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Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic salmon by 4-nonylphenol and 17β-estradiol

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Abstract

Sex steroids are known to interfere with the parr-smolt transformation of anadromous salmonids, and environmental estrogens such as nonylphenol have recently been implicated in reduced returns of Atlantic salmon in the wild. To determine the endocrine pathways by which estrogenic compounds affect smolt development and seawater tolerance, groups of juvenile Atlantic salmon were injected with one of five doses (0.5, 2, 10, 40 or 150 μg g^{-1}) of branched 4-nonylphenol (NP), 2 μg g^{-1} of 17β-estradiol (E_2), or vehicle, during the parr-smolt transformation in April, and the treatment was repeated 4, 8, and 11 days after the first injection. Plasma was obtained for biochemical analysis 7 and 14 days after initiation of treatment. After 14 days of treatment, additional fish from each treatment group were exposed to seawater for 24 h to assess salinity tolerance. The E_2 treatment and the highest NP dose resulted in lower salinity tolerance and decreased plasma insulin-like growth factor I (IGF-I) levels, along with elevated levels of plasma vitellogenin and total calcium. Plasma growth hormone levels were elevated at intermediate NP doses only, and not affected by E_2. After 7 days, plasma thyroxine (T_4) levels decreased in a strong, dose-dependent manner in response to nonylphenol, but after 14 days, this suppressive effect of T_4 occurred at the highest NP dose only. Similarly, E_2 decreased plasma T_4 levels at 7, but not 14 days. Plasma 3,3′,5-triodo-L-thyronine was reduced by E_2 and the highest NP dose after 7 and 14 days of treatment. Plasma cortisol levels were not affected by any of the treatments. The results indicate that the parr-smolt transformation and salinity tolerance can be compromised by exposure to estrogenic compounds. Suppression of plasma IGF-I levels is a likely endocrine pathway for the effects of estrogenic compounds on hypo-osmoregulatory capacity, and the detrimental effects of E_2 and NP on thyroid hormone levels are also likely to compromise the normal parr-smolt transformation of Atlantic salmon.
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1. Introduction

Over the past two decades there has been increasing awareness that contaminants can act through the endocrine system to have impacts on humans and wildlife. The field of endocrine disruptors has mainly focused on reproduction, identifying it as an important axis, as there is now ample evidence of pollutants which may impact animal populations through disruption of the reproductive process (Monosson, 2000; Sumpter, 1995). In a wider perspective, this suggests that other endocrine-mediated developmental events may be impacted by environmental contaminants (Colborn et al., 1993), many of which have yet to be examined in the laboratory or in nature.

The parr-smolt transformation (smolting) is an endocrine driven developmental event in which anadromous

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salmonids undergo preparatory physiological adaptations for seawater entry (Hoar, 1988). Changes in morphology, behavior, and physiology are driven by photoperiod and temperature, and mediated by hormones, primarily growth hormone (GH), insulin-like growth factor I (IGF-I), thyroid hormones [THs; thyroxine (T_4) and 3,3′,5-triodo-L-thyronine (T_3)], and cortisol (F), with prolactin (PRL) having an inhibitory effect (McCormick, 2001). In a retrospective analysis, Fairchild et al. (1999) found that Atlantic salmon populations in New Brunswick experienced declines following spraying for spruce budworm, and hypothesized that the estrogenic compound nonylphenol (NP) present in the spray formulations may have affected survival of smolts soon after seawater entry. Subsequently, Madsen et al. (1997) found that both NP and 17β-estradiol (E_2) can prevent the normal increase in salinity tolerance that occurs during smolting. Previous work has also shown that androgens can decrease downstream migratory behavior and salinity tolerance of salmon (Berglund et al., 1994; Lundqvist et al., 1989). It is clear that sex steroids and environmental estrogens can affect several aspects of smolt development. However, the mechanisms through which estrogenic compounds influence smolting are unknown. The present study was undertaken to determine the endocrine pathway(s) by which E_2 and NP affect the parr-smolt transformation of Atlantic salmon.

2. Methods

2.1. Fish rearing and treatment

Atlantic salmon (*Salmo salar*) used in these studies were the progeny of adults returning to the Connecticut River and were raised at the White River National Fish Hatchery (Bethel, VT, USA) as part of a restoration program. In the fall, parr were transferred to the Conte Anadromous Fish Research Center, Turners Falls, MA, and reared in 1.6-m diameter (1000 L water volume) tanks with freshwater at a flowrate of 6–8 L min^{-1} under natural photoperiod and fed to satiation twice daily with a specific salmon formulated dry feed (Zeigler Bros., Gardners, PA, USA).

