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Effects of water-borne 4-nonylphenol and 17β-estradiol exposures during parr-smolt transformation on growth and plasma IGF-I of Atlantic salmon (*Salmo salar* L.)

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Abstract

4-Nonylphenol (4-NP) is an endocrine disrupting substance (EDS) capable of mimicking the action of 17β-estradiol (E_2). It has been hypothesized that 4-NP in a pesticide formulation is linked to historical declines in Canadian Atlantic salmon (*Salmo salar* L.) populations, with effects being related to exposure during parr-smolt transformation (PST). To test this hypothesis, Atlantic salmon smolts were exposed to pulse-doses of water-borne 4-NP (20 ug/l), sustained doses of water-borne E_2 (100 ng/l) (positive control), or ethanol vehicle (negative control) in mid-May during the final stages of PST. Individually tagged smolts were then sampled at three times (June, July and October) to monitor subsequent growth in sea water and plasma insulin-like growth factor I (IGF-I) concentrations. Smolt weights and plasma IGF-I concentrations were both affected by E_2 and 4-NP. The effects of E_2 and 4-NP on mean smolt weights were most prominent in July and October {E_2 (*98.1 ± 2.8, *242.3 ± 10.6 g), 4-NP (*102.1 ± 3.1, 255.7 ± 9.5 g), controls (112.5 ± 2.8, 282.3 ± 8.8 g)} ($P < 0.05$), while their effects on mean plasma IGF-I concentrations were most prominent in June and October {E_2 (15.0±1.9, 28.4±1.8 ng/ml), 4-NP (*14.8±1.9, *21.6±1.7 ng/ml), controls (20.0 ± 1.1, 31.1 ± 2.0 ng/ml)} ($P < 0.05$). Additionally, results suggest that the mechanisms of action of E_2 and 4-NP involve disruption in the GH/IGF-I axis, and that they may be different from each other. The effects of E_2 and 4-NP on growth and plasma IGF-I concentrations observed in this study are ecologically significant because they evoke concerns for successful growth and survival of wild salmon smolts exposed to low levels of estrogenic substances that may occur from current discharges into rivers supporting sea-run salmon stocks.

Keywords: Atlantic salmon; Parr-smolt transformation; IGF-I; 4-Nonylphenol; Estrogen; Endocrine disrupting substances

1. Introduction

4-Nonylphenol (4-NP) is a synthetic chemical capable of mimicking the action of the natural female

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hormone 17β-estradiol (E_2) by binding to the E_2 receptor and inducing the synthesis of vitellogenin, an egg yolk precursor protein in fish (White et al., 1994; Madsen et al., 1997; Christiansen et al., 1998; Servos, 1999; Yadetie et al., 1999). Because 4-NP is capable of mimicking the action of E_2, it can be referred to as an environmental estrogen or xenoestrogen (Yadetie et al., 1999). Compounds of this type are able to cause effects in whole organisms, progeny, or populations via actions on the endocrine system, and are known as endocrine disrupting substances (EDSs) (OECD, 1999). 4-NP is a breakdown product of nonylphenol polyethoxylates (NPEs), which are broadly used non-ionic surfactants (Liber et al., 1999; Servos, 1999). NPEs are mainly used in cleaning products and industrial processing (i.e., plastics manufacturing, pulp and paper industries and textile manufacturing facilities) (Liber et al., 1999). Municipal and industrial effluents are the main sources of NPEs in the aquatic environment (Liber et al., 1999), and discharges from sewage treatment plants, as well as industrial effluents are the main sources for 4-NP (Madsen et al., 1997; Naylor et al., 1998; Servos, 1999).

It has been hypothesized that 4-NP in a pesticide formulation is linked to historical declines in Canadian Atlantic salmon (*Salmo salar* L.) populations, with effects being related to exposure during a critical developmental life stage called parr-smolt transformation (PST) (Fairchild et al., 1999). PST occurs when juvenile salmon in fresh water (FW), called parr, undergo a series of morphological and physiological changes to become sea water (SW) adapted smolts, which then migrate to sea to complete their growth and develop into adults (Hoar, 1988; Høgåsen, 1998). PST is a critical developmental process akin to amphibian metamorphosis (Dickhoff, 1993). PST is associated with complex hormonal changes and rapid growth (Høgåsen, 1998). Growth hormone (GH) plays a central role in PST because of its direct and indirect effects on stimulating somatic growth and on enhancing SW adaptation (Björnsson, 1997; Ágústsson et al., 2001). Many of the effects of GH are indirectly mediated by insulin-like growth factor-I (IGF-I) (Green et al., 1985). GH stimulates the synthesis of IGF-I by binding to GH receptors found on the cell surface of many different tissue types, with the liver being the primary site of IGF-I production (Duan et al., 1995; Björnsson, 1997).

