

## **Cohesive social behaviour shortens the stress response: the effects of conspecifics on the stress response in lake sturgeon *Acipenser fulvescens***

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An examination was made of whether social interactions can have a beneficial effect through the attenuation of the stress response in a social species. In the first experiment, one larger (mean  $\pm$  s.e.  $194.0 \pm 12.5$  g) and seven smaller ( $32.0 \pm 2.6$  g) juvenile lake sturgeon *Acipenser fulvescens* were placed in tanks to determine whether a classic dominance effect would be established based on body size ( $n = 6$ ). Large fish did not establish a territory or aggressively interact with smaller fish, as there were no significant differences in nearest-neighbour distances and an absence of aggressive behaviour (biting, chasing and pushing). In the second experiment, it was hypothesized that the presence of conspecifics would have a beneficial effect through an attenuation of the stress response. Fish in groups or isolation were stressed by a brief aerial exposure (30 s), and blood plasma was measured at regular time intervals (0, 20, 40, 60, 120 and 240 min) following the stressor *via* an implanted cannula ( $n = 9$ –11). The presence of conspecifics did not affect the peak cortisol response, however, the overall cortisol response was shorter in duration compared to fish in isolation. Furthermore, secondary stress variables (plasma ions and glucose) showed differences between fish in groups and isolation. The results of these experiments suggest that social interaction plays an important and beneficial role in regulating the stress response in cohesive social species such as *A. fulvescens*. © 2008 The Authors

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Key words: cortisol; glucose; group; ion; isolation; lactate.

### **INTRODUCTION**

The influence of social interactions on the stress response in fishes has received much recent interest (Gilmour *et al.*, 2005). Many of the advances in this area have come from studies primarily focused on aggressive, territorial species. In such species, subordinate fishes have chronically elevated cortisol concentrations (Sloman *et al.*, 2000a) that can result in reduced appetite, altered feeding

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behaviour and increased fin erosion (Gregory & Wood, 1999), elevated metabolic rate (Sloman *et al.*, 2000b; DiBattista *et al.*, 2006) and decreased growth rates (Barton *et al.*, 1987). Body size is often a factor in these intraspecific interactions, with the largest fishes generally being the most dominant (Yue *et al.*, 2006; Jacob *et al.*, 2007).

Intraspecific group interactions, however, have differing effects in fishes depending on whether the interaction is within an aggressive, territorial species or within a gregarious, shoaling species. Species that are social or gregarious by nature, may greatly benefit from social interactions. Group behaviour can lead to increased growth rates as a result of social feeding facilitation (Stirling, 1977; Peuhkuri *et al.*, 1995), reduced aerobic metabolic rates (Parker, 1973; Ross *et al.*, 1992), reduced predation risk (Roberts, 1996) and increased opportunities to find mates (Shapiro *et al.*, 1993). Very little is known, however, as to whether living in groups or isolation may have beneficial effects through reduction of the stress response.

Long-lived species are thought to be more likely to have evolved social behaviour (Ridley *et al.*, 2004). One group of long-lived species that has received little study on social interactions, but has received recent interest in their stress response, are acipenserids. This group of fishes is estimated to have diverged from its closest relatives, family Polyodontidae, close to 184 million years ago (Peng *et al.*, 2007). There has been an increasing interest in the stress response of acipenserids due to their ancestral evolutionary place within bony fishes, concern over the conservation of wild populations and their use in commercial aquaculture. The magnitude of the cortisol response, the principal glucocorticoid in acipenserids (Webb *et al.*, 2007), to various stressors has been found to be low in Acipenseriformes compared to teleost species, suggesting an evolutionary adaptation to a low stress response (Cataldi *et al.*, 1998; Barton *et al.*, 2000; Baker *et al.*, 2005b). Nonetheless, there is a measurable cortisol response that increases with handling time and severity of the stressor (Barton *et al.*, 1998, 2000; Bayunova *et al.*, 2002). It appears that the magnitude of the stress response may be somewhat species-specific (Baker *et al.*, 2005a, b; Webb *et al.*, 2007), and that it increases during nocturnal periods when levels of activity are naturally greater and the duration decreases with increasing temperature (Lankford *et al.*, 2003). Barton *et al.* (2000) suggested that the life history of *Acipenser* sp. is probably a factor in the low magnitude of the cortisol, lactate and glucose responses observed. Attributes of their life history, such as grouping behaviour, may influence the stress response. Adult and juvenile fishes are often found in groups, such as during spawning migrations, during occupancy of discrete locations in the river or during foraging or other movements (Foster & Clugston, 1997; Rochard *et al.*, 2001; Erickson *et al.*, 2002; Benson *et al.*, 2007). Similarly, juvenile lake sturgeon *Acipenser fulvescens* Rafinesque in the wild are predominantly captured in groups and rarely as isolated individuals (C. C. Barth & S. J. Peake, unpubl. data). Whether or not acipenserids, such as *A. fulvescens*, benefit from group behaviour through diminished stress levels is not known. Thus, research on *A. fulvescens* provides a potentially good model for social behaviour and its effects on the stress response.

