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Developmental timing of perchlorate exposure alters threespine stickleback dermal bone

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ABSTRACT

Adequate levels of thyroid hormone are critical during development and metamorphosis, and for maintaining metabolic homeostasis. Perchlorate, a common contaminant of water sources, inhibits thyroid function in vertebrates. We utilized threespine stickleback (*Gasterosteus aculeatus*) to determine if timing of perchlorate exposure during development impacts adult dermal skeletal phenotypes. Fish were exposed to water contaminated with perchlorate (30 mg/L or 100 mg/L) beginning at 0, 3, 7, 14, 21, 42, 154 or 305 days post fertilization until sexual maturity at 1 year of age. A reciprocal treatment moved stickleback from contaminated to clean water on the same schedule providing for different stages of initial exposure and different treatment durations. Perchlorate exposure caused concentration-dependent significant differences in growth for some bony traits. Continuous exposure initiated within the first 21 days post fertilization had the greatest effects on skeletal traits. Exposure to perchlorate at this early stage can result in small traits or abnormal skeletal morphology of adult fish which could affect predator avoidance and survival.

1. Introduction

Many endocrine disrupting contaminants have deleterious effects even at low concentrations (Carr and Patiño, 2011). Understanding the effects of these compounds during critical developmental stages is necessary for risk assessment, establishing regulations, and directing remediation efforts.

Perchlorate is a widespread inorganic anion found in ground and surface waters throughout the United States and in other countries (Brandhuber et al., 2009; USEPA, 2011). Perchlorate salts occur naturally at low levels in arid regions such as the Southwestern U.S., Antarctica and the Atacama Desert in Chile (Kounaves et al., 2010; Rajagopalan et al., 2006; Rao et al., 2007; Urbansky et al., 2001). Perchlorate is highly soluble, persistent, and stable in aqueous environments (Urbansky, 2002). These properties make perchlorate mobile and available to interact with biota in surface waters. Because it is a strong oxidizer, perchlorate has been manufactured for use in solid rocket propellant, munitions and many other industrial products (Trumpolt et al., 2005). Anthropogenic sources, such as military and manufacturing sites, are responsible for most of the environmental contamination in the U.S. (Morrison et al., 2006; Trumpolt et al., 2005).

Perchlorate competitively inhibits the uptake of iodide from the bloodstream into thyroid tissue (Carr et al., 2005; Wolff, 1998). Perchlorate has a greater affinity than iodide for the sodium/iodide symporter (NIS, alias SLC5A5) located in the basolateral membrane of thyrocytes (Urbansky, 2002; Wolff, 1998). Interruption of iodide concentration into thyroid tissue can impair synthesis of thyroid hormone (TH, which includes both thyroxine [T4] and triiodothyronine [T3]), as has been demonstrated in several vertebrate animal models (Bradford, 2011; Bradford et al., 2005; Carr et al., 2005; Goleman et al., 2002; McNabb et al., 2004; Patiño and Mukhi, 2007; Pickford et al., 2005; Yu et al., 2002). If the supply of iodide is sufficiently reduced for an extended length of time (i.e., TH stores become exhausted) hypothyroidism can develop (Wolff, 1998). Appropriate levels of TH are critical to normal

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development, growth and metabolism in vertebrates (Choksi et al., 2003; Power et al., 2001). Individuals most at risk to the effects of perchlorate exposure are iodide deficient, and/or in early development or metamorphosis (Carr and Patiño, 2011; Leung et al., 2010; Liu and Chan, 2002; Melse-Boonstra and Jaiswal, 2010; Tietge et al., 2005; Trumbo, 2010). In addition, perchlorate may have effects independent of iodide deficiency (LaRoche et al., 1966; McDougal et al., 2011). Environmental exposure in fish occurs via the respiratory and gastrointestinal epithelia, integument, and sometimes ingested food (Furin et al., 2013). Oviparous fish embryos are exposed to contaminants in the ambient water and/or pore water of sediments that can pass through the chorion.

Disruption of TH can profoundly impact teleost development and metamorphosis (Blanton and Specker, 2007; Carr and Patiño, 2011; Leatherland, 1982; Power et al., 2001). The role of TH in skeletal development has been studied in divergent fish species including zebrafish (*Danio rerio*) (Brown, 1997; Shkil et al., 2012), African barbs (*Labeobarbus intermedius*) (Shkil et al., 2012), medaka (*Oryzias latipes*) (Sekimizu et al., 2007), and Japanese flounder (*Paralichthys olivaceus*) (Okada et al., 2005, 2003). Bony structures differ in their response to hypo- and hyperthyroid conditions; some fail to develop entirely, some over-develop, and others are relatively unchanged (Power et al., 2001; Shkil et al., 2012). Effects depend on species and the character of interest with many changes being permanent. Evidence suggests that variation in relative timing of development (heterochrony) and relative rate of growth (allometry) drive these adverse effects under different TH levels (Shkil et al., 2012).

