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Effects of strain and body weight on low-oxygen tolerance of channel catfish (*Ictalurus punctatus*)

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Abstract Low-oxygen tolerance is important for aquaculture species, because exposure to hypoxia can result in heavy mortalities. This study evaluated the effects of strain, body weight, and gender on low-oxygen tolerance in channel catfish (*Ictalurus punctatus*) exposed to a lethal concentration of dissolved oxygen (0.1 mg/L). The variation in low-oxygen tolerance, assessed as the time to loss of equilibrium, of channel catfish from six strains (103KS, Kansas, KMix, Marion, Marion S, and Thompson) was examined. Catfish (15–179 g) showed a large variation in resistant time to hypoxia, ranging from 8 to 104 min, and both strain and body weight contributed significantly to this variation ($P < 0.05$). 103KS and Marion S strains had higher low-oxygen tolerance than the other strains, while the Marion strain had the poorest low-oxygen tolerance ($P < 0.05$). In addition to genetic background, body weight positively correlated with low-oxygen tolerance, but there were no significant differences between female and male catfish in low-oxygen tolerance. The results indicate that genetic background and body weight are important factors that contribute variations in low-oxygen tolerance.

Keywords Low dissolved oxygen · Hypoxia · Environmental stressor · Water quality

Introduction

Aquatic animals are living in low-oxygen environments (in the range of ~10 ppm). Under intensive aquaculture conditions, hypoxia occurs frequently due to high stocking density. Although fish are efficient in oxygen utilization in water, exposures to hypoxia can still result in high mortalities. In many instances, although fish can survive hypoxic exposures, adverse effects of hypoxia are realized through reduced metabolic rate (Aboagye and Allen 2014),

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reduced growth rate and feed conversion efficiency (Buentello et al. 2000), and increased susceptibility to diseases (Welker et al. 2007). All these negative effects of hypoxia can lead to slow response to other environmental stresses and low survival ability for fish.

Many factors have been reported to affect low-oxygen tolerance in fish including gender, body size, as well as genetic background. Sexual dimorphism is common in various performance traits of fish including low-oxygen tolerance. For instance, male mosquitofish (*Gambusia affinis*) were reported to have higher mortalities than females under the extreme hypoxic conditions (Cech et al. 1985). Fish size as reflected in body weight is another factor affecting low-oxygen tolerance. As reported for largemouth bass (*Micropterus salmoides*), fish with larger body weight tend to be less tolerant to hypoxia due to smaller ratio of gill surface area to total body volume (Burleson et al. 2001). However, opposite observations were also reported. For instance, Almeida-Val et al. (2000) and Sloman et al. (2005) reported that larger fish were more tolerant to hypoxia than smaller ones in Oscar cichlid (*Astronotus ocellatus*). Genetic background was reported to affect growth rate, feed conversion efficiency, and disease resistance; all these effects, in turn, may affect low-oxygen tolerance in fish (Gjerde et al. 2011; Henryon et al. 2002). Strains were reported to contribute to the variation in low-oxygen tolerance of channel × blue hybrid catfish (Dunham et al. 2014).

Channel catfish is the primary aquaculture species in the USA. Many studies have been conducted to reveal the effects of hypoxia on catfish. For instance, cardiovascular activity was found to be altered by hypoxia in channel catfish (Burleson and Silva 2011). Hypoxia was also found to affect the growth, yield, and feed consumption in channel × blue hybrid catfish (Green et al. 2012). Identification of multiple quantitative trait locus (~~QTLs~~) associated with Q1 low-oxygen tolerance suggested a complex genetic architecture of channel catfish in response to hypoxia (Wang et al. 2017). To investigate the effects of both genetic and environmental factors on low-oxygen tolerance, this study was conducted to evaluate the effects of strain, body weight, and gender on the variation in low-oxygen tolerance using survival analysis.

