Comparative Biochemistry and Physiology, Part C 152 (2010) 379–384

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Survival and iono-regulatory performance in Atlantic salmon smolts is not affected by atrazine exposure

Jacquie Matsumoto [a], Alan J. Hosmer [b], Glen Van Der Kraak [a,*]

[a] Department of Integrative Biology, University of Guelph, Guelph Ontario, Canada N1G 2W1
[b] Syngenta Crop Protection Inc., Greensboro, North Carolina 27409, USA

ARTICLE INFO

Article history:
Received 4 December 2009
Received in revised form 15 June 2010
Accepted 16 June 2010
Available online 25 June 2010

Keywords:
Atrazine
Na+/K+-ATPase
Risk assessment
Smoltification

ABSTRACT

This study was conducted to determine the potential effects of atrazine exposure on survival and physiological performance in Atlantic salmon (*Salmo salar*) during the period of smoltification. This study involved two separate experiments in which juvenile Atlantic salmon were exposed to atrazine for a four day period in freshwater after which the fish were transferred to 50% seawater for two days and then to 100% seawater for five more days. The nominal concentrations of atrazine tested (1, 10 and 100 μg/L) were representative of and exceeded the levels measured in the North American freshwater environment. After seven days in seawater, fish were weighed, bled for the determination of plasma electrolyte levels, euthanized and samples collected for the determination of gonadosomatic index, muscle water content and gill Na+/K+-ATPase activity. Measured atrazine concentrations during the freshwater exposure period were 76–99% of nominal levels. There were no mortalities attributed to atrazine exposure. There were also no statistically significant differences in body weight, plasma sodium, potassium, magnesium and chloride levels, muscle water content or gill Na+/K+-ATPase activity between control and atrazine treated fish. Measurement of testis and ovary weights showed that there were no treatment effects on relative gonad size in male or female fish. These studies have shown that short term exposure to atrazine during the freshwater phase of their lifecycle had no effects on subsequent survival, body weight, relative gonad size or various measures of iono-regulatory performance in juvenile Atlantic salmon upon transfer to seawater. The concentrations of atrazine tested exceed those likely to be experienced in the natural aquatic environment suggesting that short term exposure to atrazine does not pose a risk to Atlantic salmon during the period of smoltification.

1. Introduction

Smoltification in salmonids includes the behavioural, developmental and physiological changes that accompany the parr-smolt transformation and the ability of the fish to initiate their downstream migration and the successful transition from life in freshwater to seawater (Hoar, 1988). The development of seawater tolerance results from a reorganization of the major osmoregulatory organs including the gill, gut and kidney and is accompanied by an increase in gill Na+/K+-ATPase activity and the ability to regulate plasma ions following seawater transfer. A number of hormones including thyroid hormones, prolactin, corticosteroids, and growth hormone play pivotal roles in smoltification (Barron, 1986; McCormick, 2001; Ojima et al., 2009).

Recently, attention has focused on the possibility that exposure to anthropogenic chemicals found in the aquatic environment during the period of smoltification may be a contributing factor to declines in the numbers of Atlantic salmon (*Salmo salar*) in both Europe and North America. Several studies have shown that smoltification is sensitive to chemicals that interfere with the endocrine system (Madsen et al., 2004; McCormick et al., 2005; Lerner et al., 2007). Some of these studies have examined the triazine herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) owing to its common use in agriculture, moderate water solubility and occurrence in the aquatic environment. While the responses observed have been highly variable, there are reports that atrazine has effects on salinity tolerance and iono-regulatory performance in Atlantic salmon smolts (Waring and Moore, 2004; Moore et al., 2003, 2007, 2008; Nieves-Puigdoller et al., 2007). For example, Waring and Moore (2004) reported that exposure of Atlantic salmon smolts to atrazine at concentrations of 2–22.7 μg/L for up to seven days led to elevated mortality upon transfer to seawater and contributed to increases in plasma osmolarity, sodium, chloride and cortisol levels and a significant decrease in gill Na+/K+-ATPase activity. In one study, 100% mortality was reported upon transfer to seawater after exposure to atrazine at 0.1 μg/L for 72-h in freshwater (Moore et al., 2008).

In contrast, several other studies have reported that Atlantic salmon smolts were far less sensitive to atrazine at similar or greater concentrations than those reported in the aforementioned reports

* Corresponding author. Tel.: +1 519 824 4120x53424; fax: +1 519 837 2075.
E-mail address: gvanderk@uoguelph.ca (G. Van Der Kraak).

1532-0456/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2010.06.005

(e.g., Moore et al., 2003, 2007; Nieves-Puigdoller et al., 2007). These studies showed that substantially higher levels of atrazine were required to cause fish mortality, perturbations of ion homeostasis in freshwater, some loss of salinity tolerance, and a transient decrease in growth in seawater. For example, Nieves-Puigdoller et al. (2007) reported low mortality (9%) of smolts exposed to 100 μg atrazine/L for 21 days in freshwater and that this treatment did not influence survival following a seawater water challenge for 24 h. In addition, there were no mortalities in control or low atrazine (10 μg/L) treatment groups. There were also no differences in the weight or length of the control or atrazine treated fish after a three month grow-out period in seawater (Nieves-Puigdoller et al., 2007).

As reported by Waring and Moore (2004) and Moore et al. (2008), the effects of atrazine on survival and measures of iono-regulatory performance were seen at concentrations which may be observed in the Canadian freshwater environment (Wan et al., 2006a; PMRA, 2007). In contrast, the research by Nieves-Puigdoller et al. (2007) and others (Moore et al., 2003, 2007) suggests that the concentration at which responses to atrazine were observed would rarely be experienced in the environment, especially in lotic systems inhabited by salmonids. Overall, these studies indicate considerable uncertainty to the risks posed by atrazine to Atlantic salmon during smoltification.

