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The effects of temperature and body size on immunological development and responsiveness in juvenile shortnose sturgeon (*Acipenser brevirostrum*)

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ABSTRACT

Sturgeon are an important evolutionary taxa of which little is known regarding their responses to environmental factors. Water temperature strongly influences growth in fish; however, its effect on sturgeon immune responses is unknown. The objective of this study was to assess how 2 different temperatures affect immune responses in shortnose sturgeon (*Acipenser brevirostrum*) relevant immune organs such as the meningeal myeloid tissue, spleen, thymus and skin. These responses were studied in 2 different sizes of same age juvenile sturgeon kept at either 11 °C or 20 °C (4 treatment groups), before and after exposure to an ectoparasitic copepod (*Dichelesthium oblongum*). Based on a differential cell count, temperature was found to strongly influence immune cell production in the meningeal myeloid tissue, regardless of the fish sizes considered. Morphometric analysis of splenic white pulp showed a transient response to temperature. There were no differences between the groups in the morphometric analysis of thymus size. Splenic IRF-1 and IRF-2 had similar expression profiles, significantly higher in fish kept at 20 °C for the first 6 weeks of the study but not by 14 weeks. In the skin, IRF-1 was significantly higher in the fish kept at 11 °C over the first 6 weeks of the study. IRF-2 had a similar profile but there were no differences between the groups by the end of the trial. In conclusion, higher water temperatures (up to 20 °C) may have beneficial effects in maximizing growth and improving immunological capacity, regardless of the fish sizes considered in this study.

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1. Introduction

Sturgeon are harvested for their meat and eggs (sold worldwide as caviar) and sturgeon aquaculture has increased considerably in the last two decades. These phylogenetically primitive fish are among the most economically important [1] and are considered to be a transition between major taxa, thus useful in the understanding of vertebrate evolution. Little is known about immunological development and response to environmental factors in sturgeon. A better understanding of these interactions is important to optimize aquaculture rearing conditions and potentially aid wildlife conservation programs.

Life history and migration patterns expose sturgeon to significant temperature fluctuations [2], with temperature tolerance increasing with age and size. Temperature influences many of the behaviors and activities of sturgeon including spawning, egg development and seasonal movements [3]. Water temperature is believed to be the single most important factor in development and is known to influence all physiological functions in fish [4]. Many studies have focused on how environmental temperatures affect the immune system in teleosts. An overall decreased immune response at lower compared to higher water temperatures has been

Abbreviations: IRF-1, interferon regulatory factor 1; IRF-2, interferon regulatory factor 2; MMP-9, matrix metalloproteinase 9; TRI Reagent, Trizol Reagent; IFN, interferon.

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reported for different fish species [5] and fish reared at lower temperatures rely more heavily on innate immunity [6,7]. Both cellular and humoral specific immune responses are temperature dependent in fish [8,9]. These defense mechanisms are shown to be suppressed by temperatures averaging 10 °C below the host optimal growth temperature [10]. According to a study done in Atlantic cod (*Gadus morhua*), a temperature increase appeared to accelerate the spleen immune transcriptome in response to a viral mimic antigen [11], and to a lesser degree a response to a bacterial antigen. This supports the possibility that the impacts of high temperatures on the cod's immune response may be pathogen dependent [12]. No such studies have been done for shortnose sturgeon (*Acipenser brevirostrum*), but data suggests they prefer and perform optimally at 25 °C and lower water temperature conditions [13]. Therefore it would be relevant to understand how they respond to different pathogens under different water temperatures.

Size also influences functional immune responses in fish. Research on salmonids supports a stronger and more developed immunological response in larger fish within a group of animals of the same age. Such study assessed the onset of fish immunity (survival rate) after vaccination against bacterins [14]. Although studies on juvenile shortnose sturgeon show rapid growth of 14–30 cm during the first year at river temperatures ranging from 9 °C to 15 °C [2], immunological development and competence over this time is unknown. Culture of white sturgeon, *Acipenser transmontanus* [15–17] and other sturgeon species [18] has been hampered by viral infections of juvenile fish. Despite evidence of strong antibody responses against viruses in white sturgeon [19], a lack of basic understanding of innate and antiviral responses in these fish prevents effective minimization of the impact such pathogens have on sturgeon aquaculture and conservation efforts. Several studies [16,18] have described stocking density and husbandry practices in multiple sturgeon species as a risk factor for mortality and viral skin infections, but it is not known how environmental conditions affect leukocyte populations, antiviral mechanisms and skin healing in sturgeon.

