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PCBs can diminish the influence of temperature on thyroid indices in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The influence of PCBs on the thyroid status of rainbow trout was assessed at various temperatures to identify if PCB mixtures, as well OH-PCBs produced via biotransformation of parent PCBs, can illicit thyroid effects in fish. Juvenile rainbow trout (*Oncorhynchus mykiss*) held at 8, 12 or 16 °C were exposed to dietary concentrations of an environmentally relevant mixture of PCBs for 30 days followed by a depuration phase. Two additional treatments at 12 °C included higher concentrations of PCBs (congeners 77, 126 and 169) known to induce CYP1A in fish (referred to as CYP1A treatment) and PCBs (congeners 87, 99, 101, 153, 180, 183 and 194) known to induce CYP2B in mammals (referred to as CYP2 treatment), to assess the influence of more biologically relevant PCB congeners on thyroid indices in fish. Growth rate and liver somatic index varied with water temperature ($p < 0.05$) but did not differ between PCB exposed and control fish ($p > 0.05$) and mortality was low in all treatments. Changes in some measures of thyroid status were apparent in PCB-exposed fish held in the 12 and 16 °C treatments while other measures showed no change in any treatment. The natural inverse relationship between thyroid epithelial cell height (TECH) and temperature, was diminished after 30 days of exposure to PCBs as the epithelial cell height in PCB-exposed fish was significantly augmented in the 12 and 16 °C treatments compared to controls at these temperatures ($p < 0.05$). However, after 20 days of depuration, TECH values in the PCB exposed fish returned to control values. The natural linear gradient between T_4 outer-ring deiodinase activity (ORD) and temperature was also diminished after 30 days of exposure to PCBs. PCB-exposed fish from the 16 °C treatment had significantly lower deiodinase activities ($p < 0.05$) compared to controls at this temperature, but deiodinase activities returned to normal by day 20 of depuration. No differences were observed in T_3 inner-ring deiodinase (IRD) activities and plasma concentrations of T_3 and T_4 in any of the treatments ($p > 0.05$). EROD activity in fish from the CYP1A and CYP2 treatments were elevated compared to control and high dose PCB-exposed treatments ($p < 0.05$), but the inclusion of CYP inducing congeners did not appear to influence any index of thyroid status. Results of this study suggest that exposure of rainbow trout to high concentrations of PCBs and/or OH-PCBs may alter some indices of thyroid status when water temperatures are high, but these changes are within the compensatory scope of the thyroid system based on no change in circulating hormone concentrations, growth rates or mortality.

Keywords: Thyroid hormones; Fish; PCBs; OH-PCBs; Temperature; Epithelial cell height; Deiodinase activity

1. Introduction

Awareness regarding environmental contaminants and their effects on endocrine function in fish is on the rise (Kime, 1998; Harvey and Johnson, 2002). Thyroid hormones are of particular interest as they regulate many metabolic processes (McNabb, 1992; Capen, 1997; Colborn, 2002; Zoeller, 2002) and are paramount in early development, growth and reproduction (Eales and Brown, 1993; Leatherland, 1993). Natural variation in thyroid status of fish has been demonstrated in response to developmental state and/or age (McLeese and Eales, 1998), water temperature (Eales and Fletcher, 1982; Eales et al., 1982, 1986; Johnston and Eales, 1995) and nutritional status (Eales and Brown, 1993; Sweeting and Eales, 1992a; Leatherland and Farbridge, 1992) and, in many cases, these situations call for an increase in thyroid activity. Disruption of the thyroid endocrine system due to exposure of fish to environ-

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mental contaminants during such these periods may compromise overall fitness and survival as the thyroid axis may not be able to compensate for additional thyroid hormone requirement during times of stress.

Concern about the influence of PCBs on the thyroid systems of Great Lakes organisms has been present for decades. Effects of PCBs on the thyroid status in mammals and birds have been extensively reviewed and demonstrate that PCBs increase the metabolism and excretion of thyroid hormones (TH) and lower circulating thyroxine (T_4) concentrations (McClain, 1989; Sonstegard and Leatherland, 1979; Brouwer et al., 1989). In addition, in mammals, PCBs can cause thyroid gland anomalies (Byrne et al., 1987; Kasza et al., 1978) such as thyroid microfollicular hyperplasia. Thyroid microfollicular hyperplasia and large goiters have been observed in Great Lakes salmonids for more than 30 years (Leatherland, 1998), although this has not definitively been linked to PCB exposure. A minimal number of studies have assessed PCB mixture exposure on thyroid status in fish (Leatherland and Sonstegard, 1978, 1980; Folmar et al., 1982; Besselink et al., 1996), which suggest that PCBs can alter some indices of thyroid status in fish, but that their mode of action is not well understood.

Several recent studies have focused on the effects of coplanar, also called non-*ortho*, PCBs (PCBs 77, 126 and 169), known to induce cytochrome P4501A1 activity, on the thyroid status in fish (Brown et al., 2004; Adams et al., 2000; Palace et al., 2001). These studies have demonstrated that, in some cases, exposure to coplanar PCBs can elevate plasma T_4 concentrations or the clearance of plasma T_4 but there is little evidence of change in hepatic T_4 outer-ring deiodination (T_4ORD), triiodothyronine (T_3) concentrations or T_3-glucuronidation. Adams et al. (2000) also noted an increase of T_4ORD in American plaice (*Hippoglossoides platessoides*) exposed to PCB 77 through i.p. injection. In juvenile rainbow trout, exposure to dietary concentrations of PCB 126 accelerated the growth-related decline in muscle concentrations of T_3 and T_4, however thyroid histology was unaffected (Brown et al., 2002). A long-term study (up to 61 weeks) that exposed lake trout (*Salvelinus namaychus*) to PCB 126 by i.p. injection or oral gavage (40 μg/kg) increased thyroid epithelial cell height, plasma T_4 concentrations and plasma T_4 clearance (Brown et al., 2004). However, to date, no studies in fish have examined the effect of PCB congeners known to induce CYP2B activity in mammals (congeners 87, 99, 101, 153, 180, 183 and 194) on thyroid function in fish. While no CYP2B proteins have been found in fish (see, for example, Iwata et al., 2002 or Shlezinger and Stegeman, 2001) other CYP2 proteins (e.g., 2 K) have been found (Katchamart et al., 2002; Wang-Buhler et al., 2005). However, the relationship between CYP2 proteins in fish and their relationship with contaminants, i.e., ability to induce/inhibit CYP2 proteins, including those known to induce CYP2B in mammals, is unclear (Stegeman, 1993; Iwata et al., 2002). There is evidence that contaminants may up- or down-regulate CYP1A and CYP2 protein metabolic activities (Katchamart et al., 2002), which may affect other endpoints such as thyroid function.

