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Juvenile sturgeon exhibit reduced physiological responses to exercise

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Summary

Experiments were conducted to determine the physiological responses to exercise of Atlantic sturgeon (*Acipenser oxyrhynchus*) and shortnose sturgeon (*A. brevirostrum*). We measured the rates of oxygen consumption and ammonia excretion in both species and a variety of physiological parameters in both muscle (e.g. lactate, glycogen, pyruvate, glucose and phosphocreatine concentrations) and blood (e.g. osmolality and lactate concentration) in juvenile shortnose sturgeon following 5 min of exhaustive exercise.

In both species, oxygen consumption and ammonia excretion rates increased approximately twofold following exhaustive exercise. Post-exercise oxygen consumption rates decreased to control levels within 30 min in both sturgeon species, but post-exercise ammonia excretion rates remained high in Atlantic sturgeon throughout the 4 h experiment. Resting muscle energy metabolite levels in shortnose sturgeon were similar to those of other fish species, but the levels decreased only slightly following the exercise period and recovery occurred within an hour. Under resting conditions, muscle lactate levels were low ($<1\ \mu mol\ g^{-1}$) but they increased to approximately $6\ \mu mol\ g^{-1}$ after exercise, returning to control levels within 6 h. Unlike similarly stressed teleost fish, such as the rainbow trout, plasma lactate levels did not increase substantially and returned to resting levels within 2 h. Plasma osmolality was not significantly affected by exercise in shortnose sturgeon.

Taken together, these results suggest that shortnose and Atlantic sturgeon do not exhibit the physiological responses to exhaustive exercise typical of other fish species. They may possess behavioural or endocrinological mechanisms that differ from those of other fishes and that lead to a reduced ability to respond physiologically to exhaustive exercise.

Key words: shortnose sturgeon, Atlantic sturgeon, exercise, metabolite, blood, muscle, oxygen consumption, ammonia excretion, *Acipenser oxyrhynchus*, *Acipenser brevirostrum*.

Introduction

The physiological and biochemical responses to exhaustive exercise in fish have been well described over the past 40 years (for reviews, see Wood, 1991; Milligan, 1996; Kieffer, 2000). Much of this research has been carried out using salmonid fishes (Milligan, 1996; Kieffer, 2000), mainly because of their large capacity for burst activity, their availability and their commercial importance (Moyes and West, 1995; Milligan, 1996). Despite the valuable information, both basic and applied, acquired by studying a single group (Kieffer, 2000), research comparing the physiological responses to exercise of different groups of fish has revealed other important information about exercise-related mechanisms (Wood, 1991). For example, it has been shown that the differences in the rate of recovery from exhaustive exercise span several orders of magnitude when species are compared (Milligan and Wood, 1987; Nelson, 1990; Boutilier et al., 1993), and these differences reflect the ecological requirements, morphology and behaviour of a given species (Kieffer, 2000).

In contrast to the growing literature on the physiological responses to exhaustive exercise of teleosts, very few studies have been conducted on freshwater fishes outside this taxonomic grouping. Boutilier et al. (1993) examined the physiological responses to exhaustive exercise of the sea lamprey (*Petromyzon marinus*) and found that these organisms possess anaerobic capacities that rival the response recorded for salmonids. Unlike salmonids, however, it has been shown that lampreys recover quickly from the large metabolic disturbance associated with exhaustive exercise (Boutilier et al., 1993; Wilkie et al., 2001). Whether these differences are related to the complex life-history or to the phylogenetic position of lampreys is not well understood. In general, there is a lack of information relating evolutionary aspects of exhaustive exercise performance in fish (Bennett, 1991).

The sturgeons (Genus *Acipenser*) are freshwater chondrostean fish in the Suborder Acipensiformes that have existed since the Jurassic period (approximately 150 million years ago). Sturgeon have retained many ancestral body characteristics and ways of living that distinguish them as relict

fishes (Bemis et al., 1997). Among these primitive characteristics are the protective 'armour' or scutes, a largely cartilaginous skeleton (Kynard, 1997), the presence of a notochord and the shark-like heterocercal tail (Scott and Crossman, 1973).

A growing body of literature exists on the ecology and status of sturgeon species (Smith, 1985; Bain, 1997; Kynard, 1997); however, less is known about their physiology. Of the available research, most has focused on osmoregulatory (McEnroe and Cech, 1985; LeBreton and Beamish, 1998; Krayushkina, 1998; Altinok et al., 1998) and respiratory (Maxime et al., 1995; Thomas and Piedrahita, 1997) physiology. More recently, researchers have focused on indices of stress in several species of hatchery-reared sturgeon (Barton et al., 1998, 2000). Despite some research on the stress response in sturgeon species, there is a paucity of information about the exercise physiology of these fish species (Peake et al., 1995; Adams et al., 1997, 1999). Given the complex life-history of these fish (Kynard, 1997) and the environmental challenges they face (temperature, salinity) while migrating within the river system for foraging and reproductive purposes (Smith, 1985; Kynard, 1997), it is likely that these animals experience situations that may challenge both their aerobic and anaerobic capacities.

