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Size dependent early salinity tolerance in two sizes of juvenile white sturgeon, *Acipenser transmontanus*

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ABSTRACT

The objective of this study was to investigate the influence of size on salinity tolerance in 1 year old juvenile white sturgeon. Two sizes of sturgeon (10 and 30 g) from the same spawning event (thus reducing confounding effects of genetic make-up and size) and reared in the same environment were exposed to a salinity of 0, 8, 16, 24, or 32 ppt for up to 120 h. Both 10 and 30 g fish exhibited >93% mortality within 24 h after transfer to 24 or 32 ppt, regardless of whether they were transferred directly from freshwater (FW) or following a 48 h pre-treatment period at 16 ppt. Direct transfer from FW to 16 ppt was associated with 25 to 30% mortality, indicating that these fish have some ability to tolerate large changes in salinity for up to 5 days at this stage. Following exposure to 8 and 16 ppt, an elevation in plasma osmolarity, $[Na^+]$, and $[Cl^-]$ was observed between 24 and 72 h in both 10 and 30 g sturgeon, but plasma ions and osmolarity in surviving fish at 120 h were not significantly different between groups held at 0, 8, and 16 ppt. Despite being unprepared for either direct or stepwise transfer to salinities of 24 ppt or greater, size confers some ionoregulatory advantage, as mortality occurred more slowly and the degree of ionoregulatory perturbation was less in 30 g than 10 g fish over the course of the exposures. It is not known whether the apparent advantage of size is related to a size-dependent development of ionoregulatory capacity or due to social status which can also influence ionoregulatory capacity, but age and genetic differences did not likely contribute to this size effect.

1. Introduction

Categorizing sturgeon species by patterns of anadromy has been based on actual distributions of sturgeon as collected from the wild (e.g., Auer, 1996; Boreman, 1997; Bain, 1997; Krayushkina, 1998). However, the absence of collection of small sturgeon in marine or estuarine environments does not preclude the possibility that individuals are exploiting these resource-rich areas (Bain, 1997). Two problems are associated with attempting to classify endemic populations by pattern of anadromy. The first is that many populations have been prevented from accessing the ocean due to dam construction. This limitation imposes a potadromous life cycle in populations that may historically have exhibited other patterns. The second problem is that smaller juvenile sturgeons are very difficult to catch (Wilson and McKinley, 2004). While this difficulty is obvious from a cursory examination of the distribution literature on many sturgeon species (e.g., Kynard, 1997), the reasons for this are not as clear. However, they may be related to the phototropic response of young sturgeon (Cech and Doroshov, 2004), or the tendency of sturgeon to hide in gravel or under rocks (e.g., Peake, 1999). On the other hand, it is possible that sampling has either not occurred where these animals are, or failed to target sturgeon of these small sizes.

White sturgeon (*Acipenser transmontanus* Richardson, 1836) has been classified as a semi-anadromous species found along the Pacific coast of North America in the watersheds of the Fraser, Columbia, Sacramento, and San Joaquin rivers (Cech and Doroshov, 2004). Despite this classification, some land locked populations exist in freshwater reservoirs and tributaries of the Columbia River (Doroshov et al., 1997). Lower Columbia and Sacramento populations are thought to spend their adult life in the sea (salinity between 22 and 33‰), but migrate to fresh water during spawning periods (Bemis and Kynard, 1997; Krayushkina, 1998; Wilson and McKinley, 2004), in a pattern qualitatively similar to some salmonid species, albeit over a much longer period, as females may not return to spawn until 15–25 years old. Although sturgeons are chondrosteans, they are thought to exhibit most key teleost features of osmoregulation (Potts and Rudy, 1972), with similar osmoregulatory mechanisms (Gershanovich et al., 1991; Cech, 2000; Wright, 2007) for adapting to saline environments. As with many other species of sturgeon, little is known about early life history of this species: for example, it is not clear at what developmental stage white sturgeon enter estuaries. However, it appears that juvenile white sturgeon may remain in fresh water for

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several months or years (Doroshov, 1985; McEnroe and Cech, 1985). Resolving questions about the physiological preparation and responses of the osmoregulatory organs (e.g., gills) to a saline environment is a crucial step in consideration of this sturgeon species for aquaculture rearing in salt water (Kynard, 1997; Jarvis et al., 2001).