In addition to PCBs, PCB metabolites such as hydroxylated PCBs (OH-PCBs) have demonstrated thyroid hormone-like activity (Shiraishi et al., 2003; Kitamura et al., 2005). OH-PCBs bind to transthyretin (TTR) in mammals and exert effects on thyroid-hormone dependent function in mammals (Meerts et al., 2002; Purkey et al., 2004; Kimura-Kuroda et al., 2005). While the role of TTR in fish is not clear, hydroxylated PCBs resemble thyroid hormones and may cause thyroid effects in fish via other mechanisms. A recent study (Buckman et al., 2007), using the same samples as the present study, demonstrated a linear increase in bioformation of OH-PCBs with increasing temperature in fish exposed to Aroclor mixtures of PCBs. Exposure to PCB congeners known to induce CYP2B in mammals also caused an increase in PCB metabolism and OH-PCB formation in rainbow trout (Buckman et al., 2007), indicating a potential concern for OH-PCB-related thyroid effects in these organisms.

While it is believed that factors other than PCBs may cause thyroid disruption of the Great Lakes salmonids (Leatherland, 1993), there have been no studies examining the interplay between natural stress events, such as temperature, in combination with exposure to PCB mixtures, PCBs of biological concern and OH-PCBs. We hypothesized that the combination of PCB exposure and variation in water temperature could result in altered fish thyroid homeostasis.

2. Methods and materials

2.1. Chemicals and food preparation

Aroclors 1242, 1254 and 1260, cytochrome P4501A inducing PCB mix (including PCB congeners 77, 126 and 169), cytochrome P4502B inducing PCB mix in mammals (including PCB congeners 87, 99, 101, 153, 180, 183 and 194) and PCB congeners 202 and 209 were purchased from AccuStandard (New Haven, CT, USA). All solvents (pesticide grade) were obtained from Caledon Laboratories (Georgetown, Ont., Canada). Potassium phosphate, sodium phosphate, sucrose, dithiothreitol (DTT), ethylenediamine-tetraacetic acid (EDTA), potassium iodide, 2-mercapto-1-methylimidazole (MMI) and the radioinert thyroid (TH) hormone standards thyroxine (T_4), 3,5,3′-triiodothyronine (T_3), 3,3′,5′-triiodothyronine (rT_3) and 3,5-diiodothyronine (T_2(3,5)) were purchased from Sigma–Aldrich Canada Ltd. (Oakville, Ont., Canada). HPLC grade water was purchased from Fisher Scientific (Ottawa, Ont., Canada). The radioinert 3,3′-diiodothyronine (T_2(3,3)) was purchased from Toronto Research Chemicals (Toronto, Ont., Canada) and radioactive tracers L-(^{125}I)-thyroxine (T_4^*) and L-3,5,3′-(^{125}I)-triiodothyronine (T_3^*) were purchased from Perkins-Elmer (Waltham, MA, USA).

Food for the PCB-exposed treatment fish was spiked by dissolving a known quantity of each PCB Aroclor standard (1:1:1 ratio) and PCB 202 and 209 (10 μg/g of each Aroclor mixture and 0.5 μg/g of each of PCB 202 and 209) in 500 ml of hexane with 250 g of commercial fish food (3 Vigor Sinking Fish Feed, Corey Feed Mills, Fredericton, NB, Canada). For treatments including CYP inducing PCBs described below (referred to as the CYP1A and CYP2 treatments for simplicity; note CYP2B proteins have not been found in fish although

other CYP2 proteins have been found), an additional mixture of either CYP1A inducing PCB congeners (PCBs 77, 126 and 169; 10 ng/g each) or congeners known to induce CYP2B in mammals (PCBs 87, 99, 101, 153, 180, 183 and 194; 10 ng/g each) were added to the CYP1A and CYP2 treatments, respectively. Food and solvent mixtures were evaporated to dryness under reduced pressure in a roto-evaporator. Food was air dried for 24 h in a fume hood and stored at 10 °C in stopper sealed jars. Control treatment food and food used for the depuration phase were treated in an identical manner but without addition of the PCBs.

2.2. Fish husbandry

Juvenile rainbow trout (initial weights ~80 g; Rainbow Springs Trout Hatchery, Thamesford, Ont., Canada; Stevenson strain) were held in 8 separate aquaria (300 l), in de-chlorinated, flow-through water at either 8, 12 or 16 °C and a 12-h light:12-h dark cycle. Each aquarium contained activated charcoal in nylon bags to absorb any dissolved PCBs or metabolites in the water. Trout were maintained on non-spiked food at a feeding rate of 1.5% of the average body weight of the fish, for 1 month prior to the experiment. Upon commencement of experimentation, fish were transferred to treatment food at a rate of 1.5% of the average body weight corrected to the new mean body weight of each treatment after each sampling period. Canadian Council on Animal Care and Institutional Guidelines were adhered to for fish husbandry.

2.3. Exposures

All fish were tagged with individual pit tags and weighed 20 days prior to commencement of exposure. Eight treatments were established ($n = 85$ fish per treatment): three control treatments (no PCBs added to food), three PCB-exposed treatments (fed PCB-spiked food), a CYP1A treatment and a CYP2 treatment. Control and PCB-exposed fish were held at 8, 12 or 16 °C while CYP1A and CYP2 treatment fish were held only at 12 °C due to space limitations. PCB exposed, CYP1A and CYP2 treatment fish were fed PCB-spiked food (treatment food described above) for 30 days (uptake phase), followed by 300 days of non-spiked food (depuration phase). Control fish were fed non-spiked food throughout the experiment. On days 0, 5, 10, 20 and 30 of the uptake phase and days 5, 10, 20, 40, 80, 160 and 300 of the depuration phase, six fish from each treatment were sacrificed by cervical section and sampled for determination of PCB concentrations. Blood was taken from the caudal vein (approximately 3 ml) from each fish prior to sacrifice. Whole fish and liver were weighed for growth rate analysis and liver somatic index analysis (LSI). Tissues were collected and separated into liver, gastrointestinal tract (GI = stomach, intestines, spleen and associated fat), thyroid, brain, muscle and remaining carcass for PCB analysis. On days 0 and 30 of uptake and day 50 of depuration, six fish were sampled from each treatment for thyroid histology, ORD and IRD, plasma T4 and T3 concentrations and liver EROD analysis. Samples were stored at −80 °C until analyzed.

2.4. PCB analysis

Methods for PCB analysis are identical to those published elsewhere (Buckman et al., 2006, 2007). Briefly, samples were homogenized using an AE-G225 food processor (American Eagle, Chicago, IL, USA) and extracted on a Dionex ASE 200 (Sunnyvale, CA, USA). PCBs 30 and 204 were added prior to extraction as recovery standards. Lipids were determined gravimetrically using 10% of the extract and were removed by acidified silica gel chromatography. PCBs were eluted with 15% DCM in hexane (150 ml) and evaporated to 1 ml final volume. Concentrations of PCBs in food were determined using the same analytical techniques used for tissue.

All samples were analyzed on a Hewlett-Packard (Wilmington, DE, USA) 5890 gas chromatograph (GC) with a ^{63}Ni-electron capture detector equipped with a 60 m DB-5 column (J&W Scientific, Folsom, CA, USA). Sample quantification was performed using multiple external standards obtained from the National Laboratory for Environmental Testing (1997). Percent recoveries and method detections limits (MDLs are reported in Buckman et al., 2007).

