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Exposure to sublethal levels of PCB-126 impacts fuel metabolism and swimming performance in rainbow trout☆

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ABSTRACT

Polychlorinated biphenyls (PCBs) are recognized physiological stressors to fish which over time may impair individual performance and perhaps fitness by inducing changes that could have population-level consequences. PCB-126 (3,3′,4,4′,5-pentachlorobiphenyl) accumulates in lipids and can subsequently be released into the bloodstream during periods of high activity that involve the mobilization of stored fuels to meet with increasing energy demands. The goal of this study was to determine if a sublethal exposure to PCB-126 altered the content of tissue energy supplies (carbohydrates, proteins, amino acids, triglycerides) and impaired swimming performance as well as oxygen consumption in rainbow trout (*Oncorhynchus mykiss*). Trout were injected intraperitoneally with a single Low (100 μg kg^{-1}) or High (400 μg kg^{-1}) dose of PCB-126 then swimming performance and metabolic rates from 1 to 9 days post-injection were compared to Control (non-dosed) fish. Liver ethoxyresorufin-O-deethylase (EROD) activity was assessed as an indication of PCB-126 intoxication while plasma and white muscle tissue metabolites were analyzed as an index of physiological disturbance. Swimming performance, assessed using two successive modified critical swimming speed (U_{crit}) tests, was highest for fish in the High PCB-126 treatment; however, their initial condition factor (K) was also higher, largely due to their greater body mass. Trout in the High and Low PCB-126 treatments exhibited impaired recovery following intense exercise as they swam comparatively poorly when provided a second challenge. PCB-exposed fish exhibited reduced spleen somatic indices as well as muscle glucose and glycogen contents; whereas plasma cortisol and glucose levels were elevated, indicating higher metabolic costs during recovery and muscle restoration. Overall, this research provides insights into the sublethal effects of a toxic organic compound on swimming performance in trout.

1. Introduction

Aquatic environments serve as sinks for anthropogenically-produced toxic compounds (Van der Oost et al., 2003). Evidence exists that the deteriorating conditions of these ecosystems are resulting in species loss and contributing to the decline in freshwater biological diversity (Stabeneau et al., 2008; Zhou et al., 2010). Fish provide a wide range of ecosystem services in aquatic ecosystems (Holmlund and Hammer, 1999) and thus it is important to investigate the impacts of these contaminated environments on the health of fish. Knowledge generated by such studies could reveal mechanistic pathways by which stress acting on individuals can alter population-level processes (Calow and Forbes, 1998) which might inform risk assessment screening (Calow and Forbes, 2003) and facilitate regulatory actions (Russell and Gruber, 1987).

Freshwater fish often experience both natural and anthropogenically-induced conditions that result in fluctuating energy availability (Bureau et al., 2002; Lapointe et al., 2014). Given that energy is the currency of life, the ability of fish to acquire, transform, store and efficiently use energy is essential for their survival (Beyers et al., 1999a). It is well documented that exposure to stressors including pollutants can impact tissue biochemical and physiological responses by altering energy allocation and disrupting various aspects of metabolism from whole-organism responses (e.g., metabolic rates, swimming performance) to specific tissue responses (e.g., metabolite availability, enzyme activity) which are energy-demanding and costly in terms of metabolic resources (Beyers et al., 1999a,b; Calow, 1989; Gourley and Kennedy, 2009; Iwata et al., 1994; McKenzie et al., 2007; Wendelaar Bonga, 1991; 1997). Over time, such responses can decrease individual performance and perhaps fitness by inducing changes in foraging, migration and escape behaviors (Hopkins et al., 2003; Kolok et al., 2001;

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McKenzie et al., 2007; Stead et al., 2005). These effects could subsequently translate to the population level in terms of reproductive capacity (Calow and Forbes, 1998; De Coen and Janssen, 2003; DuRant et al., 2007).

Swimming performance is considered a crucial factor in determining the survival or fitness of a fish species within its environment (Claireaux et al., 2007; Nelson et al., 2002). Swimming involves the coordinated efforts of numerous physiological processes across multiple levels of biological organization (e.g., molecule, cell, organ, body systems); hence swimming performance can be viewed as a reliable index of general health and stress in fish (Kolok, 1999; 2001). Locomotion is required for most vital activities including foraging and escaping predators, as well as for reproductive behaviors including spawning, migrations, habitat shifts and dispersal (Plaut, 2001). Swimming performance is generally assessed using a critical swimming speed (U_{crit}) test; however, numerous modifications of the U_{crit} test have been used in the literature as reviewed by Farrell (2008). Using these tests, repeat swimming performance measurements can then be compared by using recovery ratios (RRs), which are an indication of the individual's capacity to return to its full swimming ability after an initial bout of fatiguing exercise (Jain et al., 1998; Farrell, 2008).

