences were found between any of the groups, $p < 0.05$. Sample sizes are indicated in parentheses at the bottom of each column.


Plasma Cortisol (ug/dL)
18
16
14
12
10
8
6
4
2
0
(10)
(11)
(12)
(8)
GM
Snye
GM
Snye
21 Days
41 Days

sampled after 21 days. Further, PCB exposed fish at the General Motors site had lower glucose than was found at time 0 (Fig. 4). Time 0 fish had high plasma lactate levels and all other groups showed a decrease from time 0 (Fig. 5) except for the 21 day PCB exposed General Motors group. Lactate levels at the GM sites were higher than those at the Snye site after 21 days of exposure, but lower after 41 days. Lactate levels dropped between the 21 and 41 day samplings at the General Motors site.

Plasma ions (Table I) varied considerably between the different sites and throughout the experimental period. In general, plasma chloride levels rose from time 0 control levels of 138.4 $\pm$ 3.84 mM to values between 162.9 to 167.7 mM at all sites and all later time periods, but no significant differences between sites were found. Conversely, plasma potassium levels decreased significantly from a time 0 level of 8.50 $\pm$ .779 mM to levels between 2.80 and 4.30 mM throughout the experiment. No differences were found between sites or sampling times.

Sodium values showed no definite trend, though all values except for the 21 day sample at the experimental General Motors site were significantly lower from time 0 controls. Similar to plasma lactate levels, sodium values at the General Motors site were higher than control site values after 21 day PCB exposure, but lower following 41 day exposure. Sodium levels in experimental fish also decreased significantly over the course of the experiment (Table I).

Fig. 4. Plasma glucose concentrations (± SEM) of *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Glucose concentration prior to exposure to pollutants is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are indicated by: * = different from Time 0 and + = different from previous sampling at the same site. Sample sizes are indicated in parentheses at the bottom of each column.

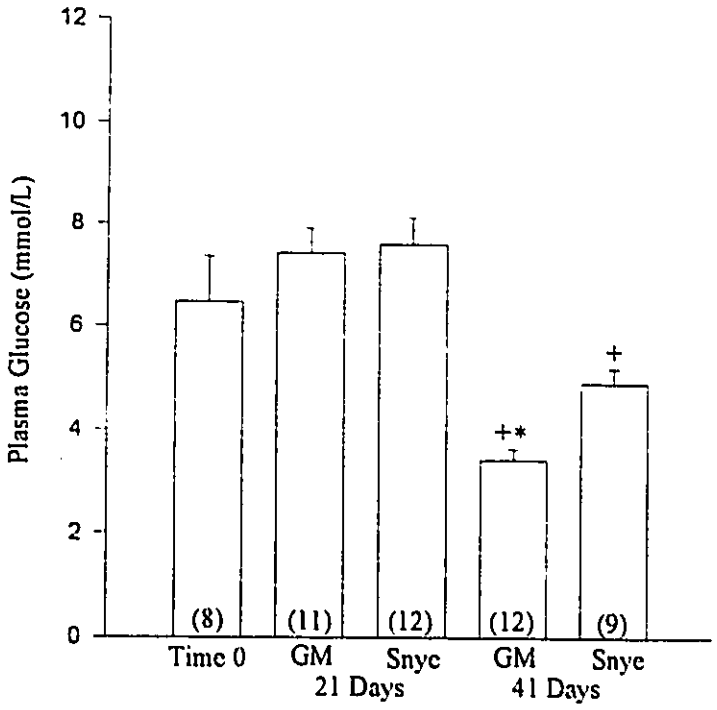
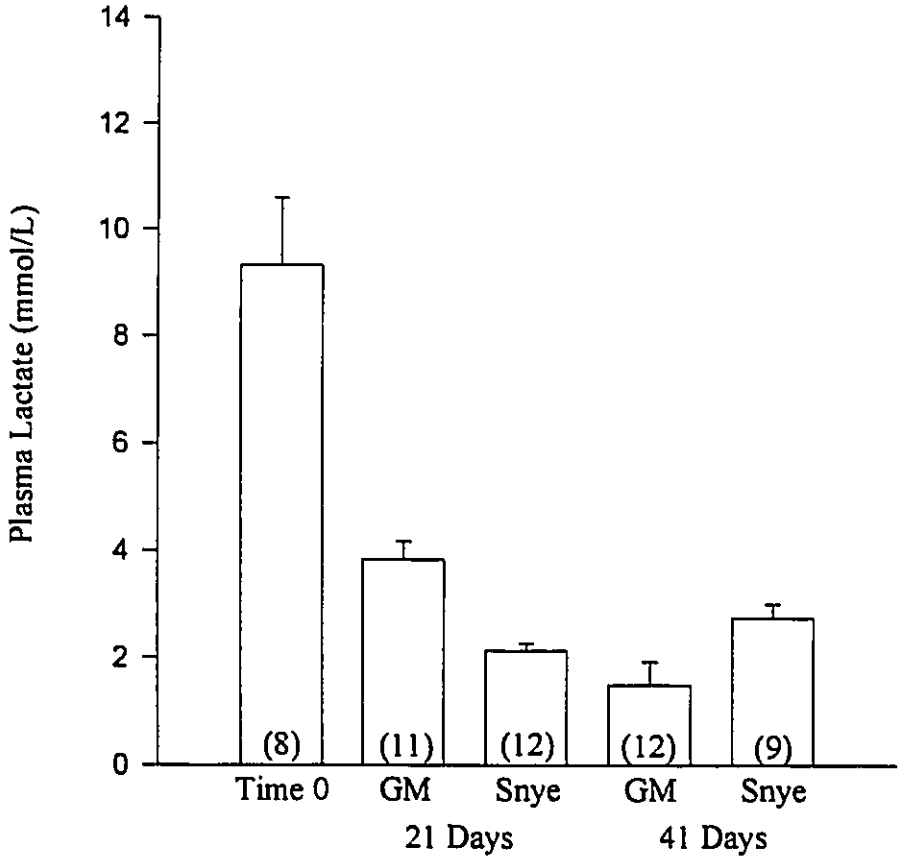

Plasma Glucose (mmol/L)
12
10
8
6
4
2
0
(8)
(11)
(12)
(12)
(9)
+*
+
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 5. Plasma lactate concentrations (± SEM) of *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Lactate concentration prior to exposure to pollutants is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are indicated by: * = different from Time 0, ! = different from control (Snye) site and + = different from previous sampling at the same site. Sample sizes are indicated in parentheses at the bottom of each column.


Plasma Lactate (mmol/L)
14
12
10
8
6
4
2
0
(8)
(11)
(12)
(12)
(9)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Group	Chloride	Calcium	Potassium	Sodium
Time 0	138.4 ± 3.84	5.74 ± .432	8.50 ± .779	180.9 ± 6.51
GM 21	162.9 ± 3.33*	4.62 ± .147	3.27 ± .220*	168.7 ± 3.69!
Snye 21	167.1 ± 5.32*	4.63 ± .084*	2.80 ± .278*	152.7 ± 2.85*
GM 42	164.1 ± 4.77*	3.86 ± .133*!+	4.28 ± .225*	146.0 ± 3.74*!+
Snye 42	167.7 ± 4.83*	4.28 ± .128*	4.30 ± .200*	158.1 ± 4.33*

Table I. Plasma electrolyte levels (± SEM) of *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. All values are expressed as mM concentration. Electrolyte levels after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are indicated by: * = different from Time 0, ! = different from control (Snye) site and + = different from previous sampling at the same site. Sample sizes are indicated in parentheses at the bottom of each column

Plasma total calcium levels in all but 21 day PCB exposed trout were also significantly different from time 0 levels. No differences between groups were found after 21 days, but 41 day samples at the experimental site were significantly lower than levels at both the control site and those at the experimental site after 21 days.

Bone Calcium

Bone calcium levels decreased significantly from time 0 in both control and experimental groups after 21 days, but recovered and were not different from time 0 in control fish after 41 days. Bone calcium in fish exposed to high levels of PCBs remained lower over the course of the experiment (Fig. 6).

Muscle Water Content

Muscle water content varied substantially and showed no significant change between any of the groups or over the course of the experiment (Fig. 7).

Gill Histology

Chloride cell fractional area (CCFA) decreased between Time 0 and day 21 in fish exposed to high levels of PCBs at the General Motors site after 21 days, but exhibited no other differences (Fig. 8; see also Fig.9).

Liver Parameters

Pyruvate kinase activity decreased from Time 0 in all samples except for those obtained at the General Motors site after 21 days exposure and were higher at the General Motors than at the Snye site after 41 days (Fig. 10). Fig. 11 shows that phosphoenolpyruvate carboxykinase (PEPCK) levels were unchanged from Time 0 at all sites and times except for the 21 day Snye sample. PEPCK values also increased from this

Fig. 6. Bone calcium content (± SEM) of *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs Bone calcium content after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are indicated by: * = different from Time 0, ! = different from control (Snye) site and + = different from previous sampling at the same site. Sample sizes are indicated in parentheses at the bottom of each column.

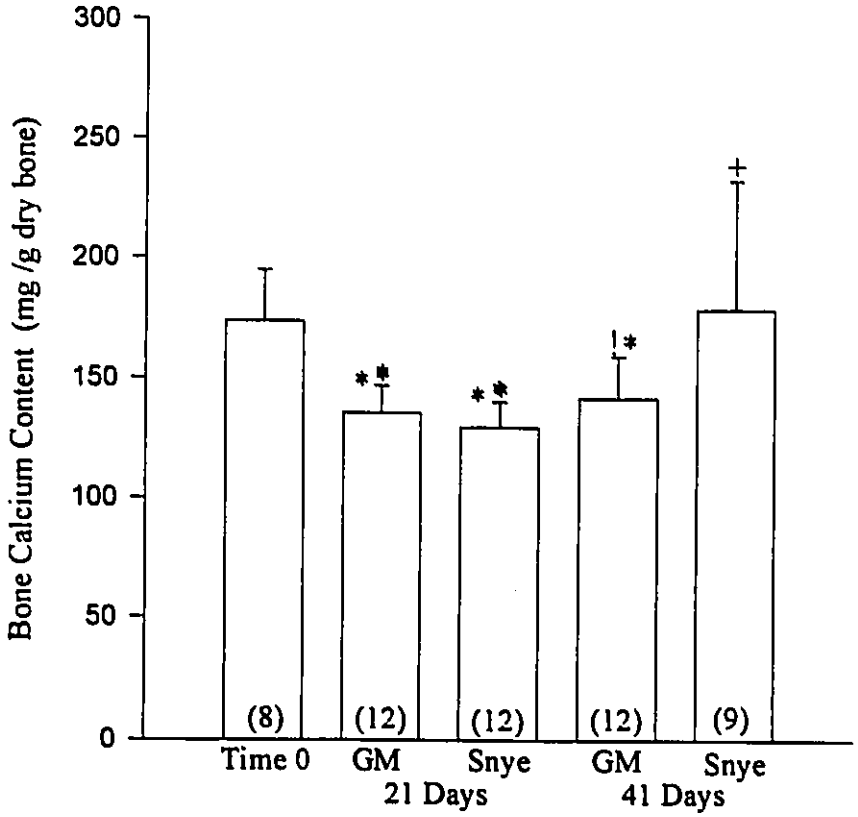

Bone Calcium Content (mg /g dry bone)
300
250
200
150
100
50
0
(8)
(12)
(12)
(12)
(9)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 7. Muscle water content (± SEM) of *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Muscle water content after background PCB exposure is denoted by Time 0 samples. No differences were found between any of the treatments ($p < 0.05$). Sample sizes are indicated in parentheses at the bottom of each column.

