

# Cardiorespiratory responses of white sturgeon to environmental hypercapnia

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**Crocker, Carlos E., Anthony P. Farrell, A. Kurt Gamperl, and Joseph J. Cech, Jr.** Cardiorespiratory responses of white sturgeon to environmental hypercapnia. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R617–R628, 2000.—Cardioventilatory variables and blood-gas, acid-base status were measured in cannulated white sturgeon (*Acipenser transmontanus*) maintained at 19°C during normocapnic and hypercapnic ( $Pw_{CO_2} \sim 20$  Torr) water conditions and after the injection of adrenergic analogs. Hypercapnia produced significant increases in arterial  $PCO_2$ , ventilatory frequency, and plasma concentration of cortisol and epinephrine, and it produced significant decreases in arterial pH and plasma concentration of glucose but no change in arterial  $PO_2$ , hematocrit, and concentration of lactate or norepinephrine. Hypercapnia significantly increased cardiac output (Q) by 22%, mean arterial pressure (MAP) by 8%, and heart rate (HR) by 8%. However, gut blood flow (GBF) remained constant. In normocapnic fish, phenylephrine significantly constricted the splanchnic circulation, whereas isoproterenol significantly increased Q and produced a systemic vasodilation. During hypercapnia, propranolol significantly decreased Q, GBF, MAP, and HR, whereas phentolamine significantly decreased MAP and increased GBF. These changes suggest that cardiovascular function in the white sturgeon is sensitive to both  $\alpha$ - and  $\beta$ -adrenergic modulation. We found microspheres to be unreliable in predicting GBF on the basis of our comparisons with simultaneous direct measurements of GBF. Overall, our results demonstrate that environmental hypercapnia (e.g., as experienced in high-intensity culture situations) elicits stress responses in white sturgeon that significantly elevate steady-state cardiovascular and ventilatory activity levels.

cardiac output; gut blood flow; blood pressure; *Acipenser*; acid-base

IN THE WILD, FISH CAN BE EXPOSED to environmental fluctuations in  $O_2$  and  $CO_2$  levels. In addition, the rearing of fish under intensive culture conditions is associated with major alterations in dissolved gas concentrations. For example, the high-density ( $\sim 75$  kg/m<sup>3</sup>) rearing of white sturgeon with the use of  $O_2$  injection and water recirculation results in extremely high water- $CO_2$  levels (25–35 Torr  $PCO_2$ ) because of the metabolic produc-

tion of large amounts of  $CO_2$  by the fish.  $CO_2$  is extremely soluble in both water and plasma, and it diffuses rapidly across gill membranes. Thus although the increased arterial  $PCO_2$  ( $Pa_{CO_2}$ ) induces hyperventilation (11), this response is ineffective in reducing  $Pa_{CO_2}$ , and the resulting respiratory acidosis may significantly affect the cardiovascular system. Although the negative inotropic effects of hypercapnic acidosis on the teleost myocardium are well documented in vitro (13, 20), little is known of these effects in vivo where the possibility of ameliorative responses exists. Perry et al. (32) recently published a study on cardiovascular responses of rainbow trout *Oncorhynchus mykiss* to environmental hypercapnia, but there have been no reports on cardiovascular function in chondrosteans (paddlefish and sturgeon) fishes when these species are exposed to stressful gas concentrations.

Teleosts and elasmobranchs respond to hypoxia and hypercapnia by releasing catecholamines (33) and corticosteroids (9, 26). Although we know much about the adrenergic control of the cardiovascular system under normoxia and hypoxia (13, 17, 18, 29, 31), scant data are available on the adrenergic control of cardiovascular function in fishes under hypercapnic conditions. On the basis of in vitro studies, adrenergic stimulation of the teleost myocardium is protective of inotropic status under hypercapnic acidosis (13). In contrast, high levels of circulating catecholamines are likely to increase systemic resistance and to alter regional blood distribution. Of particular interest in the present study was the regulation of gut blood flow (GBF) because a general problem found in high-intensity aquaculture operations, including white sturgeon, is reduced growth and survival rates (42). Early work on fishes demonstrated that blood sampling from the intestinal vein was difficult during periods of high swimming activity, after stress, and after injection of catecholamine agonist drugs (34). The conclusions drawn from these observations were that GBF was very labile and that a potent  $\alpha$ -adrenergic vasoconstrictory mechanism existed in the gut (7). These conclusions were confirmed

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in vivo on sea raven *Hemitripterus americanus* (2), and later work has further increased our knowledge of the control of GBF in both elasmobranch and teleost examples (e.g., Refs. 21, 41). In the species examined, brief stresses cause rapid decreases in GBF, whether the fish is in a nonfeeding or postprandial state. Nothing is known about GBF and its control in white sturgeon and, therefore, it is possible that reduced growth rate under hypercapnia is a result of poor GBF limiting the digestive process.

The present study focused on comparing the cardiovascular status of white sturgeon during exposure to normocapnic and hypercapnic conditions. We hypothesized that the stress associated with hypercapnia would lead to significant changes in cardiac output (Q) and its distribution such that GBF would be significantly reduced. To begin to test this hypothesis, we measured Q and GBF with ultrasonic flow probes and regional blood flow with colored microspheres. Colored microspheres are regarded as a safe alternative to radiolabelled microspheres to examine regional blood flow in fish (4, 7, 23). Shifts in blood flow patterns were induced by hypercapnia and by adrenergic agonist and antagonist drug injections. To our knowledge, this is the first study involving simultaneous measurements of Q, GBF, blood pressure (BP), and blood gas, acid-base status in sturgeon.

## MATERIALS AND METHODS

**Animals.** White sturgeon (*Acipenser transmontanus*; 1.5–2.8 kg) were collected from a commercial sturgeon farm (Sierra Aquafarms; Elverta, CA) and quickly (<1 h) transferred to the University of California, Davis. Fish were transported in an oxygenated tank of well water from the farm and were placed outdoors in 1.3-m diameter fiberglass tanks (750 liter) receiving a continuous flow of aerated well water ( $19 \pm 0.5^\circ\text{C}$ ). The fish were fed twice daily (Silvercup trout pellets) and were allowed at least 2 wk to recover from any transport-related stress.

**Surgical procedures and recovery.** Cardiovascular data were collected from 28 fish ( $1.8 \pm 0.1$  kg) that were subjected to one or more of the following surgical manipulations. Before surgical procedures, the fish were dip-netted and placed into a buffered (pH 7.0 with the use of  $\text{NaHCO}_3$ ) anesthetic water bath [3-aminobenzoic acid ethyl ester (MS-222), Sigma; 0.2 g/l] until ventilatory movements ceased. Fish were then weighed and placed on an operating table (dorsal recumbency), and retrograde ventilation was begun with an oxygenated, buffered anesthetic solution (MS-222; 0.1 g/l).

**Cannulation of the dorsal aorta.** Skin sutures (3–0 silk, Ethicon; Somerville, NJ) were placed between the first and third gill arches on the dorsal aspect of the buccal cavity and on the inside of the mouth. A 19-gauge hypodermic needle (Becton Dickinson Labware; Franklin Lakes, NJ) was used to puncture the cartilage between the second and third gill arches. Thereafter, a 1-m length of heparinized polyethylene tubing (PE-50, 0.58-mm ID, 0.965-mm OD; Clay Adams; Parsippany, NJ) with an indwelling stainless steel wire was inserted into the dorsal aorta (DA). The wire was then removed, and the cannula was anchored in place with the preplaced sutures and led out of the mouth through a small-bore hole. The cannula was filled with heparinized saline (10 IU/ml, 0.9% NaCl) and regularly flushed to prevent clot formation. The DA cannula was used for arterial BP mea-

surements, drug injections, and blood sampling. All 28 fish had a DA cannula, and of these, three had only a DA cannula.

**Cardiac output.** After dorsal aortic cannulation, the fish was placed on its side, and the gills and operculum were gently retracted to allow implantation of the ventral aorta (VA) flow probe for Q measurement. Access to the VA was achieved without disrupting the pericardium. A 1-cm incision was made in the isthmus parallel to the VA. The subdermal musculature and connective tissue were carefully teased apart with the use of blunt dissection to expose the VA, and an ultrasonic flow probe (3SB or 4SB, Transonic Systems; Ithaca, NY) was placed loosely around the vessel. Single silk sutures anchored the probe head onto the musculature of the isthmus, and the probe led to the inside of the opercular cavity and at two locations on the dorsal aspect of the body. Of the 28 fish tested, eight had a VA flow probe (DA + Q).

