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Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors

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Abstract

The effect of isoeugenol sedation (2.5 mg l^{-1}) on plasma cortisol, glucose, and lactate dynamics in channel catfish (*Ictalurus punctatus*), relative to two controls, was examined during confinement, exposure to high unionized ammonia, and acute oxygen depletion. The positive control (PC) for each treatment was no sedation, and the negative control (NC) was sedation with metomidate hydrochloride (1.5 mg l^{-1}). Isoeugenol sedated catfish had 60% lower ($P<0.05$) plasma cortisol levels than PC fish after 15-min of confinement. No differences ($P>0.05$) in plasma levels of glucose or lactate were observed between treatments during 45-min of confinement. Fish exposed to high ammonia for 24-h had elevated ($P<0.05$) cortisol levels in PC and isoeugenol treatment groups. Sedation with isoeugenol during exposure to high ammonia had no effect on plasma glucose or lactate levels relative to PC fish. Levels of plasma cortisol, glucose, and lactate all increased significantly ($P<0.05$) in PC fish following acute oxygen depletion for 30 min. Plasma glucose levels were similar ($P>0.05$) between isoeugenol and control treated fish throughout oxygen stress and recovery. Sedation with isoeugenol significantly suppressed the resulting plasma cortisol and lactate response by 74% and 46%, respectively, compared to PC fish. Plasma cortisol levels in NC fish were below detection limits for all stress treatments. As a sedative, isoeugenol had suppressive effects on channel catfish plasma cortisol levels during confinement and low oxygen conditions, and reduced the plasma lactate response to acute oxygen depletion.

Keywords: Isoeugenol; Catfish; Metomidate; Confinement; Ammonia; Dissolved oxygen; Stress

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1. Introduction

Pond culture of channel catfish (*Ictalurus punctatus*) inherently involves some degree of environmental and physical stress to the fish. The challenge for the producer is keeping fish healthy and producing a quality product. Different culture techniques and handling procedures can greatly affect the degree of fish stress. Currently, commercial harvesting techniques and handling procedures for both fingerlings and foodfish do not involve the use of chemical sedatives. However, as concern for fish health and product quality increases, so does interest in potential use of sedatives to reduce fish stress during intensive handling procedures.

Chemical anesthetics are often used in aquacultural research settings to ease handling and reduce fish stress. In addition to preventing physical injury, certain anesthetics may reduce or block activation of the hypothalamo–pituitary–interrenal (HPI) axis associated with handling stress. Failure to suppress activation of the HPI axis during stress results in a release of cortisol which in turn causes various secondary stress responses, including increases in circulating levels of glucose and lactate (Rotllant et al., 2001; Skjervold et al., 2001). Stress induced elevations in plasma cortisol are known to suppress immunological capacity in salmonid fish (Maule et al., 1989; Pickering and Pottinger, 1989) and channel catfish (Davis et al., 2002, 2003), and increases in plasma glucose and lactate, indicative of glycogen mobilization and breakdown, have been associated with poor quality and rigor development of fish fillets (Skjervold et al., 1999, 2001; Silva and Nunez, 2001).

High stocking densities and intensive handling during harvesting and transport are common to aquaculture, and often result in fish being exposed to high ammonia and low dissolved oxygen levels (Boyd, 1982; Fries et al., 1993; Abdalla and Romaire, 1996; Torrans et al., 2003), both of which result in fish stress. Tomasso et al. (1981a) found plasma corticosteroid levels in channel catfish rapidly increased in response to acute oxygen depletion, and exposure to high ammonia concentrations over a 24-h period significantly elevated levels of circulating corticosteroids (Tomasso et al., 1981b). The stress response of channel catfish to poor water quality is further exacerbated by a separate stress response to confinement. Davis et al. (1984) demonstrated a significant increase in plasma cortisol within the first 10 min of net confinement across a broad range of water temperatures, and Davis et al. (2002) showed a concurrent increase of plasma glucose levels in response to confinement stress.

