Cortisol Responsiveness to Stress in Juvenile Channel Catfish Influences Susceptibility to Enteric Septicemia of Catfish

Nagaraj G. Chatakondi, *USDA ARS Warmwater Aquaculture Research Unit, P. O. Box 38, Stoneville, MS 38776*

Brian C. Peterson, *USDA ARS Warmwater Aquaculture Research Unit, P. O. Box 38, Stoneville, MS 38776*

Abstract: Stress is unavoidable in aquaculture and hence strains of fish that are resilient and adaptable to stress need to be developed. In teleosts, cortisol is considered the primary stress hormone and often increases in cortisol concentration correspond to a stress response. The objective of this study was to assess if cortisol responsiveness to stress in channel catfish (*Ictalurus punctatus*) influences susceptibility to Enteric Septicemia of Catfish (ESC) caused by *Edwardsiella ictaluri* under controlled conditions. Juvenile channel catfish were subjected to standardized hypoxia stress (1.8 mg L^{-1} of dissolved oxygen) to classify them as either low responders (LR) or high responders (HR) based on their plasma cortisol concentration. Fish in both groups were held either in individual or co-cultured in 80-L aquaria and were challenged with virulent *E. ictaluri* by an *in situ* bath immersion to evaluate their susceptibility to the pathogen. At the end of the 21-day challenge, mean percent mortality of LR fish (38.1%) was significantly lower than that of HR fish (59.0%). Mean mortality of channel catfish was positively related to their mean plasma concentration of cortisol. An increase in susceptibility of HR fish to ESC may be the result of their higher responsiveness to standardized stress. Hence, the results of the present study suggest LR fish may be more resilient and adaptable to stressful conditions than HR fish.

Key words: mortality, *Edwardsiella ictaluri*, stress tolerance

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Fish are the largest and most diverse group of vertebrates, and they have adapted and survived in a broad range of environments. Fish grown in intensive culture conditions are often exposed to acute or chronic stressors which can affect growth, immunocompetence, and flesh quality (Barton and Iwama, 1991, Pottinger and Carrick 2001, Overli et al. 2006) among other things. The non-neurological stress response involves behavioral, endocrine, and metabolic responses (Crespel et al. 2011). In all vertebrates, including fish, an increase in concentration of corticosteroid hormones in plasma is a measure of stress. Activation of the hypothalamus-pituitary-interrenal (HPI) axis elevates the levels of corticosteroid in blood (Wendelaar-Boonga 1997). Cortisol is considered the principal corticosteroid in teleosts (Kime 1977), and concentrations of cortisol increase rapidly following a stress event (Small et al. 2008). Cortisol is a casual factor in many of the deleterious effects of chronic stress (Pankhurst and Van Der Kraak 2000, Bernier 2006), and cortisol response to stress is an individual characteristic that can be consistent over multiple exposures to stressors (Pottinger and Carrick 2001).

Disease is considered the greatest cause of reduced productivity in aquaculture production systems (Plumb and Vinitnantharat 1993), and culture intensifications in the catfish industry has exacerbated disease susceptibility (Small and Bilodeau 2005). Similar to any aquaculture industry, production conditions and practices for channel catfish (*Ictalurus punctatus*) can result in stressful conditions that affect their physiology. Enteric Septicemia of Catfish (ESC) caused by *Edwardsiella ictaluri* is one of the leading causes of fingerling mortalities in the U.S. farm-raised catfish industry (Wise et al. 2015). Losses to ESC-related mortalities have surpassed US$40–60 million annually (Shoemaker et al. 2009). In general, stress from low dissolved oxygen (DO) concentrations on fish farms escalate susceptibility of channel catfish to diseases (Welker et al. 2007). Because of the varied but consistent stress response among individuals, post-stress changes in cortisol concentration has been the basis for developing a strain of rainbow trout (*Oncorhynchus mykiss*) having high tolerance to stress (Pottinger and Carrick 1999). Fish exhibiting a low stress response to disease conditions have been observed to survive better (Trenzado et al. 2003, Martins et al. 2006). In addition, faster growing families of channel catfish have also exhibited a lower stress response (Peterson et. al. 2008).

In this study, we hypothesized that susceptibility of channel catfish to virulent *E. ictaluri* would vary according to acute standardized stress (i.e., low responders [LR] vs high responders [HR]). Thus, the objectives of the present study were to determine 1) if a measure of stress responsiveness of channel catfish is associated with mortality due to ESC, 2) whether communal rearing of LR and HR channel catfish affect their disease resistance to ESC, and 3) if cortisol responsiveness is correlated with body weight of channel catfish.

Methods

Fish

The study was conducted in 2014 using six-month-old juveniles of mixed families of Delta Select strain channel catfish developed at the U.S. Department of Agriculture, ARS Warmwater Aquaculture Research Unit, Stoneville, Mississippi. Individual fish ($n = 270$) were anesthetized with 100 mg L^{-1} tricaine methanesufonate (MS222), weighed to nearest 0.1 g, measured total length (TL, mm), and tagged with passive integrated transponder (PIT) tags inserted at the base of the pectoral fin. The fish were allowed to recover from the anesthesia in a 5-L buckets equipped with aerators. Fully recovered fish that gained complete equilibrium and were swimming normally were stocked at 10 fish per aquarium (80 L) with a flow rate of 2 L min^{-1}. Each aquarium was provided with flow-through well water (28 °C) and aerated with diffused air (DO levels of 6 mg L^{-1}). The fish were fed a 35% protein commercial catfish feed to satiation once a day for two weeks.