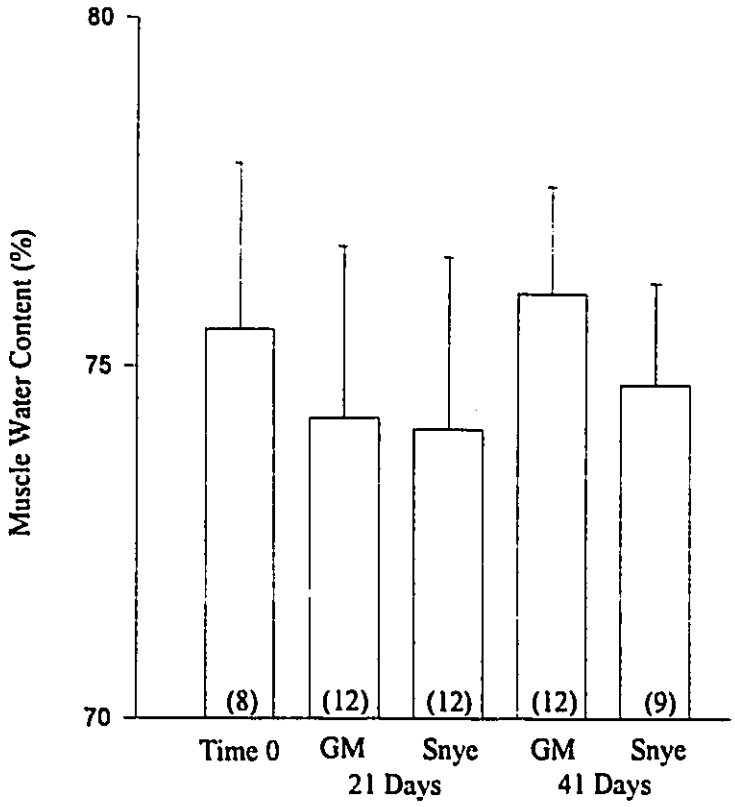

80
75
70
Muscle Water Content (%)
(8)
(12)
(12)
(12)
(9)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 8. Gill chloride cell fractional area (± SEM) in *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. CCFA after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$, * = different from Time 0 sample. Sample sizes are indicated in parentheses at the bottom of each column.

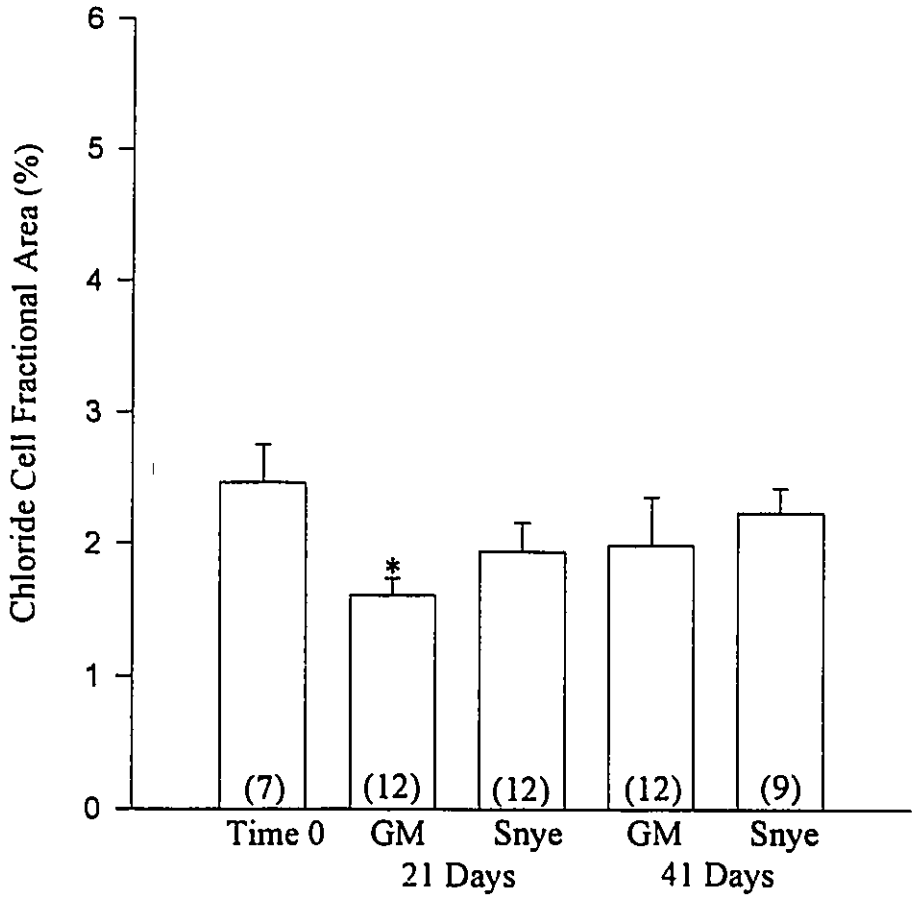

Chloride Cell Fractional Area (%)
6
5
4
3
2
1
0
*
(7)
(12)
(12)
(12)
(9)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 9. Scanning electron micrograph of gill lamellae from *Oncorhynchus mykiss* stained with osmium tetroxide. Micrographs are representative of Snye and General Motors sites and show chloride cells (cc), pavement cells (pc) and mucous cells (mc). Bar = 5 μm.

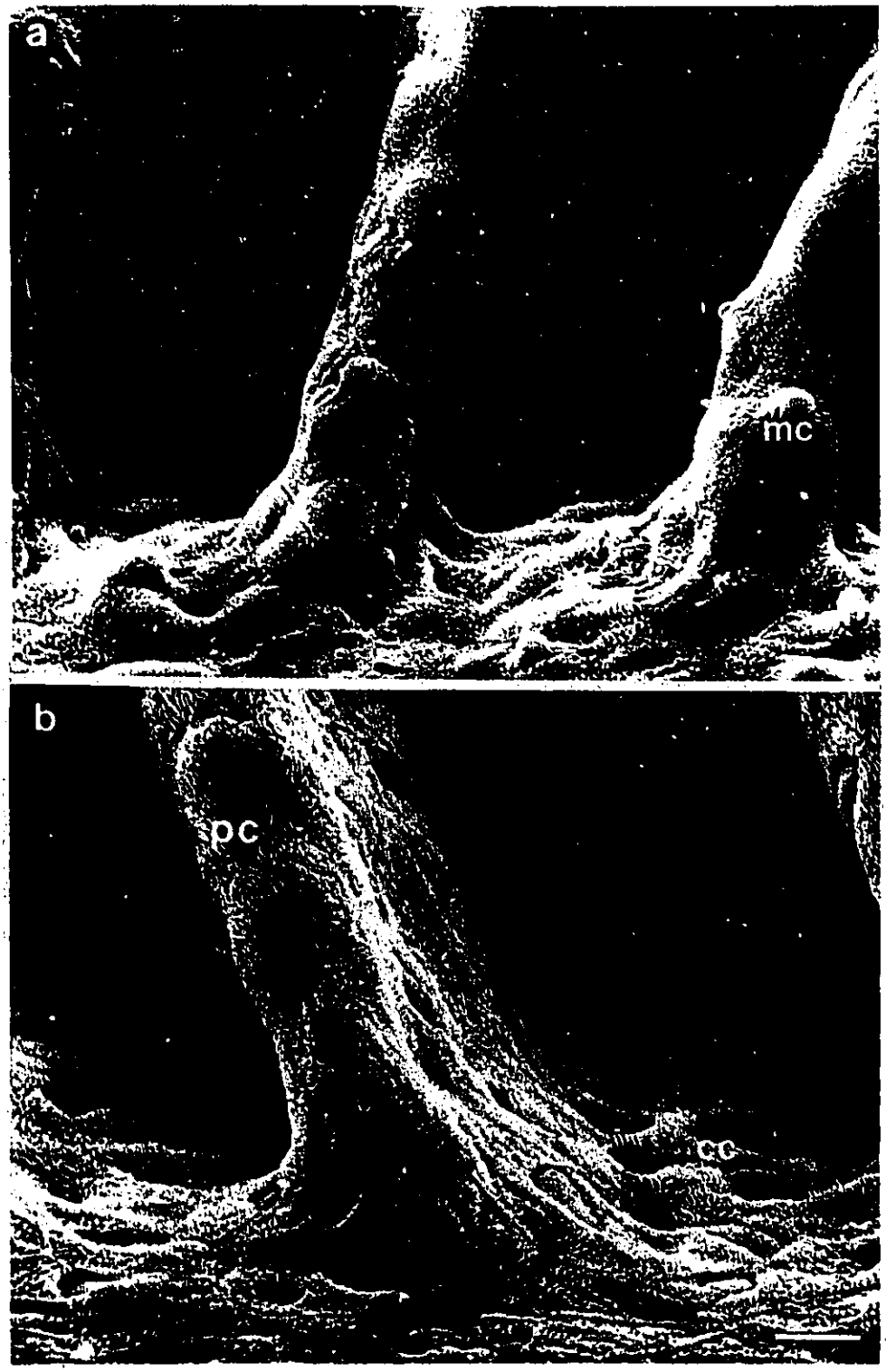

a
mc
b
pc
cc

Fig. 10. Pyruvate kinase activity (μmol/min/mg ± SEM) in *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Activity after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are denoted by: * = different from Time 0 sample and ! = different from control site. Sample sizes are indicated in parentheses at the bottom of each column.

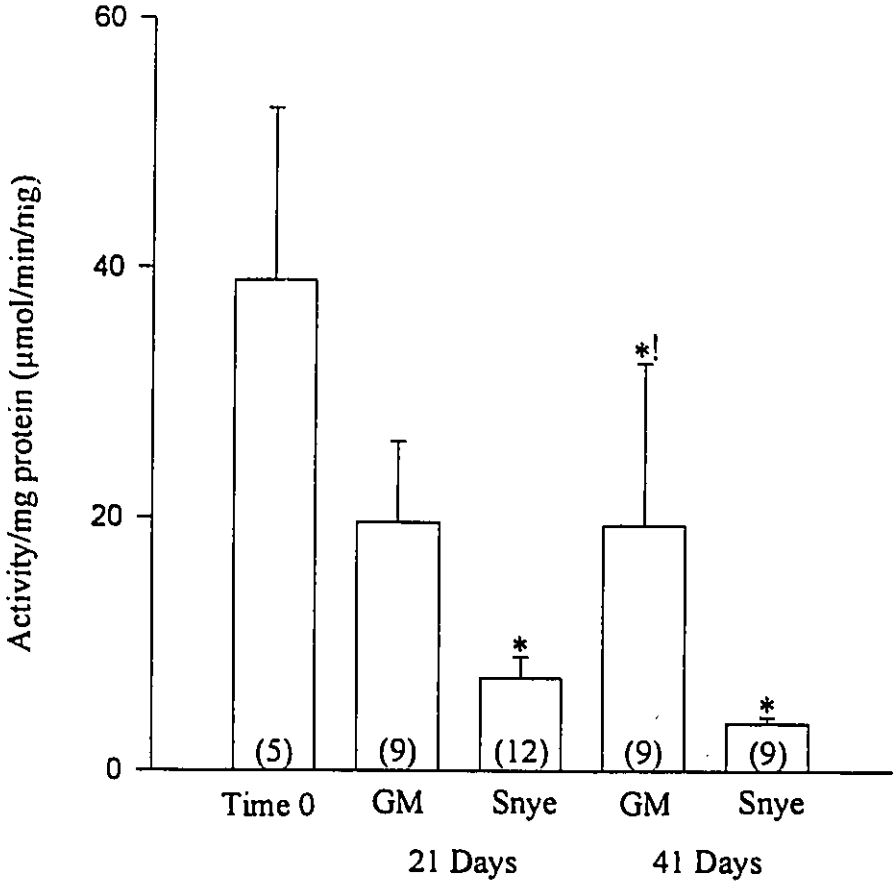
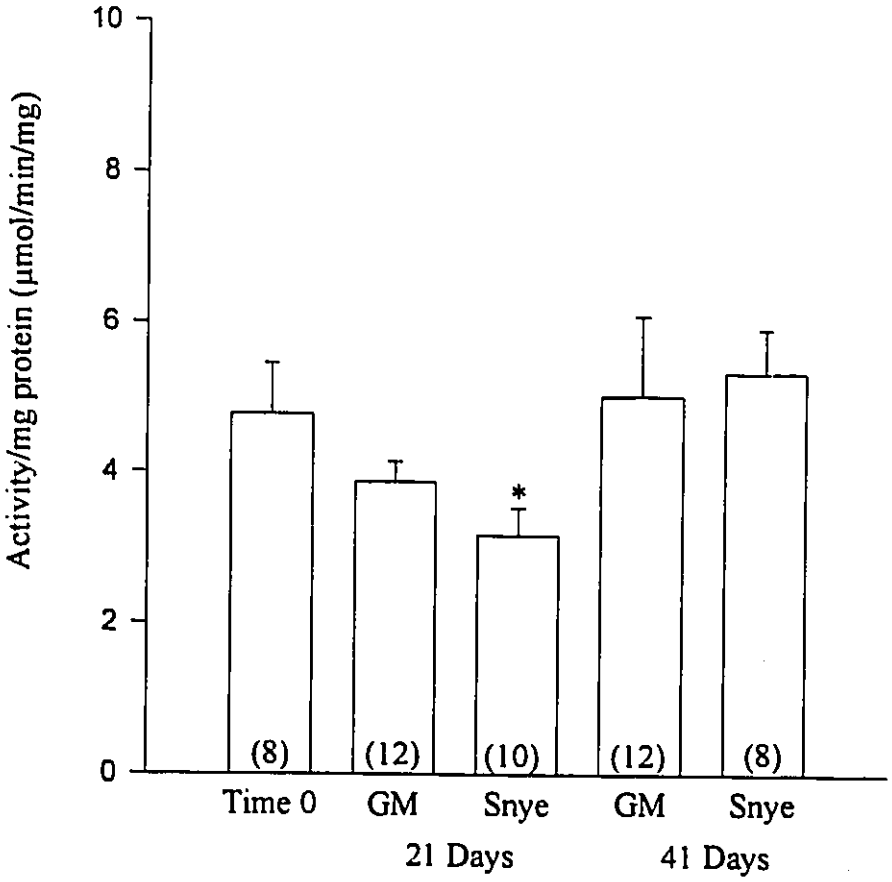

60
40
20
0
Activity/mg protein (µmol/min/mg)
(5)
(9)
(12)
(9)
(9)
*
*!
*
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 11. PEPCK activity (μmol/min/mg ± SEM) in *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Activity after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are denoted by: * = different from Time 0 sample and + = different from first sampling period. Sample sizes are indicated in parentheses at the bottom of each column.