The purpose of this research was to investigate whether *A. fulvescens* exhibit a social behaviour that results in dominance hierarchies and intraspecific

aggression when held in groups or display more gregarious, non-agonistic, cohesive behaviour. As an outcome of the initial experiment, a further purpose was to investigate whether or not living in groups influences the stress response in a social species. Based on the social behaviour observed in these fish, it was hypothesized that fish in groups would have a lower resting cortisol concentration, a lower magnitude of response to stress and a shorter duration of response than those in isolation.

## MATERIALS AND METHODS

### ANIMAL ACQUISITION AND CARE

Hatchery reared juvenile *A. fulvescens* were obtained from the Canadian Rivers Institute Field Station in Pinawa, Manitoba, and Grand Rapids Fish hatchery, Grand Rapids, Manitoba, Canada. Several months prior to experimentation, juvenile fish were transferred to tanks (170 l) housed at the University of Manitoba, Department of Zoology, where they were fed commercial trout diet (Martin Mills Ltd, Elmira, Ontario, Canada), maintained on a photoperiod of 12L:12D and supplied with 15° C flow-through de-chlorinated City of Winnipeg tap water.

### EFFECTS OF BODY SIZE ON DOMINANCE BEHAVIOUR

To determine if a size-based dominance hierarchy exists in *A. fulvescens*, two different size groups of fish were placed together in 120 l tanks at 15° C. One individual, large fish (mass range: 151–242 g) was placed with seven smaller conspecifics (mass range: 11–56 g) in a total of six tanks. Fish were acclimated to the tanks for over 1 week and fed commercial trout diet at 2% body mass day<sup>-1</sup>, which was divided into two feedings at 0830 and 1630 hours. Although information on social effects of acclimation time in *A. fulvescens* is limited, an acclimation period of 1 week has been shown to be sufficient for social experiments on isolated rainbow trout *Oncorhynchus mykiss* (Walbaum) (Schjolden *et al.*, 2005). After the acclimation period, movement and behaviour were recorded by overhead digital video cameras (Panasonic, Osaka, Japan) with 5–50 mm lenses (Pentax, Tokyo, Japan) over one full 12 h daylight period. Digital recordings were analysed using a video editing programme (VirtualDubMod v.1.5.10.2; Source Forge Inc., Mountain View, CA, U.S.A.) and image analysis software (ImageJ v.1.38x; National Institutes of Health, Bethesda, MD, U.S.A.) to reduce the number of frames analysed to one every 5 min, throughout the 12 h period. Each frame was analysed for differences in nearest-neighbour distance [concept modified from Clark & Evans (1954) for comparison of individual fish] between any part of the largest fish and any part of the closest adjacent fish. This was compared with the nearest-neighbour distance between one randomly selected conspecific fish and the closest adjacent fish at each 5 min time point. Differences in nearest-neighbour distance were also compared for feeding (30 min period following food addition) and non-feeding periods. In addition, to try and establish whether any dominant behaviour was being displayed by the larger fish, the number of aggressive events (bites, chases and pushing) was quantified by watching the entire 12 h recording.

### EFFECTS OF CONSPECIFICS ON THE STRESS RESPONSE

One week prior to experiments, different fish than used for the behavioural experiment were individually transferred to 120 l experimental tanks. Tanks held either the experimental fish in isolation ( $n = 11$ ) or housed an additional seven conspecifics ( $n = 9$ ). Tanks were supplied with flow-through, de-chlorinated tap water, 15° C,

and held under a photoperiod of 12L:12D. Lights were maintained at a moderate to low level, and the amount of light was further attenuated by semi-translucent covers over each tank. Fish were fed similarly to the behavioural experiment described above.