The objective of this study was to investigate the effect of size on salinity tolerance and the sub-lethal physiological responses to salinity in juvenile white sturgeon. Two size groups (10 g or 30 g) representing the upper and lower size range of healthy, cold water reared, one year old juvenile white sturgeon, were selected from the same spawning event, thus minimizing differences in genetic make-up and age. The 10 and 30 g sturgeon were exposed to one of five salinities (0, 8, 16, 24, and 32 ppt) for up to 120 h. Salinity tolerance was assessed by monitoring mortality, and the sub-lethal responses to salinity were assessed by measuring plasma ions ($[Na^+]$, $[Cl^-]$), osmolarity, and hematology, following exposure to the respective salinities.

2. Materials and methods

2.1. Experimental fish

All experiments were performed in August 2005, at Vancouver Island University (VIU, formerly Malaspina University College) in Nanaimo, B.C. Canada. Experimental fish were reared at MUC from eggs procured from locally-held white sturgeon, *A. transmontanus* (Fraser River origin) brood stock. Juvenile fish (14 month old, spawned from eggs of a single female and milt from two males in June of 2004) of two size classes; (11 ± 1.9 g and 30 ± 4.9 g, referred to as 10 and 30 g fish throughout) were randomly removed from different holding tanks and placed into a re-circulating system (Po_2 ~ 135 Torr, Pco_2 < 1 Torr), and left for 24 h to acclimate. Each size class of fish (10 or 30 g) was divided into five groups of twenty fish and exposed acutely to one of 0, 8, 16, 24 and 32 ppt salinity, prepared with dechlorinated fresh water and filtered (32 μm) marine water. It is important to note that 0 ppt was the salinity that fish were previously held at, and therefore represents physiological changes due solely to the transfer protocol. A further two groups were exposed to 16 ppt salinity for 48 h, and then to one of 24 or 32 ppt. All groups were subjected to identical water changes designed to disturb fish as little as possible. Sturgeons have been observed to exhibit little stress response to much more severe handling treatment (Barton et al., 2000). For each treatment, 30 g fish were held in 70 l plastic chambers while 10 g fish were held in a 10 l insert in the same chamber. The 70 l chambers were immersed in flow through troughs to keep the temperature relatively constant (±1 °C). Tanks were aerated *via* an air lift system, and water changes occurred daily. Water temperature was 16–17 °C with 7.7 to 8.5 mg/l oxygen corresponding to 84–97% O_2 saturation respectively. Water at this facility was very soft (12 ppm [$CaCO_2$], alkalinity of 13–14 ppm, pH~6.6–6.8., TDS = 27–30 and $[Na^+]$ and $[Cl^-]$ of less than 1 mM). Fish were fed Skretting NutraPlus Starter Feed (Skretting Co., B. C. Canada) mixed with gelatin up until 24 h prior to starting experiments. Fish were not fed during experimental exposures.

2.2. Sampling protocol

Fish mortality was recorded at 3, 6, 12, 24, 72 and 120 h following acute salinity exposure. Fish exposed to 16 ppt for 48 h, and then to 24 or 32 ppt were monitored for mortality at 24 h intervals. Dead fish were removed from tanks at these times and were not used for blood sampling. Fish (n = 5 for both 10 and 30 g) exposed to 0, 8, and 16 ppt salinity were removed from experimental tanks at 24, 72, and 120 h following exposure to salinity, and anesthetized with Benzocaine (in ethanol; final concentration 0.3 g/l). Blood was withdrawn from the caudal vein into 1 ml heparinized syringes. Heparinized hematocrit capillary tubes were filled in duplicate for each fish, and centrifuged at 11500 RCF for 3 min in a Damon IEC MB hematocrit centrifuge. Hematocrit values were then recorded as the portion of total blood volume that was packed red blood cells. A 10 μl microcap of whole blood was frozen in liquid nitrogen for later analysis of hemoglobin content. The remaining blood sample was centrifuged (10,000 rpm) for 3 min. Plasma was aliquoted into separate bullet tubes and frozen in liquid nitrogen and held at −80 °C for later analysis of ion content.

