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Adaptation to salinity in Atlantic cod from different regions of the Baltic Sea

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ABSTRACT

Atlantic cod (*Gadus morhua*) occur in marine water of different salinities: from oceanic waters at salinity of 35 to Baltic Sea waters where the lowest level of salinity reaches 5–6. The stress response to different salinities in the eastern and western Baltic cod populations was examined. Two genes of Na^+, K^+ -ATPase 1a (*atp1a*) and heat shock protein 70 (*hsp70*) expression, plasma cortisol and osmolality were used as markers of osmotic stress to characterize the reaction profiles of two populations of *G. morhua* from the western and eastern parts of the Baltic Sea. Atlantic cod were sampled in November 2012 from western Kiel Bight (KIEL, salinity of 18) and eastern Gdańsk Bay (GDA, salinity of 8). Live fish were transported to the Marine Station of the University of Gdańsk in Hel and were settled in tanks (3500 L). Cod were kept at 10 °C in recirculated water, which simulated the natural salinities of the geographic source region of the fish. Results showed that in the reduced and elevated salinity water of the KIEL group, we observed no change in expression of *atp1a* and slightly increased expression of *hsp70*. In the GDA group, there were no significant changes of *hsp70* expression but the level of *atp1a* was significantly increased in both salinities. In both groups, concentration of cortisol increased after exposure to elevated salinity, while in fish exposed to reduced salinity, a significantly higher concentration of cortisol was observed after 72 h. The high expression of *atp1a* that observed in the eastern group (GDA) supports the thesis of a genetic background to the adaptation to variable salinity. This adaptation may protect this species against an osmotic stress caused by daily vertical migrations and long-distance migration to spawning areas. At the same life-time, salinity is a barrier maintaining the genetic and physiological separations between *G. morhua* stocks and affecting the structure of this fish subpopulation in the Baltic Sea.

1. Introduction

Atlantic cod (*Gadus morhua*) is a species widely distributed in the North Atlantic Ocean. This species occurs in marine water of varying salinity: from oceanic waters (salinity of 35) to the Baltic Sea waters of which the lowest level of salinity reaches 5–6 in the eastern part of this sea. Fish from the Kiel Bight represent cod living in the west Baltic Sea, at salinity over 15 while fish from Gdańsk Bay (GDA) live in the inner/east Baltic Sea where salinity is at its lowest. The Baltic Sea is an enclosed, non-tidal ecosystem with steep latitudinal and vertical salinity (Tomkiewicz et al., 1998). The source of high salinity is the inflow of oceanic waters from the North Sea through the Danish Straits. Additionally, the central Baltic Sea is permanently stratified with a halocline located about 30–90 m below the surface. The halocline is dynamic due to vertical mixing. Water is mixed by number of factors such as the surface wind stress and an internal wave mixing, which erodes the halocline. Finally, water is mixed in varying intensity due to seasonal changes of temperature/thermocline (Reissmann et al., 2009). Rapid changes of salinity during vertical migration of cod and during migration to spawning areas have been observed earlier by Neuenfeldt et al. (2007, 2009). Atlantic cod from the East Baltic Sea living in low salinity waters, periodically return for the spawning season to waters of salinity over 14 (Nissling and Westin, 1991; Westin and Nissling, 1991).

Studies of Baltic cod populations have demonstrated their distinctiveness from Atlantic populations (Nielsen et al., 2003; O'Leary et al., 2007; Kijewska et al., 2011). Geographic and genetic data support the hypothesis of two separate subpopulations living in the Baltic Sea (Antoszek et al., 2011; Kijewska et al., 2009, 2011; Poćwierz-Kotus et al., 2015; Berg et al., 2015). Nonetheless, present knowledge about physiological adaptations to different salinities is still incomplete for this species. The hypoosmotic environment of the Baltic Sea influences

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the physiology and ecology of this species. Changes in physiology and genetics have been seen as responsible for interactions between the organism and the specific environment of the Baltic Sea and leading to anatomical adaptations including the width of the chorion and larger diameter (Nissling et al., 1994). The maintenance of homeostasis in suboptimal ranges of salinity requires a number of adaptations (such as a mechanism for sodium and chloride uptake, changes in excretory patterns and in metabolic enzyme activities) among which ion regulation is crucial for good condition and fertility. However, the Atlantic cod still requires salinity higher than the average in the Baltic Sea for successful reproduction (Nissling and Westin, 1997).

Salinity fluctuations and exposure of fish species to different salinities (higher or lower) can affect the expression of genes involved in sodium and potassium ion regulation and physiological stress. Cutler et al. (1995) and Deane and Woo (2004) have shown that Na^+, K^+-ATPase 1a (*atp1a*) is fundamental for the osmoregulation and ion exchange and its expression increases during salinity changes in European eel (*Anguilla anguilla*) and sea bream (*Sparus sarba*). Heat shock protein 70 (*hsp70*) gene plays a major role in cell protection from the damaging effects of osmotic stress (Deane et al., 2002; Deane and Woo, 2004). In turn, cortisol is the major corticosteroid in teleost fish, secreted and released by interrenal cells of the head kidney during activation of the hypothalamo–pituitary–interrenal (HPI) axis (Wendelaar Bonga, 1997; Mommsen et al., 1999). This hormone dramatically rises during stress and seems to be a key mediator of stress-associated responses (Vijayan et al., 1997; Mommsen et al., 1999). Cortisol may regulate osmolality, metabolism and immune response in fish (Wendelaar Bonga, 1997; Mommsen et al., 1999; Vizzini et al., 2007) and modulates the *hsp70* and *hsp90* gene expression (Celi et al., 2012) and *atp1* expression (Dang et al., 2000). Moreover, Madsen et al. (1995) have demonstrated that cortisol-induced increase in gill Na^+, K^+-ATPase activity is partially due to the expression of the Na^+, K^+-ATPase 1a mRNA.

