Environmental Pollution 236 (2018) 850–861

Contents lists available at ScienceDirect

Environmental Pollution

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

Adverse metabolic effects in fish exposed to contaminants of emerging concern in the field and laboratory☆,☆☆

James P. Meador [a, b, *], Andrew Yeh [b, 1], Evan P. Gallagher [b, 1]

[a] Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. East, Seattle, WA 98112, USA
[b] Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA 98105, USA

ARTICLE INFO

Article history:
Received 10 November 2017
Received in revised form
18 January 2018
Accepted 4 February 2018

Keywords:
Metabolic disruption
Emerging contaminants
Chinook salmon
Blood chemistry
Early indicators

ABSTRACT

Several metabolic parameters were assessed in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and staghorn sculpin (*Leptocottus armatus*) residing in two estuaries receiving wastewater treatment effluent and one reference estuary. We also conducted a laboratory study with fish dosed for 32 days with 16 of the most common contaminants of emerging concern (CECs) detected in feral fish. Several blood chemistry parameters and other indicators of health were measured in fish from the field and laboratory study that were used to assess potential metabolic disruption. The blood chemistry values observed in feral juvenile Chinook salmon were relatively consistent among fish collected from effluent-impacted sites and substantially different compared to reference site fish. These responses were more pronounced in Chinook salmon, which is supported by the disparity in accumulated CECs. The blood chemistry results for juvenile Chinook salmon collected at effluent-impacted sites exhibited a pattern generally consistent with starvation because of similarities to observations from studies of food-deprived fish; however, this response is not consistent with physical starvation but may be contaminant induced. The altered blood chemistry parameters are useful as an early indicator of metabolic stress, even though organismal characteristics (lipid content and condition factor) were not different among sites indicating an early response. Evidence of metabolic disruption was also observed in juvenile Chinook salmon that were exposed in the laboratory to a limited mixture of CECs; however, the plasma parameters were qualitatively different possibly due to exposure route, season, or the suite of CECs. Growth was impaired in the high-dose fish during the dosing phase and the low- and medium-dose fish assayed after 2 weeks of depuration. Overall, these results are consistent with metabolic disruption for fish exposed to CECs, which may result in early mortality or an impaired ability to compete for limited resources.

Published by Elsevier Ltd.

1. Introduction

Contaminants of emerging concern (CECs) are frequently associated with endocrine disruption and reproductive effects because this broad class of compounds includes very potent natural and synthetic hormones in addition to hormone mimics (Harding et al., 2016). In addition to reproductive toxicants, several of these compounds are potential metabolic disruptors (Casals-Casas and Desvergne, 2011) and can also cause adverse behavioral and immune system responses in organisms.

Wastewater treatment plants (WWTPs) are known conduits to receiving waters for a variety of pharmaceuticals, personal care products, industrial compounds, metals, and legacy compounds. Several of these chemicals have been shown to affect fish at very low concentrations (Fairchild et al., 1999; Daughton and Brooks, 2011; Schultz et al., 2012; Saaristo et al., 2017); however, few data exist on toxic responses for most of these poorly studied chemicals, especially as mixtures. To date, most studies conducted on aquatic

☆ This paper has been recommended for acceptance by Dr. Sarah Michelle Harmon.

☆☆ In this study we examined organismal and physiological parameters for feral fish exposed to contaminants of emerging concern in WWTP impacted estuaries. The plasma chemistry parameters were consistent with metabolic disruption for juvenile Chinook and appear to be useful as early indicators of adverse effects.

* Corresponding author. NOAA Fisheries, 2725 Montlake Blvd. East, Seattle, WA, USA.
E-mail addresses: james.meador@noaa.gov (J.P. Meador), ayeh3@uw.edu (A. Yeh), evang3@u.washington.edu (E.P. Gallagher).
[1] 4225 Roosevelt Way NE, Suite 100, Seattle, WA 98105–6099 USA.

https://doi.org/10.1016/j.envpol.2018.02.007
0269-7491/Published by Elsevier Ltd.

organisms have assessed reproductive effects or behavioral alterations and very few have focused on metabolic disruption. One study found reduced growth in male fathead minnow (*Pimephales promelas*) exposed to 40,000 ng/L metformin in addition to disruption of reproductive parameters (Niemuth and Klaper, 2015). Another study reported that alkylphenols (octylphenol, nonylphenol, and nonylphenol diethoxylate) inhibited growth in rainbow trout (*Oncorhynchus mykiss*) at relatively low concentrations in the range of ng/mL (Ashfield et al., 1998). Many of these compounds can affect multiple physiological pathways for a given exposure resulting in simultaneous impairment to reproductive, growth, or behavioral parameters. Additionally, these alterations may result in indirect effects such as abnormal behavior leading to reduced feeding and growth or increased predation (Painter et al., 2009).

For many endocrine disrupting compounds, there is a critical linkage between endocrine receptor agonism and activation of metabolic pathways, suggesting a commonality among metabolic abnormalities and classic endocrine disrupting responses (Chen et al., 2009). Certainly, many non-endocrine pathways may be impacted by CECs and other contaminants resulting is disruption of metabolic homeostasis. Indeed, these compounds may have fairly high specificity for their targets based on evolutionary conservation across vertebrates (Gunnarsson et al., 2008), suggesting conservation of function. Considering the range of compounds detected in WWTP effluent (Meador et al., 2016), a large number of these are potential metabolic disruptors in aquatic organisms and may act directly on metabolic or endocrine pathways, or indirectly via other receptors.

