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Effects of temperature and carbon dioxide on green sturgeon blood–oxygen equilibria

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Abstract There are three Northeast Pacific Rivers still supporting spawning populations of green sturgeon, *Acipenser medirostris*, but all have been modified hydrologically and thermally by dam construction. Age 1- to 3-year-old green sturgeon, progeny of artificially spawned, wild-caught Klamath River adults, were used to assess the effects of temperature and carbon dioxide on critical hematological parameters related to evolutionary adaptations of this species to its physical environment. In vitro measurement of the effect of temperature and carbon dioxide on blood–oxygen affinity and equilibrium curve shape yielded the following data for the respective temperature treatments (11, 15, 19, and 24°C): half-saturation values (P_{50}'s, kPa, a measure of affinity) 1.26, 1.44, 1.63, 1.69 for low-PCO_2 treatments and 2.08, 2.41, 2.74, 2.94 for high-PCO_2 treatments; Bohr factors −0.322, −0.327, −0.366, −0.536; and non-bicarbonate buffer values (slykes) −6, −3, −5, −8. Temperature sensitivities (ΔH, kJ mol O_2^{-1}) between these respective temperatures were −34.20, −15.24, −6.74 for low-PCO_2 treatments and −20.05, −27.00, and −11.55 for the high-PCO_2 treatments. These data suggest that juvenile green sturgeon may tolerate moderate environmental hypoxia, moderate aerobic activity, low to moderate hypercapnia, and moderate temperature changes in their environments.

Keywords *Acipenser* · Blood · Bohr factor · Carbon dioxide · Equilibrium · Temperature

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Introduction

Green sturgeon (*Acipenser medirostris*) are native to the northeast Pacific Ocean Basin, with a wide distribution in coastal waters (Bering Sea to those near the California–Mexico boundary, Moyle 2002). Spawning of this anadromous, chondrostean fish in North America is limited to three Pacific watersheds; Rogue River, Oregon USA, Klamath River, California USA, and the Sacramento River, California USA. All three rivers have been significantly modified (via impoundments and diversions) and the Sacramento and Klamath rivers establish the southern edge of their spawning distribution (Moyle et al. 1994). Although the Sacramento-San Joaquin Estuary green

sturgeon population size is unknown, it is estimated to be significantly smaller than that of native white sturgeon, *A. transmontanus* (Schaffter and Kohlhorst 1999), and the green sturgeon is considered a rare species in North America (Birstein 1993; Moyle et al. 1994; Campbell 1997). Green sturgeon enter these river systems in early spring to spawn, and a percentage out-migrate in early summer with the majority summer-holding in specific areas and out-migrating in the fall when river temperatures decrease (9–13°C) and river flow increases (Erickson et al. 2002, Belchik M, personal comm.[1]). Before migrating to the ocean, juvenile green sturgeon apparently remain in the Klamath River system for 1–3 years (USFWS 1979; Nakamoto et al. 1995), when they can be exposed to substantial temperature variations. Low summer flows and hot summer days increase water temperatures (>24°) in the Rogue and Klamath River systems (Erickson et al. 2002; Belchik M, personal comm.[1] ; Hillemeier 2001, personal comm.[2]).

It is known that green sturgeon aerobic metabolism and growth rates significantly increase with temperature increases from 19°C to 24°C (Mayfield and Cech 2004; Allen et al. 2006b). Because both of these aerobic processes are dependent on sufficient oxygen, it is of interest to examine the effects of temperature on green sturgeon blood–oxygen equilibria.

Blood–oxygen equilibria can be used to gain insights into a fish's functional capacity and consequently its potential environmental limits (Powers 1932; Grigg 1974; Cech et al. 1994). Typically, active fishes inhabiting oxygen-rich water have a more sigmoidal-shaped blood–oxygen equilibrium curve and larger Bohr (CO_2 and pH-related) factors (Cameron 1971; Cech et al. 1984; Dobson et al. 1986), which help maintain oxygen delivery during activity when tissue pH declines and oxygen demand increases. Fishes inhabiting low oxygen environments typically have more hyperbolic-shaped curves and very low P_{50} (half-saturation) values (Cech et al. 1979; Wood and Lenfant 1979), due to high blood–oxygen affinities.

