The Physiological Responses of Green Sturgeon (*Acipenser medirostris*) to Potential Global Climate Change Stressors

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ABSTRACT

The green sturgeon (*Acipenser medirostris*) is an anadromous species with a distinct population segment in the San Francisco Bay–Sacramento River Delta that is currently listed as threatened. Although this species is able to tolerate salinity challenges as soon as 6 mo posthatch, its ability to deal with unpredictable salinity fluctuations remains unknown. Global climate change is predicted to result in large freshwater (FW) flushes through the estuary during winter and greater tidal influence during the summer. We exposed green sturgeon acclimated to 15 (EST) or 24 (BAY) g/L salinity to a rapid FW influx, where salinity was reduced to 0 g/L in 3 h in order to simulate the effect of the "winter" scenario. Both groups survived, enduring a 10% plasma osmolality reduction after 3 h. BAY-acclimated sturgeon upregulated both Na^+, K^+-ATPase (NKA) activity and caspase 3/7 activity, but no changes were observed in the EST-acclimated fish. In addition, we exposed FW-acclimated sturgeon to a dual 12-h salinity fluctuation cycle (0–24–0 g/L) in order to simulate the effect of greater tidal influence. At 6 h, the sturgeon showed a significant increase in plasma osmolality, and branchial NKA and caspase 3/7 activities were increased, indicating an acclimation response. There was no acclimation at 18 h, and plasma osmolality was higher than the peak observed at 6 h. The second fluctuation elicited an upregulation of the stress proteins ubiquitin and heat shock 70-kDa protein (HSP 70). Sturgeon can acclimate to changes in salinity; however, salinity fluctuations resulted in substantial cellular stress.

Introduction

The green sturgeon (*Acipenser medirostris*) is a long-lived species that spends a great deal of time migrating between freshwater (FW) and seawater (SW). As an anadromous species, it hatches in FW and spends as much as 3 yr within river or estuarine habitats before migrating to the ocean as an adult (Moyle 2002). This species is widely distributed along the west coast of North America, where it is divided into two distinct population segments (DPSs). The northern DPS consists of individuals that spawn in the Klamath and Rogue Rivers of California and Oregon, and the southern DPS consists entirely of individuals that spawn in the Sacramento River system of California. The southern DPS is currently listed as threatened under the United States Endangered Species Act, and observations of this species in the San Francisco (SF) Bay–Sacramento River Delta are rare.

Green sturgeon deal with osmotic challenges in their regular movements between FW and SW, because they are out of balance with both (Allen and Cech 2007). As a result, physiological adjustments are made in order to offset osmotic water gain and diffusive ion loss in FW and osmotic water loss with diffusive ion gains in SW. This species is considered to be the most anadromous of the sturgeon species (Kelly et al. 2007), but the current status of green sturgeon populations has made studies regarding their osmo- and ionoregulatory capacity difficult. Previous work has shown that green sturgeon generally have mechanisms similar to those of teleosts with respect to osmo- and ionoregulation during 2-wk acclimation experiments (Sardella and Kültz 2009). When congeneric Adriatic sturgeon (*Acipenser naccarii*) were SW acclimated, ionocytes (ICs) were larger and more abundant than those in FW-acclimated fish (Carmona et al. 2004), and Martinez-Álvarez et al. (2005) showed that Na^+, K^+-ATPase (NKA) activity also increased following SW transfer. NKA is a basolateral membrane-bound enzyme found in high abundance within ICs, where it serves as the driving force for ion excretion and absorption in SW- and FW-acclimated fishes, respectively (Perry 1997; Marshall 2002; Dymowska et al. 2012). While there is less debate regarding the mechanisms of ion secretion in SW-acclimated fishes, there are variations in how ions are absorbed in FW-acclimated fishes (Dymowska et al. 2012; Hiroi and McCormick 2012), and the precise mechanism used by sturgeon species has yet to be described. At ages as low as 6 mo, green sturgeon can acclimate to salinity increases (Allen and Cech 2007; Sardella and Kültz 2009), but how they respond to unpredictable salinity fluctuations, such as those predicted to occur with a changing global climate, remains to be investigated.

The southern DPS of the green sturgeon primarily inhabits the SF Bay–Sacramento River Delta watershed, which represents California's primary hydrologic system. This system plays critical roles in agricultural irrigation and municipal water supply while still providing habitat for fish and wildlife. The threat

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posed by global climate change has become increasingly concerning, because variations of hydrological and meteorological factors within the SF Bay–Delta region are forecasted to complicate maintenance and/or restoration of its inhabitants (Knowles and Cayan 2002, 2004). Increased environmental temperature will have sweeping effects on the SF Bay–Delta watershed, the greatest concern being a shift in the dominant precipitation type from snow to rain at the highest altitudes of the surrounding mountains. This will result in less water storage as snow pack during winter and less runoff due to snow melt during the summer (Knowles and Cayan 2002, 2004; Schlenk and Lavado 2011; Wagner et al. 2011). The effect on the estuary will be a larger and less stable salinity range throughout the year, which will confound the direct effect of temperature. For example, greater runoff during the winter will increase overall flow and decrease salinity through the estuary. The opposite problem will occur during the spring and summer: less snow melt and FW runoff mean wider variation in salinity, resulting from a larger tidal-cycle influence (Knowles and Cayan 2002, 2004; Schlenk and Lavado 2011). Salinity and temperature are important determinants of water quality and population dynamics, so variation in these factors may lead to deleterious effects on spawning success, development, growth, and survival of fish species that inhabit sensitive areas of the system.

