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Effects of rested-harvest using the anesthetic AQUI-S™ on channel catfish, *Ictalurus punctatus*, physiology and fillet quality

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Abstract

Trials were conducted to determine effects of AQUI-S™ sedation during harvest (rested-harvest) on physiological responses and fillet quality of channel catfish, *Ictalurus punctatus*. Rested-harvest is defined as application of an anesthetic immediately before harvest to reduce fish activity associated with a normal harvest. Doses of 25–35 ppm AQUI-S™ were effective for rested-harvest of catfish (loss of equilibrium in 3 to 10 min and 100% survival following recovery). Time to loss of equilibrium and time to recovery following sedation with 35 ppm AQUI-S™ increased as water temperature decreased from 30 °C to 10 °C. Catfish exposed to 25 ppm AQUI-S™, 35 ppm AQUI-S™, 100 ppm trincaine methanesulfonate, and 8 ppm metomidate had lower blood lactate, cortisol, and glucose and higher blood pH than unsedated fish exposed to a low-water stress. Rested-harvest (RH) catfish had higher muscle and blood pH, lower blood and muscle lactate, and higher muscle ATP levels than catfish exposed to a 45 min low-water stress. Rates of muscle pH decrease, ATP decrease, and lactate increase accelerated as storage temperature decreased from 15 °C to 5 °C in RH fish acclimated to summer temperatures (33 °C), conversely these rates accelerated as storage temperature increased from 5 °C to 15 °C in RH fish acclimated to winter temperatures (7 °C). Based on physiological response (higher muscle pH, lower blood lactate, delayed time to rigor), post-sedation euthanasia by CO_2 was superior to post-sedation euthanasia by AQUI-S™ overdose (150 ppm), nitrogen gas, or electrical stunning. Compared to fillets from fish exposed to simulated industry transport conditions, fillets from RH/CO_2 euthanised fish had higher pH 1 h post-slaughter, and less drip-loss and lower L^* and a^* color values during 7 days of iced storage. RH/CO_2 and control fillets were not different for shelf-life based on bacterial counts. Rested-harvest with AQUI-S™ followed by CO_2 euthanasia has potential to improve catfish fillet quality, but AQUI-S™ approval, development of rested-harvest strategies, and demonstration of economic benefits of rested-harvest will be required for adoption of rested-harvest to commercial catfish production.

Keywords: Catfish; Rested-harvest; AQUI-S™; Meat quality

1. Introduction

The physical activity and stress associated with harvest and transport before slaughter can have negative impacts on meat quality in a wide variety of meat animal species,

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including farm-raised fish. The negative impacts of harvest and transport on meat quality are generally attributed to increased anaerobic metabolism associated with activity/stress before slaughter which leads to decreased muscle energy stores, a rapid decline in muscle pH, and accelerated denaturation/degradation of muscle proteins following slaughter (Bendall and Swatland, 1988; Offer, 1991; Rathgeber et al., 1999). The rapid pH decline and associated changes in muscle proteins can result in meat with unfavorable color, soft-texture, reduced water holding capacity, reduced shelf-life, and poor consumer acceptability. Development of techniques to minimize the negative impacts of harvest and transport will improve meat quality and benefit processors and consumers.

Channel catfish, *Ictalurus punctatus*, farming is the largest sector of the U.S. aquaculture industry with approximately 270 million kg of catfish processed in 2003 (USDA, 2004). Typical catfish harvest and transport involves seining the fish, holding the fish in a grading-sock overnight to allow sub-marketable size fish to escape, and then loading fish onto transport trucks for live delivery to processing plants the following day (Torrans et al., 2003). Catfish meat quality can be negatively affected by the activity and stress of harvest and transport and preliminary evidence (Silva and Nunez, 2001; Bosworth et al., 2004) suggests the physiological basis for these negative effects is similar to those observed in other fish and terrestrial livestock species (Froning et al., 1978; Warriss, 1990; D'Souza et al., 1998; Faucitano, 1998; Skjervold et al., 1999, 2001; Owens et al., 2000). Current methods available to reduce the negative impacts of harvest and transport on catfish meat quality are primarily limited to changes in the transport environment (e.g. manipulating the temperature, oxygen content, or ionic concentration of the transport water) and result in only minor improvements in meat quality (Bosworth et al., 2004). However, the use of AQUI-S™, a fish anesthetic approved for zero witholding in Australia, New Zealand, and Chile, has allowed development of 'rested-harvest' protocols for salmonids resulting in substantial improvements in meat quality (Kiessling et al., 2004). Rested-harvest involves exposing fish to anesthetic early in the harvest process and then killing or immobilizing the sedated fish before processing. Sedating fish early in the harvest process minimizes the activity and stress involved in typical harvest and transport and therefore results in improved meat quality.

AQUI-S™ is currently undergoing FDA review for approval for use in the U.S. as a fish anesthetic with zero witholding. AQUI-S™ is an effective anesthetic for channel catfish (Small, 2003, 2004; Small and Chatakondi, 2005), but effects of sedation during harvest on catfish meat quality have not been reported. If AQUI-S™ is approved for zero withholding usage in the U.S., development of rested-harvest techniques for catfish could improve catfish meat quality. Although implementation of rested-harvest for farm-raised catfish will require approval of AQUI-S™ (or a similar zero withholding anesthetic), development of equipment and protocols for rested-harvest, and demonstration of a cost-benefit advantage; preliminary investigations to determine the effects of rested-harvest on physiological response and meat quality of channel catfish are needed. Therefore, the objectives of this study were to: determine an effective dose of AQUI-S™ for rested-harvest of catfish, determine effects of rested-harvest on selected physiological indicators of activity/stress in catfish, and determine effects of rested-harvest on catfish meat quality.

2. Materials and methods

A series of trials were conducted to evaluate the use of AQUI-S™ for rested-harvest of channel catfish. Trials were conducted to determine the: 1) effective dose of AQUI-S™ for rested-harvest of catfish; 2) effects of water temperature on time to sedation, time to recovery, and survival for catfish following exposure to AQUI-S™; 3) physiological response of catfish to sedation with AQUI-S™, tricaine methanesulfonate (MS-222), and metomidate; 4) effects of rested-harvest of catfish with AQUI-S™ on physiological indicators of activity, stress, and anaerobic metabolism at slaughter; 5) effects of acclimation and storage temperature on post-mortem muscle metabolism in rested-harvested catfish; 6) optimal method for killing catfish following rested-harvest; and 7) effects of rested-harvest on catfish fillet drip-loss, color, and shelf-life. Analytical procedures common to the various trials are listed in Section 2.8.

Well water was used to dilute AQUI-S™ 1 to 10 according to the manufacture's directions and the resulting stock solution was shaken after mixing and prior to each use to insure adequate mixing. Metomidate and MS-222 were weighed and mixed with well water to produce unbuffered stock solutions of 10 and 100 ppt, respectively. Fresh stock solutions were made for each experiment conducted. Aquarium and tank water volumes were estimated prior to trials and the amount of AQUI-S™, metomidate, and MS-222 stock solutions required to achieve the desired concentrations were measured to the nearest 0.1 ml, mixed with a small volume of water from the tank, and quickly poured into the tank. Aeration in tanks provided sufficient water circulation to adequately mix water after addition of tranquilizers. AQUI-S™ used

in pond trials was sprayed in gradually from a hand-held tank (commonly used for home herbicide application) until fish had reached the desired stage of anesthesia. The amount of AQUI-S™ used in pond trials was estimated by the difference between the starting and ending volume in the sprayer. However, the volume of pond water treated was not precisely known and therefore the AQUI-S™ dosage listed for pond trials is a approximate value. Final concentration of AQUI-S™ in tank and aquarium trials was assumed to be accurate and concentrations were not analytically verified. Concentrations listed are given for concentration of AQUI-S™, concentration of *iso*-eugenol, the active ingredient in AQUI-S™, are one half the concentrations listed for AQUI-S™.

