The Acute Temperature Tolerance of Green Sturgeon (*Acipenser medirostris*) and the Effect of Environmental Salinity

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ABSTRACT   We investigated the effect of environmental salinity on the upper thermal tolerance of green sturgeon (*Acipenser medirostris*), a threatened species whose natural habitat is vulnerable to temperature and salinity variation as a result of global climate change. Freshwater (FW)-reared sturgeon were gradually acclimated to salinities representing FW, estuary water (EST), or San Francisco Bay water (BAY) at 18°C, and their critical thermal maximum (CTMax) was measured by increasing temperature 0.3°C/min until branchial ventilation ceased. CTMax was 34.2 ± 0.09°C in EST-acclimated fish, with FW- and BAY-acclimated fish CTMax at 33.7 ± 0.08 and 33.7 ± 0.1°C, respectively. Despite the higher CTMax in EST-acclimated fish, FW-acclimated sturgeon ventilation rate reached a peak that was 2°C higher than EST- and BAY-acclimated groups and had a greater range of temperatures within which they exhibited normal ventilatory function as assessed by Q_{10} calculation. The osmoregulatory consequences of exposure to near-lethal temperatures were assessed by measuring plasma osmolality and hematocrit, as well as white muscle, brain, and heart tissue water contents. Hematocrit was increased following CTMax exposure, most likely owing to the elevated metabolic demands of temperature increase, and plasma osmolality was significantly increased in EST- and BAY-acclimated fish, which was likely the result of a greater osmotic gradient across the gill as metabolism increased. To our knowledge, this represents the first evidence for an effect of salinity on the upper thermal tolerance of sturgeon, as well as the first investigation of the osmoregulatory consequences of exposure to near-lethal temperatures. *J. Exp. Zool. 309A:477–483, 2008.*   © 2008 Wiley-Liss, Inc.

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Variation of environmental temperature or salinity can have widespread effects on the physiology of fishes, particularly with respect to mechanisms associated with osmoregulation (Gonzalez and McDonald, 2000; Staurnes et al., 2001; Sardella et al., 2004; Sardella and Brauner, 2007b). Most fish species maintain their internal milieu at approximately one-third the concentration of seawater (SW); as a result, osmotic and ionic gradients exist between the internal and external environments in both freshwater (FW) and SW. To offset fluxes, complex mechanisms in the gill drive the uptake or excretion of ions at the expense of metabolic energy in FW- and SW-acclimated fishes, respectively (Keys and Wilmer, '32; Perry, '97; Marshall, 2002). Anadromous species such as some sturgeon (Chondrostei) must

acclimatize to both FW and SW as part of their migratory natural history, spending the early stages of life in FW and migrating to estuarine areas and open ocean as adults (Moyle, 2002). Although sturgeon typically maintain plasma osmolality and ion concentrations at lower values relative to teleosts, they are efficient osmoregulators and respond with cellular mechanisms that are similar to those of teleosts when faced with

a salinity challenge (McKenzie et al., '99; Wright, 2007).