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Temperature effects on blood–oxygen equilibria are well studied in teleost fishes (reviewed in Jensen et al. 1993), but there are far fewer accounts in the chondrosteans. The effects of temperature and CO_2 on blood–oxygen equilibria in white sturgeon (*A. transmontanus*), a related species, have been described, previously (Burggren and Randall 1978; Crocker and Cech 1997). The purpose of this study was to better understand the effect of temperature on green sturgeon's ability to bind and deliver oxygen across the range of environmental temperatures it is likely to encounter.

Materials and methods

Green sturgeon, age 1–3 (age 1: 30.4–35.6 cm total length, 0.65–1.0 kg, $n = 20$; age 2: 48.3–55.9 cm TL, 1.5–2.4 kg, $n = 48$; age 3: 78.8–86.4 cm TL, 2.3–3.5 kg, $n = 20$), used in this study were progeny of wild-caught Klamath River sturgeon that had been artificially spawned during May of 2000, 2002, and 2004 (Van Eenennaam et al. 2001). The eggs were incubated at the Center for Aquatic Biology and Aquaculture (CABA) at the University of California (UC), Davis, and the juveniles were reared in air-equilibrated water at temperatures similar to those in the Klamath River (11–15°C) during late spring. The fish were fed commercial Silvercup trout pellets at 3–5% body weight ration per day based on a feeding table for white sturgeon. Fish (after age 31 d post-hatch) were placed into round 284-l fiberglass holding tanks (ages 0–1) and 12,800-l holding tanks (ages 2–3) receiving continuous flows of air-equilibrated, 19°C well water until needed for experiments.

Fish were quickly anesthetized (10 g l^{-1} NaCl, 420 mg l^{-1} $NaHCO_3$, 350 mg l^{-1} MS-222) and bled via cardiac puncture (Houston 1990; Di Marco et al. 2000, 2001) with heparinized 3-ml syringes and 18-gauge hypodermic needles. The 24-ml blood samples either were collected from larger (3-year-old) or pooled from smaller (2 year-old) sturgeon. All fish were quickly revived and all survived the blood sampling procedure. Blood hematocrit (packed cell volume) was measured (centrifugation at 11,000*g* for 3 min, Houston 1990) and blood (6 ml) was placed into each of two rotating glass tonometers (Hall 1960) for the construction of the first blood–oxygen

equlibrium curve. The remaining blood was held in large (50-ml) Falcon tubes with air and placed horizontally on wet-ice for 60–80 min before being loaded into a second pair of tonometers (second curve). Tonometer pairs were situated in a temperature-controlled water bath (11, 15, 19, or 24 ± 0.3°C) and received either humidified air from an air pump, humidified nitrogen from a cylinder (<0.03 kPa PCO_2, for construction of ''low PCO_2'' curves, estimating arterial conditions), or humidified gas mixtures (1% CO_2 with balance either air or nitrogen) from Wostoff gas mixing pumps (1.01 kPa PCO_2, for ''high PCO_2'' curves, estimating venous conditions). Blood was equilibrated for 40–60 min before samples of oxygenated and deoxygenated blood were withdrawn from the tonometers and mixed in a 1-ml polypropylene syringe with a mixing bead (Edwards and Martin 1966; Scheid and Meyer 1978). To reach target values of 0, 20, 35, 50, 65, 80, 95, and 100% saturation proportional amounts of blood from both deoxy- and oxygenated tonometers were withdrawn and mixed as described above. The PO_2 of each sample was determined using a Cameron Instruments (models BGM200/BC202/E101) or a Radiometer PHM71 blood gas apparatus with thermostatted electrodes. Equilibration of blood, with the respective gas treatments, was defined to be complete after a measure of ≈ 0 kPa (45 min) in the deoxygenated (nitrogen) tonometer's blood. Equilibration in oxygenated tonometers, Hb oxygen saturation of 100%, was complete after 45 min, as shown by repeat sampling and a least squares linear regression analysis (45–120 min tonometry: slope = −0.01, $P = 0.29$, not significantly different than zero). The pH of blood mixtures was analyzed using either an Orion model # SA 720 or an Accumet Basic AB15 pH meter equipped with thermostatted Analytical Sensors, Inc. pH and reference electrodes, with no differences between the two systems. Whole blood lactate (mg l^{-1}) measurements, using a YSI model 2700 Select analyzer, were made on blood from each tonometer at the conclusion of each curve to test for lactacidosis. The time from completion of sampling to completion of an oxygen equilibrium curve was ≤ 2 h. Blood–oxygen equilibria data were converted from mm Hg to kiloPascals (kPa), where 1 mm Hg = 0.13332 kPa and plotted, with curves fitted using non-linear regression options within Sigma Plot 2000, SPSS, Inc. software.

