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Perchlorate exposure does not modulate temporal variation of whole-body thyroid and androgen hormone content in threespine stickleback

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ABSTRACT

Previously we showed that exposure of threespine stickleback (*Gasterosteus aculeatus*) to the endocrine disruptor perchlorate results in pronounced structural changes in thyroid and gonad, while surprisingly, whole-body thyroid hormone concentrations remain unaffected. To test for hormone titer variations on a finer scale, we evaluated the interactive effects of time (diel and reproductive season) and perchlorate exposure on whole-body contents of triiodothyronine (T_3), thyroxine (T_4), and 11-ketotestosterone (11-KT) in captive stickleback. Adult stickleback were exposed to 100 ppm perchlorate or control water and sampled at 4-h intervals across the 24-h day and at one time-point (1100 h) weekly across the reproductive season (May–July). Neither whole-body T_3 nor T_4 concentration significantly differed across the day in control or perchlorate treated stickleback. Across the reproductive season, whole-body T_3 concentration remained stable while T_4 significantly increased. However, neither hormone concentration was significantly affected by perchlorate, verifying our previous studies. The concentration of whole-body 11-KT, a major fish androgen, displayed significant diel variation and also steadily declined across the reproductive season in untreated males; perchlorate exposure did not influence the concentration of 11-KT in either diel or reproductive season schedules. Diel and reproductive season variations in 11-KT content in male stickleback are likely related to reproductive physiology and behavior. The observed increase in T_4 content across the reproductive season may be reflective of increased energy investment in reproduction near the end of the life cycle.

1. Introduction

Perchlorate, a water soluble anion, is a known inhibitor of thyroid hormone (TH) synthesis (De Groef et al., 2006; Goleman et al., 2002; Leung et al., 2010; Wu et al., 2012). Perchlorate appears in a variety of sources relevant to human health, including contaminated drinking water, milk, and leafy vegetables (Dasgupta et al., 2006; Urbansky, 2002). Environmental perchlorate largely comes from releases associated with its production, storage and use as an oxidizer for rocket fuel, artillery and a number of consumer products. The concentration of perchlorate in contaminated ground and surface water is generally in the parts per billion (ppb) range but can be in the parts per thousand range in some highly contaminated areas (Sanchez et al., 2007; Theodorakis et al., 2006). At the biochemical level, perchlorate competes with iodide at the sodium iodide symporter (NIS, also known as SLC5A5) in epithelial cells (thyrocytes) of thyroid follicles and thus disrupts normal TH synthesis, resulting in hypothyroidism in a variety of species (Lawrence et al., 2000).

Recent studies show that some fish species reorganize the architecture of their thyroid tissue in response to chronic perchlorate exposure (Furin et al., this issue; Petersen et al., 2015; Schmidt et al., 2012). Specifically, perchlorate reduces individual thyroid follicle size and increases the overall number of follicles, potentially serving as a mechanism for increasing the available surface area for NIS transporters in thyrocytes. Surprisingly, threespine stickleback (*Gasterosteus aculeatus*) that were chronically exposed to 100 ppm perchlorate from fertilization maintained normal whole-body levels of total TH (T_3 and T_4)

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(Petersen et al., 2015) despite perchlorate's known mechanism of competition with iodide at the NIS (Leung et al., 2010; Wolff, 1998). Zebrafish (*Danio rerio*) treated with perchlorate display similar changes in thyroid tissue morphology (Mukhi et al., 2007; Schmidt et al., 2012), but in contrast to stickleback, show significant reductions in whole-body T_4 concentrations (Mukhi and Patiño, 2007; Schmidt et al., 2012). Given the disparity in TH responses to perchlorate between fish species, it is possible that underlying molecular mechanisms of the response to this contaminant differ among species. An additional explanation for the failure to detect effects of perchlorate on TH levels in stickleback (Petersen et al., 2015) could be related to the experimental time-course (i.e., effects could have been masked by diel fluctuations in hormone contents).

The effects of perchlorate are not restricted to thyroid structure and function in fishes. For example, perchlorate disrupts sexual development in some species (Bernhardt and von Hippel, 2008; Bernhardt et al., 2006; Furin et al., this issue; Mukhi et al., 2007; Petersen et al., 2015). Interestingly, perchlorate-induced alteration in gonadogenesis is not consistent across fish taxa; perchlorate induces feminization in zebrafish (Mukhi et al., 2007), while in threespine stickleback it induces masculinization (Bernhardt et al., 2006). Perchlorate-induced reproductive effects in threespine stickleback also include a pronounced increase in 11-ketotestosterone (11-KT, the major fish androgen; (Borg, 1994)) in early developing fish and hyperplasia of spermatocytes (Petersen et al., 2015). Cross-talk between the hypothalamic–pituitary–thyroid (HPT) and hypothalamic–pituitary–gonadal (HPG) axes may, in part, explain the widespread phenotypic effects of perchlorate (Duarte-Guterman et al., 2014; Flood and Langlois, 2014).

