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Effects of aqueous exposure to polychlorinated biphenyls (Aroclor 1254) on physiology and behavior of smolt development of Atlantic salmon

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Abstract

Polychlorinated biphenyls (PCBs) are a widespread aquatic contaminant and are present in both wild and hatchery raised Atlantic salmon, *Salmo salar*. The possible sub-lethal alterations in smolt physiology and behavior due to PCB exposure of salmon have not been widely examined. In this study, we examined the effects of the PCB mixture Aroclor 1254 on survival and smolt development of Atlantic salmon. In separate experiments, fish were exposed as yolk-sac larvae or as juveniles just prior to the parr–smolt transformation in April to 1 $\mu g\,l^{-1}$ (PCB-1) or 10 $\mu g\,l^{-1}$ (PCB-10) aqueous Aroclor 1254 (A1254), or vehicle for 21 days. After exposure, yolk-sac larvae were reared at ambient conditions for 1 year, until the peak of smolting the following May. Juveniles were sampled immediately after exposure. Both groups were assessed for behavioral, osmoregulatory, and endocrine disruption of smolt development at the peak of smolting. PCB-1 and PCB-10 treated yolk-sac larvae exhibited significant increases in the rate of opercular movement after 14 and 21 days of exposure. At the peak of smolting, prior exposure as yolk-sac larvae to PCB-1 did not affect behavior, while PCB-10 dramatically decreased volitional preference for seawater. Neither concentration of A1254 had long-term effects on the osmoregulatory or endocrine parameters measured in animals exposed as yolk-sac larvae. Juvenile fish exposed to PCB-1 or PCB-10 during smolting exhibited a dose-dependent reduction in preference for seawater. Fish treated with the higher dose of A1254 also exhibited a 50% decrease in gill Na^+,K^+-ATPase activity and a 10% decrease in plasma chloride levels in freshwater. In addition, plasma triiodothyronine was reduced 35–50% and plasma cortisol 58% in response to exposure to either concentration; whereas plasma thyroxine, growth hormone, and insulin-like growth factor I levels were unaffected. These results indicate that the effects of exposure to A1254 may vary according to developmental stage. Exposure to A1254 in the freshwater environment can inhibit preparatory adaptations that occur during smolting, thereby reducing marine survival and sustainability of salmon populations.

Keywords: Atlantic salmon; PCB; Aroclor 1254; Salinity tolerance; Osmoregulation; Seawater preference; Smolting

1. Introduction

Since the mid-1980s, worldwide populations of wild Atlantic salmon (*Salmo salar*) have declined by 45% (WWF, 2001). These declines are attributed to anthropogenic disturbances, and anadromous salmonids pass through some of the most polluted areas of larger rivers and estuaries during their downstream migration to the sea (Parrish et al., 1998). Manufactured in mixtures called Aroclors, polychlorinated biphenyls (PCBs) are highly stable anthropogenic chemicals. Due to their historically widespread use, PCBs are ubiquitous in the aquatic environment. As a result of this significant presence and persistence, PCBs are hypothesized to be one of the factors contributing to the recent declines of wild populations of Atlantic salmon as well as failures in salmon restoration (NRC, 2004).

During the parr–smolt transformation of anadromous salmon, dramatic changes in morphology and physiology occur simultaneously with developmentally novel behaviors such as downstream migration and seawater entry. This transformation is size-dependent, driven by changes in photoperiod and temperature, and mediated by increases in endogenous hormones including thyroid hormones (THs), cortisol, growth hormone (GH), and insulin-like growth factor I (IGF-I) (Hoar, 1976; McCormick, 2001). Xenobiotics can inhibit endocrine changes

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that are critical for normal development, leading to disruption of smolt physiology (Moore et al., 2003; Jorgensen et al., 2004; McCormick et al., 2005) and behavior (Madsen et al., 2004; Lerner and McCormick, 2004).

The possible sub-lethal alterations in development due to PCB exposure are associated with disruption of neurological, immunological, reproductive, endocrine and metabolic function (Safe, 1994). While research on the effects of PCBs in animals often focuses on the interference of normal endocrine functions controlling developmental and reproductive physiology (Monosson, 2000; Pocar et al., 2003; Gauger et al., 2004), there is also evidence for their effects on a wide range of behaviors (Glennemeier and Denver, 2001; Nakayama et al., 2005; Sugawara et al., 2006; Schmidt et al., 2006; Fisher et al., 2006). Exposure of sexually mature animals to PCBs can alter a variety of behaviors including reproductive behavior of American kestrels, *Falco sparverius* (Fisher et al., 2006), swimming behavior of water fleas, *Daphnia magna* (Schmidt et al., 2006) and schooling behavior of Japanese medaka, *Oryzias latipes* (Nakayama et al., 2005). Research on the long-term effects of PCB exposure during ontogeny on future development of juvenile or adult behaviors is scarce, but the obtained data indicate that early exposure reduces activity and feeding of leopard frog tadpoles (*Rana pipiens*) and decreases maze testing success in mice (Glennemeier and Denver, 2001; Sugawara et al., 2006).

