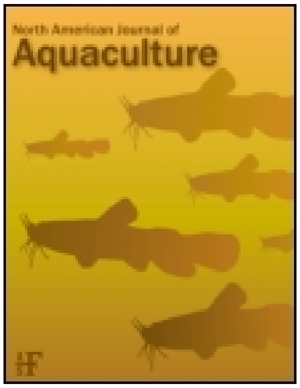

North American Journal of Aquaculture
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/unaj20

Routine Measures of Stress Are Reduced in Mature Channel Catfish during and after AQUI-S Anesthesia and Recovery

Brian C. Small [a] & Nagaraj Chatakondi [b]

[a] U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi, 38776, USA

[b] Harvest Select Farms, Post Office Box 560, Inverness, Mississippi, 38753, USA

Version of record first published: 09 Jan 2011.

To cite this article: Brian C. Small & Nagaraj Chatakondi (2005): Routine Measures of Stress Are Reduced in Mature Channel Catfish during and after AQUI-S Anesthesia and Recovery, North American Journal of Aquaculture, 67:1, 72-78

To link to this article: http://dx.doi.org/10.1577/FA04-028.1

North American Journal of Aquaculture 67:72–78, 2005

[Article]

Routine Measures of Stress Are Reduced in Mature Channel Catfish during and after AQUI-S Anesthesia and Recovery

BRIAN C. SMALL*

U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi 38776, USA

NAGARAJ CHATAKONDI

Harvest Select Farms, Post Office Box 560, Inverness, Mississippi 38753, USA

Abstract.—Mature channel catfish *Ictalurus punctatus* were exposed to water containing three different concentrations (20, 40, and 60 mg/L) of AQUI-S (50% isoeugenol) during routine handling procedures at a commercial catfish facility. Anesthetic efficacy, recovery time, and the effects of AQUI-S on routine measures of stress were compared with similar measures in a group of fish sampled prior to anesthesia (preanesthesia [PA] group) and a group anesthetized with 100 mg tricaine methanesulfonate (TMS)/L. On average, all the fish lost equilibrium at 8.0, 3.9, and 3.7 min when anesthetized in 20, 40, and 60 mg AQUI-S/L, respectively. Fish anesthetized with TMS lost equilibrium at 4.5 min. Recovery time in freshwater was 2.1, 2.8, and 5.3 min for fish anesthetized in 20, 40, and 60 mg/L AQUI-S, respectively. Recovery time after TMS anesthesia was 1.7 min. Short-term (24-h) survival was 100% for all treatments, and long-term (21-d) survival ranged from 87.5% for TMS-anesthetized fish to 98.8% for fish anesthetized in 40 mg/L AQUI-S. All anesthetic treatments significantly reduced ($P < 0.05$) plasma cortisol levels relative to PA fish, and circulating cortisol was lower ($P < 0.05$) in all AQUI-S−anesthetized fish than in TMS-anesthetized fish. Plasma glucose concentrations were significantly lower ($P < 0.05$) in fish anesthetized with 40 mg/L AQUI-S than in PA and TMS-treated fish after both anesthetization and recovery. Plasma lactate and chloride concentrations were not reduced ($P > 0.05$) in any treatment compared with the levels in PA fish. Fish anesthetized with 40 mg/L AQUI-S had lower ($P < 0.05$) plasma lactate levels after recovery than TMS-anesthetized fish, and plasma chloride levels were significantly lower ($P < 0.05$) immediately following AQUI-S anesthesia at all concentrations than in fish anesthetized with TMS. Anesthesia did not affect ($P > 0.05$) postrecovery plasma chloride concentrations. Overall, AQUI-S exhibited efficacy as an anesthetic for mature channel catfish and demonstrated stress-reducing properties during handling procedures.