Bohr factors, temperature effects, and non-bicarbonate buffer concentrations were calculated from the collected data. Bohr factors (Φ), a measure of the blood–oxygen affinity's sensitivity to pH and CO_2, were calculated using:

$$\Phi = \Delta \log P_{50} / \Delta \mathrm{pH}$$

where $\Delta \log P_{50}$ (in kPa) and ΔpH are the changes in Log PO_2 and the changes in pH, respectively, in the 50% saturated sample. The temperature effect (ΔH, kiloJoules (kJ) per mol O_2, a measure of the blood–oxygen affinity's sensitivity to temperature), was calculated using a form of the van't Hoff equation (Wood and Lenfant 1979) with kilocalories converted to kiloJoules:

$$\Delta H = 4.578(\Delta \log P_{50} / \Delta(1/T) \times 10^3)$$

where T is temperature in degrees Kelvin (Wyman 1964; Powers et al. 1979). Whole-blood, bicarbonate ion concentrations (β), in mmol HCO_3^- pH^{-1} were calculated using:

$$\beta = \Delta[HCO_3^-] / \Delta \mathrm{pH}$$

Bicarbonate ion concentration (HCO_3^-) was calculated using pH and PCO_2 data in the Henerson–Hasselbalch equation (Davenport 1974) and constants published in Boutilier et al. (1984).

Hemoglobin subunit cooperativity was estimated from slopes (n_{50}) of Hill plots:

$$\log(Y/100 - Y) \text{versus} \log(p)$$

where Y = percent saturations between 20 and 80% and $p = PO_2$ (Riggs 1970). Temperature and pH relationships were analyzed by least squares regression and correlations between measured and calculated variables were tested for significance ($P < 0.05$) using Sigma Stat, SPSS Inc. statistical software.

Similarly, 3 ml blood was pooled from several 1-year-old sturgeon for blood–oxygen capacity (CBO_2), hemoglobin, and nucleoside triphosphate (NTP) measurements. The CBO_2, (ml $O_2 dl^{-1}$ blood) data were determined using the methods and calculations outlined in Tucker (1967). Measurements from an acrylic Tucker cell, thermostatted to 37°C, were made with an E101 oxygen electrode (Analytical Sensors, Inc.) and a Radiometer PHM71 oxygen

analyzer. Total hemoglobin concentrations ([Hb], g dl^{-1} blood) were made using an assay kit (Model 525, Sigma-Aldrich Chemical Company). Whole-blood [NTP] concentrations (i.e., μmole ATP + GTP per μmole Hb) were measured from fish acclimated to 19°C and their blood equilibrated with experimental temperatures and gas mixtures and calculated using enzymatic methods outlined in Adams (1963) using polystyrene cuvettes and an Aquamate UV–VIS spectrophotometer to measure changes in absorbance at 340 nm. The NTP measures were conducted at the conclusion of tonometry (≈ 2 h) in all treatment regimes. Recovery of NTP was estimated at 86 ± 4% in samples spiked with 0.75 μmols ATP ml^{-1} whole blood (Table 1). Statistically indistinguishable P_{50} data among fish ages 1, 2, and 3 confirmed that no blood–oxygen affinity differences existed among these juvenile sturgeon.