Branched *para*-nonylphenol (CAS No. 84852-15-3, 95.3% pure) was obtained from Schenectady International (Schenectady, NY, USA). 17β-Estradiol was obtained from Sigma Chemical (St. Louis, MO). These compounds were mixed with vegetable oil at the appropriate amounts to achieve five treatment doses of NP (0.5, 2, 10, 40, and 150 μg g^{-1} fish) and one treatment dose of E_2 (2 μg g^{-1}). On March 20, fish large enough to become smolts (25–50 g) were anesthetized, length and weight were recorded, and fish were injected intraperitoneally with vehicle only or treatment at 5 μl g^{-1} fish. After recovery, fish were maintained in 1 m diameter (300 L water volume) fiberglass tanks (30 fish per tank) at 10–11 °C with a flowrate of 4 L min^{-1} and not fed during the course of the study. Fish were injected again 4, 8, and 11 days after the first injection. Seven and fourteen days after the initiation of treatment, 10 fish per group were sampled as described below. After 14 days of treatment, an additional 10 fish per group were transferred by netting to a 1 m diameter (300 L water volume) fiberglass tank with 30 ppt seawater at 10 °C with charcoal filtration and aeration for 24 h and then sampled.

Sampled fish were anesthetized with 200 mg L^{-1} tricaine methane sulfonate (neutralized and buffered with sodium bicarbonate, pH 7.0), and length and weight were measured. All fish were sampled within 6 min of first disturbing the tank. Blood was collected in heparinized syringes from the caudal vasculature, stored on ice for less than 30 min, centrifuged at 3000*g* for 5 min, and then plasma was 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, and 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.

2.2. 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*g* for 0.5 min) to remove large debris, Na^+,K^+-ATPase activity 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 μmol ADP mg^{-1} of protein h^{-1}.

Plasma GH levels were measured using a specific double-antibody salmon GH radioimmunoassay developed by Bolton et al. (1986) and modified by Björnsson et al. (1994). Plasma IGF-I levels were measured by a radioimmunoassay validated for salmonids (Moriyama et al., 1994). Plasma T_4 and T_3 were measured by a direct radioimmunoassay (Dickhoff et al., 1978) as modified by McCormick et al. (1995). Plasma cortisol was measured using an enzyme immunoassay as outlined in Carey and McCormick (1998).

Plasma vitellogenin (VTG) was measured by Western blotting. Ten micrograms of total plasma protein was heated to 70 °C for 15 min in Laemmli sample buffer and run on an 8% SDS–PAGE gel. Following electrophoresis, the proteins were transferred to PVDF Immobilon membrane (Millipore, Bedford, MA, USA) at 30 V for

14 h in 25 mM Tris, 192 mM glycine buffer at pH 8.3. PVDF membranes were blocked in phosphate-buffered saline with 0.5% Triton (PBST) and 5% powdered milk for 1 h at room temperature. Blots were probed with rabbit anti-salmon VTG polyclonal antibody (Biosense Laboratories, Bergen, Norway) at a dilution of 1:1000 in PBST for 1 h at room temperature. After washing in PBST, the blots were then probed with horseradish peroxidase (HRP) labeled goat anti-rabbit secondary antibody at a dilution of 1:2000 in PBST for 1 h at room temperature. The blots were developed using the HRP colorimetric substrate diaminobenzidine.

Plasma sodium concentration was measured on undiluted plasma using an ion selective electrode with an AVL analyzer (Roswell, GA, USA) using external standards (intra-assay coefficient of variation = 0.57%, $n = 8$). Total plasma calcium was measured on duplicate dilutions (1:100) using an atomic absorption spectrophotometer (AAnalyst 100, Perkin-Elmer, Norwalk, CT, USA; intra-assay coefficient of variation = 2.1%, $n = 6$).

2.3. Statistics

All values are reported as means ± standard error. Two-way analysis of variance (ANOVA) was used to examine the significance of treatment, time, and their interaction ($P < 0.05$). When significant treatment effects were found, Duncan's test ($P < 0.05$) was used to determine which treatments were significantly different from the vehicle group. Two-way analysis of variance at each time point of sampling was used to examine the significance of sex and treatment.