Materials and methods

Experimental fish

All procedures involving the handling and treatment of fish in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Auburn University. All catfish were Q2 produced at the E. W. Shell Fisheries Research Center, Alabama Agricultural Experiment Station, Auburn University, Alabama. One-year-old channel catfish from six strains were used in this study. Information of all experimental fish is summarized in Table 1. The ancestries of the channel catfish strains are detailed in a study by Dunham and Smitherman (1984). In brief, Kansas strain was originated from Ninnescah River in Kansas, while Kansas S has been selected for body weight for eight generations. KMix strain was obtained by crossing Kansas S with Kansas. 103KS strain was obtained by crossing NWAC 103 with Kansas S. Thompson strain was from Mississippi with an early spawning capability. Marion strain was originated from Marion National Fish Hatchery, while Marion S strain was obtained by mass selection from the original Marion population for body weight.

Experimental fish were moved to the Auburn University Hatchery Challenge Facility 1 week before the hypoxia challenge. All fish were anesthetized with MS-222 before being weighed and injected with passive integrated transponder (PIT) tags using intramuscular

Table 1 Summary of experimental fish used in this study

Strains	Female			Male		
	No. of fish	Body weight (g, mean ± SD)	Time before losing balance (min)	No. of fish	Body weight (g, mean ± SD)	Time before losing balance (min)
103KS	45	96.7 ± 31.1	62.0	36	102.9 ± 28.9	61.6
Kansas	32	57.5 ± 20.1	42.6	51	70.2 ± 18.8	43.5
KMix	57	83.1 ± 25.8	49.6	25	80.8 ± 24.3	47.6
Marion	20	52.8 ± 19.7	35.9	27	55.4 ± 22.7	33.9
Marion S	21	82.3 ± 22.8	60.6	22	99.0 ± 26.6	67.5
Thompson	40	101.6 ± 29.2	51.5	17	114.8 ± 26.5	61.9

SD standard deviation for body weight of each strain

injection. Gender of the experimental fish was determined by visual examination of body shape, dorsal fins, and genital shape. All these procedures were conducted 1 week before the hypoxia challenge. The information of PIT tag number, body weight, gender, and strain of each individual was recorded. Then the fish were randomly mixed together and kept in two 305 × 90 cm tanks at 20 °C with aerated flow-through water. The experimental fish were fed once daily, and the concentration of dissolved oxygen (DO) in water was monitored twice a day using a YSI dissolved oxygen meter.

Hypoxia challenge

All experimental fish were acclimated at ambient temperature of 20 °C in the aerated flow-through water for 72 h before challenge. Feeding was stopped 24 h prior to the hypoxia challenge. During the experiment, water temperature was allowed to fluctuate naturally from 20 to 21 °C. To reduce the DO concentration in water, the aerator was turned off, and then sodium sulfite was added to reduce the DO concentration in the water (Boyd and Tucker 2012). The DO concentration was reduced from ~9.0 to 0.1 mg/L in 1 h. DO concentration was kept at 0.1 mg/L by adding additional sodium sulfite, if needed, and constantly monitored by the YSI dissolved oxygen meter. After DO concentration reached a lethal concentration of 0.1 mg/L (starting point for measurement of resistant time), fish were monitored for the signs of losing equilibrium. Fish were removed immediately after loss of equilibrium. The resistant time and sequence of individuals were recorded along with their PIT tag number. After sampling, fish were returned to well-oxygenated water for recovery.

Statistical analysis

Low-oxygen tolerance of channel catfish was statistically analyzed with survival analysis using the "survival" package in the R software (Miller 2011; Therneau 2013). To examine the variations among stains and gender, Kaplan-Meier curves were constructed for the six strains and two gender groups using Kaplan-Meier analysis (Kaplan and Meier 1958). Comparison of different Kaplan-Meier curves was based on the log-rank test (Bland and Altman 2004). Regression analysis was conducted within each of the six strains to determine the effect of body weight on low-oxygen tolerance without the compounding effect of strain. Cox proportional hazards regression model (Cox PH model) was performed with all predictor variables (strain, body weight, gender) and their interactions simultaneously to identify the variables that significantly affected the resistant time to hypoxia (Lin and Wei 1989). Proportional hazards assumption was checked before model fitting using “Rcmdr” package in R (Fox et al. 2009). Four different Cox PH models were developed. First model was a full model with all predictor variables (strain, gender, and body weight) and their interactions. Then, three additional models were developed: one model without the “strain” variable, one model without the “body weight” variable, and one model without the “gender” variable. To determine which variables and interactions were significantly contributed to low-oxygen tolerance, the likelihood ratio test was conducted to compare these three models with the full model. In addition, auto model selection procedure was conducted to select an appropriate model. The selected stratified Cox PH model was used to determine the effect size of strains on the resistant time to hypoxia in catfish. All tests were regarded as statistically significant when $P \leq 0.05$.