2.1. Dosage trial

Juvenile channel catfish (mean weight = 73.2 g) were stocked in each of twenty seven, 72-l aquaria (10 fish/aquarium), supplied with flow through well water (1-l/min, 27 °C) and aeration. Fish were fed a commercial catfish fingerling diet once daily and allowed to acclimate for 2 weeks. Fish were not fed for 24 h prior to testing. At testing, water flow was discontinued to aquaria and AQUI-S™ was added to achieve target concentrations of 0, 5, 10, 20, 25, 30, 40, 50 and 100 ppm. Three replicate aquaria were used for each dose. Fish were exposed to AQUI-S™ in static water with aeration for 60 min, then water was drained, and flow was resumed at 10 l/min. Behavior of fish was compared to fish not exposed to AQUI-S™. Behavioral indicators recorded included: avoidance response to a hand being moved outside aquaria, ability to touch fish by hand, ability to pick fish up by hand easily compared to fully conscious fish, and loss of equilibrium. The number of fish that lost equilibrium was recorded at 3 and 10 min following addition of AQUI-S™. Recovery time (the time elapsed from start of aquarium refilling to when all fish had regained equilibrium) and survival 24 h after sedation were recorded. Effects of AQUI-S™ dose on behavior, time to loss of equilibrium, recovery time, and 24 h post-exposure survival were determined.

2.2. Effects of water temperature on induction time, recovery time, and survival following AQUl-S exposure

Juvenile channel catfish were acclimated to water temperatures of 10, 20 and 30 °C for 2 weeks and fed once daily. Water temperature was controlled by combination heater-chillers units (Model DM15D, Process Technology, Mentor, OH, USA). Three replicate 16-l plastic aquaria were used for each temperature, water flow was 0.5 l/min, and each aquarium was stocked with 10 juvenile catfish (mean weight 43.4 g). Fish were not fed for 24 h prior to testing. At testing, water flow was discontinued to aquaria and AQUI-S™ was added to achieve a target dose of 35 ppm. Percentage of fish that lost equilibrium was recorded at 3 and 10 min after addition of AQUI-S™. Fish were transferred to recovery tanks (same temperature but without AQUI-S™) 30 min after addition of AQUI-S™ and time to recovery was recorded. Effects of water temperature on time to loss of equilibrium, recovery time, and 24 h post-exposure survival were determined.

2.3. Physiological response to sedation with AQUI-S™ compared to other fish anesthetics

Juvenile channel catfish (mean weight = 81.3 g) were stocked into fifteen, 72-l aquaria (10 fish/aquarium) supplied with flow through well water (1 l/min, 27 °C) and aeration. Fish were fed daily and allowed to acclimate for 2 weeks. At testing, water flow was stopped and fish were exposed to 25 ppm AQUI-S™, 35 ppm AQUI-S™, 8 ppm metomidate, 100 ppm MS-222, or a 30 min low-water stress (3 replicate aquaria/treatment). The low-water stress was conducted by placing a 25 mm standpipe in the aquaria and allowing the water to drain, leaving the fish in approximately 20 mm of water. This amount of water was enough to cover the fish and allowed them to maintain equilibrium, but did appear to cause a 'stress' response in the fish (increased activity and increased opercular movement). Tanks with fish exposed to anesthetic treatments were drained to the same level as the low-water stress treatment 5 min after addition of anesthetic. At 30 min post-sedation or initiation of low-water stress, 3 fish from each aquaria were bled from the caudal vasculature and measured for blood pH, lactate, glucose, and cortisol. After sampling, water flow was resumed at 10 l/min and remaining fish were allowed to recover. The following day, fish in 2 replicate aquaria from each treatment were subjected to the same treatment used the previous day and 3 fish from each aquarium were sampled as before for blood pH, lactate, glucose, and cortisol. The second sampling was to determine if there was a delayed response to sedation for any of the blood parameters measured.

2.4. Effects of rested-harvest on biochemical indices of activity, stress, and anaerobic metabolism at slaughter

Three groups of channel catfish (mean weight = 430 g, $n = 8–10$ per group) were compared for physiological

indicators of activity, stress, and anaerobic metabolism at slaughter. The weight of fish used in this portion of the study is the minimum acceptable weight for processing at commercial plants. Groups included 1) fish rested-harvest with 30 ppm AQUI-S™ from a 200-l circular tank, 2) fish rested-harvest with 30 ppm AQUI-S™ from an earthen pond, and 3) fish harvested from a 200-l circular tank after a 45 min low-water stress. Tank rested-harvest fish were sedated by discontinuing water flow to the tank and adding AQUI-S™ to a concentration of 30 ppm. Pond rested-harvest fish were seined and collected in a harvest sock, the seine was then detached from the sock, and the throat of the sock was connected to a 2 m long × 1 m wide × 1 m deep tarpaulin bag. Approximately 10–15 ppm AQUI-S™ was added to the bag and fish were then gently crowded from the sock to the bag. After fish had entered the bag, additional AQUI-S™ was added until fish began to lose equilibrium (~30–35 ppm AQUI-S™). Fish were then removed from the bag, placed in coolers containing pond water with 30 ppm AQUI-S™, and transported to the laboratory for sampling (~10 min transport time). Fish in the low-water stress group had the water drained from their tank by removal of the standpipe which resulted in the fish being exposed to a water depth of ~3 cm for 45 min. Although only 8 to 10 fish were used for each treatment, the number of fish in the tank treatments was 50 fish per tank and the estimated number of fish seined, moved into the tarpaulin bag, and sedated in the pond treatment was approximately 500 fish (or approximately 100 kg m^{-3}).

All fish were killed by pithing the brain with an *iki jime* (instant death) tool (described by Fletcher et al., 2003) at harvest, bled, and sampled for white muscle. Blood and muscle samples were collected from tank rested-harvest, pond rested-harvest, and exercised catfish approximately 1 h after either the addition of AQUI-S™ or initiation of the low-water stress. Groups were compared for blood and muscle lactate and pH, blood cortisol, and muscle ATP concentrations.

2.5. Effects of acclimation and storage temperature on indices of post-mortem muscle metabolism

Market-weight fish (mean weight 680.5 g) were rested-harvested from earthen ponds using ~30 ppm AQUI-S™ in the summer (water temperature = 33 °C) and winter (water temperature = 7 °C), killed by iki jime, and then stored in water chilled to 5, 10, or 15 °C to determine effects of acclimation and storage temperature on post-mortem white muscle pH, ATP, and lactate. Fish were fed a 28% commercial catfish diet (Delta Western, Indianola, MS). Fish were fed to satiation once daily during the normal feeding season (~May through October) and on days when water temperatures were above 15 °C during the rest of the year. Fish were rested-harvested using the procedure described in the previous section for pond rested-harvest. Randomly selected fish from each group (summer $n=7$, winter $n=10$) were measured for weight, cut-surface muscle pH, blood pH, lactate, and glucose at harvest. Remaining fish were placed in tanks with water chilled to 5, 10 or 15 °C ($n=10$ fish for each storage and acclimation temperature combination). Summer acclimated fish were measured at 12 and 24 h post-harvest ($n=5$ for each storage temperature and sampling time), and winter acclimated fish were measured at 24 and 48 h ($n=5$ for each storage temperature and sampling time). Summer acclimated fish were measured at earlier time points than winter acclimated fish because visual observation indicated that the summer acclimated fish were going into rigor more quickly (and presumably the associated levels of ATP and lactate were changing more quickly also) than winter acclimated fish. Fish were measured for cut-surface muscle pH and sampled for white muscle for subsequent ATP and lactate analysis. Effect of post-harvest storage temperature on muscle pH, ATP, and lactate in winter and summer acclimated rested-harvest fish was determined.

2.6. Comparison of methods for killing fish following sedation

In order to achieve maximum benefit of rested-harvesting, fish should not be allowed to recover and become active between rested-harvest and processing. Methods for killing/immobilizing fish following rested-harvest were compared for their effects on physiology, post-mortem muscle pH, and rigor index. Channel catfish (mean weight = 308 g) were stocked in two 600 l circular tanks (10 l/min, 27 °C, 80 fish/tank), allowed to acclimate for 2 weeks, and fed once daily with a commercial catfish diet. At testing, fish in one tank were exposed to 30 ppm AQUI-S™ and fish in the other tank were exposed to a 60 min low-water stress. Fish from the rested-harvest treatment ($n=5$) and low-water stress treatment ($n=5$) were measured for blood pH, lactate, and glucose, and muscle pH 45 min after exposure to AQUI-S™ or initiation of low-water stress. Remaining sedated fish were subjected to one of the following treatments ($n=10$–12 fish per treatment): water bath (40 l) with 150 ppm AQUI-S™ (overdose), water bath with 30 ppm AQUI-S™ and saturated with N_2 gas, water bath with no AQUI-S™ and saturated with N_2 gas, water bath with 30 ppm AQUI-S™ and saturated with CO_2 gas, water bath with no

AQUI-S™ and saturated with CO_2 gas, or 40 V electrical stun and then water bath with no AQUI-S™. Nitrogen gas was used to create and expose fish to hypoxic conditions. Fish from the low-water stress treatment were killed by a percussive blow to the head and placed in a water bath without AQUI-S™. Water baths were conducted in insulated polyethylene coolers and initial water temperature was 12 °C. Either N_2 or CO_2 gas was delivered from pressurized cylinders through ceramic diffuser stones, flow was adjusted to approximately 7-l/min with a regulator valve.