3. Results

There were no mortalities in any group over the course of the study. Gill Na^+,K^+-ATPase activity was not significantly affected by 7 or 14 days of treatment (treatment effect: $P = 0.07$, one-way ANOVA). Gill Na^+,K^+-ATPase activity of 150 μg g^{-1} NP and E_2 groups at day 14 were 30 and 23% lower than controls, respectively (Fig. 1). After 14 days of treatment, 10 fish per group were subjected to a 24 h seawater challenge. Control and low dose NP groups had similar mean plasma sodium levels after seawater challenge; whereas 150 μg g^{-1} NP- and E_2-treated fish had significantly higher plasma sodium levels, indicative of reduced salinity tolerance (Fig. 1).

Total plasma calcium levels were increased by E_2 treatment by 36 and 74% after 7 and 14 days, respectively, compared with controls (Fig. 1), and the corresponding increase by 150 μg g^{-1} NP was 21 and 25%. Plasma VTG levels were clearly elevated in response to 150 μg g^{-1} NP and E_2 after 14 days of treatment (Fig. 2). Lower doses were not distinguishable from controls, with a slight, but visibly detectable band present in the 40 μg g^{-1} NP group; similar results were obtained in three other Western blots (total of $n = 4$ for each dose).

Mean plasma cortisol levels were between 2 and 7 ng ml^{-1} for all groups, indicative of unstressed levels (Fig. 1). There was no significant effect of treatment or time on plasma cortisol levels.

Plasma GH was 2–3-fold higher at intermediate NP doses relative to the vehicle group at both 7 and 14 days, but there was no effect of 150 μg g^{-1} NP or of 2 μg g^{-1} E_2 (Fig. 3). There was a 3.8-fold increase in plasma GH of the vehicle group between days 7 and 14.

Plasma IGF-I levels were substantially reduced by treatment with 150 μg g^{-1} NP and E_2 at both 7 and 14 days (Fig. 3). At day 14, plasma IGF-I was 56 and 44% lower in the 150 μg g^{-1} NP and 2 μg g^{-1} E_2 groups, respectively, compared with the vehicle group, while the lower doses of NP did not significantly affect plasma IGF-I levels at either day 7 or 14. There was a moderate 15% increase in plasma IGF-I of the vehicle group between days 7 and 14.

Plasma T_4 exhibited a strong dose-dependent decrease in response to NP after 7 days of treatment; 10, 40, and 150 μg g^{-1} NP were all significantly lower than the vehicle group (25–37%; Fig. 4). At day 14, only the 150 μg g^{-1} NP group exhibited significantly lower (30%) plasma T_4 than the vehicle group. Estradiol treatment resulted in significantly (42%) lower plasma T_4 levels at day 7 but not at day 14. There was a 37% decrease in plasma T_4 levels in the vehicle groups between days 7 and 14.

Plasma T_3 was significantly decreased at 7 and 14 days by 150 μg g^{-1} NP and 2 μg g^{-1} E_2 (Fig. 4). At day 14, mean plasma T_3 levels were 37 and 48% lower in fish treated with 150 μg g^{-1} NP or 2 μg g^{-1} E_2, respectively, compared with controls. While there were significant decreases in plasma T_3 at intermediate doses of NP at day 7, these results were not consistent. There were no significant changes in plasma T_3 levels at intermediate doses of NP at day 14. Plasma T_3 levels of the vehicle group did not change between days 7 and 14.

Sex had no significant impact ($P > 0.3$) on any of the endocrine or physiological parameters measured.

4. Discussion

The results of the present study indicate that E_2 and higher doses of NP can substantially reduce salinity tolerance of Atlantic salmon, during parr-smolt transformation, a preparatory phase critical for seawater entry and survival. The results are consistent with those of Madsen et al. (2004, 1997), who found a significant impact of injected E_2 and NP on salinity tolerance, gill Na^+,K^+-ATPase activity, and the number of gill chloride cells in Atlantic salmon smolts. The effect of estrogenic

