



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.

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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 (Aquad 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 (Metcalf 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., $\text{Na}^+/\text{K}^+-\text{ATPase}$, $\text{Na}-\text{K}-\text{Cl}$ 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 SFBED 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 (Lifeguard Automatic Fish Feeder, Lifeguard 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 \pm 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:

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

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

$$\text{CF} = 100 \times (\text{FBW} / \text{TL}^3)$$

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

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.,*

2003). An additional three fish were randomly captured and euthanized with buffered MS-222 to collect blood with a 22G needle and a vacutainer (BD Vacutainer® Ref #36664, Franklin Lakes, NJ, USA) through a puncture of the caudal vein. The blood sample was then centrifuged at 4500 g for 5 min at room temperature to collect plasma for determinations of plasma metabolites, including glucose, protein, and triacylglyceride (TAG). Each plasma metabolite was measured using a commercial assay kit (Glucose Assay Kit (Product code: GAGO20); Total Protein Kit (Product code: TP0200) and Serum Triglyceride Determination Kit (Product code: TR0100), Sigma-Aldrich, St. Louis, MO, USA).

2.3. Salinity exposure trial

Following the four-week feed restriction trial, an acute salinity exposure trial was carried out for five days (120 h). Three separate recirculating systems and one flow-through (degassed well-water) system, consisting of four tanks (97 cm diameter, ca. 160 L) per system, were used. Four replicate tanks at each one of the four salinities (0 (control), 8, 16, and 24 parts per thousand (ppt)) were used. Salinity was manipulated using synthetic sea salt (Instant Ocean, Blacksburg, VA, USA). Once water of the tanks were at treatments salinities, 72 fish (233 DPH) from each feed restriction treatment (288 total fish) were randomly and equally divided into one of the four tanks for each salinity treatment, resulting in 18 fish per salinity treatment tank. Fish were not fed for one day prior to salinity exposure and during the five-day trial. Water quality was maintained at 17.8–18.2 °C, 7.5–9.2 mg L⁻¹ (dissolved oxygen), and 0.25–0.5 mg L⁻¹ (NH₄⁺) throughout the exposure trial. No mortality was observed.

The highest salinity (24 ppt) was chosen on the basis of life history of white sturgeon juveniles (Israel et al., 2009) and of a preliminary test assessing a maximum tolerable salinity level under laboratory conditions. A brief description of this preliminary trial and outcomes are as follows. Groups of four sturgeon from the same cohort fed at 12.5% OFR or 100% OFR were acutely exposed to various salinity levels ranging from 24 to 32 ppt for five days. Two out of four optimally fed sturgeon (100% OFR) exposed to 32 ppt showed abnormal swimming during the 24-h exposure and eventually died. Two out of four feed-restricted sturgeon (12.5% OFR) showed two mortalities after 10 h post exposure to 29 ppt, and the rest died after 24-h exposure. Another four feed-restricted sturgeon exposed to 27 ppt died after 24 h. No mortalities or signs of poor health (e.g., irregular ventilation, abnormal swimming) were detected in sturgeon exposed to 24 ppt for five days.

2.3.1. Determinations of osmoregulation measurements

Samples for osmoregulation measurements, including muscle moisture, hematocrit, total blood hemoglobin, plasma glucose, lactate, osmolality, ions (Na⁺, Cl⁻, and K⁺), and cortisol, and gill and pyloric caeca (PC) Na⁺/K⁺-ATPase (NKA) activities, were collected at 12, 72, and 120 h during the salinity exposure trial. Six fish at each time point were randomly captured and euthanized with buffered MS-222 (salinity of buffered anesthetic was adjusted to the treatment level). Epaxial muscle (1–5 g depending on fish size) collected about three scutes posterior to the head, was removed, put in a pre-weighed aluminum dish, and oven-dried at 60 °C for three days for measurement of dry weight and calculation of muscle moisture. Muscle moisture (%) was calculated using the equation: $(1 - (DM_w / WM_w)) \times 100$, where the DM_w and WM_w were the dried muscle weight (g) and wet muscle weight (g), respectively. Blood samples were collected using a 22G needle and a vacutainer (BD Vacutainer® Ref #36664, Franklin Lakes) through a puncture of the caudal vein. The collected blood samples were then divided into one tube for hematocrit and hemoglobin determinations and another for plasma collection. Hematocrit was measured using a heparinized microhematocrit capillary tube (centrifuged at 12,600 g for 3 min at room temperature) with the Micro-hematocrit Capillary Tube Reader (CRITOCAPS™, Oxford Labware, St. Louis, MO, USA).

Hemoglobin was measured using a hemoglobin assay kit (Hemoglobin Reagent Set (Cat. No.: H526-480), Teco Diagnostics, Anaheim, CA, USA). Plasma samples collected from blood centrifuged at 4500 g for 5 min at room temperature were divided into three separate 2.0 mL microtubes, snap frozen in liquid nitrogen, and stored at -80 °C for determinations of glucose, lactate, osmolality, ions, and cortisol. Plasma glucose and lactate were measured using a YSI 2700 Biochemistry Analyzer (YSI Life Science, Yellow Springs, OH, USA). Plasma osmolality was analyzed using a vapor pressure osmometer (Vapro® 5520, Wescor Inc., Logan, UT, USA). Plasma Na⁺ and K⁺ concentrations were measured in a flame photometer (Model 02655-90, Cole-Parmer Instrument Company, Vernon Hills, IL, USA). Plasma Cl⁻ was analyzed using a digital chloridometer (Labconco, Kansas City, MO, USA). Plasma total cortisol was assayed with an Enzyme-linked Immuno Sorbent Assay Kit (Product code: #402710, Neogen Corporation, Lexington, KY, USA).

Filaments of a second left gill arch and a pyloric caecum were collected for the NKA activity assay (McCormick, 1993; modified for green sturgeon, Allen et al., 2009; Sardella and Kültz, 2009, and Haller et al., 2015). Tissues were rinsed with saline solution (0.9% NaCl), blotted with a paper towel, cut into small pieces, put into a separate microtube (2.0 mL) filled with 1.0 mL of homogenization buffer (85.6 µg ml⁻¹ sucrose, 3.7 µg ml⁻¹ Na₂EDTA, 3.4 µg ml⁻¹ imidazole, 0.2 µg ml⁻¹ Na deoxycholic acid), quickly immersed in liquid nitrogen, and stored at -80 °C for later NKA assays. Samples were thawed on ice, homogenized at approximately 25,000 rpm for 30 s (Polytron® PT 1200 E, Kinematica AG, Lucerne, Switzerland), and centrifuged at 5000 g at 4 °C for 1 min (Eppendorf, Hamburg, Germany). The supernatants were diluted two to five times with a homogenization buffer according to the tissue type and/or sample size to adjust their concentrations to fall within the linear range of standard curve for protein concentration measurements. For the NKA activity assay, six 10 µL aliquots of the diluted supernatants were loaded into a 96 well plate, and 200 µL of assay solution (including or excluding ouabain) was added. Measurement was performed using a microplate reader at 340 nm and 25 °C for 10 min (Synergy HT, Biotek, Winooski, VT, USA). Protein concentrations of the diluted supernatants were measured using a BCA protein assay kit (Cat. No.: PI-23223, Thermo Fisher Scientific Inc., Waltham, MA, USA). The NKA activity was expressed as µmol ADP mg protein⁻¹ h⁻¹.

2.4. Statistical analysis

2.4.1. Analysis of variance on results from the feed restriction and salinity exposure trials

Results from the four-week feed restriction trial were analyzed by one-way analysis of variance (ANOVA) accounting for the four feed restriction treatments, and comparisons between treatment means were performed through Tukey's studentized range (HSD) test.

Results from the salinity exposure trial were analyzed using two-way ANOVA to test an interaction between feed restriction and salinity or to test a main effect of feed restriction or salinity on each measurement for each time point (12, 72, 120 h). The following model was used,

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk} \quad (1)$$

where y_{ijk} was the dependent variable measured for the i th feed restriction, the j th salinity, and the k th observation where the number of observations is dependent on the response; μ is an overall constant; α_i is a fixed effect of the i th feed restriction for $i = 1, 2, 3$ and 4 (12.5%, 25%, 50%, 100% OFR); β_j is a fixed effect of the j th salinity for $j = 1, 2, 3$ and 4 (0, 8, 16, 24 ppt); $\alpha\beta_{ij}$ is an interaction for combinations of the i th feed restriction and j th salinity; and e_{ijk} is error term $\sim N(0, \sigma^2)$.

Statistical analyses were performed using the SAS software package (version 9.3, SAS Institute, Cary, NC, USA). Data was evaluated for assumptions, including normality and homogeneity of variance, using

the Shapiro–Wilk and Levene's tests, respectively. Type III sums of squares ANOVA was used to test all possible interactions or main effects. Multiple comparisons were performed using the SAS macro "pdglm800" (Saxton, 1998). When the sample sizes were unbalanced, the Tukey–Kramer test was used for comparisons. Significance was tested at $P < 0.05$. If interaction effects were detected, data were expressed as interaction effect means. Otherwise, data were presented as main effect means.

