Title: Comparison of MS222 and electronarcosis as anesthetics on cortisol levels in juvenile Atlantic sturgeon.

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Abstract

Invasive surgical procedures on sturgeon (family *Aciperseridae*) are sometimes conducted without anesthesia. We examined plasma cortisol concentrations in Atlantic sturgeon exposed to the anesthetic MS222, electronarcosis or no anesthetic 1 and 24 h after a small incision mimicking an invasive procedure (tag implantation or laparoscopy). We also determined the feasibility of using electronarcosis in the field and the effect of salinity on electronarcosis. One h after surgery under electronarcosis or MS222 anesthesia, cortisol concentrations did not differ significantly from those in untreated controls but all three were significantly lower than the no-anesthetic group. There were no significant changes between 1 and 24 h blood cortisol concentrations. We recommend electronarcosis as a method to minimize stress in fish studies involving surgical procedures because it avoids the use of toxic chemicals, and because induction and recovery are virtually instantaneous.

Key Words: Atlantic sturgeon, cortisol, electronarcosis, MS222, fish stress

Introduction

Investigators are increasingly conducting invasive procedures on sturgeon (family *Aciperseridae*) to track movements and to observe internal biological characteristics. In 2012, Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* was listed as a federally endangered species, so an increased understanding of Atlantic sturgeon life history through the use of invasive procedures is likely necessary to support future restoration efforts. However, invasive procedures are stressful to fishes, which result in increased cortisol levels (Barton 2002). Atlantic sturgeon is no exception to this general rule, and current stock status necessitates that great care be taken to minimize the stress induced from scientific studies involving invasive procedures.

Invasive procedures on sturgeon have been performed in the field without anesthesia. There is debate over whether anesthetics create more stress than procedures such as tag implantation alone (Damon-Randall et al. 2010). Before 2012 anesthetics were recommended but not required for invasive surgeries on Atlantic sturgeon (Damon-Randall 2010). Tricaine methanesulfonate (MS222), a chemical anesthetic, is commonly used for sturgeon (Damon-Randall 2010). MS222 is toxic, and in some areas the U.S. Food and Drug Administration requirements mandate fish be kept captive for a minimum of 21 days post treatment (Summerfelt and Smith 1990). Matsche (2011) measured cortisol levels of Atlantic sturgeon exposed to varying MS222 concentrations. Although Matsche (2011) demonstrated the success of MS222 as an anesthetic, dosage and toxicity of the anesthetic remains an issue for field studies, especially with larger fish. Electronarcosis, a physical anesthetic used in salmonid field studies (Roth et. al 2003, Hudson et al. 2011), has been used on captive lake sturgeon *A. fulvescens* and shortnose sturgeon *A. brevirostrum* (Henyey et al. 2002). Henyey et al. (2002) concluded the

benefits of electronarcosis over MS222 were speed of induction and recovery from anesthesia, cost, and ease of use, but more work is required to determine the physiological effects of the anesthetics. Managers are interested in the effectiveness of anesthetics. This study examined plasma cortisol concentrations in Atlantic sturgeon exposed to MS222, electronarcosis or no anesthetic during an incision mimicking an invasive procedure.

Methods

Age 2 y (n = 16, 290-385 mm fork length, 85-235 g) and 3 y Atlantic sturgeon (n = 8, 380-466 mm fork length, 379-502 g), obtained from Maryland Department of Natural Resources, were maintained at the aquatics facility at Virginia Commonwealth University for a minimum of 6 months. All fish were handled routinely on a monthly basis to collect length and weight measurements. Four fish were assigned to one of six replicate 568 L flow through tanks two months prior to experimentation. Age classes were segregated so there were two replicate tanks of 3 y old fish and four replicate tanks of 2 y old fish. Tanks were maintained at 16-18^{o}C under a 12:12 light/dark cycle, and fish were fed a pellet diet (Zeigler Bros Inc. product # 306540-18-44) once daily 5-7 times per week. Fish were fasted 24 h prior to treatment (Matsche 2011).