Selective breeding programs for channel catfish *Ictalurus punctatus* and its hybrid with the blue catfish *I. furcatus* inherently involve acute handling stressors. During selection, fish are often seined from ponds, moved to raceways, and graded. Hybridization involves additional levels of stress, such as broodfish preparation, hormonal induction of spawning, and artificial hand stripping. Numerous researchers have demonstrated that stress has the capacity to inhibit reproductive performance and viability of fish eggs (de Montalembert et al. 1978; Stacey et al. 1984; Campbell et al. 1994; Schreck et al. 2001). The challenges for the producer are keeping fish healthy and obtaining quality gametes. For this reason, chemical anesthetics may be used to ease handling and reduce fish stress.

In addition to preventing physical injury, certain anesthetics are thought to reduce the perception of stress. Some anesthetics, such as metomidate hydrochloride (dl-1-[1-phenylethyl]-5-[metoxycarbonyl] imidazole hydrochloride), are thought to block activation of the hypothalamo-pituitary-interrenal (HPI) axis associated with handling stress in fish (Olsen et al. 1995; Small 2003). Metomidate is the methyl derivative of etomidate, which has been demonstrated to affect the mitochondrial cytochrome P_{450}-dependent enzymes that catalyze the synthesis of cortisol (Vanden Bossche et al. 1984; Wagner et al. 1984). 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). Currently, tricaine methanesulfonate (TMS; MS-222) is the only anesthetic approved by the U.S. Food and Drug Administration (FDA) for use with food fish in the United States. However, even when fish

* Corresponding author: bsmall@ars.usda.gov

Received June 21, 2004; accepted September 17, 2004

are deeply anesthetized with TMS, handling procedures or simply the exposure to TMS can increase plasma cortisol concentrations (Thomas and Robertson 1991; Small 2003).

A relatively new fish anesthetic, AQUI-S, has approval for use with food fish in Australia, Chile, and New Zealand with no withholding period and has been shown to reduce the stress response of juvenile channel catfish to crowding and acute oxygen depletion (Small 2004). AQUI-S is currently in the process of evaluation for approval as a food fish anesthetic by the FDA. The purpose of this study was to determine whether anesthesia with AQUI-S during simulated broodfish selection procedures at a commercial catfish production facility reduces routine measures of stress (cortisol, glucose, lactate, and chloride) in mature channel catfish. This research provides necessary data for assessing the potential use of AQUI-S as an anesthetic to reduce channel catfish stress response to commercial handling procedures, such as those that might occur during broodfish selection and preparation.

Methods

Mature (3-year-old) channel catfish (1.1 ± 0.04 kg; mean ± SE) were seined in early spring from a 0.04-ha pond at Harvest Select Farms, Inverness, Mississippi and moved to indoor concrete raceways supplied with flowing pond water (14.5°C). The sex ratio of the catfish used in the experiment was 1.3:1 (male:female). Following 1 h of fish acclimation to the raceway environment, the efficacy of three concentrations (20, 40, and 60 mg/L) of AQUI-S as an anesthetic for rapid handling of mature channel catfish was assessed and compared to the efficacy of TMS (100 mg/L; Argent Chemical Laboratories, Redmond, Washington) under the same conditions.

To determine preanesthesia (PA) plasma concentrations of cortisol, glucose, lactate, and chloride, 12 channel catfish were individually netted from the raceway and bled without anesthesia. Blood was collected from the caudal vasculature by use of heparinized syringes, and plasma was collected by centrifugation and then stored at −20°C for subsequent analysis. For determination of anesthetic efficacy, each treatment was randomly replicated in time with four replicate groups of 20 fish each. Each replicate group was placed in a tank containing 200 L of raceway water and the respective sedative treatment. The time for all 20 fish to lose equilibrium was recorded. Immediately after all fish lost equilibrium, blood was taken from two fish per replicate, and the remaining 18 fish were transferred to 200 L of freshwater for recovery. The time for all 18 fish to regain equilibrium was then recorded. Immediately after recovery of the group, blood was collected from two fish per replicate. All fish exposed to anesthesia were heat branded after recovery to identify treatment, and were placed in a common raceway overnight. The next morning, all treated fish were moved to a single 0.04-ha pond. Mortality of each treatment group was monitored and recorded for 21 d.