**Splanchnic blood flow.** In white sturgeon, the celiacomesenteric artery (CMA) is a single, short, large-diameter vessel lying in a dorsoventral direction on the right side of the fish between the liver and pharynx. To expose the CMA, a 5- to 7-cm-long midventral incision was made posterior to the pectoral girdle, and the liver was gently retracted. GBF was measured with an ultrasonic blood flow probe (1.5 or 2 SB, Transonic Systems) that was placed around the CMA. The probe head was held in place by a silk suture in the intestinal wall and by one or two sutures in the dorsal aspect of the visceral wall. After the flow probe head was filled with acoustic gel and probe operation was verified, the incision was sutured (3–0 silk, Ethicon) closed with a slightly everted closure pattern while antibiotic powder (erythromycin) was dusted onto the surgical field. The probe lead was then anchored once to the skin on the fish's ventral surface and twice along the lateral aspect of the body. Of the 28 fish, 13 had a splanchnic flow probe, and 10 of these also had a VA flow probe for simultaneous measurement of Q and GBF (DA + Q + GBF).

**Postoperative care.** Surgery generally lasted <1.25 h (0.75- to 1.75-h range), and recovery from anesthesia was initiated by artificial ventilation with the use of aerated, anesthetic-free water. Once ventilatory activity returned (generally <5 min), the fish was placed into a Plexiglas holding chamber (triangular in cross section, 30 liter,  $20 \times 20 \times 91$  cm) that accommodated the extended pectoral fins of the sturgeon. The holding chamber was fully submersed in an insulated fiberglass tank (250 liter), and both the tank and holding chamber received a continuous flow of aerated well water ( $\sim 5$  liter/min). Continuous flows of water over and under the fish were achieved with the use of two vertically positioned inlet ports at the anterior end of each chamber (10 cm apart). The dorsoventral pattern of water flow through the chamber was used because it appeared to minimize confinement stress. Each chamber was partially covered with black plastic to shield the animal from laboratory activity. Fish were allowed at least 24 h to recover from surgery before experiments.

Cannulas were flushed regularly with heparinized saline (100 IU/ml, 0.9% NaCl) to minimize clotting and to avoid the formation of thrombi. To determine whether our cannulation technique caused significant tissue damage in the fish, we performed postexperimental examination of the cannulas' position. No animals were found with significant tissue damage resulting from cannulation.

**Experimental protocol.** During normocapnia, cardiovascular variables [Q, GBF, BP, and heart rate (HR)] and ventilatory frequency ( $V_p$ , determined visually by counting opercular movements) were continuously measured both before and after the injection of two vasoactive substances (Sigma; St. Louis, MO): the  $\alpha$ -adrenergic agonist phenylephrine hydro-

chloride (PEPH, 0.1 mg/kg) and the  $\beta$ -adrenergic agonist isoproterenol hydrochloride (Iso, 1.0  $\mu$ g/kg). In preliminary experiments, cardiovascular responses to lower doses of PEPH and Iso (0.025 and 0.05 mg/g) produced inconsistent responses, whereas higher doses had prolonged cardiovascular effects. When fish struggled in the chambers, all cardiorespiratory parameters were allowed to return to baseline levels before drug injection. The cannula was flushed after all drug injections (1.0 ml, 0.9% NaCl), and each drug injection was followed by a recovery period of no less than 15 min, sufficient time for all cardiovascular variables being measured (i.e., HR, Q, or BP) to return to preinjection levels. After all drug injections in normocapnia, a blood sample was withdrawn for analysis of various hematological parameters.

Hypercapnia was initiated 30 to 40 min after the Iso injection during normocapnia. The desired  $Pw_{CO_2}$  level ( $Pw_{CO_2} \sim 20$  Torr, simulating conditions in high-density aquaculture tanks) was achieved gradually (over a 45-min period) during which time cardiovascular variables were monitored continuously. After 2 h of steady-state hypercapnia, a second sequence of drug injections was initiated. Cardiovascular variables and  $V_f$  were remeasured before and after the sequential injection of four vasoactive substances: PEPH, 0.1 mg/kg; Iso, 1.0  $\mu$ g/kg; the  $\alpha$ -adrenergic antagonist phentolamine hydrochloride (Phent, 0.1 mg/kg); and the  $\beta$ -adrenergic antagonist propranolol hydrochloride (Prop, 2 mg/kg). With the exception of the Prop treatment, a recovery period of at least 15 min was allowed after each drug injection. The responses to the antagonist Prop were measured after 30 min when cardiovascular variables stabilized. A second blood sample was taken for hematologic analysis after the Iso injection during hypercapnia ( $\sim 4$  h after the initiation of hypercapnia).

Water- $CO_2$  levels in the fish holding chambers were controlled with an equilibration column with the use of counterflows of water and gas upstream from the chambers. Normocapnic water was equilibrated with air, whereas hypercapnic water was produced with a 10%  $CO_2$ -90% air mixture. The mixture came from either premixed gas cylinders or by combining pure gases with an electronic gas mixer (Cameron Instrument, model GF-2; Port Aransas, TX). This method yielded the same  $Pw_{CO_2}$  of 20 Torr.

**Measurement and calculation of cardiorespiratory parameters.** During experiments, BP and HR were monitored by connecting the fish's DA cannula to a Statham P23B (Oxnard, CA) pressure transducer, and Q and GBF were monitored by connecting the flow probes to a two-channel small animal blood flowmeter (T-206, Transonic Systems). Signals from the pressure transducers and the flowmeter were displayed on a Gilson (Middleton, WI) ICT-5H multichannel recorder, and these recordings were used directly for the measurement of all cardiovascular parameters.

The factory-calibrated flow probes were periodically checked by securing each probe around a short section of polyethylene tubing (Transonic Systems) and pumping saline through the tubing with the use of a multistaltic pump (model 426-2000, Haake Buchler Institute; Saddlebrook, NJ). Probes used in this study were in excellent agreement (e.g.,  $r^2 = 0.995$ ) with the known flows generated by the peristaltic pump. Pressure transducers were calibrated daily with the use of a static water column.

Values for HR, arterial BP, and  $V_f$  were determined by measuring 30-s intervals. Mean arterial pressure (MAP, mmHg) was calculated as  $MAP = \text{diastolic pressure} + 1/3 \text{ pulse pressure}$  (pulse pressure = systolic pressure - diastolic pressure). Chart records of 5-min periods of Q and GBF were digitized and integrated to obtain blood flow values (in ml  $\cdot$

$\text{min}^{-1} \cdot \text{kg}^{-1}$ ). Cardiac stroke volume (SV; in ml  $\cdot \text{beat}^{-1} \cdot \text{kg}^{-1}$ ) was calculated as  $SV = Q/HR$ , and systemic vascular resistance ( $R_{sys}$ ; in mmHg  $\cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) was calculated with the use of the formula  $R_{sys} = MAP/Q$ . Splanchnic vascular resistance ( $R_{spl}$ ; in mmHg  $\cdot \text{ml}^{-1} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) was determined with the use of  $MAP/GBF$ . Relative GBF (rGBF) was calculated as  $(GBF/Q) \times 100$ , and it is the percentage of Q going to the liver, spleen, stomach, and intestine. Artifacts associated with struggle episodes were avoided when selecting traces to measure control HR, MAP, and blood flows, by allowing sufficient time for all cardiovascular variables being measured (i.e., HR, Q, or BP) to return to prestruggle levels. However, the data after each struggle were analyzed to evaluate the effect of struggling on cardiovascular function.

**Regional blood flow measurements.** Colored microspheres [NuFlow microspheres, Interactive Medical Technologies (IMT); Los Angeles, CA] were used to measure regional blood flow distribution in white sturgeon ( $n = 5$ ,  $1.4 \pm 0.3$  kg) at 19°C. Microspheres were suspended in a solution of saline and 0.01% Tween 80 to a final concentration of  $2.5 \times 10^6$  ml, and 200- $\mu$ l samples of the solution were injected into the fish. Before the injections, which occurred at the same time of day for each fish ( $\sim 1130$ , 1530, and 1630), the solutions were vigorously shaken for 1 min. Colored microspheres (25  $\mu$ m in diameter) were injected via the DA cannula during normocapnia (control, violet), after 2-h exposure to hypercapnia (pink), and 5 min after Phent injection during hypercapnia (blue). A 1.0-ml reference blood sample was taken 10 min after the injection of each of the colored microspheres. At the end of the experiment, fish were humanely killed (overdose of MS-222), and the tissues and organs of interest were dissected from the animal. The head, white and red muscles, skeleton, ventricles, liver, spleen, stomach, intestine, kidney, gonads, and a reference blood sample were individually weighed. The heart, kidney, and reference blood samples were placed into 15-ml polypropylene centrifuge tubes (weighed tissue <5 g). Organs >10 g (liver, muscle, stomach, intestine, and gonads) were minced, and subsamples ( $n \leq 4$ ) were placed into 50-ml polypropylene centrifuge tubes (weighed tissue <8 g). Each tube was filled with diluted alkaline solution (IMT), stored at room temperature for 1 wk, and shipped to IMT for tissue processing and sphere extraction and counting. GBF (ml  $\cdot \text{min}^{-1} \cdot \text{g}^{-1}$  tissue) with the use of colored microspheres was determined by converting sphere deposition values (percent of total spheres recovered) to spheres/g tissue and correcting for Q with the use of:  $GBF = Q \times \text{wt} \times FQ$ , where Q is the mean cardiac output, wt is the body weight of the fish (kg), and FQ is the fraction of Q/g tissue (5). The sum of individual flows for the liver, spleen, stomach, and intestine represented total GBF. All procedures were approved by the University of California Davis Animal Use and Care Administrative Advisory Committee.