Most studies investigating the effects of PCBs on early development are relatively short-term and therefore do not evaluate the potential long-term effects on the development and behavior of subsequent life-stages. There is evidence that exposure to the PCB mixture Aroclor 1254 (A1254) can negatively affect mechanisms involved in smolt development and migration of Arctic charr (*Salvelinus alpinus*) (Jorgensen et al., 2002, 2004), and that PCBs can disrupt normal metabolic and immune processes in Atlantic salmon (Rees et al., 2003; Iwanowicz et al., 2005). In light of dramatic declines in Atlantic salmon populations, investigations into the effects of PCBs on smolt physiology and behavior, as well the importance of developmental timing on the impacts of exposure, are warranted. The aim of the study was thus to elucidate the developmental, physiological, and behavioral impacts on smolt development after aqueous exposure of Atlantic salmon to environmentally relevant concentrations of A1254 as yolk-sac larvae or during smolt development.

2. Materials and methods

2.1. Fish rearing

Atlantic salmon were obtained from the White River National Fish Hatchery (Bethel, VT, USA) and transferred to the Conte Anadromous Fish Research Center (Turners Falls, MA, USA) as parr in October 2002 or eggs in February 2003. Parr were reared in 1.6 m diameter tanks with Connecticut River water at a flow rate of 6–8 l min^{-1} at ambient temperature with supplemental aeration, under natural photoperiod and ambient temperature (2–18 °C). From December 7th to January 21st, water temperatures were reduced to 4 ± 1 °C to provide parr with a "winter" event. Animals were fed to satiation twice daily (Bio-Oregon Inc., Warrenton, OR) until A1254 exposure just prior to the parr–smolt transformation.

Eggs were maintained until hatching in covered egg trays under natural photoperiod with a flow rate of 1–2 l min^{-1} using dechlorinated city water at 7 ± 1 °C. After A1254 treatment and upon absorption of the yolk-sac, larvae were placed in 1 m diameter tanks, provided dechlorinated city water at 10 ± 1 °C at a flow rate of 2–4 l min^{-1}, with supplemental aeration and fed twice daily to satiation. In September, fish were transferred to 1.6 m diameter tanks with Connecticut River water at a flow rate of 6–8 l min^{-1} at ambient temperature with supplemental aeration, under natural photoperiod and fed twice daily to satiation. These fish were maintained in this manner (except from December 7th to January 21st when water temperatures were reduced to 4 ± 1 °C to provide parr with a "winter" event) until the peak of smolting in May 2004 at which time they were sampled as described below.

2.2. Yolk-sac larvae exposure

During March 2003, 21-day post-hatch (dph) Atlantic salmon yolk-sac larvae were exposed to 1 or 10 μg l^{-1} (PCB-1 or PCB-10, respectively) of A1254 (Lot # 124-191-B; Accustandard, New Haven, CT) or vehicle control (0.0001% methanol) in replicate tanks, each containing 500 yolk-sac larvae, for 3 weeks. Concentrated solutions were delivered via a peristaltic pump (Cole-Parmer Instrument Co., Vernon Hills, IL, USA) and mixed in head tanks with dechlorinated city water to deliver the desired concentrations of aqueous A1254 on a continuous basis and under flow-through conditions to the rearing tanks as described above. During the exposure, water temperature was maintained at 7 ± 1 °C. After exposure, animals were held for 1 year as described above until sampling.

2.3. Smolt exposure

During April 2003 replicate tanks of 36 fish large enough to become smolts (fork length > 11.0 cm) were exposed to 1 or 10 μg l^{-1} (PCB-1 or PCB-10, respectively) of A1254 or vehicle control (0.0001% methanol) in their rearing tanks for 3 weeks. Chemical delivery was the same as described above for yolk-sac larvae. During the exposure, water temperature was maintained at 10 ± 1 °C and animals were fed a maintenance diet (3% body weight) once every other day. Food was withheld 24 h prior to sampling. Fish were sampled immediately following exposure as described below.

2.4. Fish sampling

On the 14th and 21st days of contaminant exposure, 12 yolk-sac larvae per treatment group were recorded using a handheld video camera over their rearing trays. The number of opercular movements for each individual was recorded for 10 min. Means for individual opercular movements were used to calculate group means, and presented as mean opercular movements per minute.

During the peak of smolt development in early May (2003 for fish exposed as juveniles and 2004 for fish exposed as yolk-sac

larvae), 10 (fish exposed as yolk-sac larvae) or 12 (fish exposed during smolting) fish from each treatment group (5 or 6 fish from each of two replicate tanks) were either assessed in a behavioral assay for their preference for seawater (SW), transferred to 30‰ SW for 24 h to assess ion regulatory ability in SW (SW challenge), or sacrificed from their freshwater (FW) rearing tanks to examine potential effects on physiological indices related to smolting.

