

ENVIRONMENTAL ATRAZINE: PHYSIOLOGICAL EFFECTS ON ATLANTIC SALMON (*Salmo salar*) SMOLTS IN FRESH WATER AND AFTER SEAWATER EXPOSURE.

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Introduction

With the reported decline in numbers of returning adult salmonids in many rivers in the U.K., Europe and North America, there is increasing concern that at least part of this decline may be the result of xenobiotic contaminants entering salmonid rivers. Some pollutants have been reported to directly affect the spawning activity and spawning success of adult salmonids and also affect the survival of eggs and larvae which may directly affect population recruitment. However, in migratory salmonid species pollutants may also significantly impact upon populations by affecting the migratory success of smolts.

One class of compounds which have rarely been investigated for physiological affects on fish are the triazines. Triazines are persistent, highly water soluble, and are widely used in agriculture as herbicides. One triazine compound, atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) has been one of the most frequently used ingredients of herbicides in the last 20 years, and has been detected in rainwater at concentrations up to $3.5 \mu\text{g l}^{-1}$ (Braun et al., 1987), although the main route for its contamination of fresh waters is via runoff after rainfall due to its high mobility through soil. However, what effects this compound has on fresh water fish at low, environmentally realistic, water concentrations has only comparatively recently been studied.

The water atrazine concentrations used in this study were based on measurements made in various rivers and streams in the U.K. (data supplied by the National Rivers Authority) during the spring months. Salmon smolts were exposed to various concentrations of atrazine ($0\text{-}22.7 \mu\text{g l}^{-1}$) for 5 days in fresh water and then sampled and a second group of fish were sampled 24 h after seawater exposure after being pre-exposed to atrazine in fresh water for 5 days. The effect of atrazine exposure on the physiology of smolts was evaluated by measuring a variety of parameters. Plasma cortisol, thyroxine (T_4) and triiodothyronine (T_3) concentrations were measured in order to gauge the impact of atrazine exposure on the endocrine system of smolts, and these hormones have been found previously to be responsive to various forms of stress in salmonids. Plasma osmolarity and the water content, sodium (Na^+), chloride (Cl^-), and potassium (K^+) contents of the plasma

and white muscle to determine the ability of smolts to osmo- and iono-regulate after atrazine exposure.

Results

Atrazine Exposure in Freshwater

The range of atrazine concentrations that salmon smolts were exposed to for 5 days were sublethal, since no mortalities were recorded. Atrazine at low water concentrations had no effect on plasma cortisol concentrations in salmon smolts, and plasma concentrations of cortisol were only elevated at water atrazine concentrations at and above $6.5 \mu\text{g l}^{-1}$ (Table 1). The plasma concentrations of the thyroid hormones, T_4 and T_3 , were unaffected by atrazine exposure (Table 1). Plasma osmolarity was elevated in smolts at atrazine concentrations at and above $6.5 \mu\text{g l}^{-1}$, and the plasma concentrations of Na^+ , K^+ and Cl^- were elevated at these atrazine concentrations, although these significant elevations were inconsistent, whereas plasma water content was unaffected (Table 1). The smolts ability to maintain tissue osmo- and ionoregulatory balance in fresh water was unaffected by atrazine exposure since muscle water content and Na^+ , Cl^- , and K^+ concentrations were not significantly altered (Table 1).

Table 1. Plasma cortisol (ng ml^{-1}), T_4 (ng ml^{-1}), T_3 (ng ml^{-1}), osmolarity (mOsm l^{-1}), water (%), Na^+ (mM), Cl^- (mM), and K^+ (mM) concentrations and muscle water (%), Na^+ ($\text{mM Kg H}_2\text{O}^{-1}$), Cl^- ($\text{mM Kg H}_2\text{O}^{-1}$), and K^+ ($\text{mM Kg H}_2\text{O}^{-1}$) contents in salmon smolts exposed to various concentrations of atrazine in fresh water. * $p < 0.05$ compared to controls ($0 \mu\text{g l}^{-1}$).