The present study is aimed at investigating the physiological responses to exercise of two species of sturgeon, the Atlantic sturgeon (*Acipenser oxyrhychus*) and the shortnose sturgeon (*Acipenser brevirostrum*). Both species exist in the Saint John River system, Canada (R. S. Hardy and M. K. Litvak, unpublished results), but their distribution is known to extend southwards along the North American Atlantic coast to Florida (Scott and Crossman, 1973; Smith, 1985). There has been heightened interest in these species because population numbers have suffered a recent decline in eastern North America as a result of natural and anthropogenic factors such as the construction of dams, water pollution and over-fishing. At present, shortnose and Atlantic sturgeon are either on or are being considered for the endangered species list.

In the first experiment, we assessed the respiratory physiology (e.g. oxygen consumption rates, ammonia excretion rates) of juvenile Atlantic and shortnose sturgeons before and after exhaustive exercise. In the second experiment, we assessed the muscle and blood metabolite levels (e.g. energy metabolites, metabolic end-products) in juvenile shortnose sturgeon following exhaustive exercise.

Materials and methods

Animal husbandry

Juvenile shortnose (*Acipenser brevirostrum*) and Atlantic (*A. oxyrhynchus*) sturgeon were obtained from the hatchery at the University of New Brunswick (Saint John). These fish were raised from eggs originating from St John River sturgeon stock. After the fish had hatched and had been weaned onto artificial diets, juveniles were moved to larger grow-out tanks until they reached the appropriate size for the experiments (see below). While in these tanks, the fish were fed once in the morning and once in the late afternoon daily to satiation. Water temperatures for the experiments were 12±1 °C for series I and 16±1 °C for series II. The tank water had the following composition: pH≈6.5; total hardness≈60 mg l^{-1} $CaCO_3$.

Series I: post-exercise oxygen consumption and ammonia excretion rates of juvenile shortnose and Atlantic sturgeon

Oxygen consumption measurements

To reduce possible dietary influences on metabolite status, fish were not fed on the day before the start of experiments (Kieffer and Tufts, 1998). Fish were removed from the holding tank, weighed (N=8, average approximately 9 g) and held overnight in small flow-through respirometers. These were blackened 60 ml syringe barrels, fitted with three-way stopcocks at the inflow and outflow, and were perfused with water at 12 °C at a flow of approximately 0.6–0.8 l h^{-1} by a series of custom-built flow restrictors. To prevent fish from slipping from their resting positions (i.e. on the rounded bottom of the respirometer), each respirometer was fitted with a small plastic mesh floor kept in place by clear aquarium silicone.

The following morning, several 1–2 ml water samples for control measurements from non-exercising fish were removed from the inflow and outflow of the respirometer using gas-tight Hamilton syringes. These samples were measured immediately with a Cameron Instruments E101 P_{O_2} electrode connected to a Cameron Instruments OM 2000 oxygen meter. Partial pressures of oxygen (P_{O_2}) were converted to total O_2 content using solubility tables provided by Boutilier et al. (1984). The fish were then removed from the respirometers and individually exercised by manual chasing for 5 min. Manual chasing is a standard technique used in most fish exercise studies (Wood, 1991; Milligan, 1996). Fish were considered to be physically exhausted when they lost equilibrium and were no longer responsive to manual chasing. It should be noted that the work done during exercise was not quantified (e.g. exact time to fatigue). Instead, the fish were exercised to a behavioural state of exhaustion, and the magnitudes of the physiological disturbances and recovery characteristics were examined (for details, see Kieffer et al., 1994).

Following exercise, fish were returned to the respirometers. Preliminary experiments indicated that, to obtain a representative P_{O_2} measurement, a 5 min mixing period was required. Thus, the first post-exercise measurement was made 5 min after the end of exercise (when the fish was placed back into the respirometer). Water samples, therefore, were drawn from the inflow and outflow of the respirometer at 5, 10, 15, 30, 45, 90, 120, 180 and 240 min post-exercise. The P_{O_2} values of these water samples were determined as outlined above. Gas-exchange rates before and during recovery from exercise were calculated as the product of the flow rate through the respirometer and the difference in gas content between inflow and outflow water samples and were expressed on a wet body mass basis.

Ammonia flux experiments

Juvenile shortnose (N=6) and Atlantic (N=6) sturgeon (approximate mass 9 g) were placed individually in black

Perspex boxes (volume 450 ml) for at least 24 h prior to experimentation. Each box was fitted with multi-perforated tubing mounted on the inside portion of the box. This provided a means of both aerating and mixing the water in the box.

For control flux measurements, the inflow and the outflow of the boxes were closed. Water samples (20 ml) were then taken over the 1 h period prior to exercise (resting flux). Next, individual fish were quickly removed from the flux box and exercised to exhaustion by 5 min of manual chasing in a circular tank. The fish were then returned to the boxes, and water samples (20 ml) were taken at 0.5, 1, 2 and 4 h after exercise. These samples were immediately placed in a freezer at –20 °C for later analysis of ammonia concentrations (for methods, see Verdouw et al., 1978). It should be noted that fluxes could not be measured during the 5 min exercise period. This measurement, with the above sampling schedule, allowed us to calculate the net ammonia flux during the periods before exercise and over the 0–0.5, 0.5–1, 1–2 and 2–4 h post-exercise periods.