The use of anesthetics as a tool in aquacultural management to reduce fish stress and improve product quality may become more prominent in the future. AQUI-S™, a relatively new fish anesthetic comprised of the active ingredient isoeugenol, has foodfish approval in Australia, Chile, and New Zealand with no withholding period, and has been adopted by New Zealand salmon producers as a sedative for rested (low stress) harvesting (Dr. Alistair Jerrett, New Zealand Institute for Crop and Food Research, personal communication). The purpose of this study was to determine the effect of sedation with isoeugenol on the plasma dynamics of cortisol, glucose, and lactate in channel catfish exposed to confinement, high ammonia, and low dissolved oxygen. This research provides necessary data for assessing the potential use of isoeugenol as a sedative to reduce the stress response of channel catfish to common aquaculture related conditions.

2. Materials and methods

Two weeks prior to sampling, juvenile catfish (61.8 g±11.3; mean weight±S.E.M.) of the NWAC103 strain were stocked into 72-l aquaria (25 fish per aquarium). Each aquarium was supplied with well water (temperature, 26 °C; pH, 8.6; total hardness, 120 ppm; alkalinity, 410 ppm; total ammonia nitrogen, <1.5 ppm; nitrite nitrogen, 0 ppm) at a flow rate of 8-l min^{-1}. Fish were fed a 36% protein, floating, catfish fingerling starter (Farmland Industries, Kansas City, MO, USA) daily to satiety during the 2-week acclimation period, and photoperiod was held constant at 12-h light/12-h dark. All fish were fasted the day prior to sampling.

To determine the effect of isoeugenol (IE) sedation on the response to confinement, high ammonia, and acute oxygen depletion, channel catfish in each respective challenge were sedated with 2.5 mg isoeugenol l^{-1}(AQUI-S™ (5.0 mg l^{-1}) AQUI-S New Zealand, Lower Hutt, New Zealand) during administration of the stress challenges. This concentration was chosen based on unpublished preliminary efficacy results from our laboratory. Plasma cortisol, glucose, and lactate concentrations from fish sedated with isoeugenol were compared to positive control (PC) and negative control (NC) treatment groups. Catfish in the PC group received no anesthetic during the challenges. Catfish in the NC group were sedated with metomidate hydrochloride (DL-1-(1-phenylethyl)-5-(metoxycarbonyl)imidazole hydrochloride; Janssen Pharmaceutica, Beerse, Belgium) at a concentration of 1.5 mg l^{-1} throughout the challenges. Metomidate hydrochloride has been shown to completely block cortisol release into circulation in channel catfish (Small, 2003). Isoeugenol and metomidate hydrochloride, respectively, were added immediately prior to initiation of the stress challenges.

The respective stress challenges (confinement, high ammonia, and acute oxygen depletion) were conducted separately over three consecutive days, and each challenge was repeated three times (n=3). During each stress challenge, sampling of six fish per treatment (IE, PC, NC) per time point was done rapidly without anesthesia. Blood was collected from the caudal vasculature into syringes coated with heparin for cortisol and glucose analyses, and with sodium fluoride/potassium oxalate for lactate analyses. Plasma was separated by centrifugation, immediately analyzed for lactate, and stored frozen for later analysis of cortisol and glucose. Plasma lactate concentrations were determined by the lactate oxidase procedure (Pointe Scientific, No. L7596, Lincoln Park, MI), and plasma glucose concentrations were determined by the glucose oxidase procedure (Pointe Scientific, No. G7519, Lincoln Park, MI). Plasma cortisol was determined by a time-resolved fluoroimmunoassay which has been validated for channel catfish (Small and Davis, 2002).

2.1. Confinement stress

Confinement stress was accomplished by inserting a plastic grate with 1.5 cm square mesh perpendicular into the aquarium, and confining the 25 catfish in a 6700 cm^3 area at the rear of the aquarium. Water flow to the aquaria was stopped throughout the challenge, and continuous aeration was provided. Fish were sampled every 15 min for 45 min during the confinement stress.

2.2. Ammonia stress

Ammonia stress was accomplished by adding ammonium chloride to each aquarium to achieve an un-ionized ammonia-nitrogen (NH_3-N) concentration of 1 mg l^{-1}, similar to Tomasso et al. (1981b). Water flow to the aquaria was stopped, and aeration was provided throughout the 24-h challenge. Fish were sampled at time zero and after 24-h of ammonia stress. Verification of ammonia concentration was carried out by direct nesslerization for total ammonia-nitrogen [TAN; American Public Health Association (APHA) et al., 1989]. Un-ionized ammonia-nitrogen was calculated as a function of TAN, temperature, and pH (Emerson et al., 1975).