The green sturgeon (*Acipenser medirostris*) inhabits the San Francisco (SF) Bay-Delta Watershed of California, which is the state's primary hydrologic system (Knowles and Cayan, 2002, 2004). This species is also distributed along the Pacific coast from Alaska to Mexico, spawning within the Sacramento, Klamath, and Rogue River systems of California and Oregon (Moyle, 2002). Across this distribution, sturgeon inhabit a range of salinities and temperatures varying with both geography and season. Variation of ambient temperature or salinity requires physiological adjustments for sturgeon to maintain a consistent internal milieu. McEnroe and Cech ('85) showed that white sturgeon (*A. transmontanus*), a sympatric, congeneric species, maintained plasma ion concentrations during acclimation to salinities ranging from 0 to 26 g/L, and McKenzie et al. ('99) showed a similar response from Adriatic sturgeon (*A. naccarii*) following FW to SW transfer. With respect to temperature adaptation, the thermal optimum for sturgeon has been shown to vary as a function of age/size and geographical distribution. Van Eenennaam et al. (2001) found that 17°C was at or near the upper limit of the thermal optimal zone for FW green sturgeon embryos, whereas large juveniles that are typically found downstream in estuarine areas had a thermal optimum ranging as high as 19°C (Mayfield and Cech, 2004). As sturgeon migrate from FW to SW, changes in ambient salinity are typically concurrent with changes in ambient temperature, as FW regions within the SF Bay-Delta system are typically colder (Mayfield and Cech, 2004). Despite the importance of temperature and salinity to the life history of sturgeon, the effects of their combination on sturgeon physiology have received little attention. Studies using teleost fishes have definitively shown that the effects of combined stress are greater than when either stress was imposed alone (Al Amoudi et al., '96; Gonzalez and McDonald, 2000; Handeland et al., 2000; Jian et al., 2003; Magill and Sayer, 2004; Sardella et al., 2004; Sardella and Brauner, 2007a).

The green sturgeon is currently listed as threatened; furthermore, the changes in hydrological and meteorological factors that have been forecasted to take place within the SF Bay-Delta system as a result of global climate change (see Knowles and Cayan, 2002, 2004) may complicate the maintenance and/or restoration of local populations. If salinity within the system becomes less stable, it may have consequences on a host of physiological traits, including thermal tolerance. An understanding of the sublethal responses of sturgeon to environmental perturbations may be useful in management efforts. The purpose of this study was to investigate how environmental salinity affects the acute thermal tolerance of green sturgeon and to characterize the osmoregulatory consequences of exposure to near-lethal temperatures.

MATERIALS AND METHODS

Fish

Green sturgeon were spawned, and the resulting progeny were reared at the Center for Aquatic Biology and Aquaculture at the University of California, Davis in the spring of 2007. Larvae were matured in FW until reaching a mass of 58.4 ± 2.5 g, at which time they were deemed capable to survive and acclimate to changes in environmental salinity (McEnroe and Cech, '85). Groups of 12 individuals were acclimated to three salinities, FW, which ranged between 0–1 g/L salinity, estuarine water (EST) with a salinity of approximately 10 g/L, and SF Bay water (BAY) with a salinity of approximately 24 g/L. Salinity was manipulated by adding Instant Ocean synthetic sea salt to UC Davis well water and measured using a light refractometer. For acclimation, sturgeon were removed from flow-though rearing tanks and placed into recirculating systems that were filtered, aerated, and regularly monitored to keep ammonia accumulation negligible. During rearing and acclimation, water temperature was held constant at 18°C and tanks were held under a natural photoperiod. Acclimations were carried out by stepwise increases of 5 g/L through two-thirds water changes every 4 day until reaching target salinities. Fish were then given 1 week to acclimate at 10 and 24 g/L salinity for EST and BAY groups, respectively.

Critical thermal maxima (CTMax) measurement

Following acclimation, six sturgeon were placed into individual flow-through chambers (approx. 10 L) and allowed to familiarize with the chamber overnight. To measure CTMax, water temperature was increased by 0.3°C/min (Fangue and Bennet, 2003). Opercular movements were monitored constantly, and ventilation rate was

recorded in increments of 2°C as ventilations per minute (VPM). The endpoint indicating the CTMax was cessation of ventilation, which is commonly used in assessing the CTMax of fishes (Beitinger et al., 2000). Once CTMax was determined, fish were removed from the chambers and sampled (see below). An additional six individuals, continuously held at 18°C, were sampled to contrast with those exposed to CTMax and characterize the osmotic consequences of exposure to near-lethal temperature.

