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Effects of Transport Water Temperature, Aerator Type, and Oxygen Level on Channel Catfish *Ictalurus punctatus* Fillet Quality

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Farming of channel catfish *Ictalurus punctatus* is the largest sector of the U.S. aquaculture industry with approximately 270 million kg of catfish processed in 2001 (USDA 2002). Catfish are typically grown in earthen ponds, harvested with a size-selective net, transported to processing plants, processed, and sold in various product forms. Catfish processors adhere to strict regulations and protocols throughout processing to ensure delivery of a safe, high-quality product. However, research with salmonids (Sigholt et al. 1997; Skjervold et al. 1999, 2001) and terrestrial livestock (Froning et al. 1978; Warriss 1990; D'Souza et al. 1998; Faucitano 1998; Owens et al. 2000) has demonstrated that the pre-processing environment, in particular ante-mortem stress and activity, can affect subsequent meat quality.

Stress associated with transport can result in meat with a rapid decline in pH, lighter color (higher 'L' color values), increased drip-loss (water loss during storage), and softer texture (Faucitano 1998; Skjervold et al. 2001). The negative impacts of transport stress on meat quality are generally attributed to increased anaerobic metabolism, which leads to 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). Although the effects of ante-mortem stress on meat quality have been evaluated for other species, there is little information available about the effects of transport environment on subsequent catfish fillet quality.

Catfish are typically transported to processing plants at high densities (0.7 to 1.0 kg/L) in order to minimize transport costs. For short transport times (1–4 h), transport water aeration is usually provided by a diesel powered regenerative blower that forces large volumes of ambient air through the transport tanks and generates considerable turbulence. Use of blower aeration during the summer increases transport water temperature by injecting large volumes of warm ambient air. The combination of increased water temperature, which decreases oxygen solubility (Wetzel 1983), and high fish densities reduces dissolved oxygen levels in catfish transport tanks. Water temperatures can exceed 32 C and DO levels are generally less than 4 ppm during the summer months in transport tanks aerated by blowers (personal observation).

High water temperatures, turbulence, and reduced oxygen levels during transport may add to the general stress associated with fish harvest and transport. In addition, water temperature and oxygen levels may impact other water quality parameters (ammonia, nitrite, pH, and CO_2) that could influence the level of stress experienced by fish during transport (Amend et al. 1982; Erikson et al. 1997; Patterson et al. 2003). Thus, the objective of this study was to determine effects of transport water temperature, aerator type, and oxygen level on other transport water quality parameters (ammonia, nitrite, pH, and CO_2) and serum cortisol and fillet quality (fillet drip-loss, color, and pH) of channel catfish.

Materials and Methods

Market weight catfish (0.5–1 kg) were seined from a commercial catfish pond twice in July and twice in August, weighed in groups, and placed into each of four fiberglass transport tanks (79 cm long × 55 cm wide × 79 cm deep) at loading densities of ~0.8 kg/L (similar to stocking densities used by the industry during the summer). Fish were held in transport tanks for approximately 3.5 h.

All combinations of two initial transport water temperatures (10 C or 20 C) and three aerator type/dissolved oxygen (DO) levels (blower/DO at 4–5 ppm, pure O_2 diffuser/DO at 4–5 ppm, or pure O_2 diffuser/DO at 9–10 ppm) were tested. Two tanks were supplied with aeration from a 1-hp regenerative blower (Gast, Denton Harbor, Michigan, USA) that forced ambient air through a series of 0.6-cm diameter holes cut into a 2.54-cm diameter metal pipe in the shape of a 'T' laying in the tank bottom. The spacing of the holes and volume of air used were similar to those used in commercial catfish transport tanks. The volume of air dispersed into the tanks was controlled by a valve system. Two tanks were each supplied with aeration through a rectangular diffuser stone (15.25 cm × 3.80 cm × 3.80 cm) connected to a pure oxygen cylinder. Oxygen flow to each tank was adjusted through a regulator on the oxygen tank and a separate valve on the airline to each diffuser stone to maintain DO of either 4–5 or 9–10 ppm depending on treatment assignment. Water temperature and DO level were monitored in all tanks every 15 min during transport, and flow to diffuser stones was occasionally adjusted to maintain DO levels set for each treatment. Four replicate trials were conducted, but because only four transport tanks were available not all treatment combinations could be used during each replicate trial. The blower/10-C and blower/20-C treatments were used in all four replicates, but the diffuser 9–10 ppm/10 C, diffuser 9–10 ppm/20 C, diffuser 4–5 ppm/10 C, and diffuser 4–5 ppm/20 C were used in two replicate trials each.