A recent study has shown that plasma IGF-I concentrations are good indicators of smolt quality and have significant relationships with smolt-to adult returns (SAR) in hatchery reared Chinook salmon (*Oncorhynchus tshawytscha*) (Beckman et al., 1999). Smolts of poor quality (i.e. behaviorally dysfunctional, physiologically compromised and disease prone) were found to have poor post-release survival and lower plasma IGF-I concentrations than higher quality smolts (Beckman et al., 1999). Plasma IGF-I concentrations in smolts could therefore be considered as good indicators of tertiary level responses (i.e. changes in growth or development: Munkittrick and McCarty, 1995). Tertiary level responses result from an integration of multiple physiological responses (Munkittrick and McCarty, 1995). Additionally, tertiary level responses are often irreversible and long lasting and have high ecological relevance because they can lead to population level effects (Munkittrick and McCarty, 1995).

A recent experiment investigating the effects of water-borne concentrations of E_2 and 4-NP on PST indicated that a portion of the Atlantic salmon smolts treated with E_2 and 4-NP experienced compromised growth and did not survive the transition from FW to SW (Fairchild et al., 2000). However, the mechanisms by which E_2 and 4-NP influence smolt growth and survival are unknown. Growth is an important factor when considering smolt survival at sea (Friedland et al., 1993). Low level exposure to estrogenic substances during the smolt runs to sea may be one of a number of factors responsible for the decline in catches of adult Atlantic salmon.

The objectives of this study were two-fold. The first objective was to identify the most sensitive temporal window for determining the effects of E_2 and 4-NP exposures on growth. The second objective was to determine if the mechanism responsible for poor growth in hatchery reared Atlantic salmon smolts exposed to E_2 and 4-NP during the final stages of PST was related to a disruption in the GH/IGF-I axis by measuring plasma IGF-I concentrations, an indicator shown to be directly linked to smolt growth and SAR. This study represents a novel line of research examining the effects of EDSs on smoltification, which is an endocrine-sensitive developmental process that is critical to long-term growth and survival of salmon.

2. Materials and methods

2.1. Fish rearing

Fourteen-month post-hatch Atlantic salmon parr were obtained from the Huntsman Marine Science Centre Chamcook Hatchery, St. Andrews, NB, Canada, on 5 January 1999. Parr (75–80 g) were anesthetized in 1% *tert*-amyl alcohol and individually tagged with passive integrated transponder (PIT) tags (Biomark, Biose, Idaho). Fish were randomly distributed into 18 fiberglass tanks (400 l, 50 fishes per tank) and allowed to acclimate in dechlorinated St. Andrews, NB, municipal water at ambient temperature for 3 months prior to treatments. The tanks were covered and enclosed in individual compartments, each with their own water supply and light. The flow rate was maintained at approximately 5 l/min. Photoperiod was regulated to simulate natural photoperiod. Except on treatment and sampling days, the fish were fed by hand twice daily to satiation with a premium quality open formula diet [Moore-Clark (a division of Nutreco Canada Inc.), St. Andrews, NB].

2.2. Treatments

In spring 1999, prior to commencing treatments, the 18 individual tanks were each randomly assigned to be one of the two replicate tanks, of one of three treatments (i.e. control, E_2, 4-NP), within one of three temporal treatment windows (Early, Middle, Late) (Fig. 1). Then, salmon parr were exposed to waterborn E_2, 4-NP or ethanol vehicle (i.e. control) on one of three different occasions (Early temporal window: 12–16 May, Middle temporal window: 26–30 May, Late temporal window: 9–13 June) during the later stages of PST, according to the temporal window in which they were assigned. The test substances were dissolved in ethanol and diluted with water such that ethanol represented 10% of the delivery solution. Two replicate tanks were treated with an environmentally-relevant (Fairchild et al., 1999) nominal concentration of 4-NP (20 μg/l), E_2 (100 ng/l; positive control) or 10% ethanol vehicle (controls). Treatments occurred in freshwater over 5 days. The treatment regimes started by adding the appropriate amount of test substances (dissolved 10% ethanol) required to bring each tank up to the desired concentrations. 4-NP

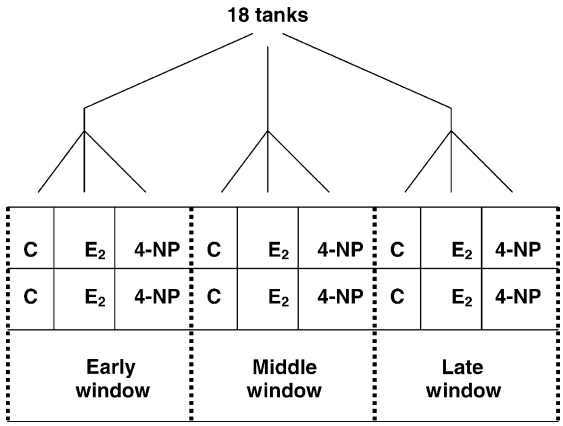

Fig. 1. Experimental design schematic. A total of 18 individual tanks were each randomly assigned to be one of the two replicate tanks, of one of three treatments (i.e. control, E_2, 4-NP), within one of three temporal treatment windows (Early, Middle, Late). *Note*: For schematic clarity purposes, the 18 tanks are shown as being grouped in order per treatment duplicate, per window. However, the 18 tanks were actually in a randomly mixed order during the course of the experiment. See text for further details.