Forty-eight hours prior to the induction of the stressor, experimental fish were removed from the tank, and a 0.58 mm inner diameter polyethylene cannula was inserted *via* blind puncture into the caudal vasculature and secured with two tie-down points (Crocker & Cech, 1998). During the surgical procedure, fish were initially anaesthetized using 150 mg l<sup>-1</sup> tricaine methanesulphonate in a water-bath with 170 mM NaCl and 96 mM NaHCO<sub>3</sub>. Fish were then placed in a supine position in an acrylic surgery trough, where gills were continuously irrigated from a temperature-controlled water-bath containing 75 mg l<sup>-1</sup> tricaine methanesulphonate buffered with the same salt mixture. Following the surgical procedure that lasted *c.* 15 min, fish were placed back into experimental tanks and closely monitored until the affects of anaesthesia had subsided. Because there were no size-based dominance relationships found in the behaviour experiment, fish of two different sizes were used for cannulation purposes, and the larger fish rather than the smaller conspecifics (experimental fish wet mass range 146–308 g and conspecific fish wet mass range 34–100 g) were analysed. The morning of sampling, a 0.3–0.4 ml blood sample was taken from the resting fish, and this was considered the time 0 prestress sample. Following this, a standardized aerial exposure was administered to all tanks by draining and re-filling the holding tanks, with the stressor applied to all fish within a 30 min period. Total time for the process was *c.* 45 s with *c.* 30 s air exposure for the fish. Following the stressor blood sampling from the experimental fish occurred at 20, 40, 60, 120 and 240 min post-stressor. After every time point but time 0 min, fish were re-infused with an equal volume of *A. fulvescens* ringer solution: 126 mM NaCl, 2.2 mM KCl, 0.45 mM CaCl<sub>2</sub>, 3 mM MgCl<sub>2</sub>, 4.6 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.2 mM KH<sub>2</sub>PO<sub>4</sub> and pH 7.6, which was based on LeBreton & Beamish (1998) and Dettlaff *et al.* (1993). Collected blood was placed immediately on ice, a sub-sample was measured for haematocrit, and the remainder was centrifuged within 30 min at 5000 *g* for 5 min, and plasma was subsequently stored at –80° C until analyses for cortisol, lactate, glucose and ions were conducted. All experiments were conducted under University of Manitoba approved animal protocols, F04-041 and F05-021.

## CORTISOL ASSAY

Cortisol assays were conducted using a radioimmunoassay protocol modified from van Anholt *et al.* (2003). Rabbit anti-cortisol antibody (IgG-F-2; Campro Scientific GmbH, Berlin, Germany), <sup>3</sup>H-cortisol tracer (1,2,6,7-<sup>3</sup>H-cortisol; GE Healthcare, Chalfont St Giles, U.K.) and a cortisol standard curve from synthetic cortisol (Q3880; Steraloids Inc., Newport, RI, U.S.A.) were used. The standard curve and all samples were run in triplicate, and there was an interassay coefficient of variation (c.v.) of 9.8% and an intra-assay c.v. of 5.5%.

## PLASMA ASSAYS

Colorimetric assays were used to measure lactate (A-108L; Biomedical Research Service Centre, University of Buffalo, Buffalo, NY, U.S.A.) based on the reduction of a tetrazolium salt in an NADH-coupled enzymatic reaction to formazan and glucose (DIGL-200; BioAssay Systems, Hayward, CA, U.S.A.) using an *o*-toluidine method modified from Powell & Djuh (1971). Plasma osmolality was measured using a vapour pressure osmometer (Vapro 5520; Wescor Inc., Logan, UT, U.S.A.). Plasma sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), chloride (Cl<sup>-</sup>), bromide (Br<sup>-</sup>), sulphate (SO<sub>4</sub><sup>2-</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>) were measured by ion-exchange chromatography (Metrohm-Peak, Herisau, Switzerland). The cation eluent was 4 mM tartaric acid and 0.75 mM dipicolinic acid, and the anion eluent was 3.6 mM Na<sub>2</sub>CO<sub>3</sub> with CO<sub>2</sub> suppression by 100 mM H<sub>2</sub>SO<sub>4</sub> followed by CO<sub>2</sub> free air. All assays were run in duplicate.

## DATA ANALYSIS

Data were transformed to meet normality (Shapiro–Wilk test) and homogeneity of variance (Levene’s test) using  $\log_{10}$  transformations when necessary. For the behaviour experiment, a paired  $t$ -test was used to compare nearest-neighbour distances between small and large fish from the same tank,  $n = 6$ .  $t$ -test statistics were used to compare feeding *v.* non-feeding nearest-neighbour distances for small or large fish. For the conspecific stress experiment, within a treatment, differences between time points were tested for using a one-way repeated measures ANOVA followed by a Fisher least significant difference (LSD) test for means comparisons,  $n = 8$ – $11$ .  $t$ -test statistics were used to compare treatments for each time point. A paired  $t$ -test was used to compare haematocrit data pooled from both treatments at the start *v.* the completion of the experiment. Data presented are means  $\pm$  s.e. and all analyses were considered significant if  $P < 0.05$ . All statistical analyses were conducted using JMP 4.0.4 software (SAS Institute Inc., Cary, NC, U.S.A.), except the repeated measures ANOVA, which was analysed on SigmaStat 3.0 software (SPSS Inc., Chicago, IL, U.S.A.).