The objective of this study was to assess how temperature influences the development and responsiveness of immune organs in shortnose sturgeon, focusing on the meningeal myeloid tissue, spleen, thymus and skin. In order to characterize these organs, both morphological and immune gene expression analysis were performed in 4 groups of 1-year old fish divided according to size and temperature (smaller and larger fish, kept at either 11 °C or 20 °C). As a measure of immunocompetence, the fish were exposed to a common sturgeon ectoparasitic copepod (*Dichelesthium oblongum*). Given that temperature influences immune responses in fish [4], our hypothesis was that the fish kept at 11 °C should rely more heavily on innate immunity (with a higher production of innate immune cells) when compared to the corresponding fish kept at 20 °C, regardless of size. Since a stronger immunological response has been observed in larger fish within a group of animals of the same age, it was also hypothesized that the larger animals would have a more responsive immune system when compared to the smaller fish, at a given temperature.

2. Materials and methods

2.1. Fish husbandry and parasite exposure

All experimental protocols followed the guidelines given in 2005 by the Canadian Council on Animal Care (http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf) and were approved by the UPEI Animal Care Committee.

2.1.1. Fish

In April of 2012, 240 five to twenty-gram 7 month-old shortnose sturgeon were transported from the Acadian Sturgeon and Caviar Inc. (Carter's Point, New Brunswick, Canada) to the Aquatic Animal Facility (AAF) of the Atlantic Veterinary College (AVC), UPEI. After a 2-week acclimation period at 11 °C, the fish were divided in 2 groups according to size and distributed among eight 150 L fresh water flow-through circular tanks, with 30 fish per tank and 12 h light/dark circadian cycles. The temperature in four of the tanks was increased to 20 °C at a rate of 2 °C/day [20] and the four other tanks were kept at 10.8 ± 0.07 °C (mean ± SD, measured weekly throughout the trial). After the fish were acclimated to the tanks at either of the temperatures, two replicate tanks were assigned per treatment (smaller or larger sturgeon, at each of the two temperatures). The fish were fed a regular sturgeon diet twice daily (2 mm pellet Corey diet, 1% body weight/day during the first 10 weeks of the trial and then 2.5% for the last 4 weeks of the trial). Water quality parameters such as concentrations of ammonia (0.04 ± 0.02 ppm), nitrites (0.006 ± 0.005 ppm), nitrates (1.58 ± 0.81 ppm) and pH (7.6 ± 0.12) were checked regularly (mean ± SD). Total gas saturation (<100%) and dissolved oxygen (g/L) levels were also measured regularly (11.08 ± 0.21 g/L and 9.03 ± 0.06 g/L at 11 °C and 20 °C respectively, mean ± SD). The selected temperatures were chosen based on the temperature at which the sturgeon were raised at the hatchery (11 °C) and on the fact that water temperatures exceeding 20 °C are contraindicated for propagation of sturgeon fry due to problems associated with balancing optimum growth rates [21]. Ten weeks after the beginning of the temperature trial, two tanks from each temperature group were exposed to adult *D. oblongum* as described in the next section. One week later the fish were checked for attached parasites, re-exposed to *D. oblongum* copepodids and then re-checked 3 weeks later.

2.1.2. Parasites

D. oblongum adults were randomly collected from wild sturgeon in the Saint John River, NB, and kept at the AVC in fresh water for 24 h until placed in the experimental tanks (7 adult female parasites per tank). Flow was kept off for up to 5 min in each tank during exposure until all female *D. oblongum* had been taken up by the fish. Egg strings from adult female parasites were also collected and hatched in a seawater hatchery system, with 33–36 parts per thousand of seawater salt concentration, at 11±1 °C (mean ± SD) [22]. The salinity tolerance of this parasite is unknown, but while adult females survive on hosts for over 1 month in fresh water, development of copepodid stages in the laboratory was inhibited below 20 parts per thousand in previous experiments (Fast, personal observation). Therefore, egg strings were maintained in saltwater for 1 week until development into copepodid stages was achieved. They were then transferred to fresh water for 2 days to ensure their survival at a low salinity prior to being introduced in the fish tanks (50 copepodids per tank). In summary, 7 female adult parasites and 50 copepodids were introduced in each of the "parasite-exposed" tanks.

2.2. Sample collection

The fish were kept off feed 24 h prior to each sampling day to allow for their guts to be emptied before sampling. This reduces stress and improves water quality at the time of sampling and minimizes contamination of tissues by gut content upon sample collection. After the initial 2-week acclimation period, two fish per tank were sampled for reference and euthanized with 0.2 g/L of tricaine methanesulfonate (TMS), followed by cervical separation. The spleen and skin samples (dorsocaudal to the pectoral fin and

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