2.4.2. Regression analysis on the relationship between a response variable and body size

The rationale for this analysis was that the large but homogenous variations in fish sizes within and among the feed restriction treatments was detected in datasets of osmoregulatory measurements so that we were allowed to investigate the effect of body size on the measurements responding to various salinities at each time point. Because of a positive correlation between body size change and nutrient partitioning in association with energy availability, this analytical approach may provide us insights to evaluate the effect of nutritional status on osmoregulatory performance and to estimate a body size range where white sturgeon can maximize osmoregulatory capacity.

Each dataset of osmoregulatory measurements, consisting of a combination of the four feed restrictions (12.5, 25, 50, 100% OFR) and four salinities (0, 8, 16, 24 ppt), at each time point (12, 72, 120 h) was broken up into different salinity treatments for each time, and a regression format was used to determine the effect of feed restriction on the relationship between a response variable (osmoregulatory measurement) and body weight. Interest was mainly on the slopes of the different feed restrictions but intercepts were also analyzed.

A sequence of models was evaluated where the first model was the full model described as:

$$y_{ij} = \mu + FR_i + \beta_0 x_{ij} + k_i x_{ij} + e_{ij} \quad (2)$$

where y_{ij} is the response variable for the i th feed restriction (FR) and the j th fish, μ is the overall constant, FR_i is the i th FR for $i = 1, 2, 3$ and 4 (12.5, 25, 50, 100% OFR), x_{ij} is the body weight for the i th FR for the j th fish, β_0 is the overall regression coefficient for x , k_i is the regression coefficient of x for the i th FR, and e_{ij} is the error term $\sim N(0, \sigma^2)$. In R (version 3.0.1, R Development Core Team, 2013), a model of this form results in μ representing the intercept of the first FR and FR_i represents the differences of the intercepts for the 2nd, 3rd, and 4th FR from the first FR or μ , then k_i represents the difference of the slopes for the 2nd, 3rd, and 4th FR from the slope of the first FR or β_0 . This allows evaluation of significant intercepts or slopes.

If both slopes and intercepts are significant ($P < 0.01$), the following model can be used to directly estimate the intercepts and slopes:

$$y_{ij} = FR_i + k_i x_{ij} + e_{ij} \quad (3)$$

where FR_i represents the intercepts of the four FR and k_i represents the slopes of the four FR. If further testing of differences of intercepts or differences in slopes are of interest then contrast statements ($P < 0.01$) can be used.

If the intercepts are not different from each other and only the slopes are important, then the following model is required:

$$y_{ij} = \mu + k_i x_{ij} + e_{ij} \quad (4)$$

where μ represents the overall intercept and k_i represents the four slopes. If further testing of differences in slopes are of interest then contrast statements can be used.

If the slopes are similar but the intercepts are different for the FR, then the following model is appropriate:

$$y_{ij} = FR_i + \beta_0 x_{ij} + e_{ij} \quad (5)$$

where FR_i represents the four FR intercepts and β_0 represents the overall slope. If further testing of differences in intercepts are of interest then contrast statements can be used.

If there are no differences between slopes or intercepts then the following model is appropriate:

$$y_{ij} = \mu + \beta_0 x_{ij} + e_{ij} \quad (6)$$

where μ and β_0 are the overall intercept and slope, respectively.

3. Results

3.1. Effects of feed restriction on nutritional status

The four-week feed restriction trial resulted in significant alterations in nutritional status of juvenile white sturgeon, including growth performance (SGR, FCR, CF, and HSI), body composition (moisture and lipid), body energy, and plasma metabolites (protein and TAG) (Table 1). Specific growth rate, CF, and HSI significantly decreased with increasing feed restriction. The most feed-restricted group (12.5% OFR) showed a slight loss in body weight (SGR: $-0.07 \pm 0.05\%$; mean \pm SEM) and a negative FCR. Body lipid and body energy significantly decreased with increasing feed restriction; however, body moisture exhibited an inverse relationship with respect to body lipid and body energy. No significant effect of feed restriction on body protein was found. Plasma protein and TAG significantly decreased with increasing feed restriction; however, no significant effect of feed restriction on plasma glucose was detected.

3.2. Effects of nutritional status on salinity exposure

Results of two-way ANOVA test, using model [1], for effects of feed restriction and salinity on osmoregulatory measurements (muscle moisture, hematocrit, hemoglobin, plasma glucose, lactate, osmolality, Na^+ , Cl^- , and K^+ , and gill and PC NKA activities) are shown in Table 2.

Overall, no mortality was detected during the 120-h salinity exposure trial. As expected, increasing salinities resulted in significant alterations in osmoregulatory indices consistent with hyperosmotic exposure; however, the most feed-restricted group (12.5% OFR) showed slower recovery from exposure to 24 ppt than did the non-feed-restricted group (100% OFR).

3.2.1. Muscle moisture

After 12 h of salinity exposure, a significant main effect of salinity on muscle moisture was detected (Table 2) with muscle moisture decreasing with increasing salinity (Table 3). At 72 h, a significant interaction between feed restriction and salinity was observed. Although muscle moisture of most of the feeding groups (25%, 50%, 100% OFR) exposed to 16 or 24 ppt was not significantly different from the same feeding groups exposed to 0 ppt, the content of the most feed-restricted group exposed to 24 ppt ($77.2 \pm 0.3\%$) was still significantly lower than that of the most feed-restricted group exposed to 0 ppt ($78.5 \pm 0.2\%$). At 120 h, neither a significant interaction nor main effect was detected.

3.2.2. Hematocrit and hemoglobin

A significant main effect of feed restriction and salinity on hematocrit values was observed at 72 h only (Table 2), showing that feed restriction (12.5%, 25%, 50% OFR) and salinity (16, 24 ppt) treatments significantly decreased hematocrit values (Fig. 1 top).

A significant main effect of salinity on hemoglobin values was detected at each time point (Table 2, Fig. 1 bottom). The hemoglobin values of groups exposed to 8, 16, or 24 ppt were significantly higher at 12 h and relatively lower at 72 and 120 h compared to the group exposed to 0 ppt.

Table 1
Measurements of nutritional status of juvenile white sturgeon after the four-week feed restriction trial.¹

Measurements	Optimum feeding rate (%)				P-value
	12.5	25	50	100	
<i>Growth performance</i>					
Specific growth rate (%)	−0.07 ± 0.05 ^d	0.28 ± 0.04 ^c	0.99 ± 0.01 ^b	1.55 ± 0.02 ^a	<0.0001
Feed conversion ratio	−1.49 ± 0.92 ^b	1.45 ± 0.28 ^a	0.73 ± 0.00 ^a	0.89 ± 0.02 ^a	0.0116
Condition factor	0.45 ± 0.02 ^b	0.46 ± 0.01 ^b	0.50 ± 0.01 ^{ab}	0.56 ± 0.02 ^a	0.0018
Hepatosomatic index	1.60 ± 0.05 ^c	2.09 ± 0.02 ^{ab}	1.92 ± 0.02 ^b	2.26 ± 0.09 ^a	0.0001
<i>Body composition and energy²</i>					
Moisture (%)	74.8 ± 0.4 ^a	73.6 ± 0.2 ^{ab}	72.9 ± 0.1 ^b	72.2 ± 0.4 ^b	0.0026
Crude protein (%)	13.0 ± 0.2	12.7 ± 0.4	13.1 ± 0.2	13.6 ± 0.1	NS ⁴
Crude lipid (%)	9.0 ± 0.3 ^c	10.3 ± 0.3 ^b	10.8 ± 0.1 ^{ab}	11.8 ± 0.2 ^a	0.0002
Energy (kJ g ^{−1})	6.9 ± 0.1 ^c	7.3 ± 0.1 ^b	7.6 ± 0.0 ^b	8.0 ± 0.1 ^a	0.0002
<i>Plasma metabolites³ (24-h post prandial)</i>					
Glucose (mg dL ^{−1})	85.4 ± 3.0	94.8 ± 1.5	88.3 ± 1.7	103.1 ± 9.0	NS
Total protein (g L ^{−1})	17.6 ± 0.4 ^b	21.8 ± 1.0 ^{ab}	22.6 ± 0.5 ^a	24.0 ± 1.6 ^a	0.0089
Triglycerides (mg dL ^{−1})	643.5 ± 81.4 ^b	967.6 ± 80.6 ^{ab}	989.1 ± 83.7 ^{ab}	1648.0 ± 303.3 ^a	0.0165

¹ Values are means ± SEM from three replicate tanks per treatment ($N = 3$ tanks). Different superscripts within each row demarcate significant ($P < 0.05$) differences according to the Tukey's HSD test based on one-way analysis of variance.

² $N = 3$ tanks (average value of three randomly selected fish within a tank was considered as a replicate tank). Average initial body compositions (%) were: moisture 73.2 ± 0.3, crude protein 12.3 ± 0.5, and crude lipid 11.2 ± 0.3. 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., 2003).

³ The same sample size for body composition and energy.

⁴ No significant difference ($P > 0.05$).