The SW preference tank was constructed of 1.25 cm thick PVC sheets supported by a wood frame. Lighting was provided by four 23 W, 5100 K (degrees Kelvin) full spectrum bulbs producing 1600 lms each. The tank was divided into four chambers (two sets of parallel chambers connected by a PVC bridge) allowing two treatment groups to be tested simultaneously. The parallel chambers were separated by a perforated wall so that water was shared between them. To conduct the behavioral assay the tank was filled with FW and SW (32‰) into their respective sides. Each chamber was filled to just below the bridge. Animals were allowed to acclimate after netting into the FW chambers for 2 h. The FW side was then filled and allowed to spill over into the SW chambers until there was 7.6 cm of water over the bridge. Once the aqueous bridge was formed, activity was videotaped from a central location above the chambers for 2 h. Water temperature, salinity, and dissolved oxygen (DO) was measured before the addition of fish and at the end of the assay. Air was bubbled into each of the four chambers to maintain DO. Both SW and FW were chilled to $11.5 \pm 1\,^{\circ}C$. Over the course of 2 h, water temperature increased by approximately 1.5 °C, the salinity of the FW chamber increased to approximately 2.5–3.5‰, while the surface and bottom salinity of the SW chambers decreased to approximately 31.5–28.5‰. Videotape was analyzed for presence of fish in SW at 30 s intervals for 120 min, and is presented as percent of fish in SW. Trials were conducted in replicate using 10 fish from each PCB concentration and 10 vehicle controls.

The SW challenge was conducted by rapidly netting fish from the FW tank and transferring them directly into 1.6 m diameter tanks with recirculating SW (30‰) at 10 °C with charcoal filtration and aeration. Fish were sampled according to the protocol below 24 h after transfer.

Sampled fish were anesthetized with $200\,mg\,l^{-1}$ tricaine methane sulphonate (neutralized and buffered with sodium bicarbonate, pH 7.0) and length and weight measured. All fish were sampled within 6 min of first disturbing the tank to ensure that baseline (unstressed) levels of cortisol were measured. Blood was collected in heparinized syringes from the caudal vasculature, centrifuged at $3000 \times g$ for 5 min, then plasma removed and frozen at −80 °C. A gill biopsy (approximately six to eight primary gill filaments) was taken and placed in 100 μl of SEI (250 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3) on ice for determination of Na^+,K^+-ATPase activity. Samples were frozen within 30 min and stored at −80 °C until analysis.

2.5. Analytical methods

Gill Na^+,K^+-ATPase activity was measured according to the microassay protocol of McCormick (1993). Gill filaments were homogenized in SEI buffer containing 0.1% sodium deoxycholate. Following centrifugation ($3000 \times g$ for 5 min) to remove large insoluble material, Na^+,K^+-ATPase activity of the supernatant was determined by linking ATP hydrolysis to the oxidation of nicotinamide adenine dinucleotide (NADH), measured at 340 nm for 10 min at 25 °C, in the presence and absence of 0.5 mM ouabain. Protein content in the gill homogenate was measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA), and specific activities were expressed as $\mu mol\,ADP\,mg^{-1}$ of protein h^{-1}.

Plasma T_3 and T_4 levels were measured by a direct radioimmunoassay (Dickhoff et al., 1978). Plasma cortisol levels were measured using an enzyme immunoassay as outlined in (Carey and McCormick, 1998). Plasma GH levels were measured using a specific double-antibody salmon GH radioimmunoassay (Björnsson et al., 1994). Plasma IGF-I levels were measured by a radioimmunoassay validated for salmonids (Moriyama et al., 1994). Plasma chloride concentration was measured using silver titration chloridometry (Labconco, Kansas City, MO) with external standards.

Fish carcasses were frozen at −80 °C, and whole fish body burden concentrations of A1254 determined (Spectrum Analytical Inc., Agawam, MA, USA) using gas chromatography (EPA method # SW846 8082) after ultrasonic extraction (EPA method # SW846 3550B). The surrogate compounds 4,4-DB-octafluorobiphenyl and decachlorobiphenyl, which have similar chemical composition and behavior as A1254 in the analytical process, were spiked into all blanks, standards, and samples prior to analysis. Percent recoveries of these compounds ranged from 40 to 140%.

2.6. Statistics

All physiological data were initially examined for differences between replicate tanks. Significant differences were not found, therefore data were statistically analyzed and reported based on combined tanks within treatment ($n = 5$ fish per replicate tank treated as yolk-sac larvae; total = 10 fish per treatment and $n = 6$ fish per replicate tank treated during smolting; total = 12 fish per treatment). All statistics were analyzed using Statistica (Version 7; Statsoft Inc., Tulsa, OK, USA).

All values for physiological parameters are reported as means ± standard error. Analysis of variance (ANOVA) was used to examine the significance of treatment on physiological parameters. When significant treatment effects were found, Newman–Keul's tests ($P < 0.05$) were used to determine which treatments were significantly different from vehicle control.