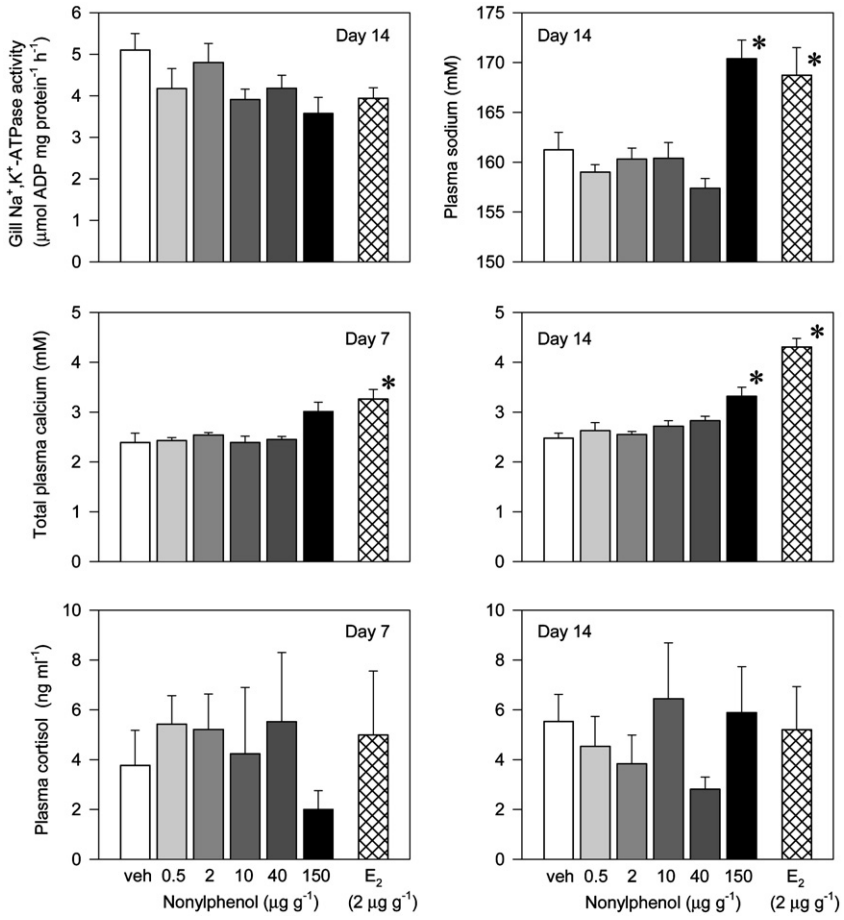

Fig. 1. Gill Na^+,K^+-ATPase activity after 14 days treatment in freshwater (upper left), plasma sodium after 14 days of treatment in freshwater followed by a 24 h seawater challenge (upper right), total plasma calcium after 7 and 14 days treatment in freshwater (middle), and plasma cortisol after 7 and 14 days treatment in freshwater (lower) in juvenile Atlantic salmon treated with nonylphenol (NP, 0.5–150 $\mu g\,g^{-1}$) or estradiol (E_2, 2 $\mu g\,g^{-1}$). Values are means ± standard error. Asterisks indicate a significant difference from vehicle group ($P < 0.05$, Duncan's test). There was a significant effect of treatment, time and a significant interaction on plasma calcium ($P < 0.05$, two-way ANOVA). There was no significant effect of time or treatment on plasma cortisol.

compounds on salinity tolerance is not limited to salmonids, as Vijayan et al. (2001) recently found that the euryhaline Mozambique tilapia (*Oreochromis mossambicus*) also has reduced hypo-osmoregulatory capacity following E_2 treatment. Rainbow trout exposed to 10 $\mu g\,L^{-1}$ NP for four 10-day periods over four months had elevated chloride cell gill surface area (Stoffel et al., 2000), a change associated with acclimation to ion poor water. These results indicate that estrogenic compounds may cause a general shift toward increased capacity for ion uptake (Na, Cl, and Ca), and in euryhaline species, a shift away from ion secretory mechanisms.