3.2.3. Plasma glucose and lactate

A significant main effect of feed restriction on plasma glucose concentrations was detected at 72 and 120 h (Table 2), showing that plasma glucose concentrations of the most feed-restricted group were relatively low compared to the non-feed-restricted group (Fig. 2 top). A significant main effect of salinity on plasma glucose concentrations was observed at each time point (Table 2). At 12 h, a significant elevation in plasma glucose concentrations was detected in the group exposed to 24 ppt only (Fig. 2 top). At 72 and 120 h, plasma glucose concentrations of groups exposed to 16 and 24 ppt were significantly lower than those of the group exposed to 0 ppt.

A significant main effect of feed restriction on plasma lactate concentrations was observed at 72 and 120 h (Table 2), showing that plasma lactate concentrations of the most feed restricted group were significantly lower than those of the non-feed-restricted group (Fig. 2 bottom). A significant main effect of salinity on plasma lactate concentrations was detected at 12 and 120 h (Table 2). At 12 h, plasma lactate concentrations of groups exposed to 16 and 24 ppt were

significantly lower than those of the group exposed to 0 ppt although conversely, plasma lactate concentration of the group exposed to 24 ppt was significantly higher than that of the group exposed to 0 ppt at 120 h (Fig. 2 bottom).

3.2.4. Plasma osmolality and ions

A significant interaction between feed restriction and salinity on plasma osmolality was detected at each exposure time (Table 2). At 12 h, plasma osmolality of both feed-restricted and non-feed-restricted groups was significantly elevated by 16 and 24 ppt compared to 0 and 8 ppt (Table 4); however, plasma osmolality of the more feed-restricted groups (12.5%, 25% OFR) exposed to 24 ppt was relatively low compared to the less feed-restricted (50% OFR) and non-feed-restricted groups exposed to the same salinity. At 72 h, plasma osmolality of all feeding groups exposed to 0 and 16 ppt showed no significant difference; however, plasma osmolality of all feeding groups exposed to 24 ppt was still significantly higher than that of the same feeding groups exposed to 0 ppt. Notably, plasma osmolality of the most feed-restricted

Table 2
Results of the two-way analysis of variance tests on osmoregulatory measurements of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure.

Exposure time (h)	Sources	Measurements										
		MM ³ (%)	Hct ⁴ (%)	Hb ⁵ (g dL ^{−1})	Glucose (mg dL ^{−1})	Lactate (mg dL ^{−1})	Osmolality (mOsm kg ^{−1})	Na ⁺ (mEq L ^{−1})	Cl [−] (mEq L ^{−1})	K ⁺ (mEq L ^{−1})	Gill NKA ⁶	PC ⁷ NKA
12	FR ¹	NS	NS	NS	NS	NS	*	NS	NS	***	**	***
	Salinity ²	***	NS	***	***	***	***	***	***	***	NS	NS
	FR × Salinity	NS	NS	NS	NS	NS	*	NS	*	NS	NS	NS
72	FR	*	*	NS	*	***	*	NS	***	**	***	***
	Salinity	***	**	*	**	NS	***	***	***	***	***	***
	FR × Salinity	*	NS	NS	NS	NS	***	**	**	NS	*	*
120	FR	NS	NS	*	*	**	*	NS	*	***	*	***
	Salinity	NS	NS	***	***	**	***	***	***	***	***	***
	FR × Salinity	NS	NS	NS	NS	NS	***	*	***	**	NS	NS

NS: no significant difference ($P > 0.05$); *: $P < 0.05$; **: $P < 0.001$; ***: $P < 0.0001$.

Plasma cortisol data was measured only for samples collected from fish at the 12-h salinity exposure (significant main effect of salinity was only detected; $P < 0.0001$).

¹ Feed restriction at 12.5, 25, 50 and 100% of optimum feeding rate (% body weight per day).

² Levels of 0, 8, 16 and 24 ppt.

³ Muscle moisture.

⁴ Hematocrit.

⁵ Hemoglobin.

⁶ Na⁺/K⁺-ATPase (μmol ADP mg protein^{−1} h^{−1}).

⁷ Pyloric caeca.

Table 3
Interaction effect means of muscle moisture of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure.¹

Salinity (ppt)	OFR ² (%)	Muscle moisture (%)		
		12 h	72 h	120 h
0	12.5	78.6 ± 0.2 ^a	78.5 ± 0.2 ^{ab}	78.8 ± 0.3
	25	78.3 ± 0.4 ^{ab}	78.5 ± 0.2 ^{ab}	79.0 ± 0.1
	50	78.3 ± 0.1 ^{ab}	78.0 ± 0.3 ^{abc}	78.8 ± 0.2
	100	78.0 ± 0.1 ^{abc}	78.5 ± 0.1 ^{ab}	78.5 ± 0.2
8	12.5	77.6 ± 0.4 ^{abc}	78.8 ± 0.3 ^a	78.9 ± 0.2
	25	78.3 ± 0.2 ^{ab}	78.5 ± 0.1 ^{ab}	78.9 ± 0.2
	50	77.8 ± 0.2 ^{abc}	78.1 ± 0.2 ^{abc}	78.9 ± 0.1
	100	78.0 ± 0.1 ^{abc}	78.1 ± 0.1 ^{abc}	78.8 ± 0.1
16	12.5	77.1 ± 0.3 ^{bcd}	78.6 ± 0.2 ^a	78.6 ± 0.5
	25	76.9 ± 0.2 ^{cd,†}	78.5 ± 0.1 ^{ab}	78.3 ± 0.4
	50	77.1 ± 0.2 ^{bcd}	78.0 ± 0.2 ^{abc}	79.0 ± 0.2
	100	77.2 ± 0.2 ^{bc}	78.1 ± 0.2 ^{abc}	78.2 ± 0.2
24	12.5	75.6 ± 0.3 ^e	77.2 ± 0.3 ^c	78.1 ± 0.3
	25	75.9 ± 0.1 ^{de}	77.8 ± 0.2 ^{abc,†}	78.4 ± 0.2
	50	75.6 ± 0.4 ^e	77.6 ± 0.2 ^{bc}	78.8 ± 0.3
	100	75.5 ± 0.2 ^e	78.1 ± 0.1 ^{abc}	78.8 ± 0.1

¹ Means ± SEM (N = 6; †N = 5). Different superscripts within each time point (column) demarcate significant (P < 0.05) differences according to the Tukey's HSD test based on two-way analysis of variance.

² Optimum feeding rate.

group exposed to 24 ppt was the highest among all feed-restricted groups within the same salinity. Overall responses at 120 h were similar to those observed at 72 h as well as plasma osmolality of the more feed-restricted groups (12.5%, 25% OFR) exposed to 24 ppt was still significantly higher than those of the less feed restricted (50% OFR) and non-feed-restricted groups.

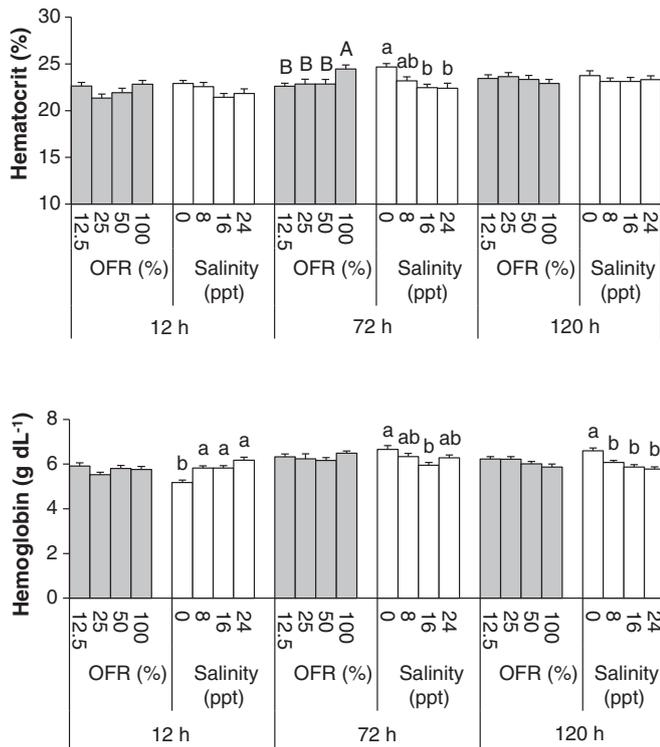


Fig. 1. Means of blood hematocrit (top) and hemoglobin (bottom) of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure for the main effects of four optimum feeding rates (OFR, %) and four salinities at three time points. Upper and lower case letters on the standard error bars represent significant differences (Tukey's HSD test based on two-way analysis of variance; P < 0.05) within the main effect of feed restriction (filled bars) and the main effect of salinity (open bars) treatments, respectively, at the same time point.

