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Effects of feed restriction on salinity tolerance in white sturgeon (*Acipenser transmontanus*)

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ABSTRACT

A multistressor study was conducted to investigate interactive effects of nutritional status and salinity on osmoregulation of juvenile white sturgeon. Our hypothesis was that lower nutritional status would decrease the salinity tolerance of juvenile white sturgeon. A four-week feed restriction (12.5%, 25%, 50%, 100% of optimum feeding rate: OFR defined as the rate (% body weight per day) at which growth is maximal) trial was performed, and relevant indices of nutritional status were measured. Following the trial, sturgeon were acutely exposed to various salinities (0, 8, 16, 24 ppt) for 120 h, and relevant osmoregulatory measurements were made at 12, 72, and 120 h post-salinity exposures. The feed-restriction trial resulted in a graded nutritional response with the most feed-restricted group (12.5% OFR) showing the lowest nutritional status. The salinity exposure trial showed clear evidence that lower nutritional status decreased the salinity tolerance of juvenile white sturgeon. Increasing salinities resulted in significant alterations in osmoregulatory indices of all feeding groups; however, a significantly slower acclimatory response to 24 ppt was detected in the most feed-restricted group compared to the non-feed-restricted group (100% OFR). Furthermore, evaluation of the effect of nutritional status on the relationship between osmoregulatory measurements and body size showed that there was a significant negative relationship between osmoregulatory performance and body size within the most feed-restricted group. This suggests that there is a certain body size range (200–300 g based on our finding) where juvenile white sturgeon can maximize osmoregulatory capacity at a salinity of 24 ppt.

1. Introduction

White sturgeon (*Acipenser transmontanus*) are an ecologically, economically, and recreationally important fish species that inhabits the pacific coast of North America (Lee et al., 2014; Moyle, 2002). White sturgeon possess unique evolutionary and life history characteristics including highly conserved morphology (i.e., living fossils; Gardiner, 1984), a long-life span (ca. 100 years), late sexual maturity (10–12 years for male, 12–16 years for female), and infrequent spawning, dependent upon environmental conditions (Moyle, 2002). White sturgeon are also semi-anadromous, spending most of their lives in estuaries of large rivers (e.g., Sacramento and Columbia rivers in the USA, Fraser river in Canada) and migrate into freshwater to spawn (Doroshov, 1985; Israel et al., 2009; Wilson and McKinley, 2004). Population declines of this valuable and primitive species are mainly attributable to anthropogenic activities (e.g., habitat loss, invasive species, contamination, overfishing). Currently, white sturgeon are listed as State S2 status (low abundance, restricted range, and potentially endangered species) in the California Natural Diversity Database (2009) and are classified as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2011).

Projected aquatic environmental alterations in the San Francisco Bay Delta (SFBD), including increasing water temperature and increasing salinity driven by global and local climate change (Cayan et al., 2008a, b; Cloern et al., 2011; Knowles and Cayan, 2002, 2004), may threaten the sustainability of white sturgeon populations native to this area. Recent evidence suggests that modifications to food web dynamics (e.g., decline in phytoplankton production, disruption of trophic linkages between phytoplankton and zooplankton) occur due to increasing water temperature (Auad et al., 2006; Boyce et al., 2010; Winder and Schindler, 2004) and that white sturgeon diets can shift to reflect availability and abundance of prey items (Kogut, 2008). The recent shift to Asian clams, white sturgeon's major prey species, will affect the qualitative and quantitative nature of their diets. Fish with reduced nutritional status are not only more vulnerable to predation (Metcalfe and Steele, 2001; Metcalfe et al., 1998) and disease (Oliva-Teles, 2012) but also to unfavorable environmental conditions (Deng et al., 2009; Haller et al., 2015; Han et al., 2011) because physiological performance to overcome these stresses is energy-dependent. Given the anticipated

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climate change impacts on white sturgeon, conservation of this species is dependent on understanding their physiological performance when faced with multiple environmental stressors.