Plasma cortisol was determined by a time-resolved fluoroimmunoassay that has been validated for channel catfish (Small and Davis 2002). Plasma glucose concentrations were determined by the glucose oxidase procedure (number G7519; Pointe Scientific, Inc., Lincoln Park, Michigan). Plasma lactate concentrations were determined by the lactate oxidase procedure (number L7596; Pointe Scientific, Inc.). Plasma chloride concentrations were determined by the mercuric thiocyanate procedure (number 461-3; Sigma Diagnostics, Inc., St. Louis, Missouri).

Anesthesia and recovery times and plasma cortisol, glucose, lactate, and chloride concentrations were subjected to analysis of variance (ANOVA) mixed-model procedures that contained anesthetic treatment as the fixed effect and replicate within treatment as the random effect. The ANOVAs were performed in the Statistical Analysis System version 8.0 (SAS 1996). Assumptions for homogeneity of variance and normality of the data were tested by examination of correlation between absolute residuals and predicted values, and the Shapiro–Wilk test for normality. Cortisol levels at the time of anesthesia were log transformed prior to ANOVA to meet the assumptions of normality. When significant differences were found by the ANOVA, pairwise contrasts in the least-significant-difference test were used to identify significant differences at the 0.05 level.

Results

Anesthetization and recovery times were significantly ($P < 0.05$) affected by anesthetic type and concentration (Table 1). Anesthetization in a 20-mg/L solution of AQUI-S resulted in the longest time for channel catfish to lose equilibrium. Both the 40- and 60-mg/L AQUI-S concentrations were similar ($P > 0.05$) to the TMS treatment in terms of anesthetization time. Recovery times were shortest ($P < 0.05$) for fish in both the TMS and 20-mg/L AQUI-S treatments. Fish in the 60-mg/L AQUI-S treatment had the longest ($P < 0.05$)

TABLE 1.—Effect of anesthetic type (tricaine methanesulfonate [TMS] or AQUI-S) and concentration on channel catfish anesthesia time (loss of equilibrium), recovery time, and survival. Mean anesthetization and recovery times with different letters are significantly different ($P < 0.05$).

Treatment (concentration)	Anesthetization time (min; ± SE)	Recovery time (min; ± SE)	21-d survival (%)
TMS (100 mg/L)	4.5 ± 0.8 z	1.7 ± 0.2 z	87.5
AQUI-S (20 mg/L)	8.0 ± 0.6 y	2.1 ± 0.2 yz	95.0
AQUI-S (40 mg/L)	3.9 ± 0.1 z	2.8 ± 0.2 y	98.8
AQUI-S (60 mg/L)	3.7 ± 0.2 z	5.3 ± 0.4 x	97.5

recovery time. Postanesthesia survival was 100% for all treatments after 24 h, and 21-d survival ranged from 87.5% (TMS group) to 98.8% (40-mg/L AQUI-S group) (Table 1).

Plasma cortisol concentrations were significantly ($P < 0.05$) and rapidly reduced by anesthesia (Figure 1). Fish anesthetized with TMS had reduced ($P < 0.05$) cortisol levels compared to PA fish but had higher cortisol levels than fish anesthetized with 20, 40, or 60 mg/L AQUI-S. No differences in plasma cortisol were observed among fish anesthetized in the three AQUI-S concentrations.

Plasma glucose levels also responded quickly to anesthesia (Figure 2). Fish anesthetized in 40- and 60-mg/L solutions of AQUI-S demonstrated reduced ($P < 0.05$) plasma glucose concentrations upon loss of equilibrium compared to plasma glucose concentrations in PA fish. Upon recovery, only fish in the 40-mg/L treatment continued to have significantly reduced plasma glucose levels.

Plasma lactate concentrations were unchanged ($P > 0.05$) upon anesthetization relative to the plasma levels of PA fish (Figure 3). Channel catfish anesthetized with TMS had significantly ($P < 0.05$) higher lactate levels following recovery from anesthesia compared to fish anesthetized with 40 mg/L AQUI-S.