Series II: muscle and blood experiments

Animal husbandry

For these experiments, juvenile shortnose sturgeon were used (approximate mass 18–20 g; 17–19 cm in total length). Fish were held in large flow-through tanks continuously supplied with fresh, well-aerated, dechlorinated, St John municipal water (pH≈6.5; total hardness≈60 mg l^{-1} $CaCO_3$; temperature 16±1 °C). Sturgeon were fed a commercial trout food twice daily to satiation but were not fed on the day before the start of experiments.

Exercise protocol and tissue collection

Individual fish were quickly netted and removed from their general holding tank and transferred to a smaller tank filled with system water. Care was taken not to disturb the other fish in the holding tank when netting fish. Fish were immediately exercised to exhaustion (except control fish; see below) by manual chasing (see above for details). Exhausted fish were placed in separate, darkened, flow-through Perspex boxes. Each fish box was supplied with well-aerated water maintained at 16 °C.

Samples of white muscle (N=7–11) were taken immediately following exercise (0 min of recovery) or 1, 2 or 6 h later, and samples of blood (N=5–7) were taken immediately following exercise (0 min of recovery) or after 2 or 6 h of recovery in blackened Perspex boxes. Fish sampled immediately after exercise (i.e. time 0) were placed directly into anaesthetic prior to removal of muscle or blood. For all the other sampling times, the water flow to the Perspex box was stopped and a buffered solution (pH 7) of tricaine methanesulphonate (MS-222) was added. After 2–3 min, during which the fish remained quiescent, the sturgeon were fully anaesthetized. We chose to anaesthetize the fish prior to muscle and blood sampling because this method has been shown to reduce any metabolic and acid–base changes associated with the handling of a conscious animal (Wang et al., 1994b).

A sample of muscle was removed from the anaesthetized fish, immediately frozen by clamping in pre-cooled aluminium tongs and stored under liquid nitrogen. Control (i.e. resting) values were obtained in a manner similar to that described above, except that individual fish were isolated in separate Perspex boxes for at least 24 h prior to sampling and were not exercised. The samples of frozen muscle tissues were analyzed for lactate, glycogen, phosphocreatine (PCr), glucose, pyruvate and water content.

For the blood experiments, a similar protocol was used on a separate group of fish, except that in this case blood was removed from the fish. Blood was taken from the caudal vasculature with a 1 ml syringe by caudal puncture. The samples were immediately centrifuged (4 min at 10 000 ***g***), and 40 µl of the resultant clear plasma was withdrawn and added to individually labelled Eppendorf tubes (500 µl) containing 80 µl of chilled 8 % perchloric acid (PCA). The acidified plasma was quickly shaken, flash-frozen in liquid nitrogen and stored under liquid nitrogen for subsequent determination of plasma lactate concentrations. Any remaining plasma was drawn off, placed in labelled microcentrifuge tubes, flash-frozen and stored under liquid nitrogen for osmolality measurements (see below).

Analytical techniques: tissue extraction and metabolite levels

Samples of sturgeon muscle were ground to a fine powder under liquid nitrogen using a pre-cooled mortar and pestle. Approximately 1 g of powder was transferred to a tared 15 ml vial to which 4 vols of ice-cold PCA solution containing 1 mmol l^{-1} EDTA was added. This mixture was vortexed briefly to form a slurry and placed on ice for a 10 min extraction period. The tissue slurry was briefly vortexed at 1–2 min intervals to increase the efficiency of the PCA extraction. Equal amounts of the homogenate were then distributed into 1.5 ml Eppendorf tubes and centrifuged (10 000 ***g***) for 5 min. The clear supernatant was collected, placed in a new 10 ml tube and neutralized with a known volume of 2 mol l^{-1} potassium hydroxide (containing 0.4 mmol l^{-1} KCl and 0.4 mmol l^{-1} imidazole) and immediately centrifuged for 45 s in a clinical centrifuge set at high speed. The neutralized supernatant was placed into labelled microcentrifuge tubes, flash-frozen and stored under liquid nitrogen. Concentrations of all metabolites, except glycogen, were determined enzymatically on neutralized PCA extracts according to the methods of Lowry and Passonneau (1972). White muscle glycogen was isolated using the methods of Hassid and Abraham (1957). All metabolites were determined in duplicate using chemicals, standards and enzymes purchased from Sigma (Sigma, St Louis, Missouri, USA).