2.3. Oxygen stress

Acute oxygen depletion was accomplished by stopping the water flow to the aquaria, and bubbling nitrogen gas into the aquaria. Dissolved oxygen (DO) concentration in the water was measured with a Model 58 DO meter (YSI, Yellow Springs, OH). Dissolved oxygen levels at the start of the challenges measured 6.4 ± 0.1 mg l^{-1} (mean$\pm$S.E.M.). Diffusion of nitrogen into the water was stopped when DO levels were reduced to 0.5 mg l^{-1} (approximately 10-min). Thirty minutes after the start of the challenge, DO in the aquaria had dropped to 0.1 mg l^{-1}, and aeration was again provided. Thirty minutes later DO levels averaged 6.5 ± 0.1 mg l^{-1}, and flow of fresh water was resumed at a rate of 8-l min^{-1}. Fish were sampled at time zero, 30-min after the start of oxygen depletion, 30-min after resumption of aeration, and 5.5-h after resumption of aeration.

2.4. Statistics

Plasma cortisol, glucose, and lactate concentrations were subjected to analysis of variance (ANOVA) mixed-model procedures with treatment as the fixed effect and tank within treatment as the random effect using the SAS software system version 8.00 (SAS Institute, Cary, NC, USA). When significant differences were found using ANOVA, pairwise contrasts were made using an LSD test to identify significant differences at the 5% level.

3. Results

Sedation (Schoettger and Julin, 1969) was achieved in all fish receiving the NC and IE treatments. No fish mortalities were observed throughout the experiments. Pre-stress concentrations of cortisol, glucose, and lactate were similar among all treatments for each stress challenge (Figs. 1–3). Overall, fish receiving the PC and NC treatments responded to the various stress challenges as expected. Fish in the PC treatment consistently responded to the stressors with increased circulating levels of cortisol. NC treatment fish demonstrated no increase in plasma cortisol during the stress challenges. For statistical purposes, plasma cortisol levels below the assay standard