Photoperiod strongly affects the regulation of multiple endocrine axes in stickleback, including the HPT and HPG axes (Kitano et al., 2010; O'Brien et al., 2012). Seasonal change in photoperiod is a potent environmental cue for regulating a suite of physiological responses, including patterns of hormone synthesis and secretion. Recent work in fishes and other species has demonstrated that TH production and secretion is responsive to changes in photoperiod and can vary on a seasonal basis (Comeau et al., 2000; Dardente et al., 2014; Nakane and Yoshimura, 2014; Yoshimura, 2013). Likewise, increased day-length initiates reproduction in many fishes and is associated with seasonal increases in the synthesis of 11-KT, a primary driver of secondary sexual characteristics, sexual behavior, and spermatogenesis (Borg, 1994). Alaskan populations of threespine stickleback, our study species, experience extreme seasonally-dependent changes in photoperiod. The early summer season in south-central Alaska is characterized by a long photophase (19 h), a correspondingly truncated scotophase (5 h), and is coincident with the peak of reproduction in this species (Bell and Foster, 1994). We targeted threespine stickleback as our platform of investigation because it is an established aquatic model for ecotoxicology (including perchlorate exposure) that is broadly distributed across the northern hemisphere and has abundant molecular and genetic resources available for studying in-depth mechanisms of toxicity.

The interaction of temporal (diel or reproductive season) variation in hormone concentration and perchlorate exposure is not well understood in threespine stickleback. The objectives of this study were to characterize the variation in whole-body TH and 11-KT contents within a day and across the reproductive season in captive adult stickleback exposed to natural changes in lighting, and to determine if exposure to perchlorate modifies patterns of hormone variation. Due to perchlorate's known effects on the HPT and HPG endocrine axes in stickleback, we hypothesized that perchlorate is an effective modulator of whole-body TH and 11-KT contents.

2. Methods

2.1. Fish collections and housing conditions

Wild anadromous threespine stickleback (hereafter, stickleback) were collected from Westchester Lagoon, Alaska (N 61.207815°, W 149.924987° and N 61.204378°, W 149.912140°) in May 2012. Fish were caught using 0.64 cm unbaited minnow traps and adults (~2 years old) were separated by sex based on nuptial coloration and housed separately in outdoor 1514 L static system pools at the University of Alaska Anchorage. Overhead tarps protected pools from rain, but still allowed natural daylight to enter the water column. Due to the protracted periods of civil twilight of this latitude in summer, complete darkness was never observed for the duration of the experiment. Water quality measurements (temperature (10 ± 3 °C), pH (6.5 ± 0.5), ammonia (~0)) were collected using a YSI multiprobe (Yellow Springs, OH) and API water testing kits (Mars Fishcare, Chalfont, PA). Daily fluctuations in water temperature within the outdoor experimental pools were relatively negligible and are representative of summer temperatures recorded at the field collection sites. Water chemistry was tested once per week for the duration of the experiment and was adjusted with water changes if deviations beyond the aforementioned ranges were observed. All animal protocols were approved by the UAA Institutional Animal Care and Use Committee (IRB reference # 159870-1). Field work was conducted under an Alaska Department of Fish & Game scientific collection permit (SF-2010-029).

2.2. Experimental design and exposures

Adult stickleback were chronically exposed to either 100 ppm sodium perchlorate ($NaClO_4$, Acros Organics, ⩾99% purity, Pittsburgh, PA) dissolved in dechlorinated and biologically conditioned tap water or to dechlorinated and biologically conditioned tap water alone (control). The exposure level of perchlorate (100 ppm) selected for this study is environmentally-relevant to some contaminated areas of the US. The fish were subsequently sampled over two experimental regimens: diel (0300, 0700, 1100, 1500, 1900, 2300 h on four independent days, Fig. 1) and across an entire reproductive season (22 May–3 July 2012, one sample each of seven weeks at the 1100 h time-point, Fig. 1). Perchlorate concentration was monitored weekly with an Acorn Ion 6 meter (Oakton Instruments, Vernon Hills, IL) with a perchlorate ISE electrode (Cole-Parmer, Vernon Hills, IL). Perchlorate concentrations measured in control tanks were below the minimum detection limit (0.7 ppm) of the electrode. Males and females were

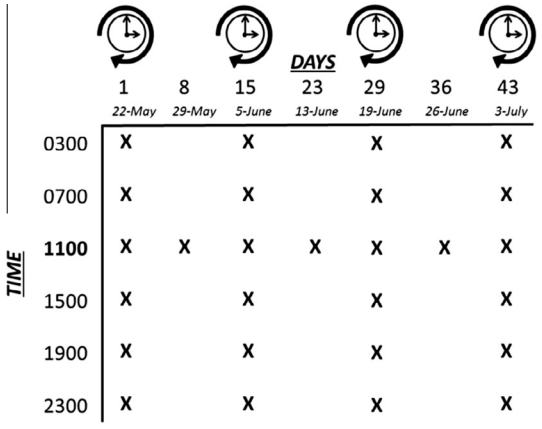

Fig. 1. Experimental sampling schedule with diel and reproductive season components. Clock icons indicate days with continuous sampling at 4 h intervals.

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