Plasma osmolality was determined on single 20 µl samples of plasma with a advanced micro-osmometer (model 3300 Advanced Instruments, USA). White muscle water content was determined by placing approximately 0.25–0.5 g of muscle tissue on a dried and tared aluminium weighing dish. Following the determination of wet mass, the samples were placed in a drying oven at 60 °C and weighed daily until there was no change in

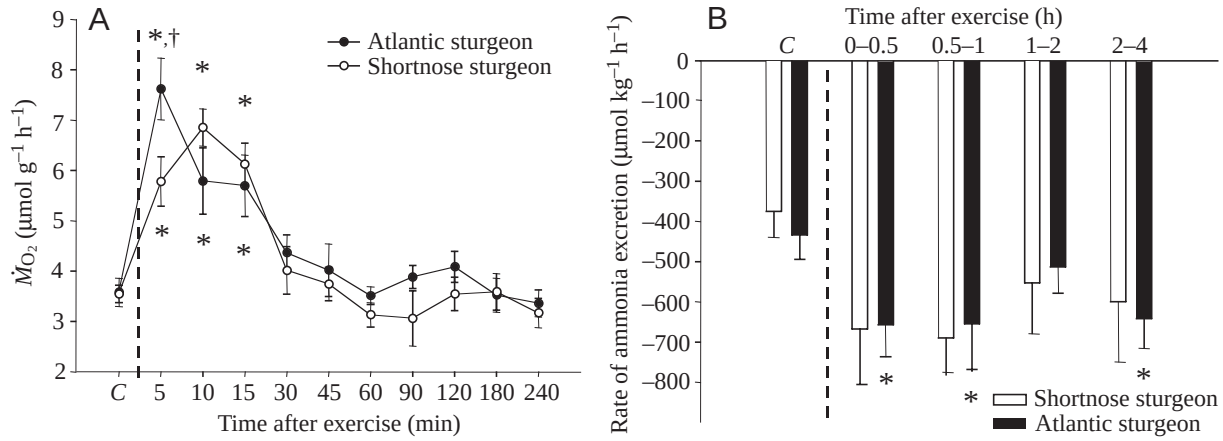

Fig. 1. Rates of oxygen consumption ($\dot{M}_{O_2}$; A) and net ammonia excretion (B) prior to and following exhaustive exercise in shortnose and Atlantic sturgeon. Asterisks denote significant differences (P<0.05) from resting values (C), and a dagger indicates a significant difference (P<0.05) between shortnose and Atlantic sturgeon at a particular sampling time. Negative values indicate net ammonia losses from the fish (net efflux). Values are means ± S.E.M. (N=8, for each species for $\dot{M}_{O_2}$; N=6 for each species for ammonia excretion).

mass for any sample (approximately 3–5 days). The percentage water content was calculated using the following equation:

$$\%H_2O = 100 - [100(\text{dry mass}/\text{wet mass})] .$$

Statistical analyses

Data were tested for normality (Proc Univariate) and homogeneity of variances (F_{max}-test) (Sokal and Rohlf, 1981) to ensure that they met parametric assumptions. Two-way repeated-measures analysis of variance (Proc ANOVA; species and time as main effects) were run for both oxygen consumption and ammonia excretion experiments (series I). Interaction P-values were set at 0.2 following Winer (1971); all other tests of significance were set at a type I error of P=0.05. When a significant interaction occurred, we ran both separate ANOVAs at each time, to detect differences between species, and one-way repeated-measures ANOVAs over time separately for each species. When an interaction between species and time was not significant, we re-ran the repeated-measures ANOVA pooling for species (Keppel, 1982). One-way ANOVAs were also used to assess the differences in responses for the shortnose sturgeon muscle and blood experiments (series II). One-way Dunnett's multiple-comparison tests were used as an *a posteriori* paired comparison of controls with all times within each temporal series.

Values are presented as means ± S.E.M.

Results

Series I

Oxygen consumption rates

Resting oxygen consumption rates ($\dot{M}_{O_2}$) for both sturgeon species were relatively low and averaged 3.5 μmol g^{-1} h^{-1} (Fig. 1A). Five minutes of exercise caused $\dot{M}_{O_2}$ to increase twofold to approximately 7.0 μmol g^{-1} h^{-1} in both species of sturgeon. This increase was immediate in Atlantic sturgeon but was slightly delayed (by approximately 5–10 min) in shortnose sturgeon. For between 5 and 15 min post-exercise, $\dot{M}_{O_2}$ remained significantly elevated, approximately twofold in both species, but these values returned to pre-exercise levels within 30 min and remained consistent and not significantly different from pre-exercise levels for the remainder of the experiment.

Ammonia excretion rates

At rest, the net ammonia excretion rates were approximately 400 μmol kg^{-1} h^{-1} in both species of fish (Fig. 1B). Following exercise, there was an approximately twofold increase in the net ammonia excretion in both groups of fish. However, because of the large variability in the shortnose sturgeon ammonia excretion rate data, these differences were not significant. In the Atlantic sturgeon, however, the ammonia excretion rates remained elevated throughout the experiment (with the exception of the 1–2 h values). There were no overall differences in the ammonia excretion rates between the two species of sturgeon (two-way ANOVA, P>0.05).

Series II: muscle fuel and metabolite levels in shortnose sturgeon

Resting muscle phosphocreatine (PCr) concentrations were approximately 11 μmol g^{-1} (Fig. 2A), while glycogen concentrations approached 15 μmol g^{-1} (Fig. 2B). Although exercise led to a significant 50 % drop in phosphocreatine levels, there was a rapid replenishment of this metabolite by 1 h post-exercise (Fig. 2A). Muscle glycogen levels decreased by approximately 30 % following exercise (Fig. 2B), but the decrease was not statistically significant. Overall, glycogen levels did not change significantly over the experimental period.