## RESULTS

### EFFECTS OF BODY SIZE ON DOMINANCE BEHAVIOUR

In the behavioural experiment, there was no evidence of a size-based dominance hierarchy. The distance between large fish and the nearest conspecific was not greater than the distance between randomly selected fish and the nearest conspecific (paired  $t$ -test,  $n = 6$ ; Fig. 1). On the contrary, small fish were slightly farther from other fish than large fish from other fish. Nearest-neighbour distance was not related to feeding or non-feeding times in either large or small fish ( $t$ -test,  $n = 6$ ; Fig. 1). There were no discernable aggressive interactions as quantified by bites, chases or aggressive pushes. There was some contact during swimming, with an average of 6.7 collisions per 12 h. This did not appear, however, to be different from small fish, and it was noted that

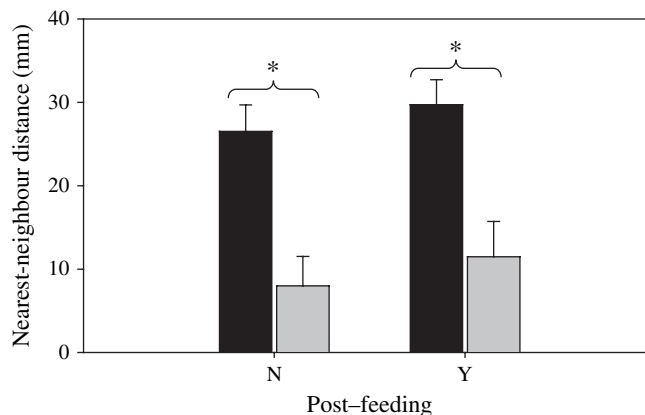


FIG. 1. Differences in mean  $\pm$  s.e. nearest-neighbour distance based on size and feeding status in juvenile *Acipenser fulvescens* (small fish (■) and large fish (▒), Y, 30 min post food addition; N, all other time points) \*, a significant difference (paired  $t$ -test between fish sizes;  $t$ -test within treatment between feeding status,  $P < 0.05$ ,  $n = 6$ ).

in general, the large fish avoided bumping into smaller fish by regularly swimming around or over them, even when they were directly in front of them.

### EFFECTS OF CONSPECIFICS ON THE STRESS RESPONSE

Following aerial exposure, plasma cortisol concentrations significantly increased in both treatment groups (repeated measures ANOVA,  $n = 9-11$ ), peaking at 20 min, although there was no difference between treatment groups at this time [ $t$ -test,  $n = 9-11$ ; Fig. 2(a)]. From 40 to 240 min post-stressor, plasma cortisol concentrations decreased in both treatments. At 40 and 60 min, however, cortisol concentrations were significantly greater in isolated fish than in group fish [ $t$ -test,  $n = 9-11$ ; Fig. 2(a)]. Cortisol concentrations returned to baseline by 120 min in fish in groups, but not until 240 min in isolated fish. Therefore, fish in groups did not have a lower baseline concentration of cortisol or a greater magnitude in the cortisol stress response, but they did have a shortened cortisol stress response as compared to fish in isolation.

There were no significant differences in plasma lactate, glucose or osmolality between treatments ( $t$ -test,  $n = 8-11$ ), although all mean values for lactate were