Results and discussion

The DO concentration in water was reduced to 0.1 mg/L and kept for the remaining period of the hypoxia challenge. Under the hypoxic condition, the experimental fish reduced their movement and occasionally swim up to the surface of water for higher DO concentration. The first fish lost its balance at 8 min after DO concentration reached 0.1 mg/L, while the last fish lost balance at 104 min. A broad range of variations in resistant time to hypoxia was observed in channel catfish.

Large variation in resistant time to hypoxia was identified among six strains by comparing Kaplan-Meier curves of these strains (Fig. 1). Apparently, the most tolerant strains are Marion S and 103KS, followed by Thompson, KMix, Kansas, and Marion strains. Results of pairwise comparison between these curves using log-rank test demonstrated that the resistant time to hypoxia for 103KS and Marion S strains was significantly longer than Kansas, Kmix, and Marion strains, whereas Thompson strain had a

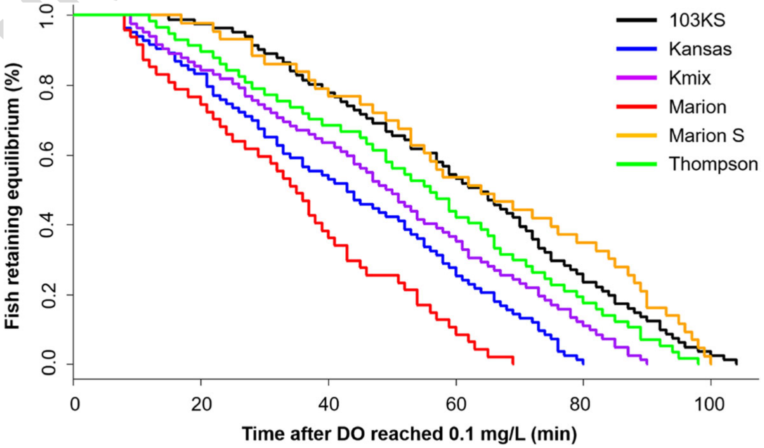


Fig. 1 Variation in resistant time to hypoxia among six channel catfish strains. Kaplan-Meier curves were constructed with resistant time to hypoxia for six strains using Kaplan-Meier analysis

Q3 **Table 2** Pairwise comparison of low-oxygen tolerance among six channel catfish strains using log-rank test

Strains	103KS	Kansas	KMix	Marion	Marion S	Thompson
103KS		26.68	12.19	36.48	0.48	5.05
Kansas	<0.0001*		2.49	0.03	25.94	10.45
KMix	0.0072*	0.8390		3.14	10.24	2.20
Marion	<0.0001*	1.0000	0.6971		36.50	14.39
Marion S	1.0000	<0.0001*	0.0204*	<0.0001*		3.08
Thompson	0.3118	0.0182*	0.8920	0.0022*	0.7100	

The numbers above the diagonal are the chi-squared values for pairwise comparison, while the numbers below the diagonal are the corresponding *P* values. Asterisks (*) indicate significant differences ($P < 0.05$)

significantly longer resistant time to hypoxia when compared with Kansas and Marion strains (Table 2). Similarly, Kaplan-Meier curves of female and male catfish were established to determine gender effect on low-oxygen tolerance (Fig. 2). Results of log-rank test between Kaplan-Meier curves of males and females revealed no significant difference between males and females ($P = 0.471$), suggesting that gender is not an important effector for low-oxygen tolerance.

To further verify the effects of strain, gender, and body weight on low-oxygen tolerance, both Cox PH model and likelihood ratio test were conducted. Results of likelihood ratio test of four Cox PH model demonstrated that (1) both strain and body weight had significantly effects on resistant time to hypoxia with a *P* value of 0.002 and $3.997e^{-15}$, respectively, whereas (2) gender had no significant effect on resistant time to hypoxia with a *P* value of 0.574. These results further confirmed the results from log-rank test on different Kaplan-Meier curves. Taken together, strain and body weight are two important effectors which significantly affect low-oxygen tolerance in catfish.