**Blood analysis.** Blood samples (0.7 ml) were taken with the use of a gas-tight glass syringe to determine blood gas and hematologic parameters. Arterial pH ( $pH_a$ ) was measured with the use of an acid-base analyzer and thermostated electrodes (Radiometer PHM73/G297/K497; Copenhagen, Denmark) calibrated with temperature-corrected precision buffers (Radiometer). Arterial  $PO_2$  ( $Pa_{O_2}$ ) and  $Pa_{CO_2}$  were measured with the use of the acid-base analyzer with thermostated electrodes (Radiometer E5046/D616 and E5036/D616, respectively). These electrodes were calibrated with the use of humidified  $N_2$  and air, and with a 10%  $CO_2$ -90% air mixture before each measurement, respectively. Hematocrit (percent packed red cells after centrifugation for 3 min at 11,000 g) and whole blood concentration of lactate (mM) and glucose (mM) (YSI 2700 Select analyzer) were immediately measured. The remaining blood



was centrifuged at 4,500 *g* for 5 min, and the plasma was extracted, preserved with an equal volume of sodium metabisulfite (1 mM, an antioxidant), and stored (<30 days) at -70°C until analyses were performed.

Plasma catecholamines (epinephrine and norepinephrine) were analyzed at California State Polytechnic University at Pomona on a Bioanalytical Systems (West Lafayette, IN) HPLC system with electrochemical detection. Catecholamines were bound to aluminum oxide at pH 8.6 (1.5-M Tris-EDTA buffer), the aluminum oxide was washed twice with ice-cold distilled water, and the catecholamines were then eluted with 0.1 M HClO<sub>4</sub>. An internal standard (3,4-dihydroxybenzylamine) was placed in all samples to control for minor variations in extraction-elution efficiency between tubes. The samples were then centrifuged, and the eluate was injected into the HPLC system circulating a mobile phase consisting of 0.15 M monochloroacetic acid, 1 mM Na<sub>2</sub>EDTA, and 1 mM sodium octylsulfate in 1.5% acetonitrile, maintained at pH 3.0. The temperature in the system was maintained at 35°C. The column used in catecholamine analysis was a 7-μm BAS Phase II ODS reverse-phase column, and the flow rate of the mobile phase was 1.0 ml/min. Plasma cortisol (ng/ml) was determined at the same laboratory with the use of a commercial assay kit (Coat-A-Count kit, Diagnostic Products; Los Angeles, CA) and a gamma counter (Beckman Instruments; Fullerton, CA).

**Water analysis.** Water PO<sub>2</sub> and PCO<sub>2</sub> were measured with use of the Radiometer acid-base analyzer. Water pH was measured with a hand-held pH meter (Corning PS-15; Corning, NY) that was calibrated with commercial buffer solutions (Fisher Scientific; Pittsburgh, PA) before each measurement. Water temperature was measured daily with the use of a mercury thermometer and maintained at 19 ± 1°C with the use of chillers (Elkay ER-10; Lanark, IL) and thermostated (YSI 72; Yellow Springs, OH) submersible heaters. Water quality [Cl<sub>2</sub>] and [NH<sub>3</sub>/NH<sub>4</sub><sup>+</sup>] was measured daily with the use of Cl<sub>2</sub> (Hach; Loveland, CO) and ammonia (Chemetrics; Calverton, VA) test kits, respectively. Values were always <0.01 and 0.2 parts/million, respectively.

**Statistical analysis.** Normocapnic and hypercapnic values for blood gas, acid-base, stress hormone, and respiratory data in resting sturgeon were compared with the use of paired *t*-tests. Control values (values before drug injections or struggle episodes) for hemodynamic data (Q, HR, SV, MAP, R<sub>sys</sub>, GBF) during normocapnia and hypercapnia were compared with the use of a one-way, repeated-measures ANOVA followed by the Bonferroni multiple comparisons procedure (SigmaStat statistical software, Jandel Scientific; San Rafael, CA). Cardiovascular responses to drug injections were compared with the control values preceding the injection with the use of a paired *t*-test. In all cases, statistical significance was taken as *P* < 0.05. All data presented in figures and throughout the text are means ± SE.

## RESULTS

**Normocapnia.** Normocapnic sturgeon had a pH<sub>a</sub> of 7.76 ± 0.18, a Pa<sub>CO<sub>2</sub></sub> of 2.8 ± 0.7 Torr, and a Pa<sub>O<sub>2</sub></sub> of 116.6 ± 4.1 Torr. In addition, plasma concentration of lactate was 0.52 ± 0.10 mM, plasma concentration of glucose was 5.2 ± 0.4 mM, and hematocrit was 21.6 ± 0.6%. Plasma stress-hormone concentrations under normocapnic conditions were 41.5 ± 5.5 nM epinephrine, 96.5 ± 12.0 nM norepinephrine, and 215.8 ± 38.5 ng/ml cortisol. Some fish underwent more extensive surgery than others, and this is reflected in the mea-

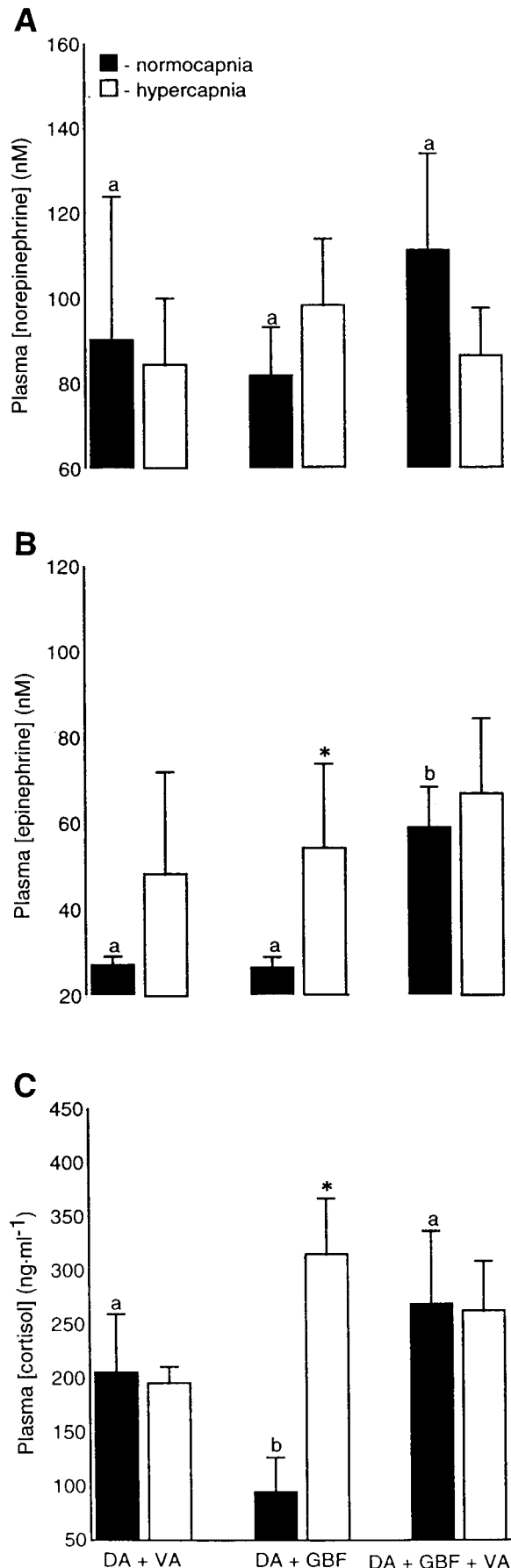
sured values of the concentration of epinephrine and cortisol (Fig. 1, *B* and *C*). Plasma concentrations of epinephrine and cortisol were significantly lower for DA + GBF fish than for DA + Q + GBF fish. V<sub>f</sub> was 58.5 breaths/min in normocapnic fish, and Q, HR, and SV were 36.1 ± 2.3 ml · min<sup>-1</sup> · kg<sup>-1</sup>, 48.0 ± 1.2 beats/min, and 0.83 ± 0.1 ml · beat<sup>-1</sup> · kg<sup>-1</sup>, respectively (Fig. 2, *A–C*). GBF in normocapnic fish was 8.9 ± 1.1 ml · min<sup>-1</sup> · kg<sup>-1</sup> (Fig. 3*A*). This represented ~20% of Q. Control MAP and R<sub>sys</sub> were 21.9 ± 0.7 mmHg and 0.66 ± 0.03 mmHg · ml<sup>-1</sup> · min<sup>-1</sup> · kg<sup>-1</sup>, respectively (Figs. 4, *A* and *B*).