was then delivered continuously in two 24 h pulses (days 1 and 5) and E_2 was delivered continuously over the 5-day period. The tank concentrations were kept constant throughout the treatment periods with a Mariotte bottle system calibrated to drip the treatment solutions at a flow rate of 1 ml/min. The treatment solutions dripped directly into the incoming water supply of the tanks to assure complete mixing. The Mariotte bottle drip flow rates were verified several times daily and adjusted if required. Beginning 12–14 days after the onset of treatment within each temporal window the fish were gradually acclimated to filtered sea water (Brandy Cove, Passamaquoddy Bay, NB) over a 5-day period and the flow rate was maintained at approximately 5 l/min.

2.3. Sampling

Post-treatment terminal-sampling for all three temporal windows occurred on three occasions (16–17 June, 20–21 July, 13–14 October 1999). At each sampling event, eight fishes were removed from each tank (16 fishes per treatment). Fish were stunned with a sharp blow to the head. Blood was then drawn from the caudal veins into a 3 ml heparinized syringe with a 21-gauge needle and centrifuged at $2000 \times g$ for 10 min

at 4 °C. Plasma was aliquoted and stored at −75 °C. Fork length to the nearest millimeter and weight to the nearest 0.1 g were recorded. Following the July sampling, all the remaining fish in each of the 18 tanks were weighed. The weights of the duplicate treatment tanks were then statistically tested (Wilcoxon test, two tailed, $P < 0.05$) to verify the possibility of tank differences. Because there were no statistical weight differences between any of the duplicate treatment tanks, the fish within each duplicate were considered to be individual treatment sampling units. At that point, an equal number of fish from duplicate tanks were randomly assigned to one of two large tanks (3000 l) for long-term seawater grow-out from July to October. This was done in order to eliminate potential tank density problems or tank effects that could occur in individual tanks during the lengthy grow-out period. In October, the fish were sampled from only one of the two large grow-out tanks. The second tank served as a back-up in the case one of the two tanks would have been lost during the grow-out period.

2.4. IGF-I determination

Plasma samples were extracted by the method described by Daughaday et al. (1980). Plasma samples (25 μl) were thoroughly mixed with 100 μl of an acid–ethanol (A/E) mixture (87.5% ethanol and 12.5% 2 M HCl, v/v) and incubated at room temperature for 30 min. The A/E extract mixture was then neutralized by adding 50 μl of Tris base (0.855 M). The tubes where then centrifuged at 2000 × *g* for 30 min at 4 °C. The supernatant (25–35 μl) was then directly transferred to 12 mm × 75 mm polystyrene tubes and assayed for IGF-I.

Recombinant salmon IGF-I (rsIGF-I) (GroPep Pty Ltd., Adelaide, Australia) was iodinated by a chloramine-T procedure described by Moriyama et al. (1994). The iodination was performed by Amersham Laboratories, Little Chalfont, Buckinghamshire, England.

Plasma IGF-I was determined by the validated radioimmunoassay (RIA) procedure described by Shimizu et al. (2000). The assay buffer was 20 mM phosphate buffered saline (PBS), pH 7.4, containing 150 mM NaCl, 1.0% BSA and 0.05% Triton X-100. RsIGF-I (GroPep Pty Ltd.) was used for standard material, and was serially diluted in the assay buffer and assayed in triplicate (0.01–2.5 ng per tube). Standards (triplicates, 100 μl) and plasma extracts (duplicates, 25–35 μl) were transferred to 12 mm × 75 mm polystyrene tubes. Anti-recombinant barramundi IGF-I (100 μl, GroPep Pty Ltd.) was added to each assay tube. Primary dilution of the anti-serum in assay buffer varied between 1:6000 to 1:8000 depending on the age and condition of the label. The anti-recombinant barramundi IGF-I displays 100% cross-reactivity with salmon IGF-I, less than 0.5% cross-reactivity with recombinant human IGF-I and less than 1% cross-reactivity with recombinant human IGF-II (GroPep Pty Ltd.). After a 24 h incubation at 4 °C, each tube received 100 μl of iodinated rsIGF-I (approximately 8 000 cpm) and incubated for another 24 h at 4 °C. The antibody-bound hormone complexes were separated from free tracer by the addition of 100 μl of 0.5% Pansorbin (Calbiochem-Novabiochem Corp., La Jolla, CA). After incubation with Pansorbin for 24 h at 4 °C, 250 μl of 20 mM PBS, pH 7.4, containing 150 mM NaCl, was added to each tube. The tubes were then centrifuged at 2000 × *g* for 30 min at 4 °C. The supernatant was aspirated and the bound fraction was counted on an LKB-Wallace gamma counter for 2 min. The intra-assay coefficient of variation (CV) of the mean of three duplicates was 2.6% and the inter-assay CV of the mean of seven duplicates was 7.1%.