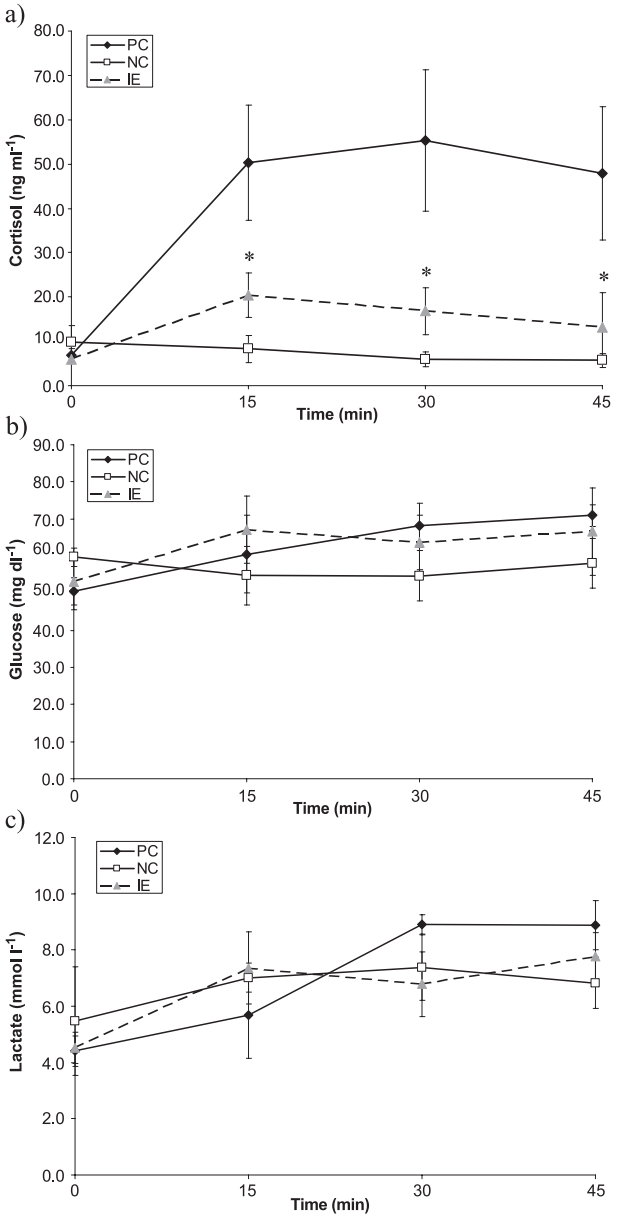

Fig. 1. Effects of confinement for 45-min without sedation (PC), sedation with metomidate hydrochloride (NC), or sedation with isoeugenol (IE) on plasma cortisol (a), glucose (b), and lactate (c) of channel catfish. The points and error bars denote means and standard error of the means (n=3), respectively. Significant differences (P<0.05) between IE and PC treatments within time are indicated by a single asterisk (*). No significant differences (P>0.05) between IE and NC treatments were observed.

curve were conservatively assigned a value of 2.7 ng ml^{-1}, the lowest standard concentration. A summary of the effects of isoeugenol sedation during stress is presented in Table 1.

3.1. Confinement stress

Significant differences ($P<0.05$) were determined between the IE and PC treatments for plasma cortisol levels during administration of the confinement stress (Fig. 1a). After 15 min of confinement, PC fish demonstrated an average 7.4-fold increase in plasma cortisol levels, and NC fish showed no increase in plasma cortisol concentration. Sedation with isoeugenol significantly reduced, but did not completely block the cortisol response to confinement stress. After 15 min of stress, isoeugenol sedated fish had nearly a 2.5-fold increase in plasma cortisol levels above pre-stress levels. Plasma cortisol levels were not significantly different ($P>0.05$) between IE and PC treatments. Plasma glucose and lactate concentrations were not significantly different ($P>0.05$) among fish from all three treatment groups during the confinement stress (Fig. 1b and c).

3.2. Ammonia stress

Exposure to NH_3-N concentrations of 1 mg l^{-1} for 24 h resulted in increased ($P<0.05$) plasma cortisol concentrations relative to pre-stress levels in both PC and IE treatment fish (Fig. 2a). No differences ($P>0.05$) in plasma glucose or lactate concentrations were observed between PC and IE treatment fish (Fig. 2b and c). Glucose levels in NC treatment fish were significantly ($P<0.05$) lower than IE and PC treatment fish after the 24-h ammonia challenge.

3.3. Oxygen stress

Plasma cortisol concentrations increased significantly ($P<0.05$) in PC fish as a result of acute oxygen depletion over a 30-min period (Fig. 3a). Cortisol levels of PC fish averaged 55.6 ± 5.1 ng ml^{-1} compared to 14.5 ± 1.3 ng ml^{-1} for isoeugenol sedated fish. Plasma cortisol levels of IE treatment fish returned to prestress levels within the 30-min recovery

Table 1
Effects of isoeugenol sedation at a dose of 2.5 mg l^{-1} during three different stress challenges on plasma concentrations of cortisol, glucose, and lactate in channel catfish

Response variable	Stressor		
	45-min confinement	24-h high ammonia	30-min oxygen depletion
Cortisol	–[a]	=	–
Glucose	=[b]	=	=
Lactate	=	=	–

[a] Significantly ($P<0.05$) reduced plasma concentration when compared to positive control (PC) fish (unsedated catfish).
[b] Not significantly ($P>0.05$) different from PC fish.