PCB-126 is a coplanar non-*ortho* substituted polychlorinated biphenyl (PCB) congener commonly found at high environmental concentrations (Bhavsar et al., 2007) as a result of its long half-life, high assimilation efficiency, and high bioaccumulation capacity (Giesy and Kannan, 1998). PCBs are industrial contaminants that are no longer produced but are still considered among the most widespread and hazardous synthetic pollutants in the environment due to their highly non-reactive nature as well as their resistance to chemical and biological degradation (Buckman et al., 2004). These toxic organic compounds are recognized stressors to fish as they have the potential to alter energy allocations by disrupting various aspects of their metabolism (Beyers et al., 1999a,b).

This study examined the effects of a polychlorinated biphenyl congener, PCB-126 (3,3′,4,4′,5-pentachlorobiphenyl), on rainbow trout (*Oncorhynchus mykiss*) by testing the hypothesis that PCB-126 exposure affects rainbow trout energy allocation and swimming performance. This should be demonstrated by a decrease in tissue fuel supplies, critical swimming speed, recovery ratios and metabolic rates with an increasing concentration of PCB-126 compared to non-exposed fish.

2. Materials and methods

2.1. Fish

Adult female rainbow trout of approximately 300 g were obtained from Linwood Acres Trout Farms Ltd (Campbellford, ON, Canada) and placed in a 1300-L re-circulating fiberglass holding tank located in the uOttawa Aquatic Care Facility. Salmonids are the target species of numerous swim performance studies for practical reasons including their availability and maintenance in the laboratory. These species also generally perform well in steady-state and burst exercise throughout laboratory studies (Moyes and West, 1995). In addition, salmonids are among the most economically valuable species on the planet (Holmlund and Hammer, 1999) and many studies (e.g., Brown et al., 2002; Buckman et al., 2004; Nault et al., 2012) demonstrate that rainbow trout respond to a PCB exposure by altering metabolic demands. All fish were acclimated to laboratory conditions for at least 3 weeks before use. All tanks received dechloraminated City of Ottawa tapwater at 13 °C which was monitored daily for pH (7.4 ± 0.2), water hardness (85.5–102.6 mg L^{-1} $CaCO_3$) and ammonia (<0.05 mg L^{-1}) levels to ensure that these parameters remained within the range of optimal conditions for rainbow trout. Photoperiod was set to a 12 h light:dark cycle. The tank systems were operated at a recycle rate where 12% of the circulating flow was continually replaced. This recycled water was denitrified by biofiltration and re-oxygenated or degassed by ambient air gravity pass-through columns. Experiments were all conducted between August 1 and November 15. All experiments were approved by the University of Ottawa Animal Care Protocol Review Committee and adhered to published guidelines of the Canadian Council on Animal Care for the use of animals in teaching and research.

2.2. Experimental design

PCB-126 (99% purity) was obtained from Ultra Scientific (North Kingstown, RI, USA); the entire contents of the vial (5 mg) was diluted in 1 mL iso-octane and sonicated for 30 min in an ultrasonic bath. Three solutions were prepared in warmed coconut oil: Control (800 μL coconut oil + 200 μL iso-octane), Low (100 μg kg^{-1} or 960 μL coconut oil + 40 μL iso-octane/PCB-126; 0.309 μmol kg^{-1}) and High (400 μg kg^{-1} or 840 μL coconut oil + 160 μL iso-octane/PCB-126; 1.24 μmol kg^{-1}) treatments. These concentrations were based on values previously found in wild fish or used in other aquatic studies for this congener (Brown et al., 2002; Luk, 2000; MOE, 2009; Nault et al., 2012; WHO, 2003).

The fish holding system consisted of five 115-L fiberglass tanks. Each tank held a group of nine fish (3 per PCB treatment) that were randomly selected and distributed. Fish of a given tank were processed according to their post-injection swim period: 1, 3, 5, 7, or 9 days. A total of 45 fish were thus used in this study. In preparation for the intraperitoneal (IP) injection, feeding was discontinued for 24 h prior to the IP injection and was withheld throughout the experimental period. This ensured a post-absorptive state during the swimming challenges (Farbridge and Leatherland, 1992) and allowed for examination of the role played by body energy stores to meet energy demands during swimming. Each individual fish was lightly anesthetized (benzocaine, 0.01 g mL^{-1}) while initial body mass (g) and total length (cm) from the tip of the snout to the longest part of the caudal fin were measured. Each fish was then IP injected once using a 1 mL syringe (26 G 3/8 in. needle) with the appropriate treatment solution. IP injection is a standard method of toxicant exposure to ensure that the contaminant is contained within the fish and to minimize contamination of holding tanks. The volume injected was proportional to fish mass (i.e., 100 μL 0.2 kg^{-1}) in order to yield a similar body burden of toxicant. Each fish was identified using a distinctive vinyl anchor tag placed into the muscle mass just below the dorsal fin. The fish were returned to their respective experimental holding tanks until their prescribed swim period.

2.3. Critical swimming speed (U_{crit}) and recovery ratio (RR)

Fish were removed from their experimental holding tank and placed into a 56-L intermittent flow, modified Blazka-type respirometer. Fish were given an overnight acclimation period to the tunnel at a nominal and constant water velocity of 10 cm s^{-1} (Kieffer, 2010; Plaut, 2001) before the start of the swim trial. All swim trials were conducted between 0900 and 1800 h to account for diurnal variations in swimming performance.