The drop (4 μmol g^{-1}) in glycogen concentration corresponded to a sixfold increase in muscle lactate concentration, which exceeded 6 μmol g^{-1} immediately after exercise (Fig. 3A). By 2 h into the recovery period, 50 % of the accumulated load had been eliminated, and by 6 h, muscle lactate concentrations had returned to resting levels (Fig. 3A).

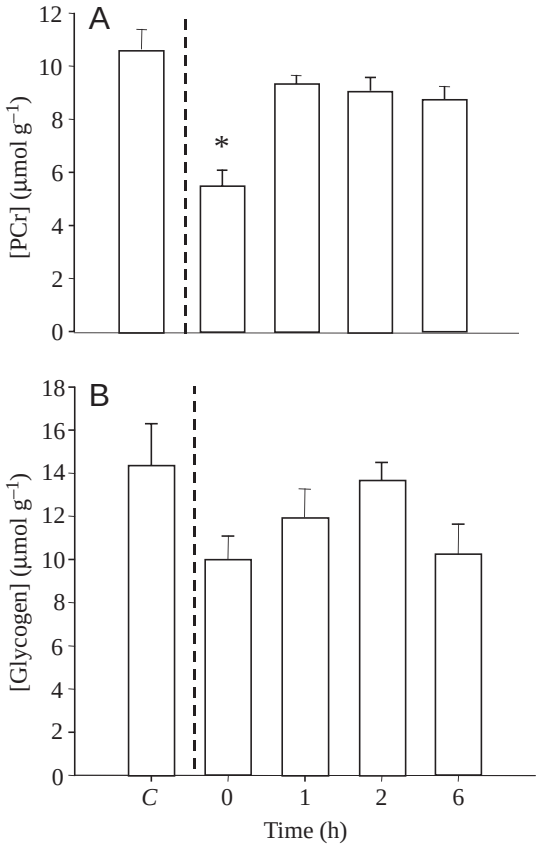

Fig. 2. Phosphocreatine (PCr; A) and glycogen (B) concentrations (μmol g^{-1}) in the muscle of shortnose sturgeon at rest (*C*) and following 0, 1, 2 and 6 h of recovery after exhaustive exercise. An asterisk denotes a significant difference (P<0.05) from the resting value. Values are means + S.E.M. (N=7–11).

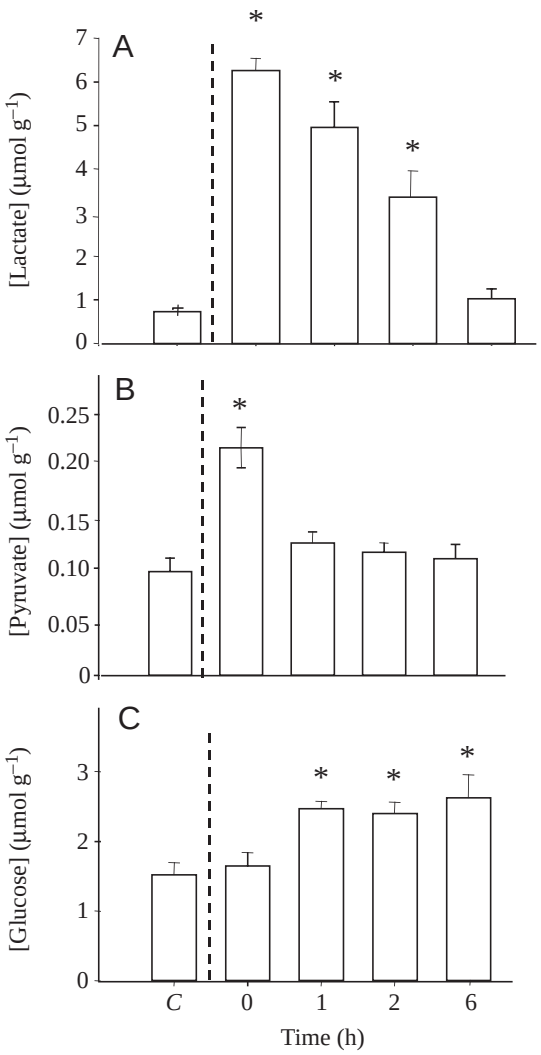

Fig. 3. Lactate (A), pyruvate (B) and glucose (C) concentrations (μmol g^{-1}) in the muscle of shortnose sturgeon at rest (*C*) and following 0, 1, 2 and 6 h of recovery after exhaustive exercise. An asterisk denotes a significant difference (P<0.05) from resting values. Values are means + S.E.M. (N=7–11).

Muscle pyruvate concentrations were approximately 0.1 μmol g^{-1} under resting conditions (Fig. 3B) and increased to 0.2 μmol g^{-1} immediately after exhaustive exercise. One hour into the recovery period, pyruvate had returned to resting levels (Fig. 3B).