Results

Temperature effects

Blood–oxygen affinity showed small, mean decreases (i.e., increased P_{50}) with increases in temperature. Both 19°C and 24°C equilibrated blood (low-PCO_2 treatment) showed a statistically significant decrease in blood–oxygen affinity ($P < 0.05$), compared with that at 11°C, with no significant difference between other low-PCO_2 treatments (Fig. 1). In high-PCO_2 treatments the 11°C O_2 affinity was significantly greater ($P < 0.05$) than those of both the 19°C and 24°C treatments, and the 15°C treatment showed a similar, statistically significant difference with the 24°C treatment. Least squares regressions of P_{50} versus temperature conditions yielded positive slopes (0.04 kPa $°C^{-1}$ [$r^2 = 0.94$]) for the low-PCO_2 treatment and (0.06 kPa $°C^{-1}$ [$r^2 = 0.95$]) for the high-PCO_2 treatment. Blood pH tended to decrease with increases in temperature in both low-PCO_2 (−0.016 pH units $°C^{-1}$ [$r^2 = 0.92$]) and high-PCO_2 treatments (−0.004 pH units $°C^{-1}$ [$r^2 = 0.08$] Fig. 1B). Higher (absolute values) ΔHs showed measurable temperature sensitivity of green sturgeon hemoglobin loading/unloading of O_2 between 11°C and 15°C (especially in the low-PCO_2 treatment) and between 15°C and 19°C (especially the high-PCO_2 treatment) with comparatively lower sensitivities observed between 19°C and 24°C in both low- and high-PCO_2 treatments (Table 2). The CBO_2 measurements showed little apparent trend with temperature, although the 11°C low-PCO_2 treatment mean was significantly greater ($P < 0.05$) when compared with the other treatment groups (Table 3). Mean Bohr factors, Hill's plot slopes (n_{50}), and β showed no statistically significant relationships with temperature ($P > 0.2$), although Bohr factors showed a trend of increasing (absolute value) with temperature throughout the 11–24°C range (Table 2).

Carbon dioxide effects

Within a temperature group high-PCO_2 treatments significantly decreased oxygen affinity, compared with low-PCO_2 treatments, shifting all curves to the right with exposure to increased levels of CO_2 ($P < 0.01$, Fig. 2). The high-PCO_2 curves, with corresponding decreases in pH, facilitated oxygen unloading from hemoglobin under anticipated conditions at sites of metabolically active tissues. There were no significant pH differences ($P > 0.28$ for both low- and high-PCO_2) of the 100% saturated samples within a PCO_2 treatment across temperatures

Table 1 Mean (with SE) nucleoside triphosphate, μmole ATP + GTP per μmol Hb, concentrations in age-1 green sturgeon whole-blood samples equilibrated with low- and high-PCO_2 gas mixtures at experimental temperatures, 11, 15, 19, and 24°C

Temp.	Low-PCO_2		High-PCO_2		Sample	Spike	% Recovered
	Air	N_2	Air + 1% CO_2	N_2 + 1% CO_2			
11	1.19 (0.05)	1.19 (0.07)	1.47 (0.01)	1.39 (0.04)			
15	0.90 (0.06)	0.82 (0.04)	0.83 (0.02)	0.59 (0.02)			
19	0.36 (0.05)	0.38 (0.11)	0.40 (0.08)	0.29 (0.15)			
24	1.18 (0.05)	0.81 (0.07)	1.14 (0.16)	1.02 (0.01)			
QA/QC					0.64 (0.08)	1.33 (0.04)	86 (0.04)