Plasma osmolarity was measured using a Wescor 5500 V.P. osmometer on duplicate 10 μl volumes. Plasma $[Cl^-]$ was measured using a digital chloridometer (Model 442-5000, HBI Inc.), and plasma $[Na^+]$ was determined by flame spectrophotometer (Model 664, Corning). Whole blood total hemoglobin concentration (Hb) was measured using a Sigma total hemoglobin assay kit (#D 5941) at an absorbance of 540 nm. Mean cell hemoglobin concentration (MCHC) was calculated from hemoglobin concentrations and hematocrit as described by Houston et al. (1991).

2.3. Statistical analysis

The effect of size on mortality was determined by Chi square analysis (size [10 or 30 g] × status [i.e., dead or alive], α = 0.05). Analysis of Variance (ANOVA, 3-way, time × salinity × size) was used to determine the effects of size, salinity and time. In the case of a significant interaction term, three 2 way ANOVAs were used to compare pairs of factors (i.e., size × salinity for each time, salinity × time for each size, size × time for

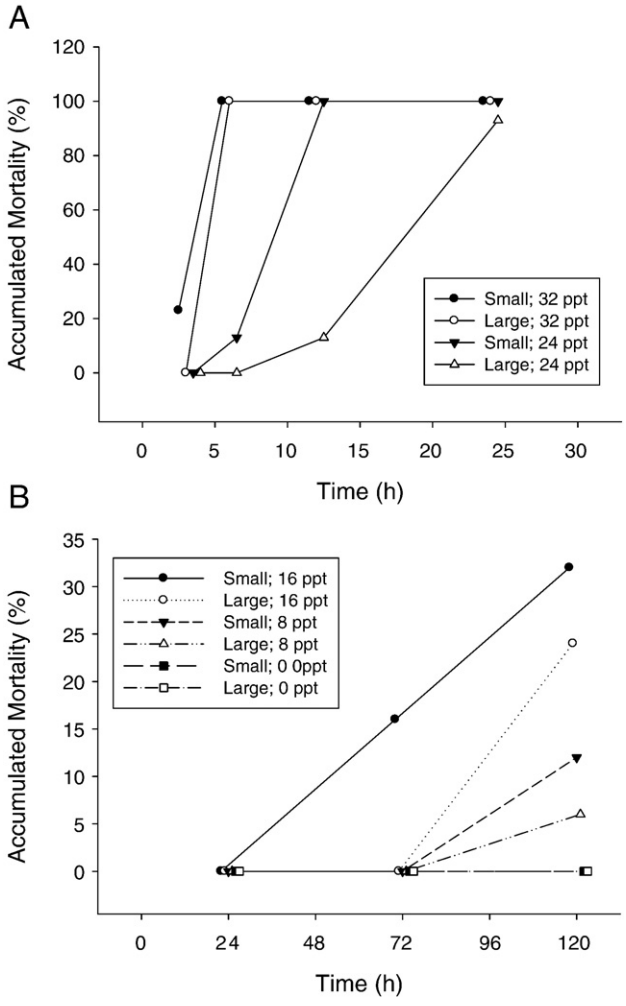

Fig. 1. Percent mortality of 10 gram (black symbols) and 30 gram (white symbols) white sturgeon exposed to (A) salinities of 24 and 32 ppt over 24 h, and (B) salinities of 0, 8, 16 ppt over 120 h.