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Russian sturgeon cultured in a subtropical climate shows weaken innate defences and a chronic stress response

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ABSTRACT

Russian sturgeon (*Acipenser gueldenstaedtii)* has been successfully farmed in Uruguay for the past ten years. However, during the Uruguayan summer fish endure high water temperatures and increased bacterial infections that threaten aquaculture. Our understanding of sturgeon's immune system and its interplay with environmental factors like temperature is almost unknown. This study analysed the way in which seasonal variations affect enzymatic blood components of Russian sturgeon's innate defences, including the serum alternative complement pathway (ACP), ceruloplasmin (Cp) and lysozyme activities. Results showed that summertime conditions in the farm altered these defences in different ways, inducing a significant decrease in ACP and Cp, and an increase in lysozyme. In addition, serum levels of total protein and cortisol decreased in summer, suggesting a chronic stress response was induced in parallel. Subsequently, we analysed whether the increase in water river temperature during summer could account for the observed results. To that end, we acclimated juvenile sturgeons to mild (18 °C) or warm (24 °C) temperatures for 37 days. Like in summer, sturgeons exposed to 24 °C showed lower levels of serum ACP, Cp and total proteins, together with a progressive decrease in body weight and increased fish mortality. Administration of an immunostimulant containing Se and Zn slightly reverted the temperature-induced effects on sturgeon's defences. Altogether, our study provides novel data on various physiological parameters of the Russian sturgeon and highlights the impact warm temperature has on stress and innate immunity in this chondrostean fish.

1. Introduction

Sturgeon is the common name of fish species belonging to the *Acipenseridae* family. In this family, the genus *Acipenser* has attracted attention because of its ecological and economical relevance. Sturgeons are distributed in cold and temperate ecosystems all over the northern hemisphere, which is likely a result that their maturation, reproduction, spawning and egg development generally occur at temperatures below 20 °C [1,2]. Unfortunately, habitat alterations (i.e. due to dams or pollution) and overfishing for meat and caviar have led to drastic declines in their natural populations, causing sturgeon species to be classified as endangered animals. For this reason, sturgeon aquaculture has gained importance over the past years. Among other species, *Acipenser gueldenstaedtii* (Russian

Abbreviations: ACP, alternative complement pathway; ACH50, ACP haemolytic activity; Cp, serum ceruloplasmin; HEL, hen egg lysozyme; PPD, *p*-phenylenediamine; RaRBC, rabbit red blood cells; SGR, specific growth rate; VBS, veronal-buffered saline.

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sturgeon) is one of the most cultured sturgeon species worldwide, being reared in about 16 countries [3]. In Uruguay, one of the major caviar-producers nowadays, Russian sturgeon was successfully introduced. Despite this success, during summer farmed sturgeons face increased bacterial infections (caused mainly by *Aeromonas hydrophila* and other opportunistic bacteria), which correlates with prolonged exposure to warm water temperatures (24 °C in average). This observation raises the question as to whether summertime temperatures can alter farmed sturgeons defences making them more susceptible to infections.

Systematic studies to determine the optimal water temperature for Russian sturgeon aquaculture are not available in the literature. However, studies that analyse the effect of temperature on fish growth, development and/or metabolism in other *Acipenser* indicate that the optimal temperature may be close to 20 °C, although for each sturgeon species it varies according to several factors like age, size and feeding rate, among others [4–8]. It has been well demonstrated that water temperature has profound implications on teleost fish physiology, including the immune response [9–11], but little is known about temperature effects on sturgeon's immunity. The immunological capacity of juvenile shortnose sturgeons (*A. brevirostrum*) seems to be strongly modulated by temperature regardless of fish size, showing higher immune cell production in relevant myeloid tissues at 20 °C than at 11 °C [12]. This agrees with observations made in teleosts, which show that high water temperatures (within the physiological range of the fish species) lead to stronger specific immune responses [13]. However, temperature effects on innate defences seem to be quite variable, since the increment in temperature can cause both down- and up-regulation of innate components [14–16].