Muscle glucose concentrations were low (1.5 μmol g^{-1}) under resting conditions (Fig. 3C) and did not increase significantly immediately after exhaustive exercise. By 1 h into the recovery period, muscle glucose levels had increased by approximately 60 %, and the levels remained stable for the remainder of the experiment (Fig. 3C).

Plasma metabolite levels in shortnose sturgeon

Plasma lactate concentrations were very low (<0.5 mmol l^{-1}) under resting conditions (Fig. 4A). These values increased to 1.3 mmol l^{-1} immediately after exercise and returned to resting values within 2 h (Fig. 4A). Similarly, plasma osmolalities were extremely low (approximately 250 mosmol kg^{-1}) under resting conditions (Fig. 4B). These levels increased slightly, but not significantly, immediately after exhaustive exercise but were reduced again 2 h post-exercise and remained low and stable at 6 h, post-exercise (see Fig. 4B). Overall, osmolality did not change significantly over the experimental period.

Discussion

Both sturgeon species exhibited a physiological stress response to physical disturbances, as indicated by changes in whole-body oxygen consumption and ammonia excretion rates (Fig. 1). Changes in levels of some of the muscle and blood metabolites were also observed in the shortnose sturgeon (Figs 2–4). These responses, however, are considerably reduced compared with those previously documented for many teleostean fishes (Moyes and West, 1995; Milligan, 1996; Barton, 2000; Kieffer, 2000). The findings of the present study, however, are consistent with those from other chondrostean species subjected to different acute stressors (Barton et al., 1998, 2000).

Oxygen consumption and ammonia excretion rates

The resting oxygen consumption rates for both species of

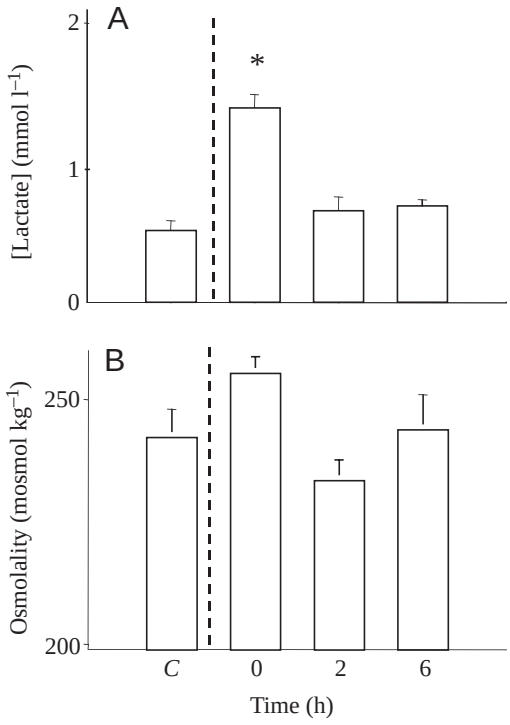

Fig. 4. Lactate concentration (mmol l^{-1}) (A) and osmolality (mosmol kg^{-1}) (B) in the plasma of shortnose sturgeon at rest (*C*) and following 0, 2 and 6 h of recovery after exhaustive exercise. An asterisk denotes a significant difference (P<0.05) from the resting value. Values are means + S.E.M. (N=5–7).

sturgeon were 10–20 % lower than those of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) of a comparable size (Scarabello et al., 1992) (A. M. Wakefield and J. D. Kieffer, unpublished data) and were similar to those of other sedentary, non-pelagic fish including other sturgeon species (Nonnotte et al., 1993) (see also Burrgren and Randall, 1978). Whether the observed differences reflect species differences or the fact that sturgeon remain relatively still in the respirometers could not be determined from the current analysis.

Post-exercise oxygen consumption rates of juvenile sturgeon increased by approximately twofold over resting levels, which is a very small increase compared with more active fish species such as trout and salmon (Wieser et al., 1985; Scarabello et al., 1992; McDonald et al., 1991; Gonzalez and McDonald, 1994). Our findings for shortnose and Atlantic sturgeon are similar to those of Khakimullin (1989), who found that the Siberian sturgeon *Acipenser baeri* did not increase its rate of oxygen consumption significantly with increasing swimming speed. Khakimullin (1989) noted that the physiological basis for these low oxygen consumption rates was related to the nature of swimming in this species. For example, these sturgeon actively resisted swimming at high speeds (probably by anchoring themselves on the bottom of the swimming apparatus with their long fins) and only swam in spurts (also noted for juvenile pallid sturgeon) (Adams et al., 1999). The results obtained by Khakimullin (1989) also support our findings that post-exercise oxygen consumption rates decreased rapidly following the swimming bout (see Fig. 1A). Thus, sturgeon appear to have a small excess post-exercise oxygen consumption, which is recovered quickly relative to other species (Scarabello et al., 1992) (A. M. Wakefield and J. D. Kieffer, in preparation). Taken together, these findings suggest that sturgeon have a low factorial scope; however, this does not appear to influence metabolic recovery from exhaustive exercise (see below).