Sampling

Fish were sacrificed in accordance with a UC Davis IACUC-approved protocol by spinal transection, and blood was collected by severing the caudal region and draining the contents of the vessels into heparinized microhematocrit capillary tubes. Blood was then centrifuged in a Damon IEC MB microhematocrit centrifuge at $11,000\,g$ for $3\,min$ and hematocrit was measured. Capillary tubes were cut, plasma was collected, and plasma osmolality was measured immediately using a freezing point depression osmometer (Advanced Instruments Inc., Norwood, MA). Sturgeon were dissected, and the heart, the brain, and approximately $500\,mg$ of epaxial muscle were removed, patted dry and placed into preweighed pieces of aluminum foil. These tissues were weighed before and after 96–120 hr in a 65°C oven to determine percent water content.

Statistics

The effect of acclimation salinity on the CTMax of sturgeon was tested by one-way analysis of variance (ANOVA), followed by a post hoc Tukey HSD test. To measure the effects of acclimation salinity and CTMax exposure on ventilation rate, plasma osmolality, and tissue water contents, we used a two-way ANOVA, followed by a post hoc Holm-Sidak multiple means comparison. The increases in ventilation rate were also analyzed by linear regression to determine the rate of ventilation increase with temperature. All statistical analyses were conducted using SigmaStat version 3.0 with significance based on an α level of 0.05, and plots were made using SigmaPlot version 9.0.

RESULTS

There was a significant effect of salinity on the CTMax of green sturgeon acclimated to 18°C ($F = 5.89$, df = 2, $P = 0.01$). Sturgeon acclimated to the EST water had a CTMax of 34.2 ± 0.09°C, whereas sturgeon in FW and BAY treatments had a CTMax of 33.7 ± 0.08 and 33.7 ± 0.10°C, respectively. Ventilation rate increased rapidly with temperature peaking at 182 ± 2.25 VPM in FW, 172 ± 4.25 in EST water, and 189 ± 3.04 in BAY water (Fig. 1a; Salinity $F = 26.2$, df = 2, $P < 0.001$; temperature $F = 141.263$, df = 6, $P < 0.001$; interaction $F = 6.86$, df = 12, $P < 0.001$). The ventilation rate of FW-acclimated fish increased at a slower rate and peaked at a higher temperature relative to those in EST and BAY-acclimated sturgeon, whereas the temperature coefficients (Q_{10}) of FW, EST, and BAY-acclimated sturgeon was 1.65, 2.07, and 2.34, respectively (Fig. 1b).

Hematocrit was affected by CTMax exposure ($F = 40.1$; df = 1, $P < 0.001$) and was significantly increased after exposure in all salinity groups (Table 1). There were also clear effects of both CTMax exposure and acclimation salinity on sturgeon plasma osmolality (Table 2; salinity: $F = 170.9$, df = 2, $P < 0.001$; temperature: $F = 90.1$, df = 1, $P < 0.001$; interaction: $F = 12.7$, df = 2, $P < 0.001$). In FW, osmolality did not change as a result of CTMax exposure, but in EST and BAY groups it was increased by 6.0 and 10.0%, respectively. Muscle water content was higher in the EST- and BAY-acclimated sturgeon ($F = 30.9$, df = 2, $P < 0.001$), but CTMax exposure had no effect (Table 2). Finally, the water content of the heart was affected by exposure to CTMax ($F = 15.8$, df = 1, $P < 0.001$), regardless of acclimation salinity or any changes in plasma osmolality it was increased (Table 2). In the brain there was no effect of salinity or CTMax exposure on water content (data not presented).