Swim performance was assessed using a modified critical swimming speed protocol (Beamish, 1978; Brett, 1964). Each fish was subjected to two subsequent U_{crit} tests where the water velocity was increased by 10 cm s^{-1} every 5 min under constant water flow until the fish could not remove itself from the rear grid and resume swimming within 5 s. These parameters were chosen based on preliminary trials for the same fish size. After both swim trials, the maximal attained swimming speed was calculated using the equation $U_{crit} = u_i + (t_i\, t_{ii}^{-1} \times u_{ii})$, where u_i = highest velocity maintained for the entire swimming interval (cm s^{-1}), t_i = time fish swam at fatigue velocity (min), t_{ii} = prescribed interval time (min) and u_{ii} = velocity increment (cm s^{-1}).

The U_{crit} tests were separated by a 1 h recovery period in which the fish remained within the respirometer, swimming at the nominal velocity of 10 cm s^{-1} and constant water flow. The recovery ratio was calculated by dividing U_{crit2} by U_{crit1} (Jain et al., 1998), where a ratio near 1

indicates an unchanged swimming performance. This value is unit-less meaning that any size-related differences between individuals from the various treatments do not influence the RR.

2.4. Oxygen consumption rates (M_{O2})

Rates of oxygen consumption were calculated from the decline in dissolved oxygen concentration while the tunnel was placed in stop-flow mode and at a nominal water velocity of 10 cm s^{-1}. A routine metabolic rate (RMR) was assessed in the morning following the overnight acclimation period, followed by a first maximum metabolic rate (MMR_1) measurement immediately upon completion of U_{crit1}. A M_{O2} measurement was also performed following the 1h recovery period separating U_{crit1} and U_{crit2} ($M_{O2recovery}$), and a second MMR_2 was calculated immediately following completion of the U_{crit2} swim challenge. Dissolved oxygen values were recorded every 5 min for a period of 30 min using a YSI-85 dissolved oxygen meter, and metabolic rates were calculated as: M_{O2} = [ΔO_2 concentration (mg L^{-1}) × respirometer respirometer volume adjusted for fish volume (L)] / [fish mass (kg) × time (h)]. An oxygenation column was used to ensure that the dissolved oxygen level within the respirometer was maintained at or above 80% saturation; dissolved oxygen values recorded throughout the experiments ranged from 251 to 311 μmol L^{-1} (13 °C).

2.5. Somatic indices and visual examination

All fish were sacrificed within 5 min following the measurement of the final metabolic rate after U_{crit2} with a lethal dose of benzocaine (>0.1 g mL^{-1}). Fish final body mass (g) and total length (cm) were recorded and the condition factor (K) was calculated [K = 100 × body mass (g) × $length^{-3}$(cm)]. Liver, heart and spleen masses were measured and tissue-somatic indices for liver (HSI), heart (HESI), and spleen (SSI) calculated [Index = 100 × organ mass × $body\ mass^{-1}$]. An external and internal visual examination was conducted to assess general fish health but no apparent differences existed between groups.

2.6. Plasma analysis

Blood samples were collected immediately following anesthesia by caudal vessel puncture using a syringe coated with ethylenediaminetetraacetic acid (EDTA) to reduce coagulation. Samples were placed in small 1 mL centrifuge tubes kept on ice, centrifuged for 5 min at 14,000 × *g* and the plasma obtained was divided into aliquots and frozen at −80 °C until analyses were conducted. Glucose concentrations were measured enzymatically according to Moon et al. (1999). Amino acids were analyzed according to the methods described by Troll and Cannan (1953) while lactate was determined according to the Gutmann and Wahlefeld (1974) protocol. Total protein levels were determined using the bicinchoninic acid (BCA) assay (Sigma Aldrich, MO, USA), cortisol levels by a radioimmunoassay RIA kit (MP Biomedicals, CA, USA) and triglyceride levels were analyzed using the TECO Diagnostics Triglyceride GPO reagents all according to the manufacturer's instructions.

2.7. White muscle tissue analysis

White muscle tissue frozen in liquid nitrogen was ground to a powder using a mortar and pestle then sonicated in 4 vol/w 70% perchloric acid (PCA) for 1–2 min, centrifuged at 14,000 × *g* for 5 min. The supernatant was collected for subsequent assays. Glucose, protein and amino acid levels were estimated as mentioned above while glycogen levels were measured using amyloglucosidase according to Keppler and Decker (1975).