The goal of this study was to measure survival and the sublethal responses of green sturgeon when exposed to the forecasted conditions of global climate change in the SF Bay–Delta watershed. We simulated a "winter" scenario, by reducing salinity over a 3-h period to FW and monitored the response of sturgeon acclimated to 15- or 24-g/L salinity for 24 h. To simulate a "summer" scenario, fish acclimated to FW were exposed to two 12-h cycles of salinity fluctuation, from 0 to 24 and back to 0 g/L. Sturgeon were sampled throughout the exposures in order to investigate which sublethal indicators of osmotic stress were prevalent and to what degree sturgeon were capable of tolerating the varying salinity.

Material and Methods

Salinity Acclimation

Captive adult green sturgeon housed at the University of California, Davis (UCD) Center for Aquatic Biology and Aquaculture were artificially spawned in the spring of 2007. The resulting progeny were reared in FW and grown to a size of 121 $\pm$ 5.0 g before experiments were conducted. Seventy-five individuals were removed from the flow-through rearing tanks and divided into three FW recirculation systems at the start of the experiment. In two tanks, salinity was increased to 15 g/L (EST, i.e., Delta) or 24 g/L (BAY) in stepwise fashion via 50% water change (4 g/L per day) until the target salinity was reached and then held for 2 wk; the remaining acclimation tank was kept at 0 g/L (FW). Acclimation tanks were filtered by mechanical, chemical, and biological filters, and water quality was closely monitored. We used slow-timed automatic feeders to provide small quantities of food during the acclimation period, and feeding was stopped 24 h before the experiments. Salinity was manipulated with Deep Ocean synthetic sea salt and measured with a light refractometer. After two weeks of acclimation, sturgeon were transferred to experimental tanks.

Salinity Manipulation Experiments

The "winter" scenario was simulated by an FW flush over a 3-h period, and the "summer" scenario was simulated as two salinity fluctuation cycles within 24 h. The FW-flush experiments were carried out in circular 1.22-m (diameter) fiberglass tanks initially containing either EST or BAY water and green sturgeon acclimated to the respective salinities. At start time (0 h), FW flow was initiated at specific rates, adjusted on the basis of both calculations and previous trials, in order for salinity to reach 0 g/L in both tanks at precisely 3 h. We sampled both EST- and BAY-acclimated sturgeon at 0, 3, 6, 12, and 24 h following initiation of FW influx. The "summer" scenario was simulated with FW-acclimated sturgeon in circular 1.22-m fiberglass tanks. At start time (0 h), we initiated flow of concentrated SW (90 g/L) into the tank at a rate of 200 mL/h. With constant mixing, the salinity of the exposure tank increased steadily and peaked at 24 g/L salinity at 6 h, when FW input was then reinitiated. With consistent flow and constant mixing, we were able to steadily decrease salinity back to 0 g/L at 12 h. This manipulation was repeated for a complete 24-h cycle, and sturgeon were removed and terminally sampled at 0 (FW), 6 (SW), 12 (FW), 18 (SW), and 24 h (FW).

Sturgeon were killed with a lethal dose of MS-222, as approved by the UCD Institutional Animal Care and Use Committee (protocol 12649) and sampled. Blood was quickly collected directly into heparinized capillary tubes after the tail was severed. After blood collection, we perfused the ventral aorta with ice-cold phosphate-buffered saline (PBS) in order to clear blood from the gills. Gills were then dissected, the second arch was rapidly frozen in liquid nitrogen, and the third arch was fixed with PBS-buffered paraformaldehyde (4%). To determine muscle hydration, a section of the dorsal epaxial muscle (approx. 0.5 g) was dissected, and the skin was removed. The muscle was washed with distilled water quickly, patted dry, and placed into preweighed aluminum foil to dry for 96 h. The difference in weights before and after drying was expressed as percent water content, as described by Sardella et al. (2004*a*).

Enzyme Assays

Gill tissue was homogenized in 1 mL of SEID buffer (250 mM sucrose, 10 mM EDTA·Na_2, 50 mM imidazole, pH 7.3, deoxycholic acid [0.05%]). NKA activity was measured as the oubain-inhibited fraction of total adenosine triphosphate (ATP) hydrolysis, as described by McCormick (1993), and expressed as micromoles of adenosine diphosphate per hour. Similarly, vacuolar-type H^+-ATPase (VHA) activity was measured as the fraction of total activity inhibited by *N*-ethylmaleimide, as described by Lin and Randall (1993). Gill caspase 3/7 activity was measured with the Promega CaspaseGlo 3/7 luminescent assay. Gill tissue was first homogenized in 10 mM TRIS-HCl 7.5, 100