To determine the effect of body weight on low-oxygen tolerance without the compounding effect of strain, regression analysis was also conducted within each of the six strains. As presented in Fig. 3, body weight had a positive correlation with resistant time to hypoxia within each of six strains, although the correlation coefficient *R* varied between 0.23 (Marion

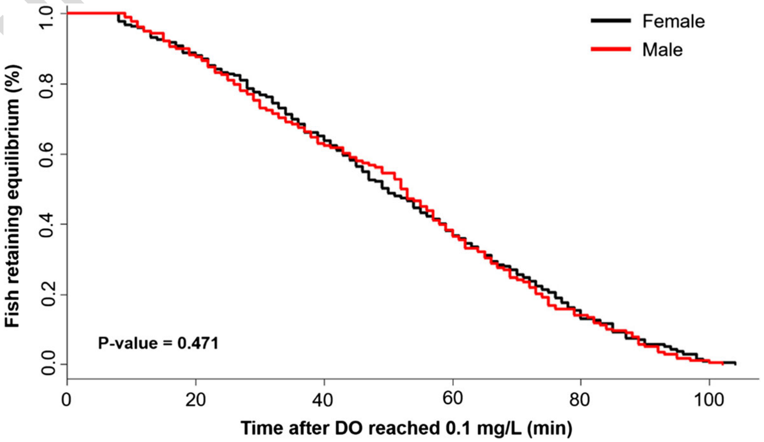
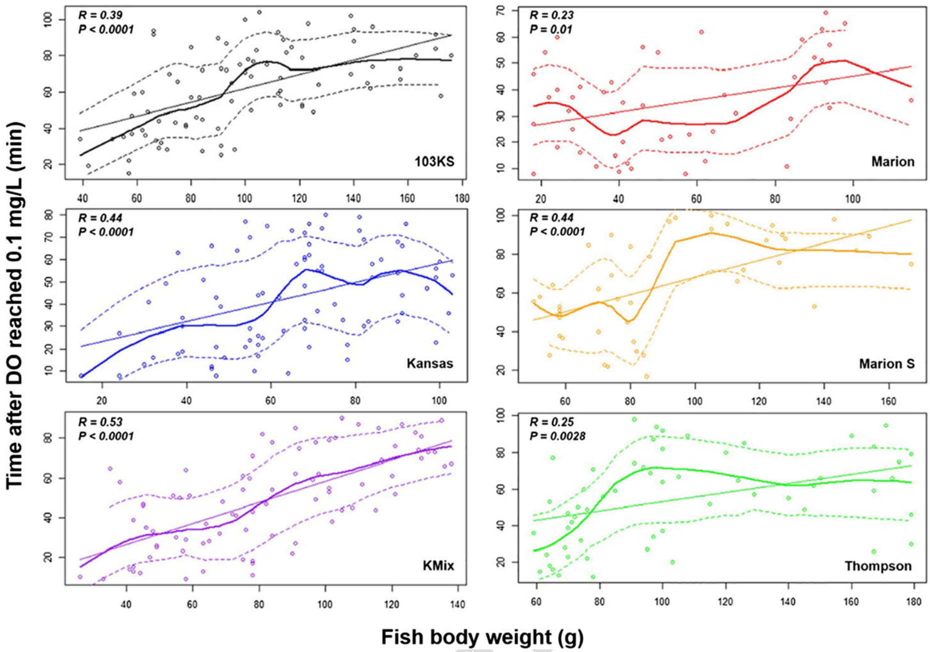


Fig. 2 Comparison of resistant time to hypoxia between female and male channel catfish. Kaplan-Meier curves were constructed for female and male catfish using Kaplan-Meier analysis



Q4 **Fig. 3** Correlation of body weight with low-oxygen tolerance within strains. Linear regression analysis was conducted using body weight and resistant time to hypoxia within each of the six strains

strain) to 0.53 (KMix strain). This result suggests that fish with larger body weight tend to be more tolerant to hypoxic stress, but the extent of body weight effect on low-oxygen tolerance is varied among strains.