2.5. Specific growth rates

The specific growth rates based on weight (SGRW) were calculated for the time period between January and July 1999. The SGRWs were calculated using the following formula: $\mathrm{SGRW} = (Y_2 - Y_1) \times (t_2 - t_1)^{-1}$, where Y_2 is the weight in July, Y_1 the weight in January, t_2 the Julian day in July, t_1 is the Julian day in January.

2.6. Statistical analysis

Weight, SGRW and plasma IGF-I data were rank transformed prior to statistical analysis because some groups were not normally distributed, and/or had high variances and/or low sample numbers.

The best temporal window for SW transfer, and therefore most sensitive temporal window for determining the effects of E_2 and 4-NP exposures on

growth and plasma IGF-I concentrations, was identified by conducting comparisons between the weights and SGRWs of the control smolts from the three temporal windows, using a non-parametric one-way analysis of variance test (NPAR-1-ANOVA), followed by a Tukey multiple comparison test (Zar, 1999). Additionally, a Kolmogorov–Smirnov test (KS test) was used to conduct comparisons between the weight and SGRW frequency distributions of the control smolts from the three temporal windows (Zar, 1999). The data sets used for the weight and SGRW analyses described above comprised of all the control smolts per temporal window available in July ($n \sim 50$).

Within the temporal window identified as the most sensitive one for determining the effects of E_2 and 4-NP exposures on growth and plasma IGF-I concentrations, a NPAR-1-ANOVA, followed by a Dunnett's multiple comparison test was used to compare the weights and plasma IGF-I concentrations of the control smolts with those of the E_2 and 4-NP treated smolts. The data sets used for the July and October weight analyses comprised of all the controls, E_2 and 4-NP treated smolts available in July ($n \sim 50$) and October ($n \sim 35$), whereas only a sub-sets of these samples ($n =\sim$ 10–12 per treatment) were collected for IGF-I determination. Additionally, a KS test was used to compare the July weight frequency distributions of the control smolts with those of the E_2 and 4-NP treated smolts. SPSS SAS version 8.01 (SAS Institute Inc., Cary, NC, USA) was used to conduct all of the statistical analyses.

3. Results

3.1. Identification of the most sensitive temporal window for determining the effects of E_2 and 4-NP exposures on growth and plasma IGF-I concentrations

Comparisons between the weights and SGRWs of the controls from the three temporal windows showed that the timing of SW transfer of each window had significant effects on control weights and SGRWs. July weight frequency histograms (Fig. 2) and weight frequency distributions (Fig. 3) show how the weights of the Middle and Late window controls were affected relative to those of the Early window. July weight frequency distributions of the Middle ($P < 0.001$) and Late ($0.002 < P < 0.005$) window controls were significantly different from the Early window's (KS test) (Fig. 3), and similar results were obtained for the SGRWs (Figures not shown).

Additionally, the mean July weights and SGRWs of the Early window control smolts (112.4 ± 2.8, 0.353 ± 0.014 g per day, $n = 53$) were both significantly higher than those of the Middle ($^*98.5 \pm 2.4$, $^*0.297 \pm 0.012$ g per day, $n = 43$) and Late windows ($^*101.8 \pm 2.9$, $^*0.300 \pm 0.014$ g per day, $n = 48$) ($P < 0.05$) (NPAR-1-ANOVA, Tukey).

Based on the July weight and SGRW data analysis of the control smolts, the Early window was considered to be the best temporal window for SW transfer and therefore the most sensitive window for determining the effects of E_2 and 4-NP exposures on growth and plasma IGF-I concentrations (Section 3.2). Analysis of the effects of the E_2 and 4-NP exposures within the Middle and Late windows were not conducted due to the significant negative effects that the later SW transfer times were shown to have on the weights and SGRWs of their control smolts.