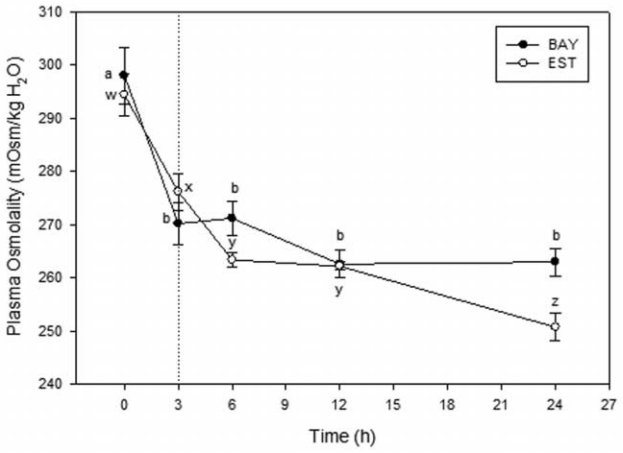

Figure 1. Effect of a 3-h freshwater flush on EST- and BAY-acclimated green sturgeon plasma osmolality. Values represent mean ± SE; letters denote significant differences with time as measured by one-way ANOVA ($\alpha = 0.05$; $n = 5$ for all experiments). See text for specific P values. EST = 15-g/L salinity; BAY = 24-g/L salinity.

mM NaCl, 0.1 mM EDTA, and 0.2% TRITON-X. Homogenates were diluted 1 : 100 with buffer and then mixed 50 : 50 with CaspaseGlo reagent in triplicate. Assays were incubated at room temperature (~20°C) for 1 h before the relative luminescence was measured. All enzyme activities were standardized by the amount of total protein, which was measured as absorbance at 560 nm following 30 min of incubation at 37 °C with a 50 : 1 solution of bicinchoninic acid to $CuSO_4$.

Tissue Microarrays

Tissue microarrays (TMAs) were constructed for gill tissue as described previously (Lima and Kültz 2004; Sardella et al. 2008). Array sections were blocked with 1% bovine serum albumin in PBS (PBA) for 30 min, followed by 60 min of incubation in PBA containing antibodies to heat shock 60-, 70-, and 90-kDa proteins (HSP 60, 70, and 90), as well as ubiquitin. HSP antibodies were purchased from Sigma and were diluted 1 : 1,000 with PBA, while anti-ubiquitin antibodies were diluted 1 : 50. Sections were stained for 60 min with a secondary antibody covalently bound to Alexa 688 (Molecular Probes, Eugene, OR) and diluted 1 : 1,000 with PBA. Finally, sections were counterstained for 10 min with the nuclear stain propidium iodide diluted 1 : 500 with deionized water and mounted.

Tissue Microarray Analysis

A laser scanning cytometer was used to quantify protein concentrations per unit area within randomly assigned areas of tissue on TMAs, as described by Lima and Kültz (2004; also Sardella et al. 2008). Immunohistochemical analysis of TMAs was conducted with a 40× objective (UPlanFL 40x/0.75/∞/0.17, Olympus, Melville, NY), in combination with an ultraviolet laser (400 nm). Event detection for TMAs was optimized for the phantom protocol, as described by Sardella et al. (2008).

Statistical Analysis

We used a one-way ANOVA, followed by Tukey's HSD post hoc test when results were significant ($\alpha = 0.05$; $n = 5$). Statistical tests were performed with SigmaStat, version 3.0, and plots were made with SigmaPlot, version 9.0.

Results

Experiment 1: FW Flush

In both acclimation trials, we achieved a steady salinity decrease and reached 0 g/L at 3 h. The rapid reduction resulted in immediate decreases of plasma osmolality in both EST- and BAY-acclimated sturgeon (both $P < 0.001$; fig. 1). Plasma osmolality stabilized in the BAY-acclimated fish after 3 h but continued to decrease in EST-acclimated sturgeon throughout the 24-h period. As plasma osmolality decreased, muscle water content remained unchanged, and there was a significant decrease in hematocrit in the EST group at 24 h ($P = 0.03$; fig. 2). NKA activity was significantly increased in BAY-acclimated sturgeon at 12 h by nearly 80% ($P < 0.01$), but there were no increases in the EST group (fig. 3), while no changes were seen in VHA activity in any experiment (data not presented). The same pattern was observed with caspase 3/7: activity in BAY-acclimated fish at 24 h was nearly 300% that at 0 h ($P < 0.01$) but was not increased in EST-acclimated fish (fig. 4). There were no differences in HSP or ubiquitin abundances throughout the exposure relative to those at 0 h (data not presented).

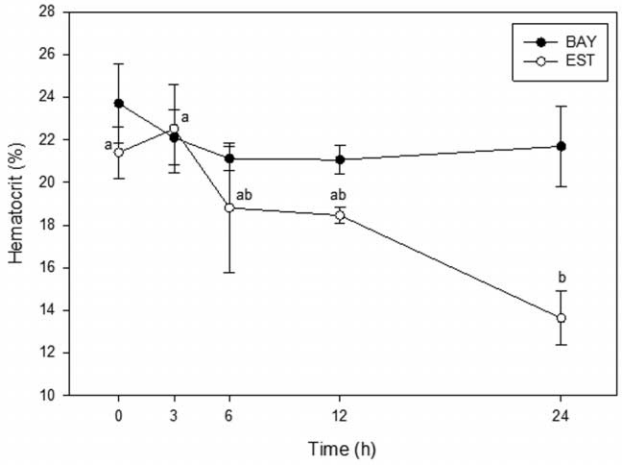

Figure 2. Effect of a 3-h freshwater flush on EST- and BAY-acclimated green sturgeon hematocrit. See figure 1 for statistical details. EST = 15-g/L salinity; BAY = 24-g/L salinity.