DISCUSSION

CTMax values estimate the nonlethal thermal tolerance during an acute temperature increase, where the rate of increase was slow enough to track body temperature, but rapid enough to prevent the animal from acclimating (Fangue and Bennet, 2003). The CTMax for 18°C-acclimated green sturgeon was approximately 0.5°C greater in EST-acclimated sturgeon relative to those acclimated to FW or BAY water. This finding was the first measurement of upper thermal tolerance for green sturgeon, and showed that they were capable of tolerating increases of approximately 15°C over an acclimation temperature of 18°C; an acclimation temperature of 18°C is within the optimal range for growth, oxygen
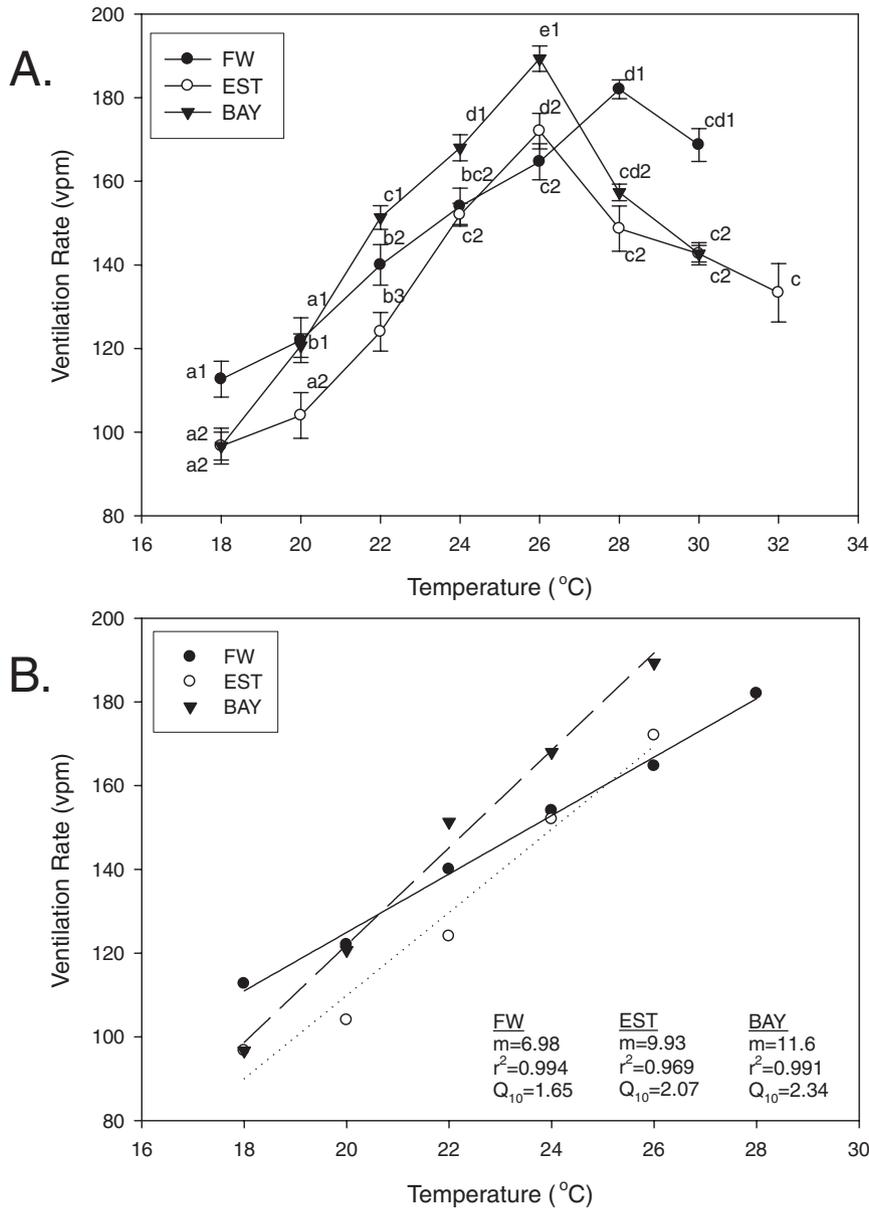
Fig. 1. (**A**) Changes in mean ventilation rate ($\pm$SE) with increasing temperature during CTMax trials, ($n = 6$). (**B**) Regression analysis of the increase in ventilation rate measured from 18°C to peak value for each salinity, see figure for statistical details. (FW: freshwater, EST: estuarine water, BAY: SF Bay water).