The plasma chloride concentrations of anesthetized and recovered fish were similar ($P > 0.05$) to the plasma chloride concentrations of PA fish (Figure 4). Significant differences ($P < 0.05$) in plasma chloride levels were observed between TMS- and AQUI-S-treated fish immediately after loss of equilibrium; however, no differences ($P > 0.05$) between anesthetic treatments were observed for plasma chloride concentrations of recovered fish.

Discussion

The criteria for determining anesthetic efficacy are often based on the behavioral responses de-

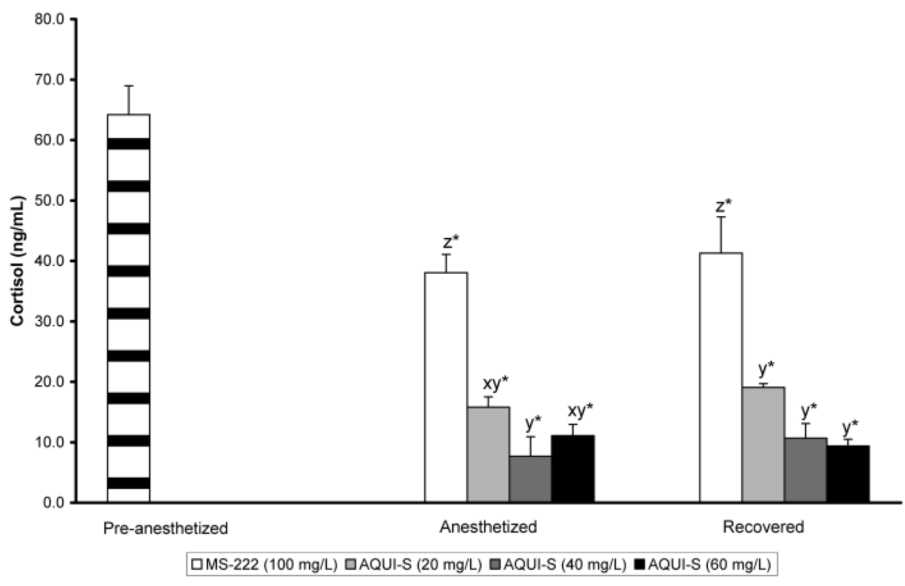

FIGURE 1.—Effect of anesthetic type (tricaine methanesulfonate [MS-222] and AQUI-S) and concentration on channel catfish plasma cortisol concentration measured immediately after loss of equilibrium (anesthetized) and immediately after recovery in freshwater (recovered), compared with plasma concentrations prior to anesthesia (preanesthetized). Mean (±SE) cortisol concentrations within a time point that have different letters are significantly different ($P < 0.05$). An asterisk denotes a significant difference ($P < 0.05$) from the preanesthetic mean concentration.