Bernhardt et al. (2011) determined that chronic exposure to perchlorate reduces development of bony structures in young threespine stickleback (*Gasterosteus aculeatus*, hereafter: stickleback) in a concentration-dependent manner. They found that stickleback exposed to greater than 12 mg/L perchlorate exhibited phenotypic abnormalities. Of the 25 measured bony characters, 24 were significantly modified, and gross abnormalities occurred such as missing lateral plates and lack of skin pigments. Exposed fish had reduced fitness with abnormal locomotion and reproduction (Bernhardt and von Hippel, 2008; Bernhardt et al., 2006, 2011). These results raise the question of the developmental time frame during which perchlorate exerts its effects on these phenotypes.

The current study used controlled variations in timing and duration of perchlorate exposure to further quantify the effects of perchlorate on stickleback skeletal development. Concentration and critical developmental windows are considered in light of development of dermal skeleton features, with a focus on defensive traits already determined to be affected by perchlorate (Bernhardt et al., 2011). Based on the morphological changes with perchlorate exposure observed by Bernhardt et al. (2011), and changes in thyroid endpoints during development (Furin et al. p. XX, this issue), we hypothesized that perchlorate exposure during the first three weeks of embryonic and larval development will lead to reduced growth and smaller skeletal armor traits in adults.

2. Materials and methods

2.1. Experimental design

Using a static renewal experiment (i.e., partial, periodic replacement of treatment solutions (USEPA, 2002)), stickleback were exposed to one of two different concentrations of sodium perchlorate (30 and 100 mg/L) at different time points for varying durations across their development. Embryos started in either perchlorate treated water or control water. At 0, 3, 7, 14, 21, 42, 154 (5 months) or 305 (10 months) days post fertilization (dpf), fish were transferred into or out of contaminated water. Fish that began in contaminated water and subsequently moved into clean water were in the downshift (rescue) exposure regime, and those moved from clean water to contaminated water were in the upshift exposure regime (Fig. 1). Fish were raised in their respective treatments until approximately 1 year of age when they were collected and processed for morphological analysis. Due to differential survivorship and use for other experimental endpoints, sample sizes varied (Table 1).

2.2. Fish collection and experimental procedures

Stickleback were collected from Rabbit Slough, Alaska (61.534° N, 149.266° W) with un-baited wire-mesh (0.64 cm) minnow traps on 4 June, 2008. They were transported to the University of Alaska Anchorage in aerated coolers where they were kept in outdoor 1600-L pools, in de-chlorinated city water with Instant Ocean© added to 3 g/L.

On 10 June, 2008, a mass cross was performed to generate a representative study population with genetic variation randomly distributed throughout the treatments. Testes were collected from 40 males and eggs from 40 females. Egg clutches from all females, and testes from all males, were combined to randomize the genetic pool before fertilizing batches in Petri dishes (100 × 200 mm) for all treatments. Sterilized reverse osmosis (RO) purified water with Instant Ocean© added to 4 g/L was used as the embryo medium. Sodium perchlorate (>98% purity, Sigma–Aldrich, St. Louis, MO, USA) dried in an oven at 90 °C before weighing, was added to produce embryo medium at 30 and 100 mg/L. Water was changed daily and dead embryos were removed. Day 0 downshift fish were fertilized in perchlorate treated water and then moved into uncontaminated water after 15 min. Within 3 dpf, embryos in the initial Petri dishes were divided into three replicates with approximately 100 embryos each (3 replicates × 100 embryos = 300 embryos/treatment).

Embryos were kept in an incubator held at 20 ± 0.5 °C for the first ten days and then transferred to aerated 56-L aquaria (60 cm × 31 cm × 32 cm) with an AZOO© multi sponge filter (65 mm diameter). Water levels were adjusted in aquaria to maintain a ratio of at least 1-L water per 1 cm of fish, and Bacta-pur© N3000 (IET-Aquaresearch Ltd., Quebec, Canada) was added to control nitrogenous waste. Water was changed every 2 weeks or as needed. Sodium perchlorate salt was not added directly to aquaria and was only used when making fresh treatment water in separate holding tanks, which was added as needed on the two week schedule. Dead fish were removed daily. RO water was added weekly to replace evaporative loss and maintain desired perchlorate concentrations. The lighting cycle mimicked the natural diurnal cycle for Anchorage, Alaska and the average water temperature during the

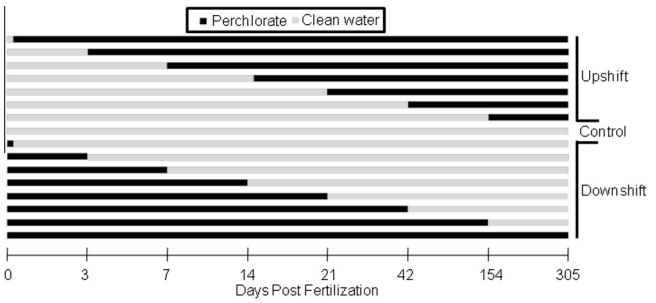

Fig. 1. Perchlorate exposure regime. This protocol was used for both 30 and 100 mg/L exposures with three replicates at each dpf. Zero dpf downshift fish were exposed during fertilization and for 15 min post fertilization before their transfer to clean water.

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