3.2. Effects of E_2 and 4-NP exposures on growth and plasma IGF-I concentrations of the Early window smolts

Early window smolt weights were affected by both E_2 and 4-NP exposures, though E_2 had an overall greater effect on mean smolt weights across time than 4-NP (Fig. 4). The most prominent effects of E_2 and 4-NP on mean smolt weights were seen in July {E_2 ($^*98.1 \pm 2.8$ g, $n = 49$), 4-NP ($^*102.1 \pm 3.1$ g, $n = 50$), controls (112.5 ± 2.8 g, $n = 53$)} and October {E_2 ($^*242.3 \pm 10.6$ g, $n = 28$), 4-NP (255.7 ± 9.5 g, $n = 29$), controls (282.3 ± 8.8 g, $n = 32$)} ($P < 0.05$) (NPAR-1-ANOVA, Dunnett's) (Fig. 4).

July weight frequency histograms (Fig. 5) and weight frequency distributions (Fig. 6) show how the weights of the E_2 and 4-NP treated smolts were affected relative to the controls. July weight frequency distributions of E_2 ($P < 0.001$) and 4-NP ($P < 0.05$) treated smolts were significantly different from the controls (KS test) (Fig. 6).

Smolt plasma IGF-I concentrations were both affected by E_2 and 4-NP, though 4-NP had an overall greater effect on mean plasma IGF-I concentrations

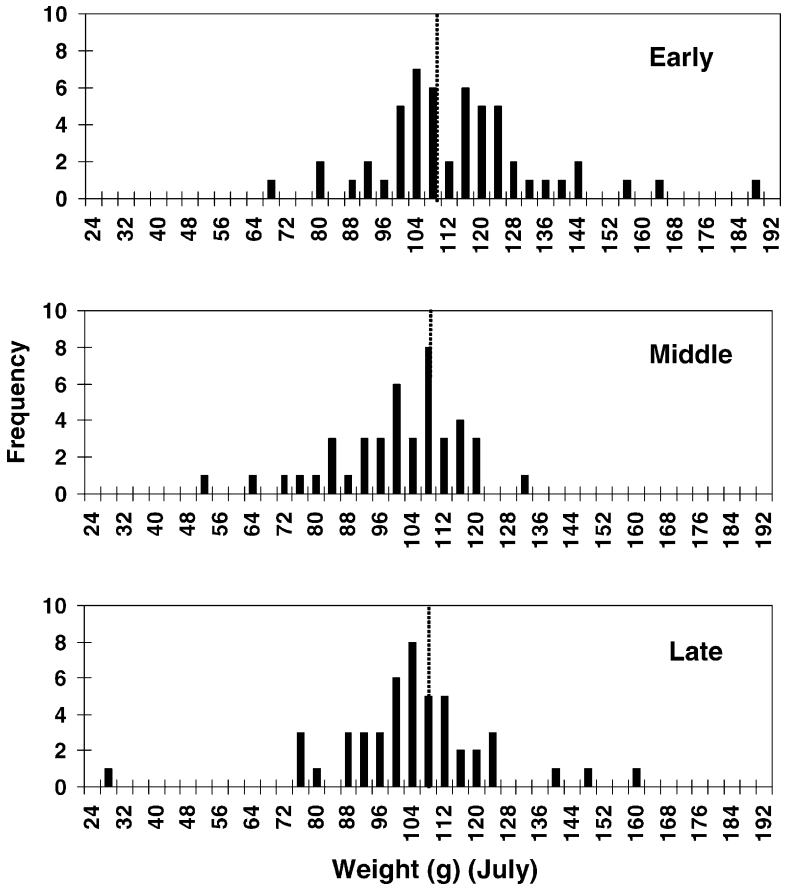

Fig. 2. July weight frequency histograms of the Early, Middle and Late window control smolts. Dotted line represents the median weight (i.e. 108.9 g) of the Early window controls.

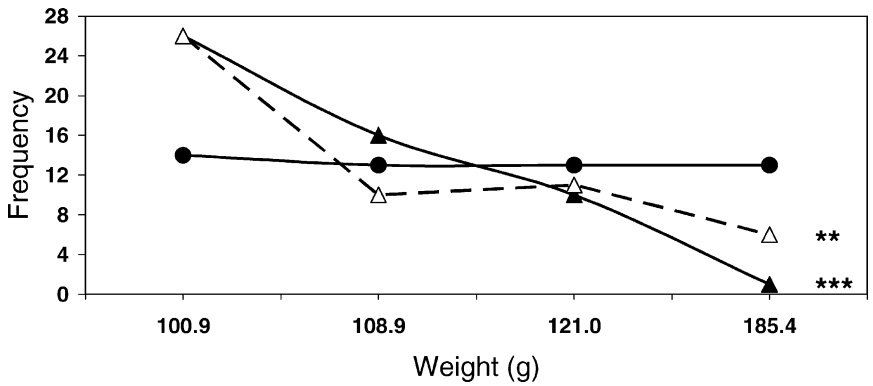

Fig. 3. July weight frequency distributions of the Early (●), Middle (▲) and Late (△) window control smolts. Tick marks on the *X*-axis are the upper-limit of each Early window control weight quartile. *Y*-axis gives the frequency defined by the Early window control weight quartiles. Asterisks indicate significant differences from the Early window control weight frequency distribution ($^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$) (KS test).