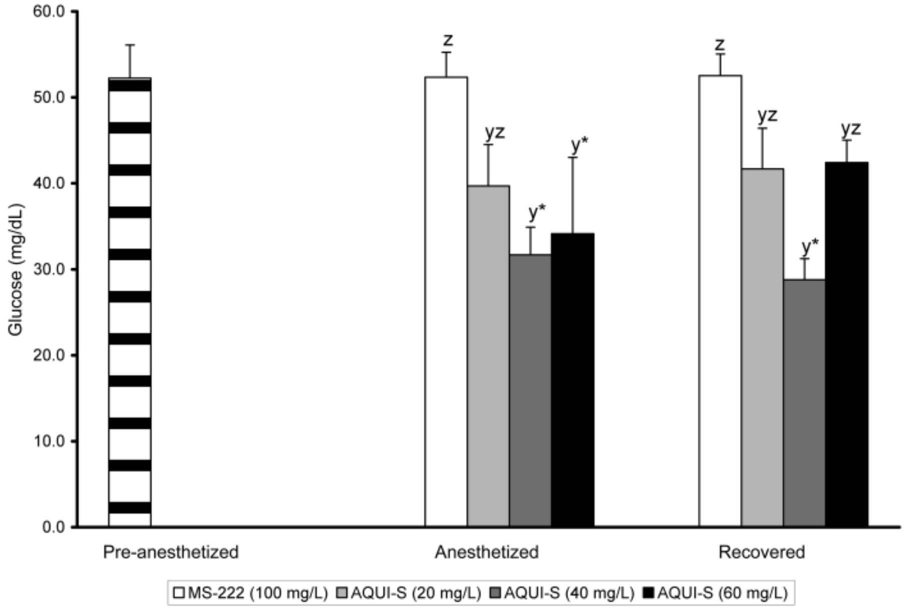

FIGURE 2.—Effect of anesthetic type (tricaine methanesulfonate [MS-222] and AQUI-S) and concentration on channel catfish plasma glucose concentration measured immediately after loss of equilibrium (anesthetized) and immediately after recovery in freshwater (recovered), compared with plasma concentrations prior to anesthesia (preanesthetized). Mean (±SE) glucose concentrations within a time point that have different letters are significantly different ($P < 0.05$). An asterisk denotes a significant difference ($P < 0.05$) from the preanesthetic mean concentration.

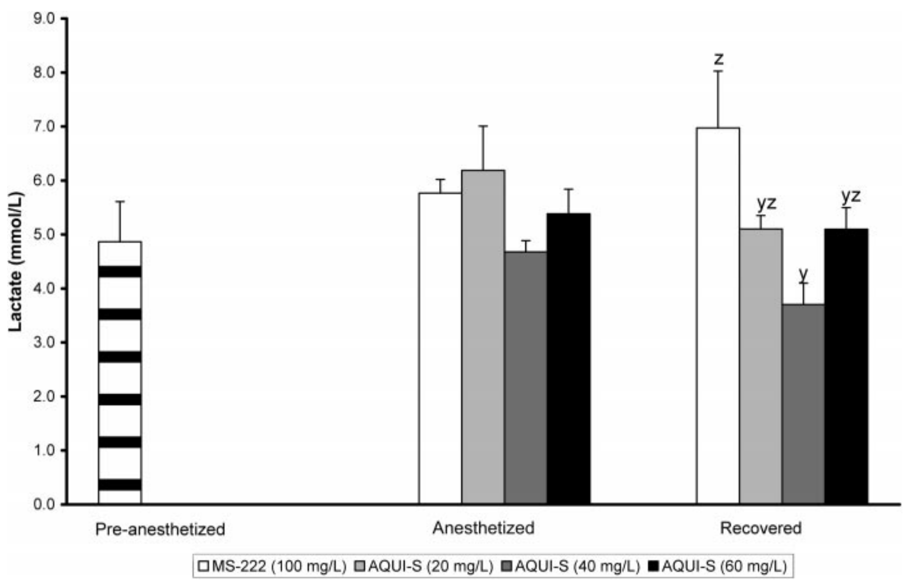

FIGURE 3.—Effect of anesthetic type (tricaine methanesulfonate [MS-222] and AQUI-S) and concentration on channel catfish plasma lactate concentration measured immediately after loss of equilibrium (anesthetized) and immediately after recovery in freshwater (recovered), compared with plasma concentrations prior to anesthesia (preanesthetized). Mean (±SE) lactate concentrations within a time point that have different letters are significantly different ($P < 0.05$).

