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The immune and stress responses of Atlantic cod to long-term increases in water temperature

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Abstract Sea-caged cod are limited in their movements in the water column, and thus can be exposed to large seasonal (~0–20 °C) temperature fluctuations. To investigate the physiological response of Atlantic cod to summer-like increases in temperature, we exposed 10 °C acclimated juvenile cod to a graded thermal challenge (1 °C increase every 5 days) and measured: (1) plasma cortisol and glucose levels; (2) the respiratory burst activity of blood leukocytes; and (3) the expression of specific immune-related genes [MHC Class I, Interleukin-1β (IL-1β), β_2-microglobulin (β_2-M), Immunoglobulin M (IgM)-light (L) and -heavy (H) chains] in the blood using quantitative reverse transcription–polymerase chain reaction (QRT–PCR). The experiment was stopped at 19.1 °C, with 26.7% of the fish surviving to this point. Plasma glucose levels increased slightly at 16 and 18 °C (by 1.39- and 1.74-fold, respectively), in contrast, cortisol levels were elevated significantly (by 2.9-fold) at 16 °C but returned to control levels thereafter. The effect of increasing temperature on the expression of immune related genes in blood cells (leukocytes) was variable and depended on the gene of interest. The expression of IgM-H remained stable for the duration of the experiment. In contrast, IL-1β expression was increased significantly (by ~25-fold) at 19 °C as compared to time-matched control fish, and changes in the expression of β_2-M, MHC Class I and IgM-L followed a pattern similar to that seen for cortisol: increasing at 16 °C (by 4.2-, 5.3- and 17-fold, respectively), but returning to pre-stress levels by 19 °C. Interestingly, increasing temperatures had no effect on respiratory burst activity. This study is the first to examine the effects of a chronic regimen of increasing temperature on the stress physiology and immunology of a marine teleost, and suggests that immune function is influenced by complex interactions between thermal effects and temperature-induced stress (elevated circulating cortisol levels).

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Introduction

Atlantic cod (*Gadus morhua* L.) aquaculture is a growing industry in Canada, the United States and Europe [1—3], and there are significant efforts underway in several countries to identify cod with economically beneficial production traits and to initiate selective breeding programs (e.g. Atlantic Cod Genomics and Broodstock Development Project; www.codgene.ca). When cultured in sea-cages, Atlantic cod are limited in their movement in the water column, and thus can be exposed to water temperatures that exceed their thermal preferenda [3]. Given that temperature has a considerable influence on fish biochemistry, physiology and behavior [4], and elevated temperatures can have a negative influence on fish health and lead to decreased growth and increased mortality [5], it is important to understand the effects of elevated water temperatures on cod biology so that: (1) appropriate cage-sites can be selected, and (2) breeding programs can identify genotypes/phenotypes that are best suited for cage-site locations where high summer temperatures are a concern.

Over the past 4 decades, there has been a considerable amount of work on cod thermal biology. However most of this work has focused on determining preferred temperatures [6—8], optimal temperatures for growth and feed conversion [9—11], or the effects of temperature on metabolism [12—16]. Interestingly, very few studies have focused on the effects of temperature on the immune system of Atlantic cod, and those that did failed to address the potential impact of high temperatures (i.e. >15 °C). For example, Magnadottir et al. [17] only exposed fish to constant temperatures of 14 °C or below, and compared immune parameters in wild cod captured at 0—4 °C vs. 6—10 °C [18].

Studies on cod immune function are clearly needed, as while it is generally accepted that high temperatures enhance the specific immune response of fishes [19,20], measurements of circulating IgM in the Nile tilapia (*Oreochromis niloticus*) [5] suggest that some species may have an optimal thermal range for immune function. Further, the effect that elevated water temperature has on the fish's innate immune system is quite variable. For example, while respiratory burst was reported to be more intense at higher acclimation temperatures when rainbow trout (*Oncorhynchus mykiss*) were reared between 5 and 20 °C [21], Le Morvan et al. [20] reported that high temperatures resulted in decreased respiratory burst activity in common carp (*Cyprinus carpio*), and Ndong et al. [22] found that respiratory burst increased but lysozyme activity decreased when Mozambique tilapia (*Oreochromis mossambicus*) were acclimated to temperatures between 19 and 35 °C.

In addition to the paucity of published studies pertaining to the impact of temperature on cod immune function, the only data on cortisol levels in cod during exposure to high temperatures (15 °C or greater) have been generated using acute experimental protocols [23,24]. Information on the effects of chronic high temperature on this hormone are potentially critical to managing cod aquaculture operations because cortisol is the predominant corticosteroid in teleost fish [25], and exerts a modulatory effect on a diversity of fish immune parameters. For example: (1) *in vitro* studies on winter flounder (*Pseudopleuronectes americanus*) indicate that long-term cortisol administration reduces the primary immune response of lymphocytes [26]; (2) gilthead seabream (*Sparus aurata* L.) head-kidney leukocytes exposed to high doses of cortisol have decreased respiratory burst activity [27]; and (3) intraperitoneally injected cortisol increases circulating IgM levels in gilthead seabream [28] and can suppress head kidney leukocyte interleukin-1β expression in common carp and rainbow trout [29—31].

Given the lack of information on the responses of Atlantic cod immunology and stress physiology to chronically high (>15 °C) temperatures, we examined the impact of a chronic regimen of slowly increasing water temperature (which mimicked changes in seawater temperatures previously recorded at Newfoundland sea-cage sites) on this species' stress response and various immune parameters. The cod's stress response to chronically elevated temperatures was assessed by measuring plasma cortisol and glucose levels. Effects on components of the innate and adaptive immune system were examined by measuring blood leukocyte respiratory burst (RB) activity, as well as the expression of major histocompatibility complex (MHC) Class I, Interleukin-1β (IL-1β), β_2-microglobulin (β_2-M), and immunoglobulin M-light (IgM-L) and -heavy (IgM-H) chains using quantitative, real-time reverse transcription—polymerase chain reaction (QRT—PCR).

Materials and methods

These studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (Protocol # 05-07-KG).

Animals

Fish for these experiments were obtained from the Aquaculture Research and Development Facility (ARDF) of the Ocean Sciences Centre, Memorial University of Newfoundland, and were from a single spawning of Cod Genomics Project broodstock. These fish were selected for study based on their excellent growth and low rates of mortality, and were reared (at 10—11 °C) in the same production tank following standard rearing protocols in place at the ARDF. Interestingly, despite their high levels of performance in culture, some of the fish in this population were later identified as being carriers of nodavirus (by PCR and SSN-1 cell culture). However, we are confident that their carrier state had no influence on their critical thermal maximum (CTM), temperature-related physiology or immune function. These fish never showed any clinical signs of the disease (erratic swimming behavior, floating belly up due to over-inflation of the swim bladder, lethargy, change of pigmentation etc. [32,33]), and it has recently been shown that nodavirus carrier status does not influence the constitutive expression of immune-relevant genes, or their expression following polyriboinosinic polyribocytidylic acid (pIC) injection in cod (as assessed by QRT—PCR analysis) [34].

Experiments were performed using juvenile Atlantic cod of ~40 g initial average wet mass and six 250-L tanks. The experimental tanks were randomly assigned to 1 of 2 treatments: (1) control, where the water temperature remained