For SW preference behavior analyses, a binomial distribution was used to compute Z-values for the observed number of cases where the number of vehicle controls in SW was greater than the number of A1254-treated fish in SW. The associated tail probability for that Z-value was used to estimate P and significance assigned when $P < 0.05$. Replicate trials were pooled due to a lack of significant differences between them ($n = 20$).

3. Results

3.1. Yolk-sac larvae exposure

Aqueous exposure of yolk-sac larvae led to an accumulation of whole body A1254 in a dose-dependant fashion. The sensitivity of detection was $11.5 \pm 0.3\ \mu g\ kg^{-1}$, and no detectable levels of A1254 were measured in the vehicle control group. Mean A1254 concentration in the treated groups were 139 ± 27 and $516 \pm 19\ \mu g\ kg^{-1}$ for PCB-1 and PCB-10, respectively.

There were no differences in mortality between groups, which was <8% during the 21-day A1254 exposure and <10% during yolk-sac absorption and the onset of first feeding. Opercular movements increased significantly by A1254 exposure at 14 and 21 days ($P < 0.001$; Fig. 1). There was no difference between the A1254 treatments and no treatment interaction with time.

Early exposure to PCB-1 did not affect smolt behavior. Time for the first fish to enter SW was not significantly different between the groups (vehicle control = 18.5 min and PCB-1 = 20.5 min) ($Z = 1.32$, $P = 0.3254$; Fig. 2) and over the entire 2 h bioassay the number of fish in SW were not different ($Z = 2.44$, $P = 0.2553$; Fig. 2). Time to first entry into SW was not significantly different between the vehicle control and PCB-10 groups (31 and 34.5 min, respectively) ($Z = 3.24$, $P = 0.1882$; Fig. 2), but exposure to PCB-10 severely impacted preference for SW ($Z = 11.21$; $P < 0.0001$; Fig. 2).

There was no detectable effect of prior A1254 exposure on gill Na^+,K^+-ATPase activity and plasma chloride levels (data not shown). Similarly, plasma levels of GH, IGF-I, THs and cortisol were unaffected (Table 1).

3.2. Smolt exposure

Aqueous exposure of smolts led to an accumulation of whole body A1254 in a dose-dependant fashion. A1254 measured in the vehicle control group was $12.9 \pm 2.1\ \mu g\ kg^{-1}$; similar, but slightly greater than the limit of detection. Mean

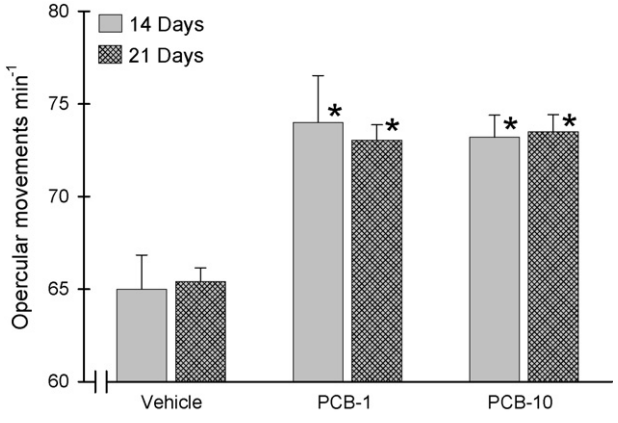

Fig. 1. Opercular movements of Atlantic salmon yolk-sac larvae 14 and 21 days during aqueous exposure to 1 or $10\ \mu g\ l^{-1}$ Aroclor 1254 (PCB-1 or PCB-10, respectively). Asterisks (*) indicate significant differences from vehicle control (one-way ANOVA followed by Newman–Keul's multiple range test; $P < 0.05$). Error bars represent the standard error from the mean; $n = 12$ fish per treatment.