Activity/mg protein (µmol/min/mg)
10
8
6
4
2
0
(8)
(12)
(10)
(12)
(8)
*
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

value at the Snye site following 41 days. Glutamate-pyruvate transaminase levels at the General Motors site after 41 days were different from Time 0, the 41 day Snye site and the 21 day General Motors site (Fig. 12). Lactate dehydrogenase activity at the General Motors site were higher than at the Snye site after 21 days, but no significant differences were found after 41 days (Fig.13). Liver glycogen values were lower than Time 0 in fish held at the Snye site for 21 days, and these values were different from the 41 day samples. No other differences occurred (Fig. 14).

Following the writing of this thesis, it was suggested that performance of correlations between physiological parameters and muscle PCB levels of specific fish might prove valuable. Correlations were performed and evaluated using the Pearson Product method. Significance was accepted at $p < .05$ and found only for muscle water content and GPT activity. The data can be found at the end of the thesis in Appendix A (p. 85).

Fig. 12 GPT activity (µmol/min/mg ± SEM) in *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Activity after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are denoted by: * = different from Time 0 sample, ! = different from control site and + = different from first sampling period. Sample sizes are indicated in parentheses at the bottom of each column

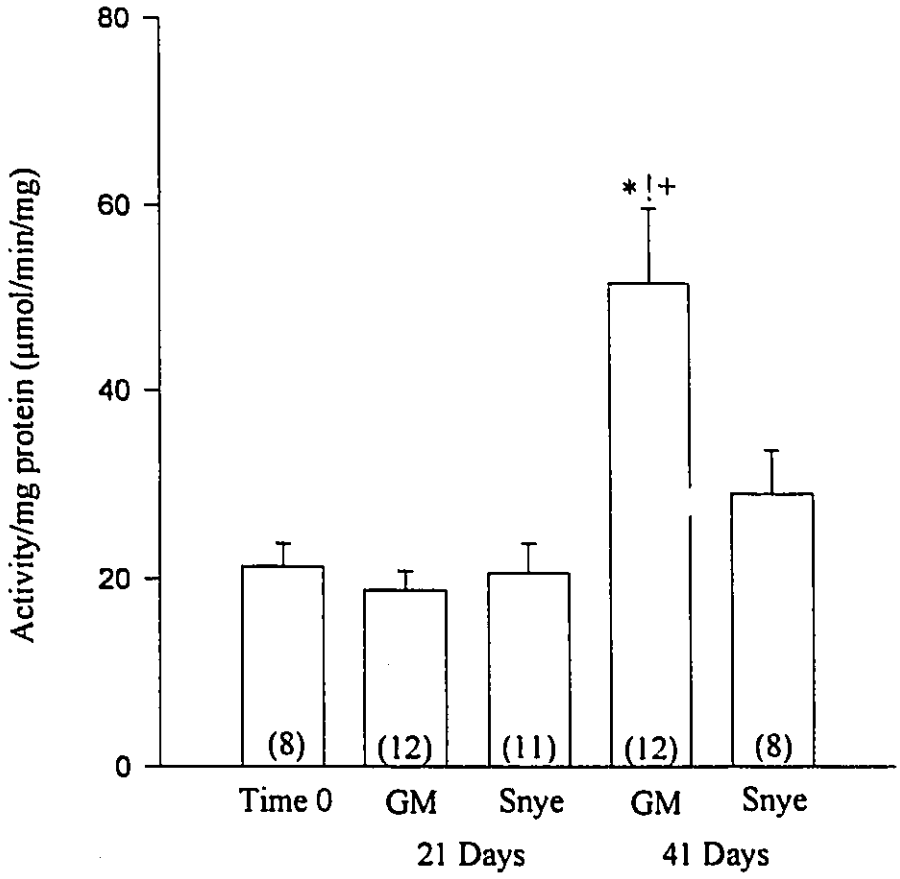

80
60
40
20
0
Activity/mg protein (µmol/min/mg)
*!+
(8)
(12)
(11)
(12)
(8)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 13. Lactate dehydrogenase activity (μmol/min/mg ± SEM) in *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Activity after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are denoted by: * = different from Time 0 sample, ! = different from control site and + = different from first sampling period. Sample sizes are indicated in parentheses at the bottom of each column

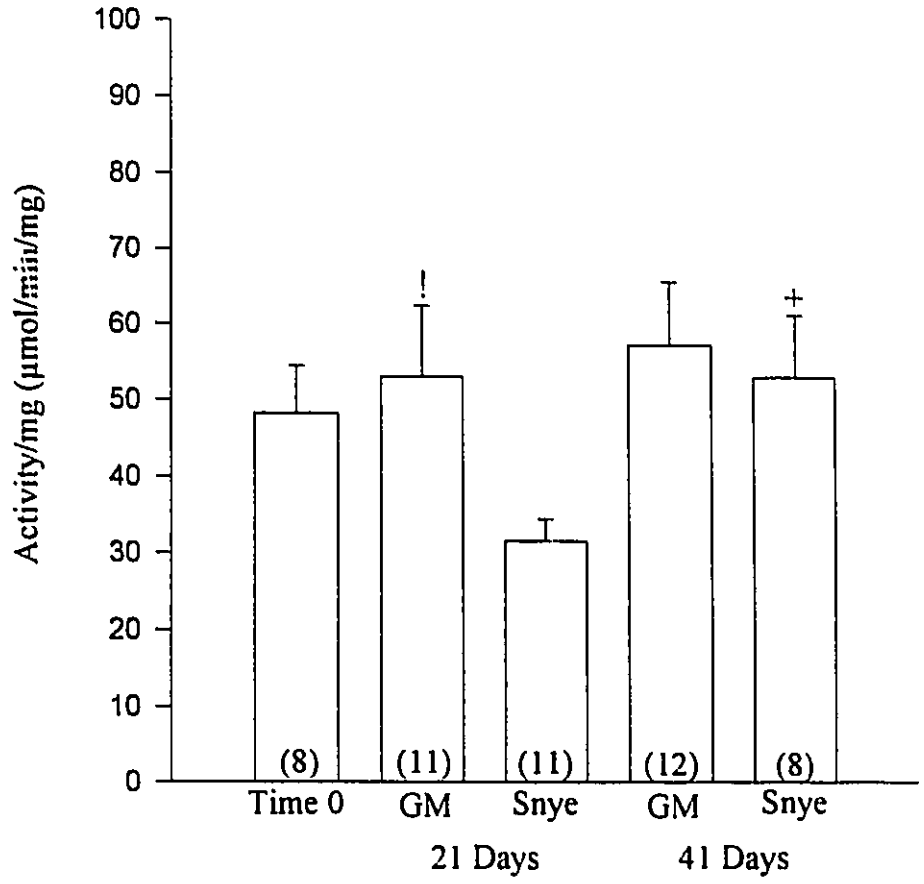
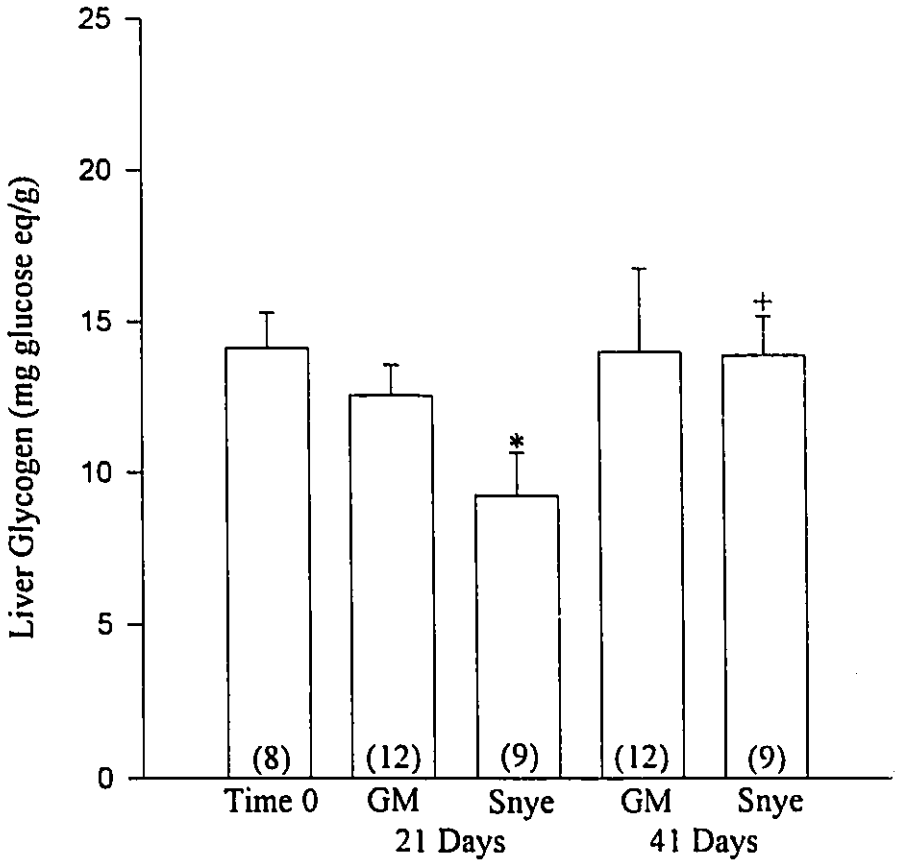

Activity/mg (µmol/min/mg)
100
90
80
70
60
50
40
30
20
10
0
!
+
(8)
(11)
(11)
(12)
(8)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Fig. 14. Liver glycogen levels (mg glucose equivalents/g liver ± SEM) in *Oncorhynchus mykiss* following 21 and 41 day exposure to low (Snye site) and high (GM site) concentrations of waterborne PCBs. Activity after background PCB exposure is denoted by Time 0 samples. Differences were accepted at $p < 0.05$ and are denoted by: * = different from Time 0 sample and + = different from first sampling period. Sample sizes are indicated in parentheses at the bottom of each column


Liver Glycogen (mg glucose eq/g)
25
20
15
10
5
0
*
+
(8)
(12)
(9)
(12)
(9)
Time 0
GM
Snye
GM
Snye
21 Days
41 Days

Discussion

The primary goal of this study was to identify and to assess some physiological variables with a view to using them as bioindicators of the toxic effects of PCBs in teleost fish. To be a candidate for the measurement of stress in this study, two absolute criteria were established. First, the selected parameter must previously have been demonstrated to consistently undergo change in response to stress in more than one species of teleost fish. Secondly, the parameter must be practical to measure given that sampling would have to occur under field conditions. The eventual parameters chosen encompassed a variety of plasma variables, specifically, the level of the hormone cortisol, various ion concentrations, plasma glucose and plasma lactate levels. Additionally, and for reasons which will be discussed later, certain secondary effects of the stress hormone cortisol (e.g. changes in muscle water content, bone calcium content and gill chloride cell fractional area) were also quantified. Further, certain liver enzymes associated with metabolism in general and plasma glucose and lactate were also measured. It was hypothesized that the measurement of these variables would provide one or more bioindicators useful for the examination of stress within fish populations in the St. Lawrence River with the aim of using them to track the success of any remedial efforts.