E_2 and the high dose of NP affected both the salinity tolerance of juvenile Atlantic salmon and circulating IGF-I at both days 7 and 14. Previous work has established that IGF-I can increase salinity tolerance in salmonids (McCormick, 1996; McCormick et al., 1991), and it therefore seems likely that the substantial declines in salinity tolerance induced by NP and estradiol are caused by the effect of these estrogenic compounds on plasma IGF-I levels. Estrogen has been shown to reduce liver IGF-I mRNA and circulating IGF-I levels in rats (Borski et al., 1996), and Riley et al. (2002) demonstrated a similar effect of E_2 on IGF-I in the Mozambique tilapia. Arsenault et al. (2004) have recently shown that aqueous exposure of Atlantic salmon smolts to NP and E_2 followed by seawater exposure results in long term depression of growth and circulating IGF-I. These data along with the present study suggest that the capacity of estrogenic compounds to decrease plasma IGF-I levels may be a common phenomenon among vertebrates. As neither E_2 nor high doses of NP caused changes in plasma GH levels, it seems likely that the effects of these compounds on plasma IGF-I levels are not through their impact at the pituitary level, but rather at the hepatic level. In mammals, there is now compelling evidence for circulating IGF-I primarily being of hepatic origin (Sjögren et al., 1999), and both in fish and mammals, GH has been shown to be the major secretagogue of hepatic IGF-I (Duan, 1998). Both E_2 and NP increase plasma VTG and total calcium (Ca_T) levels, a clear indication of an induction of hepatic VTG production. It

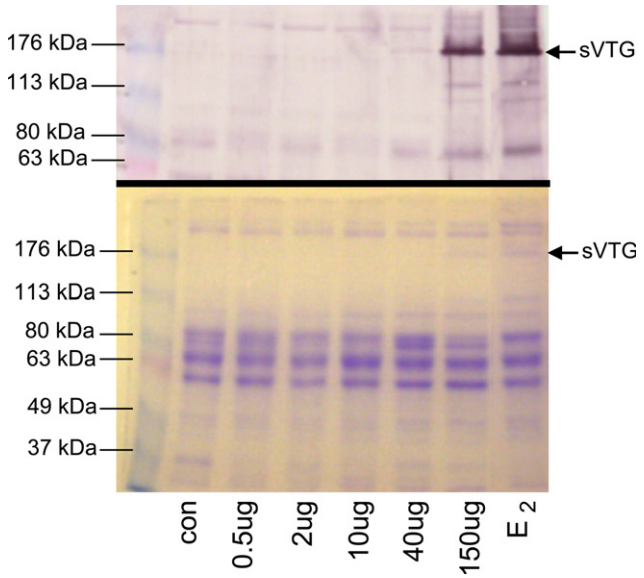

Fig. 2. Representative Western blot of plasma vitellogenin in juvenile Atlantic salmon treated with nonylphenol (NP, 0.5–150 μg g^{-1}) or estradiol (E_2, 2 μg g^{-1}) for 14 days in freshwater.

may be speculated that such a shift in hepatic cellular activity may, directly or indirectly, affect the hepatic GH–IGF-I secretory pathway, although these concomitant events may also be fully independent. Of several non-exclusive explanations for decreased plasma IGF-I levels, it can be hypothesized that hepatic GH receptors are downregulated by E_2 and NP in Atlantic salmon. Estradiol inhibits GH receptor mRNA levels in rabbit liver (Domene et al., 1994), whereas E_2 treatment stimulates hepatic GH receptor gene expression in rat (Bennett et al., 1996). Intracellular signaling pathways could also be affected, so that binding of GH to its receptor either leads to less IGF-I gene expression and/or less translation and secretion of the protein. However, even in mammals, the chain of intracellular events from GH stimulation to IGF-I secretion is not well elucidated.

The speculations on the mechanisms for how IGF-I levels are affected by steroid action are compounded by the apparent inconsistent effect of NP and E_2 on plasma GH levels. While neither steroid affects GH levels at high doses, there is a stimulatory response to NP at a lower dose of 2 μg NP g^{-1}, both after 7 and 14 days, and to a dose of 40 ng NP ml^{-1} after 14 days (Fig. 3). As only one dose of E_2 was used, it cannot be excluded that such a 'bell-shaped' GH response could also be found for lower E_2 doses, as seen for NP, especially at day 7. A dose of 5 μg g^{-1} E_2 elevated plasma GH levels of male tilapia (Riley et al., 2002) and 7.5 μg g^{-1} E_2 increased plasma GH levels in fed and fasted immature rainbow trout (Holloway and Leatherland, 1997). The mechanisms through which E_2 and NP affect plasma GH levels are not clear. Treatment of juvenile Atlantic salmon for 3 days with doses of NP and E_2 similar to those used in the present study did not affect pituitary GH mRNA levels (Yadetie and Male, 2002), an indication that GH synthesis rate is not greatly changed. On the other hand, E_2 can decrease the levels of circulating somatostatin and hypothalamic preprosomatostatin mRNA in rainbow