Fish in coolers were observed for signs of movement, and temperature and dissolved oxygen of water baths were measured periodically with a dissolved oxygen meter (YSI 550A, YSI, Yellow Springs, OH, USA). After approximately 1 h in the water bath, 3–4 fish from each treatment were given a percussive blow to the head and measured for blood pH, lactate, and glucose, and muscle pH. Remaining fish from each treatment were given a percussive blow to the head (even though most fish appeared to be dead), marked to treatment with a unique fin clip, and placed in a common tank with water chilled to 12 °C. Fish were measured for muscle pH and rigor index 16 h post-harvest. Two replicate trials were conducted.

2.7. Effects of rested-harvest on fillet drip-loss, color, pH, and shelf-life

Three replicate trials were conducted during August and September (considered late summer). During each trial, market-weight channel catfish (mean weight 847 g) were seined from earthen ponds and approximately 100 fish were placed in each of two concrete raceways (7 m long × 2.1 m wide × 1 m deep). Raceways were supplied with flow-through well water (~27 °C) and aeration, fish were allowed to acclimate for 2 weeks and fed a commercial catfish diet to satiation once daily. At harvest, fish in one raceway were gently crowded to one end of the raceway with a crowding panel, a weighted plastic sheet was dropped in front of the crowding panel to isolate the water in the sectioning retaining the fish, and then ~30 ppm AQUI-S™ was added to sedate the fish. Ten minutes after exposure to AQUI-S™, fish were removed with dip-nets and placed in slush ice-water baths saturated with CO_2 at 7-l/min. This comprised the rested-harvest treatment. Fish in the other raceway were gently crowded to one end, removed with dip-nets, and placed in a fiberglass transport tank at a density of ~0.7 kg/l. Dissolved oxygen in transport water was maintained at 3–4 ppm by aeration with a regenerative blower. This treatment was referred to as simulated industry transport.

Five fish from each treatment were removed at 2 h post-harvest, killed by a percussive blow to the head, and measured for blood pH, lactate, and glucose, and muscle pH. At 2.5 h post-harvest, simulated transport and rested-harvest fish were processed. Prior to processing, simulated industry transport fish were removed from the transport tank by netting and immobilized by a 40 V electrical shock. Rested-harvest fish were immobilized (displaying no opercular or body movement) and therefore stunning before processing was not required. Fish were mechanically deheaded and filleted (Baader 166 catfish heading machine and Baader 184 catfish filleting machine, Baader, Lubeck, Germany) and fillets were trimmed by hand.

Left-side fillets from 40 fish/treatment were placed individually in sealed polyethylene bags and placed in an ice-water slurry in a insulated cooler. After 1 h in ice-water, 10 fillets from each treatment were removed from bags, patted dry with an absorbent paper towel, weighed to the nearest 0.1 g, and measured for CIE color values (L^*, a^*, b^*) on the inner surface of the fillet between the dorsal fin insertion and the midline of the fillet. The 30 remaining fillets from each treatment were patted dry, weighed, placed individually in polyethylene bags, and stored on ice. Ten fillets from each treatment were removed from ice-storage at 1, 4, and 7 days post-processing and measured for weight, color, and cut surface pH as described above.

During the last replicate trial, 15 right-hand-side fillets from each treatment were transported to the Aquaculture Systems Research Unit, USDA-ARS (Pine Bluff, AR) for determination of bacterial counts during 12 days iced storage. Colony forming units (c.f.u.)/g of fillet was determined for one fillet/treatment/day from days 1 to 12 of storage on ice in polyethylene bags. Effects of rested-harvesting on fillet pH, drip-loss, color, and bacterial counts were determined.

2.8. Analytical methods

Muscle pH was measured on freshly cut portions of white muscle with a pH surface electrode (Model 450-C, Sensorex, Garden Grove, CA, USA) and blood pH was measured with a 6 mm diameter pH electrode (Model S900C, Sensorex) connected to a pH/mV meter (Sension1, Hach, Loveland, CO, USA). Blood was collected from the caudal vasculature with a heparin-rinsed syringe. Muscle lactate, blood lactate, and blood glucose were measured with portable meters (Accusport Lactate Analyser and Accucheck Advantage Blood Glucose Meter, Boherniger–Manheim, Manheim, Germany). Transverse sections of white muscle (~3 g) were collected from the dorsal musculature below the adipose fin, immediately freeze-clamped between blocks of

aluminum cooled in liquid nitrogen, wrapped in aluminum foil, and stored at −80 °C until analysis. Muscle ATP and lactate analysis were performed on perchloric acid extracts of white muscle prepared according to Jerret et al. (1996). ATP content of white muscle was determined using an ultraviolet spectrophometric analysis kit (catalog # 366-A, Sigma Diagnostics, Sigma Diagnostics, St. Louis, MO, USA). Details on the development of standard curves and equations used to determine concentrations of ATP and lactate on an μmol/g of muscle basis are described in detail in Black (2002). CIE L^*, a^*, b^* color measurements were recorded with a Minolta colorimeter (CR10, Minolta, Osaka, Japan). L^* values indicate whiteness (+values= whiter), a^* values indicate redness (+values=more red), and b^* values indicate yellowness (+values=more yellow). Serum cortisol was measured using a fluoroimmunoassay validated for use with channel catfish (Small and Davis, 2002). Fish and fillet weights were recorded with a platform balance (Model QC5DCE-S, Sartorius, Edgewood, NY, USA). Fillet percent drip-loss during storage was calculated as 100*(initial fillet weight−final fillet weight)/initial fillet weight. Rigor index was determined by resting the head of the fish on a table with the rest of the body extending off the table and estimating the degree of departure from parallel, similar to methods described for measuring rigor in tilapia, *Oreochromis aureus* (Korhonen et al., 1990) Fish were classified as 25, 50, 75, or 100% in rigor based on the angle that the body deviated from parallel. Bacterial counts were made by placing approximately 30 g of catfish fillet in a Whirl-Pak blender bag and adding Butterfields buffer (Biotrace International Bioproducts, Bothell, WA) to a total weight of 300 g. The mixture in the bag was then homogenized with a masticator (IUL, Barcelona, Spain) for 2 min. Further decimal dilutions, as required, were made with the same diluent, and solution was plated in quadruplicate on plate count agar (Biotrace International Bioproducts, Bothell, WA), and incubated at 30 °C for 48 h (ICMSF 1978). Viable colonies were counted and reported as log colony forming units (c.f.u.)/g of fillet.