TABLE 1. *The effects of acclimation salinity and CTMax exposure on the mean hematocrit ($\pm$SE) of green sturgeon*

	Hematocrit (%)	
	18°C	CTMax
FW	22.6$\pm$0.65	29.2$\pm$0.91*
EST	26.0$\pm$0.86	28.7$\pm$1.0*
BAY	25.1$\pm$0.59	28.9$\pm$0.94*

*Denotes significant difference following CTMax exposure. See text for statistical details.

consumption, and overall bioenergetic performance (Mayfield and Cech, 2004). The increase in temperature had clear effects on metabolism, which is evident by both increased branchial ventilation throughout the trials, and an increased hematocrit following CTMax exposure. Elevated hematocrit is typically mediated through splenic contraction under conditions of elevated metabolism to increase the capacity for oxygen and carbon dioxide transport (Pearson and Stevens, '91).

TABLE 2. *The effects of acclimation salinity and CTMax exposure on the mean plasma osmolality, muscle water content, and heart water content (±SE) of green sturgeon*

	Plasma osmolality (mOsm/kg H_2O)		Muscle water content (%)		Heart water content (%)	
	18°C	CTMax	18°C	CTMax	18°C	CTMax
FW	268 ± 1.4[a]	273 ± 0.48*	81.2 ± 0.19[a]	81.2 ± 0.25	82.9 ± 0.36	84.8 ± 0.37*
EST	274 ± 2.6[a]	291 ± 1.1*	84.9 ± 0.61[b]	84.1 ± 0.37	82.6 ± 0.45	84.7 ± 0.76*
BAY	296 ± 1.6[b]	326 ± 4.2*	84.1 ± 0.50[b]	85.5 ± 0.29	82.9 ± 0.52	83.8 ± 0.32*

*Denotes significant difference following CTMax exposure. Letters denote significant difference among control groups at 18°C. See text for statistical details.

The initial increase in ventilation rate was linear, reaching peak rates at 26°C in EST and BAY sturgeon, and at 28°C in FW sturgeon. We calculated the temperature coefficient (Q_{10}) for ventilation rate in each group across the range of temperatures from control to that which elicited the peak in ventilation rate (18–26°C for EST and BAY; 18–18°C for FW). The Q_{10} value represents the factor by which biological rates increase as a result of a 10°C temperature increase (Hochachka and Somero, 2002). For ectotherms, a Q_{10} near 2 is expected when an organism is within normal physiological limits (Hochachka and Somero, 2002). Our Q_{10} values for the increase in ventilation rate were approximately two in all groups, although the range of temperatures eliciting this response was greater in FW-acclimated sturgeon by a full 2°C. The Q_{10} values for ventilation rate following the peak temperature (down slope; all <1) indicated that the temperature increase was resulting in damage to the system (Hochachka and Somero, 2002). FW-acclimated sturgeon were able to maintain a normal physiological function to a higher peak temperature relative to EST and BAY fish and had a greater range of temperature tolerance before damage to the system occurred. Despite the better performance of FW-acclimated sturgeon in functional tolerance, CTMax estimates assessed by cessation of ventilation were highest in the EST-acclimated sturgeon. Which value is of greater significance is open to debate, but it should be considered that functional temperature range is more crucial to the natural history of an organism. Better performance by sturgeon in FW may be a reflection of the age and size class used in this study (<3 yr), which typically inhabits FW naturally (Moyle, 2002). In contrast, the higher CTMax value measured in EST-acclimated sturgeon may reflect the lower energy demand that has been associated with acclimation to near-isosmotic water, where osmoregulatory mechanisms are

thought to be functioning minimally (Morgan and Iwama, '91). Regardless of which endpoint is considered to better represent thermal tolerance, our data indicate that sturgeon thermal tolerance varies with environmental salinity. Our findings on tolerance in this study are supported by previous studies that have shown that thermal optima for sturgeon also vary based on environmental salinity and age/size (McEnroe and Cech, '85; Van Eenennaam et al., 2001).