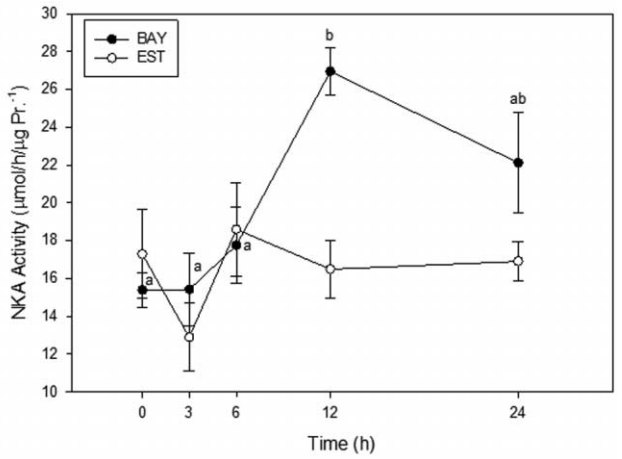

Figure 3. Effect of a 3-h freshwater flush on EST- and BAY-acclimated green sturgeon branchial Na^+, K^+-ATPase (NKA) activity. See figure 1 for statistical details. EST = 15-g/L salinity; BAY = 24-g/L salinity.

Experiment 2: Salinity Fluctuation

Both salinity cycles resulted in a significant plasma osmolality increase relative to levels at 0 h ($P < 0.001$), and the peak value at 18 h was approximately double that at 6 h (fig. 5*A*). There was a significant decrease in muscle water content at 6 h, which recovered to near-control level by 12 h, where it remained ($P < 0.01$; fig. 5*B*). NKA activity was also significantly increased at 6 h before returning to control level at 12–24 h ($P < 0.01$; fig. 6). Similarly, caspase 3/7 activity was increased by as much as 400% at 12 h but then recovered to control levels by 24 h ($P < 0.001$; fig. 7). Finally, no changes in HSP 60 or 90 were measured, but both HSP 70 and ubiquitin abundances were significantly increased at 18 h (both $P < 0.001$; fig. 8).

Discussion

Despite what we predicted to be a very stressful series of 24-h challenges, the sturgeon tolerated and survived the effects of both experiments. We did not observe any mortality, but there were substantial sublethal effects that indicated that the "summer" scenario was the more stressful of the two challenges.

FW Flush

The plasma osmolality of the EST and BAY groups decreased approximately 10% within the first 3 h of FW influx. Interestingly, in our previous experiments, the difference between FW and EST or BAY-acclimated green sturgeon over longer-term exposure was also approximately 10%; however, the absolute values are below what has been previously documented for FW-acclimated sturgeon of this size (Allen and Cech 2007; Sardella and Kültz 2009), which may indicate that these individuals were not fully acclimated to the ambient salinity by 24 h. The maintenance of muscle water content despite a reduced plasma osmolality was observed in this experiment as well as in longer-term acclimation experiments (Sardella and Kültz 2009). How sturgeon maintain intracellular osmotic balance has not been described, but these experiments combined show that they have some mechanism in place to defend this compartment and that it can be upregulated under osmoregulatory stress.

The responses from hour 3 to hour 24 varied between the EST- and BAY-acclimated sturgeon. The plasma osmolality of the BAY-acclimated fish stabilized after 3 h and remained consistent throughout the rest of the 24-h period, while values from EST-acclimated sturgeon continued to decline (fig. 1). A rapid drop in plasma osmolality was seen in the first 3 h, after which time it remained steady at a level similar to what was observed previously with SW-versus-FW acclimation studies (Sardella and Kültz 2009). Differences in plasma osmolality between the two salinities have been seen in this species previously (Allen and Cech 2007). An increase in NKA activity occurred following the stabilization of the osmolality (figs. 1, 2), which suggests that the osmolality may have decreased further without upregulation of this enzyme. Caspase 3/7 activity also increased, with its peak lagging behind the peak of NKA activity (fig. 3). The stabilization of plasma osmolality at the reduced level in the BAY group may be attributed to the increased NKA activity. An increase in the activity of this enzyme is typical of many euryhaline species following a salinity challenge (Jensen et al. 1998; Rodríguez et al. 2002; Imsland et al. 2003), and the increase we have observed 12 h after an FW influx was a good indicator of acclimation to a new ambient salinity (Imsland et al. 2003). A rapid activation of previously synthesized NKA, via either posttranslational modification of enzyme or posttranscriptional modification of messenger RNA, has been described for some euryhaline species, such as the common killifish (*Fundulus heteroclitus*; Towle et al. 1977; Mancera and McCormick 2000; Tipsmark and Madsen 2001), but the time interval for those mechanisms was shorter. Therefore, we suggest that the increased NKA activity was indicative of a