In a review by Todgham and Stillman (2013), they stressed the importance of considering multiple stressors in physiological research and built a conceptual framework for understanding three possible interactive effects of two stressors, including "additive" (independent influence on physiological performance), "antagonistic" (reduced interactive influence), and "synergistic" (enhanced interactive influence). Although multistressor studies are difficult to conduct because of their overall complexity, their findings can greatly enhance our predictability of physiological performance responses (Todgham and Stillman, 2013). In the current study, nutritional status and salinity were selected as the environmental stressors for investigation of their interactive effect on physiological performance of white sturgeon. Hyper- or hypoosmoregulation is the maintenance of homeostasis of organisms' inner fluids from osmotic pressure, including ionic balances through transporters (e.g., Na^+/K^+-ATPase, Na–K–Cl cotransporter), hormonal regulation (e.g., prolactin, cortisol), water flux, synthesis and degradation of relevant proteins (see a review by Evans, 2008; Grosell, 2006; Tseng and Hwang, 2008). While salinity levels increase, these energy-demanding osmoregulatory mechanisms cause elevation in costs of basal metabolism, depletion in energy reserves, and reduction in energy expenditures for activity, growth, and reproduction (i.e., physiological trade-offs; Sokolova, 2013). Due to concurrent alterations in the prey base as well as in environmental salinity, white sturgeon will likely be under a difficult circumstance (i.e., catch-22), facing a lack of energy storage and a higher energetic demand for stress responses simultaneously, eventually leading to unfavorable physiological conditions for their survival.

Previous studies report that energy restriction significantly altered osmoregulatory responses in tilapia (*Oreochromis mossambicus*, Kültz and Jürss, 1991; Vijayan et al., 1996), gilthead sea bream (*Sparus auratus*, Polakof et al., 2006), and green sturgeon (*Acipenser medirostris*, Haller et al., 2015). To our knowledge, no investigation of an interactive effect between nutritional status and salinity on osmoregulation in white sturgeon has been performed. Thus, we tested the hypothesis that lower nutritional status would decrease the salinity tolerance of juvenile white sturgeon. Results from the current study can provide important insights for predicting possible integrative responses of white sturgeon to the projected environmental changes and enhance our understanding for conservation and management of this important species inhabiting the rapidly changing SFBD ecosystem.

2. Materials and methods

2.1. Animal acquisition and maintenance

White sturgeon larvae (3 days post-hatch: DPH) from one domesticated female (46 kg and 12 years old) and four males (in average, 28 kg and 8 years old), donated by a local farm (Lazy Q Fish Ranch LLC, Dixon, CA, USA), were transported (April 23rd, 2012) to the Center for Aquatic Biology and Aquaculture (CABA) at the University of California, Davis, CA, USA. Fish were reared in circular fiberglass tanks (152 cm diameter, 45 cm height, ca. 750 L water volume) supplied with flow-through degassed well-water (18–19 °C) throughout the rearing period. Once the larvae started exogenous feeding (10–14 DPH), they were fed a commercial starter feed (Soft-Moist #0 crumble, Rangen, Buhl, ID, USA) with 24-h automatic feeders (Lifegard Automatic Fish Feeder, Lifegard Aquatics, Cerritos, CA, USA). Fish were fed a variety of commercial feeds (Soft-Moist #1, #2 and #3 crumble, Rangen; SCD 1.0 and 2.0 mm sinking pellet, Skretting, Tooele, UT, USA) while they grew up to the desired initial weight (173.2 ± 0.6 g; mean ± SEM) for the experiment. Fish were maintained according to the animal protocol approved by the Campus Animal Care and Use Committee (Protocol Number 16541).