2.9. Statistical analysis

Data from the dosage trial (Section 2.1.), temperature trial (Section 2.2), and comparison of different anesthetics (Section 2.3) were analyzed by one-way ANOVA with replicate aquarium as the experimental unit and aquarium within treatment as the error term in tests of significance. Data from the effects of rested-harvesting on biochemical indices at slaughter (Section 2.4) effects of acclimation and storage temperature (Section 2.5), and comparison of methods for euthanizing/immobilizing fish (Section 2.6) were analyzed by one-way ANOVA with replicate fish as the experimental unit and fish within group as the error

Table 1
Effects[1] of AQUI-S™ dose on behavioral responses, percentage of fish losing equilibrium at 3 and 10 min post-exposure, time to recovery, and post-exposure survival following a 60-minute exposure

Dose	Behavioral response	Loss of equilibrium at 3 min (%)	Loss of equilibrium at 10 min (%)	Time to recovery (min)	Survival after 60 min exposure (%)
5 ppm AQUI-S™	Reduced response to hand movement outside aquaria	–	–	–	100
10 ppm AQUI-S™	Fish could be touched	–	–	–	100
15 ppm AQUI-S™	Fish could be touched, some picked up by hand	–	–	–	100
20 ppm AQUI-S™	Fish picked up by hand, fish begin to lose equilibrium	16.7 ^{C}	33.3 ^{C}	0.8 ^{C}	100
25 ppm AQUI-S™	Fish begin to lose equilibrium	33.3 ^{C}	66.7 ^{B}	1.0 ^{C}	100
30 ppm AQUI-S™	Loss of equilibrium	70.0 ^{B}	96.7 ^{A}	1.0 ^{C}	100
40 ppm AQUI-S™	Loss of equilibrium	100 ^{A}	100 ^{A}	4.6 ^{B}	100
50 ppm AQUI-S™	Loss of equilibrium	100 ^{A}	100 ^{A}	13.2 ^{A}	100
100 ppm AQUI-S™	Loss of equilibrium	100 ^{A}	100 ^{A}	–	0
S.E.		8.4	7.1	1.8	–

[1] Values within a column with different superscript letters are different at $P \leq 0.05$.

term in tests of significance. Data from the trial comparing effects of rested-harvest on fillet drip-loss, color, and pH (2.7) were analyzed by one-way ANOVA with replicate raceway as the experimental unit and treatment × replicate interaction as the error term in tests of significance. Log_{10} c.f.u./g for rested and simulated industry transport fillets were compared by linear regression with log_{10} c.f.u./g as the dependent variable and day of storage as the independent variable. Statistical analysis was performed with SAS software, version 8.0 (SAS Institute Inc., Cary, NC, USA). Differences among treatments were declared significant at $P<0.05$.

3. Results

3.1. Effects of AQUI-S™ dose on behavior, loss of equilibrium, recovery, and survival

Juvenile channel catfish exhibited behavioral changes at all AQUI-S™ dosages tested. Response to hand movements outside the aquarium were reduced at 5 ppm, fish could be touched at 10 ppm, fish could be picked up by hand and began to lose equilibrium at 20 ppm (Table 1). Percentage of fish losing equilibrium at 3 and 10 min, and time to recovery (regaining equilibrium) increased as the dosage of AQUI-S™ increased. At dosages of >40 ppm all fish lost equilibrium by 3 min post-exposure. Survival 24 h post-exposure was 100% after 60 min exposure to dosages <50 ppm, but no fish recovered after a 60 min exposure to 100 ppm AQUI-S™. Dosage trials were conducted at water temperatures of 27 °C.

3.2. Effects of water temperature on time to loss of equilibrium, recovery, and survival

Percentage of fish that lost equilibrium after 3 min exposure to 35 ppm AQUI-S™ was highest at 30 °C (90.0%, S.E. used for mean comparison = 7.9) but not different at 20 °C (33.3%) or 10 °C (23.3%). All fish had lost equilibrium at all temperatures after 10 min exposure to 35 ppm AQUI-S™. Time to recovery following a 30 min exposure to 35 ppm AQUI-S™ was fastest at 30 °C (3.29 min, S.E. used for mean comparison = 0.16), intermediate at 20 °C (6.94 min), and slowest at 10 °C (19.47 min). Survival 24 h post-exposure was 100% at all temperatures.

3.3. Physiological response to sedation with AQUI-S™ compared to other fish anesthetics

Blood lactate, serum cortisol, and blood glucose were higher and blood pH was lower for low-water stress treatment fish than fish from any anesthetic treatments (Table 2). Blood lactate and serum cortisol were not different among fish exposed to 25 ppm AQUI-S™, 35 ppm AQUI-S™, 8 ppm metomidate, and 100 ppm MS-222. Blood glucose was higher for fish exposed to 25 or 35 ppm AQUI-S™ than for fish exposed to metomidate or MS-222. Blood pH was highest for fish exposed to 25 and 35 ppm AQUI-S™, intermediate for fish exposed to 8 ppm metomidate, and lowest for fish exposed to 100 ppm MS-222. The pattern of response in blood pH, glucose, lactate, and cortisol to the same treatments 24 h after the initial

Table 2
Mean[1] blood pH, lactate, cortisol, and glucose of channel catfish juveniles following 30 min exposure to 25 ppm AQUI-S™, 35 ppm AQUI-S™, 8 ppm metomidate, 100 ppm MS-222, or low-water stress

Treatment	Blood pH	Blood lactate (mmol/L)	Serum cortisol (ng/ml)	Blood glucose (mmol/L)
1st exposure				
25 ppm AQUI-S™	7.52 ^{A}	0.32 ^{B}	5.63 ^{B}	1.44 ^{B}
35 ppm AQUI-S™	7.48 ^{A}	0.78 ^{B}	3.43 ^{B}	1.64 ^{B}
8 ppm metomidate	7.40 ^{B}	0.95 ^{B}	3.45 ^{B}	0.93 ^{C}
100 ppm MS-222	7.30 ^{C}	0.50 ^{B}	7.99 ^{B}	0.86 ^{C}
Low-water stress	7.19 ^{D}	4.14 ^{A}	53.63 ^{A}	2.70 ^{A}
SE	0.024	0.36	3.05	0.18
2nd exposure 24 h delay				
25 ppm AQUI-S™	7.52 ^{A}	0.90 ^{B}	2.8 ^{B}	1.44 ^{BC}
35 ppm AQUI-S™	7.47 ^{A}	0.87 ^{B}	2.9 ^{B}	1.59 ^{B}
8 ppm metomidate	7.41 ^{AB}	1.02 ^{B}	3.3 ^{B}	0.96 ^{C}
100 ppm MS-222	7.29 ^{C}	1.00 ^{B}	5.6 ^{B}	0.93 ^{C}
Low-water stress	7.19 ^{D}	4.26 ^{A}	29.4 ^{A}	2.69 ^{A}
S.E.	0.03	0.34	3.5	0.18

[1] Values within a column time and exposure trial with different superscript letters are different at $P \leq 0.05$.

Table 3
Mean[1] blood pH, lactate, glucose, cortisol; and muscle pH, ATP, and lactate at slaughter for channel catfish that were rested-harvested from tanks, rested-harvested from earthen ponds, or exposed to a 45 min low-water stress in tanks

Group	Blood pH	Blood lactate (mmol/L)	Blood glucose (mmol/L)	Blood cortisol (ng/ml)	Muscle pH[1]	Muscle ATP[1] (μmol/g)	Muscle lactate[1] (μmol/g)
Tank-rested	7.58 ^{A}	0.72 ^{B}	2.1 ^{B}	22.6 ^{B}	7.68 ^{B}	4.2 ^{A}	8.9 ^{B}
Pond-rested	7.57 ^{A}	0.90 ^{B}	2.8 ^{B}	9.2 ^{B}	7.79 ^{A}	3.9 ^{A}	10.6 ^{B}
Low-water stress	6.80 ^{B}	13.16 ^{A}	6.5 ^{A}	82.2 ^{A}	6.79 ^{C}	1.1 ^{B}	25.6 ^{A}
S.E.	0.05	1.04	0.6	10.1	0.04	0.47	2.3

[1] Values within a column with different superscript letters are different at $P \leq 0.05$.

treatment was similar to that observed following the initial treatment (Table 2). Behaviorally, fish that were sedated with AQUI-S™ frequently exhibited a single body flexion when they were picked up by hand that was not observed in fish sedated with MS-222 or metomidate.

3.4. Effects of rested-harvest on biochemical indices of activity, stress, and anaerobic metabolism at slaughter

Tank and pond rested-harvest catfish had higher blood pH, muscle pH, and muscle ATP; and lower blood lactate, blood glucose, blood cortisol, and muscle lactate than low-water stress fish (Table 3).