In contrast to oxygen consumption rates, sturgeon excrete amounts of ammonia (approximately 400 μmol kg^{-1} h^{-1}) similar to those excreted by teleost fishes under resting conditions (Wood, 1988; Alsop and Wood, 1997). This trend changed with exercise, however; sturgeon only increased their ammonia excretion rates approximately 1.5-fold, while post-exercise excretion rates for rainbow trout (Wood, 1988; Kieffer and Tufts, 1996) typically increased at least twofold over resting levels. The lower post-exercise ammonia excretion rates for sturgeon may reflect their ability to excrete larger amounts of urea relative to ammonia. Most teleosts are ammoniotelic, with ammonia forming 75–100 % of the nitrogenous waste excreted. Recently, however, Gershanovich and Pototskij (1992) showed that the sturgeon species *Acipenser ruthenus* excreted a large proportion of their nitrogenous waste as urea (1.4 parts ammonia:1 part urea). Whether this pattern of nitrogen excretion is common among all sturgeon species and whether urea excretion rates in sturgeon change with exhaustive exercise is not known but would provide an interesting area for study.

Muscle and blood metabolite levels before and after exercise in shortnose sturgeon

The resting levels of PCr and glycogen are within the range noted for another teleost species of similar size, the rainbow trout (McDonald et al., 1998; Milligan, 1996), and the observed utilization and recovery patterns for PCr were within the range reported for salmonids (Milligan, 1996). However, in sturgeon, glycogen usage and recovery were markedly different from those of other species (Milligan, 1996). For example, glycogen levels decreased slightly, but not significantly, following exercise (Fig. 2B) and approached resting values within the first hour post-exercise. The rapid rates of restoration of PCr and glycogen (within 1 h) following exhaustive exercise by shortnose sturgeon matched recovery rates measured in the muscle of adult sea lampreys (Boutilier et al., 1993) and the rates of muscle glycogen replenishment reported in exhaustively exercised juvenile trout and herring (Scarabello et al., 1992; Franklin et al., 1996). A possible explanation for the rapid recovery of muscle PCr and glycogen levels may reflect the nature of the muscle tissue. Although nothing is known about the muscle types involved in swimming in sturgeon, there is some evidence that the muscle mass of sturgeon is made up of the same three zones noted in teleosts (Kiessling et al., 1993). A more plausible explanation may be that, since the metabolic disturbance is minimal following exhaustive exercise, relatively little time is needed

Table 1. *Maximum muscle lactate concentrations following exhaustive exercise in various species of juvenile fish*

Species	[Muscle lactate]* (μmol g^{-1} wet tissue)	Reference
Juvenile rainbow trout (*Oncorhynchus mykiss*)	30	McDonald et al. (1998)
Juvenile Atlantic salmon (*Salmo salar*)	22	McDonald et al. (1998)
Juvenile largemouth bass (*Micropterus salmoides*)	23	Kieffer et al. (1996)
Juvenile brook trout (*Salvelinus fontinalis*)	15	Kieffer et al. (1996)
Juvenile yellow perch (*Perca flavescens*)	15	McDonald et al. (1991)
Juvenile winter flounder (*Pleuronectes americanus*)	9	J. D. Kieffer and J. Frank (unpublished results) (whole-body value)
Juvenile Smallmouth bass (*Micropterus dolomieu*)	8	McDonald et al. (1991)
Roach (*Rutilus rutilis*)	6	Dalla Via et al. (1989)
Juvenile shortnose sturgeon (*Acipenser brevirostrum*)	6	Present study

*Values are approximate.

for full recovery of these metabolites. This is supported by the rapid repayment of the oxygen debt in this species (Fig. 1A).

Consistent with most studies on exhaustive exercise, there were notable changes in muscle glucose, pyruvate and lactate concentrations following exhaustive exercise (for a review, see Kieffer, 2000). However, the magnitude of these changes (particularly those for lactate) was much lower in shortnose sturgeon than in teleost fish of a similar size (see Table 1). For example, McDonald et al. (1998) found that rainbow trout (weighing 16 g) produced nearly eight times more lactate after exercise than did control fish. Even very small Atlantic salmon (approximately 4 g body mass) produced much higher levels of lactate (McDonald et al., 1998) than the shortnose sturgeon used in the present study. Studies on other sedentary fish show that post-exercise levels of lactate are approximately the same as the values noted for shortnose sturgeon (for a review, see Kieffer, 2000). The increases in lactate levels following exercise agree well with the decrease in muscle glycogen levels (i.e. 2 molecules of lactate produced per molecule of glycogen used) (compare Fig. 2B with Fig. 3A). However, the recovery of lactate appeared to be uncoupled from glycogen replenishment. Despite the speed at which muscle glycogen is replenished, recovery appears to occur without any utilization of muscle lactate, since concentrations of the latter remain relatively unchanged during this time (see Fig. 3A). Increases in muscle glucose content (Fig. 3C) and decreases in pyruvate content (Fig. 3B) may play a role in the early replenishment of muscle glycogen, even though the process of replenishment is unknown. It is also possible that other fuel sources, such as protein and lipids, may play a large role in the metabolic processes of these fish (Singer et al., 1990).