2.8. EROD activity

Hepatic EROD (ethoxyresorufin-O-deethylase) activities were assessed as a sensitive indicator of PCB exposure (Whyte et al., 2000). Fresh liver tissue was minced with scissors, homogenized in ice-cold homogenization buffer (10 mM HEPES, 1 mM EDTA, 10% (*v*/*v*) glycerol, pH 7.4) and centrifuged at 9000 × *g* for 20 min at 4 °C. The supernatant was removed and re-centrifuged at 100,000 × *g* for 60 min using a Beckman L7-65 ultracentrifuge at 4 °C. The supernatant was discarded and the microsomal pellet was re-suspended in buffer (50 mM Tris, 1 mM EDTA, 10% (*v*/*v*) glycerol, pH 7.4), aliquoted and stored at −80 °C until used. The assay was conducted using a plate reader and 48-well plates as described in Nault et al. (2012). Reactions (total volume 250 μL) were initiated by adding (final concentration) 0.5 mM NADPH to 2 μM 7-ethoxyresorufin (in 1.5% methanol, 98.5% Tris–HCl, pH 7.2), and 50 μg microsomal protein; reactions were terminated after 15 min using acetonitrile and resorufin production was compared against a resorufin standard curve fluorometrically at 530 nm excitation and 590 nm emission (SpectraMax GeminiXS; Molecular Devices, Sunnyvale, CA, USA). Activities are defined as pmol resorufin produced per min per mg protein (determined as above).

2.9. Statistical analysis

Statistical analysis of the data was performed using IBM SPSS Statistics version 22 (IBM Corporation, Armonk, NY). All tests used the $p < 0.05$ significance level. The normality and homogeneity of variance assumptions were verified using the Shapiro–Wilk and Levene Median tests, respectively. A two-way ANOVA was conducted to examine the effect of days post-injection (DPI; 1, 3, 5, 7, 9 days), treatment (Control, Low, High), and their interaction on fish mass, total length, K, HSI, HESI, SSI, EROD activity, as well as plasma and white muscle metabolites. A two-way ANCOVA was performed to examine the effect of the same fixed factors (DPI, Treatment, DPI * Treatment) on U_{crit1}, U_{crit2}, RMR, MMR_1, $M_{O2recovery}$, and MMR_2, with total length as the covariate for U_{crit1} and U_{crit2} and $\log_{10}$ fish mass as the covariate for the metabolic rates (which were also $\log_{10}$ transformed for the purpose of this analysis). For both analyses, when a significant effect of DPI or Treatments was observed, *a posteriori* comparisons were performed using the Holm Sidak test. For variables where no significant effect of DPI * treatment interaction were found, the analyses were performed without the interaction term in the models. For variables where no significant effect of DPI was found, data are presented as estimated marginal Means ± S.E.M. for each treatment (i.e. the post-injection time periods were ignored and all individuals of a given treatment were pooled). When the assumptions were violated (i.e. RR, plasma cortisol, as well as white muscle glucose, glycogen, and protein), the effect of DPI on a variable, for a given treatment group, was assessed using a Kruskal Wallis test. If DPI had no significant effect, the individuals of a given treatment group were pooled, and differences between treatment groups were subsequently examined using a Kruskal Wallis test. *A posteriori* comparisons were performed using the Tukey HSD test. Temporal trends for variables where individuals of all DPI were pooled are presented as Supplementary data. Comparisons between U_{crit1} and U_{crit2}, as well as between MMR_1 and MMR_2, and between RMR and $M_{O2recovery}$, for fish of a given treatment group, were performed using paired t-tests.

3. Results

3.1. Body parameters and somatic indices

Mean total length of fish differed between Low and High treatment groups, whereas fish in the High treatment group had a larger body mass and therefore condition factor than those of fish in the other two treatments (Table 1).

Table 1
Morphometric characteristics and tissue-somatic indices of rainbow trout for the three treatment groups (Control, Low, High). Values are shown as Mean ± S.E.M. (n = 15 for each group; except for mass of Control and Low where n = 14, and total length of High where n = 13). K: condition factor; SSI: spleen somatic index; HESI: heart somatic index (see Materials and methods for definition). As no significant effects of days post-injection existed (see Supplementary table S1), individual data for each treatment group were combined. Different letters indicate a significant difference ($p < 0.05$) between treatments. The absence of letters indicates that all treatment groups were statistically similar.

	Control	Low	High
Mass (g)	287.1 ± 5.2^{a}	294.2 ± 5.2^{a}	319.4 ± 5.0^{b}
Total length (cm)	31.0 ± 0.2^{ab}	30.7 ± 0.2^{a}	31.5 ± 0.2^{b}
K	1.13 ± 0.03^{a}	1.13 ± 0.03^{a}	1.24 ± 0.03^{b}
SSI	0.18 ± 0.01^{a}	0.17 ± 0.01^{a}	0.11 ± 0.01^{b}
HESI	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01

A significant decrease was noted in the value of SSI in the High group but not HESI (Table 1). The HSI was significantly different in the High-dosed group, while this index significantly increased between days 1 and 9 post-injection (Table 2). This increase in HSI was 107, 120 and 140% for the Control, Low and High treatment groups, respectively.