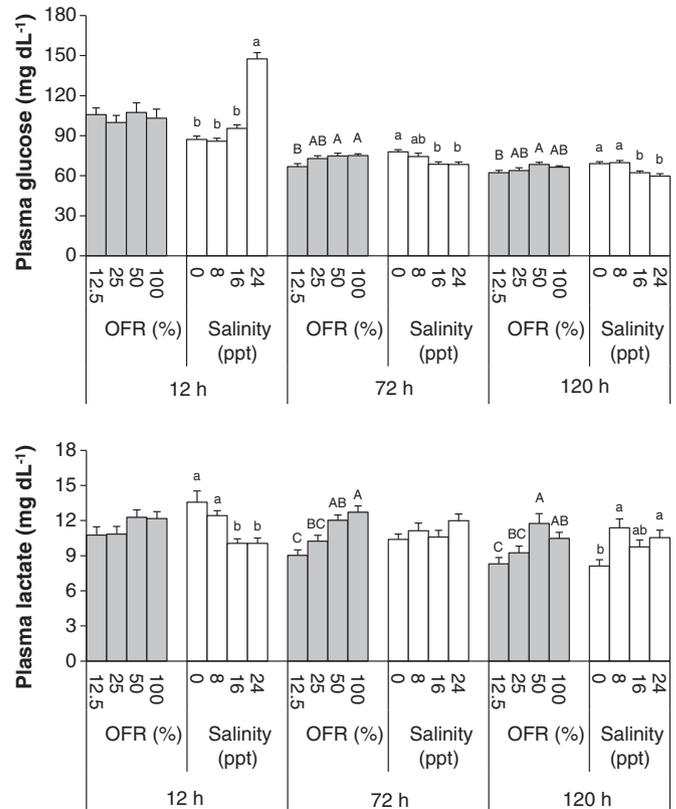


Fig. 2. Means of plasma glucose (top) and lactate (bottom) concentrations of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure for the main effects of four optimum feeding rates (OFR, %) and four salinities at three time points. Upper and lower case letters on the standard error bars represent significant differences (Tukey's HSD test based on two-way analysis of variance; P < 0.05) within the main effect of feed restriction (filled bars) and the main effect of salinity (open bars) treatments, respectively, at the same time point.

The overall time course of changes in plasma ions (Na⁺, Cl⁻, K⁺) responding to various salinities was similar to that of plasma osmolality with the exception of a distinct response in plasma Na⁺ and K⁺ levels to feed restriction in comparisons to plasma osmolality and Cl⁻ at 120 h and 24 ppt (Tables 2, 5). At 120 h, plasma osmolality and Cl⁻ levels of

Table 4
Interaction effect means of plasma osmolality of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure.¹

Salinity (ppt)	OFR ² (%)	Plasma osmolality (mOsm kg ⁻¹)		
		12 h	72 h	120 h
0	12.5	263 ± 2 ^d	264 ± 2 ^e	264 ± 4 ^d
	25	260 ± 1 ^d	261 ± 3 ^e	259 ± 3 ^d
	50	264 ± 1 ^d	264 ± 3 ^e	262 ± 2 ^d
	100	264 ± 3 ^d	260 ± 2 ^e	259 ± 1 ^d
8	12.5	265 ± 1 ^d	263 ± 3 ^e	259 ± 3 ^d
	25	265 ± 2 ^d	263 ± 2 ^e	258 ± 2 ^d
	50	264 ± 2 ^d	266 ± 1 ^e	268 ± 4 ^{bcd}
	100	267 ± 2 ^d	262 ± 2 ^e	257 ± 2 ^d
16	12.5	285 ± 2 ^c	269 ± 2 ^e	270 ± 2 ^{bcd}
	25	295 ± 3 ^c	272 ± 3 ^{de}	267 ± 2 ^{cd}
	50	291 ± 2 ^c	267 ± 1 ^{e,†}	272 ± 2 ^{bcd}
	100	290 ± 5 ^c	273 ± 2 ^{de}	271 ± 2 ^{bcd}
24	12.5	316 ± 3 ^b	315 ± 6 ^a	304 ± 8 ^a
	25	319 ± 4 ^{ab}	297 ± 5 ^{bc,†}	299 ± 4 ^a
	50	333 ± 4 ^a	298 ± 3 ^b	281 ± 2 ^{bc}
	100	330 ± 6 ^{ab}	284 ± 2 ^{cd}	282 ± 3 ^b

¹ Means ± SEM (N = 6; †N = 5). Different superscripts within each time point (column) demarcate significant (P < 0.05) differences according to the Tukey's HSD test based on two-way analysis of variance.

² Optimum feeding rate.

Table 5
Interaction effect means of plasma Na⁺, Cl⁻, and K⁺ concentrations of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure.¹

Salinity (ppt)	OFR ² (%)	Plasma Na ⁺ (mEq L ⁻¹)			Plasma Cl ⁻ (mEq L ⁻¹)			Plasma K ⁺ (mEq L ⁻¹)		
		12 h	72 h	120 h	12 h	72 h	120 h	12 h	72 h	120 h
0	12.5	138.5 ± 2.2 ^h	139.5 ± 1.4 ^{cd}	141.6 ± 1.4 ^{cd}	119.9 ± 1.3 ^{fg}	123.8 ± 1.3 ^{fg}	122.8 ± 1.2 ^{de}	2.4 ± 0.1 ^g	2.5 ± 0.1 ^{ef}	3.4 ± 0.1 ^{ab}
	25	139.9 ± 1.3 ^{gh}	142.1 ± 2.3 ^{cd}	140.8 ± 2.1 ^d	120.3 ± 1.0 ^{fg}	121.3 ± 1.5 ^{hi}	119.5 ± 0.6 ^e	2.8 ± 0.2 ^{defg}	2.3 ± 0.1 ^f	2.4 ± 0.1 ^g
	50	138.8 ± 1.8 ^{gh}	142.9 ± 2.5 ^{cd}	138.8 ± 1.0 ^d	121.2 ± 1.5 ^{fg}	122.1 ± 1.1 ^{ghi}	122.5 ± 1.0 ^{de}	2.7 ± 0.1 ^{defg}	2.8 ± 0.1 ^{bcde}	2.7 ± 0.1 ^{efg}
	100	135.9 ± 1.3 ^h	139.0 ± 2.1 ^{cd}	140.1 ± 0.8 ^d	116.8 ± 0.9 ^g	118.3 ± 0.8 ⁱ	121.3 ± 0.7 ^e	3.1 ± 0.2 ^{cde}	2.9 ± 0.1 ^{abcde}	2.7 ± 0.1 ^{efg}
8	12.5	141.4 ± 0.9 ^{efgh}	139.0 ± 1.1 ^{cd}	140.5 ± 2.8 ^d	126.0 ± 0.9 ^{ef}	126.5 ± 0.7 ^{efgh}	123.1 ± 1.0 ^{de}	2.4 ± 0.1 ^g	2.7 ± 0.1 ^{def}	3.0 ± 0.1 ^{abcde}
	25	136.9 ± 4.0 ^h	140.4 ± 1.1 ^{cd}	143.0 ± 1.7 ^{bcd}	127.7 ± 1.0 ^{def}	127.0 ± 1.7 ^{efgh}	124.5 ± 0.8 ^{cde}	2.5 ± 0.1 ^{fg}	2.6 ± 0.1 ^{def}	2.5 ± 0.1 ^{fg}
	50	139.7 ± 2.5 ^{efgh}	137.2 ± 1.2 ^d	145.2 ± 0.9 ^{bcd}	124.9 ± 0.3 ^{fg}	124.3 ± 1.2 ^{efghi}	129.7 ± 1.4 ^{bcd}	2.6 ± 0.2 ^{efg}	2.6 ± 0.1 ^{def}	2.8 ± 0.1 ^{defg}
	100	140.8 ± 1.5 ^{efgh}	141.2 ± 1.5 ^{cd}	141.9 ± 2.3 ^{bcd}	126.8 ± 0.9 ^{def}	123.6 ± 0.6 ^{fghi}	125.0 ± 0.6 ^{cde}	2.9 ± 0.1 ^{cdefg}	2.8 ± 0.2 ^{bcdef}	2.7 ± 0.1 ^{efg}
16	12.5	150.8 ± 1.2 ^{cde}	141.7 ± 1.8 ^{cd}	142.5 ± 1.0 ^{bcd}	134.3 ± 1.8 ^{cde}	130.8 ± 1.4 ^{ef}	130.8 ± 0.6 ^{bc}	2.8 ± 0.1 ^{defg}	2.9 ± 0.1 ^{abcde}	3.1 ± 0.2 ^{abcde}
	25	156.1 ± 1.9 ^{bcd}	145.9 ± 2.5 ^{cd,†}	140.6 ± 1.2 ^d	140.8 ± 2.1 ^{bc}	131.9 ± 1.4 ^{de,†}	129.8 ± 1.2 ^{bcd}	3.0 ± 0.1 ^{cdef}	2.9 ± 0.1 ^{abcde,†}	3.0 ± 0.1 ^{cdef}
	50	149.2 ± 1.9 ^{defg}	141.0 ± 1.5 ^{cd}	144.3 ± 1.3 ^{bcd}	137.0 ± 1.9 ^c	129.8 ± 0.9 ^{ef}	130.9 ± 1.1 ^{bc}	3.0 ± 0.1 ^{cdef}	3.0 ± 0.0 ^{abcde}	3.4 ± 0.1 ^{abc}
	100	150.3 ± 1.8 ^{cdef}	143.7 ± 1.4 ^{cd}	145.0 ± 2.2 ^{bcd}	135.5 ± 3.2 ^{cd}	129.3 ± 1.3 ^{efg}	131.1 ± 1.0 ^{bc}	3.2 ± 0.1 ^{bcd}	3.0 ± 0.1 ^{abcde}	3.2 ± 0.1 ^{abcde}
24	12.5	162.2 ± 2.0 ^{ab}	160.5 ± 2.7 ^a	156.1 ± 2.2 ^a	149.6 ± 1.6 ^{ab}	154.5 ± 3.4 ^a	149.6 ± 4.3 ^a	3.1 ± 0.1 ^{bcd}	3.3 ± 0.1 ^{ab}	3.4 ± 0.1 ^{abc}
	25	160.1 ± 2.5 ^{abc}	156.1 ± 2.9 ^{ab,†}	155.2 ± 2.9 ^a	149.6 ± 2.0 ^{ab}	141.3 ± 2.7 ^{bc,†}	146.3 ± 2.4 ^a	3.3 ± 0.1 ^{bc}	3.0 ± 0.1 ^{abcde,†}	3.0 ± 0.1 ^{bcd}
	50	167.4 ± 3.2 ^a	159.5 ± 1.4 ^a	150.1 ± 0.9 ^{abc}	158.3 ± 2.4 ^a	147.5 ± 1.7 ^{ab}	136.7 ± 1.1 ^b	3.7 ± 0.1 ^{ab}	3.3 ± 0.1 ^a	3.5 ± 0.1 ^a
	100	165.3 ± 1.8 ^{ab}	147.6 ± 1.6 ^{bc}	150.5 ± 1.1 ^{ab}	155.6 ± 3.0 ^a	139.8 ± 1.3 ^{cd}	136.4 ± 1.0 ^b	4.0 ± 0.1 ^a	3.2 ± 0.1 ^{abc}	3.3 ± 0.1 ^{abc}