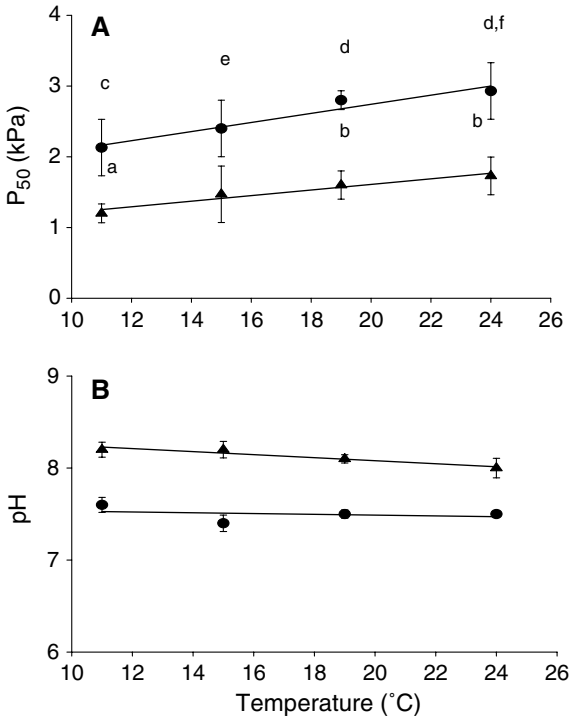

Fig. 1 (**A**) In vitro effects of temperature and CO_2 on mean (±SE) PO_2 at the 50% saturation level (P_{50}) in green sturgeon blood equilibrated with low-PCO_2 (*solid triangles*) and high-PCO_2 (*solid circles*) gas mixtures with least squares regression lines shown. (**B**) In vitro effects of temperature and CO_2 on whole-blood pH equilibrated with low (*solid triangles*) and high (*solid circles*) PCO_2 gas mixtures with least squares regression lines shown. Consecutive letters (within a treatment) indicate a significant difference between respective experimental groups (one-way ANOVA; $P < 0.05$)

(mean ± SE values for low-PCO_2: 8.07 ± 0.22, for high-PCO_2: 7.46 ± 0.10). PCO_2 increases (from <0.03 kPa to 1.01 kPa) statistically decreased ($P < 0.001$) whole-blood pH (Table 2). There were no significant relationships between CBO_2 and CO_2 treatments, and, therefore, no Root effect (Root 1931). Also, there were no significant differences in either HCT or [Hb] within or between treatment groups. Lactate measurements showed no significant relationship with low-PCO_2 (mean ± SE: 171 ± 67 mg l^{-1}) or high-PCO_2 (167 ± 61 mg l^{-1}) treatments across experimental temperatures.

Repeat measures ($n = 3$) of NTP in age-1 green sturgeon blood equilibrated with low- and high-PCO_2 and at experimental temperatures showed no discernable effect pattern in the 100% oxygen-saturated samples, (Table 1), with a possible trend of decreased NTP in the deoxygenated blood (i.e., nitrogen treatments).

Discussion

Our data suggest that green sturgeon have moderate oxygen binding (gills) and oxygen unloading (tissue sites) responses regarding temperature and CO_2 effects. Moderately high blood oxygen affinities, non-bicarbonate buffer values, and moderate n_{50}s may indicate that this species is capable of inhabiting slightly hypoxic- and/or slightly hypercapnic-environments. The CBO_2 and Bohr factors suggest

Table 2 Green sturgeon blood hematological parameters derived from tonometered blood used in blood–oxygen equilibria experiments. Temperature in (°C), PCO_2 (kPa), P_{50} (kPa), HCT = hematocrit (%), β = whole-blood non-bicarbonate buffer value (slykes), Φ = Bohr factor, n_{50} = hemoglobin subunit cooperativity, ΔH = temperature effect (kJ mol O_2^{-1}) in low- and high-PCO_2 treatments, n = number of experimental replicates