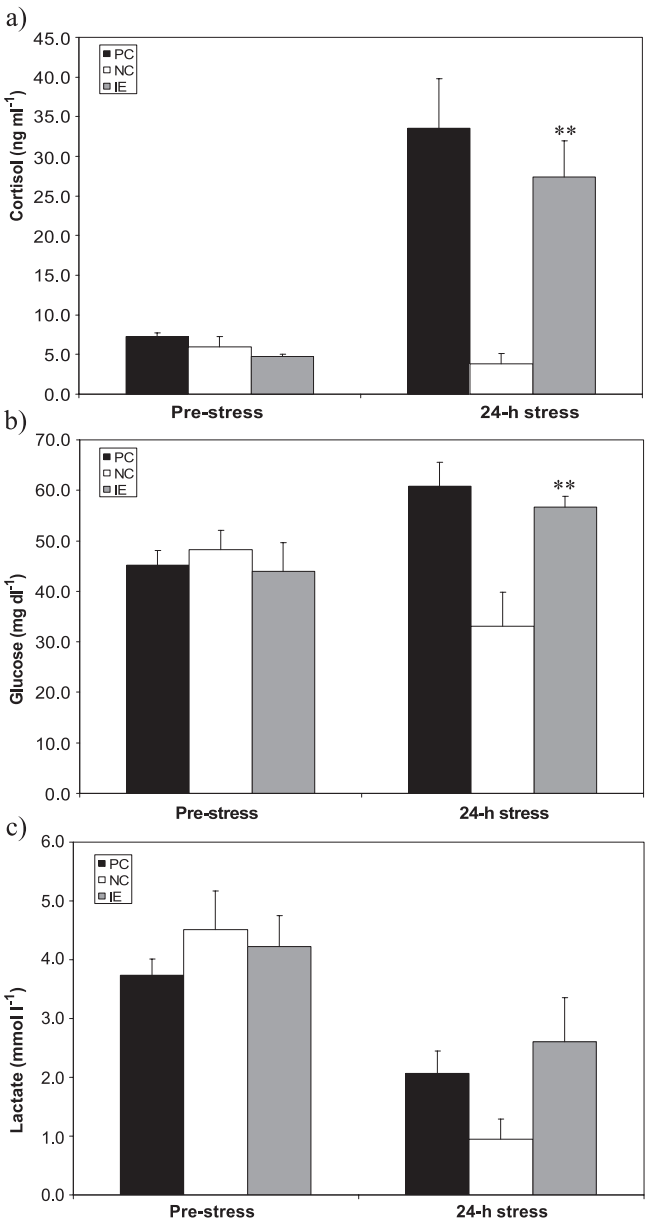

Fig. 2. Effects of exposure to 1 mg l^{-1} unionized ammonia for 24-h without sedation (PC), sedation with metomidate hydrochloride (NC), or sedation with isoeugenol (IE) on plasma cortisol (a), glucose (b), and lactate (c) of channel catfish. The bars and error bars denote means and standard error of the means (n=3), respectively. Significant differences (P<0.05) between IE and NC treatments within time are indicated by double asterisks (**). No significant differences (P>0.05) between IE and PC treatments were observed.