3.5. Effects of acclimation and storage temperature on indices of post-mortem muscle metabolism

Muscle ATP and pH decreased, and muscle lactate increased over time at all storage temperatures in both winter and summer acclimated channel catfish (Figs. 1, 2, and 3). However, the magnitude of the changes was greater, and the rate of changes was faster for muscle pH, ATP, and lactate for summer acclimated fish than for winter acclimated fish. The pattern of changes in muscle ATP, pH, and lactate during storage of winter and summer acclimated fish were affected differently by post-mortem storage temperature. Muscle ATP and pH of summer acclimated fish were lower and muscle

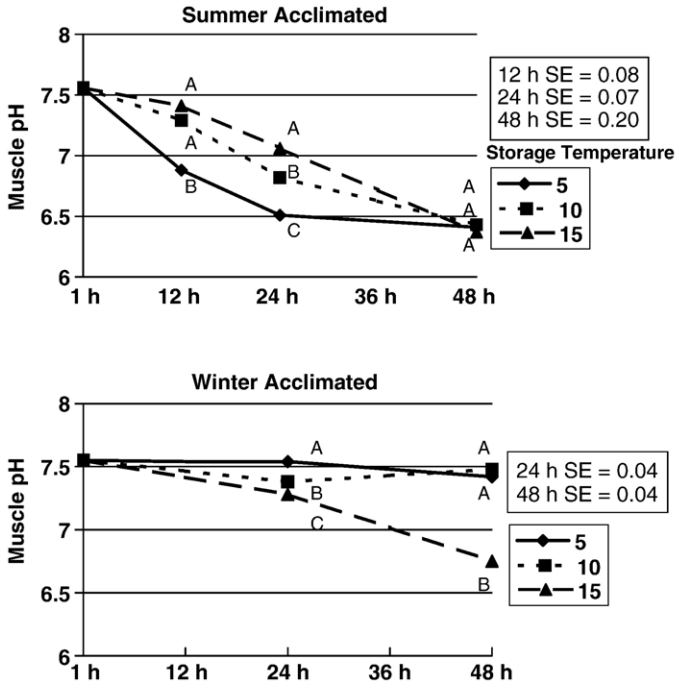

Fig. 1. White muscle pH measured for rested-harvested channel catfish acclimated to summer (33 °C) and winter (7 °C) water temperatures and stored at 5, 10, or 15 °C.

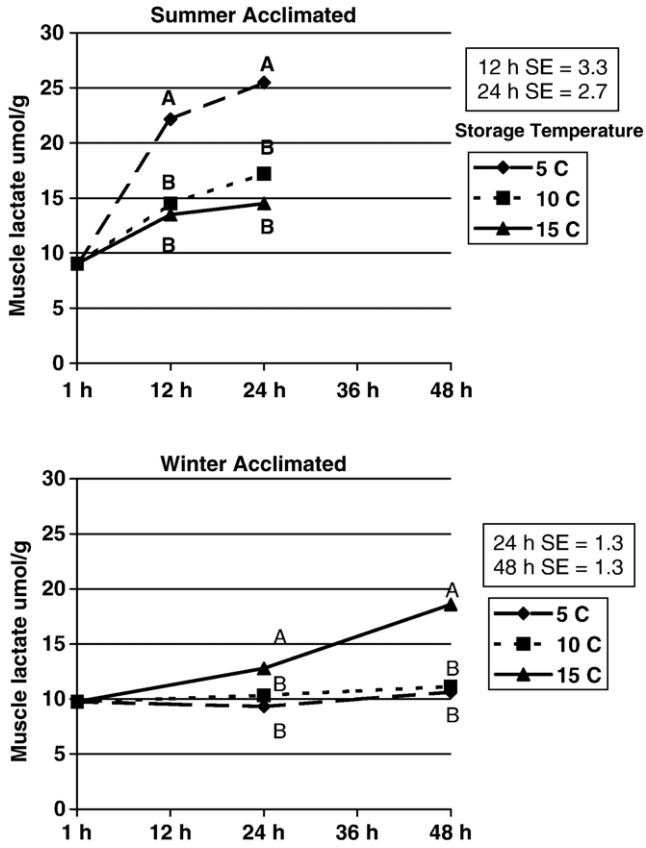

Fig. 2. White muscle ATP levels (μmol/g) measured for rested-harvested channel catfish acclimated to summer (33 °C) and winter (7 °C) water temperatures and stored at 5, 10, or 15 °C.

lactate was higher at 12 and 24 h for fish stored at 5 °C than for fish stored at 10 or 15 °C. Conversely, muscle ATP and pH of winter acclimated channel catfish were lower and muscle lactate was higher at 24 and 48 h post-slaughter for fish stored at 15 °C than for fish stored at 5 or 10 °C.

3.6. Comparison of methods for killing/immobilizing fish following sedation

The lower blood lactate and glucose, and the higher blood and muscle pH of rested-harvest fish relative to low-water stress fish at harvest (Table 4) indicated that rested-harvest was effective in reducing activity/stress in the fish used for this trial. Muscle pH 1 h post-harvest was highest for fish sedated with 30 ppm AQUI-S™ then exposed to CO_2 saturation either with or without 30 ppm AQUI-S™ in the waterbath, AQUI-S™ overdose, electrical stun, and nitrogen saturation with 30 ppm AQUI-S™; intermediate in fish exposed to nitrogen saturation without AQUI-S™ in the waterbath, and lowest in low-water stress fish. Blood lactate at 1 h post-harvest was lowest for fish from both CO_2 treatments and electrical stun; intermediate for fish from nitrogen saturation with or without AQUI-S™, AQUI-S™ overdose; and highest in low-water stress fish. Blood glucose at 1 h post-harvest was lowest in fish from the nitrogen with AQUI-S™ treatment; intermediate in fish from both CO_2 treatments, nitrogen without AQUI-S™, and electrical stun; and highest in fish from AQUI-S™ overdose and low-water stress treatments. Blood pH was lower for fish from low-water stress and both CO_2 treatments than for fish from other treatments. Muscle pH at 16 h post-harvest was highest in fish from both CO_2 treatments, nitrogen with AQUI-S™, and AQUI-S™ overdose; intermediate in fish from electrical stun and nitrogen without AQUI-S™; and lowest for low-water stress fish. Rigor at 16 h post-harvest was most advanced for low-water stress fish, intermediate for fish from the N_2 without AQUI-S™ treatment, and less advanced for other treatments.

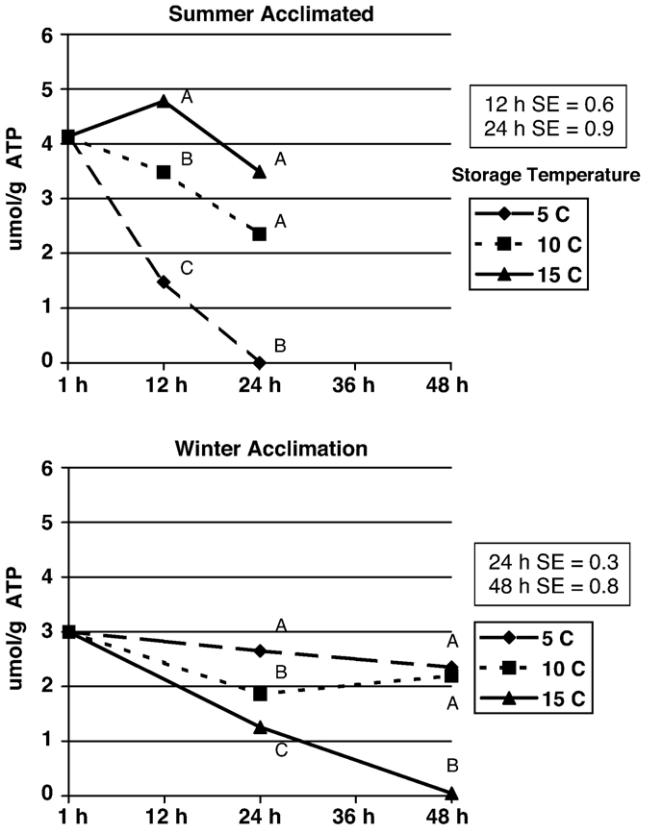

Fig. 3. White muscle lactate levels (μmol/g) measured for rested-harvested channel catfish acclimated to summer (33 °C) and winter (7 °C) water temperatures and stored at 5, 10, or 15 °C.