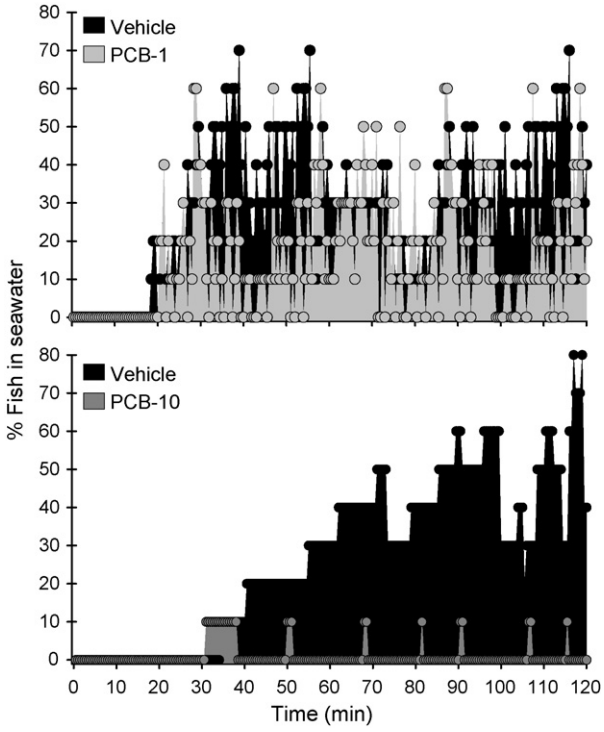

Fig. 2. Seawater preference of Atlantic salmon smolts 1 year after aqueous exposure as yolk-sac larvae to 1 or $10\ \mu g\ l^{-1}$ Aroclor 1254 (PCB-1 or PCB-10, respectively) for 21 days. For the behavioral analyses a binomial distribution was used to compare the number of cases where percent of vehicle controls in SW outnumbered treated, and significance assigned when $P < 0.05$ ($n = 12$ individuals per group in two replicate tests; error bars removed for clarity).

A1254 concentrations in the treated groups were 173 ± 34 and $529 \pm 13\ \mu g\ kg^{-1}$ for PCB-1 and PCB-10, respectively. There was no mortality in any group during exposure.

Vehicle-treated control fish were first observed in SW 13.5 min after the aqueous bridge was formed, whereas fish exposed to PCB-1 exhibited a significant latency to enter SW; no fish entered until 25.5 min after bridge formation ($Z = 4.37$, $P < 0.0001$; Fig. 3). Over the entire 2 h bioassay, significantly fewer PCB-1 treated fish entered SW ($Z = 10.7$, $P < 0.0001$; Fig. 3), and a statistical comparison of fish present in SW from 30 to 120 min indicates that this difference was not dependent on the initial delay to enter ($Z = 9.77$, $P < 0.0001$; Fig. 3). In contrast, the time of first entry into SW was not significantly different between vehicle control- and PCB-10-treated fish ($Z = 1.21$, $P = 0.2249$; Fig. 3) and the number of fish in SW was not significantly different from 0 to 64.5 min ($Z = 0.34$, $P = 0.7357$; Fig. 3). Over the entire 2 h bioassay, significantly fewer PCB-10 treated fish entered SW ($Z = 10.79$, $P < 0.0001$; Fig. 3) and this difference was due to the SW preference behavior of PCB-10 fish in the last hour of the assay. From 65 to 120 min of the bioassay, less than 10% of the PCB-10 treated fish were in SW, compared with approximately 60% of the control fish ($Z = 9.14$, $P < 0.0001$; Fig. 3).

Gill Na^+,K^+-ATPase activity was not affected by PCB-1 and was decreased 50% in fish exposed to PCB-10 ($P < 0.001$; Fig. 3). Similarly, PCB-1 did not affect plasma chloride levels, which was lower in the PCB-10 group in FW ($P < 0.001$; Fig. 3).