Another interesting feature of sturgeon physiology is the low post-exercise plasma lactate concentration. For example, plasma lactate levels were elevated to only 1.5 mmol l^{-1} immediately after exercise, and these levels quickly returned to pre-exercise levels (within 2 h) (see Fig. 4A). Such low levels contrast with those seen in most teleost fish, which normally accumulate a large amount of lactate in their bloodstream, peaking between 1 and 2 h post-exercise (Wood, 1991; Milligan, 1996). Given that sturgeon have similar blood volumes to those of other fish (Olson, 1992), these low plasma lactate levels probably reflect the low levels of lactate leaking from the muscle into the blood. The absence of a 'stress-induced' elevation of plasma lactate levels appears to be constant across most sturgeon species subjected to various physical disturbances (Barton et al., 1998, 2000).

Under resting conditions, plasma osmolalities were approximately 250 mosmol kg^{-1} in shortnose sturgeon. These values are lower than those typical of most teleost fishes (range 270–310 mosmol kg^{-1}), but are consistent with the values for many sturgeon species (Citaldi et al., 1998; LeBreton and Beamish 1998; Altinok et al., 1998), including shortnose sturgeon (Krayushkina, 1998). Post-exercise osmolality did not increase significantly in shortnose sturgeon (an increase of approximately 10 mosmol kg^{-1}) (Fig. 4B). Citaldi et al. (1998) showed a similar trend for Adriatic sturgeon (*Acipenser naccarii*) following crowding and handling stress. Our osmolality measurements suggest that there is probably no major ionic (Na^+ and Cl^-) disturbance in sturgeon similar to that noted for many other fish species subjected exercise and stress (Wood, 1991). Indeed, Barton et al. (1998) and Citaldi et al. (1998) found that plasma Cl^- concentrations were not modified by stress in sturgeon species. Thus, overall, our data suggest that the osmoregulatory function of sturgeon may not be modified following exercise.

Although Atlantic and shortnose sturgeon responded to manual chasing with a typical series of behavioural responses (e.g. increased ventilation, rolling over, tiring), they did not show responses to physical disturbances of a magnitude similar to those observed in most teleosts of a similar size. The reasons for these differences are not known, but the available data for several sturgeon species suggest that chondrostean fish may be physiologically less responsive to physical stressors than are teleosts in general (Barton et al., 1998, 2000).

Differences in physiological response to stress between teleosts and chondrosteans may be related to their evolutionary histories, with sturgeon being relatively ancient fish. Sturgeon, in general, are considered to be ancestral fish that have changed relatively little during evolutionary time. This lack of morphological change coupled with other features necessary

for their life history (e.g. feeding strategy, habitat requirements, lack of natural predators) may have led to a reduced need for sturgeon to develop strategies to deal with certain stressors compared with teleosts. For example, it is possible that, since sturgeon are well armoured (large dorsal scutes) and generally lack natural predators, they did not need to develop a capacity for exhaustive exercise or to respond physiologically to stress. It may also be possible that sturgeon are able to meet all their swimming requirements through aerobic processes. Although this is not known for shortnose sturgeon, Peake et al. (1995) found that lake sturgeon (*Acipenser fulvescens*) were poor swimmers in all categories (e.g. endurance and burst swimming) relative to salmonid species. Adams et al. (1999) also noted that pallid sturgeon demonstrated a reduced swimming performance compared with other fishes. The lower metabolic rate of sturgeon (i.e. oxygen consumption rates) could partially account for their depressed swimming ability. In addition, poorer performance at burst swimming speeds could indicate that anaerobic processes are less efficient in sturgeon than in salmonids (Peake et al., 1995).

The differences in the stress response between sturgeon and teleosts may also reflect differences in the anatomy of the interrenal cells of the kidney. For example, the interrenal tissue, which produces corticosteroids (e.g. cortisol), is dispersed throughout the kidney in chondrosteans, whereas in teleosts these cells are located in the anterior portion of the kidney (Barton et al., 2000). Differences in interrenal tissue between these two groups of fish may explain the lack of cortisol secretion post-stress in sturgeon (Barton et al., 2000). It has recently been documented that post-exercise elevations in cortisol levels and the rate of cortisol clearance dramatically influence the recovery process in exhaustively exercised salmonids (Milligan et al., 2000). More research into the cortisol response and mechanisms of stress hormone production in sturgeon species is needed because other factors, such as hypothalamic-pituitary-interrenal sensitivity, at other levels and receptor activity could also be involved.

In conclusion, our results clearly show that the stress response in sturgeon is small in magnitude relative to that of other species of fish (Wood, 1991; Barton et al., 2000; Barton, 2000; Kieffer, 2000). The reasons for this reduced response could be because of behavioural, morphological, ecological, evolutionary or physiological differences between sturgeon and other types of fishes. Determining an evolutionary link (correlation) will be difficult because it has been shown that a more primitive fish, the sea lamprey, exhibits a physiological response to burst exercise that rivals the response of salmonids (Boutilier et al., 1993). In addition, differences in laboratory exercise protocols and animal husbandry (e.g. water quality) could mask certain physiological responses. More research is needed in these areas to further our understanding of why sturgeons have a response to stress different from that of other fishes.

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