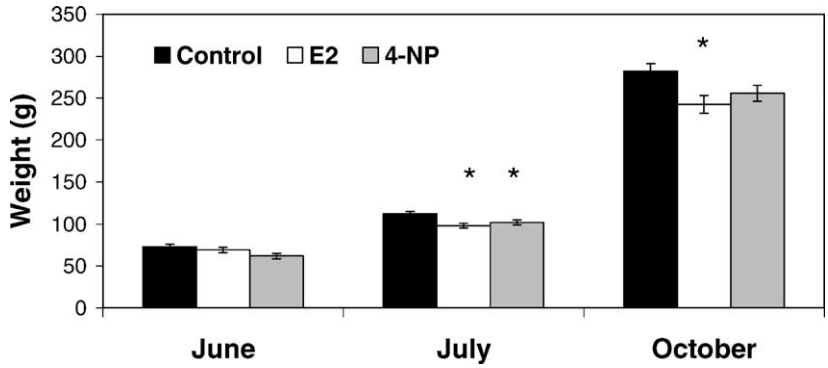

Fig. 4. Mean weights and standard errors of Early window controls, E_2 and 4-NP treated smolts, across time (June, July and October). Asterisks indicate significant differences from controls ($^*P < 0.05$) (NPAR-1-ANOVA, Dunnett's).

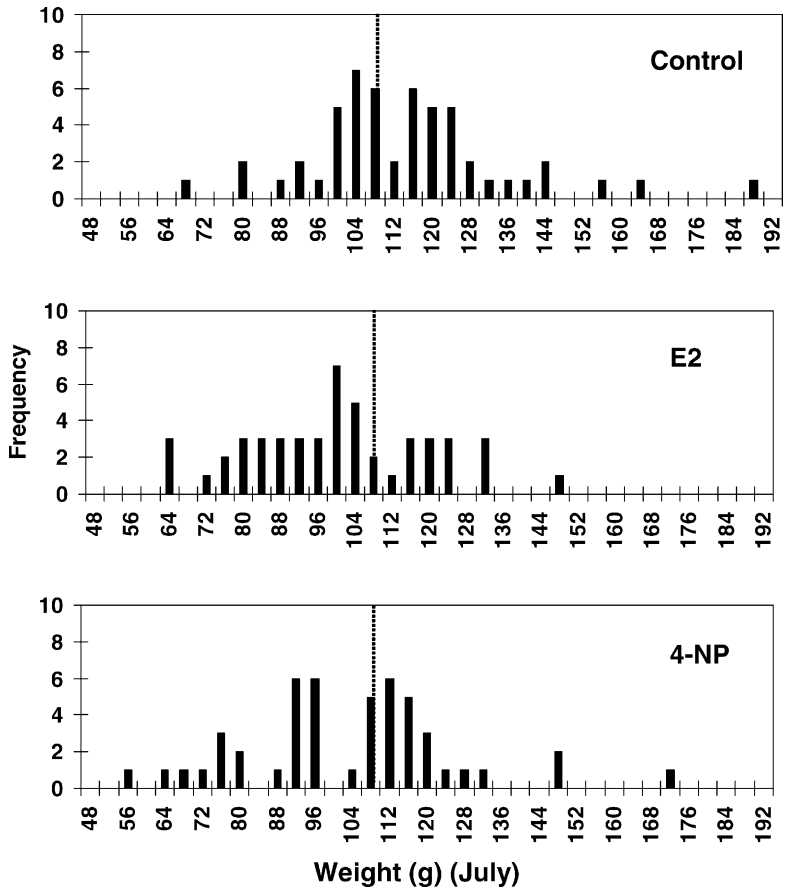

Fig. 5. July weight frequency histograms of Early window controls, E_2 and 4-NP treated smolts. Vertical dotted line represents the median control weight (i.e. 108.9 g).

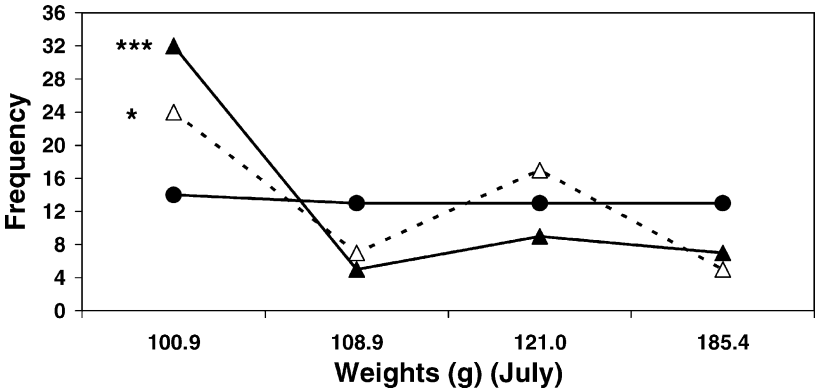

Fig. 6. July weight frequency distributions of Early window controls (●), E_2 (▲) and 4-NP (△) treated smolts. Tick marks on the *X*-axis are the upper-limit of each control weight quartile. *Y*-axis gives the frequency defined by the control weight quartiles. Asterisks indicate significant differences from the control weight frequency distribution ($^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$) (KS test).

