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Biomarker response and hypothalamus-pituitary-interrenal axis functioning in Arctic charr from Bjørnøya (74°30′N), Norway, with high levels of organohalogenated compounds

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ABSTRACT

The populations of Arctic charr (*Salvelinus alpinus*) residing in Lake Ellasjøen at Bjørnøya Island in the Norwegian Arctic (74° 30′N, 19° 00′E) possess substantially higher levels of organohalogenated compounds (strongly dominated by polychlorinated biphenyls, PCBs) than conspecifics residing in other, proximate lakes on the island. In the present study we sampled large (< 400 g), immature charr from Lake Ellasjøen (high PCB levels) and Lake Laksvatn (reference lake, low PCB levels) by hook and line for an immediate blood sampling, and blood and tissue sampling after a 1 h confinement stressor. This was done in order to investigate possible effects of pollutants on an acute stress performance in a high-latitude fish species by comparing muscle PCB levels, hepatic cytochrome P4501A (CYP1A) biomarker activation and functioning of the hypothalamus-pituitary-interrenal (HPI) axis between these two populations of Arctic charr. As expected sum PCB muscle levels were 8-fold higher on a wet weigh basis, and 19-fold higher on a lipid weight basis, in charr from Ellasjøen than in charr from Laksvatn. This was accompanied by a 3.5-fold higher liver *cyp1a* mRNA abundance in the Ellasjøen charr compared to Laksvatn charr. Brain transcript levels encoding glucocorticoid receptor 1 and 2 (*GR2*) and corticotropin-releasing factor, and pituitary transcript levels encoding *GR2* and proopiomelanocortin A1 and A2 were higher in Ellasjøen charr than in Laksvatn charr, while interrenal transcript levels encoding melanocortin 2 receptor and steroidogenic acute regulatory protein were lower. There were no differences in plasma cortisol concentration between the two charr populations immediately after capture and one hour after confinement. The strong biomarker response to OHCs and altered mRNA abundances of key genes related to HPI axis functioning in the Ellasjøen charr suggest endocrine disruptive effects of OHCs in this charr population. Possible ecological implications are not known, but it cannot be excluded that a slower growth rate in Ellasjøen charr compared to Laksvatn charr due to an increased metabolic demand associated with the activation of xenobiotic defense and detoxification systems may have contributed to the lower body mass of Ellasjøen charr compared to Laksvatn charr.

1. Introduction

High concentrations of organohalogenated compounds (OHCs), including hexachlorobenzene (HCB), chlordanes (ΣCHLs), mirex, dichlorodiphenyltrichloroethanes (ΣDDTs) and polychlorinated biphenyls (ΣPCBs) have been measured in the sediment and biota from the remote Lake Ellasjøen located at Bjørnøya Island (74° 30′N, 19° 00′E) in the Norwegian Arctic (Evenset et al., 2004, 2005). Recently, PCB-levels (sum of 12 congeners) of up to 409 ng/g wet weight (ww) were recorded in muscle tissues of Arctic charr (*Salvelinus alpinus*) residing

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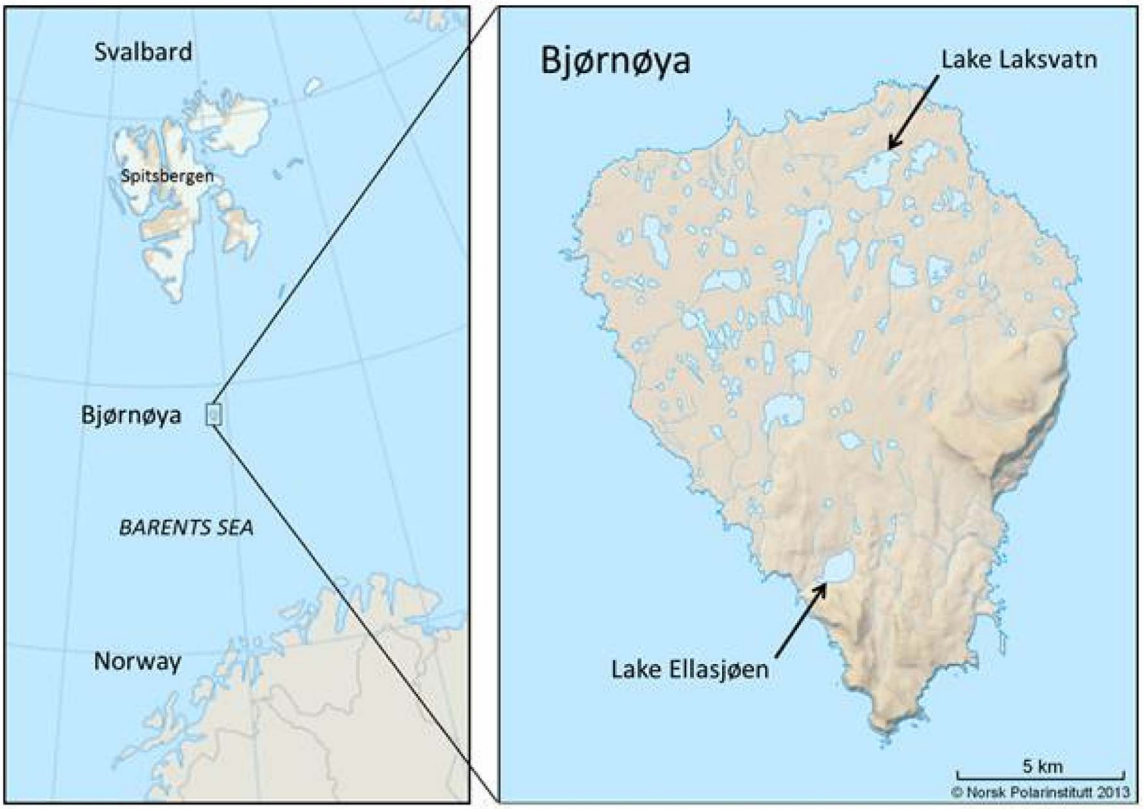