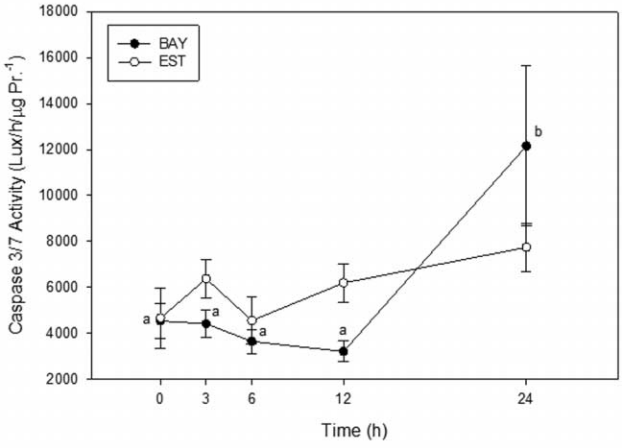

Figure 4. Effect of a 3-h freshwater flush on EST- and BAY-acclimated green sturgeon branchial caspase 3/7 activity. See figure 1 for statistical details. EST = 15-g/L salinity; BAY = 24-g/L salinity.

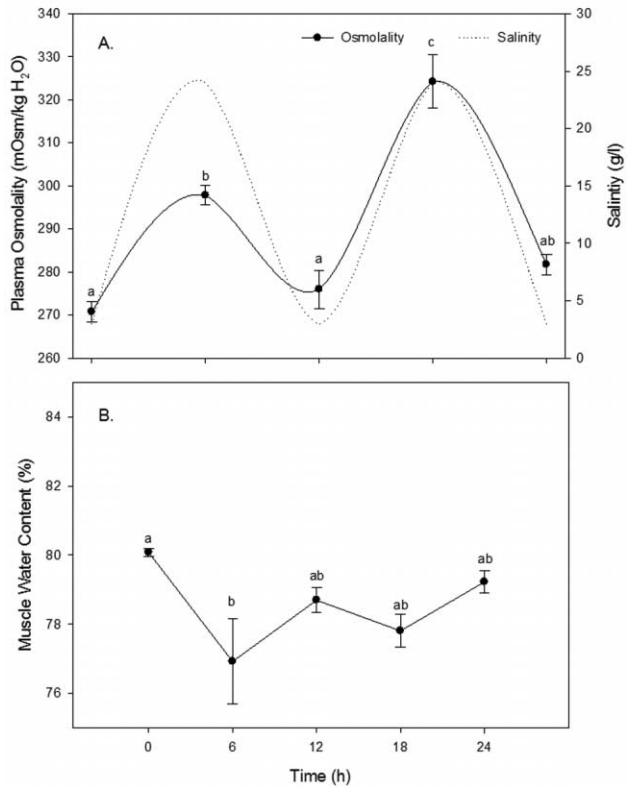

Figure 5. Effect of a dual 12-h 0–24–0-g/L salinity fluctuation cycle on green sturgeon plasma osmolality (*A*) and muscle water content (*B*). Dotted line in *A* marks ambient salinity. See figure 1 for statistical details.

switch between SW and FW regulation mechanisms, an idea that is further supported by the large increase in branchial apoptosis at 24 h in the BAY group. An increase in apoptosis following salinity challenges has been previously associated with accelerated cellular turnover in the gill as well as a restructuring of the branchial epithelium during transfer (Wendelaar Bonga and van der Meij 1989; van der Heijden et al. 1997; Sardella et al. 2004*b*).

Interestingly, VHA activity, which plays a key role in some of the proposed mechanisms of Na^+ absorption in FW-acclimated fishes (Perry 1997; Marshall 2002; Dymowska et al. 2012), did not change within 24 h of the FW flush. VHA is one potential mechanism for Na^+ absorption in FW-acclimated fishes, whereby the transport of protons at the expense of ATP provides an electromotive force to drive Na^+ movement through an apical channel (Perry 1997; Marshall 2002; Dymowska et al. 2012; Hiroi and McCormick 2012), but our previous work also showed that VHA activity and abundance were unchanged in green sturgeon following acclimation to salinities ranging from 0 to 24 g/L for 2 wk (Sardella and Kültz 2009), indicating that this enzyme may not play a role in short- or longer-term acclimation. The role of this enzyme in acid-base regulation and the possibility that an apical Na^+/H^+ exchanger may be present in sturgeon gills must also be considered. The precise mechanism of ion absorption in sturgeon has received little attention thus far.

There were no changes in the abundances of stress proteins in the gills; HSP 60, 70, and 90 all remained unchanged, as did ubiquitin. We conclude, on the basis of the lack of stress-protein response, that the BAY-acclimated fish responded by acclimating to the new ambient salinity, while the EST-acclimated fish were somehow tolerant of it, despite what appeared to detrimental effects on blood parameters. In the EST-acclimated sturgeon, plasma osmolality continued to decrease without upregulation of osmo- and ionoregulatory mechanisms (fig. 1), and at 24 h, when osmolality was at its lowest value, hematocrit was also dramatically reduced. Hematocrit reached a low value of 13% in the EST-acclimated fish at 24 h (fig. 2), possibly indicating cell lysis due to the hypotonic effect of such dilute plasma. The reason for the lack of osmoregulatory response remains unclear but interesting. One possibility is that the overall magnitude of salinity change was too small (from 15 to 0 g/L in EST vs. from 24 to 0 g/L in BAY) to elicit a regulatory response, although the similarities in plasma osmolality between the two groups at 3 h convolutes this idea. Regardless of the explanation, the difference between the two responses is an interesting contrast for additional studies.