Individuals in each tank were randomly assigned as a control (no surgery), surgery with no anesthesia, surgery with MS222, or surgery with electronarcosis so each replicate tank had each treatment. To replicate an invasive procedure, a ~1 cm incision was made in the abdomen and closed with two double surgeon knot sutures (Mohler 2004). Incisions were sutured within 20 seconds of being made. The control, no anesthesia, and MS222 procedures were conducted in12 L tanks. Electronarcosis was conducted in a 16 L tank of similar length and width but deeper to allow cathode and anode screens. To minimize possible daily-cyclic cortisol

fluctuation two groups of four fish were processed per day: one group started at 10:00 and the second at 10:30. Treatments were conducted on four consecutive Tuesdays.

All four fish per tank were removed simultaneously and placed in a treatment tank. The control fish were placed in the experimental tank for 1 minute and returned to their home tank. The no anesthesia group was immobilized with straps on a stretcher during the procedure. The MS222 fish were exposed to100 mg/L with 200mg/L of sodium bicarbonate, and time to reach stage 4 anesthesia was recorded, after which fish were placed on a support stretcher for the surgical procedure. Stage 4 anesthesia is characterized by complete loss of spinal reflexes and slowed opercular movement (Summerfelt and Smith 1990). After surgery freshwater was run over the gills and recovery time was recorded for fish to exhibit typical forward taxis before returning individuals to their home tank.

Electronarcosis was conducted using a 0-60VDC, 1.5A (BK Precision: Model 1623A) power supply. Positive and negative electrodes were attached to 6.35 mm mesh galvanized hardware cloth. Fish to be anesthetized by electronarcosis were placed between the two electrodes with their head towards the anode (positive) electrode (Hudson et al. 2011). Previous trials were conducted on similar sized Atlantic sturgeon to get an estimated range of voltage required for loss of spinal reflexes. Using a dial voltage (V) was increased (average 0.54 V/cm, range 0.46-0.57; average 0.08 amps, range 0.08-0.10) until level 4 anesthesia was reached although opercular motion remained constant (Summerfelt and Smith 1990, Henyey et al. 2002). When surgery was complete the voltage was turned off using the dial. Electronarcosis anesthesia was instantaneous; there were no induction or recovery times unless you consider the time taken to turn the dial which was not timed but typically took less than two seconds (Virginia Commonwealth University IACUC AD20127).

Plasma cortisol levels typically peak 1 h after stress events (Iwama et al. 2006, Matsche 2011). Blood samples were taken 1 h and 24 h after initial removal from the tank. Samples (2.5 ml) were collected via caudal vein using a 21-gauge needle, and transferred to heparinized vacutainer tubes with a plasma separation layer (BD Diagnostics, Franklin Lakes, New Jersey). Within 10 minutes of collection blood samples were centrifuged at 5 ^{o} C and 1,000 X g for 15 min. Plasma samples were stored at -80 ^{o} C until cortisol extraction.

Plasma cortisol was extracted and assayed with the Cortisol Express EIA Kit (Cayman Chemical Company, Ann Arbor, Michigan, Item No. 500370) following manufacturers protocols. Assays w ere run in triplicate at 1:2 and 1:4 dilutions or 3:1 concentration and final values read on a Bio-Tek μQuant Universal Microplate reader at a 410 nm wavelength. Triplicate values were averaged. Samples were rerun if any readings varied by more than 10% of the samples average.

Blood samples were separated by time (1 h and 24 h) for statistical analysis. ANCOVAs were run accounting for treatment, tank, and a fish parameter. Fish parameters were fish fork length, fish weight, or fish condition factor:

$$\frac{\text{Weight}}{\text{Fork Length}^3} \times 100000$$

with weight in kilograms and fork length in centimeters. ANCOVA models were first compared using an *F*-test to identify the most parsimonious model. Post-hoc comparisons among specific treatments from the appropriate model were then conducted using Tukey's HSD test.