2.2. Feed restriction trial

A random distribution of 840 white sturgeon juveniles (173.2 ± 0.6 g) into 12 fiberglass tanks (ca. 750 L) resulted in 70 fish per tank. During an eight-day acclimation period, fish were fed at 1.8% body weight per day with commercial feed (SCD 2.0 mm sinking pellet, Skretting). Proximate composition of the feed (%), as determined through the Association of Official Analytical Chemists (AOAC) method (Jones, 1988), was 8.7 moisture, 42.0 crude protein, and 26.7 crude lipid. At the end of the acclimation period, the 12 experimental tanks were randomly assigned to one of the four feed restriction treatments (12.5%, 25%, 50%, 100% of optimum feeding rate (OFR) determined by an OFR model equation developed for white sturgeon (Lee et al., 2014)), resulting in three tanks per treatment. The OFR is defined as the rate (% body weight per day) at which growth is maximal. The average initial body weight of fish in all tanks was 204.5 ± 0.9 g (197 DPH). The feed restriction trial was carried out for four weeks. At the middle of the trial (two weeks), all fish in each tank were batch weighed, and the amount of feed per tank was adjusted according to the weight change. Every morning between 9:00 and 10:00 AM, feed was loaded on 24-h belt feeders (Zeigler Brothers Inc., Gardners, PA, USA) located on top of each tank cover. The tank covers had a rectangular hole (ca. 10 cm width, 33 cm length) for the feed to fall through. After the feed loading for all tanks was complete, water inside the tank was quickly drained to ca. 50% of total volume to remove fecal matters and uneaten feed once a day. Water quality was measured daily and was maintained at 18.1–18.7 °C and 7.5–9.0 mg L^{-1} (dissolved oxygen) throughout the trial. Total ammonia levels and pH were recorded weekly, and their levels were 0.1–0.2 mg L^{-1} (NH_4^+) and 7.6–8.0 (pH). The experimental tanks were located outdoors, exposing the fish to a natural photoperiod through the feeding hole. The feed restriction trial started on Nov 3rd, 2012 and ended on Dec 3rd, 2012.

2.2.1. Determinations of nutritional status measurements

After the four-week feed restriction trial, the determination of nutritional status, including growth performance, body composition, body energy, and plasma metabolites was conducted. All fish in each tank were weighed, then their weights were averaged for calculations of specific growth rate (SGR) and feed conversion ratio (FCR). Three randomly selected fish from each tank were measured individually for weight and total length for calculation of condition factor (CF). Livers from the same fish were dissected and weighed for the calculation of hepatosomatic index (HSI). Individually calculated CF and HSI values were then averaged for fish from each replicate tank. These indices were calculated using the following equations:

$$\mathrm{SGR}(\%) = 100 \times (\ln(\mathrm{FBW}) - \ln(\mathrm{IBW}))/\text{days of the trial}$$

$$\mathrm{FCR} = \text{total amount of feed given}/(\mathrm{FBW}-\mathrm{IBW})$$

$$\mathrm{CF} = 100 \times \left(\mathrm{FBW}/\mathrm{TL}^3\right)$$

$$\mathrm{HSI} = 100 \times (\text{liver weight}/\mathrm{FBW})$$

where FBW, IBW, and TL were the final body weight (g), initial body weight (g), and total length (cm), respectively.

After the final weighing, fish were not fed for 24 h prior to sampling. Three fish from each tank were randomly selected and euthanized with an overdose of buffered MS-222 (6 g NaCl, 0.42 g $NaHCO_3$, and 0.5 g tricaine methanesulfonate per L, Argent Inc., Redmond, WA, USA). The three fish per tank were then pooled, put in a 15 L plastic bag, and kept at −20 °C for later body proximate composition analysis. This composition, consisting of moisture, crude protein, lipid, and ash, was determined through the AOAC method. Body energy was calculated using the following values: crude protein 23.6 kJ g^{-1}, crude lipid 39.3 kJ g^{-1}, and nitrogen-free extract (NFE) 17.7 kJ g^{-1} (Deng et al.,