Unfortunately, although some of the parameters measured in the present work may, following further examination, prove to be useful bioindicators of PCB stress in rainbow trout, most of the parameters did not respond according to the expectations of this study. Further experiments are clearly indicated to better study some of the variables

examined here as well as other variables not included here in order to provide a reliable bioindicator of PCB stress in rainbow trout in the St. Lawrence River.

In spite of the fact that the rainbow trout used in this study were clearly subjected to elevated levels of PCBs, there was little indication of stress as there were few changes in the measured parameters. Indeed, no reliable bioindicator was unambiguously identified. The expected increase in cortisol did not occur. While this observation may have resulted from some masking action, it is clear that cortisol levels per se are not a useful bioindicator under the conditions of this study. This is further supported by the observation that several of the other candidate parameters, known to respond to elevated cortisol titers, did not change. Bone calcium, however, did drop following exposure to PCBs and remained low for the duration of the study. Though no bioindicators were positively and indisputably identified in this study, bone calcium content was identified as a possible sensitive indicator of PCB stress. Confirmation of this observation is clearly indicated.

As a result of its great size and the strain which it has undergone, the recovery of the St. Lawrence River will be protracted and involved. The first step of any remedial action program is the diagnosis of the problems within the ecosystem so that they may be addressed in the most appropriate and effective manner. The first step in this process is to complete a profile of the stressed system and to identify possible ways in which to ascertain which areas are stressed by human activity and require action to alleviate anthrogenic impacts. This study was performed in the hope of providing some simple, practical and reliable tools for the diagnosis of stress in fish resulting from PCB exposure

in the St. Lawrence River. A secondary goal of this study was the examination of opportunities for aquaculture in certain parts of the St. Lawrence River adjacent the St. Regis Mohawk Nation. By maintaining fish in the St. Lawrence River for long periods of time, evaluations of the state of health of the fish and their suitability for consumption could be examined.

As predicted from previous work (Kadlec, 1994), exposure to high levels of PCBs in the water column resulted in significant increases in muscle PCB levels at the General Motors site when compared to both the control and the Time 0 samples. This is based on the fact that since all of the animals in this study were provided with the same uncontaminated commercial food pellets, and because contact with sediments except those suspended in the water column during the dredging operation at the General Motors site was prevented through the use of floating cages, substantial PCB uptake was still observed. Conversely, Makarewicz *et al* (1993) reported that neither black bullheads, *Ameiurus melas*, nor rainbow trout, incorporated significant amounts of Mirex, a lipophilic pesticide, directly from the water column. This discrepancy may be a result of interspecies differences in the case of the bullhead, the chemical properties of Mirex, or both.

Levels of PCBs at the control site in the Snye channel also provided results with implications important to aquaculture in the St. Lawrence River. Though neither the 21 nor the 41 days samples showed levels different from Time 0 values, PCB accumulation did occur between the 21 and the 41 day sampling times. This result agrees well with Madenjian *et al* (1994), who observed a correlation between body burden of PCBs and

time of exposure to PCB contaminated waters. Locating culture sites at large distances from known sites of PCB contamination would lessen this effect as PCB body burden was shown to correlate negatively with distance from PCB contaminated sites (Stow, 1995). Since pisciculture would naturally involve long term exposure to the waters of the St. Lawrence River, periodic monitoring of the PCB levels of farmed fish will be necessary to prevent the marketing of fish containing PCB loads greater than the present governmental guidelines of 2 ppm (Government of Canada, 1991). Therefore, prior to investment in full scale aquaculture in the St. Lawrence River, further long term studies of rainbow trout held at the proposed aquaculture sites are necessary to determine the site specific kinetics of PCB loading.

Though animals at the General Motors site were clearly exposed to elevated levels of PCBs, no change in levels of plasma cortisol were noted. It is possible that internal mediation of the stress response had occurred. Animals at the General Motors site may have exhibited an initial cortisol increase as a result of the stress posed by high PCB exposure, but they could have adapted to this stress before the first sampling after 21 days. Over the course of the experiment, animals may have moved into the second stage of the General Adaptation Syndrome (GAS) (reviewed by Barton and Iwama, 1991) and, as a result, cortisol levels could have decreased to basal levels before the first sampling period. Such a masking response was reported previously in rainbow trout, following a period of acclimation to crowding stress (Laidley and Leatherland, 1988). However, this response may be species dependent. For example, Pankhurst *et al* (1992), reported that wild blue mao mao, captured by angling and held for a period of twenty one days exhibited

continually elevated cortisol levels. Since domesticated rainbow trout were bred for greater docility, it is possible that wild fish exposed to similar stress exhibit a larger cortisol mediated response and are less able to adapt to chronic stresses (Pickering, 1993) such as PCB exposure. This contention is supported by a study of the territorial wild fish northern pike and yellow perch. These fish were captured within areas highly polluted by PCBs, polyaromatic hydrocarbons (PAHs) and mercury (Hontela *et al.*, 1992) and showed that, possibly due to chronically elevated activity of the adrenal corticotropes, they could no longer respond to stress. Thus cortisol may provide a better bioindicator of pollution stress in wild fish than in domesticated fish such as the trout used in this study or other species which will likely be cultured.

Other difficulties, however, complicate the use of cortisol as a bioindicator. Although plasma cortisol levels may be elevated, it is possible that the effect at the tissue level is minimal. It was shown that in the coho salmon, *Oncorhynchus kisutch*, down regulation of cortisol receptor concentration occurred in response to both cortisol implants and stress (Shrimpton and Randall, 1994). Thus, even if cortisol levels are increased in the plasma, their effects at the level of the tissue could be significantly ameliorated. Under these circumstances, high cortisol levels would become less representative of the stress regimen experienced by the fish. In the present study, however, increases in cortisol were not seen in any of the sample groups, and thus down regulation of the receptors is unlikely to have occurred. The possibility remains, however, that the initial high levels of cortisol in the fish remained high for a period of time at the General Motors site. Analysis of blood samples taken during the first few days of a similar

study would serve to remove any doubt surrounding this issue. Similarly, changes in the metabolic clearance rate (MCR) of cortisol must be considered. The level of cortisol in the plasma at a single point in time is not completely representative of utilisation of cortisol within the body (Brown *et al*, 1986). Large quantities of cortisol may be secreted into the plasma, but if there is a concomitant increase in the MCR, the increased secretion may not translate into increased plasma concentrations. This would render plasma cortisol concentration of little value in response to the stressor. Indeed, increased MCR was observed in response to both exercise in rainbow trout (Nielsen *et al*, 1994) and to seawater acclimation in Atlantic salmon (Nichols and Weisbart, 1985).

Cortisol levels, then, are at present somewhat unreliable as a bioindicator of stress. In this study, although fish held at the General Motors site were exposed to significantly higher body PCB loads, no differences between the control and experimental sites were observed. This result may be due to an increase in the metabolic clearance rate of cortisol or an adaptation to PCB imposed stress in fish held at the General Motors site prior to the initial sampling after 21 days. It is also possible, though unlikely in the short time span of this study, that the HPI axis of trout held at General Motors became exhausted, resulting in the observed low cortisol levels. Though no significant differences among plasma cortisol levels were found in the present study, there was a trend for cortisol to be lower at the General Motors site at both the 21 and 41 day sampling periods in this study. Exhaustion of the HPI axis may be one explanation for this trend. Histological studies of the interrenal tissue or a longer term experiment to determine whether fish at the General Motors site would be able to respond to stress such as handling following extended

periods of time would elucidate this question. Because of the uncertainty of how reflective cortisol levels actually are of stress and the fact that they are also very sensitive to sampling stress (Brown *et al*, 1986), it is concluded from the present study that cortisol is not a useful bioindicator of PCB related stress in the present study. Further studies are required to examine if adaptation to PCB stress occurs in rainbow trout, and, if adaptation does occur, the time course over which this process takes place should be determined.

Examination of lactate levels in experimental fish showed differences throughout the time course of the experiment, suggesting that lactate production, utilisation, or both may be sensitive to PCB stress. This result agrees with studies performed by Pankhurst and Dedual (1994) who reported an increase in lactate levels in rainbow trout following handling stress. But Gadomski *et al* (1994), working with the chinook salmon, observed that stress induced cortisol increases did not result in any change in lactate levels. Cortisol induced increases in lactate concentration were observed in the red snapper, but the cortisol injections were of pharmacological proportions (Bollard *et al*, 1993). It is unlikely that either cortisol or PCB mediated changes in lactate were observed in the present study. Cortisol levels did not, through the time course of the study, change significantly, suggesting that cortisol would have little if any effect on lactate levels in the plasma. Since the observed changes in lactate were inconsistent, exhibiting decreases at 21 days PCB exposure and increases following 41 day exposure, it is not likely that these values are reflective of PCB stress. These changes were likely artefactual and probably resulting from the physical exercise and anaerobic activity within the white muscle mass associated with the capture and handling of the fish during sampling. Increases in lactate

were observed at all sampling times as more fish were removed from cages and netting required greater effort and a longer period of time Similar changes in lactate levels were reported previously following extreme exercise in both the brown (Pickering *et al*, 1982), and the rainbow trout, probably as a result of anaerobic activity in the white muscle mass following short handling periods (Pankhurst and Dedual, 1994). It is likely that the changes seen in this study also occurred as a result of physical disturbance rather than as a physiological response to PCB exposure. However, it is also possible that fish at both sites utilised lactate as a substrate for gluconeogenesis and thus lowered lactate concentrations following the suspension of feeding after day 31. Certainly, Vijayan *et al* (1991) demonstrated such an occurrence in fasted brook charr. As such, the results here suggest reasons other than PCB exposure for the observed changes in lactate concentration. Further study is warranted to determine if, in the long term, feeding fish exposed to PCBs exhibit any changes in lactate concentrations as a result of pollutant exposure.

Plasma glucose presents a scenario similar to that of lactate. There were definite differences in the data, with both General Motors and Snye sites showing decreased plasma glucose titers following 41 days of exposure. However, these reductions in glucose levels were likely the result of the cessation of feeding of the animals at both sites from day 31 until the end of the study as opposed to a stress mediated response to PCBs within their environment. Previously it was shown in the rainbow trout (Moon *et al*, 1988) that plasma glucose decreased significantly in starved fish when compared to fed fish. This result agrees with the observed reduction of plasma glucose at both control and experimental sites seen in the present study following the 10 day fasting period. However,

it has been shown that fish under stress do not feed well (Vijayan *et al*, 1990) and it is necessary to address this problem through further long term studies where suspension of feeding does not occur.

Though differences in concentrations of plasma ions were observed, it is unlikely that these variations were a result of PCB stress. Cortisol was shown to decrease plasma sodium concentrations and increase plasma potassium concentrations in coho salmon. These changes were, however, season dependent and were likely responses to the seawater adaptation (Redding *et al*, 1991). Gadomski *et al* (1994) demonstrated similar ionoregulatory disturbance in the plasma of juvenile chinook salmon following descaling and attributed the changes to stress. Using rainbow trout, Nielsen *et al* (1994) observed increases in plasma potassium following exercise as a result of potassium efflux from the tissue. In the present study, plasma ion values changed but there was no clear cut relationship to PCB stress. Time 0 values were likely the result of transfer stress and are probably unrepresentative of normal physiological values. Neither plasma chloride nor plasma potassium concentrations varied during the study and sodium values showed no clear trend. The possibility exists that calcium values may provide a possible bioindicator of PCB stress. Plasma calcium values at the General Motors site decreased through the course of the study and were significantly lower than control and Time 0 values at the 41 day sampling.