2.5. OH-PCB analysis

The method used for OH-PCB extraction, clean-up and analysis of PCBs and OH-PCBs in plasma is detailed previously (Sandau et al., 2000; Buckman et al., 2007). Briefly, plasma samples were thawed, spiked with $^{13}C_{12}$-labelled recovery standards and non-labeled PCB internal standards (PCBs 30 and 204), mixed and allowed to equilibrate. Proteins were denatured, and contaminants were extracted using a conventional liquid:liquid extraction technique. Organic and aqueous fractions were reduced to a final volume of approximately 1000 and 100 μl, respectively. Volume correction was determined by weight for both aqueous and organic fractions. The resulting aqueous phase extracts were analyzed by high resolution GC/MS on a Micromass Ultima mass spectrometer coupled to an Agilent 6890 GC equipped with a CTC A200s autosampler (Mississauga, Ont., Canada). Forty-seven OH-PCB congeners were commercially available as authentic standards at the time of analysis. The quantification of OH-PCBs for which standards were available (identified OH-PCBs) was conducted using external calibration curves. The high selectivity of the high resolution HRGC/HRMS permitted the identification of numerous unknown peaks as OH-PCBs. Unknown peaks that matched the isotopic ratio of primary and secondary ions were determined as unidentified OH-PCB homologues. The quantification of OH-PCB homologues was accomplished using an average response factor based on the native standard within the same chlorine-substituted homolog group. Five ^{13}C internal standards (^{13}C 4-OH-12, ^{13}C 4-OH-29, ^{13}C 4-OH-61, ^{13}C 4-OH-120 and ^{13}C 4-OH-187) from various homologue groups were used to determine the recovery of OH-PCBs in the plasma. OH-PCB concentration data were recovery corrected in order to compare homologue group levels. Percent recoveries for individual OH-PCBs and MDLs are reported in Buckman et al. (2007). No OH-PCBs were detected in the food.

2.6. Growth and liver somatic index

Individual growth rates were determined by taking the natural log of the difference in fish weight (final weight − initial weight) and dividing by the day of experiment plus 20 (because fish were tagged and weighed 20 days before experimentation began). Liver growth rates between temperature treatments were calculated and compared by fitting all liver weight data (days 0–320 of experimentation) to an exponential model (ln liver weight = $a + b \times$ time (days), where a is the intercept and b is the liver growth rate) due to the fact that we could not calculate an initial liver weight. Liver somatic indices (reported as %LSI) were calculated by dividing the liver weight by the whole fish weight and multiplying by 100.

2.7. Histological examination of thyroid

Following cervical section, rainbow trout were opened to expose the internal organs and the jaws were cut at the corners to expose thyroid tissue. Thyroid tissue was excised from the trout and fixed in Bouin's fixative for 72 h and stored in 70% ethanol. Tissues were dehydrated using an ethanol/butanol series and embedded in paraffin. Thyroid tissues were then sectioned at 7 μm. Thyroids were stained for light microscopy and basic histopathological analyses with hematoxylin and eosin. The protocol employed followed the methods described elsewhere (Brown et al., 1998). To perform morphological measurements, microscope images were projected onto a Summagraphics Bit Pad (Summagraphics, Fairfield, CT, USA) and measured with Sigma-Scan Version 3.90 (Jandel Scientific, Corte Madera, CA, USA). The cell height of the thyroid epithelium was measured in a total of 15 follicles per fish. Measurements were made at four points within each follicle at 90° from one another. Cell height is reported as the arithmetic mean ± 1S.E.

2.8. Deiodinase assay

Deiodination assays were based on the methods described elsewhere (Shields and Eales, 1986) with a few modifications. Microsomal fractions were prepared following established methodology (Eales et al., 1999) and stored at −80 °C until analysis. Microsomal fractions were thawed on ice and diluted (∼1:25) with 0.1 M Tris–HCl buffer (pH 7.2) containing 10 mM of DTT and 1 mM of EDTA to achieve a final protein concentration of 0.2–0.8 mg/ml. A volume of 500 μl of diluted microsomal fraction was added to methanol rinsed culture tubes and equilibrated for 30 min in a shaking water bath (12 °C; 140 rpm). The reaction was started by adding 10 μl of TH (100,000 cpm of ^{125}I-labeled $T_4^* + T_4$ inert (at 5 K) or ^{125}I-labeled $T_3^* + T_3$ inert (at 5 K)) to each tube to be assayed. The reaction was stopped after exactly 60 min for each tube by adding 10 μl of 2 M KI and 550 μl (equal volume to the assay tube solution) of 0.001 M MMI/methanol and vortexing. All samples are centrifuged for 5–10 min at 16,000 rpm and 700 μl of sample is transferred to amber HPLC vials. Samples were analyzed on a Waters HPLC system (Mississauga) with a Waters 996 Photodiode array detector (at 254 nm) and a B-Ram model 3 radiometric detector with a NaI cell. To confirm chromatography retention times, a standard mixture of five inert hormones (T_2 (3,3′), T_2 (3,5), T_3, rT_3, T_4: 10 ng/μl) (Brown et al., 1991) and the radioactive T_3^* and T_4^* (at 100,000 CPM in 50 μl) counts was run at the start of the HPLC analysis and after every 12 samples. Methods for analysis followed those described previously (Eales et al., 1999; Sweeting and Eales, 1992b) with a few modifications. HPLC analysis of deiodination products was conducted using a C18, 5μ 150 mm × 46 mm Econosphere (Alltech) column. The mobile phase was acetonitrile (ACN) containing 0.1% trifluoroacetic acid (TFA) and HPLC grade water containing 0.1% TFA, which were sparged at 30% with ultra pure He during the assay. The runs were 20 min at a flow rate of 1 ml/min: isocratic from 0 to 10 min (40% ACN) increasing linearly to 45% ACN at 15 min and then decreasing to 40% ACN at 15.1 min. Deiodination (ORD (measured as T_4 outer-ring deiodination) or IRD (measured as T_3 inner-ring deiodination)) activity was expressed as pmol TH (T_4, T_3 or rT_3) deiodinated/mg protein/h.

2.9. Plasma thyroid hormone concentrations

Radioimmunoassays for T_3 and T_4 in plasma followed previously established procedures (Brown and Eales, 1977).

2.10. EROD analysis

Analysis of liver samples for EROD enzyme activity was carried out with microsomal fractions (same as used for deiodinase assays) and is described previously (Hodson et al., 1996) with modifications (Fragoso et al., 1998).

2.11. Data analysis

Bartlett's test for homogeneity of variance was applied to the data. For clarity of presentation, arithmetic means and their standard errors are given in results. The SYSTAT statistical package (SYSTAT, 2004) was used to analyze the data. Differences between whole body growth rate constants and depuration rates were tested for homogeneity of slope and parallelism in an analysis of covariance using general linear model. Tukey's honestly significant difference (HSD) test was used to compare percent lipid differences between treatments. Three-way ANOVA using temperature, dose and day for comparison of PCB exposed and controls and two-way ANOVA using dose and day for comparison of all treatments at 12 °C, including CYP1A and CYP2B treatments, was computed for all thyroid effects data. Tukey's studentized range test was used to evaluate differences between temperature, day and dose group means. Probabilities (p) of <0.05 were considered significant.