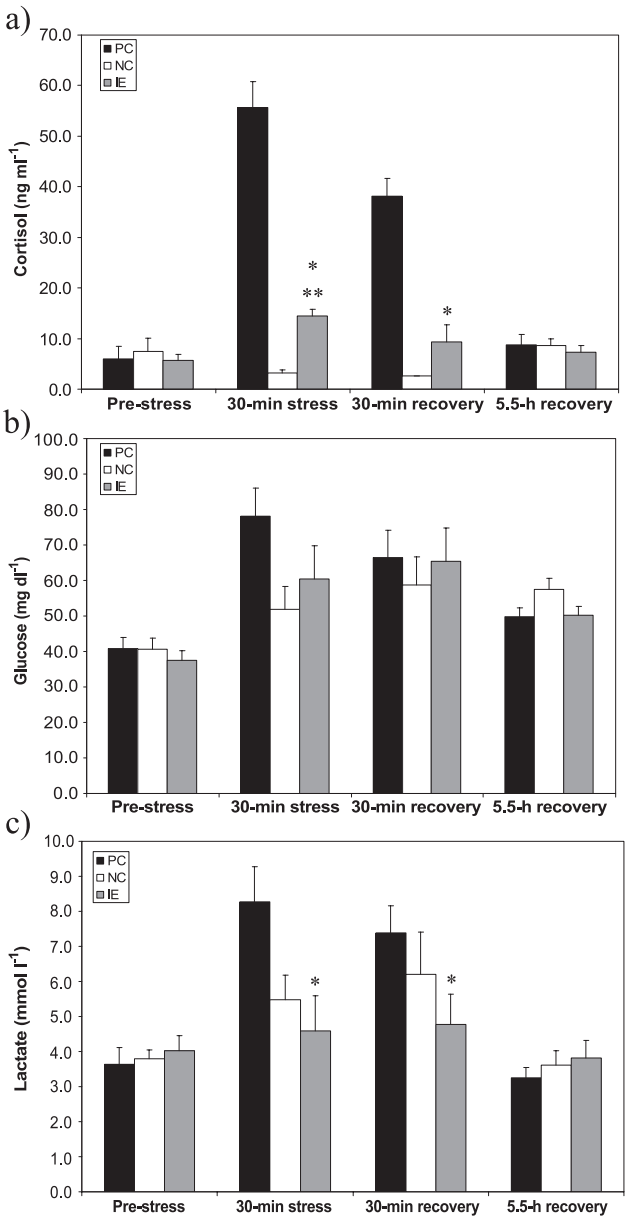

Fig. 3. Effects of acute oxygen depletion for 30-min without sedation (PC), sedation with metomidate hydrochloride (NC), or sedation with isoeugenol (IE), followed by oxygen repletion for 30-min, then recovery in flowing aerated water on plasma cortisol (a), glucose (b), and lactate (c) of channel catfish. The points and error bars denote means and standard error of the means (n=3), respectively. Significant differences (P<0.05) between IE and PC treatments within time are indicated by a single asterisk (*), and significant differences (P<0.05) between IE and NC treatments within time are indicated by double asterisks (**).

period. Plasma cortisol levels in PC fish, however, remained elevated, returning to pre-stress levels 5.5-h post-stress. Flushing of sedative during the extended recovery phase did not increase plasma cortisol levels in any treatment group.

Plasma glucose concentrations were significantly ($P<0.05$) elevated as a result of acute oxygen depletion (Fig. 3b). Isoeugenol sedated fish had glucose levels intermediate of PC and NC fish after 30-min of stress, with PC fish having significantly ($P<0.05$) higher levels than NC fish. No differences ($P>0.05$) between treatments were observed for plasma glucose during recovery.

Plasma lactate levels in PC fish followed a similar trend to glucose throughout (Fig. 3c). Isoeugenol sedated fish had lactate levels similar ($P>0.05$) to NC fish after 30-min of stress, with PC fish having significantly ($P<0.05$) higher levels than IE and NC treatment fish. Plasma lactate returned to prestress levels in all treatments groups by 5.5-h post stress.

4. Discussion

The effects of confinement, exposure to high ammonia, and acute oxygen depletion on channel catfish corticosteroid dynamics have been previously reported (Davis et al., 1984; Tomasso et al., 1981a,b). Each of these conditions is inherent to aquaculture, and have been found to elicit a significant stress response. In assessing various biotic and abiotic influences on corticosteroid rhythms in channel catfish, Davis et al. (1984) observed increased plasma corticosteroid concentrations when fish were stressed by confinement. Plasma corticosteroid levels of undisturbed channel catfish were reported to be less than 10 ng ml^{-1}. Channel catfish stressed by net confinement demonstrated significant increases in plasma corticosteroids within 10-min of being confined at a range of temperatures from 15 to 35 °C, and corticosteroids tended to plateau throughout the remainder of the 50-min confinement. Similar results are reported for PC fish in the present study, conducted at 26 °C. At all time-points after the initiation of stress, isoeugenol significantly reduced the cortisol response to confinement relative to PC fish. Fish sedated with metomidate hydrochloride (NC) demonstrated no cortisol response as expected.

Regardless of the stressor, metomidate hydrochloride effectively blocked the cortisol response in the present study. Metomidate hydrochloride is a rapid acting water-soluble non-barbiturate hypnotic in several species (Mattson and Riple, 1989; Thomas and Robertson, 1991; Knoph, 1995; Massee et al., 1995), and has been used in fish research for its ability to block cortisol synthesis (Thomas and Robertson, 1991; Olsen et al., 1995; Small, 2003). Small (2003) found metomidate hydrochloride to be efficacious as an anesthetic for channel catfish research; however, it does not have approval for commercial use. Preclusion of metomidate hydrochloride from acceptance for commercial use may be due to an absence of analgesic properties, as suggested by Iversen et al. (2003).