3.2. Swimming performance tests

Swimming performance during the first challenge tended to increase with PCB-126 exposure ($F = 11.151$; $p < 0.001$) (Fig. 1). The U_{crit1} performance increased by 13 and 38% compared to that of Controls for fish exposed to the Low and High treatments, respectively. No influence of treatment on swimming performance was observed during the second trial ($F = 3.047$; $p = 0.059$) (Fig. 1). The U_{crit} performance of all three treatment groups was higher during the first swim challenge than the second trial (Control: $t = 3.268$, $p = 0.006$; Low: $t = 7.846$, $p < 0.0001$; High: $t = 15.891$, $p < 0.0001$).

The recovery ratio, which is size independent, decreased with increasing levels of PCB-126 ($H = 37.493$; $p < 0.0001$); RR was 7 and 16% lower for fish of the Low and High treatments compared to that of the Control treatment, respectively (Fig. 1).

3.3. Oxygen consumption rates

Routine metabolic rate increased with increasing level of PCB exposure ($F = 18.385$; $p < 0.0001$) (Fig. 2). Mean RMR increased by 12 and 27% for fish exposed to the Low and High treatments, respectively, compared to that of Controls.

Maximum metabolic rate determined following U_{crit1} was higher in fish of the High treatment compared to that of fish from the Control treatment ($F = 7.647$; $p = 0.002$) (Fig. 2). Mean MMR_1 was 4 and 11% higher for fish from the Low and High treatments, respectively, compared to MMR_1 measured for fish of the Control group. Treatment had no effect on MMR_2 ($F = 2.187$; $p = 0.13$). For fish of the three treatments, MMR_2 was significantly higher than MMR_1 ($p \leq 0.001$).

Metabolic rate determined during the recovery period between the two U_{crit} challenges increased following exposure to PCB-126 ($F = 11.082$; $p < 0.0001$) (Fig. 2). Mean $M_{O2recovery}$ for fish from the Low and High treatments was respectively 11 and 22% higher than that of fish from the Control group. For fish of the three treatments, $M_{O2recovery}$ was significantly higher than RMR ($p < 0.001$).

Table 2
Hepato-somatic index (HSI) of rainbow trout for the three treatment groups (Control, Low, High) determined 1, 3, 5, 7, and 9 days post-injection (DPI). Values are shown as Mean ± S.E.M. (n = 3; except for HSI High dose 7 days post-injection where n = 2). HSI was affected by treatment ($F = 10.585$; $p < 0.001$) and DPI ($F = 3.504$; $p = 0.019$). Overall, HSI was significantly lower in rainbow trout of the High treatment compared to those of the Control and Low treatments. Different letters indicate a significant difference ($p < 0.05$) between DPI or treatments.

HSI	Days post-injection				
	1^{a}	3^{b}	5^{ab}	7^{ab}	9^{b}
Control[a]	0.90 ± 0.06	1.01 ± 0.06	0.96 ± 0.06	1.01 ± 0.06	0.96 ± 0.06
Low[a]	0.85 ± 0.06	1.02 ± 0.06	0.99 ± 0.06	0.92 ± 0.06	1.02 ± 0.06
High[b]	0.65 ± 0.06	0.82 ± 0.06	0.85 ± 0.06	0.81 ± 0.07	0.91 ± 0.06

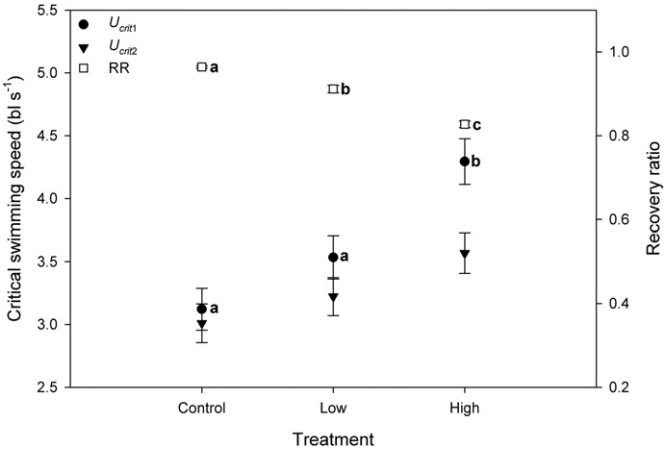

Fig. 1. Critical swimming speed obtained during two successive trials (U_{crit1} and U_{crit2}), and recovery ratios (RRs) of rainbow trout of the three treatment groups (Control, Low, High). Data for U_{crit1} and U_{crit2} are shown as estimated marginal Means ± S.E.M. adjusted for fish length using the common value of the covariate (31.05 cm). Data for RRs are shown as Mean ± S.E.M. Different letters indicate statistical significance ($p < 0.05$). The absence of letters indicates no significant difference. See Supplementary table S2 for individual data.