3. Results

3.1. Growth, liver size and survival

Whole fish and liver growth rates were significantly different among temperature treatments, with significantly faster growth rates at the higher temperatures (Table 1). No differ-

Table 1
Growth rates, lipid content, liver somatic index and percent mortality (mean ± 1S.E.; $n = 6$ per treatment and day) of juvenile rainbow trout exposed to PCBs at three temperatures and at 12 °C to treatments containing additional cytochrome P450-inducing PCB congeners (CYP1A in fish (CYP1A exposed) and CYP2B (CYP2 exposed) in mammals)

Treatment	Growth rate constants ($day^{-1} \times 10^{-3}$)				Lipid[a] (%)	Liver somatic index			Mortality (%)
	Whole fish[b] (day 0)	Whole fish[b] (day 30)	Whole fish[b] (day 50)	Liver[c]	Day 30	Day 0	Day 30	Day 50	
8 °C									
Control	9.1 ± 0.8^{A}	8.8 ± 0.7^{A}	8.7 ± 0.8^{A}	$4.9 \pm 0.4\ (0.68)^{A}$	8.6 ± 0.5^{A}	2.7 ± 0.2^{A}	$1.7 \pm 0.1^{A*}$	$1.8 \pm 0.3^{A*}$	5.1
PCB exposed[d]	9.1 ± 0.7^{A}	9.2 ± 0.5^{A}	7.7 ± 1.0^{A}	$5.4 \pm 0.5\ (0.71)^{A}$	8.7 ± 1.1^{A}	2.2 ± 0.1^{A}	$1.8 \pm 0.2^{A*}$	$1.6 \pm 0.1^{A*}$	1.3
12 °C									
Control	11 ± 1.0^{AB}	10 ± 0.4^{AB}	10 ± 0.9^{AB}	$5.2 \pm 0.5\ (0.69)^{AB}$	8.3 ± 0.4^{A}	1.7 ± 0.1^{B}	1.5 ± 0.1^{AB}	1.6 ± 0.1^{A}	2.2
PCB exposed[d]	9.9 ± 0.2^{AB}	9.5 ± 0.2^{AB}	9.6 ± 1.0^{AB}	$5.7 \pm 0.5\ (0.74)^{AB}$	8.9 ± 0.6^{A}	1.8 ± 0.1^{B}	1.6 ± 0.1^{AB}	1.5 ± 0.1^{A}	2.2
CYP1A exposed[e]	9.6 ± 0.4^{AB}	10 ± 0.8^{AB}	11 ± 0.6^{AB}	$5.6 \pm 0.5\ (0.68)^{AB}$	8.4 ± 0.4^{A}	1.7 ± 0.1^{B}	1.4 ± 0.1^{AB}	1.6 ± 0.1^{A}	2.2
CYP2 exposed[f]	9.9 ± 0.8^{AB}	9.0 ± 0.8^{AB}	9.6 ± 0.6^{AB}	$5.6 \pm 0.4\ (0.74)^{AB}$	8.4 ± 0.5^{A}	1.8 ± 0.2^{B}	1.2 ± 0.4^{AB}	1.5 ± 0.1^{A}	4.2
16 °C									
Control	13 ± 0.9^{B}	11 ± 0.6^{B}	11 ± 0.8^{B}	$7.1 \pm 0.6\ (0.70)^{B}$	8.5 ± 0.5^{A}	1.5 ± 0.2^{B}	1.4 ± 0.1^{B}	1.5 ± 0.1^{A}	4.2
PCB exposed[d]	12 ± 1.0^{B}	12 ± 1.4^{B}	11 ± 0.5^{B}	$6.3 \pm 0.6\ (0.65)^{B}$	8.8 ± 0.3^{A}	1.5 ± 0.4^{B}	1.3 ± 0.1^{B}	1.5 ± 0.1^{A}	6.0

Tukey's honestly significant difference (HSD) test was used to compare differences between treatments (columns). Capital letters (A and B) represent statistically significant similarities or differences ($p < 0.05$) within columns.

[a] Lipid percentage is the average lipid percentage of all fish in each treatment.

[b] Whole fish growth rate calculated from the equation ln weight = $a + b$ day, where b is the growth rate ± 1 standard error. Coefficient of determination in brackets (R^2).

[c] Liver growth rate calculated from the equation ln weight = $a + b$, where b is the growth rate ± 1 standard error. Coefficient of determination in brackets (R^2).

[d] PCB exposed = 10 μg/g food of Aroclors 1242:1254:1260 in a 1:1:1 ratio.

[e] CYP1A exposed = same as PCB exposed + 10 ng/g food of CYP1A inducing congeners (PCBs 77, 126 and 169).

[f] CYP2 exposed = same as PCB exposed + 10 ng/g food of CYP2B inducing congeners (PCBs 87, 99, 101, 153, 180, 183 and 194).

[*] A statistically significant difference ($p < 0.05$) between sampling day of interest and day 0 within the same treatment.

ences in whole fish or liver growth rates were found between PCB exposed and control juvenile rainbow trout held at the same temperatures (Table 1). Liver somatic index (LSI) was significantly different ($p < 0.05$) among temperature treatments at day 0 with LSI decreasing with increasing temperature. However, LSI of fish exposed to PCBs after 30 days were not significantly different from controls ($p > 0.05$) (Table 1). Liver somatic indices also decreased over the course of the experiment in both the control and PCB-exposed fish in the 8 °C treatment ($p < 0.05$). Mortality was minimal and showed no trend across treatments (Table 1).

3.2. PCB and OH-PCB concentrations

Background PCBs were measured in control food and fish carcass at levels more than an order of magnitude lower than the PCBs in exposed treatments (Table 2). PCB concentrations in the control and PCB-exposed treatment fish (carcass—liver and GI tract) did not vary with temperature, but were higher in the CYP1A treatment fish (Table 2). PCB and OH-PCB concentrations were present in the plasma of fish from all treatments including control fish but at much lower levels than the PCB-exposed fish (Table 2). A full list of individual PCB and OH-PCB congener concentrations in fish from this study including MDL values are reported in Buckman et al. (2007). OH-PCB concentrations, but not PCB concentrations, increase with temperature ($p < 0.05$) and exposure to PCB congeners known to induce CYP2B in mammals, the CYP2 treatment ($p < 0.05$) (Table 2).

3.3. Thyroid epithelial cell height (TECH)

Histological analysis of TECH in rainbow trout showed significant differences ($p < 0.05$) among temperature regimes at day 0 with larger TECH in the 8 °C and decreasing with temperature, but this relationship between TECH and temperature was not apparent in PCB-exposed fish at day 30 (Fig. 1 and Table 3). Significant differences ($p < 0.05$) were found between control and PCB-exposed rainbow trout in the 12 and 16 °C treatments and between control and CYP2B-treatment fish in the 12 °C treatments at day 30 of exposure (Table 3). TECH values in the 8 °C control and PCB-exposed treatments did not vary on any collection day, but after 30 days of exposure to PCBs TECH levels in the 12 and 16 °C treatments were higher ($p < 0.05$) than the respective control treatments at these temperatures. However, visual analysis of histology slices of the thyroid tissue after 30 days of PCB exposure in these treatments did not suggest evidence of microfollicular hyperplasia (data not shown). After 20 days of depuration (day 50), TECH values in the 12 and 16 °C PCB-exposed fish declined to levels observed in control fish at these temperatures (Table 3) and the inverse relationship between TECH and temperature returned in PCB-exposed fish as observed in control treatments (Fig. 1). TECH in the 12 °C CYP1A treatment fish after 20 days of depuration also declined and returned to TECH values observed in the control fish at this temperature (Table 3).