Confinement stress has been shown to induce increases in plasma glucose levels in several species of fish (Robertson et al., 1987; Reubush and Heath, 1997; Rotllant et al., 2001; Skjervold et al., 2001; Davis et al., 2002). Davis et al. (2002) showed a concurrent increase in glucose and cortisol in channel catfish following a 2-h low-water confinement. In the present study, we also observed significant increases in plasma glucose levels of PC fish after 30-min of confinement. Isoeugenol appeared to have no effect on plasma glucose

levels relative to control fish during the 45-min confinement stress. Plasma lactate increased among all treatment groups as a result of confinement, and was unaffected by sedation with isoeugenol or metomidate hydrochloride. Confinement stress, without sedation, has been observed to increase plasma lactate levels in Atlantic salmon (*Salmo salar* L.; Iversen et al., 1998; Skjervold et al., 2001), common carp (*Cyprinus carpio* L.; Pottinger, 1998; Ruane et al., 2002) and gilthead seabream (*Sparus aurata* L.; Arends et al., 1999; Rotllant et al., 2001). The effects of isoeugenol on plasma lactate during confinement of these and other species is presently unknown.

High NH_3-N concentration in the culture water caused a significant increase in plasma cortisol concentrations in both PC and isoeugenol treated catfish. These results confirm the corticosteroid response to high ammonia observed by Tomasso et al. (1981b). In their research, Tomasso et al. (1981b) found plasma corticosteroids increased four to five-fold as a result of exposing channel catfish to NH_3-N levels of approximately $0.5-1.0$ mg l^{-1}. In the present study, NC fish were observed to have lower post-stress glucose and lactate relative to PC and isoeugenol treated fish. In red drum (*Sciaenops ocellatus*), metomidate hydrochloride has also been shown to prevent stress-related increases of plasma glucose (Thomas and Robertson, 1991). No differences in glucose metabolism, however, were observed between Atlantic salmon anesthetized with isoeugenol or metomidate hydrochloride with minimal handling (Iversen et al., 2003).

Tomasso et al. (1981a) observed a significant increase in plasma corticosteroids of channel catfish in response to acute oxygen depletion. In their research, plasma corticosteroid levels returned to control levels within 30-min of increasing DO. Tomasso et al. (1981a) also reported a second increase in plasma corticosteroid levels after 5.5-h of increasing DO. The results of acute oxygen depletion in the present study confirm the initial cortisol response of unsedated catfish; however, a second response was not observed in any of the treatment groups after 5.5-h of increasing the DO content of the water. Isoeugenol sedated fish had significantly lower plasma cortisol levels compared to PC fish after 30-min of acute oxygen depletion, and after 30-min of increasing the DO, had plasma cortisol levels similar to NC fish. Plasma cortisol levels of PC fish were reduced after 30-min of recovery, but had not reached pre-stress levels at that point. Plasma glucose and lactate also increased in PC fish as a result of acute oxygen depletion, while isoeugenol sedation significantly reduced lactate response.

Occasional problems in the catfish industry with red blotches in fillets, referred to as "red spot syndrome", are thought to be a result of pre-slaughter stress (Jenson and Brunson, 1992). Short-term stress in Atlantic salmon has been associated with a rapid decline in fillet pH (Love, 1980; Korhonon et al., 1990), and appears to result in poor fillet quality (Skjervold et al., 2001). Anaerobic conditions caused by stress are known to result in muscle glycogen and lactate breakdown, with some of the lactate being released into circulation (Thomas et al., 1999). Accumulation of lactic acid in the muscle causes a decline of muscle pH. The research presented here suggests isoeugenol sedation reduces plasma lactate levels in channel catfish relative to unsedated (PC) fish during acute oxygen depletion. Further research is needed to determine what effect this may have on channel catfish fillet quality.

Data on the stress-reducing capacity of isoeugenol in fish is mostly limited to salmonid species. Anesthesia of rainbow trout with isoeugenol (AQUI-S™) does not

appear to be effective for reducing stress from crowding (Davidson et al., 2000). In Atlantic salmon, Iversen et al. (2003) report a reduction in cortisol response with AQUI-S™ concentrations of 20 mg l^{-1} and above, observing no improvement at lower concentrations. The results presented here for channel catfish, suggest that at a concentration of only 5 mg AQUI-S™ l^{-1} (2.5 mg isoeugenol l^{-1}), cortisol release is reduced relative to unsedated fish during both confinement and acute oxygen depletion. Sedation with isoeugenol also had a reducing effect on plasma lactate levels during stress from acute oxygen depletion, but did not appear to reduce primary or secondary stress responses in channel catfish exposed to a high level of unionized ammonia. From these observations, it can be concluded that isoeugenol, and thus AQUI-S™, shows promise as a stress-reducing sedative for channel catfish under certain stressful conditions, and suggests a need for further investigation.

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