3.4. Plasma and white muscle metabolites

Estimated plasma metabolites were relatively constant across treatments and no effect of DPI or DPI * treatment interaction existed. However, glucose and cortisol levels both increased with increasing PCB-126 dose ($H = 37.3$ and 27.836 for cortisol and glucose, respectively; $p < 0.0001$). Cortisol was 92 and 229% higher in fish of the Low and High treatment groups compared to Controls, respectively, whereas fish exposed to the Low and High PCB treatments experienced an increase of 15 and 32% in glucose, respectively, in comparison with the concentration measured in fish of the Control group (Table 3). Plasma

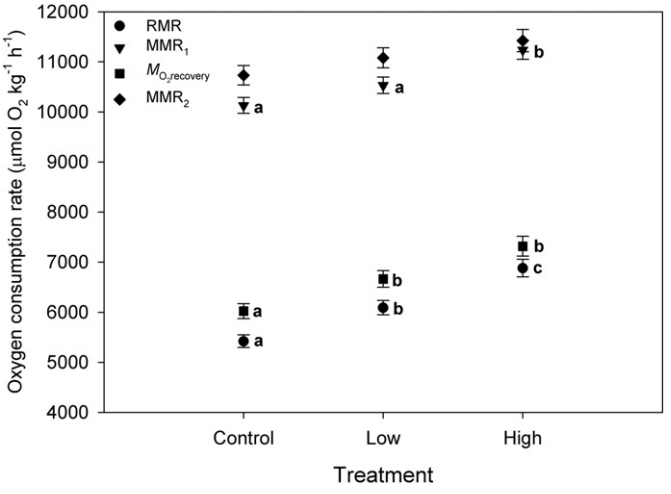

Fig. 2. Routine metabolic rate (RMR), M_{O2} during the recovery period separating U_{crit1} and U_{crit2} ($M_{O2recovery}$), and maximum metabolic rates (MMR_1 and MMR_2) of rainbow trout of the three treatment groups (Control, Low, High). Data are presented as estimated marginal Means ± S.E.M. adjusted for fish mass using the common value of the covariate (299.36 g). Different letters indicate statistical significance ($p < 0.05$). The absence of letters indicates no significant difference. See Supplementary table S3 for individual data.

Table 3
Plasma parameters measured in rainbow trout of the three treatment groups (Control, Low, High). Values are shown as Mean ± S.E.M.; n = 15 (except for cortisol in High group where n = 13). As no significant effects of days post-injection existed (see Supplementary table S4), individual data for each treatment group were combined. Different letters indicate a significant difference ($p < 0.05$) between treatments. The absence of letters indicates that all treatment groups were statistically similar.

	Control	Low	High
Cortisol (ng ml^{-1})	6.3 ± 0.5[a]	12.1 ± 1.6[b]	21.6 ± 1.6[c]
Glucose (mmol L^{-1})	5.3 ± 0.1[a]	6.1 ± 0.1[b]	7.0 ± 0.1[c]
Lactate (mmol L^{-1})	2.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.2
Total protein (mg ml^{-1})	37.4 ± 1.2	36.3 ± 1.2	36.8 ± 1.2
Amino acids (mg ml^{-1})	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.01

triglyceride levels did not significantly differ between treatments but did decrease between days 1 and 9 post-injection (Table 4).

No significant effect of DPI or interactions between DPI * treatment existed for white muscle tissue metabolites, but significant variation between treatments was observed for all tested metabolites (Table 5). Metabolite levels in PCB-injected fish were significantly below those of the Controls ($p < 0.0001$), with the exception of amino acids that increased with PCB exposure. Fish exposed to the Low and High PCB treatments experienced a respective decrease of 16 and 49% in glucose, 11 and 24% in glycogen, and 28 and 41% in total protein, in comparison to fish of the Control group. The increase in amino acids was 8 and 13% for fish of the Low and High treatment groups, respectively, compared to the level measured in Controls (Table 5).

3.5. EROD activity

Liver EROD activities increased with increasing PCB exposure dose ($H = 39.13$; $p < 0.0001$). EROD activities for the Control, Low, and High treatments were 2.2 ± 0.17, 208.1 ± 8.44, and 475.8 ± 11.6 pmol min^{-1} mg $protein^{-1}$, respectively. As noted above, no time dependent changes in EROD activities were observed over the 9 day experimental period (see Supplementary table S6).

4. Discussion

The purpose of this study was to determine if a sublethal exposure to PCB-126 altered rainbow trout energy allocation, and consequently compromised swimming performance and exercise recovery relative to Control fish. The significant increase in EROD activity following exposure to PCB-126 confirmed that the fish responded to the pollutant by activating liver detoxification enzymes (i.e., CYP1A). Thus the trout responded to the PCB-126 injection, allowing us to conclude that changes observed in physiological parameters, swimming performance and metabolic rate are a response to PCB exposure. What was particularly remarkable was that despite somewhat small samples sizes, a number of notable significant differences were observed which reflects the fact that effect sizes were reasonable. Nonetheless, overall power tended to be low such that our ability to detect statistically significant differences was limited. Our sample sizes were diluted by the fact that we sampled at so many time points post-injection. For many analyses we were able to combine those data given lack of differences among days post-injection.