Table 2
Concentrations (ng/g, lw; mean ± 1S.E.) of PCBs in food ($n = 5$) and rainbow trout carcass ($n = 6$ per treatment; minus liver and gastrointestinal tract) and OH-PCBs ($n = 3$; pools of two fish) in blood plasma from rainbow trout exposed to PCBs at three temperatures and at 12 °C to treatments containing additional cytochrome P450-inducing PCB congeners (CYP1A in fish (CYP1A exposed) and CYP2B (CYP2 exposed) in mammals)

Chemical, matrix	Control 8 °C	Control 12 °C	Control 16 °C	[a]PCB exposed 8 °C	[a]PCB exposed 12 °C	[a]PCB exposed 16 °C	[b]CYP1A treatment 12 °C	[c]CYP2 treatment 12 °C
∑PCB, food	180 ± 2.1	180 ± 2.1	180 ± 2.1	6,600 ± 550	6,600 ± 550	6,600 ± 550	10,000 ± 260	10,000 ± 760
∑PCB, carcass	1,000 ± 330^{A}	930 ± 210^{A}	902 ± 132^{A}	28,500 ± 10,600^{B}	28,700 ± 3,780^{B}	30,000 ± 5,100^{B}	36,000 ± 4,000^{B}	28,000 ± 4,400^{B}
∑PCB, plasma	400 ± 100^{A}	300 ± 50^{A}	280 ± 32^{A}	1,700 ± 48^{B}	1,800 ± 120^{B}	2,200 ± 120^{B}	2,200 ± 290^{B}	2,400 ± 560^{B}
∑OH-PCB, plasma	0.530 ± 0.092^{A}	0.500 ± 0.013^{A}	0.590 ± 0.037^{A}	0.770 ± 0.033^{B}	2.60 ± 0.330^{C}	5.50 ± 0.520^{D}	2.70 ± 230^{C}	5.90 ± 0.600^{D}

Capital letters (A and B) represent statistically significant ($p < 0.05$; ANOVA and Tukey's test) differences within rows.
[a] PCB exposed = 10 μg/g food of each of Aroclor (1242, 1254 and 1260) and 0.5 μg/g food of PCBs 202 and 209.
[b] CYP1A exposed = same as PCB exposed with additional CYP1A inducing PCB congeners (PCBs 77, 126 and 169 (10 ng/g food each)).
[c] CYP2 exposed = same as PCB exposed with additional CYP2B inducting PCB congeners (PCBs 87, 99, 101, 153, 180, 183 and 194 (10 ng/g food each)).

Table 3
Indices of thyroid status including epithelial cell height, liver deiodinase activity and thyroid hormone concentration (mean ± 1S.E.; $n = 6$ per treatment and day) of juvenile rainbow trout exposed to PCBs at three temperatures and at 12 °C to treatments containing additional cytochrome P450-inducing PCB congeners (CYP1A in fish (CYP1A exposed) and CYP2B (CYP2 exposed) in mammals)

Indices of thyroid status	8 °C		12 °C				16 °C	
	Control	[a]PCB exposed	Control	PCB exposed	[b]CYP1A treatment	[c]CYP2 treatment	Control	PCB exposed
Epithelial cell height								
Sampling day 0	8.8 ± 0.78	8.7 ± 0.71	6.9 ± 0.56	6.5 ± 0.28	6.8 ± 0.78	6.3 ± 0.44	5.5 ± 0.35	5.0 ± 0.40
Sampling day 30	8.3 ± 0.46	8.0 ± 0.48	6.6 ± 0.38	7.5 ± 0.29*	7.1 ± 0.30	7.4 ± 0.32*	5.4 ± 0.25	7.2 ± 0.40*
Sampling day 50	8.2 ± 0.75	8.0 ± 0.45	6.6 ± 0.30	6.7 ± 0.14	7.1 ± 0.29	6.6 ± 0.40	5.4 ± 0.28	5.8 ± 0.25
Outer-ring deiodinase activity (pmol/mg protein/min) ($\times 10^{-3}$)								
Sampling day 0	3.6 ± 0.75	3.6 ± 0.36	4.5 ± 1.1	4.8 ± 0.44	4.9 ± 0.98	5.0 ± 0.65	6.0 ± 0.84	6.2 ± 0.49
Sampling day 30	3.7 ± 0.64	4.6 ± 0.41	4.4 ± 0.65	4.7 ± 0.81	4.5 ± 0.69	4.7 ± 0.42	5.5 ± 0.47	4.5 ± 0.40*
Sampling day 50	4.3 ± 0.41	4.8 ± 0.46	5.6 ± 0.57	6.0 ± 0.83	6.9 ± 0.86	6.1 ± 0.49	6.1 ± 0.90	6.9 ± 0.73
Sampling day 70	4.6 ± 0.88	5.2 ± 0.45	5.4 ± 1.1	5.3 ± 1.4	4.7 ± 0.70	7.2 ± 0.80	6.2 ± 0.51	6.6 ± 0.58
Inner-ring deiodinase activity (pmol/mg protein/min) ($\times 10^{-3}$)								
Sampling day 0	0.72 ± 0.58	0.14 ± 0.14	0.72 ± 0.54	1.3 ± 0.74	1.8 ± 0.42	0.34 ± 0.34	0.38 ± 0.38	1.4 ± 0.71
Sampling day 30	1.2 ± 0.33	0.45 ± 0.31	0.48 ± 0.16	0.76 ± 0.26	0.31 ± 0.17	0.32 ± 0.16	1.9 ± 0.59	1.5 ± 0.56
Sampling day 50	0.98 ± 0.46	1.4 ± 0.32	0.74 ± 0.32	1.9 ± 0.32	1.2 ± 0.24	1.5 ± 0.27	0.91 ± 0.28	1.3 ± 0.37
Sampling day 70	1.1 ± 0.38	1.9 ± 0.42	1.1 ± 0.38	0.23 ± 0.29	0.91 ± 0.91	1.8 ± 0.92	1.4 ± 0.79	0.84 ± 0.38
T4 plasma concentrations (nmol/l)								
Sampling day 0	1.6 ± 0.43	1.8 ± 0.81	2.7 ± 0.55	1.5 ± 1.1	2.2 ± 0.45	2.6 ± 0.80	3.2 ± 0.53	3.6 ± 0.54
Sampling day 30	2.6 ± 0.20	2.5 ± 0.34	2.9 ± 0.22	3.4 ± 0.56	2.4 ± 0.37	2.0 ± 0.54	2.6 ± 0.30	3.7 ± 0.40
Sampling day 50	2.5 ± 0.76	2.7 ± 0.19	3.0 ± 0.24	3.0 ± 0.25	2.2 ± 0.48	2.3 ± 0.44	2.5 ± 0.42	3.4 ± 0.43
T3 plasma concentrations (nmol/l)								
Sampling day 0	1.5 ± 0.84	1.8 ± 0.81	2.7 ± 1.1	1.5 ± 1.0	1.6 ± 0.51	2.6 ± 0.80	2.6 ± 0.96	2.7 ± 0.85
Sampling day 30	2.6 ± 0.38	1.6 ± 0.49	3.4 ± 0.56	2.2 ± 0.90	2.0 ± 0.54	2.4 ± 0.37	1.9 ± 0.46	1.6 ± 0.54
Sampling day 50	2.5 ± 0.76	3.7 ± 0.54	3.2 ± 0.51	2.8 ± 0.41	2.2 ± 0.48	2.3 ± 0.44	3.4 ± 0.43	2.5 ± 0.42