Changes in bone calcium over the course of the experiment suggest that this variable may be a viable bioindicator of PCB stress as bone calcium content decreased at both sites following 21 days exposure but returned to time 0 values only at the control

site. Fish at the General Motors site exhibited decreased bone calcium levels following both the 21 and 41 day sampling intervals. Since bone calcium is a relatively slowly changing variable, and not subject to rapid fluctuations as a result of sampling stress, this result is likely real and may provide a useful bioindicator of PCB stress , though further experiment is required as there is a paucity of literature on this subject..

Bone cells are known to concentrate ionic calcium, both directly from the external medium and to lesser extent, from the diet (Simmons, 1971). Deposition of calcium occurs at regions of the bone matrix, resulting in mineraiisation. Increases in concentrations of acid phosphatases, proteolytic enzymes and citrate result in the mobilisation of the deposited calcium into the extracellular medium of the fish (Simmons, 1971). Thus, bone calcium content is a dynamic variable, reacting to changes within the body of teleost fish.

Though no studies could be found specifically on the relationship between PCB stress and bone demineralisation, stress previously was shown to act upon the bone matrix. For example, in the white sucker, exposure to acidified water resulted in a decrease in bone calcium content, possibly as a result of an attempt to regulate extracellular pH by utilising the mineral components of the bone (Fraser and Harvey, 1982). In the present study, differences in electrolytes were found over the course of the experiment and the possibility exists that attempts to regulate extracellular ion concentrations through demineralisation of bone may have occurred. Indeed, ionoregulatory disturbance in cortisol injected fish were reported previously in the eel, *Anguilla anguilla*, in the form of declines in serum Na^+ (Chan *et al*, 1969). Similarly, Flik

and Perry (1989) demonstrated an increase in plasma calcium concentration following cortisol injections in the rainbow trout, though they concluded the source of calcium to be the external medium as opposed to the bone. Based on these observations, it appears that a relationship between stress and bone calcium content may exist and may provide an indicator of PCB stress in the rainbow trout. This indeed may be the most useful bioindicator discovered in the current study.

Though the present study shows that calcium changes in response to PCB stress, it would be interesting to elucidate exactly how these changes occur. The endocrine control of body calcium homeostasis in fish is well understood (c.f. review by Simmons, 1971). Increased activity in the interrenal gland, which secretes cortisol, resulted in a decrease in the muscle calcium levels of freshwater adapted American eels and adrenocorticotropic hormone (ACTH) injection resulted in decreases in serum calcium in the killifish, *Fundulus kansae*. Deposition of calcium was observed in fry of the Japanese medaka after ingestion of ground thyroid powder. It is clear, then, that calcium regulation is controlled primarily by the action of hormones.

Of most pertinence to the present study are observations on the effects of the ultimobranchial bodies, which secrete calcitonin, and the thyroid. Both calcitonin and thyroid hormones were shown to affect calcium in a manner similar to that seen in fish held at the General Motors site. Increased levels of calcitonin were shown in several studies to decrease the levels of calcium in the body of teleost fish (Simmons, 1971). It is postulated that rainbow trout caged at the General Motors site may have exhibited a hyperactivity of the ultimobranchial bodies resulting in a reduction in the overall body

calcium content and hence, reduced bone calcium content. Similarly, thyroidectomised young rainbow trout demonstrate impaired ossification of the skull, suggesting a role for the thyroid in calcium deposition (Simmons, 1971). The possibility exists that PCB exposure in rainbow trout causes either a hyperactivity of the ultimobranchial bodies resulting in increased levels of calcitonin and bone demineralisation, a depression of the thyroid leading to a decrease in bone mineralisation, or a combination of both. Certainly, there is ample evidence for the effect on thyroid tissue but the possible involvement of the ultimobranchial bodies requires study.

Effects of pollutants on endocrine function previously were demonstrated in highly polluted areas of the St. Lawrence River. Hontela *et al* (1995) observed that, in wild yellow perch, long term exposure to PCBs, PAHs, and a variety of heavy metals including mercury, cadmium, arsenic and zinc resulted in decreases in blood levels of both cortisol and thyroxine. Further, microscopic examination of the corticotropes of pollutant exposed animals showed that adrenal corticotropes were atrophied, suggesting a cause for the observed dysfunction (Hontela *et al*, 1992). Though the authors suggested that the atrophy resulted from prolonged hyperactivity of the corticotropes, it may have been induced directly by pollutant exposure. A direct effect of PCBs on the thyroid gland of the rainbow trout held at General Motors may have resulted in a depression of the activity of the gland and the concomitant reduction in bone calcium content. Such depression would explain decreases seen in thyroxine levels following pollutant exposure in the yellow perch (Hontela *et al*, 1995). However, Leatherland (reviewed by Leatherland, 1993), in a series of experiments examining the effects of dietary PCBs on the thyroid of

the rainbow trout, found no changes within the gland. Conversely PCBs in the present study were absorbed across body surfaces. It is possible that the mode of entry of PCBs to the body may have a different effect on the thyroid gland. A similar effect on the corticotropes may explain the lack of elevation observed in plasma cortisol in animals at the site. Further study on bone calcium levels and histological changes in the thyroid and corticotropes following exposure to waterborne PCBs are required to answer this question.

The analysis of liver enzymes yielded few differences, except at the General Motors site after 41 day exposure. Pyruvate kinase (PK) levels at the Snye site were lower than Time 0 at both sampling times, while the General Motors site was lower only following 41 day exposure. It is possible that Time 0 samples are reflective of a mobilisation of body energy stores by liver glycogenolysis as a result of stress due to handling and transfer. At 21 days, fish held at the Snye site demonstrated PK levels reflective of the unstressed state values while fish held at General Motors demonstrated levels indicative of high glycolytic flux. Previous whole animal studies have shown regulation of PK activities by stress (Wright *et al.*, 1989). Increased levels of lactate dehydrogenase (LDH) at the General Motors site at the 21 day sampling period further support this hypothesis. Since LDH catalyses the breakdown of pyruvate, the observed activation of the enzyme is indicative of increased glycolytic flux. Thus it is possible that fish exposed to high levels of PCBs alter their pattern of energy partitioning, at least in the short term, to utilise glycogen stores in the liver.

Glutamate-pyruvate transaminase (GPT) levels were inconclusive. GPT levels after 41 day exposure at the General Motors site were significantly higher than all other levels. Such an increase is indicative of the use of amino acids for metabolic fuel. Although reported data are inconsistent, it appears that the skeletal muscle of teleost fish can be used as a large reserve of amino acids which can be mobilised for gluconeogenesis under conditions of stress (Vijayan *et al.*, 1990; reviewed by Van der Boon *et al.*, 1991). However, the observed increases in GPT activity are confused by the unchanged levels of PEPCK. Were both of these enzymes activated, it would indicate a shift to gluconeogenesis, but this was not seen. The strong GPT response at 41 days is, therefore, likely a spurious result and does not provide a valid bioindicator. However, the GPT activity does correlate well with muscle PCB levels and may provide a valid bioindicator following further study. The significant correlation between muscle water content and PCB level provides support for this observation. Since GPT is an aminotransferase, and associated with gluconeogenesis, muscle water content would increase as a result of the mobilisation of amino acids from this tissue.

Liver glycogen remained unchanged throughout the experiment except for a decrease after 21 days at the Snye site. This result is inconsistent with alterations in enzyme activity of animals in the present study and may have resulted from the method glycogen analysis used. Similar to the enzymes analysed in this study, reports of stress effects on liver glycogen are inconsistent (Van der Boon *et al.*, 1991). Though the liver glycogen values observed in the present study may be poorly representative of actual

values, this variable and how it is affected by PCB induced enzyme activity require further study.

Impairment of the cortisol response may explain the observed lack of change in both muscle water content and chloride cell fractional area. Increases in plasma cortisol were previously demonstrated to result in increased levels of muscle water in the coho salmon (Redding *et al.*, 1991). Chloride cell proliferation was also observed following an increase in plasma cortisol levels (Bindon *et al.*, 1994). But, in the present study, neither of these events occurred since cortisol levels remained unchanged. The failure of cortisol levels to change may have resulted from impairment of corticotropes as a direct effect of PCBs. Thus, for the present, each of these variables must be disqualified as possible bioindicators of PCB stress in rainbow trout.

Though the present study was not designed primarily to examine the feasibility of aquaculture in the St. Lawrence River, it did raise some questions with respect to PCB contamination of cultured fish. Though PCB levels at the Snye site were never different from those found in hatchery raised fish, PCB burdens increased from the 21 day to the 41 day sampling period. Before implementation of commercial aquaculture in the St. Lawrence River, it will be necessary to perform purely aquacultural studies at proposed sites to ensure that contamination is not an issue. Further studies must determine both the accumulation rates of PCBs in cultured fish and ascertain that PCB levels remain lower than government guidelines for human consumption.

Though the present study does not provide any unambiguous physiological parameters which can serve as bioindicators of PCB exposure in rainbow trout, some of

the parameters show promise. Plasma variables, such as lactate, glucose, and cortisol levels were shown to be unreliable and subject to artefactual values as a result of sampling. Similarly, chloride cell fractional area and muscle water content, which are mediated by increases in cortisol, did not change in response to PCB exposure and do not provide useful bioindicators. Conversely, the observed depression of bone calcium content, which may indicate either a depression of the thyroid or a hyperactivity of the ultimobranchial bodies shows a great deal of promise as this parameter is unaffected by the acute stress associated wit.. sampling. Histological studies to clarify the effects of PCBs on these tissues are clearly indicated by the present study. Similarly, further study on the alteration of the energy metabolism of PCB exposed fish is indicated, particularly with respect to pyruvate kinase. A clear understanding of changes in the metabolic pathways utilised for energy mobilisation may provide enzymatic bioindicators of PCB stress in rainbow trout. Future studies on these parameters to fully elucidate their role in the response of the rainbow trout to PCB imposed stresses could provide a simple diagnostic tool in remedial action programs.

References

Andersen, D.E., Reid, S.D., Moon, T.W. & Perry, S.F. (1991). Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish. Aquat. Sci.* **48**, 1811-1817.

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

Barton, B.A., Schreck, C.B. & Barton, L.B. (1987). Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Dis. Aquat. Org.* **2**, 173-185.

Bindon, S.D., Gilmour, K.M., Fenwick, J.C.F. & Perry, S.F. (1994). The effects of chloride cell proliferation on respiratory function in the rainbow trout, *Oncorhynchus mykiss*. *J. Exp. Biol.* **197**, 47-63.

Bollard, B.A., Pankhurst, N.W. & Wells, R.M.G. (1993). Effects of artificially elevated plasma levels on blood parameters in the teleost fish *Pagrus auratus* (Sparidae). *Comp. Biochem. Physiol.* **106A (1)**, 157-162.

Brown, S.B., Eales, J.G. & Hara, T.J. (1986). A protocol for estimation of cortisol plasma clearance in acid-exposed rainbow trout (*Salmo gairdneri*). *Gen. Comp. Endocrinol.* **62**, 493-502.

Bush, B., Streeter, R.W. & Sloan, R.J. (1989). Polychlorobiphenyl (PCB) congeners in striped bass (*Morone saxatilis*) from marine and estuarine waters of New York determined by capillary gas chromatography. *Arch. Environ. Contam. Toxicol.* **19**, 49-61.

Campbell, P.M., Pottinger, T.G. & Sumpter, J.P. (1992). Stress reduces the quality of gametes produced by rainbow trout. *Biology of Reproduction*, **47**, 1140-1150.

Casillas, E. & Smith, L.S. (1977). Effects of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). *J. Fish Biol.*, **10**, 481-491.

Chan, D.K.O., Rankin, J.C. & Jones, I.C. (1969). Influences of the adrenal cortex and the corpuscles of Stannius on osmoregulation in the European eel (*Anguilla anguilla* L.) adapted to freshwater. *Gen. Comp. Endocrinol. Suppl.* **2**, 342-353.

Chrousos, J.P. & Gold, P.W. (1992). The concepts of stress and stress and stress system disorders. Overview of physical and behavioural homeostasis. *J. Am. Med. Assoc.* **267**, 1244-1252.

Conklin, D.J., Mowbray, R.C. & Gingerich, W.H. (1992). Effects of chronic exposure to soft, acidic water on gill development and chloride cell numbers in embryo-larval brook trout, *Salvelinus fontinalis*. *Aquat. toxicol.* **22**, 39-52.

De Guise, S., Lagace, A. & Beland, P. (1994). Tumours in St. Lawrence Beluga Whales (*Delphinapterus leucas*). *Vet. Pathol.* **31**, 444-449.