Tidal Fluctuation

Exposure of green sturgeon to two cycles of salinity fluctuation resulted in dramatic sublethal effects. Plasma osmolality spiked at 6 h and then again to a greater magnitude at 18 h and was 11.0% and 18.0% greater, respectively, relative to that at 0 h. The first peak in osmolality at 6 h corresponded with a drop in muscle water content (fig. 4*b*), indicating an osmotic water loss to the plasma (Brauner et al. 1992). A loss of muscle water

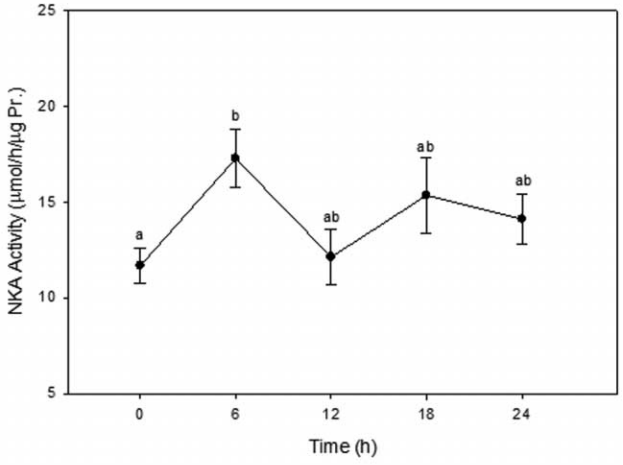

Figure 6. Effect of a dual 12-h 0–24–0-g/L salinity fluctuation cycle on green sturgeon branchial Na^+, K^+-ATPase (NKA) activity. See figure 1 for statistical details.

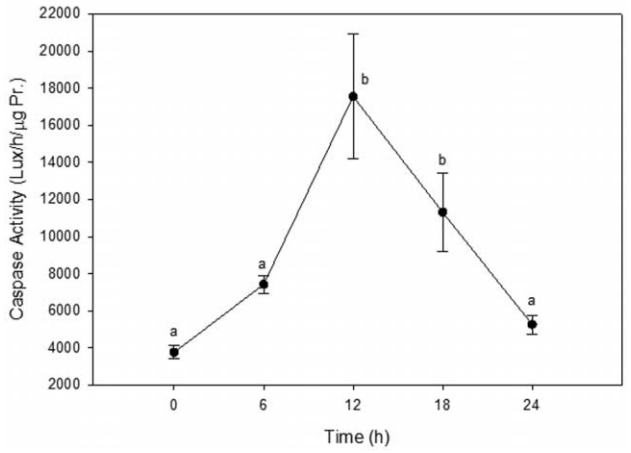

Figure 7. Effect of a dual 12-h 0–24–0-g/L salinity fluctuation cycle on green sturgeon branchial caspase 3/7 activity. See figure 1 for statistical details.

was not evident at 18 h despite an even larger apparent osmotic gradient. As mentioned above, sturgeon defended the muscle compartment when plasma osmolality was reduced. Under this fluctuating salinity, sturgeon were able to defend the muscle compartment on the secondary exposure to elevated osmolality, which may indicate that the osmotic gradient between the muscle and plasma was eliminated by a regulatory mechanism.

During the first cycle of salinity fluctuation, sturgeon responded by upregulating osmo- and ionoregulatory mechanisms. This again involved increased branchial NKA activity at 6 h and increased caspase 3/7 activity at 12 h. The peak in apoptosis lagged slightly behind the peak of NKA activity, as we observed when BAY-acclimated fish were exposed to the FW-flush experiment. Again, these responses indicate acclimation to changing environmental salinity (Wendelaar Bonga and van der Meij 1989; van der Heijden et al. 1997; Jensen et al. 1998; Rodríguez et al. 2002; Imsland et al. 2003; Sardella et al. 2004*b*). By the completion of the second cycle, the acclimatory mechanisms appeared to be either complete or interrupted by osmotic stress, as there were no increases of NKA or caspase 3/7 activity. Whether the sturgeon had fully acclimated or were simply attempting to tolerate the secondary increase in salinity, they appeared to be experiencing stress on the cellular level. We observed a dramatic stress response: spikes in the abundances of HSP 70 (fig. 8*A*) and ubiquitin (fig. 8*B*), coupled with a much greater increase in plasma osmolality. HSP 70 has been characterized as a crucial molecular chaperone and is typically associated with osmotically induced stress (Werner et al. 2007), among other factors. While HSP 70 was likely upregulated to salvage damaged proteins (Iwama et al. 1999), the increase in ubiquitin indicated that there were also damaged proteins being targeted for destruction (Wickner et al. 1999). We did not observe an increased level of stress-protein abundance during the first salinity cycle, where once again the response was more typical of acclimation, but during the second cycle our data indicated that there was substantial protein damage coincidental with severe plasma dehydration. Although we cannot fully know whether the sturgeon were fully acclimated to an SW condition at the time of the secondary exposure, this study clearly demonstrates the additive stressful effect of repeated salinity fluctuations.