Significant differences between PCB exposed or CYP treatments (CYP1A or CYP2) and control fish within the same temperature and sampling day are indicated by an asterisk. Tukey's test for temperatures and Dunnet's test comparing exposed fish to control fish at the significance level of $^{*}p < 0.05$.

[a] PCB exposed = 10 μg/g of Aroclors 1242:1454:1260 in a 1:1:1 ratio.

[b] CYP1A exposed = same as PCB exposed + 10 ng/g of CYP1A inducing congeners (PCBs 77, 126 and 169).

[c] CYP2 exposed = same as PCB exposed + 10 ng/g of CYP2B inducing congeners (PCBs 87, 99, 101, 153, 180, 183 and 194).

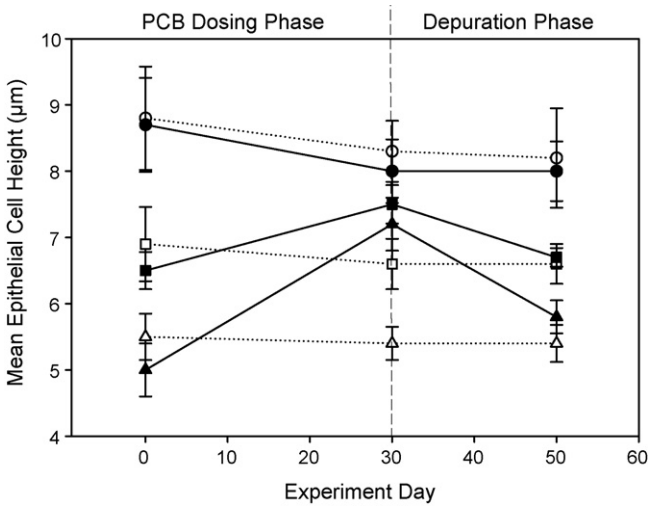

Fig. 1. Thyroid epithelial cell height (TECH) measured via thyroid histology in rainbow trout (*Oncorhynchus mykiss*) held at 8, 12 and 16 °C fed a PCB Aroclor mixture (1248:1254:1260; 1:1:1) spiked in fish food for 30 days, followed by a depuration phase where fish were fed non-spiked food. Circles represent fish held at 8 °C, squares represent fish held at 12 °C and triangles represent fish held at 16 °C. Dotted lines represent control treatment fish, solid lines represent PCB-exposed fish and each symbol is mean ± 1 SE, $n = 6$.

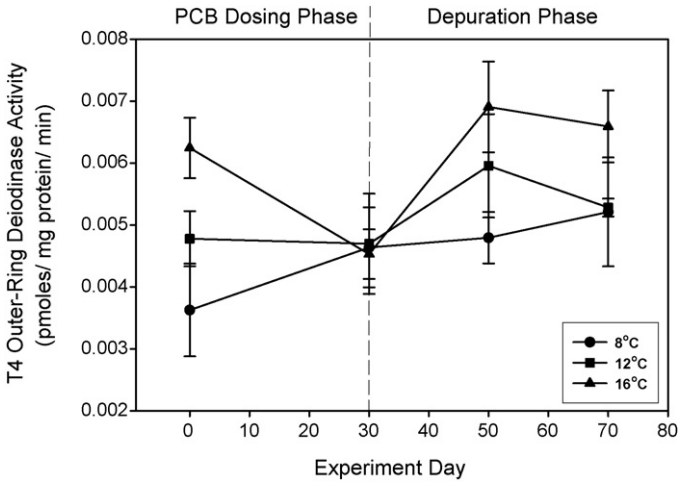

Fig. 2. Outer-ring T4 deiodinase activity (ORD) in liver hepatocytes of rainbow trout (*Oncorhynchus mykiss*) held at 8, 12 and 16 °C fed a PCB Aroclor mixture (1248:1254:1260; 1:1:1) spiked in fish food for 30 days, followed by a depuration phase where fish were fed non-spiked food. Circles represent fish held at 8 °C, squares represent fish held at 12 °C, triangles represent fish held at 16 °C and each symbol is mean ± 1 SE, $n = 6$.

3.4. Liver deiodinase activity

Significant differences ($p < 0.05$) were found in T_4ORD activity between PCB-exposed fish and control fish only in the 16 °C treatment at day 30 of exposure but not at any other sampling day or temperature treatment (Table 3). Exposure to PCBs known to induce CYP (i.e., CYP1A and CYP2 treatments) did not influence T_4ORD in the 12 °C treatment on any day (Table 3). No significant differences ($p > 0.05$) were found in T_3IRD activity between PCB-exposed fish or fish from the CYP1A or CYP2 treatments and control fish at any sampling day or temperature treatment (Table 3). Temperature was a significant factor in the amount of T_4 ORD activity (Fig. 2), but not T_3IRD activity (data not shown) in rainbow trout. Outer-ring deiodinase activity was greater at higher temperatures at day 0 (Fig. 2). While there were no changes in T_4ORD activity in control treatments over the course of the experiment, T_4ORD activity was influenced by PCB exposure in 16 °C treatment and the relationship between T_4ORD activity and temperature was diminished in the PCB-exposed fish at day 30 of exposure relative to controls (Fig. 2 and Table 3). At day 30, levels of ORD in the 16 °C PCB exposed fish were not significantly different ($p > 0.05$) than ORD activity in the 8 or 12 °C control and PCB-exposed fish while control fish from the 16 °C treatment had elevated ORD activity relative to 8 °C fish similar to observations at day 0. At day 50 of the experiment (20 days of depuration), levels of T_4ORD activity in the PCB-exposed fish from the 16 °C treatment returned to levels observed in the control fish at this temperature (Fig. 2 and Table 3).

3.5. Plasma thyroid hormone concentrations

There were no significant differences ($p > 0.05$) in plasma thyroid concentrations (T_3 and T_4 concentrations) among PCB treatments, temperature treatments or day (Table 3). Mean T_4 plasma concentrations in the trout across all treatments was 2.86 ± 0.13 nmol/l, whereas plasma T_3 concentrations were below the method detection limit (MDL) (0.05 nmol/l) in most fish. Mean concentrations of T_3 found in fish with levels above the MDL were 1.60 ± 0.16 nmol/l.