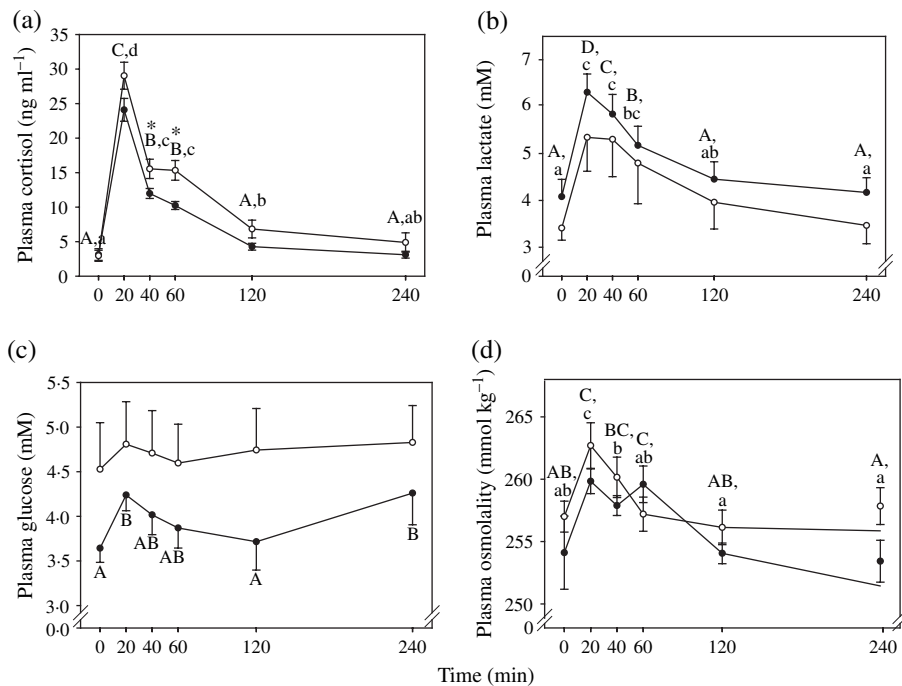


FIG. 2. Mean  $\pm$  S.E. plasma (a) cortisol, (b) lactate, (c) glucose and (d) osmolality concentrations in grouped (●) or isolated (○) juvenile *Acipenser fulvescens* subjected to an acute, aerial-exposure stressor. Different letters represent significant differences between points within a treatment group (upper case, group; lower case, isolated; one-way repeated measures ANOVA, Fisher LSD means comparison,  $P < 0.05$ ,  $n = 8-11$ ). \*, a significant difference between treatments within a time point ( $t$ -test,  $P < 0.05$ ).

greater in grouped fish, and all mean values for glucose were greater in isolated fish. In both treatments, plasma lactate significantly increased post-aerial exposure, with a peak at 20–40 min similar to plasma cortisol, and a return to baseline concentrations by 120 min [repeated measures ANOVA,  $n = 8-9$ ; Fig. 2(b)]. Plasma glucose increased in grouped fish at 20 min, returned to baseline from 40 to 120 min, and increased again at 240 min [repeated measures ANOVA,  $n = 8-9$ ; Fig. 2(c)].

Plasma osmolality increased post-aerial emersion stressor, peaking from 20 to 60 min in grouped fish and at 20 min in isolated fish [repeated measures ANOVA,  $n = 9-11$ ; Fig. 2(d)]. In both treatments, osmolality gradually returned to initial baseline concentrations. In grouped fish, osmolality concentrations were indistinguishable from baseline values at 40, 120 and 240 min. In isolated fish, osmolality concentrations were indistinguishable from initial baseline concentrations from 40 min onwards. There were no significant differences in haematocrit between the start and end of the experiment (repeated measures ANOVA,  $n = 8-9$ ) and between treatment groups ( $t$ -test,  $n = 8-9$ ). The pooled haematocrit for both treatments was  $15.7 \pm 1.3\%$  at time 0 min and  $12.1 \pm 1.0\%$  at time 240 min, which was significantly different (paired  $t$ -test). This indicated a slight drop in red blood cell (RBC) concentration, which would be expected for the volume of blood removed (estimated 15% of fish's total blood volume) although *A. fulvescens* Ringer's solution was re-infused after each time point except time 0 min to counteract any losses in blood volume, as described previously.

For plasma ions, data generally paralleled plasma osmolality, obtaining highest concentrations near 20–40 min followed by a return to baseline concentrations (repeated measures ANOVA,  $n = 8-11$ ; Table I). Plasma  $\text{Cl}^-$  was highest at 20 min and was significantly greater than concentrations at 120 and 240 min in grouped fish. Plasma  $\text{Cl}^-$  was also significantly greater at 20 min in isolated fish than grouped fish ( $t$ -test,  $n = 9-11$ ). Plasma  $\text{Br}^-$  was significantly greater in grouped fish than isolated fish at 240 min ( $t$ -test,  $n = 9-11$ ). Plasma  $\text{PO}_4^{3-}$  was highest at 20 min in both treatments and returned to baseline or lower concentrations thereafter (repeated measures ANOVA,  $n = 8-9$ ). Similarly, plasma  $\text{SO}_4^{2-}$  was highest at 20–40 min and returned to baseline or lower concentrations afterwards (repeated measures ANOVA,  $n = 9-11$ ), although there were no significant differences in grouped fish. Plasma  $\text{Na}^+$  was significantly higher in resting isolated fish than grouped fish (time 0;  $t$ -test,  $n = 9-11$ ). Plasma  $\text{K}^+$  concentrations decreased after time 0 (repeated measures ANOVA,  $n = 8-11$ ), although concentrations were not significantly different in isolated fish. There were no significant differences in plasma  $\text{Ca}^{2+}$  between time points (repeated measures ANOVA,  $n = 9-11$ ). Plasma  $\text{Mg}^{2+}$  was significantly greater at times 0–60 min than time 240 min in grouped fish (repeated measures ANOVA,  $n = 9-11$ ). Concentrations were also significantly greater in isolated fish than grouped fish at 240 min ( $t$ -test,  $n = 9-11$ ).