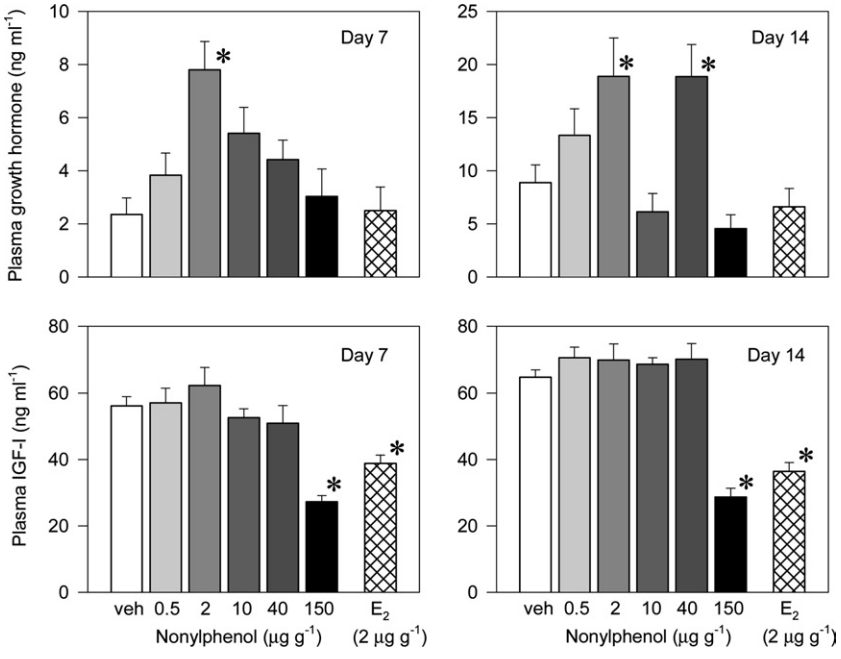

Fig. 3. Plasma growth hormone (upper) and plasma insulin-like growth factor I (lower) in juvenile Atlantic salmon treated with nonylphenol (NP, 0.5–150 μg g^{-1}) or estradiol (E_2, 2 μg g^{-1}) and sampled after 7 and 14 days in freshwater. Values are means ± standard error. Asterisks indicate a significant difference from vehicle group ($P < 0.05$, Duncan's test). There was a significant effect of treatment, time and a significant interaction on plasma growth hormone and plasma insulin-like growth factor I ($P < 0.05$, two-way ANOVA).