¹ Means ± SEM (N = 6; †N = 5; ‡N = 4). Different superscripts within each time point (column) demarcate significant (P < 0.05) differences according to the Tukey's HSD test based on two-way analysis of variance.

² Optimum feeding rate.

the most feed-restricted group exposed to 24 ppt were significantly higher than those of the non-feed-restricted group exposed to the same salinity; however, no significant feed restriction effect on plasma Na⁺ and K⁺ levels was found within the same time point (120 h) and salinity (24 ppt).

3.2.5. Gill and PC NKA activities

While no significant main effect of salinity on gill NKA activity at 12 h was observed, a significant main effect of feed restriction was detected, showing a decrease in activity with increasing feed restriction (Tables 2, 6). At 72 h, a significant interaction between feed restriction and salinity on gill NKA activity was observed. Although gill NKA activity of the more feed-restricted groups (12.5%, 25% OFR) exposed to 24 ppt was still not significantly different from the more feed-restricted groups exposed to 0 ppt, the activity of the less feed-restricted (50% OFR) and non-feed-restricted groups exposed to 24 ppt were significantly higher than that of the same feeding groups exposed to 0 ppt. At 120 h, a significant main effect of feed restriction and salinity was observed. The activity decreased with increasing feed restriction; however, the activity increased with increasing salinity except for that at 8 ppt. Although no statistical comparison of gill NKA activities between 72 and 120 h was

made, the change in gill NKA activity between 72 and 120 h appeared to differ between feed-restricted and non-feed-restricted groups. Gill NKA activity of the feed-restricted groups exposed to 24 ppt at 120 h were relatively upregulated in comparison to the activity at 72 h (in 12.5% OFR, from 5.1 ± 0.2 μmol ADP mg protein⁻¹ h⁻¹ at 72 h to 6.3 ± 0.4 μmol ADP mg protein⁻¹ h⁻¹ at 120 h; in 25% OFR, from 5.8 ± 0.6 to 7.1 ± 0.7; in 50% OFR, from 7.1 ± 0.6 to 8.5 ± 0.4). Conversely, gill NKA activity of the non-feed-restricted group exposed to 24 ppt at 120 h was relatively down-regulated with respect to the activity at 72 h (from 9.1 ± 0.5 to 6.9 ± 0.5).

The overall time course of changes in PC NKA activity responding to various salinities was comparable to that observed in gill NKA activity; however, a distinct pattern between gill and PC NKA activities was found (Tables 2, 6). At 12 h, no significant salinity effect on PC NKA activity was detected, which is comparable to gill NKA activity observed at the same time point. At 72 h, a significant interaction between feed restriction and salinity on PC NKA activity was found. Although it was shown that PC NKA activity of both the feed-restricted and non-feed-restricted groups exposed to 24 ppt was significantly higher than those of the same feeding groups exposed to 0 and 8 ppt, the more feed-restricted groups (12.5%, 25% OFR) exposed to 16 ppt was

Table 6
Interaction effect means of gill and pyloric caeca Na⁺/K⁺-ATPase (NKA) activities of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure.¹

Salinity (ppt)	OFR ² (%)	Gill NKA activity (μmol ADP mg protein ⁻¹ h ⁻¹)			Pyloric caeca NKA activity (μmol ADP mg protein ⁻¹ h ⁻¹)		
		12 h	72 h	120 h	12 h	72 h	120 h
0	12.5	4.9 ± 0.2 ^{ab}	3.9 ± 0.3 ^{cdef}	3.7 ± 0.3 ^{efgh}	5.0 ± 0.4 ^{ab}	3.3 ± 0.6 ^f	4.1 ± 0.1 ^{efg}
	25	4.8 ± 0.2 ^{ab,†}	3.4 ± 0.7 ^{def}	4.0 ± 0.5 ^{efgh}	4.2 ± 0.5 ^{ab,†}	3.4 ± 0.1 ^f	3.9 ± 0.2 ^{efg}
	50	4.6 ± 0.4 ^{ab}	4.7 ± 0.3 ^{cdef}	4.6 ± 0.5 ^{def}	3.2 ± 0.3 ^f	3.2 ± 0.3 ^f	3.8 ± 0.3 ^{fg}
	100	5.5 ± 0.5 ^{ab}	4.7 ± 0.4 ^{cdef}	5.1 ± 0.5 ^{cdef}	5.7 ± 0.4 ^a	4.1 ± 0.3 ^{ef}	3.3 ± 0.1 ^g
8	12.5	4.5 ± 0.4 ^{ab}	2.9 ± 0.3 ^f	2.1 ± 0.2 ^h	4.4 ± 0.3 ^{ab}	5.3 ± 0.2 ^{def}	6.1 ± 0.9 ^{cde,†}
	25	5.1 ± 0.2 ^{ab}	3.0 ± 0.3 ^{ef}	2.3 ± 0.4 ^{gh}	3.4 ± 0.2 ^b	4.3 ± 0.5 ^{ef}	5.8 ± 0.3 ^{cde}
	50	4.6 ± 0.5 ^{ab}	2.7 ± 0.3 ^f	2.1 ± 0.2 ^h	5.1 ± 0.2 ^{ab,†}	3.7 ± 0.2 ^f	4.8 ± 0.3 ^{defg,†}
	100	5.4 ± 0.4 ^{ab}	4.2 ± 0.6 ^{cdef}	3.1 ± 0.4 ^{fgh}	4.9 ± 0.2 ^{ab}	4.6 ± 0.4 ^{ef}	4.4 ± 0.3 ^{efg}
16	12.5	4.2 ± 0.1 ^b	4.8 ± 0.4 ^{cdef}	4.2 ± 0.3 ^{efg}	4.7 ± 0.4 ^{ab}	9.1 ± 0.6 ^{bc}	7.9 ± 0.3 ^{c,†}
	25	4.0 ± 0.2 ^b	5.1 ± 0.2 ^{bcd}	4.5 ± 0.4 ^{def}	3.9 ± 0.6 ^{ab}	7.3 ± 0.6 ^{cd}	7.3 ± 0.4 ^{c,†}
	50	5.7 ± 0.8 ^{ab}	5.8 ± 0.4 ^{bc}	5.1 ± 0.2 ^{bcd}	5.6 ± 0.4 ^a	5.8 ± 0.3 ^{def}	7.7 ± 0.6 ^c
	100	5.4 ± 0.5 ^{ab}	5.3 ± 0.4 ^{bcd}	5.0 ± 0.3 ^{cdef}	4.7 ± 0.3 ^{ab,†}	6.6 ± 0.4 ^{cde}	6.6 ± 0.3 ^{cd}
24	12.5	4.4 ± 0.6 ^{ab,†}	5.1 ± 0.2 ^{bcd}	6.3 ± 0.4 ^{bcd}	4.7 ± 0.7 ^{ab}	13.5 ± 0.9 ^a	14.5 ± 0.6 ^a
	25	4.7 ± 0.3 ^{ab}	5.8 ± 0.6 ^{bc}	7.1 ± 0.7 ^{ab}	3.9 ± 0.2 ^{ab}	11.0 ± 0.6 ^{ab,†}	14.8 ± 0.5 ^a
	50	5.6 ± 0.4 ^{ab}	7.1 ± 0.6 ^{ab,†}	8.5 ± 0.4 ^a	5.4 ± 0.5 ^a	12.0 ± 0.5 ^{ab,†}	13.2 ± 0.5 ^{ab}
	100	6.4 ± 0.3 ^a	9.1 ± 0.5 ^a	6.9 ± 0.5 ^{abc}	4.9 ± 0.2 ^{ab}	11.3 ± 0.7 ^{ab}	12.1 ± 0.4 ^b

¹ Means ± SEM (N = 6; †N = 5). Different superscripts within each time point (column) demarcate significant (P < 0.05) differences according to a Tukey's HSD test based on two-way analysis of variance.