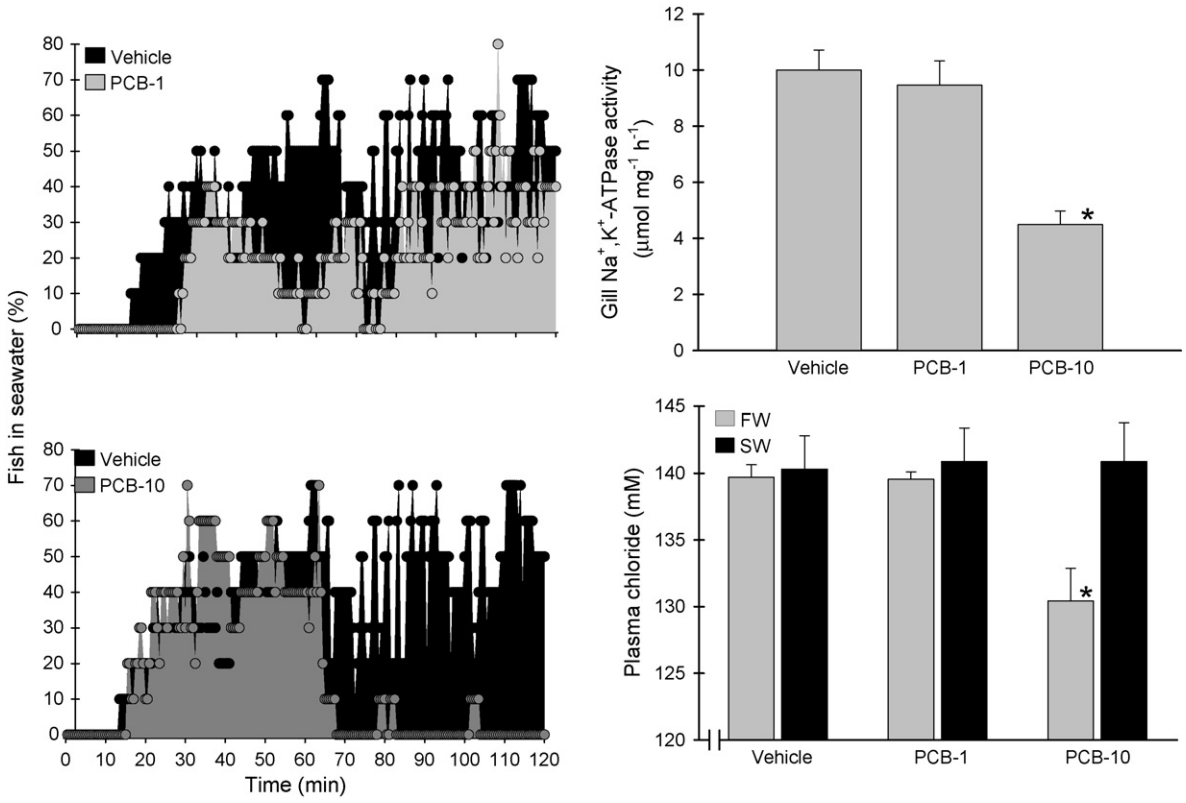

Fig. 3. Seawater preference (top and bottom left), gill Na^+,K^+-ATPase activity (top right), and plasma chloride (bottom right) of Atlantic salmon smolts directly after aqueous exposure during smolting to 1 or 10 $\mu g\,l^{-1}$ Aroclor 1254 (PCB-1 or PCB-10, respectively) for 21 days. For the behavioral analyses a binomial distribution was used to compare the number of cases where percent of vehicle controls in SW outnumbered treated, and significance assigned when $P < 0.05$ ($n = 12$ individuals per group in replicate tests; error bars removed for clarity). For the physiological analyses asterisks (*) indicate significant differences from vehicle control (one-way ANOVA followed by Newman–Keul's multiple range test; $P < 0.05$). Error bars represent the standard error from the mean; $n = 12$ fish per treatment.

There was no effect of exposure to either PCB concentration on plasma chloride levels of fish in SW (Fig. 3).

Plasma thyroxine levels were unaffected by aqueous A1254 exposure, but plasma triiodothyronine levels were reduced 35–50% in A1254-exposed smolts (PCB-1, $P < 0.001$; PCB-10, $P = 0.0023$) (Table 1). Plasma cortisol levels were 57 and 59% lower after exposure to PCB-1 and PCB-10, respectively ($P < 0.001$; Table 1). There was no effect of A1254 on plasma GH and IGF-I levels (Table 1).

4. Discussion

Total body burden of fish exposed to PCBs in this study are in the range of levels reported in wild salmon from contaminated FW sites (Coleman, 2001). In addition, PCBs have been detected in adult farmed and wild salmon returning from residence in the ocean (Hites et al., 2004). Furthermore, there is evidence that similar levels of exposure can reduce time spent in SW in striped bass (*Morone saxatilis*) (Zlokovitz and Secor, 1999). Finally, several field and laboratory studies have shown that these levels of exposure can have sub-lethal reproductive and developmental effects in teleost fish (Monosson, 2000). Collectively these studies demonstrate that the concentrations used in the present study are environmentally relevant and below the lethal limits.

The present study demonstrates that aqueous exposure to environmentally relevant levels of A1254 can disrupt behavioral preference for SW during smolt development of Atlantic salmon. Interestingly, exposure of yolk-sac larvae to PCB-10 decreases SW preference 1 year after exposure without altering other aspects of smolt development. In comparison to the long-term effects, fish exposed to PCB-10 during smolt development exhibit decreased preference for SW in conjunction with a decrease in at least one indicator of physiological preparedness for SW, i.e. gill Na^+,K^+-ATPase activity. Plasma T_3 and cortisol levels were also lower in smolts exposed to PCB-10. The effects of PCB-1 during smolting was more subtle, resulting in delayed onset of entry into SW in addition to an overall reduction in preference behavior, reductions in plasma T_3 and cortisol levels, and no effect on gill Na^+,K^+-ATPase activity or ion regulatory capacity. These results suggest that the impacts of A1254 on SW preference behavior are dependent on dose and developmental stage, and that early exposure can have long-term impacts on behavior.

In anadromous salmonids TH levels increase prior to smolting (Dickhoff et al., 1978), are associated with increased migratory behavior (Hoar, 1988) and have previously been implicated in induction of preference for SW in coho salmon parr (*Oncorhynchus kisutch*) (Iwata et al., 1990). The current study provides evidence for significant reductions of circulating T_3 levels in fish exposed to aqueous A1254 during smolting. This has been demonstrated in other teleost fish including American plaice (*Hippoglossoides platessoides*) injected with 5 or

Table 1
Endocrine effects of developmental timing of exposure to PCBs on plasma GH, IGF-I, T_3, T_4 and cortisol during smolting