Struggles during normocapnia significantly increased Q (by 29%) and caused an abrupt and large decrease in absolute GBF (to 2.5 ± 0.5 ml · min<sup>-1</sup> · kg<sup>-1</sup>) and rGBF (to 3.3% of Q). GBF was normally restored to control levels within 4 to 6 min after a struggle during normocapnia unless another struggle ensued. An example of the effect of a struggle on GBF in a hypercapnic sturgeon is shown in Fig. 5.

PEPH injections had no significant effect on Q, HR, or SV (Fig. 2). However, PEPH injections caused MAP and R<sub>sys</sub> to increase by similar amounts (30 and 31%, respectively; Fig. 4). This result suggests that α-adrenoceptors are involved in vasoconstrictory control of the systemic circulation. Because PEPH injections significantly increased R<sub>spl</sub> by threefold (Fig. 3*B*), decreasing GBF by 40% and rGBF from 20 to 10%, it appears that a significant proportion of the α-adrenergic control of MAP was located within the splanchnic circulation. Iso injections significantly increased Q (by 12%), HR (by 9%), and SV (by 13%) (Fig. 2), and it significantly decreased R<sub>sys</sub> and MAP (by 12 and 14%, respectively) (Fig. 4). These results suggest that β-adrenoceptors are involved in vasodilatory control of the systemic circulation and stimulation of cardiac activity. In contrast to PEPH, Iso did not affect R<sub>spl</sub>, GBF, or rGBF.

**Hypercapnia.** Hypercapnia resulted in a respiratory acidosis in white sturgeon. Four hours of hypercapnic exposure significantly increased Pa<sub>CO<sub>2</sub></sub> (to 19.5 ± 0.7 Torr) and significantly decreased pH<sub>a</sub> (to 7.36 ± 0.08), without affecting plasma concentration of lactate (0.35 ± 0.1 mM). As expected, hypercapnia stimulated a 60% increase in V<sub>f</sub> (to 95.5 ± 1.7 breaths/min). There was a small but statistically significant reduction in plasma concentration of glucose (to 4.5 ± 0.3 mmol/l) and significant increases in concentrations of cortisol (DA + GBF fish and mean values of all fish, Fig. 1) and of epinephrine (DA + GBF fish only, Fig. 1) with hypercapnic exposure. On the other hand, Pa<sub>O<sub>2</sub></sub>, concentration of norepinephrine, and hematocrit were unaffected during hypercapnia.

Before initiating hypercapnia, values for Q, HR, SV, MAP, and R<sub>sys</sub> were not significantly different from those measured at the start of the experiments. Two hours of hypercapnia significantly increased Q (by 31%), HR (8%), and SV (41%) (Fig. 2). Initially, (~0.5 h) hypercapnia-mediated increases in MAP (to 22.6 ± 0.8 mmHg) reflected increases in Q not alterations in R<sub>sys</sub>. However, after 2 h of hypercapnia, MAP remained elevated (at 22.5 ± 0.8 mmHg; Fig. 3) despite a signif-



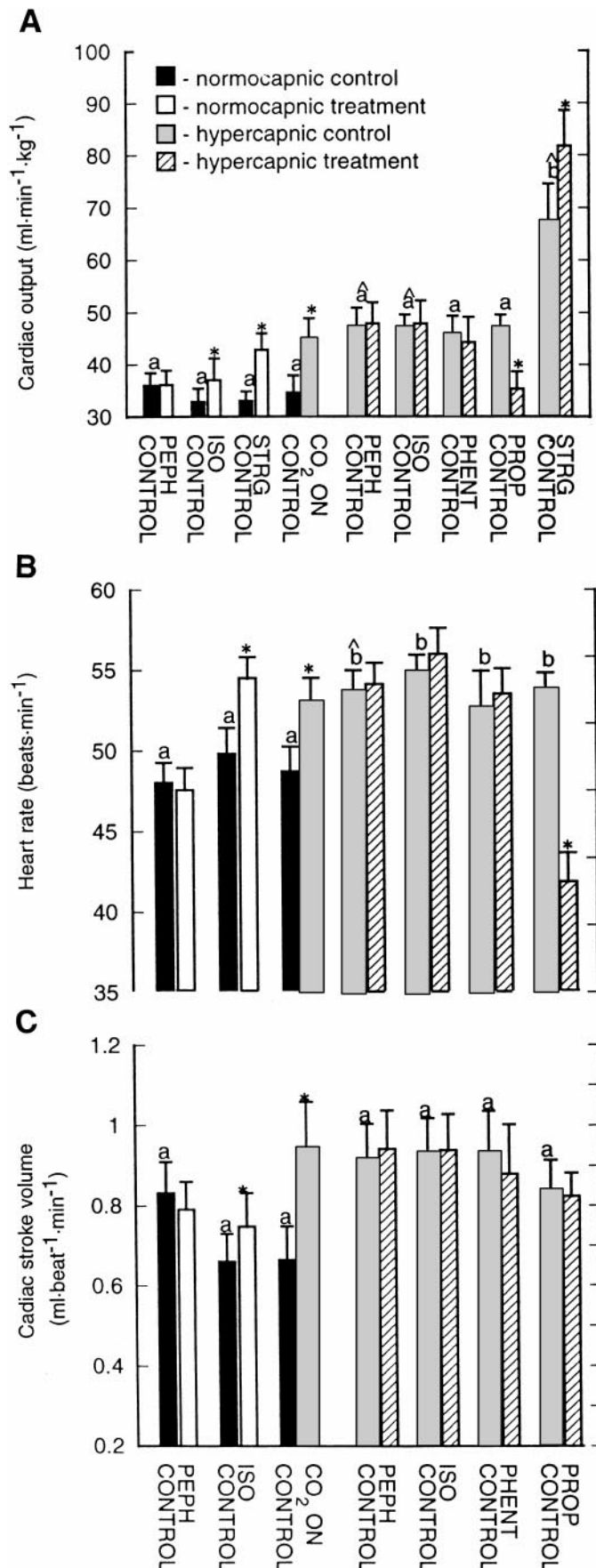
ificant (20%) decrease in  $R_{sys}$ . Hypercapnia did not significantly affect GBF, rGBF, or  $R_{spl}$ .

Struggles were more frequent during hypercapnia than during normocapnia. Possibly as a result, values for  $Q$  recorded before struggling episodes were significantly higher ( $67.2 \pm 7.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) than the other control values during hypercapnia (Fig. 2A). Struggles during hypercapnia significantly increased  $Q$  (by  $14.6 \pm 4.5 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ). Although hypercapnic struggles did not reduce absolute GBF or rGBF to levels ( $2.9 \pm 1.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  and 9%, respectively) that were lower than observed during normocapnia, struggles that reduced GBF by 50% were more frequent (2.2 h during hypercapnia compared with 0.82 h during normocapnia) and significantly longer (10–14 min during hypercapnia compared with 4–6 min during normocapnia). During hypercapnia, GBF was on average disturbed to some degree for 22 to 31 min in any given hour (2.2 struggles/h  $\times$  10–14 min in duration). In contrast, the disturbance to GBF (3–5 min) was almost an order of magnitude less during normocapnia (0.8 struggles/h  $\times$  4–6 min in duration).

PEPH injections produced qualitatively and quantitatively similar responses under hypercapnia. There was no effect on  $Q$ , SV, or HR, but  $R_{sys}$  and MAP increased significantly (by 17 and 24%, respectively). Similarly,  $R_{spl}$  increased by 2.3-fold, absolute GBF decreased by 42%, and rGBF decreased from 12 to 5% (Fig. 3). Furthermore, Phent injections significantly decreased  $R_{sys}$ , MAP, and  $R_{spl}$  by 22, 25, and 34%, respectively, without affecting  $Q$ . These results suggest that the systemic circulation is under tonic  $\alpha$ -adrenergic vasoconstriction and that a significant component of this  $\alpha$ -adrenergic tone is found in the splanchnic circulation. Struggle responses after Phent were not frequent, and the magnitude of the reduction in GBF (22%) was considerably less than that after PEPH injection (data not shown).