Results

MS222 anesthesia induction time averaged 6 min (range 4 -8 minutes) and 5 min (range 3-8 minutes) for recovery. The ANCOVAs indicated no significant difference between the models when blocking for tank so the simpler model with just treatment and fish parameter was chosen for statistical inference. The ANCOVAs accounting for fish length, weight, or condition factor found significant differences between the same treatment types for both time periods so only the fish parameter is described. The ANCOVA indicated a significant difference in plasma cortisol levels at 1 h among treatments ($F_{3,20}$=9.86, p=3.8 x 10^{-4}). Post-hoc testing revealed no significant differences between the control, electronarcosis, and MS222 1 h blood cortisol levels, but all three were significantly lower than the no-anesthesia group (Figure 1). Cortisol in the no-anesthesia group was four fold higher than the control group (p=2.3 x 10^{-4}) and twice as high as electronarcosis (p=6.4 x 10^{-3}) and MS222 (p=0.03). At 24 h after surgery, plasma cortisol differed significantly among treatments ($F_{3,20}$=3.14, p=0.04). Post-hoc testing determined only the control and no-anesthesia (p=0.03) groups were significantly different (Figure 1). All fish were observed feeding on pellet diet within 2 h of the 24 h blood sample. Varying salinity in captivity indicated that freshwater is required for electronarcosis with our equipment. A mild sluggish behavior was observed at 1 ppt, and there was no response in 2 ppt. At 12 ^{o}C anesthesia and recovery with electronarcosis was still instantaneous.

Discussion

This study demonstrates that electronarcosis and MS222 anesthesia have equivalent effects on plasma cortisol concentrations after short duration surgical procedures. One h blood cortisol levels with no anesthetic were significantly elevated over control, electronarcosis, and MS222 groups. One h samples without anesthetic had levels elevated four fold over controls.

The 1 h electronarcosis and MS222 samples appeared to have a bimodal distribution with three from both groups having blood cortisol levels similar to controls and three almost double. The slightly higher plasma cortisol levels in half of the anesthesia samples could not be accounted for by fish parameter or tank. The higher values cannot be definitively explained with the context of this study; however, the increased values were lower than the average no-anesthesia group. Control cortisol levels from this study were similar to baseline values found by Matsche (2011) in captive juvenile Atlantic sturgeon and Barton et al. (2000) in captive juvenile pallid sturgeon *Scaphirhynchus albus*. However, our treatment results were lower than found by Matsche (2011) and Barton et al. (2000) for handling and confinement treatments, perhaps because our fish had been handled extensively prior to the experiment.

Although our results suggest that electronarcosis and MS222 have equivalent effects on stress, we suggest there are advantages to electronarcosis. Electronarcosis allows instantaneous induction and recovery from anesthesia for fish of various sizes and across a range of temperatures (12-27^{o}C) in a laboratory or field setting (personal observation). MS222 anesthesia induction time increases in colder water and with increased fish size (Henyey et al. 2002, Matsche 2011). For example, stage 4 anesthesia in a 74 kg Atlantic sturgeon required 17 min at 14 ^{o}C, which is a considerable delay and inefficient in the field. Although excessive electrical current causes erratic opercule movements and mouth protrusion in sturgeon, voltage can be decreased to safe levels immediately. MS222 overdose is more difficult to determine and problematic to correct. Furthermore, MS222 introduces toxic chemicals into the fish and environment, particularly for large fish, and in some areas MS222 is prohibited for use due to potential consumption of fish by humans. Unfortunately, the electronarcosis system used in this study is ineffective in brackish water. However, Atlantic sturgeon commonly move between

fresh and marine water and transfer of estuarine or marine sturgeon to a freshwater tank for brief procedures should not be harmful. Future research is needed to compare stress of fish if moved from saline water to freshwater for electronarcosis anesthesia compared to MS222 anesthesia maintained in saline water. Power supplies specifically designed for electronarcosis are commercially available that are described as being able to work in saline waters.

The National Institutes of Health allows surgical procedures without an anesthetic if a state of anesthesia "would defeat the purpose of the experiment" (NIH 1985). The National Oceanic and Atmospheric Administration currently require an anesthetic for invasive procedures on Atlantic sturgeon but not for other sturgeon species. Given the four fold increase in cortisol levels with no anesthesia, it will be increasingly difficult to justify field procedures on non-anesthetized fish. Construction of an electronarcosis setup is relatively inexpensive, and we recommend this technique for future field work on Atlantic and other sturgeon species in freshwater.