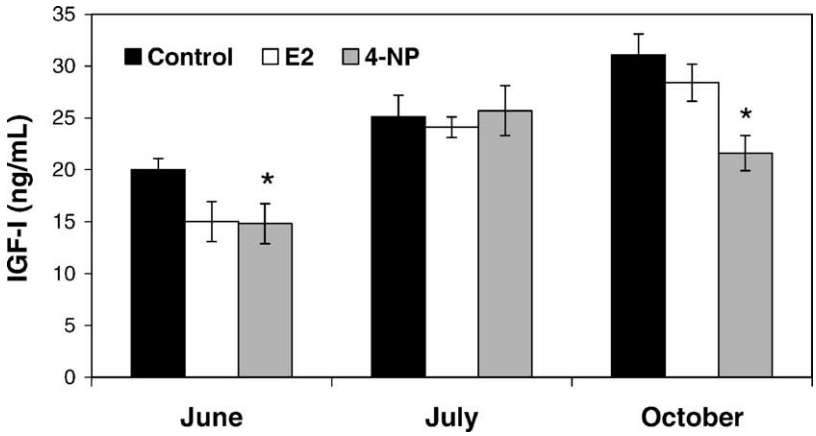

Fig. 7. Mean plasma IGF-I concentrations and standard errors of Early window controls, E_2 and 4-NP treated smolts, across time (June, July and October). Asterisks indicate significant differences from controls ($^{*}P < 0.05$) (NPAR-1-ANOVA, Dunnett's).

across time than E_2 (Fig. 7). The most prominent effects of E_2 and 4-NP on mean plasma IGF-I concentrations were seen in June {E_2 (15.0 ± 1.9 ng/ml, $n = 11$), 4-NP ($^{*}14.8 \pm 1.9$ ng/ml, $n = 9$), controls (20.0 ± 1.1 ng/ml, $n = 10$)} and October {E_2 (28.4 ± 1.8 ng/ml, $n = 12$), 4-NP ($^{*}21.6 \pm 1.7$ ng/ml, $n = 15$), controls (31.1 ± 2.0 ng/ml, $n = 13$)} ($P < 0.05$) (NPAR-1-ANOVA, Dunnett's) (Fig. 7).

4. Discussion

In this study, short-term water-borne E_2 and 4-NP exposures were conducted during three temporal windows (Early, Middle and Late) within the final stages of PST. The Early window was considered to be the most sensitive window for determining the effects of E_2 and 4-NP exposures on growth and plasma IGF-I concentrations, based on the growth performance of its control smolts compared to those of the Middle and Late windows. The effects of E_2 and 4-NP on growth in the Middle and Late windows would be difficult to interpret because they would be confounded by the additional concomitant effects of the later SW transfer times on growth.

The impaired growth performance of the Middle and Late window control smolts relative to those of the Early window is likely a result of being held in

FW longer. Smolts from the Middle and Late windows were probably transferred to SW beyond their optimal SW transfer period and began to desmoltify and revert back to a parr-like state (Clarke, 1982), a condition termed parr-revertant in which fish lose their ability to hypo-osmoregulate efficiently in SW (Folmar et al., 1982). Some parr-revertants are not able to maintain the required growth once transferred to SW. Growth and development are likely compromised to maintain an adequate osmoregulatory function (Folmar et al., 1982). The mechanism responsible for the impaired growth in the Middle and Late window control smolts is uncertain, but could be linked to depressed thyroidal status. SW adapted parr-revertants have lower plasma thyroxine concentrations relative to SW-adapted smolts (Folmar et al., 1982). Depressed thyroidal status can lead to hepatic GH resistance and hence depressed hepatic IGF-I synthesis (Pérez-Sánchez and LeBail, 1999).