Cardiovascular responses to Iso injection during hypercapnia were different than those observed during normocapnia (Figs. 2–4). Iso injection during hypercapnia did not affect  $Q$ , MAP, or  $R_{sys}$  and may have resulted because  $\beta$ -adrenergic stimulation of the heart was approaching its maximum. Prop significantly decreased  $Q$  (16%), HR (22%), and MAP (30%) without affecting  $R_{sys}$  (Fig. 2, A and B; Fig. 4, A and B). In contrast to normocapnia, Iso injection reduced  $R_{spl}$  (by 28%) and significantly increased both absolute GBF (by 40%) and rGBF (from 19 to 28%). Prop did not affect either  $R_{spl}$  or rGBF. Therefore, the significant decrease in GBF (35%) after Prop injection must have resulted

Fig. 1. Plasma stress-hormone concentrations (A, norepinephrine; B, epinephrine; C, cortisol) during normocapnia (solid bars) and hypercapnia (open bars) for fish ( $n = 8$ –10) subjected to 2 or 3 surgical manipulations [DA, dorsal aortic cannulation; GBF, gut blood flow probe placed on the celiacomesenteric artery (CMA); VA, flow probe placed on the ventral aorta]. \*Statistically significant (paired  $t$ -test,  $P < 0.05$ ) differences between normocapnia and hypercapnia means. a and b indicate significant differences between normocapnic fish subjected to different surgical manipulations. Values are means  $\pm$  SE.



from the decrease in  $Q$ . During normocapnia and hypercapnia, we occasionally observed short-term (<10 min) sinusoidal oscillations in MAP and GBF (Mayer waves). During these periods, the pressure and blood flow patterns were in phase, and the frequency of oscillations was always slower than  $V_f$ . An example of the sinusoidal oscillation of GBF is shown in Fig. 6.

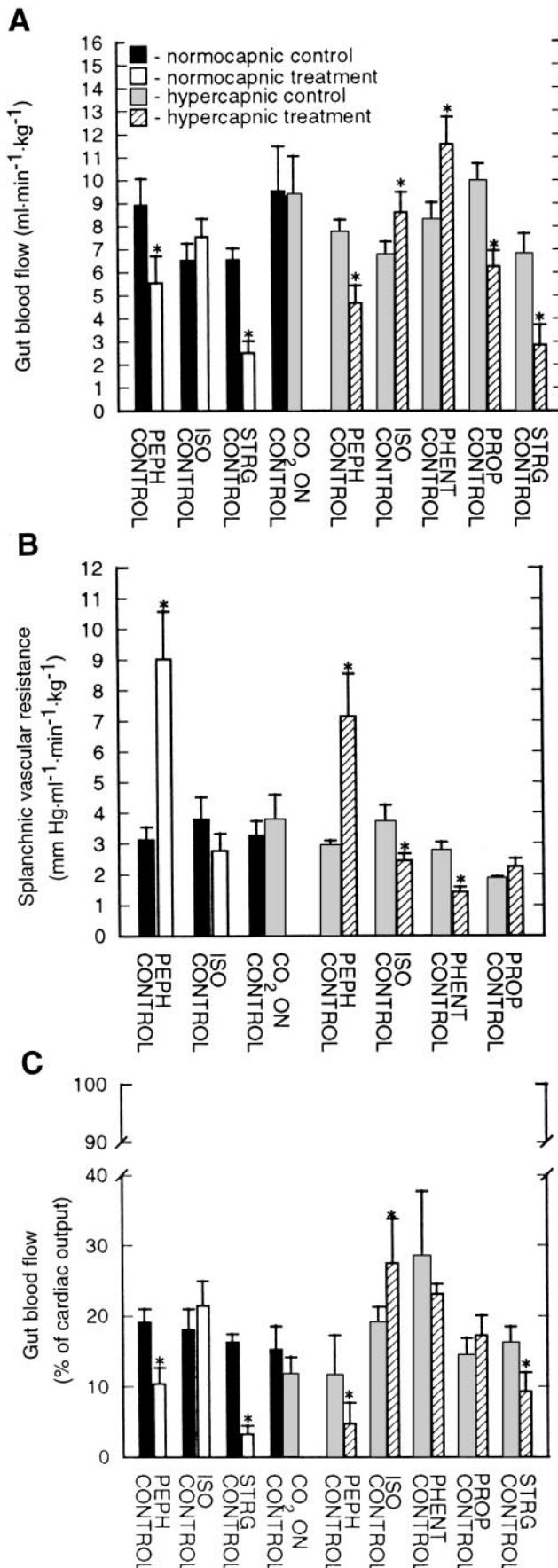
**Regional blood flow.** Organs and tissues (wet masses, including blood) were dissected from 20 sturgeon ( $1.6 \pm 0.10$  kg) to indirectly assess regional blood flow. The head, skeleton, and skin comprised 59% of body mass but were not processed for microspheres. These tissues were extremely difficult to digest so that microspheres could not be counted. Combined red and white muscle represented 35% of body mass, whereas viscera and remaining blood constituted the remaining 6%. The muscles, heart, gills, gonads, and kidney were individually processed for microspheres, but regional blood flow ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ) to these tissues was not significantly altered by our experimental protocol (data not shown). The regional blood flow results for the splanchnic organs (liver, spleen, stomach, and intestine) are presented in Fig. 7. Unfortunately, the microsphere data provided a pattern of total GBF that was qualitatively and quantitatively different from the direct measurements obtained with the ultrasonic flow probes (Fig. 3). Normocapnic GBF (total) measured with the use of microspheres was  $\sim 3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , nearly  $5 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  less than the value measured simultaneously with the ultrasonic flow probes (Fig. 3A). Although hypercapnia did not affect GBF when measured with the use of flow probes, microsphere counts indicated that total blood flow to the splanchnic organs increased by  $11 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ . Finally, after Phent injection, total blood flow to the splanchnic organs ( $20 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) was much greater than that measured directly with the implanted flow probes.

## DISCUSSION

**Normocapnic state.** In a preliminary in vivo study at Sierra Aquafarms, we found that white sturgeon had high  $\text{Pa}_{\text{CO}_2}$  and low blood pH values (C.E. Crocker and J.J. Cech, Jr. unpublished data). Thus we were concerned that residual effects from these rearing conditions would influence the responses of our fish to acute,

Fig. 2. Cardiac output ( $Q$ ; A), heart rate (B), and cardiac stroke volume (C) in white sturgeon ( $n = 8-25$ ) during normocapnia and hypercapnia, before and after the injection of adrenergic agonists/antagonists or a volitional struggle. Solid bars are control (preinjection, pretreatment) values for normocapnic fish, and open bars are the normocapnic treatment response values. CO<sub>2</sub> ON (gray bars) are values measured 2 h after the initiation of hypercapnia. The other gray bars are control values in hypercapnic fish, and the hatched bars are the hypercapnic treatment response values. \*Statistically significant (paired  $t$ -test,  $P < 0.05$ ) difference between the treatment value and the corresponding control value. a and b: control values during normocapnia and hypercapnia that are statistically different from each other (ANOVA, repeated measures;  $P < 0.05$ ). ^Control value that is significantly different ( $P < 0.05$ ) compared with the first control value during normocapnia. Values are means  $\pm$  SE. PEPH, phenylephrine; Iso, isoproterenol; Strg, struggle; Phent, phentolamine; and Prop, propranolol.





hypercapnic exposure. However, it appears that the 2-wk (minimum) acclimation period at the University of California in Davis was sufficient to clear any residual volatile or fixed acid loads and that our fish were physiologically similar to those used in other studies. The control arterial  $P_{O_2}$ ,  $PCO_2$ , pH, hematocrit, and  $V_f$  data are consistent with the values reported for quiescent normocapnic-raised white sturgeon by Crocker and Cech (19°C; Ref. 11), and our arterial pH was similar to that of normocapnic Adriatic sturgeon *A. naccarii* (25°C; Ref. 34). In addition, the arterial pH, plasma concentration of lactate,  $Pa_{O_2}$ , plasma concentration of glucose, and hematocrit reported for our sturgeon are characteristic of teleost fish (42). Whether some of the responses of our fish to hypercapnia reflected hypercapnic preconditioning as a result of the rearing conditions will require further study.