	0	Atrazine 1.1	($\mu\text{g l}^{-1}$) 6.5	13.9	22.7
Plasma					
Cortisol	18.3 ± 1.0	21.8 ± 2.8	$32.1 \pm 2.2^*$	$35.9 \pm 3.1^*$	$37.2 \pm 4.5^*$
T_4	3.9 ± 0.6	3.5 ± 0.2	4.3 ± 0.3	3.6 ± 0.2	3.7 ± 0.5
T_3	1.1 ± 0.1	1.4 ± 0.2	1.4 ± 0.2	1.0 ± 0.2	1.5 ± 0.2
Osmolarity	304 ± 2	310 ± 1	$318 \pm 2^*$	$320 \pm 3^*$	$325 \pm 3^*$
Water	94.5 ± 0.5	92.9 ± 0.6	94.6 ± 0.5	94.1 ± 1.1	94.8 ± 0.8
Na^+	147 ± 1	150 ± 1	153 ± 1	151 ± 1	$155 \pm 1^*$
Cl^-	134 ± 2	139 ± 1	$143 \pm 2^*$	140 ± 2	136 ± 3
K^+	2.0 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	$2.5 \pm 0.1^*$	$2.6 \pm 0.1^*$
Muscle					
Water	77.6 ± 0.3	76.9 ± 0.3	78.0 ± 0.9	78.3 ± 0.6	78.4 ± 0.5
Na^+	15.1 ± 0.3	15.9 ± 0.4	15.3 ± 0.2	15.5 ± 0.5	15.5 ± 0.2
Cl^-	9.8 ± 0.2	9.8 ± 0.2	9.8 ± 0.2	9.7 ± 0.2	9.7 ± 0.2
K^+	169 ± 2	171 ± 2	166 ± 3	170 ± 3	168 ± 4

Seawater-Challenge Test after Fresh Water Atrazine Exposure

The 24 h seawater-challenge caused no mortalities in the control, 1.1 and the 6.5 $\mu\text{g l}^{-1}$ atrazine group, but the salinity change caused 14 and 28 % mortality in the 13.9 and 22.7 $\mu\text{g l}^{-1}$ atrazine groups respectively. Plasma cortisol and T_4 concentrations were significantly elevated over control concentrations in the fish that had been exposed to 6.5 $\mu\text{g l}^{-1}$ atrazine and above in freshwater whereas plasma T_3 concentrations were unchanged (Table 2). The control fish exhibited elevated plasma osmolarity and plasma monovalent ion concentrations compared to fresh water values and the osmolarity and monovalent ion concentrations were significantly higher in the fish that had been exposed to atrazine, especially in the 13.9 and 22.7 $\mu\text{g l}^{-1}$ groups (Table 2). Plasma water contents in sea water-exposed salmon were similar in all groups and were unaffected by previous atrazine exposure in fresh water (Table 2). Although plasma ions showed marked effects of atrazine exposure after the seawater-challenge, muscle water, K^+ and Cl^- contents were relatively stable and muscle Na^+ concentrations were only significantly elevated in the fish from the 22.7 $\mu\text{g l}^{-1}$ group (Table 2).

Table 2. Plasma and muscle variables in salmon smolts after a 24 h seawater-challenge after pre-exposure to atrazine in freshwater. Units are as in Table 1. * $p < 0.05$ compared to control ($0 \mu\text{g l}^{-1}$).

	0	Atrazine 1.1	($\mu\text{g l}^{-1}$) 6.5	13.9	22.7
Plasma					
Cortisol	21.6 \pm 1.3	29.6 \pm 4.6	41.4 \pm 2.2*	57.5 \pm 6.3*	74.7 \pm 7.7*
T_4	4.0 \pm 0.2	4.3 \pm 0.3	6.3 \pm 1.0*	6.1 \pm 0.5*	6.7 \pm 0.8*
T_3	1.4 \pm 0.2	1.2 \pm 0.1	0.9 \pm 0.1	1.0 \pm 0.2	1.4 \pm 0.2
Osmolarity	357 \pm 2	356 \pm 2	360 \pm 1	394 \pm 2*	400 \pm 4*
Water	93.6 \pm 0.7	93.0 \pm 0.6	93.9 \pm 0.6	94.9 \pm 0.4	95.4 \pm 0.6
Na^+	185 \pm 1	184 \pm 1	185 \pm 2	200 \pm 1*	205 \pm 2*
Cl^-	157 \pm 1	161 \pm 1	165 \pm 1	173 \pm 3*	183 \pm 3*
K^+	2.4 \pm 0.1	2.7 \pm 0.1	3.6 \pm 0.1*	3.4 \pm 0.1*	4.0 \pm 0.1*
Muscle					
Water	76.9 \pm 0.3	77.2 \pm 0.2	77.7 \pm 0.3	77.6 \pm 0.2	76.9 \pm 0.2
Na^+	17.2 \pm 0.4	17.0 \pm 0.3	17.1 \pm 0.4	17.0 \pm 0.2	18.4 \pm 0.3*
Cl^-	10.8 \pm 0.4	10.2 \pm 0.2	10.5 \pm 0.3	10.5 \pm 0.3	10.8 \pm 0.4
K^+	179 \pm 2	175 \pm 2	177 \pm 2	181 \pm 3	180 \pm 3