Table 4
Plasma triglyceride levels (mmol L^{-1}) of rainbow trout for the three treatment groups (Control, Low, High) determined 1, 3, 5, 7, and 9 days post-injection (DPI). Values are shown as Mean ± S.E.M. (n = 3). Plasma triglyceride levels varied with DPI ($F = 9.067$; $p < 0.0001$) but not between treatments ($F = 3.212$; $p = 0.054$). DPI (all groups considered) with different superscript letters differ significantly.

	Days post-injection				
	1[a]	3[a]	5[ab]	7[b]	9[b]
Control	5.8 ± 0.2	5.5 ± 0.2	5.2 ± 0.2	4.8 ± 0.2	4.7 ± 0.2
Low	5.4 ± 0.2	5.2 ± 0.2	4.9 ± 0.2	4.5 ± 0.2	4.4 ± 0.2
High	5.4 ± 0.2	5.2 ± 0.2	4.9 ± 0.2	4.4 ± 0.2	4.3 ± 0.2

Table 5
White muscle parameters measured in rainbow trout of the three treatment groups (Control, Low, High). Values are shown as Mean ± S.E.M.; n = 15 (except for amino acids of the High group where n = 12). As no significant effects of days post-injection existed (see Supplementary table S5), individual data for each treatment group were combined. Different letters indicate a significant difference ($p < 0.05$) between treatments.

	Control	Low	High
Glucose (mg g^{-1})	0.37 ± 0.02[a]	0.30 ± 0.01[b]	0.19 ± 0.01[c]
Glycogen (mg g^{-1})	0.66 ± 0.02[a]	0.58 ± 0.01[b]	0.50 ± 0.01[c]
Total protein (mg g^{-1})	7.8 ± 0.1[a]	5.6 ± 0.2[b]	4.6 ± 0.2[c]
Amino acids (μg g^{-1})	40.6 ± 0.8[a]	43.9 ± 0.8[b]	47.2 ± 0.8[c]

Our results support an effect of PCB-126 on swimming performance and recovery in rainbow trout. The significant increase in our modified U_{crit1} performance test observed in High PCB-exposed fish compared to Control fish is not consistent with the impaired swimming performance generally observed following a sublethal exposure to organic and inorganic toxicants (Baltz et al., 2005; Beamish, 1978; Beaumont et al., 1995; Heath, 1995, Jain et al., 1998; Little and Finger, 1990; McKenzie et al., 2007; Nikl and Farrell, 1993; Wood et al., 1996). Body mass and condition factor of rainbow trout of the High-dose treatment were significantly higher than those of trout from the other treatments (Table 1), an undesirable outcome of random sampling. Body condition has been shown to positively influence U_{crit} performance (Lapointe et al., 2006; Martinez et al., 2004), thus it appears that the greater body mass of rainbow trout from the High-PCB treatment group may at least partially explain the higher swimming performance they achieved during the first trial compared to that of fish from the Control treatment. The High-dose trout were unable to repeat their U_{crit} performance during the second trial (e.g., no significant difference in U_{crit2} among treatments; Fig. 1), suggesting that exposure to elevated levels of PCB-126 impaired their ability to recover from the fatiguing exercise used here. Considering that the U_{crit} performance relies on both oxidative and glycolytic capacities, the greater U_{crit1} performance of the High-dose fish suggests that these better conditioned fish took advantage of their relatively larger white muscle mass, yielding a greater contribution of the glycolytic metabolism to prolong swimming performance. This is consistent with the greater depletion of glucose, glycogen and proteins measured in the white muscle of this group (Table 5).

Exposure to PCB-126 impaired recovery in rainbow trout. The U_{crit} performance achieved during the second trial represented a decrease of 8 and 17% for the Low and High PCB treatments, respectively, in comparison to the first swimming trial, suggesting an impairment of their capacity to recover with increasing level of PCB exposure following fatiguing exercise (Fig. 1). This is shown as a significant decrease in RR across the 3 groups of fish. Recovery ratios are considered sensitive indicators of metabolic disturbance, and reductions were also documented in sockeye salmon (*Oncorhynchus nerka*) exposed to sublethal concentrations of dehydroabietic acid (DHA; 0.12–0.77 mg L^{-1}) and subjected to two swim challenge tests (Jain et al., 1998), as well as in other studies on various stress factors (Farrell et al., 1998; MacNutt et al., 2004; Peake and Farrell, 2004; Tierney and Farrell, 2004). These decreased RRs support a reduced ability to recover with increasing level of exposure, implying that PCB-exposed trout require longer periods to adjust following fatiguing exercise or during stressful conditions than unexposed fish. It is important to note that RR is a unit-less parameter so the difference in mass between the two treatment groups is removed.

Rates of oxygen consumption were also affected following exposure to PCB-126. We observed an increase in RMR with exposure to PCB-126 (Fig. 2), which may be associated with the depuration or detoxification processes (Billiard et al., 2002; Brown et al., 2002) as EROD activities were higher in the Low and High PCB groups than the Controls. A similar

trend was observed for maximal metabolic rates (Fig. 2). Generally metabolism is higher following fatiguing exercise, but it has also been observed that oxygen consumption can increase further when exposed to different stressors including the presence of predators, air exposure, temperature, light intensity, and pollutants (Farrell et al., 1998; Thompson et al., 2008).