We made every effort to minimize disturbance of the fish during data collection. Nevertheless, concentrations of plasma with concentrations of epinephrine, norepinephrine, and cortisol indicate that our sturgeon were experiencing a significant level of stress under control conditions. Gamperl et al. (19) report that resting catecholamine levels in cannulated teleosts and elasmobranchs are typically <10 nM. In addition, resting hormone levels reported in this study are higher than those reported for cannulated white sturgeon (11), Siberian sturgeon *A. baeri* (25), and Adriatic sturgeon (35). In particular, the mean cortisol concentration during normocapnia was significantly higher than concentrations previously reported for normocapnic white sturgeon (<40 ng/ml; Ref. 11) and for normocapnic Siberian sturgeon (<10 ng/ml; Ref. 25). We suspect that the elevated levels of stress hormones in our fish resulted from the invasive surgery required to make the cardiovascular measurements. Therefore, although we report the first measurements of  $Q$  and GBF in any species of sturgeon, these values should be considered representative of moderately stressed fish. We anticipate that as surgical procedures and holding aquaria become refined, resting values of stress hormones will be reduced significantly.

A primary objective of this study was to make the first direct measurements of  $Q$  in white sturgeon. Agnisola et al. (1) studied the effects of dietary polyunsaturated fatty acids on cardiac performance in Adriatic sturgeon and reported *in vivo*  $Q$  values of  $\sim 13 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  in anesthetized fish ( $\sim 1 \text{ kg}$ ;  $23 \pm 1.0^\circ\text{C}$ ).

Fig. 3. Absolute gut blood flow (GBF; A), splanchnic vascular resistance (B), and rGBF (% of  $Q$ ; C) in white sturgeon ( $n = 4\text{--}22$ ) during normocapnia and hypercapnia, before and after the injection of adrenergic agonists/antagonists or a volitional struggle. Solid bars are control (preinjection, pretreatment) values for normocapnic fish, and open bars are the normocapnic treatment response values.  $CO_2$  ON (gray bars) are values measured 2 h after the initiation of hypercapnia. The other gray bars are control values in hypercapnic fish, and the hatched bars are the hypercapnic treatment response values. \*Statistically significant (paired  $t$ -test,  $P < 0.05$ ) difference between the treatment and the corresponding control values. Data presented in C were obtained from fish that were fitted with ultrasonic flow probes on the VA and CMA. Values are means  $\pm$  SE.

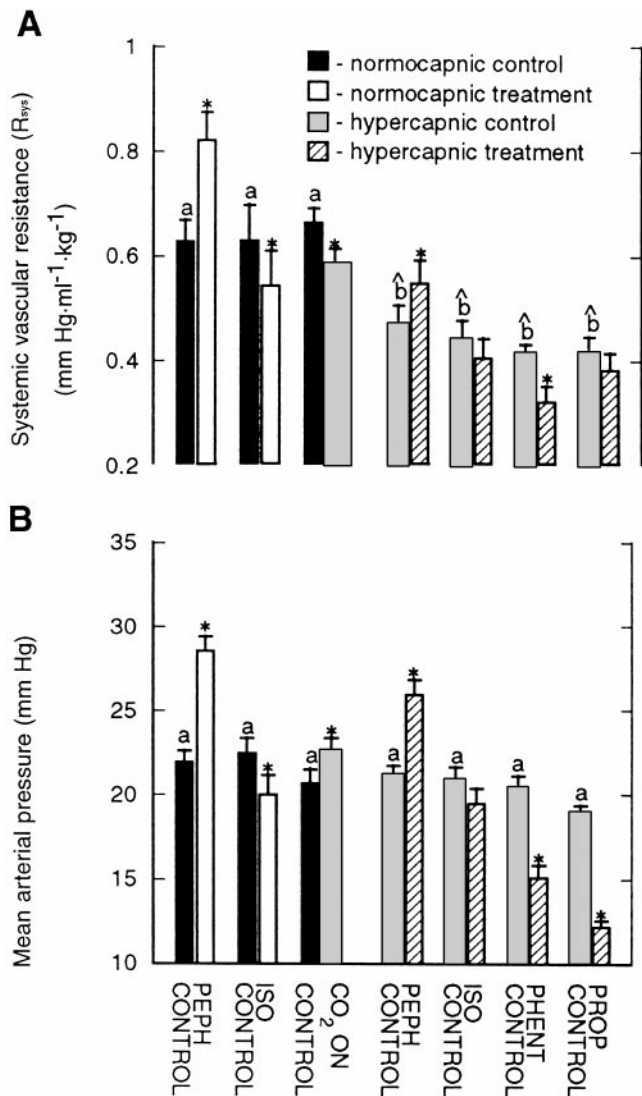


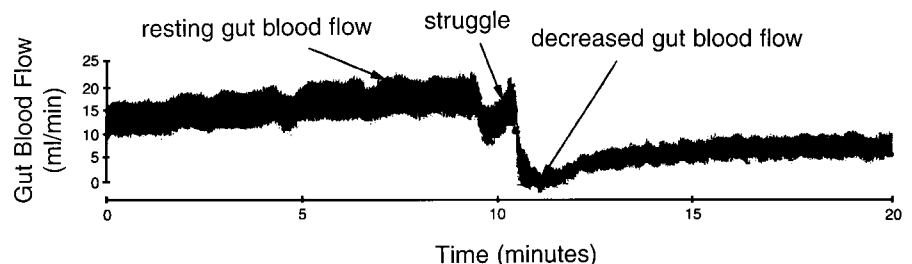
Fig. 4. Systemic vascular resistance (A) and mean arterial pressure (MAP; B) of white sturgeon ( $n = 8-25$ ) during normocapnia and hypercapnia, before and after the injection of adrenergic agonists/antagonists or a volitional struggle. Solid bars are control (preinjection, pretreatment) values for normocapnic fish, and open bars are the normocapnic treatment response values. CO<sub>2</sub> ON (gray bars) are values measured 2 h after the initiation of hypercapnia. The other gray bars are control values in hypercapnic fish, and the hatched bars are the hypercapnic treatment response values. \*Statistically significant (paired  $t$ -test,  $P < 0.05$ ) difference between the treatment value and the corresponding control value. a and b: control values during normocapnia and hypercapnia that are statistically different from each other (ANOVA, repeated measures;  $P < 0.05$ ). ^Control value that is significantly different ( $P < 0.05$ ) compared with the first control value during normocapnia. Values are means  $\pm$  SE.

Control  $Q$  in the present study ( $36.1 \pm 2.3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) was almost three times higher than this value, and poststruggle measurements of  $Q$  during hypercapnia ( $81.8 \pm 7.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) were nearly six times the resting  $Q$  reported by Agnisola et al. (1). Because control  $Q$  in the present study at  $19^\circ\text{C}$  was comparable to values reported for resting chinook salmon (*Oncorhynchus tshawytscha*,  $8-11^\circ\text{C}$ ,  $33 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ; Ref. 39) and leopard shark (*Triakis semifasciata*,  $20^\circ\text{C}$ ,  $33.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ; Ref. 24), and sixfold increases in  $Q$  have not been reported previously for fish (16), we suspect that the  $Q$  value reported for anesthetized Adriatic sturgeon greatly underestimates resting  $Q$  in sturgeon. Although, resting values for MAP and  $R_{sys}$  ( $22 \pm 1.0 \text{ mmHg}$  and  $0.63 \pm 0.0 \text{ mmHg ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ , respectively) were within the range of values reported for resting fishes (7, 15), they are closer to values found in elasmobranchs than in teleosts.

Although the control of HR in fish is accomplished by intrinsic, neural, and humoral mechanisms (15), it has been suggested that resting HR in sturgeon is set by excitatory sympathetic tone (27). For example, Agnisola et al. (1) reported that Adriatic sturgeon had an in vitro intrinsic HR of  $<30$  beats/min and an in vivo HR of 60 beats/min at  $23 \pm 1.0^\circ\text{C}$ . Control HR in the present study ( $49 \pm 1.5$  beats/min) was similar to that reported for quiescent white sturgeon ( $19^\circ\text{C}$ ; Ref. 11) and Siberian sturgeon ( $52 \pm 2$  beats/min,  $18^\circ\text{C}$ ; Ref. 25). Resting SV for our normocapnic white sturgeon ( $0.83 \text{ ml} \cdot \text{beat}^{-1} \cdot \text{kg}^{-1}$ ) was higher than values previously reported for teleost fishes but similar to those reported for elasmobranchs (14, 16). This finding is interesting because white sturgeon possess a pericardioperitoneal canal (PPC; C. E. Crocker, J. J. Cech, and J. B. Graham, unpublished observations) as do sharks (24). Apparently, the PPC allows for large SVs by permitting the displacement of pericardial fluid from the pericardial cavity into the peritoneal cavity (14, 24).