3.6. EROD activity

All treatments had similar EROD activity [2.3 ± 0.4 pmol/min/mg protein (microsomal)] at day 0 of experimentation. Treatments that included additional CYP inducing PCB congeners (CYP1A and CYP2 treatments) had elevated EROD activity at day 30 of dosing as compared to both control and PCB-exposed fish at this day ($p < 0.05$) (Table 4). After 20 days depuration (day 50), EROD in the CYP2 treatment was similar to control, however, EROD activity in the CYP1A treatment remained elevated until 40 days after switching fish to non-spiked food relative to control fish treatments. No differences were observed between PCB-exposed (fish exposed to PCBs but not to additional CYP inducing PCBs) and control fish at any day of experimentation.

4. Discussion

4.1. Growth, liver size and survival

Increasing whole body and liver growth rates in fish with increasing temperature observed in this study are not surprising and have been noted previously (Wurtsbaugh and Davis, 1977; Austreng et al., 1987; Myrick and Cech, 2000). The lack of a significant difference in growth rates between the 12 and 16 °C treatments likely reflects the fact that this temperature range is within the optimal for growth of rainbow trout (Hokanson et al., 1977). Liver somatic indices were greater in fish held at cooler

Table 4
Ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/min/mg microsomal protein) in juvenile rainbow trout exposed to PCBs at three temperatures and at 12 °C to treatments containing additional cytochrome P450-inducing PCB congeners (CYP1A in fish (CYP1A exposed) and CYP2B (CYP2 exposed) in mammals)

Day	8 °C		12 °C				16 °C	
	Control	[a]PCB exposed	Control	PCB exposed	[b]CYP1A treatment	[c]CYP2 treatment	Control	PCB exposed
Day 0	0.98 ± 0.46	2.9 ± 1.6	2.1 ± 1.0	1.1 ± 0.25	3.3 ± 1.3	4.8 ± 0.32	2.5 ± 0.64	1.6 ± 0.12
Day 30	2.8 ± 0.44	7.7 ± 1.2	4.0 ± 0.92	6.5 ± 1.5	49 ± 7.1*	31 ± 9.5*	3.2 ± 0.97	6.1 ± 1.7
Day 50	4.4 ± 0.65	11 ± 4.3	3.3 ± 0.96	6.2 ± 1.7	44 ± 9.0*	5.5 ± 1.1	2.6 ± 0.40	7.3 ± 1.1
Day 70	1.4 ± 0.37	2.3 ± 0.60	1.5 ± 0.42	2.3 ± 0.60	2.3 ± 0.80	2.1 ± 0.92	0.92 ± 0.31	0.55 ± 0.1

Significant differences between PCB treated fish and controls are indicated by an asterisk. Tukey's test for temperatures and Dunnet's test comparing exposed fish to control fish at the significance level of $^{*}p > 0.05$.

temperatures, which has also been demonstrated previously (Jürss, 1979; Jürss, 1981), but did not differ between control, PCB-exposed, and CYP1A or CYP2 treatments. Elevation of LSI accompanying exposure to high doses of CYP1A-inducing compounds is often reported (Niimi, 1996; Cleland et al., 1988), but was not observed in this study. Rainbow trout exposed to high concentrations of PCBs (300 mg/kg body weight) for 1 year had increased liver size (Cleland et al., 1988). Niimi (1996) also found that laboratory held fish exposed to PCBs in excess of 50 mg/kg wet weight caused reductions in fish weight. Lipid corrected concentrations in fish from this study were lower than but approaching those concentrations reported by Niimi (1996). The lack of differences in growth rates, LSI and mortality between control, PCB-exposed and CYP1A and CYP2 treatment fish at the same temperatures in this study suggest that PCBs did not significantly compromise the health of these fish, consistent with other studies exposing juvenile rainbow trout to PCBs at similar dietary concentrations (Buckman et al., 2004; Fisk et al., 1998).

4.2. PCB and OH-PCB concentrations

It was not surprising that PCBs were found in control food because the trout chow fed to fish in this experiment was made from fish meal and PCBs are ubiquitous in the environment. PCB levels were higher in PCB-exposed fish as expected, however the increase in PCB concentrations in fish from the CYP1A treatment at day 30 was unexpected. Although additional CYP1A inducing PCB congeners were added to the food, these levels were relatively low as compared to total PCB concentrations in the food. OH-PCBs profiles in rainbow trout from this study (reported in Buckman et al., 2007) were similar to OH-PCB profiles observed in lake trout from the Great Lakes (Campbell et al., 2003). This is relevant as PCB exposures in this experiment are similar to the pattern of PCB exposure in the environment.

4.3. Indices of thyroid status

This study was unique in that it examined the effects of environmentally relevant PCB mixtures on the thyroid status in fish at varying temperature regimes. In addition, we examined indices of thyroid status which encompass both central control (brain–pituitary axis; thyroid histology and plasma T_4 concentrations) and peripheral control (tissue thyroid hormone metabolism; plasma T_3 concentrations, ORD and IRD activity) of thyroid hormone regulation. Indices of thyroid status were highly dependent on water temperature but some indices were modulated by exposure to high concentrations of PCBs in the food after a 30-day period at higher water temperatures. Our observations of dysfunction of some indices of thyroid status only at high temperatures (>12 °C) in highly PCB contaminated fish (concentrations approximately >30,000 ng/g, lipid weight) would suggest that thyroid dysfunction due to PCBs in fish from the wild should only occur in warmer systems that are located in highly contaminated areas. Great Lakes fish remain in water temperatures that are usually well below 12 °C (Haynes, 1995) and cases of microfollicular hyperplasia are observed year-round in Great Lakes salmonids (Leatherland, 1993), although this may reflect the use of adult fish in these assessments. Further, although concentrations of PCBs in the PCB-exposed and CYP1A and CYP2 treatment fish in this study exceeded PCB concentrations in fish from the Great Lakes, we observed no microfollicular hyperplasia suggesting that microfollicular hyperplasia is likely due to factors other than PCBs. However, continued exposure to the PCB spiked food over longer periods of time (beyond 30 days) may elicit this effect.

4.4. Thyroid epithelial cell height

The inverse relationship between temperature and epithelial cell height in fish has been noted in other studies (Eales, 1964; Drury and Eales, 1968). This relationship was somewhat modulated by high PCB exposure at higher temperatures, but there was no evidence of overt goiter or microfollicular hyperplasia development, which has been observed in salmonids from the Great Lakes (Leatherland, 1993). Previous studies on the long-term exposure to dietary PCBs given as Aroclor mixtures for 4–12 weeks showed no evidence of thyroid hyperplasia in Coho salmon (Leatherland and Sonstegard, 1978) and rainbow trout (Leatherland and Sonstegard, 1979). Long term exposure of lake trout (*Salvelinus namaycush*) to PCB 126 by i.p. injection or oral gavage temporarily increased thyroid epithelial cell height (Brown et al., 2004). Single dietary doses of PCB congener 77 in Arctic grayling (*Thymallus arcticus*) did not affect thyroid histology after 30 or 90 days (Palace et al., 2001). In this study, fish exposed to PCB congeners known to induce CYP isoforms (CYP1A in fish and CYP2B in mammals) did not differ from

PCB exposed fish (10 μg/g for each Aroclor) without additional CYP inducing PCB congeners indicating that factors other than CYP inducing PCBs, and possibly PCB exposure in general, may be responsible for eliciting thyroid effects in fish. However, long-term, continuous exposure to various PCB congeners may be an important factor in eliciting thyroid effects in rainbow trout, and because fish from this study were only exposed for 30 days, it is possible that the exposure duration was too short to observe effects on TECH.