Fenwick, J.E., Davison, W. & Forster, M.E. (1994). In vivo calcitropic effect of some vitamin D compounds in the marine Antarctic teleost, *Pagothenia bernacchii*. *Fish Physiol. and Biochem.* **12(6)**, 479-484.

Flik, G. & Perry, S.F. (1989). Cortisol stimulates whole body calcium uptake and the branchial calcium pump in freshwater rainbow trout. *J. Endocrinol.* **120**, 75-82.

Foster, G.D. & Moon, T.W. (1986). Cortisol and liver metabolism of immature American eels, *Anguilla rostrata* (LeSueur). *Fish Physiol. Biochem.* **1(2)**, 113-124.

Fraser, G.A. & Harvey, H.H. (1982). Elemental composition of bone from white sucker (*Catastomus commersoni*) in relation to lake acidification. *Can. J. Fish. Aquat. Sci.* **39**, 1289-1296.

Gadomski, D.M., Mesa, M.G. & Olson, T.M. (1994). Vulnerability to predation and physiological stress responses of experimentally descaled juvenile chinook salmon, *Oncorhynchus tshawytscha*. *Environm. Biol. Fishes*, **39**, 191-199.

Government of Canada. (1991). The State of Canada's Environment. Banfield-Seguin Ltd., Ottawa. 709 pp.

Greco, A.M., Gilmour, K.M., Fenwick, J.C.F & Perry, S.F.P. (1995a). The effects of softwater acclimation on respiratory gas transfer in the rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* (in press).

Greco, A.M., Fenwick, J.C.F. & Perry, S.F.P. (1995b). The effects of softwater acclimation on gill structure in the rainbow trout *Oncorhynchus mykiss*. *Cell Tissue Res.* (in press).

Harris, G.E., Kiparissis, Y., & Metcalfe, C.D. (1994a). Assessment of the toxic potential of PCB congener 81 (3,4,4',5-tetrachlorobiphenyl) to fish in relation to other non-ortho-substituted PCB congeners. *Environm. Toxicol. Chem.* **13(9)**, 1405-1413.

Harris, G.E., Metcalfe, T.L., Metcalfe, C.D. & Huestis, S.Y. (1994b). Embryotoxicity of extracts from Lake Ontario rainbow trout (*Oncorhynchus mykiss*) to Japanese medaka (*Oryzias latipes*). *Environm. Toxicol. Chem.*, **13(9)**, 1393-1403.

Hodson, P.V., Castonguay, M., Couillard, C.M., Desjardins, C., Pelletier, E. & Macleod, R. (1994). Spatial and temporal variations in chemical contamination of American eels, *Anguilla rostrata*, captured in the estuary of the St. Lawrence River. *Can. J. Fish. Aquat. Sci.* **51**, 464-478.

Hontela, A., Dumont, P., Duclos, D. & Fortin, R. (1995). Endocrine and metabolic dysfunction in yellow perch, *Perca flavescens*, exposed to organic contaminants and heavy metals in the St. Lawrence River. *Env. Toxicol. Chem.* **14(4)**, 725-731.

Hontela, A., Rasmussen, J.B., Audet, C. & Chevalier, G. (1992). Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs and mercury. *Arch. Environ. Contam. Toxicol.* **22**, 278-283.

Hopkins, T.E. & Cech, J.E. (1992). Physiological effects of capturing striped bass in gill nets and fyke traps. *Trans. Am. Fish. Soc.* **121**, 819-822.

Kadlec, M.J. (1994). Bioconcentration of congener specific polychlorinated biphenyl (PCB) in rainbow trout (*Oncorhynchus mykiss*) exposed to the water column of the St. Lawrence River. Master's thesis, State University of N.Y. at Albany.

Laidley, C.W. & Leatherland, J.F. (1988). Cohort sampling, anaesthesia and stocking-density effects on plasma cortisol, thyroid hormone, metabolite and ion levels in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* **33**, 73-88.

Laurent, P. & Perry, S.F. (1990). Effects of cortisol on gill chloride cell morphology and ionic uptake in the freshwater trout, *Salmo gairdneri. Cell Tissue Res.* **259**, 429-442.

Laurent, P. & Perry, S.F. (1991). Environmental effects on fish gill morphology. *Physiol. Zool.* **64(1)**, 4-25.

Leatherland, J.F. (1993). Reflections on the thyroidology of fishes: from molecules to mankind. *Guelph Icthyology Reviews* **2**, 45-58.

Madenjian, C.P., Carpenter, S.R. & Rand, P.S. (1994). Why are PCB concentrations of salmonine individuals from the same lake so highly variable? *Can. J. Fish. Aquat. Sci.* **51(4)**, 800-807.

Makarewicz, J.C., Buttner, J.K. & Lewis, T.W. (1993). Uptake and retention of mirex by fish maintained on formulated and natural diets in Lake Ontario waters. *The Progressive Fish Culturist* **55**, 163-168.

McKim, J.M. & Erickson, R.J. (1991). Environmental impacts on the physiological mechanisms controlling xenobiotic transfer across fish gills. *Physiol. Zool.* **64(1)**, 39-67.

Mesa, M.G. (1994). Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile chinook salmon. *Trans. Am. Fish. Soc.* **123**, 786-793.

Moon, T.W., Foster, G.D. & Plisetskaya, E.M. (1989). Changes in peptide hormones and liver enzymes in the rainbow trout deprived of food for 6 weeks. *Can. J. Zool.* **67**, 2189-2193.

Nichols, D.J. & Weisbart, M. (1985). Cortisol dynamics during seawater adaptation of Atlantic salmon *Salmo salar*. *Am. J. Physiol.* **248**, R651-R659.

Nielsen, M.E., Boesgaard, L., Sweeting, R.M., McKeown, B.A. & Rosenkilde, P. (1994). Plasma levels of lactate, potassium, glucose, cortisol, growth hormone and triiodo-L-thyronine in rainbow trout (*Oncorhynchus mykiss*) during exercise at various levels for 24 h. *Can. J. Zool.* **72**, 1643-1647.

Pankhurst, N.W. & Dedual, M. (1994). Effects of capture and recovery on plasma levels of cortisol, lactate and gonadal steroids in a natural population of rainbow trout. *J. Fish Biol.* **45**, 1013-1025.

Pankhurst, N.W., Wells, R.M.G. & Carragher, J.F. (1992). Effects of stress on plasma cortisol levels and blood viscosity in blue mao mao *Scorpis violaceus* (Hutton), a marine teleost. *Comp. Biochem. Physiol.* **101A (2)**, 335-339.

Pickering, A.D. (1984). Cortisol-induced lymphocytopenia in brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* **53**, 252-259.

Pickering, A.D. (1993). Endocrine-induced pathology in stressed salmonid fish. *Fisheries Res.* **17**, 35-50.

Pickering, A.D. & Duston, J. (1983). Administration of cortisol to brown trout, *Salmo trutta* L., and its effects on the susceptibility to *Saprolegnia* infection and furunculosis. *J. Fish Biol.* **23**, 163-175.

Pickering, A.D. & Pottinger, T.G. (1985). Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white cell count. *J. Fish Biol.* **27**, 611-619.

Pickering, A.D. & Pottinger, T.G. (1989). Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiol. Biochem.* **7(1-4)**, 253-258.

Pickering, A.D. & Pottinger, T.G. & Christie, P. (1982). Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. *J. Fish Biol.* **20**, 229-244.

Pickering, A.D. & Pottinger, T.G. & Sumpter, J.P., Carragher, J.F. & LeBail, P.Y. (1991). Effects of acute and chronic stress on the levels of circulating growth hormone in the rainbow trout, *Oncorhynchus mykiss. Gen. Comp. Endocrinol.* **83**, 86-93.

Pottinger, T.G. & Mosuwe, E. (1994). The corticosteroidogenic response of brown and rainbow trout alevins and fry to environmental stress during a "critical period". *Gen. Comp. Endocrinol.* **95**, 350-362.

Redding, J.M., Patino, R. & Schreck, C.B. (1991). cortisol effects on plasma electrolytes and thyroid hormones during smoltification in coho salmon *Oncorhynchus kisutch. Gen. Comp. Endocrinol.* **81**, 373-382.

Rudneva-Titova, I.I. & Zherko, N.V. (1994). Effects of polychlorinated biphenyls on the activity of antioxidant enzymes and lipid peroxidation in muscle and liver of two Black Sea fish species. *Biochemistry (Moscow)* **59(1)**, 25-31.

Shrimpton, J.M. & Randall, D.J. (1994). Downregulation of corticosteroid receptors in gills of coho salmon due to stress and cortisol treatment. *Am. J. Physiol* **267** (*Regulatory Integrative Comp. Physiol.* **36**), R432-R438.

Simmons, D.J. (1971). Calcium and skeletal tissue physiology in teleost fishes. *Clin. Orthop.* **76**, 244-280.

St. Lawrence RAP team & St. Lawrence (Cornwall) Public Advisory Committee. (1994). Choices for cleanup: Deciding the future of a great river. *St. Lawrence River Remedial Action Plan Options Discussion Paper.*

Stow, C.E. (1995). Factors associated with PCB concentration in Lake Michigan salmonids. *Environ. Sci. Technol.* **29(2)**, 522-527.

Thuvander, A. & Carlstein, M. (1991). Sublethal exposure of rainbow trout (*Oncorhynchus mykiss)* to polychlorinated biphenyls: effect on the humoral immune response to *Vibrio anguillarium. Fish Shellfish Immunol.* **1**, 77-86.

Tomasso, J.R., Davis, K.B. & Parker, N.C. (1981). Plasma corticosteroid dynamics in channel catfish, *Ictalurus punctatus* (Rafinesque), during and after oxygen depletion. *J. Fish Biol.* **18**, 519-526.

Van der Boon, J., Van den Thillart, G.E.E.J.M. & Addink, A.D.F. (1991). The effects of cortisol administration on intermediary metabolism in teleost fish. *Comp. Biochem. Physiol.* **100A(1)**, 47-53.

Vijayan, M.M., Ballantyne, J.S. & Leatherland, J.F. (1990). High stocking density alters the energy metabolism of the brook charr, *Salvelinus fontinalis. Aquaculture* **88**, 371-381.

Vijayan, M.M., Ballantyne, J.S. & Leatherland, J.F. (1991). Cortisol-induced changes in some aspects of the intermediary metabolism of *Salvelinus fontinalis*. *Gen. Comp. Endocrinol.* **82**, 476-486.

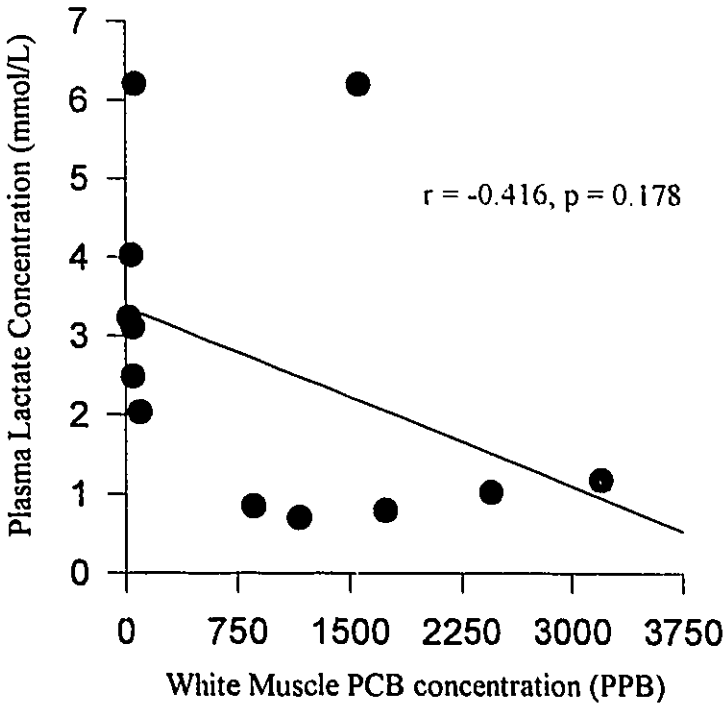
Williams, L.L., Giesy, J.P., DeGalan, N., Verbrugge, D.A., Tillitt, D.E., Ankley, G.T. & Welch, R.L. (1992). Prediction of concentrations of 2,3,7,8-tetrachloro-*p*-dioxin equivalents from total concentrations of polychlorinated biphenyls in fillets. *Environm. Sci. & Tech.* **26**, 1151-1159.