There were significant differences in plasma osmolality and muscle water content among green sturgeon acclimated to FW, EST, or BAY water earlier to CTMax assessment. Plasma osmolality was within the previously described range for this species and was slightly elevated in BAY-acclimated individuals relative to those in the EST and FW groups at 18°C. A slightly elevated plasma osmolality is typical for sturgeon acclimated to SW (McEnroe and Cech, '85; McKenzie et al., '99). Muscle water content was greater in EST and BAY sturgeon groups relative to FW at 18°C. The higher percent water content despite a higher plasma osmolality may be indicative of intracellular regulatory mechanisms, which is interesting and worthy of further study.

The collapse of osmoregulatory mechanisms was evident in the EST and BAY groups after exposure to near-lethal temperature. Temperature increases have been shown to have deleterious effects on osmotic balance, which are mediated by altering enzyme kinetics (Handeland et al., 2000), varying phospholipid membrane fluidity/integrity (Johnston and Cheverie, '85), and/or altering metabolic rate (Barron et al., '87; Hayton and Barron, '90). Plasma osmolality was significantly increased following CTMax exposure in the EST and BAY groups, indicating an increased osmotic gradient across the gill as blood flow and gill ventilation rate increased. These effects have been shown to exacerbate the effects of the osmorespiratory compromise at the gill, and result in

greater ion flux (Randall et al., '72; Gonzalez and McDonald, '92, '94; Sardella and Brauner, 2007b). FW-acclimated sturgeon were able to maintain plasma osmolality during CTMax exposure, although ventilation rate was dramatically increased. The intracellular tight junctions in the branchial epithelium of FW-acclimated fishes are deeper and tighter relative to those in SW-acclimated fishes (Sardet et al., '79; Stevenson, '98; Marshall, 2002; Matter and Balda, 2003), where the leakiness of intercellular tight junctions plays a crucial role in the excretion of Na^+ (Marshall, 2002). The greater leakiness in the hyperosmotic-acclimated groups (EST and BAY) likely contributed to the loss of osmotic balance as ventilation rate increased and may have played a role in the shorter functional range of temperatures for these groups compared with FW.

The increased plasma osmolality in the EST and BAY groups did not result in a loss of tissue water in the heart, brain, or white muscle following CTMax exposure. In the heart, tissue water content was increased following exposure to CTMax regardless of salinity or plasma osmolality. The increase was significant in all salinities, although it was more subtle in the BAY-acclimated sturgeon (the increase in BAY was 50% of those in FW and EST). The increased contraction rate resulting in an impaired repolarization may have lead to an osmotic disruption that drew water into the tissue. The high plasma osmolality in the BAY fish following CTMax may explain why the heart water gain was more subtle in that group relative to FW and EST fish. We do not have enough evidence to suggest that heart failure was responsible for the failure of these sturgeon at CTMax, but this is an interesting idea for further study.

The typical range of salinities and temperatures that sturgeon encounter are likely to expand as a result of global climate change (Knowles and Cayan, 2002, 2004). The variable salinity and temperature tolerances that have been observed in this and other studies become a concern when it is considered that the main deleterious effect of climate change will be a shift in the dominant precipitation type from snow to rain at higher altitudes, resulting in less water storage within the snow pack that maintains the system. Such a shift is likely to result in a larger and less predictable salinity gradient within the estuary, confounding the direct effects of warmer temperature (Knowles and Cayan, 2002, 2004). As these abiotic factors become more unstable, the population dynamics and distribution of estuarine inhabitants will eventually be determined by their level of thermal tolerance and/or osmoregulatory capability. Salinity and temperature combinations that sturgeon are not able to acclimatize to will lead to elevated physiological stress. For example, in this study juvenile sturgeon that were of the age/size class typically found in FW were exposed to higher salinities, and as a result, had a shorter range normal physiological function as temperature was increased. Such physiological stresses resulting from changing abiotic conditions may subsequently reduce fitness and/or force relocation to suboptimal regions within the system, both of which may negatively affect population dynamics and distribution of an already threatened species.

ACKNOWLEDGMENTS

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