Summary

Green sturgeon have the capacity to inhabit various salinities over long-term exposures (Sardella and Kültz 2009), and according to current data, acclimation may take place as soon as within 24 h of exposure to a salinity challenge. However, the addition of a second cycle of fluctuation resulted in a substantial stress response, as opposed to upregulation of osmo- and ionoregulatory mechanisms. Therefore, an unpredictable cycling of salinity should be of concern with respect to wild green sturgeon populations. Unfortunately, widespread variations of temperature and salinity are forecasted to occur as a result of global climate change (Knowles and Cayan 2002, 2004), and the exact pattern and magnitude of these variations have yet to be fully realized. It is clear from our data that decreased

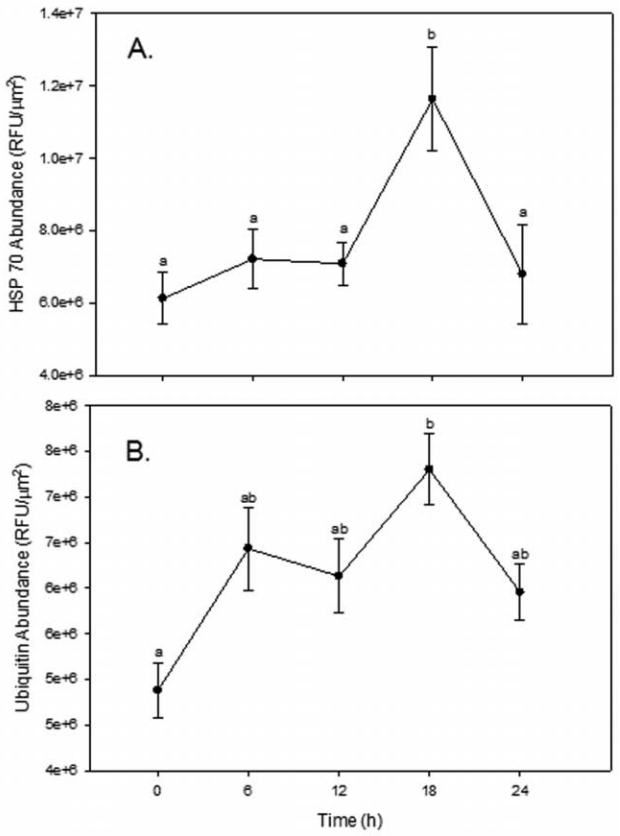

Figure 8. Effect of a dual 12-h 0–24–0-g/L salinity fluctuation cycle on green sturgeon branchial heat shock 70-kDa protein (HSP 70) abundance (*A*) and branchial ubiquitin abundance (*B*). See figure 1 for statistical details. RFU = relative fluorescence units.

stability of abiotic factors within the system will be deleterious to sturgeon, especially for the southern DPS, which is currently threatened. While the salinity variations that wild sturgeon must cope with will most likely not match the magnitude that we have imposed during our experiments, it is important to consider that wild sturgeon will be chronically exposed to these factors; thus, we believe that the bioindicators described here should fit a model for longer-term exposure to a lower grade of stress. As these variables destabilize, a physiological marker such as a high stress-protein expression may be a useful bioindicator of local population status. Unfortunately, as temperature and salinity begin to fluctuate, we can predict that sturgeon in the SF Bay–Delta region will either be forced to cope with high levels of physiological stress or, more likely, be forced to relocate to areas that may be less optimal with respect to factors such as foraging or fishing pressure. Either consequence could complicate restoration or maintenance of a species that is already in decline.

Acknowledgments

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Literature Cited

Allen P.J. and J.J. Cech Jr. 2007. Age/size effects on juvenile green sturgeon (*Acipenser medirostris*), oxygen consumption, growth, and osmoregulation in saline environments. Environ Biol Fishes 79:211–229.

Brauner C.J., J.M. Shrimpton, and D.J. Randall. 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance of coho salmon (*Oncorhynchus kisutch*) parr. Can J Fish Aquat Sci 49:2399–2405.

Carmona R., M. García-Gallego, A. Sanz, A. Domezain, and M.V. Ostos-Garrido. 2004. Chloride cells and pavement cells in gill epithelia of *Acipenser naccarii*: ultrastructural modifications in seawater-acclimated specimens. J Fish Biol 64: 553–566.

Dymowska A.K., P.P. Hwang, and G.G. Goss. 2012. Structure and function of ionocytes in the freshwater fish gill. Respir Physiol Neurobiol 184:282–292.

Hiroi J. and S.D. McCormick. 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. Respir Physiol Neurobiol 184:257–268.

Imsland A.K., S. Gunnarsson, A. Foss, and S.O. Stefansson. 2003. Gill Na^+, K^+-ATPase activity, plasma chloride and osmolality in juvenile turbot (*Scophthalmus maximus*) reared at different temperatures and salinities. Aquaculture 218: 671–683.

Iwama G.K., M.M. Vijayan, R.B. Forsyth, and P.A. Ackerman. 1999. Heat shock proteins and physiological stress in fish. Integr Comp Biol 39:901–909.

Jensen M.K., S.S. Madsen, and K. Kristiansen. 1998. Osmoregulation and salinity effects on the expression and activity of Na^+, K^+-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). J Exp Zool 282:290–300.

Kelly J.T., A.P. Klimley, and C.E. Crocker. 2007. Movements of green sturgeon, *Acipenser medirostris*, in the San Francisco Bay estuary, California. Environ Biol Fishes 79:281–295.