² Optimum feeding rate.

significantly higher than the same feeding groups exposed to 0 and 8 ppt. This was the opposite response to that observed in gill NKA activity at the same treatments (72 h, 24 ppt). At 120 h, a significant main effect of feed restriction and salinity on PC NKA activity was detected, showing that the activity significantly increased with increasing feed restriction and salinity. Although salinity had a similar effect on both gill and PC NKA activities at 120 h, a trend was shown as an inverse effect of feed restriction on PC NKA activity with respect to gill NKA activity.

3.2.6. Plasma cortisol

At 12 h, a significant main effect of salinity was detected, showing a significantly elevated plasma cortisol in the group exposed to 24 ppt only (Table 2, Fig. 3).

3.2.7. Regression analysis on the relationship between a response variable and body size

Datasets of all osmoregulatory measurements were tested using model [2] in order to evaluate the relationship between the measurement (response variable) and body weight (predictor variable). Significant outcomes were observed for plasma osmolality and Cl^- at 24 ppt only. Details of the outcomes described below are for plasma osmolality only because the pattern of responses of the two variables was similar, and plasma Cl^- values influence plasma osmolality values.

Although no difference between slopes or intercepts of all possible pairs of feed restriction treatments (i.e., 12.5% OFR vs 25% OFR; 12.5% vs 50%; 12.5% vs 100%; 25% vs 50%, 25% vs 100%, 50% vs 100%) was found at 12 h (Fig. 4A), a significant effect of feed restriction on the relationship between plasma osmolality and body weight was detected at 72 and 120 h (Fig. 4B and C, respectively). At 72 h, pairwise comparisons, using contrast statements with the use of model [3] (when both slopes and intercepts are significant), showed that the slope and intercept of the most feed-restricted group (12.5% OFR) were significantly different from those of the less feed-restricted (50% OFR) and non-feed-restricted groups (100% OFR). In addition, the slope (-0.32 ± 0.06 ; estimate \pm SEM) of the most feed-restricted group was the only treatment significantly different from 0 (P -value = 0.0002). At 120 h, the pairwise comparisons using model [3] showed that the slope and intercept of the most feed-restricted group were significantly different from those of the non-feed-restricted group. As well, the slope (-0.48 ± 0.12) of the most feed-restricted group only was significantly different from 0 (P -value = 0.0007).

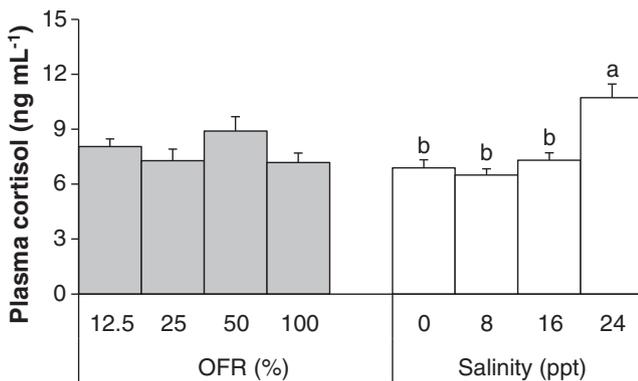


Fig. 3. Means of plasma cortisol of juvenile white sturgeon after the four-week feed restriction trial and five-day salinity exposure for the main effects of four optimum feeding rates (OFR, %) and four salinities at 12 h. The 12-h exposure data was shown only because no significant difference was detected in the other time exposures. Lower case letters on the standard error bars represent significant differences (Tukey's HSD test based on two-way analysis of variance; $P < 0.05$) within the main effect of salinity treatments (open bars).

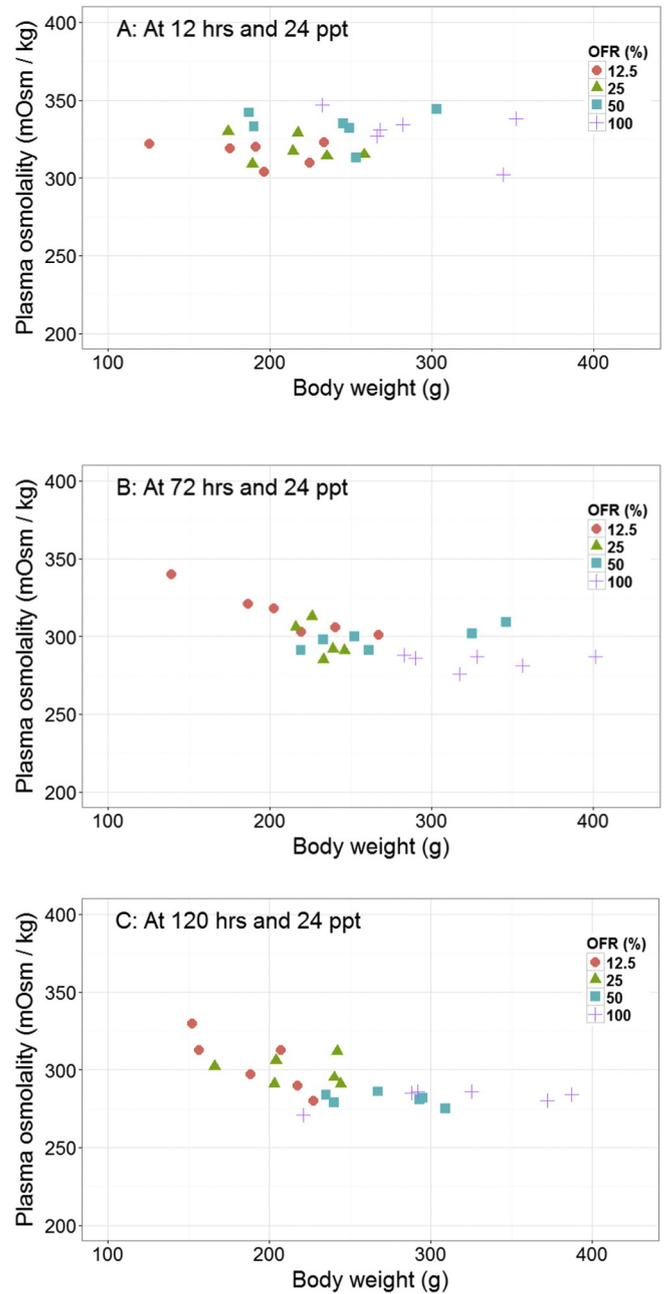


Fig. 4. Plots showing the relationship between plasma osmolality and body weight observed in feed-restricted (12.5%, 25%, 50% of optimum feeding rate (OFR) and non-feed-restricted (100% OFR) groups, acutely exposed to 24 ppt for 12, 72, and 120 h. At 12 h (top; A), no significant difference between slopes or intercepts of all possible pairs of feed restriction treatments (i.e., 12.5% vs 25%; 12.5% vs 50%; 12.5% vs 100%; 25% vs 50%; 25% vs 100%; 50% vs 100% OFR) were found. At 72 h (middle; B), the slope and intercept of the most feed restricted group (12.5% OFR) was significantly different from those of the less feed-restricted (50% OFR) and non-feed-restricted (100% OFR) groups. The slope (-0.32 ± 0.06 ; estimate \pm SEM) of the most feed-restricted group only was significantly different from 0 (P -value = 0.0002). At 120 h (bottom; C), the slope and intercept of the most feed-restricted group were significantly different from those of the non-feed-restricted group. The slope (-0.48 ± 0.12) of the most feed-restricted group only was significantly different from 0 (P -value = 0.0007).

4. Discussion

4.1. Effects of feed restriction on nutritional status

In general, when fish are food limited, ingested foods and energy reserves are primarily mobilized and catabolized to meet the requirements for maintenance and other activities (e.g., foraging,

reproduction). As a result, nutritional status indices, including growth performance, body composition, body energy, and plasma metabolites, are altered. In the current study, the four-week feed restriction significantly altered SGR, FCR, CF, HSI, body moisture and lipid, body energy, and plasma protein and TAG in juvenile white sturgeon. Furthermore, the most feed-restricted group (12.5% OFR) exhibited a negative SGR and FCR, indicating that energy expenditures were devoted mainly for maintenance instead of growth or energy accumulation. The decreased CF and HSI values with increased feed restriction are likely related to a reduced anabolism of protein and lipids in muscle and of glycogen and lipids in liver (Bar and Volkoff, 2012; Hung et al., 1997; Lee et al., 2015). Similar alterations caused by feed restriction have been observed in other sturgeon species (Haller et al., 2015; Hung and Lutes, 1987; Hung et al., 1997; Lee et al., 2015) and in fishes, including Arctic charr (*Salvelinus alpinus*; Miglavs and Jobling, 1989), Atlantic salmon (*Salmo salar*; Einen et al., 1999), Atlantic cod (*Gadus morhua* L.; Hatlen et al., 2007), European sea bass (*Dicentrarchus labrax* L.; Eroldoğan et al., 2004), gilthead sea bream (Company et al., 1999), rainbow trout (*Salmo gairdneri* Richardson; Storebakken and Austreng, 1987), striped bass (*Morone saxatilis*; Hung et al., 1993), and tropical bagrid catfish (*Mystus nemurus*; Ng et al., 2000).