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Figure Captions

Figure 1. Plasma cortisol concentrations (ng/ml) in juvenile Atlantic sturgeon. Blood was collected from the caudal vein in control fish (Con) and in fish 1 h (dark bar) or 24 h (light bar) after surgery performed under anesthesia with electronarcosis (EN), tricaine methanesulfonate (MS) , or with no anesthetic (No). Bars represent means ± SE. Letters (1 h samples) and numbers (24 h samples) above the bars indicate significant differences derived from the ANCOVA accounting for various fish parameters and treatment as factors.

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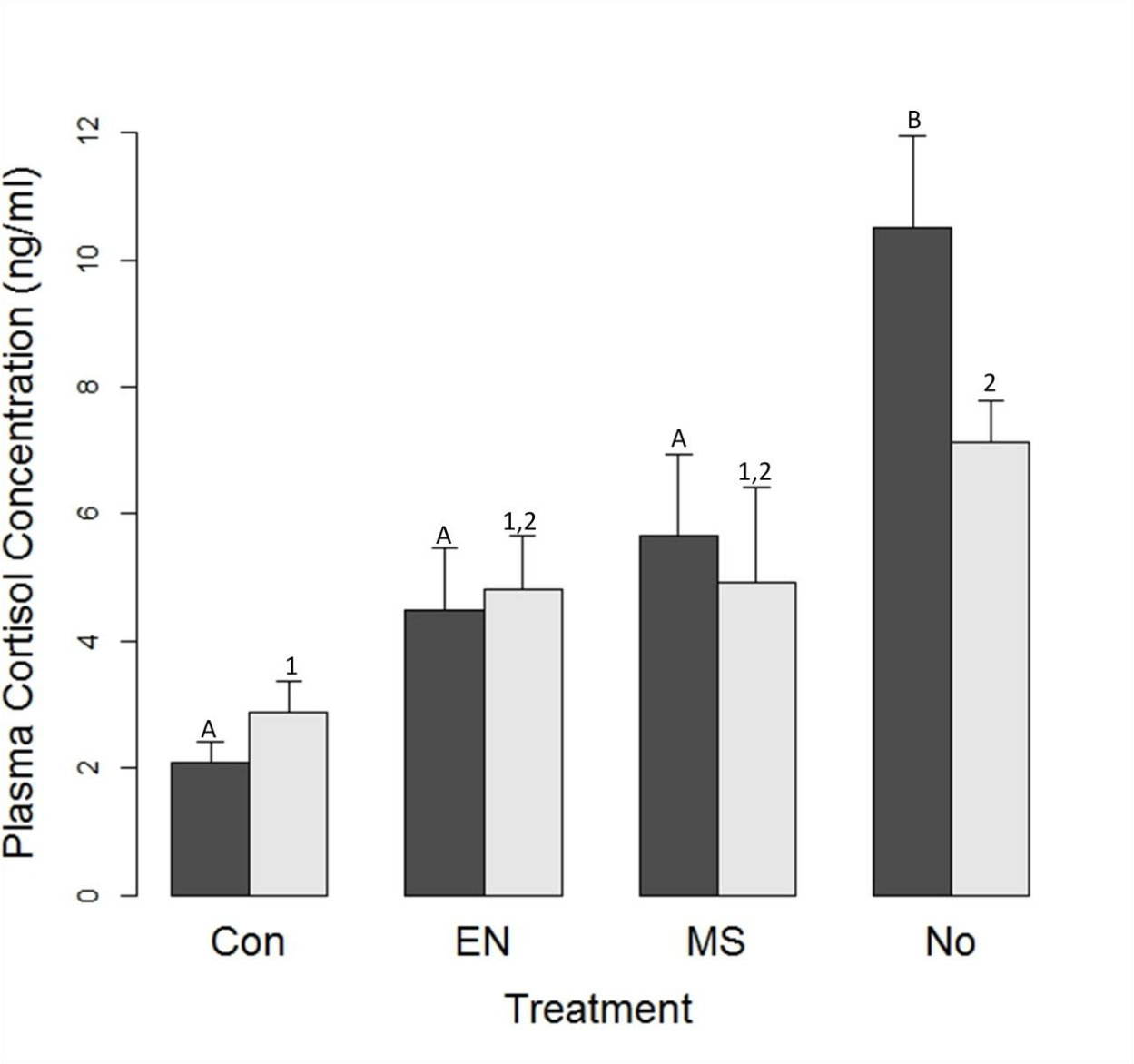

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