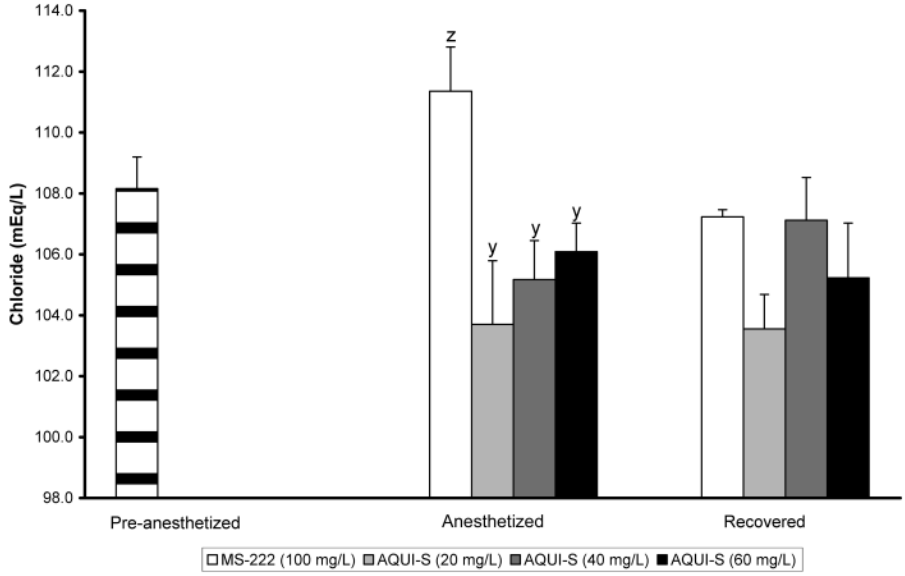

FIGURE 4.—Effect of anesthetic type (tricaine methanesulfonate [MS-222] and AQUI-S) and concentration on channel catfish plasma chloride concentration measured immediately after loss of equilibrium (anesthetized) and immediately after recovery in freshwater (recovered), compared with plasma concentrations prior to anesthesia (preanesthetized). Mean (±SE) chloride concentrations within a time point that have different letters are significantly different ($P < 0.05$).

scribed by Schoettger and Julin (1967) and later modified by the same authors (Schoettger and Julin 1969). Their guidelines establish the optimum anesthetic dose for general fish handling as the minimum dose producing the desired effects of rapid immobility (without medullary collapse) and rapid recovery. For most general fish handling procedures, rapid immobility can be defined as a total loss of equilibrium in all fish within 2–5 min. Recovery time is generally of less concern to fish handlers than is fish survival. In general, recovery should also be rapid and should occur within 5–10 min. In the present study, only fish anesthetized in a 60-mg/L AQUI-S solution took greater than 5 min to recover. The 40-mg/L treatment was the only AQUI-S concentration tested that yielded anesthetization and recovery times less than 5 min. In this regard, Schoettger and Julin (1967) recommended 100 mg/L TMS as the optimal anesthetic dose for channel catfish. The results presented here support the use of 100 mg/L TMS as an efficacious anesthetic for channel catfish.

Anesthetic type and concentration can have significant effects on fish stress response. Small (2003) demonstrated that anesthesia alone, without prior handling, could result in increased plasma cortisol levels in channel catfish. In that study, fish anesthetized with 100 mg/L TMS demonstrated a nearly sixfold increase in plasma cortisol levels within the first 10 min of anesthetization. In that same study, channel catfish anesthetized with clove oil prior to handling demonstrated no significant increase in circulating cortisol levels. Clove oil appears to be a viable anesthetic for aquacultural use (Taylor and Roberts 1999), and was found to be efficacious as an anesthetic for channel catfish (Waterstrat 1999). Clove oil is comprised of three active ingredients; eugenol is the primary (85–95%) component, and isoeugenol and methyleugenol make up the remaining 5–15% (FDA 2002). AQUI-S is reported to contain 50% isoeugenol as the active ingredient (Ross and Ross 1999). The research presented here is the first to describe the effects of AQUI-S anesthesia on circulating cortisol and other stress indices in mature channel catfish during simulated commercial broodfish handling procedures.

Although stress-induced changes in plasma glucose concentrations in fish are generally thought to occur at a much slower rate than changes in cortisol, the glucose response in channel catfish appears to be relatively rapid (Davis et al. 2002; Small 2004). In the present study, differences in plasma glucose concentrations were detected immediately after fish lost equilibrium, similar to the results observed for the cortisol response. Anesthetic concentration, however, apparently mediates the glucose response to stress. At a sedative concentration of AQUI-S (5 mg/L), Small (2004) observed no changes in channel catfish plasma glucose levels during crowding, oxygen, and ammonia stress conditions.