Discussion

Although exposure to atrazine, up to 22.7 $\mu\text{g l}^{-1}$, in freshwater was sublethal for salmon smolts, the fish were clearly physiologically stressed at the higher water atrazine concentrations as evidenced by their increased plasma cortisol concentrations and osmolarity and monovalent ion concentrations. Gluth and Hanke (1985) also found elevated plasma cortisol concentrations in carp, *Cyprinus carpio*, exposed to atrazine, but at much higher water concentrations (100 $\mu\text{g l}^{-1}$). At water atrazine concentrations at and above 6.5 $\mu\text{g l}^{-1}$, the increased plasma cortisol concentrations in salmon smolts were

accompanied by small increases in plasma Na^+ , Cl^- and K^+ concentrations which contributed to the increased plasma osmolarity. Tilapia, *Oreochromis mossambicus*, exposed to atrazine for 7-90 days also showed significant elevations in serum Na^+ , Cl^- and K^+ concentrations (Prasad and Reddy, 1994). These authors suggested that the increased monovalent ions in the serum of atrazine-exposed tilapia was due to increased tissue ion efflux. However, in salmon, the increased plasma monovalent ions in fish exposed to the higher water atrazine concentrations in fresh water did not originate from the muscle compartment since the contents of these ions were stable in this tissue.

Data from a variety of studies have indicated that atrazine-exposure of freshwater fish can cause a haemodilution effect as judged from decreased plasma protein concentrations (Gluth and Hanke, 1985) or increased blood or whole-body water content (Prasad and Reddy, 1994; Davies et al, 1994). Indeed, atrazine-induced haemodilution may emanate from renal damage, which occurs at relatively low water atrazine concentrations, which may result in decreased water excretion by the kidney (Fischer-Scherl et al, 1991; Oulmi et al., 1995). However, salmon smolts in the present study showed no plasma haemodilution, as evidenced by unchanged plasma water content, and the unchanged muscle water content suggests that whole-body osmotic balance was also unaltered.

Although the branchial ultrastructure show very minor alterations in fish exposed to atrazine, even at very high water concentrations (Neskovic et al., 1993), there is no data regarding the effect of water-borne atrazine on branchial ion flux activity. The fact that muscle, and presumably whole-body, water and ion contents were unaltered in salmon may suggest that the increased plasma monovalent ions in atrazine-exposed salmon smolts was due to altered branchial ion flux activity, perhaps due to decreased ion efflux. However, further work is needed to confirm this possibility.

Water atrazine concentrations of 13.9-22.7 $\mu\text{g l}^{-1}$, which were sublethal in fresh water, affected salmon smolts to the extent that on seawater transfer 14-28 % of the fish died within 24 h. This dose-dependent effect on mortality rates mirrored the dose-dependent increase in plasma cortisol concentrations on seawater transfer, indicating that the surviving fish were significantly stressed. At water atrazine concentrations which caused plasma ionoregulatory disturbances in freshwater smolts, seawater exposure clearly exacerbated this problem, although the fish regulated their tissue, at least white muscle, water and ion concentrations well.

Seawater transfer elevated plasma T_4 concentrations in the control fish compared to freshwater values. In atrazine-exposed fish, plasma T_4 , but not T_3 , concentrations were elevated even further by the seawater transfer. The significance of this is not known, since the role of thyroid hormones in seawater adaptation, if any, of salmon smolts is unclear. It may reflect a non-specific effect of stress on the thyroidal system and may not directly be related to the ionoregulatory disturbance which occurred in these fish.

In summary, although water atrazine concentrations up to 23 $\mu\text{g l}^{-1}$ were sublethal for salmon smolts in fresh water, the fish were nevertheless physiologically stressed at concentrations above 6.5 $\mu\text{g l}^{-1}$. The performance capacity of the smolts, in terms of a 24 h seawater challenge, was significantly reduced by the atrazine exposure and mortalities occurred in fish that were exposed to the higher water atrazine concentrations in fresh water. Since these water concentrations were based on environmentally-relevant concentrations, atrazine may be a hazard for salmon undergoing smoltification and subsequent migration into seawater. This latter aspect may be particularly important since

a recent study, using acoustically-tagged migrating smolts, has shown that smolts do not always require a period of saltwater acclimation before moving into seawater (Moore et al., 1995).

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