Although no statistical comparisons were made of water quality, temperatures in water baths were slightly lower for CO_2 treatments (~ 11 °C) than for the nitrogen gas treatments (~13 °C). The temperature difference was probably due to gas cooling as it was released from the CO_2 cylinder (we observed accumulation of ice around the regulator) which was not observed with the nitrogen gas cylinder. Dissolved oxygen levels dropped

Table 4
Mean[1] muscle pH, blood pH, blood lactate, blood glucose at 1 h post-harvest; and muscle pH and rigor index at 16 h post-harvest for different methods of immobilizing/euthanizing channel catfish after rested-harvest with AQUI-S™

Immobilization method[2]	Muscle pH 1 h post-harvest	Blood pH 1 h post-harvest	Blood lactate 1 h post-harvest (mmol/L)	Blood glucose 1 h post-harvest (mmol/L)	Muscle pH at 16 h post-harvest	Rigor index at 16 h (%)
CO_2 w/AQUI-S™	7.58 ^{A}	6.89 ^{C}	0.8 ^{C}	3.3 ^{BC}	7.37 ^{A}	32.5 ^{C}
CO_2 w/o AQUI-S™	7.45 ^{A}	6.84 ^{C}	0.6 ^{C}	2.9 ^{CD}	7.40 ^{A}	38.6 ^{C}
N_2 w/AQUI-S™	7.42 ^{A}	7.34 ^{B}	3.4 ^{BC}	2.0 ^{D}	7.38 ^{A}	35.0 ^{C}
N_2 w/o AQUI-S™	7.20 ^{B}	7.31 ^{B}	5.8 ^{B}	4.2 ^{B}	7.19 ^{C}	65.9 ^{B}
AQUI-S™ overdose	7.51 ^{A}	7.22 ^{B}	3.0 ^{BC}	6.3 ^{A}	7.33 ^{AB}	30.6 ^{C}
Electric stun	7.43 ^{A}	7.27 ^{B}	1.4 ^{C}	3.8 ^{BC}	7.21 ^{BC}	46.4 ^{C}
Rested-harvest control [3]	7.45 ^{A}	7.66 ^{A}	0.6 ^{C}	2.3 ^{DC}	–	–
Low-water stress control [4]	6.78 ^{C}	6.83 ^{C}	17.0 ^{A}	5.6 ^{A}	6.55 ^{D}	100 ^{A}
S.E.	0.06	0.08	1.21	0.44	0.05	0.07

[1] Values within a column with different superscript letters are different at $P \leq 0.05$.
[2] Methods included: water bath saturation with CO_2 with or without 30 ppm AQUI-S™, saturation with N_2 with or without 30 ppm AQUI-S™, overdose (150 ppm AQUI-S™), and 40 V electrical stun.
[3] Values listed for rested-harvest control were measured prior to the water bath treatments.
[4] Low-water stress treatment was used to allow comparison of rested-harvest treatments to non-rested fish.

Table 5
Mean[1] muscle pH, CIE color (L^*, a^*, b^*), and drip-loss at 0, 1, 4, and 7 days post-processing in channel catfish fillets from sedated harvest (30 ppm AQUI-S™/CO_2) or control (simulated industry transport) treatments

Day/treatment	Muscle pH	L^*	a^*	b^*	Drip-loss (%)
Day 0					
AQUI-S™/CO_2	7.38 ^{A}	49.96 ^{A}	−1.06 ^{A}	5.06 ^{A}	–
Simulated industry	6.46 ^{B}	51.28 ^{A}	−0.32 ^{A}	5.67 ^{A}	–
S.E.	0.12	0.85	0.29	0.57	–
Day 1					
AQUI-S™/CO_2	6.58 ^{A}	51.43 ^{B}	0.05 ^{B}	5.69 ^{A}	0.33 ^{B}
Simulated industry	6.36 ^{A}	55.69 ^{A}	0.93 ^{A}	7.24 ^{A}	1.34 ^{A}
S.E.	0.08	0.89	0.37	0.43	0.16
Day 4					
AQUI-S™/CO_2	6.37 ^{A}	53.07 ^{B}	−0.43 ^{A}	5.94 ^{A}	0.51 ^{B}
Simulated industry	6.37 ^{A}	54.90 ^{A}	0.52 ^{A}	6.26 ^{A}	2.44 ^{A}
S.E.	0.05	0.58	0.45	0.56	0.22
Day 7					
AQUI-S™/CO_2	6.40^{A}	53.94 ^{B}	−1.17 ^{A}	5.13 ^{A}	1.49 ^{B}
Simulated industry	6.35^{A}	54.94 ^{A}	−0.46 ^{A}	5.19 ^{A}	4.00 ^{A}
S.E.	0.06	0.39	0.21	0.25	0.43

[1] Values within a column and day with different superscript letters are different at $P \leq 0.05$.

initially and then remained at about 3 ppm in CO_2 treatments probably due to agitation associated with gas passing through the diffusers. Dissolved oxygen levels in the nitrogen treatments dropped initially but remained at about 0.2 to 0.3 ppm even after flow was reduced to minimize the agitation that might have resulted in elevation of dissolved oxygen. It is unclear if the apparent differences in temperature and dissolved oxygen between treatments affected results of this trial.

3.7. Effects of rested-harvest on fillet drip-loss, color, pH, and shelf-life

Blood lactate was lower and muscle pH was higher for rested-harvest fish (2.03 mmol/L and 7.68, respectively) than for simulated industry transport fish (14.51 mmol/L and 6.70, respectively), but blood pH was not different between rested-harvest (6.65) and simulated transport fish (6.82) just before processing. Fillet pH was higher for rested-harvest fish than for simulated transport fish at 1 h post-slaughter, but not different among treatments at 1, 4 or 7 days post-slaughter (Table 5). Fillet L^* value was lower for rested-harvest fish than for simulated transport fish at 1, 4, and 7 days. Fillet a^* value was lower for rested-harvest fish than for than for simulated transport fish at day 1, but not different at days 4 and 7. Fillet b^* value was not affected by treatment. Fillet drip-loss was lower for rested-harvest fish than for simulated transport fish at 1, 4, and 7 days post-slaughter. Temperature of fillets was not recorded, however, fillets were stored on wet ice with thin layer of ice spread over them and it is unlikely there was any difference among treatments for fillet temperature during storage.

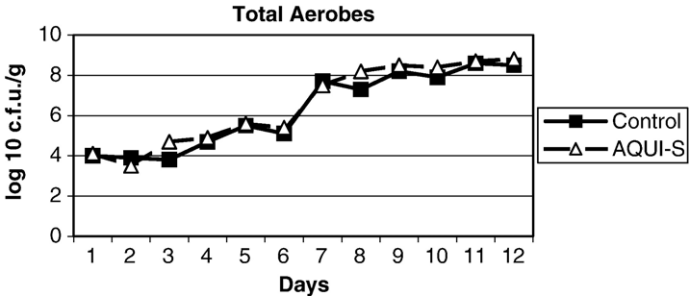

Fig. 4. Bacterial counts ($\log_{10}$ c.f.u./g) for fillets from rested-harvest and simulated industry transport channel catfish during 12 days of iced-storage.

There were no differences between treatments for total aerobic bacteria counts from 1 to 12 days of storage (Fig. 4). Bacteria numbers increased in both treatments (rested-harvest $\log_{10}$ c.f.u./g = 3.07 + day*0.53, R^2 = 0.91; control $\log_{10}$ c.f.u./g = 2.96 + day*0.51, R^2 = 0.90). Overall mean (rested-harvest and simulated industry harvest/transport combined) bacterial counts increased from 4.05 $\log_{10}$ c.f.u./g on day 1 to 8.64 $\log_{10}$ c.f.u./g on day 12.

4. Discussion

Application of rested-harvesting with AQUI-S™ to farm-raised catfish will require a short induction time for efficient harvesting of the large quantities of fish needed to supply a commercial processing plant. In addition, survival following prolonged exposure to AQUI-S™ will be important for rested-harvest of catfish because release of fish too small for processing and processing of only a portion of the fish captured during seining are common practices in the catfish industry. AQUI-S™ doses of 25–35 ppm achieved the desired properties of quick response (loss of equilibrium began within 3 min) and good survival following prolonged exposure (100% following a 60 min exposure). Our results on effective dose of AQUI-S™ for rested-harvest of channel catfish were similar to those of Stehly and Gingerich (1999) who reported channel catfish were handleable (fish could be easily held by hand) after exposure to 20 ppm AQUI-S™. Small and Chatakondi (2005) reported adult channel catfish lost equilibrium 8 min after exposure to 20 ppm AQUI-S™ and lost equilibrium 3.9 min after exposure to 40 ppm AQUI-S™, similar to our observation of loss of equilibrium by 3 min for 40 ppm AQUI-S™.