Knowles A.B. and D.R. Cayan. 2002. Potential effects of global warming on the Sacramento/San Joaquin watershed and the San Francisco Bay estuary. Geophys Res Letters 29(18):1891. doi:10.1029/2001GL014339.

———. 2004. Elevational dependence of projected hydrologic changes in the San Francisco estuary and watershed. Clim Change 62:319–336.

Lima R. and D. Kültz. 2004. Laser scanning cytometry and tissue microarray analysis of salinity effects on killifish chloride cells. J Exp Biol 207:1729–1739.

Lin H. and D.J. Randall. 1993. H^+-ATPase activity in crude homogenates of fish gill tissue: inhibitor sensitivity and environmental and hormonal regulation. J Exp Biol 180:163–174.

Mancera J.M. and S.D. McCormick. 2000. Rapid activation of gill Na^+/K^+-ATPase in the euryhaline teleost *Fundulus heteroclitus*. J Exp Zool 287:263–274.

Marshall W.S. 2002. Na^+, Cl^-, Ca^{2+}, and Zn^{2+} Transport by fish gills: a retrospective review and prospective synthesis. J Exp Zool 293:264–283.

Martinez-Álvarez R.M., A. Sanz, M. García-Gallego, A. Domezain, J. Domezain, R. Carmona, M.V. Ostos-Garrido, and A.E. Morales. 2005. Adaptive branchial mechanisms in the sturgeon *Acipenser naccarii* during acclimation to seawater. Comp Biochem Physiol A 141:183–190.

McCormick S.D. 1993. Methods for non-lethal gill biopsy and measurement of Na^+, K^+-ATPase activity. Can J Fish Aquat Sci 50:656–658.

Moyle P.B. 2002. Inland fishes of California. University of California Press, Berkeley.

Perry S.F. 1997. The chloride cell: structure and function in the gills of freshwater fishes. Annu Rev Physiol 59:325–347.

Rodríguez A., M.A. Gallardo, E. Gisbert, S. Santilari, A. Ibarz, J.J. Sánchez, and F. Castelló-Orvay. 2002. Osmoregulation in juvenile Siberian sturgeon (*Acipenser baerii*). Fish Physiol Biochem 26:345–354.

Sardella B.A., J. Cooper, R.J. Gonzalez, and C.J. Brauner. 2004*a*. The effect of temperature on juvenile Mozambique tilapia hybrids (*Oreochroms mossambicus* × *O. urolepis hornorum*) exposed to full-strength and hypersaline seawater. Comp Biochem Physiol A 137:621–629.

Sardella B.A. and D. Kültz. 2009. Osmo- and ionoregulatory responses of green sturgeon (*Acipenser medirostris*) to salinity acclimation. J Comp Physiol B 179:383–390.

Sardella B.A., D. Kültz, J.J. Cech Jr., and C.J. Brauner. 2008.

Salinity-dependent changes in Na^+/K^+-ATPase content of mitochondria-rich cells contribute to the differences in thermal tolerance of Mozambique tilapia. J Comp Physiol B 178: 249–256.

Sardella B.A., V. Matey, J. Cooper, R.J. Gonzalez, and C.J. Brauner. 2004*b*. Physiological, biochemical, and morphological indicators of osmoregulatory stress in 'California' Mozambique tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*) exposed to hypersaline water. J Exp Biol 207: 1399–1413.

Schlenk D. and R. Lavado. 2011. Impacts of climate change on hypersaline conditions of estuaries and xenobiotic toxicity. Aquat Toxicol 105:78–82.

Tipsmark C. and S. Madsen. 2001. Rapid modulation of Na^+/K^+-ATPase activity in osmoregulatory tissues of a salmonid fish. J Exp Biol 204:701–709.

Towle D.W., M.E. Gilman, and J.D. Hempel. 1977. Rapid modulation of gill sodium and potassium dependent ATPase activity during acclimation of the killifish (*Fundulus heteroclitus*) to salinity change. J Exp Zool 202:179–186.

van der Heijden A.J.H., P. Verbost, J. Eygensteyn, S.E. Wendelaar Bonga, and G. Flik. 1997. Mitochondria-rich cells in gills of tilapia (*Oreochromis mossmabicus*) adapted to fresh water or sea water: quantification by confocal laser scanning microscopy. J Exp Biol 200:55–64.

Wagner R.W., M. Stacey, L.R. Brown, and M. Dettinger. 2011. Statistical models of temperature in the Sacramento-San Joaquin Delta under climate-change scenarios and ecological implications. Estuar Coasts 34:544–556.

Wendelaar Bonga S.E. and C.J.M. van der Meij. 1989. Degeneration and death, by apoptosis and necrosis, of the pavement and chloride cells in the gills of the teleost *Oreochromis mossambicus*. Cell Tissue Res 255:235–243.

Werner R.G., J. Linares-Casenave, J.P. Van Eenennaam, and S.I. Doroshov. 2007. The effect of temperature stress on development and heat-shock protein expression in larval green sturgeon (*Acipenser medirostris*). Environ Biol Fishes 79:191–200.

Wickner S., M.R. Maurizi, and S. Gottesman. 1999. Posttranslational quality control: folding, refolding, and degrading proteins. Science 286:1888–1893.