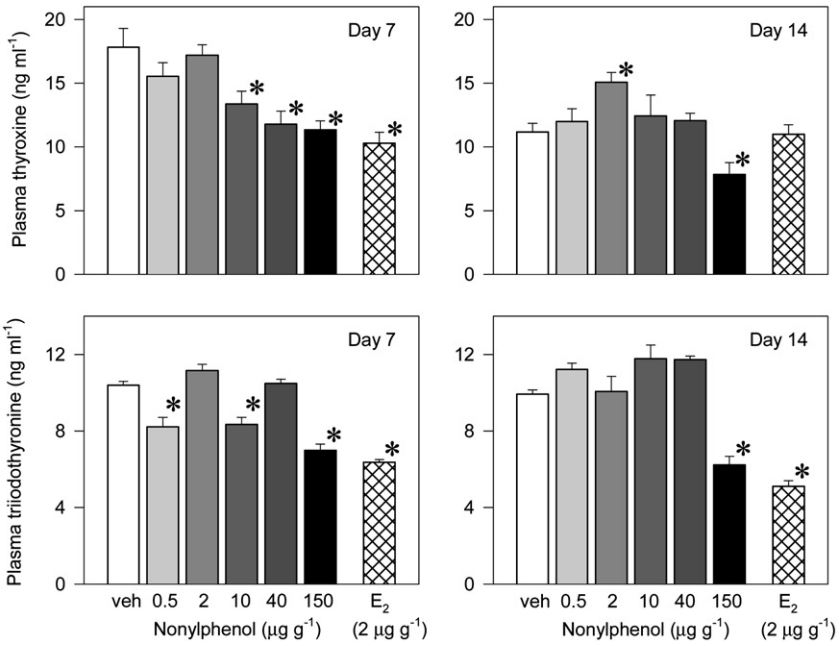

Fig. 4. Plasma thyroxine (upper) and plasma 3,3′,5-triodo-L-thyronine (lower) in juvenile Atlantic salmon treated with nonylphenol (NP, 0.5–150 μg g^{-1}) or estradiol (E_2, 2 μg g^{-1}) and sampled after 7 and 14 days in freshwater. Values are means ± standard error. Asterisks indicate a significant difference from vehicle group ($P < 0.05$, Duncan's test). There was a significant effect of treatment, time and a significant interaction on thyroxine and plasma 3,3′,5-triodo-L-thyronine ($P < 0.05$, two-way ANOVA).

trout (Holloway et al., 2000), a phenomenon that could lead to an increased pituitary GH secretory rate. While dose may be a factor, another possible explanation for the apparent lack of stimulation of plasma GH by E_2 is that the fish in the present study were in the early stages of smolting, a period during which increased photoperiod strongly stimulates GH secretion rate, leading to increased plasma GH (Agustsson et al., 2001). It is therefore possible that GH secretion is already being stimulated by natural secretagogues, so that this process is relatively insensitive to any additional stimulatory effects of estrogenic compounds, seen in other species and/or developmental stages.

In the present study, plasma thyroid hormone levels are clearly decreased by NP and E_2, and of all the physiological responses examined, plasma T_4 appears to be the most sensitive to NP, at least in the short term (first 7 days). It is possible that there is a greater sensitivity of estrogen receptors putatively involved in regulating the thyroid axis compared with those in the liver that control the vitellogenic response. However, a different dose dependency from the vitellogenic response suggests the possibility that the observed thyroid hormone effects may not be through the estrogen receptor. Nonylphenol interferes with the binding of T_3 to transthyretin in chicken and bullfrog (Yamauchi et al., 2003), and the closely related bisphenol A inhibits thyroid action by acting as an antagonist of T_3 binding to the TH receptor in mammals (Moriyama et al., 2002). Thus, it is possible that the actions of NP on circulating THs in the present study are the result of a direct interaction of this compound with components of the thyroid axis rather than through the estrogen receptor. As the presented data are the first demonstrated impacts of environmental estrogens on the thyroid axis in fish, and the relatively high sensitivity observed warrants investigation of other compounds and species.

Estradiol treatment can decrease circulating thyroid hormones in the European eel (*Anguilla anguilla*), Japanese eel (*Anguilla japonica*), masu salmon (*Oncorhynchus masou*), and rainbow trout (*Oncorhynchus mykiss*) (Leatherland, 1985; Olivereau et al., 2004; Qu et al., 2001; Yamada et al., 1993; see Cyr and Eales, 1996, for a review). In rainbow trout, plasma T_3 but not T_4 decreased after 4 and 8 weeks of E_2 treatment (Flett and Leatherland, 1989). The present study is in agreement with these results, as the effect on plasma T_3 was found at both day 7 and 14, but the effect on plasma T_4 was found at day 7 only. This suggests that the thyroid response to E_2 and estrogenic compounds will change over time, and that this may thus be an important consideration in detecting the thyroid response to contaminants. Previous studies indicate that both the route of administration and the time course can affect the response of the thyroid axis to E_2 (Leatherland, 1985). The mechanism of action of E_2 on the thyroid axis has yet to be fully established. Estradiol decreases T_4 to T_3 conversion in liver and gill and head kidney (Yamada et al., 1993), which could explain decreased plasma T_3 levels. Estrogen also decreases thyroid epithelial cell height (Leatherland, 1985; Olivereau et al., 2004; Qu et al., 2001), suggesting an effect of estrogen on thyroid

secretion. Paradoxically, E_2 increases pituitary level of thyroid stimulating hormone mRNA in the Japanese eel (Qu et al., 2001). Interpreting how environmental estrogens influence the thyroid hormones will require a more complete understanding of the normal biological actions of sex steroids on the thyroid axis of fish.

The dose of E_2 used in the present study elevated plasma VTG, and the vitellogenic response is further verified by an increase in plasma Ca_T, which rises in conjunction with VTG during normal reproduction and following hormonal induction (Björnsson et al., 1989). The highest two doses of NP also increased plasma VTG, which is consistent with previous studies of NP in salmon and other species in which the VTG response has a sharp threshold (Yadetie et al., 1999).