Ten fish were removed from each transport tank after approximately 3 h, quickly anesthetized in MS-222, bled from the caudal vasculature, and measured for core carcass temperature with a probe thermometer (Model MT 100, Cole-Palmer, Vernon Hills, IL). Blood samples were placed on ice, centrifuged, and serum was collected and frozen at −20 C and later measured for serum cortisol using a fluoroimmunoassay (Small and Davis 2002). At the end of the transport period (~3.5 h), 20 fish from each tank were stunned by electric pulse (40 volts), and processed using commercial processing equipment (Baader 166 catfish heading machine, and Badder 184 catfish filleting machine, Baader, Lubeck, Germany). The right-side fillet from each fish was placed in a separate, sealed polyethylene bag and chilled by immersion in ice-water slush. After 4 h, fillets were patted dry, weighed to the nearest 0.1 g, and a colorimeter (Minolta CR10, Minolta, Osaka, Japan) was used to measure CIE color values ('L,' 'a,' 'b') on the inner surface of the fillet between the dorsal fin insertion and the midline of the fillet. Three replicate colorimeter readings per fillet were averaged to determine color values for each fillet. 'L' values indicate whiteness (+ values = lighter), 'a' values indicate redness (+ values = more red), and 'b' values indicate yellowness (+ values = more yellow). Fillets were then placed back in polyethylene bags and stored on ice in a walk-in chiller (air temperature was 2–4 C). After 72 h of iced-storage, fillets were removed from bags, patted dry, weighed, and measured for CIE color values as described above. Percent fillet weight loss (drip-loss) during storage

[100*(4 h weight − 72 h weight)/4 h weight] was determined. Muscle pH at 72 h was determined by cutting a 1-g piece of white muscle from each fillet, grinding the muscle in five volumes of cold, 5-mM iodoacetic acid, and measuring the pH of the homogenate with a pH meter (Mettler MP220, Mettler-Toledo AB, Stockholm, Sweden). Water samples were collected from each transport tank at 30, 90, 150, 210 min after loading the fish. Water samples were analyzed for ammonia (Nessler method), nitrite (diazotization method), alkalinity (sulfuric acid titration), pH (Hach meter), and CO_2 (APHA 1989).

Water quality and fillet quality data were analyzed using an incomplete block design ANOVA with replicate trial as the blocking factor, transport water temperature as a fixed effect, aerator type/oxygen level as a fixed effect, water temperature by aerator type/oxygen level interaction as a fixed effect, and replicate transport tank within water temperature by aerator type/oxygen level interaction as a random effect. Individual transport tank was considered the experimental unit and mean square associated with replicate tank within water temperature by aeration type/oxygen level interaction was used in test of significance of fixed effects. Contrasts among various treatment combination means were used to provide insight into which factors had influenced the variables measured. ANOVA and contrasts were conducted using the Mixed Procedure of SAS (SAS Institute Inc., Cary, North Carolina, USA). A macro written for Proc Mixed (Pdmix800, Arnold M. Saxton, University of Tennessee, Knoxville, Tennessee, USA) was used to conduct multiple-range tests on fixed effect means. Pearson product-moment correlations among 4-h and 72-h 'L' values, pH, and drip-loss of fillets were determined using the Correlation Procedure of SAS. Differences were declared significant at $P < 0.05$.

Results

Although water quality was measured at several points during transport, only the means of water quality variables for the final time point (210 min) are presented since this was the time point at which water quality was the most degraded. Total ammonia-nitrogen (TAN) was higher at warmer transport water temperatures and higher in transport water aerated by diffusers than by aerators (Table 1). Transport water pH was higher in tanks aerated with blowers than those aerated with pure oxygen diffusers. Transport water TAN and pH were not affected by oxygen level. CO_2 levels were higher in tanks aerated by oxygen diffusers than those aerated by blowers, and marginally higher ($P = 0.09$) in the low oxygen diffuser aeration treatment than in the high oxygen diffuser aeration treatment. Water temperature had no effect on CO_2 levels. Transport water nitrite levels were not affected by transport conditions. Transport water alkalinity was higher at 20-C transport temperatures than at 10 C.