Body composition changes are connected to exogenous and endogenous energy availability, and studying these changes through manipulative experiments of feed ration/reduction provides a strong indication of nutritional status in fishes. Bar and Volkoff (2012) summarized sequential compositional changes associated with starvation as follows: “Phase I. A short transient stage where both protein tissues and fat reserves are mobilized”, “Phase II. A longer steady state with mobilization of fat as the main source of energy, that lasts until fat sources reach a critical threshold”, and “Phase III. A state in which the mobilization of protein tissue is largely increased as this becomes the main source of energy”. In the current study, body lipids significantly decreased with increasing feed restriction; however, no change in body protein was detected. Body moisture showed an inverse relationship with respect to body lipids. This can be explained as the maintenance of cell functionality by maintaining cell size through replacement of moisture after loss of organic matter (McCue, 2010). Body energy level followed the trend what the body lipids showed.

A previous study conducted in green sturgeon, performed in parallel to the current study (similar experimental design, determined variables, and analytical methods), showed that body composition changes observed in green sturgeon (Haller et al., 2015) were not comparable to those exhibited in white sturgeon. Juvenile green sturgeon exposed to the most feed-restricted treatment (i.e., 12.5% OFR) showed some mortality, and significant loss of body protein along with a severe shift in body lipids to a threshold level suggesting that the majority of the remaining lipids were likely phospholipids which are crucial in cell membrane structure (Haller et al., 2015). According to the aforementioned Bar and Volkoff's summary (2012), the most feed-restricted group of white sturgeon was likely transitioning into “Phase II”; however, under the same feed restriction condition green sturgeon had further transitioned into “Phase III”.

Inherent physiological differences between the two sturgeon species may explain why these distinct responses were observed. Specifically, because green sturgeon, a truly anadromous species (Moyle, 2002), undergo physiological transformations to prepare for entry to full-strength seawater (Allen et al., 2011), this physiological event would lead to relatively higher energy expenditures in comparison to white sturgeon, a semi-anadromous species (Haller et al., 2015; Moyle, 2002). Faster growth rates observed in younger green sturgeon compared to white sturgeon (Deng et al., 2002) is thought to be a strategy for ocean migration, which in turn would result in less energy accumulated as lipid reserves (Haller et al., 2015). Indeed, different growth rates were observed between the optimally fed groups in both species, showing that green sturgeon (SGR: $1.89 \pm 0.0\%$; mean \pm SEM; Haller et al., 2015) grew faster than white sturgeon (SGR: $1.55 \pm 0.02\%$; the current

study). In addition, we saw a large difference in body energy values in which the level in green sturgeon (4.3 kJ g^{-1} ; Haller et al., 2015) was almost two times lower than that in white sturgeon (8.0 kJ g^{-1} ; the current study). This can be supported by the existence of a gonadal fat body, known as a unique energy storage organ observed in many Acipenseriform fishes (Scarnecchia et al., 2007), in white sturgeon (Lee et al., 2015) but not likely in green sturgeon during the juvenile life stage (Haller et al., 2015).

Plasma metabolites can be used as another good indicator of nutritional status of fishes; however, careful interpretations are necessary because of their transiency (Congleton and Wagner, 2006; McCue, 2010). Plasma glucose level is relatively stable in comparison to other metabolites (e.g., protein, TAG) under energy restriction because glucose is an essential nutrient for functions of nervous and cellular systems and is replenished by gluconeogenic pathways (Moon and Foster, 1995). In the current study, no change in plasma glucose (24-h postprandial) was observed after the four-week feed restriction trial; however, plasma protein and TAG in the most feed-restricted group were significantly lower than those in the other groups. A previous study conducted in larger white sturgeon (initial body weight: ca. 360 g) fed at various feeding rates (0.4%–2.0% body weight per day) for 10 weeks showed comparable responses to what we saw in the current study, including no effect of feeding rate on plasma glucose levels but alterations in plasma protein and TAG at the lowest feeding rate (Lee et al., 2015).

4.2. Effects of altered nutritional status on salinity tolerance

Through the 120-h salinity exposure trial, we demonstrated that altered nutritional status decreased salinity tolerance of juvenile white sturgeon. Many of the osmoregulatory measurements, especially muscle moisture, plasma osmolality and ions (Na^+ , Cl^- , K^+), and gill and PC NKA activities, clearly showed correlations between nutritional status and osmoregulatory ability.

Maintenance of body water is a key component for fishes living in a hyperosmotic environment. To avoid dehydration from osmotic water losses, it is necessary to drink surrounding water (e.g., seawater) and eliminate excess salts, accompanied by various behavioral and physiological actions (Whittamore, 2012). Numerous studies have shown that salinity acclimation is energy-dependent, constituting a wide range (ca. 10 to >50%) of a fish's total energy budget (see a review by Boeuf and Payan, 2001). Muscle moisture has been used as an indicator of osmoregulation in sturgeon species, typically exhibiting an immediate decline after hyperosmotic exposure and recovery over time (Allen and Cech, 2007; Altinok et al., 1998; Haller et al., 2015; Martínez-Álvarez et al., 2002; Sardella and Kültz, 2014). In the current study, we observed a typical response in which muscle moisture significantly decreased with increasing salinity except for after 12 h at 8 ppt salinity exposure, and then recovered to a level of the control salinity group (0 ppt) over the 120-h exposure. Interestingly, a significant interaction between feed restriction and salinity on muscle moisture was detected after the 72-h exposure. Although muscle moisture content of feed-restricted (25%, 50% OFR) and non-feed-restricted groups exposed to 24 ppt was not different from the same feeding groups exposed to 0 ppt, the content of the most feed-restricted group exposed to 24 ppt was significantly lower than that of the most feed-restricted group exposed to 0 ppt. This indicates slower recovery of muscle moisture from osmotic water losses associated with lower nutritional status. This energy-dependent osmoregulatory ability is supported by additional findings discussed below.

Although a combination of osmotic water losses and increased plasma osmolality likely induce hematological changes (e.g., hypovolemia), inconsistent results are being reported in sturgeon species (Allen and Cech, 2007; Altinok et al., 1998; Amiri et al., 2009; Haller et al., 2015; Martínez-Álvarez et al., 2002; Penny and Kieffer, 2014; Zarejabad et al., 2010), which may be attributed to several possible reasons,

including species specificity, size/age, methodology (e.g., gradual vs acute exposure), and/or sampling time. In the current study, temporal response of hemoglobin to salinity was an initial increase (12 h) with increasing salinity, but hemoglobin decreased with increasing salinity in a longer exposure (120 h). A significant main effect of feed restriction on hematocrit was found at the 72-h exposure only, and the hematocrit value of the non-feed-restricted group was higher than that of the feed-restricted groups. On the other hand, no significant effect of feed restriction on hemoglobin was found at any time point. Given these transient and inconsistent responses to feed restriction or salinity over time points, it is difficult to explain the underlying reasons and therefore further investigation is required.

Salinity-induced energy metabolism includes a change of plasma metabolites, reflecting energy mobilizations between osmoregulatory (e.g., gill, intestine) and non-osmoregulatory (e.g., liver) organs in order to support osmoregulatory activities (Sangiao-Alvarellos et al., 2005; Tseng and Hwang, 2008). Although a transient disturbance in plasma glucose and lactate is expected after hyperosmotic exposure, the magnitude and timing of peak responses have been shown to be species specific (Haller et al., 2015; Penny and Kieffer, 2014; Sangiao-Alvarellos et al., 2005; Soengas et al., 1993). In the current study, a significant increase from 87.2 ± 2.4 (mean \pm SEM) at 0 ppt to 147.6 ± 4.7 mg dL⁻¹ at 24 ppt in plasma glucose of juvenile white sturgeon following the 12-h exposure was detected, and returned to near control levels by 72-h exposure; both comparable to previous studies (Haller et al., 2015; Penny and Kieffer, 2014; Sangiao-Alvarellos et al., 2005; Soengas et al., 1993). In addition, plasma glucose of white sturgeon exposed to 16 and 24 ppt following a 72-h exposure remained significantly lower than 0 ppt. On the other hand, no interactive effect between feed restriction and salinity on plasma glucose was detected over the different time points. This may be attributed to negligible effects from the four-week feed restriction, discussed earlier, or little effect from starvation on plasma glucose during the five-day salinity exposure, possibly explained by glucose homeostasis (Congleton and Wagner, 2006; McCue, 2010). In contrast, a parallel study conducted in juvenile green sturgeon showed a significant interaction between feed restriction and salinity on plasma glucose (Haller et al., 2015). These distinct results between the two sturgeon species may be attributed to more pronounced effects of feed restriction in green sturgeon possibly related to species specific life history strategies and associated with physiological differences. On the other hand, inconsistent plasma lactate responses of juvenile white sturgeon to feed restriction and salinity were observed over time points. This result indicates that plasma lactate played a role in supplying energy requirements derived from feed restriction and/or salinity during salinity acclimation; however, it seems difficult to make a general statement of stress-related responses in plasma lactate of juvenile white sturgeon on the basis of our study.