An increase in plasma lactate may indicate glycogen mobilization and breakdown and has been associated with poor quality of and rigor development in fish fillets (Skjervold et al. 1999; 2001). Small (2004) demonstrated a reduction in plasma lactate concentrations of channel catfish sedated with 5 mg/L AQUI-S during acute oxygen stress, but observed no effect of AQUI-S sedation on plasma lactate levels during crowding or ammonia stress. The overall effect of anesthesia on plasma lactate in the present study was not significant relative to plasma lactate levels in PA channel catfish. Anesthetization in 40 mg/L AQUI-S did, however, result in decreased plasma lactate levels relative to fish anesthetized in 100 mg/L TMS.

Electrolyte concentrations in fingerling and food-size channel catfish have been shown to be very stable during handling (Davis et al. 1993). Although plasma chloride concentrations were significantly lower in channel catfish anesthetized with AQUI-S than in fish anesthetized with TMS in the present study, plasma chloride concentrations were not different from PA levels, and there were no postrecovery differences.

The physiological stress responses of juvenile channel catfish (Small 2003, 2004), Atlantic salmon *Salmo salar* (Iversen et al. 2003), Chinook salmon *Oncorhynchus tshawytscha* (Cho and Heath 2000), and rainbow trout *O. mykiss* (Davidson et al. 2000) to AQUI-S or clove oil anesthesia have been reported; however, little is known of the effects of AQUI-S anesthesia on mature fish. Wagner et al. (2002) examined the effects of anesthetization with AQUI-S, TMS, and carbon dioxide on rainbow trout broodstock stress responses, egg survival, and sperm motility. Trout anesthetized with AQUI-S had significantly lower plasma cortisol concentrations at 1 and 7 h postimmersion relative to the other treatments. Percentage of motile sperm and egg survival was not affected by anesthetic type.

The present study demonstrates that AQUI-S can be an efficacious anesthetic for mature channel catfish. At a concentration of 40 mg/L, AQUI-S yields anesthetization times similar to 100 mg/L TMS and allows for rapid recovery. The 40-mg/L AQUI-S concentration also suppressed overall stress response in comparison to both TMS-anesthetized and PA fish. Perhaps the anesthetic blocks the release of cortisol by suppressing activation of the HPI axis, or alters the state of consciousness in a way that diminishes central cortisol release. Currently, the mechanism of AQUI-S action on circulating cortisol levels remains unknown, as does the effect of AQUI-S on channel catfish gamete quality and egg viability. Further research is needed to answer these questions. In the event that AQUI-S receives FDA approval for food fish use, the associated ease of handling and reduction of stress indices are factors worth considering when choosing an anesthetic for use with mature channel catfish.

Acknowledgments

The authors wish to acknowledge the technical assistance of Kira Johnson and Jimmie Warren of the U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit and the staff of Harvest Select Farms. This research was conducted in accordance with Investigational New Animal Drug permit number 10541-04-7. Mention of trade names, proprietary products, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products that may be suitable.

References

Campbell, P. M., T. G. Pottinger, and J. P. Sumpter. 1994. Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120:151–169.

Cho, G. K., and D. D. Heath. 2000. Comparison of tricaine methanesulfonate (MS-222) and clove oil anesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquaculture Research 31:537–546.

Davidson, G. W., P. S. Davie, G. Young, and R. T. Fowler. 2000. Physiological responses of rainbow trout *Oncorhynchus mykiss* to crowding and anesthesia with AQUI-S. Journal of the World Aquaculture Society 31:105–114.

Davis, K. B., B. R. Griffin, and W. L. Gray. 2002. Effect of handling stress on susceptibility of channel catfish *Ictalurus punctatus* to *Ichthyophthirius multifiliis* and channel catfish virus infection. Aquaculture 214:55–66.

Davis, K. B., J. Newsom, and B. A. Simco. 1993. Physiological stress in channel catfish, *Ictalurus punctatus*, harvested by lift net, vacuum pump, or turbine pump. Journal of Aplied Aquaculture 3:297–310.

de Montalembert, G., B. Jalabert, and C. Bry. 1978. Precocious induction of maturation and ovulation in northern pike (*Esox lucius*). Annales de Biologie Animale, Biochimie, Biophysique 18:969–975.

FDA (Food and Drug Administration). 2002. Guidance for industry: status of clove oil and eugenol for anesthesia of fish. Food and Drug Administration, Center for Veterinary Medicine, No. 150, Rockville, Maryland.