Our previous study reported a high percentage of analyzed CECs (61%, n = 150) in effluent, estuary water, or fish tissue collected from WWTP impacted estuarine sites (Meador et al., 2016). The current report presents an evaluation of the potential effects resulting from exposure to those compounds, which were selected as a representative group with little data available on occurrence in marine waters or toxicity for exposed fish. Our goal for this study was to assess several metabolic attributes in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and Pacific staghorn sculpin (*Leptocottus armatus*) exposed to WWTP effluent in the field and juvenile Chinook exposed in the laboratory to a model mixture of a select group of CECs quantified in whole-body feral fish inhabiting impacted sites. Our specific focus was on blood chemistry parameters as potentially useful metrics that could be utilized as early indicators of metabolic disruption.

2. Methods

2.1. Field study

Details regarding fish collections, physical-chemical parameters, and chemical analysis can be found in Meador et al. (2016) and Table S1. Briefly, fish were collected from three local Puget Sound, WA estuaries. Two of the sites (Sinclair Inlet and the Puyallup River estuary) receive effluent from WWTPs. A minimally contaminated site, the Nisqually River estuary, was selected for comparison and served as our reference site. Two fish species that commonly occur in Puget Sound estuaries were collected and sampled for blood plasma. One was a benthic species, Pacific staghorn sculpin, which is found widely in Puget Sound and U.S. west coast temperate waters. The other fish species was juvenile ocean-type Chinook salmon, which can reside for several weeks in nearshore estuaries where contaminants are often concentrated. We also collected several juvenile Chinook salmon from the Voights Creek Hatchery on the Puyallup River upstream of the estuary on 28 May 2014. As reported in Meador et al. (2016) only 7 analytes were detected in these fish, most at relatively low concentrations. All estuarine juvenile Chinook salmon were collected in the estuary within a 10 d period and were approximately the same age as most were released from upstream hatcheries approximately 3–5 weeks before capture (Table S1).

2.1.1. Field samples

Fish were collected under a Washington State Scientific Collection Permit 13–046 and ESA Section 10(a)(1)(A) permit 17798. All methods for obtaining, transporting, and tissue sampling were approved by the University of Washington Institutional Animal Care and Use Committee (protocol number 4096-01). Details of all sampling methods used in this study were reported in Meador et al. (2016).

Juvenile Chinook salmon and staghorn sculpin were obtained at each field site with a beach seine. Fish were kept alive after collection in the field and transported to the laboratory for processing in aerated site water that was maintained at 13 °C with ice packs. All samples for chemistry and plasma were taken approximately 3–6 h after capture. Fish were euthanized with tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA) for processing. Chemical analyses for CEC analytes were conducted on composite samples consisting of 3–12 whole-body salmon or 3–5 whole-body sculpin (Meador et al., 2016).

2.2. Laboratory study

The laboratory study was conducted at the University of Washington fish hatchery in Seattle, WA. Approximately 400 juvenile Chinook salmon were received from the Wallace Falls hatchery (Gold Bar, WA) on 9 Feb 2015. The average individual weighed approximately 45 g and water temperature at the hatchery was approximately 10 °C. These fish were yearling (1 + years) Chinook salmon that were a genetic cross between hatchery and wild fish (first generation) and were not treated with any chemicals at the hatchery. No mortality occurred due to handling or transit to our laboratory holding tanks. Fish (n = 20 per tank) were randomly distributed to 15 circular tanks each with a 500 L capacity. Lake Washington was the source of water to the tanks, which was supplied at approximately 1 L/m flow-through at 12 °C. Treatments were assigned at random to tanks and 3 or 4 tank replicates per CEC dose were tested. An additional tank of fish was not fed during the 32 d exposure period. Large windows allowed ambient light into the experimental area and tanks were partially covered with black plastic to give fish cover and shield them from artificial light.

The rationale for selection of the compounds comprising the CEC mixture was presented in Yeh et al. (2017) and in Table S2. Concentrated stock solutions for each CEC analyte were generated by dissolving the compounds in 50–100 mL of absolute ethanol. Calculated amounts of CEC stock solutions were added to three separate volumes of 4 L of 100% ethanol in order to generate the 0.3× (low), 1× (medium), and 10× (high) dose CEC mixtures, which were selected to mimic whole-body concentrations observed in our field-collected fish. BioClark's Fry 2.5 mm low-fat food pellets (BioOregon, Longview, WA) were dosed with the CEC mixtures by complete immersion with the ethanol mixture and taken to dryness under a fume hood. Previous studies have shown this to be an effective method for dosing fish pellets (Meador et al., 2005, 2006). The fish food used in the control diet was treated identically with the ethanol solvent minus the CEC mixture. Based on previous studies (Meador et al., 2005, 2006) fish were fed 2% body weight (bw)•day^{-1} spread over 2 feedings per day, 5 days per week, from days 0–32 for a total of 25 daily feedings (50 total). Dietary exposure for toxicity studies is a well-supported approach that is widely used. The most important aspect for toxicity characterization is the