Temp.	PCO_2	PH	P_{50}	HCT	β	Φ	n_{50}	ΔH		n
								Low-PCO_2	High-PCO_2	
11	≤0.03	8.22 (0.24)	1.26 (0.08)	22 (1)	−6 (1)	−0.322 (0.07)	1.45 (0.1)			5
11	1.01	7.56 (0.15)	2.08 (0.18)	22 (1)			1.57 (0.03)			5
								−34.20	−20.05	
15	≤0.03	8.10 (0.21)	1.44 (0.05)	19 (1)	−3 (1)	−0.327(0.01)	1.46 (0.04)			6
15	1.01	7.35 (0.15)	2.41 (0.19)	19 (1)			1.69 (0.1)			6
								−15.24	−27.00	
19	≤0.03	8.08 (0.09)	1.63 (0.09)	20 (1)	−5(1)	−0.366 (0.04)	1.40 (0.06)			5
19	1.01	7.45 (0.05)	2.74 (0.05)	20 (1)			1.73 (0.04)			5
								−6.74	−11.55	
24	≤0.03	8.03 (0.27)	1.69 (0.1)	20 (1)	−8 (1)	−0.536 (0.1)	1.49 (0.04)			8
24	1.01	7.48 (0.13)	2.94 (0.13)	20 (1)			1.73 (0.05)			8

Measurements are presented as means with standard deviations in parentheses

Table 3 Mean (with SE) total hemoglobin concentration ([Hb], g dl^{-1}), blood oxygen capacity (CBO_2, ml O_2 dl^{-1} blood), and hematocrit (HCT, %), in low- and high-PCO_2 (kPa) treatments over the experimental range of temperatures (11, 15, 19, and 24°C) in blood from age-1 green sturgeon

Temp.	PCO_2	[Hb]	CBO_2	HCT
11	≤0.03	6 (0.2)	12.6 (0.1)	19
11	1.01		10.1 (0.2)	
15	≤0.03	7 (0.1)	10.3 (0.2)	20
15	1.01		9.8 (0.1)	
19	≤0.03	6 (0.1)	11.3 (0.1)	20.5
19	1.01		11.2 (0.1)	
24	≤0.03	6 (0.3)	11.3 (0.03)	20.5
24	1.01		10.8 (0.2)	

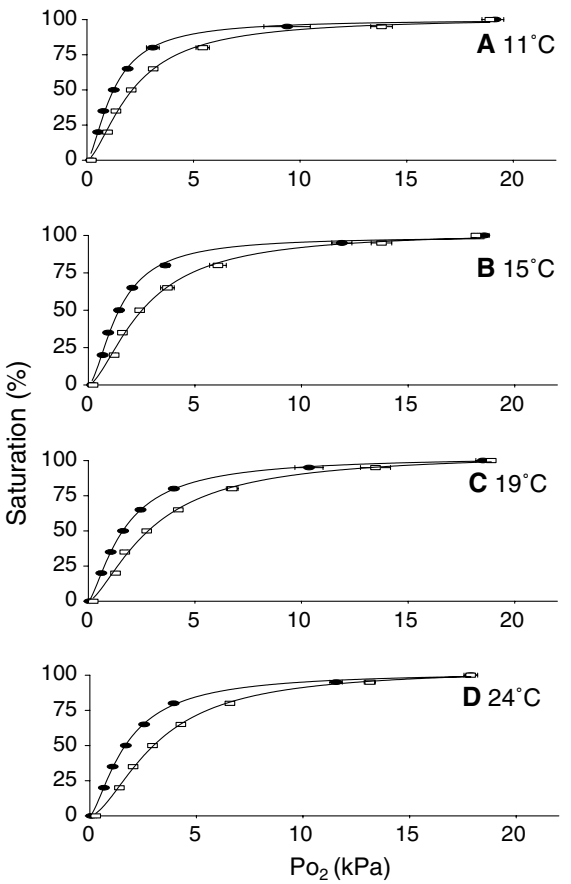

Fig. 2 Green Sturgeon blood–oxygen equilibria at low-PCO_2 (*solid elipses*, PCO_2 = 0.03 kPa) and high-PCO_2 (*open rectangles*, PCO_2 = 1.01 kPa) conditions and temperatures: (**A**) 11°C, (**B**) 15°C, (**C**) 19°C, and (**D**) 24°C. All curves were fitted to data means (± SE) with non-linear regressions, and R^2 values ranged from 0.998 to 1.000

that green sturgeon may have a moderate capacity for aerobic activity, throughout the temperature range tested.