Serum cortisol was not affected by transport water temperature, aerator type, or oxygen level. There was a trend ($P = 0.12$) for higher cortisol at lower transport temperature. However, the standard errors for cortisol were large, indicating a large amount of variation between replicate tanks within a treatment combination, making it difficult to draw any conclusions about the effects of treatment factors on cortisol levels.

Carcass temperature was lower for fish transported at 10 C than at 20 C, and there was a trend ($P = 0.09$) for lower carcass temperatures in fish transported with diffuser aeration compared to blower aeration (Table 2). 'L' values at 4 and 72 h and fillet drip-loss at 72 h were lower for fish transported at high DO levels than for fish transported at low DO. Fillet 'a' and 'b' values at 4 and 72 h and fillet pH at 72 h were not affected by treatment. Fillet drip-loss during storage was positively correlated with 4-h fillet 'L' value ($+0.43$ $P < 0.0001$), 72-h fillet 'L' value ($+ 0.47$ $P < 0.0001$), and 72-h fillet pH (-0.53 $P < 0.0001$).

TABLE 1. *Least square means (±SE) and contrasts for final transport water total ammonia-nitrogen (TAN), nitrite, pH, CO_2, and alkalinity for various combinations of transport water temperature, oxygen level, and aerator type. Within a column means with different letters are significantly different at* P ≤ *0.05.*

Treatment /temperature/ $_2$ level	TAN (ppm)	Nitrite (ppm)	pH	CO_2 (ppm)	Alkalinity (ppm)
Blower/10 C/Low O_2	4.39 a	0.021	7.95 a	6.55 a	289
Blower/20 C/Low O_2	6.22 a,b	0.025	7.81 a	8.42 a	311
Diffuser/10 C/Low O_2	7.90 a,b	0.026	7.06 b	69.5 c	303
Diffuser/20 C/Low O_2	9.60 b	0.026	7.07 b	62.8 b, c	336
Diffuser/10 C/High O_2	6.31 a,b	0.017	7.09 b	40.0 b	295
Diffuser/20 C/High O_2	8.53 a,b	0.024	7.17 b	39.5 b	322
Contrasts[1]					
20 C vs 10 C	1.91*	0.004	−0.07	−1.8	28*
Blower vs Diffuser/Low O_2	−3.44*	−0.003	0.82*	−58.7*	−14
Blower vs Diffuser/High O_2	−2.11*	0.003	0.75*	−32.2*	−14
Diffuser/Low O_2 vs Diffuser/ High O_2	1.33	0.006	−0.07	26.4	0.3
Pooled Standard Error	1.34	0.006	0.14	11.6	16
Significant Effects[2]	AT, WT		AT	AT	WT

[1] An * indicates the contrast was significant ($P \leq 0.05$).
[2] Water temperature (WT), aerator type (AT), or oxygen level (OL) had a significant effect ($P \leq 0.05$).

Discussion

The results indicate that transport water DO level influenced channel catfish fillet color and drip-loss during iced-storage. Similar associations between transport stress and meat quality (increased transport stress resulting in increased drip-loss and higher 'L' values) have been observed in poultry, swine, and salmon (Aaslyng and Barton Gade 2001; Skjervold et al. 2001; Molette et al. 2003) suggesting that the same physiological mechanisms associated with effects of stress on meat quality in other meat animals affect catfish fillet quality.