To determine the effect size of strain and body weight on resistant time to hypoxia in catfish, model selection with stepwise procedure was conducted to obtain a fitted Cox PH model (Burnham and Anderson 2004). The results of the Cox PH model are listed in Table 3. The most fitted model only included strain and body weight, indicating that strain and body weight independently contributed to the observed variation in resistant time to hypoxia ($P < 0.05$). The stratified Cox PH model was used to estimate effect size of strain as the variable body weight failed the assumption of proportionality (Grambsch and Therneau 1994; Therneau and Grambsch 2000). Based on the hazard ratio, effect size of

Table 3 Analysis of strain effects on low-oxygen tolerance with stratified Cox proportional hazards model by considering body weight variations among strains

Strain	Coefficient	Hazard ratio	*P* value
103KS	−0.5043	0.6039	0.03
Kansas	−0.1722	0.8418	0.44
Marion	0.7855	2.1935	0.02
Marion S	−0.5723	0.5642	0.05
Thompson	−0.0608	0.9410	0.81

The KMix strain was used as a reference in this model, and all the values were obtained by comparing with KMix strain

strain on resistant time to hypoxia was calculated (Table 3). Using KMix strain as a reference, Marion S strain had a 44% higher chance, 103KS strain had a 40% higher chance, Kansas strain had a 16% higher chance, Thompson strain had a 6% higher chance to maintain balance, while Marion strain had a 1.19-fold lower chance to maintain balance under hypoxic conditions. The 103KS and Marion S strains had significantly higher low-oxygen tolerance with a P value of 0.02 and 0.05, respectively. Marion strain had significantly lower level of low-oxygen tolerance ($P = 0.02$). These results suggested that 103KS and Marion S strains may be good choice for the breeding programs to improve low-oxygen tolerance.

Surprisingly, this study demonstrated that body weight was positively correlated with resistant time to hypoxia in channel catfish. This finding is the opposite of what was reported with channel × blue catfish that larger fish were less tolerant to hypoxic stress (Dunham et al. 2014). Several factors may explain this difference: (1) different catfish were used in these two studies; channel catfish from six strains were used in this study, while Dunham et al. used channel × blue catfish in their study; (2) the range of fish size was different, which could be a major cause for the opposite results. Juvenile catfish with relatively small body weight were used in this study, whereas 2-year-old hybrid catfish with larger body weight were used in the other study (Dunham et al. 2014). For juvenile fish, the "vitality" would be expected to increase with body weight. For example, fish have to rely on anaerobic ATP production (glycolysis) for survival under sever hypoxic conditions; smaller fish with higher mass-specific metabolic rate can run out of glycogen or reach lethal levels of anaerobic end products much faster than larger ones (Nilsson and Östlund-Nilsson 2008). However, once body weight increases beyond a certain threshold, the increasing of body weight can reduce the relative ratio of surface areas of gill to body size, which may lead to the observed results by Dunham et al. (2014). Future studies are required to delineate which of these, or additional reasons, accounted for these different results. Q5

Six strains used in our study have not been widely used in aquaculture. However, these strains are heavily used in catfish breeding programs for future applications in aquaculture. This study, in spite of being an initial study, suggested that the genetic architecture of low-oxygen tolerance is quite complex. The strong strain effect on low-oxygen tolerance may suggest that strain effect is needed to be consider when conducting any QTL mapping studies, as QTL identified in one strain may not be operational in another, as reported by Wang et al. (2017). Effect of fish size on low-oxygen tolerance may be variable. Taken results from our study, along with a study by Dunham et al. (2014), a quadratic relationship may exist between body weight and low-oxygen tolerance. Body weight positively affects the low-oxygen tolerance in a certain threshold, but beyond this threshold, body weight may negatively affect the low-oxygen tolerance. Future studies are warranted to determine the exact correlation of body size with low-oxygen tolerance.

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Compliance with ethical standards All procedures involving the handling and treatment of fish in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Auburn University.

Conflict of interest The authors declare that they have no conflict of interest.

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AUTHOR QUERIES

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