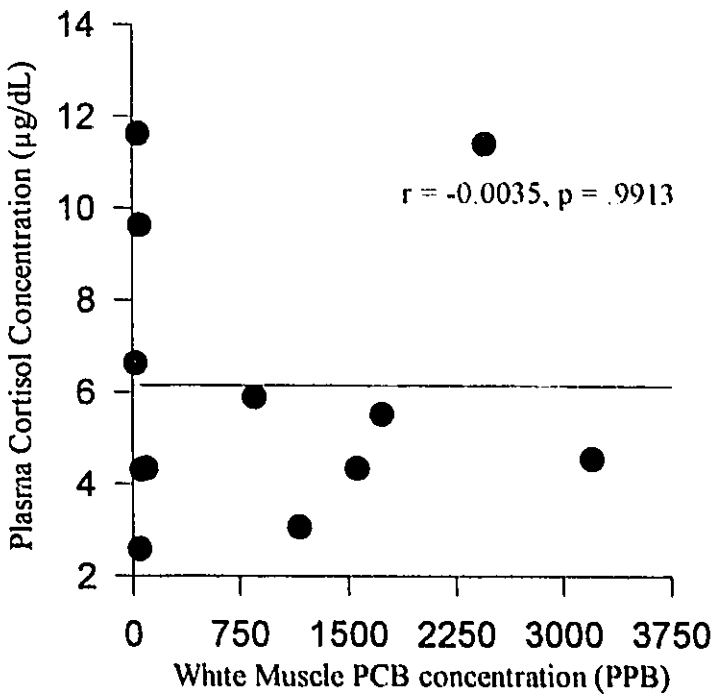
Wright, P.A., Perry, S.F. & Moon, T.W. (1989). Regulation of hepatic gluconeogenesis and glycogenolysis by catecholamines in rainbow trout during environmental hypoxia. *J. Exp. Biol.* **147**, 169-188.

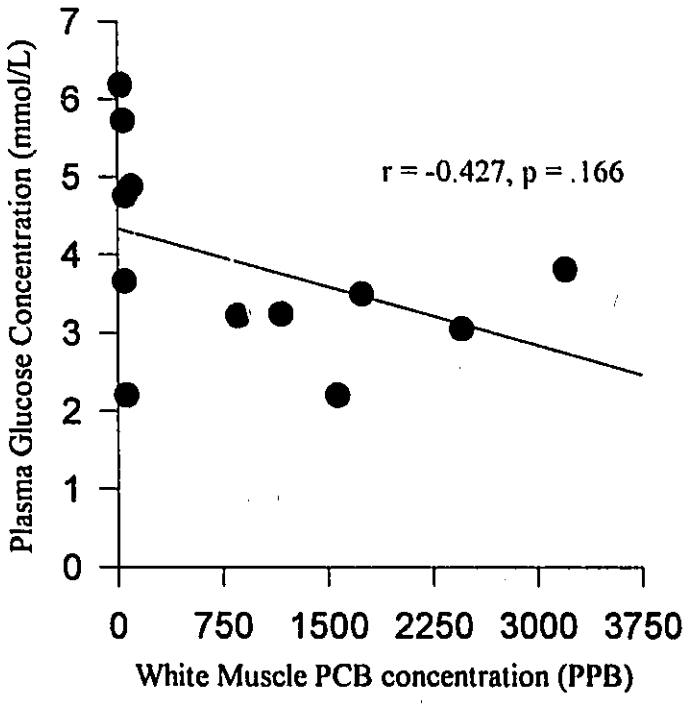
Zall, D.M., Fisher, D.M. & Garner, M.Q. (1956). Photometric determination of chlorides in water. *Anal. Chem.* **28**, 1665-1678.

Appendix A

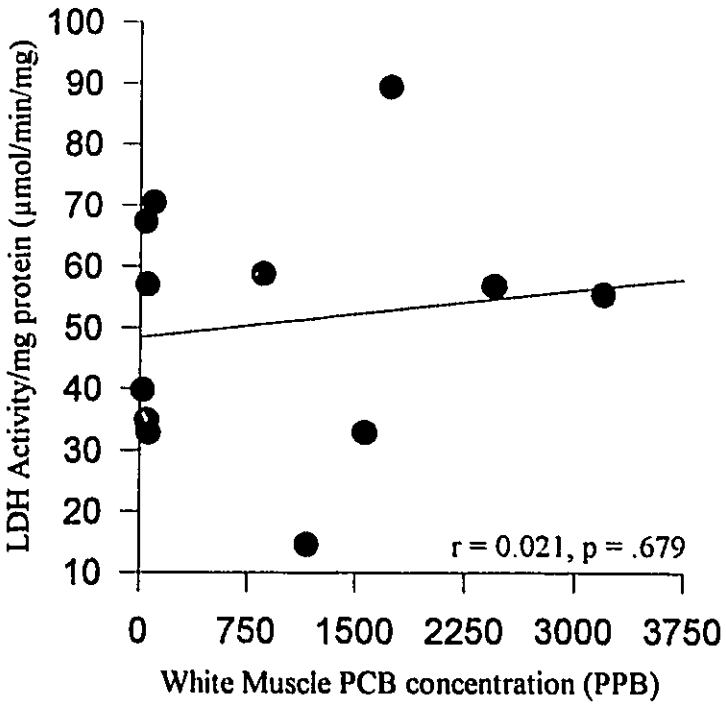
Fig. 1 Correlations between muscle PCB levels and plasma variables (A), liver enzyme activities (B), histological variables (C) and plasma ion concentrations (D). Correlation coefficients (r) and significance (p) are indicated on each graph. Significance was accepted at $p < .05$ and is indicated by an asterisk (*).

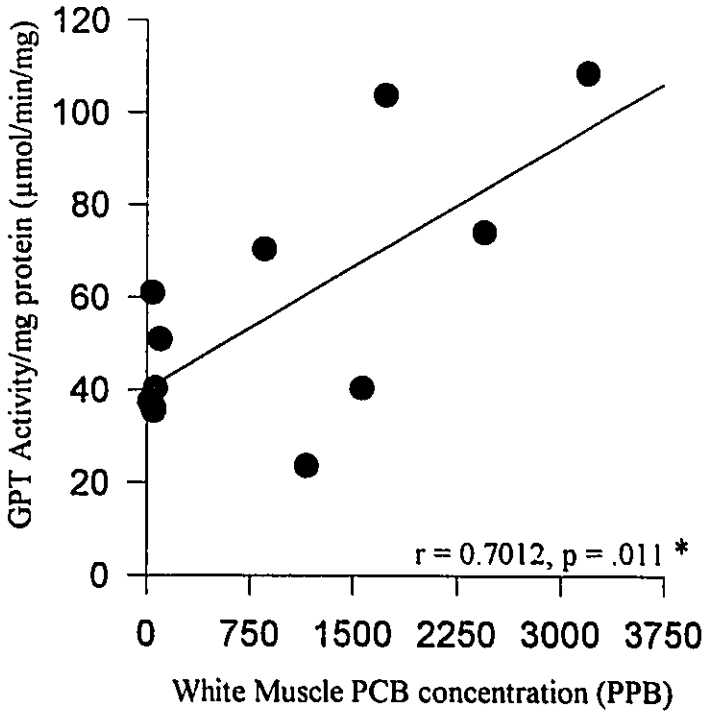

Plasma Lactate Concentration (mmol/L)
r = -0.416, p = 0.178
White Muscle PCB concentration (PPB)
0
750
1500
2250
3000
3750

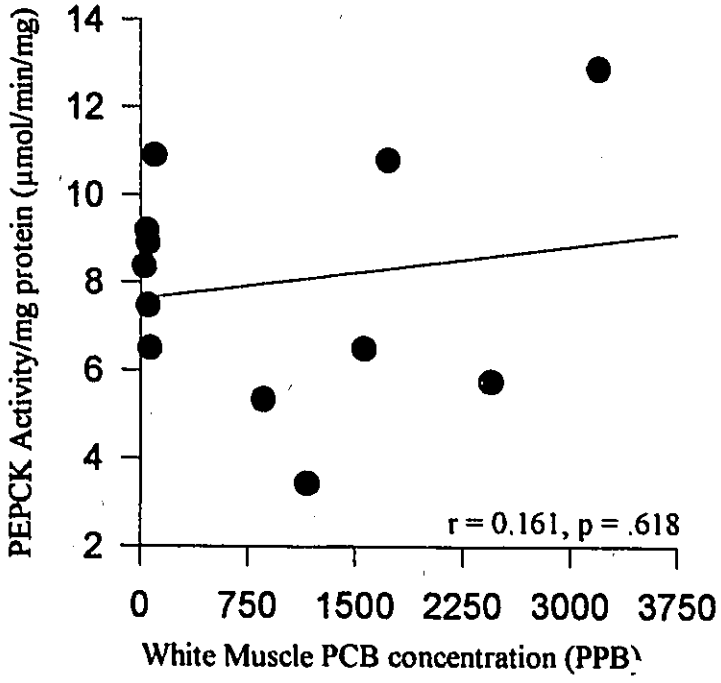

Plasma Cortisol Concentration (µg/dL)
r = -0.0035, p = .9913
White Muscle PCB concentration (PPB)

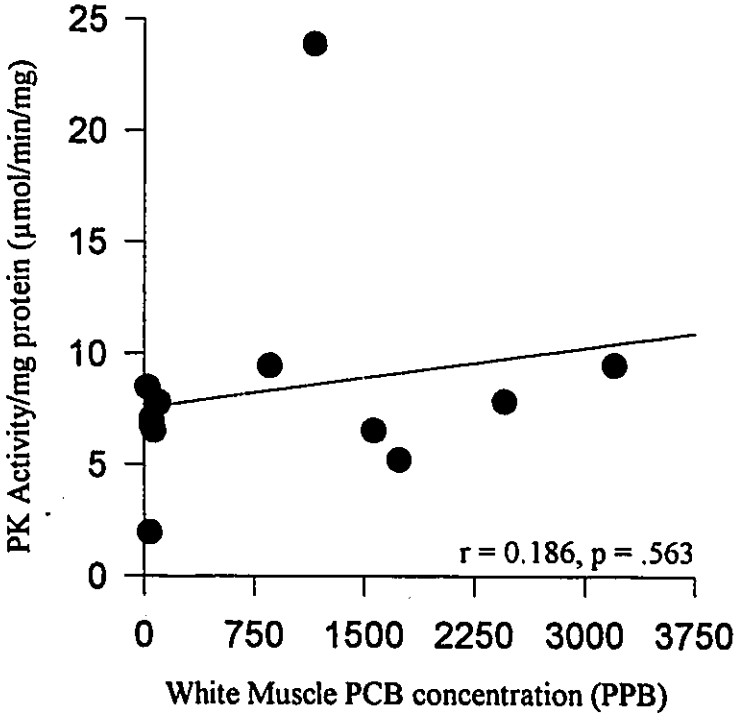

Plasma Glucose Concentration (mmol/L)
r = -0.427, p = .166
White Muscle PCB concentration (PPB)

A

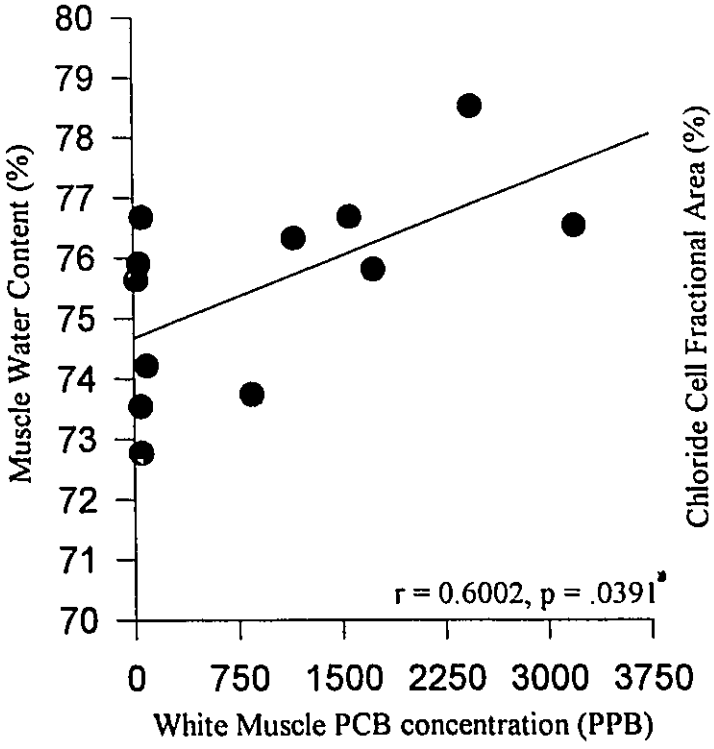

LDH Activity/mg protein (µmol/min/mg)
100
90
80
70
60
50
40
30
20
10
r = 0.021, p = .679
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