This study also demonstrated significant changes in tissue metabolites and somatic indices associated with PCB exposure. The largest PCB-induced changes in metabolites occurred in white muscle (Table 5). In general, PCB-exposed trout demonstrated relative declines in total carbohydrates (glucose + glycogen) and total protein, and an increase in free amino acids. The increase observed in plasma cortisol may have led to the observed decrease in protein and increase in amino acid content in white muscle and a cortisol-induced hepatic glucose production, consistent with the increase in plasma glucose levels observed with increasing PCB-126 exposure (Table 5), consistent with previous fish cortisol studies (see Mommsen et al., 1999). Swimming requires energy in the form of ATP generated by the oxidation of carbohydrate and lipid fuels (Kieffer, 2000). The decrease in carbohydrates observed in PCB-exposed fish might indicate that these fish experienced greater muscle depletion than the Controls, implying that more energy was used for the swimming bout, which is in agreement with the higher U_{crit} performance achieved by fish from the High PCB group at least during the first swimming challenge (Fig. 1). These lower carbohydrate levels may also suggest that fuel restoration was impeded following exposure to PCB-126, which could reduce the ability of the fish to achieve its critical swimming speed and thus reduce the recovery ratios as observed in our study. Finally, it is also plausible that PCB-exposed fish had lower levels of carbohydrates prior to the swimming challenge, but no sampling was done prior to PCB exposure.

Only slight changes occurred in plasma metabolites with PCB exposure (Table 3 and Table 4). It must be noted that blood samples were taken 35 min following the U_{Crit2} estimate, so disturbances in this compartment by exercise may not be captured. Only cortisol and glucose levels increased with increasing exposure to PCB-126, relative to the Control fish. Increased cortisol levels are often associated with increased levels of plasma glucose and lactate (Barton, 2002; Mommsen et al., 1999; Wendelaar Bonga, 1997). Cortisol is known to elevate glucose production through its action on hepatic gluconeogenic and glycogenolytic pathways (Iwama et al., 1999; Mommsen et al., 1999) to cope with the additional energy demands imposed by the stressor. Cortisol values in the plasma of salmonids are extremely variable but are often found in the range of <10 and 40–200 ng mL^{-1} for resting and stressed fish, respectively (Iwama et al., 2004). Apparently the swimming protocol with or without PCB exposure used here did not elicit a significant stress response as plasma lactate values (Table 3) did not increase and cortisol values (6–21 mg mL^{-1}) were low across all 3 groups. Generally, exposure to PCBs and other contaminants impairs cortisol responses in fish as a result of hyperactivity and interrenal exhaustion (Hontela et al., 1992; Hontela, 1998). Here, PCB exposure significantly elevated plasma cortisol, although not to values seen in stressed salmonids (Iwama et al., 2004). Thus, this PCB protocol did result in a generalized stress response which may reflect the changes noted in organ/tissue metabolites.

The HSI was lower in the High-dosed group than the other two groups, possibly related to the higher condition factor of this group (Table 2). This organ is the site of detoxification and PCB exposure generally results in larger livers (Denton and Youssef, 1976; Gingerich, 1982; Lech et al., 1982). Also, toxicants generally increase hepatic lipid stores (Hacking et al., 1978) but in this case, exposed-fish appeared to be suffering from poor nutritional conditions compared to the Control group (Heath, 1995) and mobilization of fuels (such as lipids and glycogen) had to occur in order to support swimming and meet the increasing energy demands. Nevertheless, HSI determined nine days post-injection was significantly higher than that measured one day post-injection (Table 2), suggesting a significant increase in hepatic reserves over the course of nine days following the exposure to PCB-126. The spleen is involved in immune responses and is considered a storage organ for principally red but also white blood cells (Hylland et al., 2001). The SSI decreased by 39% at the highest PCB exposure (Table 1), which was expected as the loss of red blood cells in the spleen is a common stress response that enhances the oxygen carrying capacity of the blood (Wendelaar Bonga, 1997). A decreased SSI was documented for fish exposed to pulp mill effluents (Barker et al., 1994; Couillard and Hodson, 1996), polycyclic aromatic hydrocarbons (Hart et al., 1998) and pesticides (Hart et al., 1997). The HESI remained constant across treatments (Table 1); this is understandable given the short duration of the experiment though it would be interesting to determine if chronic PCB exposure would alter this index.

This research has important implications in an ecological context, especially for long-lived species that are also considered high performance swimmers and rely heavily (compared to other fish species) upon these swimming and recovery abilities to perform reproductive duties with high energetic costs such as migration and spawning, competition, and gonadal development. The reduction in recovery ratios of intoxicated fish compared to non-exposed fish demonstrates the occurrence of a metabolic disturbance. The bases for these changes appear to be linked to modifications in recovery metabolism, especially in the white skeletal muscle. This study provides further evidence that the sublethal effects of PCBs on fish could potentially have significant repercussions at the population and ecosystem levels.

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Appendix A. Supplementary data

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