## DISCUSSION

Social interactions in fishes have generally been described in species displaying agonistic or territorial behaviours. In these species, social interactions result

TABLE I. Mean  $\pm$  s.e. plasma ion concentrations in grouped and isolated juvenile *Acipenser fulvescens* following an air exposure stressor. Different letters represent significant differences between time points within a treatment group (upper case, group; lower case, isolated); (one-way repeated measures ANOVA,  $P < 0.05$ , Fisher LSD,  $n = 8-11$ )

Ion	Treatment	0 min	20 min	40 min	60 min	120 min	240 min
Cl <sup>-</sup>	Group	108.4 $\pm$ 4.0 <sup>BC</sup>	110.9 $\pm$ 2.0 <sup>C*</sup>	105.2 $\pm$ 2.1 <sup>ABC</sup>	103.7 $\pm$ 3.5 <sup>ABC</sup>	100.3 $\pm$ 1.4 <sup>A</sup>	101.6 $\pm$ 2.3 <sup>AB</sup>
	Isolated	108.1 $\pm$ 3.9	118.4 $\pm$ 2.7	105.0 $\pm$ 4.5	111.3 $\pm$ 2.8	104.9 $\pm$ 2.7	104.8 $\pm$ 4.3
Br <sup>-</sup>	Group	0.048 $\pm$ 0.003	0.050 $\pm$ 0.002	0.057 $\pm$ 0.009	0.043 $\pm$ 0.005	0.049 $\pm$ 0.002	0.051 $\pm$ 0.001
	Isolated	0.046 $\pm$ 0.004	0.044 $\pm$ 0.003	0.041 $\pm$ 0.003	0.040 $\pm$ 0.003	0.039 $\pm$ 0.005	0.038 $\pm$ 0.004*
PO <sub>4</sub> <sup>3-</sup>	Group	2.29 $\pm$ 0.15 <sup>B</sup>	2.31 $\pm$ 0.15 <sup>B</sup>	1.89 $\pm$ 0.09 <sup>A</sup>	1.78 $\pm$ 0.19 <sup>A</sup>	1.83 $\pm$ 0.10 <sup>A</sup>	1.68 $\pm$ 0.12 <sup>A</sup>
	Isolated	2.05 $\pm$ 0.19 <sup>bc</sup>	2.92 $\pm$ 0.46 <sup>c</sup>	2.02 $\pm$ 0.19 <sup>b</sup>	1.80 $\pm$ 0.16 <sup>ab</sup>	1.94 $\pm$ 0.16 <sup>ab</sup>	1.63 $\pm$ 0.13 <sup>a</sup>
SO <sub>4</sub> <sup>2-</sup>	Group	3.10 $\pm$ 0.36	3.12 $\pm$ 0.37	3.44 $\pm$ 0.62	2.84 $\pm$ 0.31	2.58 $\pm$ 0.32	2.86 $\pm$ 0.35
	Isolated	3.18 $\pm$ 0.19 <sup>bc</sup>	3.44 $\pm$ 0.33 <sup>c</sup>	2.85 $\pm$ 0.14 <sup>ab</sup>	2.85 $\pm$ 0.19 <sup>ab</sup>	2.68 $\pm$ 0.15 <sup>a</sup>	2.85 $\pm$ 0.21 <sup>ab</sup>
Na <sup>+</sup>	Group	120.9 $\pm$ 2.5*	126.9 $\pm$ 6.0	129.4 $\pm$ 2.3	127.9 $\pm$ 2.6	127.9 $\pm$ 5.0	123.4 $\pm$ 3.6
	Isolated	131.4 $\pm$ 3.1	132.9 $\pm$ 3.9	133.4 $\pm$ 2.8	131.8 $\pm$ 2.7	132.3 $\pm$ 3.6	128.2 $\pm$ 3.6
K <sup>+</sup>	Group	2.01 $\pm$ 0.19 <sup>A</sup>	1.56 $\pm$ 0.09 <sup>B*</sup>	1.52 $\pm$ 0.06 <sup>B*</sup>	1.46 $\pm$ 0.06 <sup>B</sup>	1.62 $\pm$ 0.08 <sup>B</sup>	1.56 $\pm$ 0.04 <sup>B*</sup>
	Isolated	2.11 $\pm$ 0.10	1.89 $\pm$ 0.07	1.83 $\pm$ 0.10	1.73 $\pm$ 0.12	1.86 $\pm$ 0.09	1.83 $\pm$ 0.08
Ca <sup>2+</sup>	Group	1.58 $\pm$ 0.11	1.66 $\pm$ 0.06	1.58 $\pm$ 0.07	1.72 $\pm$ 0.18	1.46 $\pm$ 0.09	1.50 $\pm$ 0.04
	Isolated	1.66 $\pm$ 0.09	1.62 $\pm$ 0.07	1.63 $\pm$ 0.07	1.54 $\pm$ 0.05	1.47 $\pm$ 0.07	1.56 $\pm$ 0.07
Mg <sup>2+</sup>	Group	0.83 $\pm$ 0.04 <sup>A</sup>	0.84 $\pm$ 0.07 <sup>A</sup>	0.75 $\pm$ 0.09 <sup>A</sup>	0.77 $\pm$ 0.06 <sup>A</sup>	0.72 $\pm$ 0.07 <sup>AB</sup>	0.61 $\pm$ 0.04 <sup>B</sup>
	Isolated	0.82 $\pm$ 0.05	0.88 $\pm$ 0.05	0.81 $\pm$ 0.04	0.81 $\pm$ 0.02	0.71 $\pm$ 0.06	0.74 $\pm$ 0.04*