Within the Early window, E_2 and 4-NP had negative effects on smolt weights and plasma IGF-I concentrations, and the effects were apparent as of June (i.e. 4 weeks after the beginning of treatments) (Figs. 4 and 7). However, across time, E_2 had an overall greater effect on mean smolt weights than 4-NP (Fig. 4), while 4-NP had an overall greater effect on mean plasma IGF-I (Fig. 7). Depressed plasma IGF-I concentrations observed in the E_2 and 4-NP treated smolts may be responsible for delayed or stalled progression of PST, such that when the smolts were transferred to SW, their ability to adapt to SW was not optimal. Since IGF-I modulates growth as well as PST (Björnsson, 1997), the controls likely had an advantage over the E_2 and 4-NP treated smolts with regard to long-term growth as well as optimal completion of PST. These results are consistent with other studies that have reported that E_2 and 4-NP can adversely affect PST in salmonids (Miwa and Inui, 1986; Madsen and Korsgaard, 1989; Madsen et al., 1997) and have a detrimental influence on their hypo-osmoregulatory performance (Madsen and Korsgaard, 1991).

The negative effects of E_2 and 4-NP on growth (i.e. a tertiary level response: Munkittrick and McCarty, 1995) and plasma IGF-I concentrations (i.e. an indicator of smolt growth: Beckman et al., 1999) of hatchery reared Atlantic salmon smolts in this study are ecologically significant because they evoke concerns for successful growth and survival of wild Atlantic salmon smolts exposed to low levels of estrogenic substances that may occur from current discharges into rivers supporting sea-run salmon stocks. Low level exposure to estrogenic substances during the smolt runs to the sea may be one of a number of factors responsible for the decline in catches of adult Atlantic salmon. Based on the results of this study, plasma IGF-I concentrations in wild Atlantic salmon smolts could potentially be used as indicators of low-level exposures to estrogenic substances during the smolt runs to sea, as well as indicators of growth and adult returns.

Additionally, the growth and plasma IGF-I concentrations results of this study provide insight into the mechanisms by which E_2 and 4-NP influence Atlantic salmon smolt growth. The results suggest that their mechanisms of action involve a disruption of the GH/IGF-I axis, and that their pathways leading to its disruption may be different from each other. This is somewhat surprising, given the wealth of data showing that many of 4-NP's actions, such as vitellogenin induction, are via estrogen receptor binding (White et al., 1994; Madsen et al., 1997; Christiansen et al., 1998; Servos, 1999; Yadetie et al., 1999). Reproduction and PST are physiological processes that are antagonistic to each other (Aida et al., 1984; Langdon and Thorpe, 1985; Ikuta et al., 1985; Miwa and Inui, 1986; Madsen and Korsgaard, 1991; Madsen et al., 1997). Vitellogenin is normally produced by females during reproduction (Specker and Sullivan, 1994) and its induction during PST would likely impose expenditure in energy that would otherwise have been put toward growth or other physiological processes involved with PST. Other mechanisms could be related to interferences in the interactions among hormonal axes modulating PST. Studies have shown that E_2 is capable of affecting the thyroid axis (Leatherland, 1985; Cyr et al., 1988; Cyr and Eales, 1996), and Ikuta et al. (1987) suggested that E_2 could potentially interfere with the cross-talk between the thyroid and GH/IGF-I axis. Disturbances at the hypothalamus or pituitary levels could also explain our observations. Ikuta et al. (1987) suggested that E_2 could potentially affect the hypothalamus, causing inhibition of GH and/or TSH secretion from the pituitary, and Miwa and Inui (1986) showed that E_2 has a suppressive action on pituitary somatotroph function. Other potential mechanisms could involve disruption in the interrenal axis, inhibition of metabolic enzymatic functions or

others. Differences between E_2 and 4-NP could also be due to the recognized toxic effects of 4-NP on Atlantic salmon (McLeese et al., 1981; Staples et al., 1998). In summary, the mechanistic pathways of E_2 and 4-NP leading to disruption of the GH/IGF-I axis are potentially numerous and remain uncertain.

5. Conclusion

This study has shown that short term, FW exposures of hatchery reared Atlantic salmon smolts to environmentally relevant, low-level, water-borne concentrations of E_2 and 4-NP during the final stages of PST, resulted in significant negative effects on weights and plasma concentrations of IGF-I, over the course of a critical SW growth period which influences their early marine survival and subsequent return as spawning adults.

The results of this study are ecologically significant because they evoke concerns for successful growth and survival of wild Atlantic salmon smolts exposed to low levels of estrogenic substances that may occur from current discharges into rivers supporting sea-run salmon stocks. Exposure to low-levels of estrogenic substances during the smolt runs to the sea may be one of a number of factors responsible for the decline in wild Atlantic salmon adult returns. Based on the results of this study, plasma IGF-I concentrations in wild Atlantic salmon smolts could potentially be used as indicators of low-level exposures to estrogenic substances during the smolt runs to sea, as well as indicators of growth and adult returns.

Additionally, the results of this study provide insight into the mechanisms by which E_2 and 4-NP influence Atlantic salmon smolt growth. The results suggest that their mechanisms of action involve a disruption of the GH/IGF-I axis, and that their pathways leading to its disruption may be different from each other.

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