Larsen et al. (2012) analysed the expression of *hsp70* and *atp1a1* after short-term and long-term acclimatization to reciprocal salinities in *G. morhua* from the North Sea, Skagen (Danish Straits), and Baltic, Bornholm area. Significant differences were shown between these two groups, which can be considered as population-specific patterns of gene regulation depending on the origin of the population. However, the inner Baltic cod populations have not previously been studied in this respect. A profile of response to different salinities could be a useful parameter to characterize east and west Baltic cod populations due to the salinity changes in the Baltic Sea. It remains to be determined how far salinity can be considered as an environmental barrier responsible for maintaining the separation of the two Baltic populations of cod. In the present study, the expression of two genes: *atp1a* and *hsp70*, plasma cortisol and osmolality were used as markers of osmotic stress to characterize the reaction profiles of two populations of the Atlantic cod from the west and east of the Baltic Sea.

2. Materials and methods

2.1. Animals and experiment protocol

Atlantic cod were collected by fyke net and pelagic trawl in November 2012 (n = 131) from Kiel Bight (KIEL; n = 89) and Gdańsk Bay (GDA; n = 42). Live fish were transported to the Marine Station of the University of Gdańsk in Hel and were settled in tanks (2000 L). Fish were kept at 10 °C in recirculated water, which simulated the natural salinities of the geographic source of the cod [salinity of 18 (ctrl 18) and 8 (ctrl 8)]. During primary acclimatization period (over 14 days), fish were maintained at natural photoperiod and acclimated to laboratory conditions until they start feeding and displayed typical behaviour. Fish were fed once a day with fresh herrings during acclimatization and experimental periods. Cod from both geographical areas were randomly divided into 3 groups (control group, reduced salinity group and raised salinity group), transferred to separate tanks and acclimated again. Salinity was changed gradually (1/h) in order to minimize acute stress responses. Salinity value was measured every hour by conductometer (Elmetron, Zabrze, Poland). During the experiment, water temperature was about 10 °C in all tanks. The first change was the elevation of salinity to 10 (ctrl + 10) above the natural environment. Time was counted from the first hour after the salinity was modified, *e.g.* 72 h after the last change of the salinity (ctrl + 10/72 h). High salinity water was obtained by adding aquarium ocean salt (Aquarium Systems, Sarrebourg, France). The reduction in salinity in the KIEL group was from 18 to 8 (ctrl − 10). In the GDA group, salinity was reduced from 8 to 3. After 72 h, salinity was continuously elevated or decreased to extreme levels (salinities 3 and 33) except the GDA group, which was already exposed to extremely reduced salinity. Fish from all groups were caught with a landing net. Blood samples were collected by cardiac puncture during the first 1.5 min. Then, the fish were immediately sacrificed by spinal cord dissection. Each fish was measured (weight and total length) and samples for RNA (gills) and DNA analysis (pelvic fin) were collected using sterile instruments. Five fish from the KIEL group (salinity 18) were submitted to handling stress by continuous moving from tank to tank until they stopped resisting and become motionless. Thereafter, fish were sampled as described above. Another sample of six individuals from the Kiel Bight was kept 6 weeks in a reduced salinity of 7.5. Then, samples were also analysed for cortisol and gene expression as all other individuals following the protocol described above.

All experiments complied with EC Directive 2010/63/EU for animal experiments and with the guidelines of the Local Ethics Committee on Animal Experimentation (decision no. 60/2012).

2.2. Cortisol and osmolality

Blood samples were collected by cardiac puncture and then centrifuged at 3000 *g* in 4 °C for 10 min and plasma samples were stored at −70 °C prior to cortisol analysis. Plasma cortisol concentration was determined by immunoenzymatic assay (EIA) with preceding extraction procedure. Plasma samples were acetated with 3 M HCl to pH 1.5–3. Extraction of plasma samples (150 μL) was performed with methylene chloride according to the method recommended by the producer, with slight modifications. Then, the samples were frozen to allow the separation of layers. The methylene chloride layer was transported to a clean glass tube and evaporated under a gentle stream of nitrogen. This step was repeated three times. Dried extracts were stored at −20 °C for further analysis. The recovery of extraction was found in the range 89 to 110%. The assay was performed using Cayman's EIA kit No. 500360 (Ann Arbor, MI, USA). Extracts were dissolved in 2 mL of EIA buffer and 50 μL of the diluted samples were used for EIA analysis. The standard curve consisted of ten standards with the following concentrations: 20, 10, 4, 1.6, 0.64, 0.256, 0.102, 0.041, 0.0164 and 0.0066 ng mL^{-1}. The microplate was gently shaken for 15 min and then incubated overnight at 4 °C. After rinsing, the microplate was developed with Ellman's reagent at room temperature, in the dark, by shaking for 60 min. The plate was read at 412 nm using Sunrise Absorbance Reader (TECAN, Austria). All samples were assayed in duplicate. The detection limit of assay was 0.012 ng mL^{-1}. The intra-assay coefficient of variation was 0.9%. The inter-assay variation was not determined, because all samples were measured in the same plate. Plasma osmolality was measured immediately after sampling using a 5500 Vapor Pressure Osmometer (Wescor Inc., Logan, USA).

2.3. Gene expression

Gills were collected during sampling and immediately submerged in RNAlater®, according to the manufacturer's instruction (Qiagen, Hilden, Germany). Gills were stored at −80 °C prior to analysis. Before the extraction, tissues were defrosted on ice. Total RNA was extracted using the ISOLATE II RNA Mini Kit (Bioline, London, UK) and then was