Hypoxic and hypercapnic environments

Curve shape and position provide information on a species' ability to efficiently extract oxygen from water. Species with hyperbolic curves (e.g., carp with extraction efficiencies close to 80%, Lomholdt and Johansen 1979), typically extract more O_2 than species with sigmoidal curves (e.g., rainbow trout with an extraction efficiency of 50%, Davis and Cameron 1971). Hill constants showed green sturgeon blood–oxygen curves to be moderately sigmoidal (mean n_{50} = 1.45, Table 2) and are consistent with observations of sigmoidicity in other anadromous sturgeons' blood–oxygen equilibria curves (Kamshilov 2003).

A mathematical model, Malte and Weber (1987), comparing n_{50}, P_{50}, extraction efficiency, and ventilatory effort indicates that green sturgeon would fall between carp and rainbow trout with an extraction efficiency between 60 and 70%, if a comparison between teleostean and chondrostean species is warranted. Increases in P_{50} can be associated with greater ventilation effort, and increases in n_{50} may be required to offset the energetic costs associated with ventilation while maintaining extraction efficiency (Malte and Weber 1987). The green sturgeon's high oxygen affinity, with their n_{50}s of 1.40–1.49 (Table 2), presumably facilitates oxygen loading while minimizing the costs associated with ventilation.

Non-bicarbonate buffer values (β) represent the ability of blood proteins to buffer changes in PCO_2 (Davenport 1974; Wood et al. 1977). Green sturgeon βs (mean: −5.5) were comparable to those of rainbow trout (β = −5.05 to −6.36, Cameron 1971) and lower than those of white sturgeon (β = −15.27 to −15.78, Crocker and Cech 1998) with the highest β for green sturgeon noted at 24°C (Table 2). These data suggest a limited ability of green sturgeon to handle metabolically produced or increased environmental CO_2.

Temperature

The reaction of hemoglobin with oxygen is exothermic, and increases in temperature typically decrease blood–oxygen affinities (Wood and Lenfant 1979;

Powers 1980, 1983). The apparent heat of oxygenation, ΔH, represents the combined effect of the exothermic binding of oxygen and the endothermic release of intrinsic intraerythrocytic ligands (e.g., NTP and protons), providing a measure of temperature sensitivity for hemoglobin–O_2 affinity (Wood and Lenfant 1979; Jensen et al. 1993). Clementi et al. (2001) found that purified hemoglobin hemolysates from the Italian sturgeon, *Acipenser naccarii*, showed a very low temperature sensitivity across the range of pH's examined. In the present study we noted measurable changes in ΔH with the largest difference (absolute value) between 11°C and 15°C in the low-PCO_2 treatment and between 15°C and 19°C in the high-PCO_2 treatment (Table 2) with a minimal increase in temperature sensitivity between the 19°C and 24°C temperature treatments. The low temperature sensitivity of green sturgeon blood–oxygen binding between 19°C and 24°C could assist this species' binding sufficient oxygen when oxygen demands are highest (Mayfield and Cech 2004; Allen and Cech in press). The P_{50} plateau between 19°C and 24°C would exact the least blood–oxygen affinity ''penalty'' at the gills for the green sturgeon when river temperatures reach these high levels (Erickson et al. 2002).

One of the two dominant NTPs in fishes, GTP, shows a strong modulating effect on oxygen binding in purified hemoglobin hemolysates of the Italian sturgeon (Clementi et al. 2001). Although we found no temperature-associated blood [NTP] pattern in our in vitro experiments, green sturgeon may have the ability to regulate hemoglobin–oxygen affinity via the observed whole-blood NTP pools.