A 24 h seawater challenge was used in the present study to assess the impact of NP and E_2 on salinity tolerance. While this is a relatively short term measure of performance, it has been widely used in assessing smolt capacity and is strongly associated with long term performance in seawater including survival, feeding rate, growth rate, and adult return rates (see Handeland et al., 1996). Thus, the observation that NP and E_2 affect salinity tolerance (increased plasma sodium) indicates that smolts exposed to estrogenic compounds may have compromised long term performance. This is in agreement with the findings of Arsenault et al. (2004) that aqueous NP exposure in freshwater affected the subsequent growth performance of Atlantic salmon smolts in seawater, providing further evidence for the long term effects of disruption of smolting by xenoestrogens. Finally, Madsen et al. (2004) found that injections of NP and E_2 in Atlantic salmon smolts resulted in decreased downstream migratory behavior after release. Since a connection between thyroid hormones and migratory behavior has been established (Hoar, 1988), our observation of decreased plasma thyroid hormones following NP and E_2 treatment may explain these impacts.

Since the route of administration in this study was injection and thus 'artificial,' one must be cautious in extrapolating our results to exposure of these compounds in nature. Moore et al. (2003) did not find an effect of waterborne NP in the range of 5–20 $\mu g\,L^{-1}$ on salinity tolerance or gill Na^+,K^+-ATPase activity of Atlantic salmon, though it is notable that an impact was seen when NP was combined with atrazine, a commonly used agricultural herbicide. Arsenault et al. (2004) found that two pulses of 20 $\mu g\,L^{-1}$ 4 days apart during smolting affected seawater growth performance. Their results also indicate a possible impact on timing of exposure on the subsequent growth response. Since major endocrine changes during smolting occur relatively rapidly (e.g., over a 4–8 week period), it may be expected that the capacity of this developmental process to be affected by endocrine disrupting compounds will change during this period. Fish in the present study were not fed during treatment, and both the baseline hormone levels and the response to NP and E_2 may have been affected by this restricted caloric intake. Therefore, the potential impact of xenoestrogens such as nonylphenol to disrupt smolting in nature will likely depend not just on the route of administration, dose, and frequency of exposure, but will also depend on temperature, diet, developmental stage of the fish, and presence of other contaminants. More studies on water borne and dietary exposures both during smolting and at other developmental stages are warranted.

The present results strongly implicate IGF-I as the mediating factor in the reduction in salinity tolerance caused by E_2 and NP. However, the possibility cannot be ruled out that other pathways are involved in the effects of estrogenic compounds. There was a clear effect of E_2 and the high dose of NP on plasma T_3, and while there is little evidence that thyroid hormones have a direct role in controlling salinity tolerance in salmonids, they do have indirect effects on salinity tolerance (McCormick, 2001). Prolactin is important in ion uptake in freshwater, and estradiol has been implicated in controlling prolactin (Brinca et al., 2003), though we could find no published evidence that circulating levels are impacted by E_2 in any fish species. Yadetie and Male (2002) found no effect of NP or E_2 on pituitary prolactin mRNA in Atlantic salmon. It is also possible that estrogenic compounds could have direct effects on the gill or other osmoregulatory organs. We know of no studies that have examined the gills of Atlantic salmon for the presence of estrogen receptors, though Persson et al. (2000) found no evidence for their presence in rainbow trout gill. However, androgen receptors have been found in Atlantic salmon gills (Jakobsson et al., 1997), and it is possible that estrogen receptors are present as well.

In the present study, we have focused on the osmoregulatory components of smolting as a highly tractable and ecologically important developmental change in anadromous salmonids. Given the widespread endocrine changes that resulted from exposure to E_2 and NP, it is possible that other important aspects of smolt development are altered by exposure to environmental estrogens. In salmonids, the GH–IGF-I axis is not only of importance for osmoregulation, but also for growth, protein and lipid metabolism, and behavior (for reviews, see Björnsson, 1997; Björnsson et al., 2002; Jönsson and Björnsson, 2002). Thyroid hormones are also known to control several critical aspects of smolt development, including morphological changes (such as silvering), downstream migratory behavior, and imprinting (Hoar, 1988). It is concluded that environmental estrogens may be able to compromise important aspects of the smolting process and could thus have substantial impact on the survival of salmon in seawater and ultimately on salmon populations.

Acknowledgments

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