Cardiovascular control has not been studied previously in sturgeon in vivo. The present study suggests that the systemic circulation is predominantly under tonic constrictory  $\alpha$ -adrenergic stimulation, although  $\beta$ -adrenergic-mediated vasodilation and other humoral/neural influences may play a role. In addition, our findings suggest that a significant component of this vasoconstrictory activity was located in the splanchnic circulation. For example, the 17% change in  $R_{sys}$  with PEPH injection was associated with a threefold increase in  $R_{spl}$  (Figs. 3 and 4). Although  $\alpha$ -adrenergic

Fig. 5. GBF recorded in a white sturgeon during hypercapnia after a volitional struggle. In this individual, GBF was reduced to 10–20% of prestruggle levels.





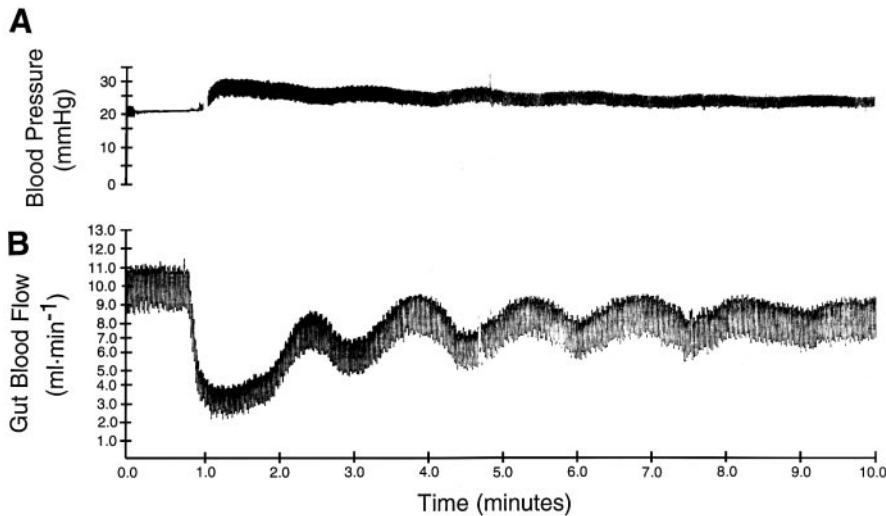


Fig. 6. Mayer waves in MAP (A) and GBF (B) recorded in a white sturgeon after the injection of PEPH during hypercapnia.

control of the white sturgeon heart appears to be either minor or absent, our results show that  $\beta$ -adrenoceptors play a significant role in stimulating cardiac function. These findings are qualitatively similar to those documented for both teleosts and elasmobranchs (7, 8, 13, 29, 30), and so the white sturgeon does not appear unusual in this regard.

No measurements of GBF in sturgeon or other chondrosteans exist in the literature. Our direct measurements of GBF in white sturgeon show that blood flow in the CMA ( $8.9 \pm 1.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) accounted for 20% of  $Q$ . Although our value for GBF is comparable to that reported for resting chinook salmon ( $12.0\text{--}14.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ; Ref. 41), anesthetized rainbow trout

( $13 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ; Ref. 28), and Atlantic cod (*Gadus morhua*,  $7.6 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ; Ref. 3), the rGBF we measured (20%) was only half that reported for the cod. The potent  $\alpha$ -adrenergic regulation of the splanchnic vasculature, as revealed by the threefold increase in  $R_{\text{spl}}$  with PEPH and the 50% decrease in  $R_{\text{spl}}$  with Phent, is also consistent with these studies on non-chondrosteans. Two pieces of evidence suggest that the pronounced decrease in GBF after a struggle was primarily mediated by an  $\alpha$ -adrenergic constriction of the splanchnic circulation. First, despite the 29% increase in  $Q$  after a struggle, GBF decreased sharply and took several minutes to recover. Second, although  $R_{\text{spl}}$  after a struggle could not be determined quantitatively because of mechanical artifacts in our pressure traces, it appeared that changes in  $R_{\text{spl}}$  after a struggle were of a similar magnitude to those measured after PEPH injection (2.36-fold). Although  $\alpha$ -adrenoreceptors are probably the predominant mechanism involved in the control of  $R_{\text{spl}}$  in white sturgeon, it is clear that other factors are involved. For example, because Prop injection into our hypercapnic sturgeon decreased GBF by 32%,  $\beta$ -adrenoreceptors must also play a role in mediating  $R_{\text{spl}}$ . The increase in  $Q$  after struggles most likely reflected  $\beta$ -adrenergic stimulation of chronotropy and inotropy as well as an enhancement of venous return due to body undulations associated with struggles.

*Regional blood flow measured with the use of colored microspheres.* Burggren and Randall (6) reported that white sturgeon decreased total energy expenditures during hypoxia and suggested that this hypometabolic response was partially accomplished by reduced ventilatory and cardiovascular work. Furthermore, they hypothesized that  $Q$  and regional blood distribution during hypoxia changed to meet the minimal oxygen demand of the "vital" organs and tissues. However, few studies have actually measured the distribution of blood flow in fish (2, 3, 7, 10, 23, 41). Our expectation was that the colored microspheres would reveal useful information on regional blood flow in white sturgeon

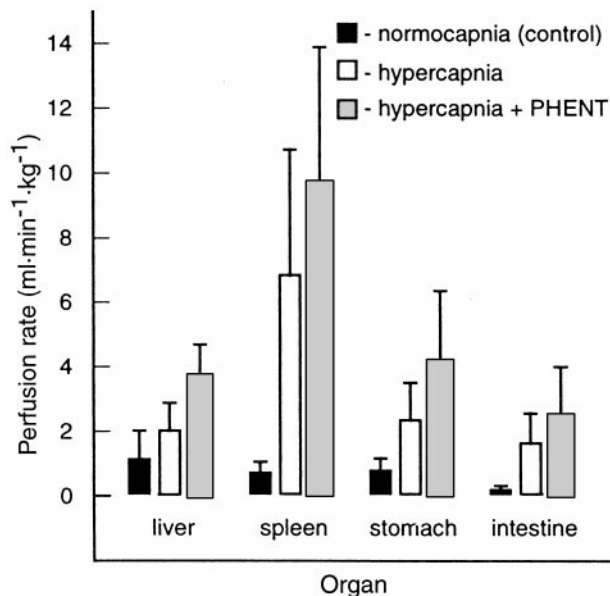


Fig. 7. Tissue perfusion rates estimated by injecting colored microspheres into white sturgeon. These organs are perfused by the CMA, and their total during each of the conditions represents GBF. Neither hypercapnia nor Phent injection affected tissue perfusion rates as measured with the use of microsphere counts. All values are means  $\pm$  SE.

under conditions of normocapnia, hypercapnia, and after  $\alpha$ -adrenergic blockade and that the indirect measurements of GBF with colored microspheres could be calibrated against the direct measurements obtained with the use of the ultrasonic flow probes. In fact, this is the first time that a direct calibration of the microsphere method has been attempted in fish. However, our expectations were not realized, and we were very disappointed with the quality of the microsphere results. First, we conducted eight more microsphere experiments than those on the five fish reported here. However, because sphere recovery values in many fish were very variable (range 14–94%), only data from tissue microsphere recovery values  $>35\%$  are reported here. Second, individual microsphere flow measurements had high variability, unlike the direct measurements of GBF. Third, when GBF was measured with the use of flow probes (directly) and microspheres (indirectly) in the same fish, the results of the two methods were substantially different. GBF measured with the use of microspheres during the control period was  $2.8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  (nearly 1/3 of the control values measured with the ultrasonic flow probes). In addition, GBF measurements during hypercapnia and after Phent treatment were 38 and 72% greater, respectively, than those directly recorded with the use of flow probes.

In reviewing blood flow distribution in fishes, Bushnell et al. (7) also noted that GBF is generally lower for microsphere studies compared with direct measurements. Furthermore, other authors have reported quality control difficulties with radiolabelled and colored microsphere studies with fish (23). On the basis of our previous experience with both direct and indirect methods of blood flow measurement, we put greater trust in the direct measurements of GBF, despite the greater degree of surgical intervention. In fact, of all the fish we have worked with, the CMA in white sturgeon is ideally located for flow probe placement. It is a single, large vessel that is the first branch off of the DA and is easily accessible. We believe that the problem with the microsphere method in fish lies with the site used for the injection of the microspheres. The microspheres have to be injected relatively fast to prevent settling in the cannula, and the most suitable site for introducing the microspheres is the DA (microspheres injected into the heart or VA would get trapped in the gills). However, the CMA, which is the largest single vascular branch of the DA, is located immediately posterior to the efferent branchial arteries. Consequently, there is an unacceptably high probability that microspheres are not distributed to the CMA in proportion to relative blood flow. Given the high variability in our GBF measurements and the tendency of the microsphere method to underestimate GBF, it would seem that another method must be sought to provide reliable data on regional blood flow in fishes. In addition, it is clear that this problem cannot be easily resolved by injecting microspheres at a site upstream of the DA. When radiolabelled microspheres were injected into the second efferent branchial artery of rest-

ing catfish, total blood flow to the spleen, liver, intestine, and stomach was estimated to be only 2.7% (38).