Activity

Anadromous species often migrate substantial distances to reach suitable spawning habitat. Green sturgeon migrate distances up to 118 km in the Rogue River, Oregon USA (Erickson et al. 2002), 425 km in the Sacramento River, California (Brown 2002), and 113 km in the Klamath River, California (Moyle 2002). Sustained aerobic activity is required for species to successfully complete upstream migrations (Hinch and Rand 2000). Aerobically active species have large CBO_2s and Φs to efficiently transport and unload O_2 to exercising red muscle (Cameron and Davis 1970; Weber and Wells 1989). Fish that are extremely active such as albacore, *Thunnus alalunga*, have exceptionally large CBO_2s (21.8 ml dl^{-1}, Cech et al. 1984). Measured green sturgeon CBO_2s fall below those of albacore but are comparable to those of rainbow trout (8.9–9.8 ml dl^{-1}, Cameron 1971) and northern pikeminnow (10.7–13.0 ml dl^{-1}, Cech et al. 1994, Table 3). The comparatively large Bohr factors in fishes characterized as being highly active, i.e., rainbow trout (−0.57, Cameron 1971) or Atlantic mackerel, *Scomber scombrus*, (−1.2, Hall and McCutcheon 1938), presumably facilitate unloading of oxygen at metabolically active tissues. In comparison to other species, green sturgeon Bohr factors (Table 2) fall between reported values for comparatively less active brown bullhead, *Ameiurus nebulosus*, (−0.31, Eddy 1971) and European flounder, *Platichthys flesus*, (−0.55, Weber and DeWilde 1975). Bohr factors for green sturgeon were comparable to those found in two species of anadromous sturgeon, Siberian sturgeon, *Acipenser baerii baicalensis*, (−0.40, Kamshilov 2003) and Amu-Darya shovelnose, *Pseudoscaphirhynchus kaufmannii*, (−0.35, Kamshilov 2003) when these fish were captured in fresh water. Swimming performance should reflect capacity for aerobic activity, and critical swimming velocity (U_{crit}) is a commonly used measure of this performance (Brett 1964). Green sturgeon $U_{crit's}$ (determined at 19°C and 20-min intervals), ranging from 44 cm s^{-1} (20 cm TL, Allen et al. 2006a, in press), to 53 cm s^{-1} (67.6 cm TL, Lankford et al. 2005), fall below the 15−min interval values (U_{crit}: 64.7 cm s^{-1}, at 16°C, 61 cm standard length) for shovelnose sturgeon, *Scaphirhynchus platorynchus* (Adams et al. 1997), but above the 30-min interval U_{crits} reported for shovelnose sturgeon (U_{crit}: 36.9 cm s^{-1}, at 20°C, 18 cm SL) and pallid sturgeon, *Scaphirhynchus albus* (U_{crit}: 35.9 cm s^{-1}, at 20°C, 18 cm SL, Adams and Adams 2003). Therefore, the comparatively large CBO_2s, high oxygen affinities, U_{crit}, and the limited effects of temperature on green sturgeon oxygen affinity, as measured in vitro, are consistent with observations of green sturgeons' abilities to maintain moderate aerobic activity (Lankford et al. 2005; Allen et al. 2006a, in press).

In conclusion, green sturgeon display in vitro blood–oxygen equilibrium characteristics that are intermediate between ''low'' and ''high'' activity-level species and include a mildly sigmoidal

equilibrium curve with relatively high oxygen affinities, low non-bicarbonate buffer values, and low temperature sensitivities. These characteristics may enhance its ability to tolerate hypoxic environments (e.g., when compared to that of rainbow trout). Its high blood O_2 capacity, low to moderate temperature sensitivity, and moderate Bohr factors suggest that green sturgeon may be capable of aerobic activity across a wide range of environmental temperatures. Finally, hydrological and thermal management of flow regimes on the remaining river habitats used by this species for reproduction should include considerations of its physiological limitations (e.g., blood–oxygen equilibrial dynamics) as well as life-stage-dependent environmental requirements (Van Eenennaam et al. 2005) to maximize the reproductive success of this rare North American sturgeon.

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