	Exposure timing	
	Yolk-sac larvae	Smolts
GH (ng ml^{-1})		
Vehicle	10.4 ± 3.4	5.1 ± 1.1
PCB-1	5.0 ± 1.0	8.7 ± 1.8
PCB-10	5.4 ± 0.7	7.8 ± 1.2
IGF-I (ng ml^{-1})		
Vehicle	49.8 ± 1.9	53.6 ± 2.4
PCB-1	53.7 ± 0.8	54.4 ± 3.8
PCB-10	49.9 ± 1.8	55.5 ± 3.0
T_3 (ng ml^{-1})		
Vehicle	4.9 ± 0.5	5.7 ± 0.3
PCB-1	6.3 ± 0.6	2.8 ± 0.5*
PCB-10	5.3 ± 0.5	3.7 ± 0.5*
T_4 (ng ml^{-1})		
Vehicle	5.0 ± 0.6	3.9 ± 0.4
PCB-1	7.6 ± 1.2	3.4 ± 0.4
PCB-10	5.8 ± 0.5	3.0 ± 0.3
Cortisol (ng ml^{-1})		
Vehicle	22.4 ± 7.6	22.5 ± 6.7
PCB-1	26.5 ± 8.6	9.6 ± 2.8*
PCB-10	29.1 ± 12.6	9.3 ± 1.4*

Values are means ± standard error; $n = 10$ for fish exposed as yolk-sac larvae and $n = 12$ for fish exposed during smolting. GH, growth hormone; IGF-I, insulin-like growth factor-I; T_3, triiodothyronine; T_4, thyroxine. There were no significant treatment effects on plasma GH or IGF-I regardless of exposure timing and no effects on plasma T_3, T_4, or cortisol in fish exposed as yolk-sac larvae. Fish exposed to PCBs during smolting exhibited significant lower plasma cortisol and T_3, not T_4. Asterisks (*) indicate significant differences from vehicle control (one-way ANOVA followed by Newman–Keul's multiple range test; $P < 0.05$).

25 ng g^{-1} PCB 77, Arctic charr fed PCBs (100 mg kg^{-1} A1254) and coho salmon fed 500 mg kg^{-1} A1254 (Leatherland and Sonstegard, 1978; Adams et al., 2000; Jorgensen et al., 2004). In the present study, A1254 did not have long-term effects on THs in fish exposed as yolk-sac larvae. However, the possibility that THs were impacted immediately following exposure in these fish cannot be ruled out. In fact, A1254-treated yolk-sac larvae exhibited increased opercular movement, which is indicative of altered metabolism and/or respiratory stress, and has been demonstrated after exposure to the insecticides ethofenprox and cypermethrin (Muniyan and Veeraraghavan, 1999; Prashanth et al., 2005). PCB mixtures and their parent congeners are known to reduce plasma TH levels in mammals, which may have significant impact on brain development (Zoeller, 2005). These effects could occur at multiple points in the thyroid cascade including synthesis, regulation, metabolism and action. To date, disruption of the thyroid axis by commercial PCB mixtures including A1254 and hydroxylated PCBs have been found in hepatic TH catabolism, antagonism of hormone transport by thyroid-binding globulin and transthyretin (mammalian and non-mammalian vertebrates, respectively), and by direct interference with thyroid hormone receptors (Cheek et al., 1999; Gauger et al., 2004; Crofton et al., 2005). While there is evidence that PCBs decrease thyroxine outer ring deiodination (T_4ORD), thereby reducing T_4 to T_3 conversion in American plaice (Adams et al., 2000), the mechanisms underlying the effects of PCBs on the thyroid axis in fish are poorly understood. Regardless of the mechanisms involved, THs have been hypothesized to play a role in controlling downstream migratory behavior and SW preference (Iwata et al., 1990; Iwata, 1995), and the observed impacts of PCBs on SW preference act through the thyroid axis. THs are also thought to effect the imprinting process (Hoar, 1988), and disruption of the thyroid axis by PCBs could have long-term effects on the homing ability of adults returning to their natal streams.

Due to their close association with the aquatic environment, the effects of contaminants in fishes often include disturbance of ion homeostasis (Bonga and Lock, 1992). Fish treated with PCB-10 during smolting exhibit reduced osmoregulatory capacity as indicated by lower gill Na^+,K^+-ATPase activity and lower plasma chloride levels in FW. This decrease in gill Na^+,K^+-ATPase activity and the reduced behavioral preference for SW in this group implies negative impacts on physiological tolerance for SW. However, plasma chloride concentration after 24 h in SW, a test commonly used to assess physiological tolerance (Blackburn and Clark, 1987), was unaffected. In addition, fish exposed to PCB-1 as smolts or PCB-10 as yolk-sac larvae also exhibit reduced preference for SW during smolting, with no impact on SW tolerance. One possibility is that the salinity of 30‰ used in this study was not a sufficient challenge to detect subtle differences in salinity tolerance.