**Hypercapnic status.** In the present study, plasma norepinephrine concentrations during normocapnia ( $96.5 \pm 12.0 \text{ nM}$ ) and hypercapnia ( $91.5 \pm 8.5 \text{ nM}$ ) were higher than plasma epinephrine concentrations ( $41.5 \pm 5.5$  and  $59.8 \pm 11.6 \text{ nM}$ , respectively), and only plasma epinephrine was significantly elevated during hypercapnia. In contrast, previous measurements of circulating catecholamines in white sturgeon (11) indicated that the predominant hormone released during rapid-onset hypercapnia was norepinephrine (transient spike followed by a return to control levels within 72 h), and this pattern of catecholamine release has been demonstrated in trout exposed to hypercapnia (39) and in dogfish (*Scyliorhinus canicula*) exposed to hypoxia (9, 29). Differences in the concentrations of plasma epinephrine and norepinephrine during hypercapnic stress may be species specific and related to the severity of hypercapnia, to catecholamine storage levels within the chromaffin tissue, or to different rates of reuptake, metabolism, and tissue binding (36). Alternatively, because our sampling interval (4 h) was longer compared with our previous study (1 h; Ref. 11), it is possible that we missed the transient norepinephrine spike.

Our results showing that hypercapnia caused considerable changes in blood-gas, acid-base, and ventilatory status in the white sturgeon are consistent with the results of our previous study (11). The significant increase in plasma concentration of cortisol observed during hypercapnia suggests that environmental hypercapnia initiated a stress response in white sturgeon. In fish, increases in plasma concentration of cortisol mobilize energy reserves (12) and will typically result in an elevation in plasma concentration of glucose. Interestingly, however, we recorded a 22% decrease in plasma concentration of glucose in our sturgeon when exposed to hypercapnia. In autoperfused hearts of the dogfish *Squalus acanthias*, hypercapnia (seawater equilibrated with 5%  $\text{CO}_2$ ) caused a vagally mediated bradycardia and a decrease in Q but no change in SV (22). However, our findings were the opposite and similar to those of Randall and Shelton (37), who reported tachycardia in hypercapnic tench *Tinca tinca* L. In hypercapnic white sturgeon, we report positive chronotropic and inotropic effects and a decrease in  $R_{\text{sys}}$ . This could have come about through sympathetic ( $\beta$ -adrenergic) activation of the heart and inactivation of  $\alpha$ -adrenergic mechanisms controlling vasomotor tone in the peripheral vasculature. This is substantiated, in part, by the observation that Iso had limited cardiovascular actions under hypercapnia but stimulated the heart and systemic vasculature under normocapnia. The adrenergic stimulation of the heart may be part of a ventilation-perfusion matching related to the hypercapnic stimulation of the respiratory center and  $\dot{V}_f$ . In addition, adrenergic stimulation could protect the myocardium from the negative inotropic effects of hypercapnic acidosis that are well documented for teleost hearts (13, 20).

$R_{spl}$  and GBF were unchanged with hypercapnia. In addition, measurements of  $rGBF$  with the use of microspheres and flow probes indicated that hypercapnia did not redistribute blood flow away from the splanchnic organs. Therefore, the change in  $R_{sys}$  associated with hypercapnia must reside elsewhere in the systemic circulation. The effectiveness of  $\alpha$ -adrenergic splanchnic vasoconstriction was retained under hypercapnia, whereas Iso reduced  $R_{spl}$  and increased GBF under hypercapnic but not normocapnic conditions. This latter result cannot be explained at this time and will require further investigation.

Cardiovascular responses and their control during hypercapnia were recently studied in rainbow trout (32) with the use of a shorter (20 min), lower ( $Pw_{CO_2} = 9$  Torr) and colder (12–14°C) hypercapnic exposure compared with the present study. A comparison of the two studies, however, reveals important similarities and differences in the responses of these two species. Under normoxia, it appears that a qualitatively similar adrenergic control exists. Perry et al. (32) found that epinephrine injection significantly increased  $R_{sys}$ , VA pressure, DA pressure, venous pressure, and  $Q$ , presumably through a mixture of  $\alpha$ - and  $\beta$ -adrenergic effects. With PEPH, we found that the  $\alpha$ -adrenergic effects were reflected in increased  $R_{sys}$ ,  $R_{spl}$ , and DA pressure (Figs. 3 and 4). With Iso, we found that the  $\beta$ -adrenergic effects were reflected in increased HR, SV, and  $Q$  with a decrease in  $R_{sys}$  (Figs. 2 and 4). Thus even though background plasma levels of epinephrine and norepinephrine were higher in white sturgeon than in rainbow trout, the sturgeon circulatory system was still responsive to adrenergic stimulation. In rainbow trout, hypercapnia resulted in increased  $R_{sys}$ , increased VA and DA pressures, bradycardia, and decreased  $Q$  but no change in branchial resistance or venous BP. For sturgeon, the responses were quite different.  $R_{sys}$  was initially unchanged but subsequently decreased, and  $Q$ , SV, and HR all increased significantly (Figs. 2 and 4). Thus the significant increase in DA BP was due solely to the increase in  $Q$ . Although we currently have no explanation for these differences, a number of possibilities exist. First, the differences could be inherent to the species and reflective of long, separated evolutionary histories. Second, there was a significant difference in temperature between the two studies (12–14 vs. 19°C), and cardiovascular responses are known to vary with temperature in fish. Third, the hypercapnic exposure period was longer in the sturgeon (120 vs. 20 min). However, it is difficult to resolve whether the duration of hypercapnic exposure had any influence on the difference in cardiovascular response in these two fishes. When exposed to incremental levels of hypercapnia (up to 9 Torr  $PCO_2$ ) that lasted for a total of 60 min, the cardiovascular response of rainbow trout was the same as seen with the single 20-min increment to 9 Torr (32). Fourth, the rainbow trout were not stressed during hypercapnia, as indicated by unchanged plasma catecholamine levels (32), perhaps reflecting a longer (inbred) culture history than that of white sturgeon. We do not think

that the elevated  $Q$  in sturgeon was solely due to an increase in plasma catecholamine levels during hypercapnia, because neither epinephrine nor norepinephrine showed significant changes (DA + VA and DA + GBF + VA groups) with hypercapnic exposure. Instead, the differences could be related to the 2.5-fold increase in the number of struggling episodes. Because struggling increased  $Q$  and HR, it is possible that  $Q$  during hypercapnia did not recover fully. Therefore, even though we selected periods between struggles that we assumed were a routine state, this may not have been the case. The decrease in  $R_{sys}$  in sturgeon is less easy to resolve with the increase in  $R_{sys}$  in rainbow trout. Foremost,  $R_{sys}$  was initially unchanged with hypercapnia even though struggling frequency had already increased. Second, stress would be expected to increase rather than decrease  $R_{sys}$ . Therefore, other than a real or temperature-related response to hypercapnia that differs with that of rainbow trout, the decrease could be similar to that seen in postexercise, hypotensive rainbow trout (40).

Our original hypothesis was that poor GBF limits the digestive process in white sturgeon during hypercapnia. Our observation that environmental hypercapnia did not cause preferential redistribution of blood away from the gastrointestinal tract and to other organs and tissues (e.g., heart, brain, and kidney) under routine conditions clearly does not support this hypothesis. However, this narrow analysis of cardiovascular status clearly ignores the cumulative effects of struggles during hypercapnia and therefore biases the assessment of overall GBF. During hypercapnia, white sturgeon were more hyperactive, as evidenced by struggles that were twice as frequent compared with normocapnic fish. In addition, each struggle during hypercapnia resulted in a much longer negative effect on GBF. Whether this longer duration reflected a harder struggle or some unknown hypercapnia-induced effect on splanchnic blood flow regulation is unclear at this time. The important point is that overall GBF was reduced as a result, and this may be related to the poor growth of white sturgeon maintained under intense, hypercapnic culture situations. Further studies, however, are needed to ascertain whether this hyperactive state persists in long-term hypercapnic situations.

In summary, we report the first comprehensive set of cardiovascular measurements in white sturgeon. The cardiovascular controls that we identified were qualitatively similar to those previously described for teleosts and elasmobranchs. The impact of hypercapnia on cardiovascular status and its control appeared to be rather small, with the exception of hyperactivity and its negative consequences on GBF.

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