The effects of stress on meat quality are generally attributed to the increased level of anaerobic metabolism associated with the stress and activity of harvest/transport which results in accumulation of lactate, accelerated muscle pH decline, and accelerated denaturation/degradation of muscle proteins (Bendall and Swatland 1988; Offer 1991; Rathgeber et al. 1999). The lower 'L' values and drip-loss of fillets from fish subjected to high oxygen during transport may be due to greater availability of oxygen to muscle during transport which would result in lower muscle lactate and a more gradual postmortem pH decline. No effect of treatment on muscle pH was observed, but pH was not measured until 72-h post-processing, at which time the pH had stabilized and was no longer decreasing (ultimate pH). Ultimate pH generally reflects the energy stores in the fish muscle at the time of death (Ofstad et al. 1996), but is not a good indicator of the rate of pH decline. However, there was a significant negative correlation between ultimate pH and drip-loss (lower pH = increased drip-loss) when individual fillet values were analyzed with all treatments combined, suggesting that there was some relationship between ultimate pH and drip-loss. Future studies should focus on factors influencing the the rate of muscle pH decline, ultimate pH, and fillet quality in catfish.

Carcass temperature was affected by initial transport water temperature as expected. In addition there was a trend towards lower carcass temperature in diffuser aeration treatments compared to blower aeration

TABLE 2. *Least square means (± SE) and contrasts for carcass temperature, fillet CIE color values (L*, a*, b*) at 4-h and 72-h post-processing and fillet weight loss (%) and pH at 72-h post-processing for various combinations of transport water temperature, oxygen level, and aerator type. Within a column means with different letters are significantly different at* P ≤ *0.05.*

Treatment	Carcass	Color values 4-h post-processing			Color values 72-h post-processing		
Aerator/Temperature/O_2 Level	temp	'L'	'a'	'b'	'L'	'a'	'b'
Blower/10 C/Low O_2	19.2 a	54.64 a	−2.12	1.06	56.15 a	−2.05	1.40
Blower/20 C/Low O_2	24.8 b	54.79 a	−2.10	1.11	56.16 a	−2.00	1.51
Diffuser/10 C/Low O_2	17.2 a	55.05 a	−2.18	1.09	56.78 a	−2.10	1.36
Diffuser/20 C/Low O_2	23.8 b	55.00 a	−2.11	1.30	56.75 a	−1.96	1.61
Diffuser/10 C/High O_2	17.3 a	52.93 b	−2.11	0.67	55.67 a, b	−2.03	1.25
Diffuser/20 C/High O_2	23.3 b	53.33 b	−2.05	1.00	54.61 b	−1.93	1.54
Contrasts[1]							
20 C vs 10 C	6.0*	0.17	0.03	0.20	−0.34	0.10	0.21
Blower vs Diffuser/Low O_2	1.6	−0.31	0.04	−0.11	−0.58	0.01	−0.03
Blower vs Diffuser/High O_2	1.7	1.59*	−0.03	0.25	1.03*	−0.04	0.05
Diffuser/Low O_2 vs Diffuser/High O_2	0.1	1.90*	−0.07	0.37	1.61*	−0.05	0.09
Pooled Standard Error	1.2	0.50	0.13	0.29	0.43	0.10	0.29
Significant Effects[2]	WT	OL			OL		

[1] An * indicates the contrast was significant ($P \leq 0.05$).
[2] Water temperature (WT) or oxygen level (OL) had a significant effect ($P \leq 0.05$).

treatments. Trials were conducted in the summer and the blower aeration treatments had large volumes of warm ambient air injected into the transport tanks, resulting in increased water temperatures that was reflected by trend towards increased carcass temperature in blower aeration treatments. However, we did not observe any effect of transport water temperature on fillet quality.

Serum cortisol is widely used as a measure of stress in fish (Barton and Iwama 1991) and the results presented on cortisol levels indicate fish in all treatments experienced stress. Previous transport trials with channel catfish have shown a large decrease in water temperature results in elevated cortisol (Bosworth et al. 2001). The results of this study indicate a similar, but nonsignificant, trend of increased cortisol when fish are removed from warm ambient temperatures and placed in much cooler transport water. However, there was no relationship between cortisol level and measures of fillet quality.