Fig. 1. Map of Bjørnøya (left) with Lake Ellasjøen and Lake Laksvatn (right).

in this lake (Bytingsvik et al., 2015). The high level of OHCs in Lake Ellasjøen is mainly due to the high presence of seabirds in this lake, and transport of contaminants from the marine environment in guano from these birds (Evenset et al., 2007b). Hence, the levels of the most persistent OHCs (DDTs and PCBs) are several times higher in Ellasjøen than in other lakes on Bjørnøya Island (Bytingsvik et al., 2015). For example, it was shown that the ww PCB concentration in the livers of charr from Ellasjøen was 15- to 25-fold higher than in livers from individuals residing in Lake Øyangen, located only 6 km north of Ellasjøen (Evenset et al., 2004; Wiseman et al., 2011). Likewise, the level of PCBs in muscle samples was 36-fold higher in fish from Ellasjøen than in fish from Lake Laksvatn, another lake on the island (Bytingsvik et al., 2015). These differences between lakes provide a natural environment for investigating whether persistent OHCs pose a threat to fish in the Arctic.

So far, only one study of potential biological responses to elevated OHC levels in Arctic charr from Ellasjøen has been carried out. In this study, it was shown that the Ellasjøen charr had 50-fold higher liver cytochrome P450 (CYP) 1A protein expression, and significantly higher brain heat shock protein 70 and liver glucocorticoid receptor (GR) protein expression than charr from Øyangen (Wiseman et al., 2011). This suggests that key components in the cellular and physiological stress response are affected by the high contaminant levels in Ellasjøen charr.

Fish respond to stressors by activating the hypothalamus-pituitary-interrenal (HPI) axis, ultimately leading to release of cortisol from interrenal cells in the head kidney (Wendelar Bonga, 1997). Activation of the HPI-axis includes production of hypothalamic corticotrophin releasing factor (CRF), which stimulates the production of proopiomelanocortin (POMC) in the anterior pituitary and release of its derived peptide, adrenocorticotropic hormone (ACTH). ACTH binds to the melanocortin 2 receptor (MC2R) in the steroidogenic cells of the head kidney and activates the steroid biosynthetic cascade leading to cortisol production and secretion (Mommsen et al., 1999; Vijayan et al., 2010). A key rate-limiting step in steroid biosynthesis involves the transport of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). Cortisol binds to GR in target tissues and activates transcriptional processes leading, among other actions, to the mobilization of energy substrates necessary for the animal to cope with the stressor (Mommsen et al., 1999; Vijayan et al., 2010).

In a series of laboratory contamination studies, an environmentally realistic PCB (Arochlor 1254) dose (1 mg/kg body ww) was shown to impact biological processes, including physiological and cellular stress responses in Arctic charr (Jørgensen et al., 2002b; Aluru et al., 2004). Also, DDTs (the second most abundant OHC in Ellasjøen charr; Bytingsvik et al., 2015) disrupted ACTH-stimulated interrenal steroidogenesis in rainbow trout (*Oncorhynchus mykiss*) in vitro (Lacroix and Hontela, 2003). These studies support the concept that the HPI-axis is a target for endocrine disruption by OHCs in teleosts and, thereby, may impair their ability to mount a stress response (Hontela and Vijayan, 2010). However, very little is known about the effect of environmental OHCs on the stress performance in wild fish residing in the Arctic ecosystem.

The Arctic charr from Bjørnøya Island is an ideal model for studying the integrity of the HPI-axis activity in response to OHCs impact given the close proximity of the contaminated and less contaminated lakes (Evenset et al., 2005; Bytingsvik et al., 2015). The goal of the present study was to compare muscle PCB levels, biomarker response (liver *cyp1a* mRNA abundance) and HPI-axis functioning between charr sampled in Ellasjøen (high OHC levels) and Laksvatn (reference lake, low OHC levels). This was carried out by monitoring the abundance of key transcripts involved in the functioning of the HPI-axis, as well as blood sampling for cortisol analysis immediately after hook-and-line capture (unstimulated basal levels) and following a one-hour capture/confinement stressor (stimulated release) in the same fish.