\*Significant differences between treatment groups within a time point ( $t$ -test,  $P < 0.05$ ).



in dominance hierarchies and potentially deleterious effects to fishes involved, particularly subordinate fishes (Gilmour *et al.*, 2005). In the subordinate fishes, these interactions have been shown to affect the stress response through chronically elevated cortisol concentrations (Sloman *et al.*, 2000a) and a general reduction in sensitivity to cortisol and catecholamines (Sloman *et al.*, 2002). In contrast, this study quantifies social interactions in what was found to be a non-agonistic species, and shows potential benefits of group interactions in terms of a shortening of the stress response as compared to isolated individuals. This shortening of the stress response was observed both at the primary (cortisol) and secondary (ion concentrations) stress response levels.

*Acipenser fulvescens* of disparate sizes showed no dominance hierarchies based on body size, and no agonistic behaviours from larger individuals on smaller individuals. The social behaviour seen in *A. fulvescens* is clearly different from most of the species studied to date for intraspecific social stress interactions but not unusual in fishes. Social aggregation behaviour is common in fishes, and does not necessarily have a dominance context for individuals involved (Pitcher & Parrish, 1993). In gregarious or social fish species, energetic benefits in terms of reduced aerobic metabolic rates have been demonstrated (Parker, 1973; Ross *et al.*, 1992), with increased growth rates occurring as a result of social feeding facilitation (Stirling, 1977; Peuhkuri *et al.*, 1995), and decreased growth rates due to decreased feed intake in isolation (Martins *et al.*, 2006). In the African catfish *Clarias gariepinus* (Burchell), the effects of group living *v.* isolation did not affect the stress response, probably because, despite being a social species, isolated fish are commonly found in the wild (Martins *et al.*, 2006). In contrast, sampling of wild populations of *A. fulvescens* has revealed that juveniles are usually found in groups, and rarely are captured as isolated individuals (C. C. Barth & S. J. Peake, unpubl. data). Similarly, wild adults are often found in dense aggregations (Thomas & Haas, 2002). In contrast to this study, one report of agonistic behaviour based on body size differences has been described in a different *Acipenser* sp., although it was not quantified (Kynard & Horgan, 2002). Differences from this study are probably due to species-specific differences, or possibly a result of the much smaller body sizes or experimental set-up differences in the other study.

Juvenile *A. fulvescens* exhibited a shortened stress response in the presence of conspecifics as compared to fish in isolation. In terms of the primary stress response (Barton & Iwama, 1991), cortisol concentrations, while similar in magnitude between the two treatment groups, returned to baseline concentrations more rapidly in grouped fish. Similarly, in humans, other mammals and birds there is evidence that social behaviour can reduce the hypothalamus–pituitary–adrenal stress response (Cohen & Wills, 1985; Hennessy *et al.*, 2008; Stowe *et al.*, 2008). Interestingly, baseline cortisol concentrations were not different between the two treatments, indicating that resting stress levels may not have been significantly different. Cortisol, the end product of the hypothalamus–pituitary–interrenal (HPI) axis, has two main functions: fuelling the long-term stress response primarily through gluconeogenesis, and restoring osmotic and ionic homeostasis (Wendelaar Bonga, 1997). This dual role was observed through the changes at the secondary stress response level in lactate, glucose, ions and osmolality, which paralleled the changes in cortisol.

Plasma glucose and lactate, while not different between treatments, may indicate a measure of chronic stress in the isolated fish. Negative feedback mechanisms that act to reduce the levels of circulating corticosteroids and resulting metabolites may be inhibited during chronic stress (Sloman *et al.*, 2002). Although baseline cortisol concentrations were not different between treatments, at every time point lactate, which increases as a result of glycolysis in the white muscle (Driedzic & Hochachka, 1978), was elevated in the grouped fish. Plasma glucose, which increases in response to catecholamine initiated hepatic glycogenolysis or cortisol mediated gluconeogenesis (Wendelaar Bonga, 1997; Mommsen *et al.*, 1999), was higher in isolated fish at every time point and was only responsive to the stressor in the grouped fish. In *Acipenser medirostris* Ayres, chronic stress has been shown to cause increased mobilization of liver glycogen resulting in elevated circulating glucose concentrations (Lankford *et al.*, 2005). Thus, the patterns of lactate and glucose between the treatments, and the lack of responsiveness in glucose post-stressor in isolated fish may indicate that isolation is a chronic stressor. Similar magnitudes of plasma lactate increases as those found in this experiment have been reported in other *Acipenser* sp. (Warren *et al.*, 2004; Baker *et al.*, 2005b), although glucose has generally not been as good of a measurement of short-term stress in sturgeons (Cataldi *et al.*, 1998, Barton *et al.*, 2000; Warren *et al.*, 2004; Baker *et al.*, 2005b).