Although aerator type did affect water pH, CO_2, and ammonia, there was no apparent, direct effect of aerator type on the fillet quality parameters measured. The differences in water quality due to aerator type were probably due, at least in part, to differences in the level of water agitation/turbulence provided by the different aeration methods and the resulting influences on water CO_2 levels. Diffusers caused very little disturbance of the water surface, likely resulting in the observed accumulation of CO_2 in diffuser aerator treatments. CO_2 accumulation would produce the observed reduction in water pH through changes in the equilibrium of the carbonate/bicarbonate buffering system (Wetzel 1983). The higher ammonia values observed at higher transport water temperatures were probably due to the higher metabolism or activity of fish at warmer temperatures. The effect of aerator type on ammonia levels (higher for diffuser aerators) is more difficult to explain but may be an indirect effect of the higher CO_2 levels. CO_2 has been used to stun salmon prior to slaughter, but has been shown to be stressful to the fish (Azam et al. 1989). Therefore, the high level of CO_2

TABLE 2. *Extended.*

Weight loss 72-h post-processing (%)	pH 72-h post-processing	Serum cortisol (ng/mL)
1.53 a	6.69	62.8
1.42 a	6.72	65.7
1.52 a	6.61	111.3
1.47 a	6.65	67.3
1.24 a, b	6.69	107.5
0.92 b	6.73	50.9
−0.17	0.04	32.5
−0.03	0.07	−25.1
0.40*	0.00	−14.9
0.42*	−0.08	10.13
0.14	0.05	23.3
OL		

may have produced a physiological response that resulted in increased ammonia production. In future tests, using some sort of agitator in combination with pure oxygen diffuser could provide increased oxygen levels while reducing CO_2 accumulation.

It was not possible in the present study to determine if observed differences in fillet quality were direct effects of aerator type/ oxygen level or indirect effects of aerator type/oxygen level on other water quality parameters, in particular CO_2 level. However, contrasts among treatment group means summed across temperature (i.e., mean for blower aeration/low oxygen level across temperatures vs. mean for pure oxygen/low oxygen level across temperatures vs. means for pure oxygen/high oxygen across temperatures) indicated that oxygen level was the primary factor affecting fillet quality in this study. Fillet drip-loss and 'L' values were not different between blower/ low oxygen and pure oxygen/low oxygen groups even though there were large differences in CO_2 levels between these groups. However, drip-loss and 'L' values were lower for the pure oxygen/high oxygen group than other groups, whereas the CO_2 values for this group were intermediate to CO_2 levels for blower/low oxygen and pure oxygen/low oxygen groups. Therefore, it appears that any effect of CO_2 on fillet quality was minor compared to the effect of oxygen level on fillet quality.

A better understanding of the interactions between transport water CO_2 and oxygen levels will be useful in determining how catfish physiology is impacted during transport and how these physiological responses affect subsequent meat quality. Although elevated CO_2 levels in transport water result in respiratory acidosis due to increased blood CO_2, CO_2 is also known to have anesthetic effect on fish (Iwama and Ackerman 1994), which could reduce activity and decrease the resulting metabolic acidosis associated with lactate accumulation during exhaustive activity in fish (Erikson et al. 1997). The negative impacts of harvest and transport stress/activity on meat quality reported in fish and other livestock are generally attributed to the lactate accumulation and the associated rapid drop in muscle pH (Bendall and Swatland 1988; Offer 1991; Rathgeber et al. 1999).

Any speculation about the effects/interactions of CO_2 and oxygen levels in the present study are well beyond the scope of data. However, it was noted that the activity level of fish in the diffuser aeration treatments (high CO_2) was low compared to the activity level in the blower aeration treatments. Other studies at our facility (Bosworth et al. 2003; unpublished data) demonstrated that sedated harvest and carbon dioxide euthanasia of catfish results in respiratory acidosis (rapid decrease in blood pH) but reduces metabolic acidosis (lower muscle lactate, slower rate of muscle pH decline) and improves meat quality (lower 'L' values and drip-loss) relative to standard hauling procedures. Determination of the influences and interactions of fish activity, transport water oxygen, and CO_2 levels on respiratory and metabolic acidosis, and resulting impacts on meat quality in catfish could provide information useful to catfish producers and processors.

Summary

The results of this study indicate that increasing transport DO levels could improve channel catfish fillet quality. A more complete understanding of the physiological basis for the observed effects on fillet quality and an economic analysis of the costs and benefits associated with increasing transport oxygen are needed to determine if changing current transport conditions is warranted.

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