The increment in fish mortality observed during the Uruguayan summer represents a severe economic burden for sturgeon farms. Therefore, the development of strategies to improve sturgeon defences, for example by means of immunostimulants, is of great interest. In teleosts, components derived from algae, plants, bacteria or fungi have been evaluated as immunostimulants [17]. Among them, yeast-derived components, mainly beta-glucans and mannan oligosaccharides, are one of the most promising immunostimulants used in aquaculture [18,19]. In particular, beneficial effects of a yeast cell wall extract derived from *Saccharomyces cerevisiae* named Actigen® (Alltech) have been observed in catfish fingerlings (*Ictalurus punctatus*). In this fish, Actigen® supplementation induced higher levels of natural immune components, like mucins and lysozyme in mucus, and promoted the resolution of inflammation induced by bacteria infection, favouring tissue repair [20]. In addition, selenium (Se) and zinc (Zn) have also been utilised as diet supplements in aquaculture since they are essential minerals that improve fish physiology, favouring stress coping mechanisms and immune response to pathogens [21]. Likewise, beneficial effects have been observed using Sel-Plex® (Alltech) in rainbow trout (*Oncorhynchus mykiss*) [22], yellowtail kingfish (*Seriola lalandi*) [23] and even in beluga sturgeon (*Huso huso*) [24]. There are no studies addressing the effect of Zn supplementation on sturgeon health, although suboptimal levels of this micronutrient has been associated with poor growth and/or high mortality in various teleosts [25–27].

Given this background, we first studied the effect of seasonal variations on innate defences of Russian sturgeons reared in a Uruguayan aquaculture farm. Since species-specific reagents for immunological studies are not available for sturgeons, we focused our analysis on some blood components that can be determined using enzymatic assays: the alternative complement pathway (ACP), the ceruloplasmin (Cp) and the lysozyme. In parallel, we evaluated the occurrence of a chronic stress response since farming conditions are usually stressful for fish, weakening their defences and threatening fish welfare [28]. To that end, we used serum cortisol and total proteins as biomarkers [28–30]. Our results show that Uruguayan summer conditions are stressful for Russian sturgeon, altering components of their natural defences. Furthermore, controlled laboratory experiments revealed that a long-term exposure to 24 °C constitutes an important environmental factor involved in these effects. Supplementation with immunostimulants (Actigen®) plus Se (Sel-Plex®) and Zn (Bioplex®) slightly reverted the temperature-induced effects on sturgeon's defences. Overall, our studies provide novel data on the innate defences of a member of the *Acipenseridae* family, whose mechanisms governing the immune response are still almost unknown.

2. Materials and methods

2.1. Fish maintenance in farming conditions

Acipenser gueldenstaedtii (Russian sturgeon) were grown and maintained in the Black River Caviar farm in Baygorria (Esturiones del Río Negro S.A., Durazno, Uruguay). This farm uses freshwater from the Río Negro River channelled via an open flow-through system of raceways rearing units with a 35 min exchange water rate. The mean annual temperature registered during 2014 in the raceways were 18.8 ± 4.8 °C. During this study, total oxygen saturation was regularly measured, varying between 80 and 100%. Fish were typically grown at a maximal stocking density of 30 kg/m^3 and fed daily with a standard pelleted diet at a feeding rate according to fish age (between 0.5 and 2% of body weight, for adult and juvenile fish, respectively).

2.2. Fish diets

All pelleted sturgeon diets were formulated and prepared by Esturiones del Río Negro S. A. (Uruguay). The composition of the standard diet was 47% protein, 12.5% lipids and 3% fibre (dry matter basis). The supplemented diet was prepared by adding 0.2% of Alltech's dietary additive Biopack, (consisting of 50% of Bioplex®, 42.5% of Actigen® and 7.5% of Sel-Plex®) to the standard diet.

2.3. Blood collection from sturgeons

Fish were fasted for 24 h before bleeding. Blood collection was undertaken according to the Ethic Committee of the Honorary Commission of Animal Experimentation (CHEA, University of Uruguay) to minimize handling stress. Blood samples (2.0 ml) were collected by caudal puncture in less than 1 min and transferred into tubes to allow clotting at 4 °C. The contracted blood clot was separated from serum by centrifuging at 2800×g for 20 min at 4 °C. Fish serum was aliquoted and stored at −80 °C until use. Serum samples were centrifuged at 10000×g for 20 min prior to use in all assays described below.

2.4. Trial for analysing effects caused by seasonal environmental changes

Blood samples were collected from 4- and 9-year-old female sturgeons (n = 10 each one) maintained at farming conditions in the raceways during 2014 summer (February) and winter (July) seasons. The mean summer and winter water temperatures recorded in the raceways were 24.3 ± 1.3 °C and 12.9 ± 1.1 °C, respectively. The serological parameters associated with innate immune and stress responses were analysed as described below.