4.5. Liver deiodinase activity

Exposure to PCBs in this study elicited effects in liver ORD but not IRD, although at concentrations approximately 10 times those currently observed in Great Lakes salmonids. The increase in ORD with increasing temperature has been noted in other studies with fish (Johnston and Eales, 1995). V_{max}/K_m for T_4ORD was 0.48, 1.62 and 1.83 at 4, 12 and 18 °C, respectively, by Johnston and Eales (1995), which were similar to values at day 0 in this study. Outer-ring deiodination in this study was decreased only in the PCB-exposed treatment at 16 °C, indicating that PCBs can reduce outer-ring deiodination at higher temperatures.

In addition, results from our study demonstrated that exposure to CYP inducing PCBs (1A in fish or 2B in mammals) had no influence on ORD relative to control fish. This is in contrast to immature female American plaice (*H. platessoides*), which showed increased liver T_4ORD activity after i.p. injection with 5–500 mg/kg of CYP1A-inducing PCBs (congener 77 or 126) (Adams et al., 2000), although concentrations were much higher than those used in this experiment. Changes in IRD activity were not observed in any treatment in this study, although IRD activity is generally low in laboratory rainbow trout (Todd and Eales, 2002).

4.6. Plasma thyroid hormone concentrations

Neither temperature nor PCB exposure influenced concentrations of plasma T_4 and T_3 in trout from this study. Temperature has been shown to have little effect on plasma T_4 and T_3 in fish (Eales et al., 1982, 1986), however there are mixed reports on the influence of PCBs on plasma T_4 and T_3 concentrations. Rainbow trout fed Aroclor 1254 (500 mg/g dry food) showed no change (Leatherland and Sonstegard, 1978) but rainbow trout fed a mixture of Aroclor 1242 and 1254 (500 mg/kg of a 1:4 ratio) had lower plasma T_4 and T_3 concentrations (Leatherland and Sonstegard, 1980); again, these concentrations were much higher than those used in this experiment and what is observed in the environment. As well, no effects were observed in fish from the CYP1A and CYP2 treatments in this study, which is in agreement with Palace et al. (2001) who reported that single dietary doses of PCB 77 did not affect plasma thyroid concentrations in Arctic grayling. However, long term exposure of lake trout (*S. namaycush*) to PCB 126 increased plasma T_4 concentrations and also increased plasma T_4 clearance, but did not change plasma T_3 concentrations, or T_3-glucuronidation (Brown et al., 2004). Adams et al. (2000) also reported increased plasma T_4 concentrations but no change in T_3 concentrations in American plaice (*H. platessoides*) injected i.p. with either PCB 77 or PCB 126.

4.7. EROD activity

Elevated EROD activity has been observed in a number of studies where fish have been exposed to PCBs, usually the coplanar PCBs (congeners 77, 126 and 169). Concentrations of Aroclor 1254, as low as 0.2 mg/kg (b.w.), lower than concentrations used in this experiment, were able to induce EROD in rainbow trout (Melancon and Lech, 1983). In addition, a 10-fold induction of EROD activity was found in rainbow trout injected with Aroclor 1254 (i.p. 150 mg/kg b.w.) (Melancon and Lech, 1983). The most potent CYP1A-inducers are the coplanar PCB congeners (McKinney and Singh, 1981; Tsuzuki and Tanabe, 1991) and the planar conformation is considered a prerequisite for AhR binding (Metcalfe and Haffner, 1995). EROD induction in the CYP1A treatment was observed in this study, as expected, but was also elevated in the CYP2 treatment fish. This would indicate that CYP1A may be induced by high concentrations of PCB congeners known to induce CYP2B in mammals or that a non-AhR mediated regulatory pathway is involved in CYP1A induction or in the EROD assay. Other studies (Sadar et al., 1996a,b) have also demonstrated induction of CYP1A gene expression in cultured rainbow trout hepatocytes exposed to congeners that induce CYP2B in mammals, although our study may be the first to definitively demonstrate this effect in vivo. Studies of PCB exposure in primates have indicated that di- and tri-*ortho* substituted PCBs (CYP2B-like inducers) which are not assumed to elicit 'dioxin-like' responses (CYP1A induction) demonstrated the capacity to induce CYP1A mRNA and enzyme activity in hepatocytes of cynomolgus monkey (*Macaca fascicularis*) (Van Der Burght et al., 1998, 1999) suggesting that the AhR may not be as selective for ligand binding as previously thought.

4.8. Influence of OH-PCBs on thyroid indices

While there is evidence in this study that exposure to PCBs diminishes the response of TECH to temperature and ORD in the fish, it is possible that OH-PCBs may be responsible for these responses. Hydroxylated PCBs, which are phase 1 metabolites of PCBs, have a –OH group substituted in the *para* position of the biphenyl ring, resemble the thyroid hormones (T_3 and T_4), and may disrupt the thyroid transport system of exposed organisms (Letcher et al., 2000). Relationships between OH-PCBs and some indices of thyroid status have been found in polar bears (*Ursus maritimus*) (Sandau et al., 2000) and rats (Brouwer et al., 1990; Cheek et al., 1999) but studies with fish are lacking. OH-PCBs were observed in the plasma of fish from all treatments in this experiment, with the greatest concentrations in the PCB-exposed 16 °C treatment and the CYP2 treatment at 12 °C (Table 2). Unfortunately, the individual effect of PCBs and OH-PCBs cannot be teased apart in this experiment, however due to the high concentrations of OH-PCBs and the lack of thyroid effects observed in the CYP2 treatment our results would suggest that OH-PCBs alone are

not responsible for the thyroid effects observed in wild fish. It is also likely that past experiments reported in literature that exposed fish to PCBs were also confounded by the presence of OH-PCBs, although this factor was not considered in those experiments.

5. Conclusion

This study not only looked at the effect of PCBs on the thyroid status in rainbow trout but also the multiple influences of temperature, exposure to CYP-inducing PCBs, and concentrations of OH-PCBs, PCB metabolites. In summary, some indices of thyroid dysfunction (TECH and T_4ORD) were observed in rainbow trout held at 12 and 16 °C but only after 30 days of PCB exposure. However, these changes are likely within the compensatory scope of the thyroid system since other indices of thyroid dysfunction (i.e., T_3IRD and circulating concentrations of T_3 and T_4) were not observed. Effects of PCB-exposure on thyroid axis of the fish were diminished after 20 days of depuration. No thyroid dysfunction was observed in control fish despite PCB concentrations similar to wild fish from the Great Lakes. These results suggest that microfollicular hyperplasia observed in salmonids from the Great Lakes is likely due to factors other than PCB exposure or due to long term exposure to PCBs.

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