Iversen, M., B. Finstad, R. S. McKinley, and R. A. Eliassen. 2003. The effect of metomidate, clove oil Aqui-S and Benzoak as anesthetics in Altantic salmon (*Salmo salar* L.) smolts and their potential stress-reducing capacity. Aquaculture 221:549–566.

Olsen, Y. A., I. E. Einarsdottir, and K. J. Nilssen. 1995. Metomidate anaesthesia in Atlantic salmon, *Salmo salar*, prevents plasma cortisol increase during stress. Aquaculture 134:155–168.

Ross, L. G., and B. Ross. 1999. Anesthetic and sedative techniques for aquatic animals, 2nd edition. Blackwell Scientific Publications, London.

Rotllant, J., P. H. Balm, J. Perez-Sanchez, S. E. Wendelaar-Bonga, and L. Tort. 2001. Pituitary and interrenal function in gilthead sea bream (*Sparus aurata* L., Teleostei) after handling and confinement stress. General and Comparative Endocrinology 121:333–342.

SAS. 1996. SAS/STAT software: changes and enhancements through release 6.11. SAS Institute, Cary, North Carolina.

Schoettger, R. A., and A. M. Julin. 1967. Efficacy of MS-222 as an anesthetic on four salmonids: U.S. Fish and Wildlife Service Investigations in Fish Control 13:1–15.

Schoettger, R. A., and A. M. Julin. 1969. Efficacy of quinaldine as an anesthetic for seven species of fish. U.S. Fish and Wildlife Service Investigations in Fish Control 22:3–10.

Schreck, C. B., W. Contreras-Sanchez, and M. S. Fitzpatrick. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3–24.

Skjervold, P. O., S. O. Fjaera, and P. B. Oestby. 1999. Rigor in Atlantic salmon as affected by crowding stress prior to chilling before slaughter. Aquaculture 175:93–101.

Skjervold, P. O., S. O. Fjaera, P. B. Oestby, and O. Einen. 2001. Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). Aquaculture 192:265–280.

Small, B. C. 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine, and clove oil anesthetized channel catfish *Ictalurus punctatus*. Aquaculture 218:177–185.

Small, B. C. 2004. Effect of isoeugenol sedation on plasma cortisol, glucose, and lactate dynamics in channel catfish *Ictalurus punctatus* exposed to three stressors. Aquaculture 238:469–481.

Small, B. C., and K. B. Davis. 2002. Validation of a time-resolved fluoroimmunoassay for measuring plasma cortisol in channel catfish *Ictalurus punctatus*. Journal of the World Aquaculture Society 33:184–187.

Stacey, N. E., D. S. MacKenzie, T. A. Marchant, A. L. Kyle, and R. E. Peter. 1984. Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*, 1. Gonadotropin, growth hormone, and thyroid hormones. General and Comparative Endocrinology 56:333–348.

Taylor, P. W., and S. D. Roberts. 1999. Clove oil: an alternative anaesthetic for aquaculture. North American Journal of Aquaculture 61:150–155.

Thomas, P., and L. Robertson. 1991. Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate, and metomidate. Aquaculture 96:69–86.

Vanden Bossche, H., G. Willemsens, W. Cools, and D. Bellens. 1984. Effects of etomidate on steroid biosynthesis in subcellular fractions of bovine adrenals. Biochemical Pharmacology 33:3861–3868.

Wagner, E., R. Arndt, and B. Hilton. 2002. Physiological stress responses, egg survival, and sperm motility with clove oil, tricaine methanesulfonate, or carbon dioxide. Aquaculture 211:353–366.

Wagner, R. L., P. F. White, P. B. Kan, M. H. Rosenthal, and D. Feldman. 1984. Inhibition of adrenal steroidogenesis by the anesthetic etomidate. New England Journal of Medicine 310:1415–1421.

Waterstrat, P. R. 1999. Induction and recovery from anesthesia in channel catfish *Ictalurus punctatus* fingerlings exposed to clove oil. Journal of the World Aquaculture Society 30:250–255.