There is evidence that environmental contaminants, especially estrogenic compounds (xenoestrogens), can disrupt salinity tolerance during smolt development and the major target of this disruption is the GH–IGF-I system (Lerner and McCormick, 2004; McCormick et al., 2005). In particular, these compounds reduce plasma IGF-I levels, which are known to play a critical role in controlling the osmoregulatory changes necessary for smolt development (McCormick, 2001). Anadromous Arctic charr fed A1254 exhibit reduced plasma GH, IGF-I, and T_3 levels (Jorgensen et al., 2004). Therefore, the absence of an effect of aqueous A1254 exposure on the GH–IGF-I system in the current study may corroborate the lack of impact on salinity tolerance. Nonetheless, the present results indicating no effect on the GH–IGF-I system and salinity tolerance reinforces the importance of the thyroid axis as a critical target for the negative consequences of A1254 on smolt development.

Cortisol is a mineralocorticoid in teleost fish, which has a direct role in promoting osmoregulatory changes during smolting of anadromous salmonids (Specker and Schreck, 1982). Cortisol is also a glucocorticoid involved in energy metabolism and the stress response (Mommsen et al., 1999). Increased plasma cortisol levels are commonly used as an indicator of stress in fish, and aquatic contaminants can activate the hypothalamus-pituitary-interrenal axis (HPI), thereby promoting increases in this corticosteroid (Bonga, 1997; Mommsen et al., 1999). With respect to their impact on the interrenal and its secretions, the effects of PCBs in fish are predominately inhibitory. The present study finds that aqueous exposure to environmentally relevant levels of A1254 during smolting reduces plasma cortisol levels. Other laboratory and field studies confirm that chronic exposure of teleost fish to PCB mixtures, including

A1254, as well as individual congeners, can decrease cortisol and the cortisol response to stress (Sivarajah et al., 1978; Quabius et al., 1997; Hontela, 1998). These authors concluded that hyperactivity of the HPI axis in response to relatively long-term exposures to contaminants results in a reduced capacity of the interrenal to secrete cortisol (Hontela, 1998) and that this disruption may be a result of direct toxic effects on the interrenal cells (Quabius et al., 1997). Through *in vitro* experiments using rainbow trout, other investigators have begun to reveal the mechanisms underlying this inhibitory role, providing evidence for reduced StAR protein and P450scc gene expression, two integral components involved in steroidogenesis (Aluru et al., 2005).

Plasma levels of cortisol exhibit a prolonged increase during smolting and coincide with an increase in SW tolerance in Atlantic and coho salmon (Specker and Schreck, 1982; Virtanen and Soivio, 1985). Exogenous cortisol has been shown to increase chloride cell number and size, which is directly related to increases in gill Na^+,K^+-ATPase activity (Richman and Zaugg, 1987; Madsen, 1990; Pelis et al., 2001). Therefore, reduced cortisol levels as a consequence of A1254 exposure during smolting may explain the observed reduction in gill Na^+,K^+-ATPase activity in the present study. However, other factors are likely involved, as a similar decrease in plasma cortisol levels were found in fish treated with PCB-1, without affecting gill Na^+,K^+-ATPase activity.

In the present study, the differential impacts on salinity tolerance and SW preference behavior of fish treated at as either yolk-sac larvae or during smolting may be due to developmental differences in endocrine function and PCB metabolism. All the basic endocrine systems are present during the yolk-sac larvae stage, though not all details are known. This lack of knowledge is due to a bias for research investigating the role of the endocrine system on juvenile and adult life-stages. For example, the role of the IGF-system in early development in anadromous salmonids remains unknown, but is well established with regard to juvenile smolt development (McCormick, 2001) Similarly, studies that have examined the biotransformation and metabolism of PCBs in fish have focused on a single life stage (Buckman et al., 2006) There is evidence, however, that metabolism of PCBs can differ according to developmental stage in frogs (Leney et al., 2006). Similar investigations in fish comparing biotransformation and clearance of PCBs throughout development would clarify the differential effects found in the present study as well as identify life-stages that are potentially more sensitive to the impacts of PCB exposure.

Volitional movement into SW is a whole organism response that integrates developmental and physiological cues, the timing of which can be altered by the external environment. The current study demonstrates that aqueous exposure of Atlantic salmon to the PCB mixture A1254 during early development or as juveniles can disrupt developmentally appropriate behavior. The long-term effects on behavior may be due to alterations in the organization of cellular complexes in the brain that control behavior (Sugawara et al., 2006). Disruption of SW preference due to A1254 exposure during smolting appears to be related to negative impacts on endocrine parameters involved in smolt development; particularly T_3 and cortisol. PCBs are widespread in the aquatic environment and found in both hatchery and wild salmon (Hites et al., 2004). Research indicates that anadromous fish are exposed to a multitude of contaminants throughout their life (Hites et al., 2004) and that combinations of contaminants often have greater impacts than any single compound (Moore et al., 2003; Crofton et al., 2005). The present data indicates that exposure to sub-lethal, environmentally relevant concentration of A1254 can affect critical aspects of smolt behavior and physiology, which in turn could impact marine survival and population sustainability.

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