Although the objective of our research was to investigate potential for rested-harvest of market-weight channel catfish with AQUI-S™, dosage trials were conducted on juveniles due to the convenience of using smaller fish in replicate aquaria. Although we did not do dosage trials with market-weight catfish, the AQUI-S™ concentrations that were effective for juveniles also appeared to work well for market-weight fish. Efficacy trials for AQUI-S™ with channel catfish conducted by the U.S. Fish and Wildlife Service demonstrated that although juvenile fish become handleable and recover faster than adult fish, effective doses for sedation of juvenile and adult channel catfish are similar (Bowker, 2004).

Our results (100% mortality after 60 min exposure to 100 ppm AQUI-S™) indicate that mortality of catfish can occur at high concentrations and extended exposure to AQUI-S™. Stehly and Gingerich (1999) reported increased mortality in juvenile channel catfish exposed to 120 ppm AQUI-S™ for 15 min and Waterstrat (1999) observed 40% mortality in channel catfish exposed to 100 ppm clove oil for 30 min. Clove oil contains active ingredients chemically similar to *iso*-eugenol, the active ingredient in AQUI-S™, but clove oil is approximately 100% active ingredient and AQUI-S™ is 50% active ingredient. However, exposure to 50 ppm AQUI-S™ for 60 min did not result in mortality in this study. Therefore, AQUI-S™ appears to be a safe and effective anesthetic for rested-harvest of catfish.

Farm-raised catfish are exposed to extreme seasonal water temperature fluctuations with summer water temperatures commonly higher than 30 °C and winter temperatures near freezing. Therefore, we felt it was important to examine effects of temperature on induction and recovery following extended AQUI-S™ exposure. We observed increased induction and recovery times for juvenile catfish exposed to 35 ppm AQUI-S™ with decreased water temperature. A similar increase in induction and recovery times with decreasing water temperature was observed for clove oil sedation of tench, *Tinca tinca* (Hamackova et al., 2004) and for Propoiscin sedation of European catfish, *Silurus glanis* (Trzebiatowski et al., 1996). In contrast to our results, Small and Chatakondi (2005) reported fairly rapid recovery time (2.8 min) for catfish sedated with 40 ppm AQUI-S™ at water temperatures of 14.5 °C. The longer recovery times we observed may be due to our holding fish in 35 ppm AQUI-S™ for 30 min following addition of AQUI-S™ whereas Small and Chatakondi removed fish to recovery tanks immediately following loss of equilibrium. In addition, Small and Chatakondi used fish from ponds that had been exposed to a natural temperature cycle and we acclimated fish to cold temperatures in tanks during a 2 week period. Differences in the acclimation process may have resulted in the differences in AQUI-S™ recovery times. Rested-harvest may be most beneficial during the warmer times of the year since catfish processors typically report fewer fillet quality problems (e.g. high drip-loss and soft texture) during the winter months when water temperatures are colder. More information on the effects of pond water temperatures on the effects of rested-harvest on catfish meat quality is needed.

All anesthetics we tested reduced indices of stress, activity, and anaerobic metabolism in juvenile catfish relative to catfish subjected to a low-water stress. Iversen et al. (2003) reported suppressed cortisol and blood glucose in Atlantic salmon smolts sedated with metomidate or AQUI-S™ compared to unsedated controls, similar to our results with channel catfish. Small (2004) reported higher blood glucose for channel catfish sedated with AQUI-S™ than for fish sedated with metomidate, in

agreement with observations from this study. However, Small and Chatakondi (2005) reported channel catfish sedated with MS-222 had higher blood glucose than catfish sedated with AQUI-S™, in contrast to our results (AQUI-S™ sedated fish had higher blood glucose than MS-22 sedated fish). Small (2003) reported cortisol was suppressed for channel catfish sedated with AQUI-S™ and metomidate, but elevated in catfish sedated with MS-222. We also observed suppressed cortisol response for catfish sedated with AQUI-S™ and metomidate, but did not observe increased cortisol in MS-222 sedated catfish. Wagner et al. (2002) reported a 24 h delayed elevation in cortisol following AQUI-S™ sedation of rainbow trout broodstock, but we did not observe a 24 h delayed response in any physiological indices measured following sedation with any anesthetics tested. Although there are slight discrepancies among studies, anesthetics typically minimize physiological indicators of stress and exercise in fish relative to unsedated fish. However, AQUI-S™ is the only chemical anesthetic currently under FDA review for use with zero witholding, an essential requirement for rested-harvesting.

Comparison of tank and pond rested-harvested catfish to catfish exposed to a low-water stress clearly demonstrates that rested-harvesting minimizes the effects of exhaustive exercise on measures of stress and anaerobic metabolism. Cortisol levels were lower for rested-harvested catfish than for low-water stressed fish, indicating that rested-harvest minimized stress response as discussed in the previous section. The higher muscle pH and ATP and lower blood and muscle lactate of rested-harvested catfish compared to low-water stress indicated that rested-harvest reduced anaerobic metabolism before slaughter. Muscle pH of rested-harvest catfish at slaughter (7.58) was similar to muscle pH values reported for rested yellow-eye mullet, *Aldrichetaa forsteri* (7.61) and New Zealand snapper, *Pagrus auratus* (7.72) (Black et al., 2004), chinook salmon, *Oncorhynchus tshawytscha* (7.64) (Jerrett et al., 2002), rainbow trout, *Oncorhynchus mykiss* (7.8) (Robb et al., 2000a,b), and Atlantic salmon, *Salmo salar* (7.3) (Erikson et al., 1999). White muscle ATP levels in rested-harvest catfish (4.2 and 3.9 umol/g in tank and pond rested-harvested fish, respectively) were similar to resting ATP levels reported for rainbow trout (5.74 μmol/g, Thomas et al., 1999; 4.99 μmol/g, Dobson et al., 1987), Atlantic salmon (4.99 μmol/g Thomas et al., 1999), and somewhat lower than those reported by Black et al. (2004) for yellow-eye mullet (7.4 μmol/g) and New Zealand snapper (9.3 μmol/g). Muscle lactate levels reported for rested-harvested catfish (8.9 and 10.6 μmol/g in tank and pond rested-harvested fish, respectively) were similar to levels reported for resting rainbow trout (7.67 μmol/g, Milligan and Girard, 1993), New Zealand snapper (~15 μmol/g, Lowe et al., 1993; 16.5 μmol/g, Black et al., 2004), and mullet (12.7 μmol/g, Black et al., 2004). Catfish exposed to low-water stress exhibited physiological responses (decreased blood and muscle pH, increased blood and muscle lactate, increased blood glucose and cortisol, and decreased muscle ATP) indicative of exhaustive in rainbow trout (Kieffer, 2000; Wang et al., 1994), Atlantic salmon (Berg et al., 1997), snapper (Lowe et al., 1993), and sea lamprey (Boutilier et al., 1993). Muscle pH for low-water stress (6.8) measured at or shortly after harvest was similar to ultimate pH (lowest pH observed) values (6.7) previously reported for catfish (Bosworth et al., 2004). Differences between ultimate pH values reported for catfish in this study (~6.35) and those reported by Bosworth et al. (2004), and the possible lower values we observed in fish harvested in the summer than those in the winter may reflect differences in glycogen levels present in fish at the start of trials.

Although groups of catfish used to determine effects of rested-harvest on biochemical indices of stress and anaerobic metabolism at slaughter were from different culture environments and may not have been treated identically prior to slaughter, all groups were collected during a 1 week period in the summer and all fish were being fed a commercial catfish diet before sampling. The consistency of physiological indices measured at slaughter for rested-harvest fish with those measured for rested-harvest fish in other trials in this study, and for other species cited above, suggest that the differences among groups at slaughter were primarily attributable to rested-harvest and other factors were not considered to have substantially affected the measured variables.