The purpose of this study was to determine the potential effects of atrazine exposure on survival and physiological performance in Atlantic salmon during the period of smoltification. This study, conducted under Good Laboratory Practices, involved two separate experiments in which juvenile Atlantic salmon were exposed to graded concentrations of atrazine for a four day period in freshwater after which the fish were transferred to 50% seawater for two days and then to 100% seawater for five more days. The nominal concentrations of atrazine tested (1, 10 and 100 μg/L) were representative of and exceeded the levels measured in the Canadian freshwater environment. After seven days in seawater, fish were weighed, bled for the determination of plasma electrolyte levels, euthanized and samples collected for the determination of gonadosomatic index, muscle water content and gill $Na+/K+$-ATPase activity. The latter is a marker of ion regulatory change that is correlated with the development of seawater tolerance in salmon smolts. The experiment was repeated in a separate study using fish from the same stock to ascertain if there were temporal differences in the responsiveness of the fish to atrazine exposure during the period of smoltification.

2. Materials and methods

2.1. Animals and routine husbandry

Atlantic salmon presmolts (2 years old; approximately 40 g each) were obtained in late April 2008 from the Mactaquac Biodiversity Facility, French Village, NB (Canada) and held at the Hagen Aqualab at the University of Guelph (Guelph, ON, Canada) in 2000 L fiberglass tanks supplied with freshwater. The fish were held in self-contained tanks supplied with re-circulated water with freshwater replaced at a rate of approximately 1500 L per day. Fish were initially held at 4 °C to reflect the water temperatures at the Mactaquac facility. Temperatures were increased over a two week period to 8 °C and then maintained at this temperature for the duration of the experiments. The photoperiod simulated conditions for French Village, NB. The photoperiod was adjusted biweekly and varied from approximately 15 h light/9 h dark to 17 h light/7 h dark over the course of the experiment. Fish were fed a commercial salmon diet (Martin Feed Mills, Elmira, Ontario) every other day to satiation. Swimming and feeding behaviour were monitored daily. Under these husbandry conditions, there was a single mortality recorded for the 600 fish transferred to the University of Guelph. This fish died within 24 h of arriving in Guelph.

2.2. Atrazine exposure and seawater challenge

The two experiments were started on May 26 and June 11, 2008, respectively. The experiments included a four day acclimation period (days 0–4), a four day exposure to graded concentrations of atrazine (0, 1, 10 and 100 μg/L) (days 4–8), transfer to non-atrazine treated seawater (50%; 17.5 ppt) for two days (days 8–10), followed by five days in 100% (35 ppt) non-treated seawater (days 10–15). Stock solutions of technical grade atrazine (10 mg/L) were prepared just prior to the initiation of both experiments and were used to dose the experimental tanks. Seawater was prepared using Crystal Sea (Marine Enterprises, Baltimore, MD, USA) and Hagen Aqualab well water. Salinity was measured in each tank using a refractometer. Additionally, samples of seawater were obtained daily for determination of sodium and magnesium concentrations.

The experiments were conducted in 125 L fiberglass tanks that were secured within a 6 foot diameter tank supplied with running water to maintain the water temperature at 8 °C. The exposures were semi-static and every 48 h fish were netted and transferred to a new tank containing new solutions of the appropriate treatment conditions. There were 16 fish per tank and four replicate tanks per treatment. Temperature, pH and dissolved oxygen levels were measured daily in each tank. Total ammonia, nitrate and nitrite were measured in water samples collected at 2–4 day intervals throughout the exposure period. The atrazine exposure concentrations were measured in water samples collected six times during the exposure period: initiation of atrazine exposure (day 4); immediately prior to solution renewal (day 6); immediately upon transfer of fish to new solutions (day 6) and on day 8 just prior to transfer to 50% seawater. Additionally, water samples were collected in the seawater tanks on days 10 and 15.

2.3. Sampling

At the end of the experiment on day 15, fish were anesthetized using MS-222. Body weight was obtained and blood was collected from the caudal vein using a heparinized syringe. Blood was immediately placed on ice. Within 2 h of collection, the plasma component of the blood matrix was separated and stored at −20 °C in aliquots for the measurement on plasma electrolytes. Fish were euthanized by cervical severance and the gonads excised and weighed. Gonadosomatic index (GSI) was determined as the ratio of gonad weight to body weight × 100. Gill filaments were removed and placed in a solution of SEI buffer (150 mM sucrose, 10 mM EDTA, and 50 mM imidazole) and immediately frozen in dry ice. Gill filaments were stored at −80 °C. Finally, a 75–125 mg piece of white muscle adjacent to the lateral line was excised and frozen for determination of water content.

2.4. Analytical methods

Atrazine concentrations in water samples were determined using a commercial enzyme-linked immunosorbent assay (ELISA) test kit (EnviroGard Triazine plate kits; Strategic Diagnostics, Newark, DE). The limit of detection (LOD) in the assay was 0.025 μg/L. The concentration of atrazine in the fish food was determined at the Laboratory Services Division of the University of Guelph using GC-MSD in single ion monitoring mode. The LOD was 0.03 μg/L. Atrazine was not detected in the fish food used in both experiments.

Total ammonia, nitrite and nitrate in water samples were measured using Hach water chemistry test kits (Hach, Loveland, CO, USA). Salinity was measured using a refractometer and confirmed by the concentration of sodium and magnesium. Sodium was measured in duplicate by flame photometry (Model PFP7, Jenway Corporation, Techne Incorporated, Burlington, NJ). Magnesium concentrations were determined in triplicate using atomic absorption spectrophotometry on a Varian 220 (Palo Alto, CA, USA). Alkalinity was determined in well water and 100%