A time course of changes in plasma osmolality of juvenile white sturgeon responding to various salinities can be compared to previous studies (Haller et al., 2015; Penny and Kieffer, 2014; Sangiao-Alvarellos et al., 2005). We observed a significant interaction between feed restriction and salinity on plasma osmolality at the 72- and 120-h exposures, showing that the most feed-restricted group recovered more slowly from osmotic stress (24 ppt only) than did the non-feed-restricted group. This agrees with findings in green sturgeon (Haller et al., 2015) as well as supports the slow recovery in muscle moisture from osmotic stress observed in the same group of white sturgeon. On the other hand, Haller et al. (2015) showed that there was no difference between 0 and 16 ppt in plasma osmolality of green sturgeon during the salinity exposure trial although a significant increase by 16 ppt was detected in white sturgeon at the 12-h exposure. In addition, no disturbance by feed restriction on recovery of plasma osmolality from salinity stress (32 ppt) was found in green sturgeon at the 120-h exposure (Haller et al., 2015), whereas a significant effect of feed restriction was still detected in white sturgeon at a lower salinity (24 ppt). In other words, regardless of relatively lower nutritional status observed in

green sturgeon ("Phase III") than white sturgeon ("Phase II"), green sturgeon showed higher tolerance to salinity stress. These distinct and interesting results may be attributed to the physiological difference between green (truly anadromous) and white (semi-anadromous) sturgeon as discussed above. However, these data do not necessary exclude the possibility that we would see similar salinity tolerance performance in green and white sturgeon if they were feed-restricted for a longer period than four weeks.

In the current study, it was shown that an overall time course of changes in plasma ions (Na⁺, Cl⁻, K⁺) of juvenile white sturgeon responding to various salinities were similar to that observed in plasma osmolality; however, a distinct response between plasma osmolality and Cl⁻, and Na⁺ and K⁺ to feed restriction was found. At the 120-h exposure, no effect of feed restriction on recovery of plasma Na⁺ and K⁺ from salinity stress (24 ppt) was detected. However, slower recovery of plasma osmolality and Cl⁻ at the same salinity exposure was found in the more feed-restricted groups (12.5%, 25% OFR). The lack of energy-dependent response observed in plasma Na⁺ and K⁺ could be associated with the essential role of NKA in ionoregulation (Allen and Cech, 2007; Haller et al., 2015).

Many studies have investigated the magnitude and timing of gill NKA activity in fishes such as Atlantic salmon (Mancera and McCormick, 2000), European sea bass (Jensen et al., 1998), gilthead sea bream (Laiz-Carrión et al., 2005; Sangiao-Alvarellos et al., 2005), and killifish (*Fundulus heteroclitus* L.; Jacob and Taylor, 1983), and the responses are highly variable. In the current study, gill NKA activity was negligibly affected by salinity at the 12-h exposure. However, gill NKA activity remained significantly upregulated at 24 ppt in comparison to that at 0 ppt after 72 h of salinity exposure. This lack of upregulation by higher salinities (16, 24 ppt) at 12 h may be the result of insufficient time required for proliferation and/or development of chloride cells (De Renzis and Bornancin, 1984; Mancera and McCormick, 2000).

On the other hand, a significant interaction between feed restriction and salinity on gill NKA activity was detected at the 72-h exposure. Furthermore, a significant main effect of feed restriction on gill NKA activity was detected at each time point, exhibiting that the activity of the most feed-restricted group was significantly lower than that of the non-feed-restricted group. This altered gill NKA activity associated with lower nutritional status agrees with previous studies in rainbow trout (Jürss et al., 1986), tilapia (Kültz and Jürss, 1991), and gilthead sea bream (Polakof et al., 2006). The slower recovery from osmotic stress found in the current study is likely linked to disturbed NKA function in osmo- and ionoregulation, associated with nutritional status. In addition, the combined effect of feed restriction and salinity, likely resulting in high energy demands in gills should also be taken into consideration (Polakof et al., 2006). Glycogen metabolism in gills and liver has been shown to play an important role during salinity acclimation in gilthead sea bream (Polakof et al., 2006) and tilapia (Chang et al., 2007). Besides, glycogen metabolism, which takes place in muscle and liver of white sturgeon (Hung et al., 1997), is also known to be readily affected by energy restriction. Further investigation on interactive effects of energy restriction and salinity on glycogen metabolism in various body parts (e.g., gills, liver, muscle) of white sturgeon will enhance our understanding on the mechanisms driving the alterations of gill NKA activity. Although an overall time course of changes in PC NKA activity of juvenile white sturgeon responding to various salinities was similar to that observed in gill NKA activity, an inverse response to feed restriction was found at the 72- and 120-h exposures. This agrees with what Haller et al. (2015) reported in green sturgeon exposed to 32 ppt in which increased PC NKA activity by feed restriction along with salinity exposure was likely associated with compensatory effect driven from elevated plasma cortisol. A speculation can be made on the basis of this unexpected and interesting finding such that upregulation in PC NKA activity associated with feed restriction could play a role in compensation for the impaired ionoregulatory capacity at the gills. Further investigation is needed to understand this possible compensatory

mechanism of PC NKA in sturgeon when faced with feed restriction and salinity stress.

Elevated plasma cortisol with salinity stress (24 ppt only) in juvenile white sturgeon can be explained by its role in metabolic regulation and acclimation to a new environment (reviewed in fishes by McCormick, 2001; Mommsen et al., 1999). Although previous studies have shown that energy restriction increased plasma cortisol concentrations, a lack of change in plasma cortisol responding to feed restriction was observed in the current study, which can be associated with gluconeogenesis for meeting energy demands (Haller et al., 2015; Mommsen et al., 1999; Polakof et al., 2006). In addition, this can be attributed to the limited sampling time points (i.e., in the current study, the first sampling time was 12 h post-salinity exposure) for detection of a time course change in plasma cortisol. A previous study conducted in white sturgeon demonstrated that a significant increase in plasma cortisol was detected in 15–30 min following exposure to various stresses (e.g., water reduction, handling) (Belanger et al., 2001).

Body size, developmental stage, and environmental cues are known to be major determinants for the development of salinity tolerance in various fish species (reviewed in Zydlewski and Wilkie, 2013). Although the transition from freshwater to seawater is supported by a suite of physiological processes, it has been shown to mainly depend on body size in tilapia (Watanabe et al., 1985), Gulf of Mexico sturgeon (*Acipenser oxyrinchus de sotoi*; Altinok et al., 1998), green sturgeon (Allen and Cech, 2007; Allen et al., 2009), white sturgeon (Amiri et al., 2009; McEnroe and Cech, 1985), and salmonid fishes (Parry, 1958). Allen et al. (2009) postulated that decreasing gill surface area with increasing body size which results in reduced diffusional surface likely alleviate osmotic stress in larger green sturgeon. A relationship between energy content and body size is possibly linked to this body size-dependent osmoregulatory ability because body size change is correlated to nutrient partitioning in different body parts in association with energy availability (Lee et al., 2015; Storebakken et al., 1991). Our aforementioned findings, showing that the lower nutritional status (i.e., smaller body weight) decreased salinity tolerance of white sturgeon, support this body size-dependent osmoregulatory ability. In addition, the regression analysis performed to test the relationship between osmoregulatory performance and body weight detected the size-dependent performance in the most feed-restricted white sturgeon such that there was a significant negative relationship between plasma osmolality and body weight (Fig. 4). Further, an ecologically important interpretation from these data can be made such that body weights of 200–300 g are necessary for juvenile white sturgeon to maximize osmoregulatory capacity at a salinity of 24 ppt.

5. Conclusion

Due to current and projected food web alterations and salinity increases in the SFBD, it is important to understand whether a lower nutritional status would decrease the salinity tolerance of indigenous white sturgeon. Through a four-week feed restriction trial, nutritionally manipulated white sturgeon showed energy-dependent osmoregulatory activity (i.e., slower recovery from osmotic stress) during acute salinity exposures. The slowest osmoregulatory recovery patterns (e.g., muscle moisture, plasma osmolality and ions (Na^+ , Cl^- , K^+), and gill and PC NKA activities) were found in the most feed restricted group exposed to 24 ppt. This energy-dependent osmoregulatory performance was further supported by regression analysis showing a significant negative relationship between plasma osmolality and body weight for the most feed-restricted white sturgeon. Little information is available on the distribution of juvenile white sturgeon in the SFBD, and it is suggested that they are present in Suisun and San Pablo Bays as well as the western delta (Israel et al., 2009). Current salinity in the Bays is 22–28 ppt (estimated in December 2013 by Peng et al., 2014), similar to the highest salinity level (24 ppt) tested in the current study. Taken together, the result of the current study suggests that

increasing salinity as well as reduced food availability in their habitats may influence (i.e., delay) the onset of transition from estuary to bay environments, which should be taken into consideration for conservation and management of this valuable species.

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