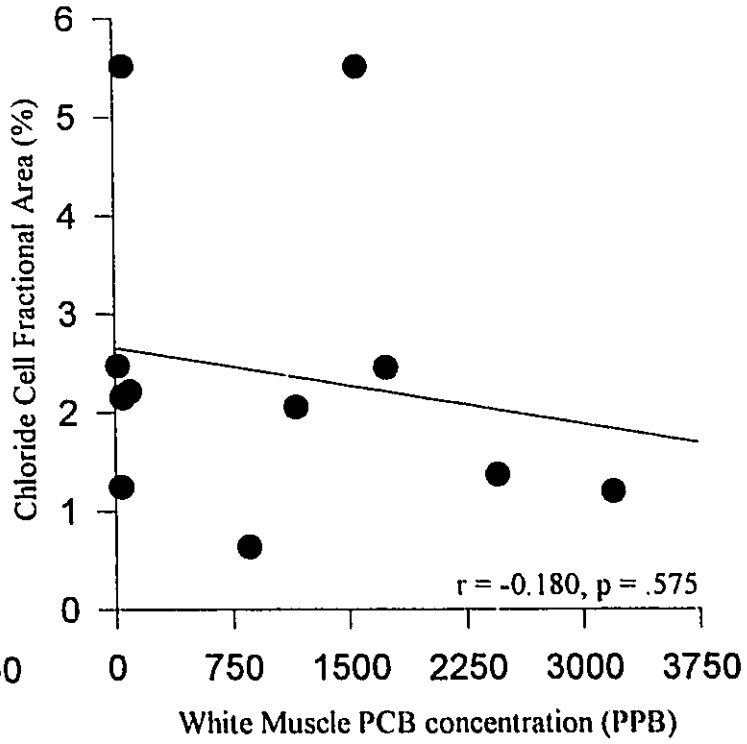

GPT Activity/mg protein (µmol/min/mg)
120
100
80
60
40
20
0
r = 0.7012, p = .011 *
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

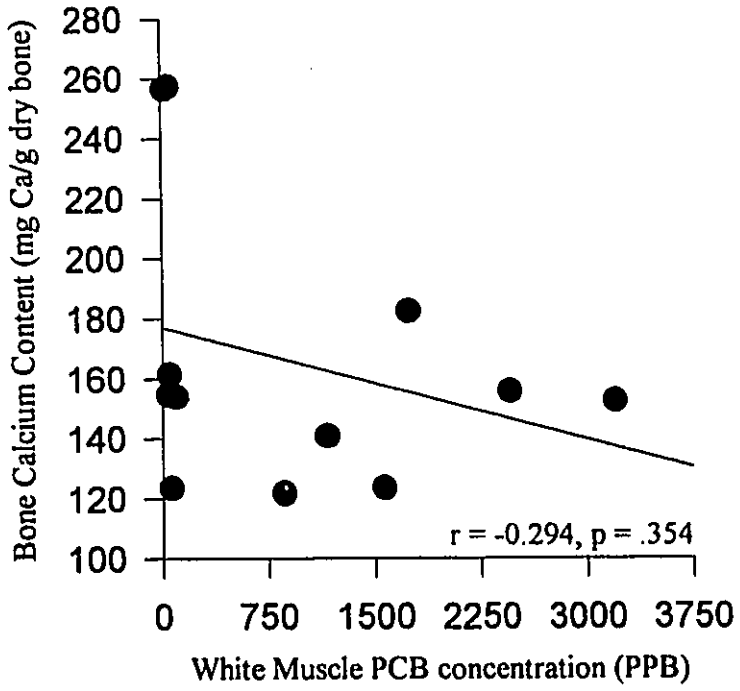

PEPCK Activity/mg protein (µmol/min/mg)
14
12
10
8
6
4
2
r = 0.161, p = .618
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)


PK Activity/mg protein (µmol/min/mg)
25
20
15
10
5
0
r = 0.186, p = .563
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

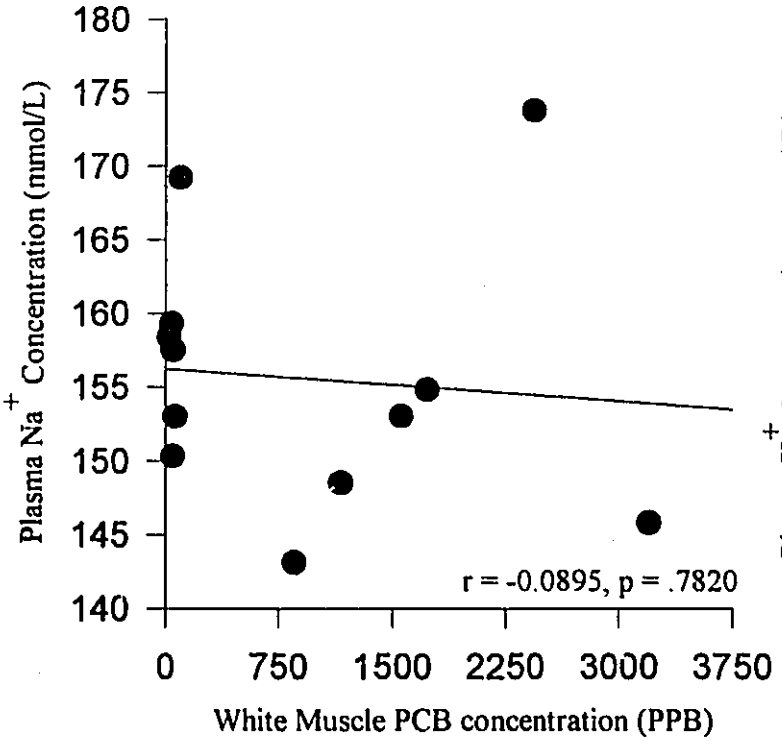
B

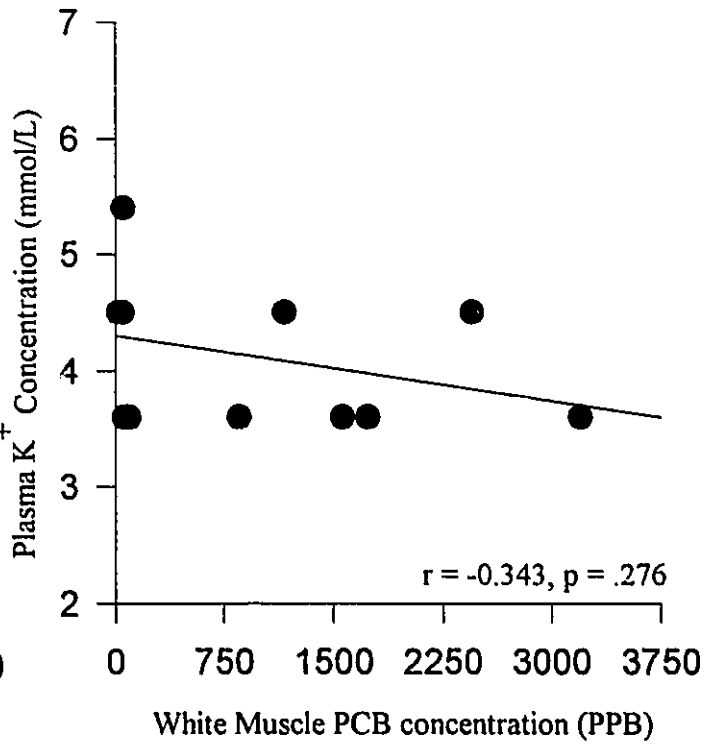

Muscle Water Content (%)
80
79
78
77
76
75
74
73
72
71
70
r = 0.6002, p = .0391*
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

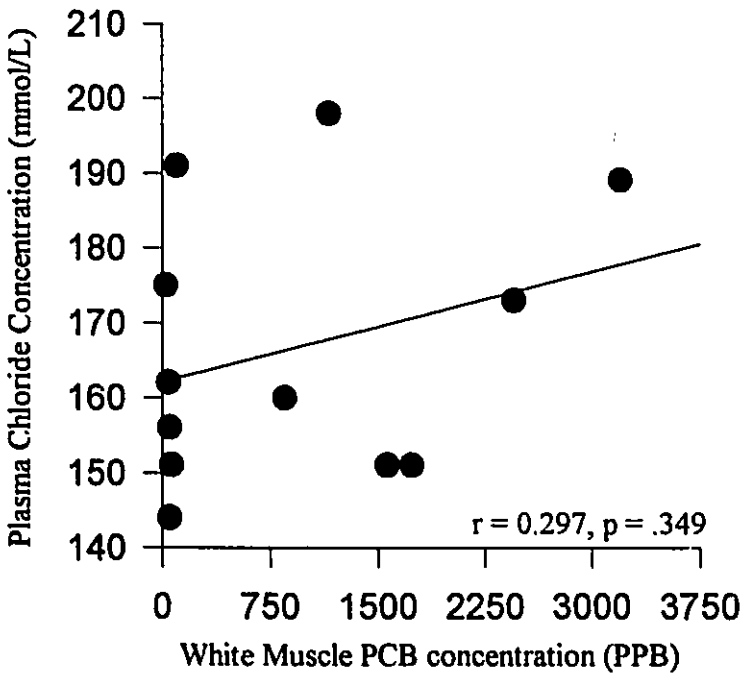

Chloride Cell Fractional Area (%)
6
5
4
3
2
1
0
r = -0.180, p = .575
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

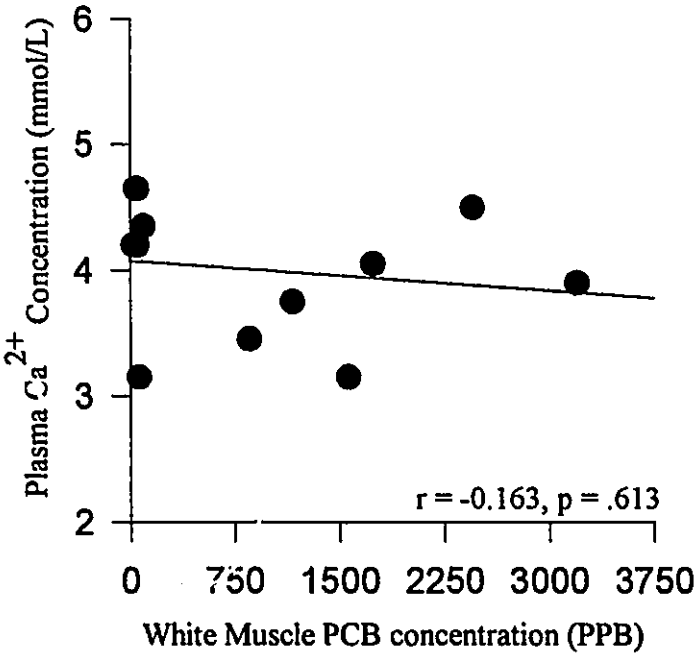

Bone Calcium Content (mg Ca/g dry bone)
280
260
240
220
200
180
160
140
120
100
r = -0.294, p = .354
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

C


Plasma Na^+ Concentration (mmol/L)
180
175
170
165
160
155
150
145
140
r = -0.0895, p = .7820
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)


Plasma K^+ Concentration (mmol/L)
7
6
5
4
3
2
r = -0.343, p = .276
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)


Plasma Chloride Concentration (mmol/L)
210
200
190
180
170
160
150
140
r = 0.297, p = .349
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)


Plasma Ca^{2+} Concentration (mmol/L)
6
5
4
3
2
r = -0.163, p = .613
0
750
1500
2250
3000
3750
White Muscle PCB concentration (PPB)

D