Plasma ionic concentrations exhibited similar temporary increases as lactate, glucose and osmotic concentrations in response to the stressor and were different between treatments. Notably, plasma  $\text{Na}^+$  was elevated in isolated fish at resting conditions, and plasma  $\text{Cl}^-$  and  $\text{K}^+$  were elevated in isolated fish as compared to fish in groups immediately after the stressor. Sloman *et al.* (2003) found that  $\text{Na}^+$  uptake rates were elevated in subordinate fish that were chronically stressed by the social interaction. In the present experiment, perhaps the stress of isolation caused an overcompensatory effect in  $\text{Na}^+$  uptake rates or affected acid–base balance, as  $\text{Cl}^-$  concentrations were similar prior to the stressor between treatments.

The transient increases in plasma osmolality and ionic concentrations ( $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$ ), as well as the trends seen for other ions ( $\text{Na}^+$  and  $\text{Br}^-$ ) are contrary to normal changes in freshwater fishes, where catecholamine-induced changes in gill permeability and branchial circulation, in order to increase gas transfer and oxygen uptake, cause decreased osmolality and ion concentrations (Wendelaar Bonga, 1997). Because the transient increase is observed in osmolality, as well as in most of the ions, it may be due to either decreased plasma volume from water moving out of the circulation and into tissues (Okimoto *et al.*, 1994), or haemoconcentration caused by increased urine flow rates (Wood & Randall, 1973; Tervonen *et al.*, 2006). In *Acipenser*, most of the studies that have measured osmotic and ionic changes were evaluated in the context of salinity changes (Allen & Cech, 2007). Few studies have measured changes in ion concentrations in relation to the stress response in *Acipenser* sp. In most studies that have, little differences between pre-stress and post-stress states have been documented (Cataldi *et al.*, 1998; Gisbert *et al.*, 2004; Baker *et al.*, 2005b). In contrast, this study found a suite of ion changes, with similar post-stress increases in osmolality and chloride

concentrations reported in stressed, wild *A. fulvescens* (Baker *et al.*, 2008). Explanations for the decreases in  $K^+$  and  $Mg^{2+}$  in response to the stressor are not as clear as the reasoning behind the changes in other ions. Other studies have found evidence for stress-induced  $K^+$  loss in Siberian sturgeon *Acipenser baerii* Brandt (Gisbert *et al.*, 2004) and  $Mg^{2+}$  and  $K^+$  loss in *O. mykiss* (Chowdhury & Wood, 2007) *via* renal routes. Therefore, decreases may be *via* renal routes either through increased urine flow rate (Tervonen *et al.*, 2006) or decreased tubular resorption. Along this line of reasoning, the decreased concentrations of  $K^+$  and  $Mg^{2+}$  in group fish as compared to isolated fish may reflect increased post-stressor renal handling, although this was not tested.

As found in this and previous studies on *Acipenser* sp., cortisol concentrations and secondary stress response measures are often correlated, but considered reduced in comparison to other fishes such as teleosts (Barton *et al.*, 1998, 2000; Lankford *et al.*, 2003, 2005; Warren *et al.*, 2004; Baker *et al.*, 2005b, 2008; Webb *et al.*, 2007; Haukenes *et al.*, 2008). As a consequence, it has been suggested that *Acipenser* sp. may have a reduced stress response in comparison to teleosts. Barton (2002) noted that it is not clear whether corticosteroid concentration is related to stress level or a different capacity to respond to stressors. Knowledge on the stimulus for release (HPI axis), carrying capacity (cortisol binding protein) and receptor abundance and affinity for cortisol in *Acipenser* sp. is limited. It is clear therefore, that further research is required to determine if the stress response in *Acipenser* sp. is indeed reduced, or whether it is mediated in a different manner than in teleosts.

In conclusion, *A. fulvescens* did not display characteristics of agonistic behaviour when placed in a group context at disparate sizes. Instead, fish appeared to have cohesive social behaviour, the apparent beneficial effects were demonstrated when a common stressor was applied to fish in isolation or in groups. The changes observed in the primary and secondary stress responses clearly showed that for gregarious species, sociality can shorten the stress response. The stress response in the *A. fulvescens* is also of added interest considering its ancestral evolutionary position, and the rather reduced stress response observed in acipenserids as compared to teleosts in studies to date. Acipenserid species, such as *A. fulvescens* provide good models for further investigation of the influence of social behaviour on the stress response and the stress response in general.

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