The levels and patterns of change for post-mortem muscle ATP, lactate, and pH in catfish appear to affected by the temperature the fish were acclimated prior to harvest and the subsequent storage temperature. Interactions between acclimation temperature and post-mortem storage temperature have been reported in other fish species (Abe and Okuma, 1991; Jerrett et al., 2002). The basis for the acclimation × storage temperature interaction for post-mortem muscle metabolism may be related to differences in protein or lipid synthesis in fish acclimated to different temperatures (Abe and Okuma, 1991) or changes in muscle cell membrane permeability associated with large differences between acclimation and storage temperatures (Hochachka, 1986; Watabe et al., 1989). Abe and Okuma (1991) reported that common carp, *Cyprinus carpio*, acclimated to 5 °C had slower post-slaughter rigor development, ATP depletion, and lactate accumulation than carp

acclimated to 30 °C when fish from both acclimation temperatures were stored at 0 °C, similar to our results with channel catfish. In addition, Abe and Okuma reported that carp acclimated to 5 °C had slower rigor development when fish were stored at 0 °C than at 10 °C, but carp acclimated to 30 °C had slower rigor development when fish were stored at 10 °C than at 0 °C, similar to the acclimation × storage temperature interaction we observed for measures related to rigor development (decreased pH and ATP, increased lactate). Jerrett et al. (2002) reported the rate of postmortem muscle metabolism of rested-harvested New Zealand snapper was accelerated when the differences between acclimation and storage temperatures increased, similar to what we observed in catfish. Effects of acclimation and storage temperature may also have been influenced by differences in energy stores between summer and winter acclimated fish since feed intake in catfish decreases at cold water temperatures and the winter acclimated fish had not eaten much for approximately 4 weeks before sampling but summer acclimated fish were being fed daily to satiation before sampling. The higher ATP at harvest and larger changes in pH and muscle lactate during storage for summer acclimated fish suggest that summer acclimated fish had higher energy stores at slaughter than winter acclimated fish. The data from this and other studies suggest that a rapid drop from warm summer acclimation temperatures to cold storage temperatures accelerates post-mortem muscle metabolism in some fish species.

To retain benefits of rested-harvest until processing, methods to euthanize or immobilize fish between harvest and processing are needed. Retention of rested-harvest benefits in salmon can be achieved by killing fish with an automated operation that delivers a percussive blow to the head (Robb and Roth, 2003), after which fish are quickly processed on site or at nearby onshore facilities. Stunning by percussive blow to the head and immediate processing is probably not feasible with catfish because centralized processing facilities receive fish from farms spread over a fairly large geographic area and time and labor costs required to stun individual fish by percussion. Although fish could be maintained in a sedated state by addition of AQUI-S™ to the transport water, this would substantially increase the cost associated with rested-harvest. Use of CO_2 gas to euthanize/immobilize catfish following sedation appears to have potential for retaining benefits of rested-harvesting.

CO_2 gas has been used for fish 'anesthesia' (Fish, 1942; Iwama et al., 1989; Pirhonen and Schreck, 2003). However, Robb et al. (2000a,b) demonstrated that CO_2 acts as a muscle relaxant but does not affect sensation of pain and therefore CO_2 narcosis would be a more appropriate term. CO_2 is commonly used as a method of euthanasia in fish and other laboratory animals (AVMA, 1993), but this use is under review. Narcosis with CO_2 is due to a decrease in brain pH resulting from the CO_2 induced reduction in blood pH (Yoshikawa et al., 1991), and euthanasia results from hypoxia after extended exposure to high environmental CO_2 and neural failure (AVMA, 1993). CO_2 was commonly used in the past to immobilize salmon prior to slaughter, but alternative methods are currently being developed because salmon and trout exhibit strong adverse reactions to high CO_2 levels resulting in high levels of stress and activity and resultant degraded meat quality (soft texture, increased drip-loss, gaping); (Robb et al., 2002; Robb and Roth, 2003; Kiessling et al., 2004). In our study, catfish were sedated before exposure to CO_2 and we did not observe adverse reaction to CO_2. The lack of response to CO_2 was consistent whether there was AQUI-S™ in the CO_2 water bath or not, suggesting that the induction of CO_2 narcosis occurred quickly before fish recovered from AQUI-S™ sedation. Catfish exposed to CO_2 following rested-harvest had low blood pH, indicative of respiratory acidosis, but blood lactate was low and muscle pH was high indicating minimal metabolic acidosis.

In contrast to CO_2 exposure, fish exposed to N_2 gas without AQUI-S™ in the water bath regained equilibrium and exhibited behavioral response to low oxygen (gulping at the water surface) and physiological indicators of increased activity, stress, and anaerobic metabolism (at slaughter and 16 h post-slaughter). Fish exposed to N_2 gas regained consciousness and were likely subsequently killed by hypoxia. However, channel catfish are fairly tolerant of low dissolved oxygen and the method we used of creating hypoxia with N_2 resulted in residual oxygen levels that probably resulted in fairly stressful conditions prior to death. Therefore it seems killing catfish by exposure to hypoxic conditions created with N_2 would not be an acceptable method. Electrical stunning, which is currently used to immobilize catfish at off-loading at processing plants, may also be useful for immobilizing rested-harvest catfish before processing. However, use of electrical stunning on or near pond sites will present worker safety issues that would need to be adequately addressed.

The main benefit of rested-harvest for channel catfish on meat quality traits we observed was the lower drip-loss for rested-harvest fillets during storage. Kiessling et al. (2004) reported a similar decrease in drip-loss in rested-harvested Atlantic salmon compared to fish stunned with CO_2. Robb et al. (2000a,b) reported higher

pH and L^* values during storage for rested-harvested vs. electrically stunned Atlantic salmon during iced-storage, similar to patterns of pH and L^* values we reported for rested vs. control catfish fillets. A rapid decrease in muscle pH is typically associated with increased drip-loss in swine (Faucitano, 1998; Monin et al., 1999) and poultry (Owens et al., 2000) and data from this and other studies (Ofstad et al., 1996; Robb et al., 2002; Mørkøre et al., 2002; Bosworth et al., 2004; Kiessling et al., 2004) suggest a similar basis for differences in drip-loss between rested and non-rested-harvested fish. Assigning economic value to drip-loss is difficult because assessment of value depends on where the drip-loss occurs (at the processing plant, retail, or consumer level) and if the drip-loss is noticed and viewed unfavorably. Future studies are needed to quantify effects of rested-harvest on catfish fillet texture and appearance and the associated economic benefits. We did anecdotally observe that rested-harvest fillets clearly had substantially firmer texture and a more iridescent flesh color than control fillets.

We observed no difference between fillets from rested-harvest and simulated industry transport catfish for total aerobic bacterial counts through 12 days of storage on ice. Similarly, Fletcher et al. (2003) reported no differences in bacterial counts between rested-harvest and control fillets of King salmon (*Oncorhynchus tshawytscha*) during 22 days of storage at 0 °C. Given the reduction in stress and activity associated with rested-harvesting, we anticipated that rested-harvest fillets may have extended shelf-life relative to control fillets. However, shelf-life is dependent on both post-mortem autolytic changes in the muscle, for which rested-harvesting is beneficial, and bacterial growth, for which the effects of rested-harvest are less clear. Rested-harvest fillets have higher initial pH and it is possible the higher pH is a more favorable environment for early bacterial growth than the lower pH of non-rested fillets. Allen et al. (1997) reported increased shelf-life in chicken breast fillets with high L values and low pH and attributed the increased shelf-life to the lower pH delaying the lag phase of spoilage bacteria. From a shelf-life perspective, rested-harvest catfish may be beneficial from a muscle energy/metabolism perspective, but not beneficial for reducing bacterial growth. It might be possible to increase shelf-life of catfish fillets by combining rested-harvesting with other strategies such as modified atmosphere packaging or use of ozone to reduce initial bacteria numbers.

Catfish processors spend considerable effort to insure a continuous supply of live fish to the plant to provide efficient use of labor and facilities while also attempting to minimize holding times in grading socks or on transport trucks to maintain good meat quality. Rested-harvesting could alleviate some of the problems with scheduling and product flow at plants since the superior meat quality of rested-harvested fish could be retained for a period of time during storage before processing.

Incorporation of rested-harvest by the catfish industry will require approval of AQUI-S™ (or a similar zero withholding anesthetic compound), development of cost-effective strategies and equipment for implementing rested-harvesting, and demonstration of a cost-benefit advantage to producers or processors. This initial research demonstrates that rested-harvest of catfish with AQUI-S™ reduces the negative effects of typical harvest and transport on catfish meat quality and may allow improvement of catfish meat quality. However, substantial work is needed to determine the logistic and economic feasibility and benefits of a rested-harvesting strategy for commercial catfish production and processing.

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