Low Dissolved Oxygen Stress Test

Fish were not fed for two days prior to the low DO stress test. At the end of this period, channel catfish were subjected to a low DO stress challenge by turning off air and water, and nitrogen gas was bubbled through the water column to displace remaining oxygen following the procedures described by Small (2004). Target DO levels for this challenge were 1.8 mg L^{-1} (25% of DO saturation at 28 °C); DO was measured with an YSI model 55 (Yellow Springs Instruments Inc., Yellow Springs, Ohio). The fish were exposed to this level of DO for 5 min and then anaesthetized in metomidate hydrochloride (6 mg L^{-1}) which effectively blocks the handling-related stress release of cortisol into circulation, thus decreasing plasma cortisol variability due to sampling (Small 2004). A 200-uL sample of blood from the caudal peduncle of individual fish was taken with a heparinized 1-mL (23-gauge) disposable syringe. The blood was dispensed into a labeled 1.5-mL microcentrifuge tube and held on ice. Blood samples were centrifuged at 10,000 relative centrifugal force units for 10 min, and the plasma was removed and stored in marked microcentrifuge tubes. Plasma samples were stored at –20 °C initially and later stored at –80 °C until assayed for cortisol following the radio-immunoassay protocol described by Small and Davis (2002).

Application of a low DO stress on channel catfish resulted in an increase in plasma cortisol concentrations in the range of 10–110 ng mL^{-1}. Selection criterion based on rank of cortisol concentration described by Weil et al. (2001) was followed. Channel catfish that were ranked 50% or lower were designated as LR and channel catfish that were ranked 51% or higher were designated as HR. Individual LR and HR fingerlings were identified by their PIT-tags and were held in individual aquarium with 20 fish per tank. Fingerlings in the aquaria were held for two weeks to recover from the low DO stress test.

ESC Disease Challenge

LR and HR catfish were either co-cultured (five aquaria with 10 LR paired with 10 HR fish per aquarium) or mono-cultured (7 aquaria with 20 fish per aquarium; three aquaria with LR and four aquaria with HR fish). LR and HR fish were acclimated for two weeks in aquaria conditions even though effects of PIT-tagging and low DO stress test would be negated in 24 h. Channel catfish fingerlings that were neither PIT-tagged nor subjected to low DO stress were held in three aquaria at the same density to serve as controls. All fish were acclimated in aquaria for two weeks to ensure stress effects in treatment fish resulting from PIT-tagging and DO-stress trials had dissipated. Small et al. (2008) found that channel catfish subjected to stress conditions returned to normal cortisol levels within 24 h of the stress event. Thus, both treatment and control fish should have been at baseline cortisol levels at the beginning of the ESC trials.

A virulent *E. ictaluri* isolate (Strain S97-773) from a natural outbreak (confirmed by Mississippi State University Fish Diagnostics Lab) was used for the ESC disease challenge under controlled conditions. Fish were challenged with virulent *E. ictaluri* (1.9×10^8 cfu mL^{-1}) by an *in situ* bath immersion for 30 minutes (Booth and Bilodeau-Bourgeois, 2009). Fish in control aquaria received broth that was devoid of pathogen (mock challenge). Feeding was suspended to challenged and control fish held in aquaria a day before the challenge and resumed a day after challenge at the normal rate described above. Dead and moribund fish (fish that were swimming in circles and upside down) were removed from aquarium once daily for 21 days and their PIT-tag number was recorded. Percent cumulative mortality of LR and HR fish were compared during the 21-day post-disease challenge period. A random sample of four dead fish was submitted to the Mississippi State University Aquatic Research and Diagnostic Laboratory for confirmation that the fish died of ESC. At the end of the study, fish remaining in all the aquaria including controls were euthanized with an overdose (0.5g L^{-1}) of MS222.

Statistical Analyses

Experimental data were subjected to analyses of variance (ANOVA) mixed-model procedures using the SAS software system version 9.11 (SAS Institute 2012). A 2×2 factorial design was employed to compare two factors: cortisol response (LR, HR) and stocking method (co-culture or mono-culture). The interaction of cortisol response and stocking method (LR, HR and LH + HR) was

considered as fixed effect, and the aquarium within a treatment was considered a random effect. Percent cumulative mortality of mean cortisol response of individual aquarium were log-transformed prior to analyses to meet the assumptions for homogeneity and normality of treatment means. When significant differences were found, Tukey's Honestly Significant Difference (HSD) test was used to identify differences at the 0.05 alpha level. Correlation among mean cortisol response of treatment groups and their percent cumulative mortality for 21 d post-disease challenge or their mean body weight or total length was determined using the CORR procedure in SAS.

Results

Tags of 30 fish of the 270 PIT-tagged fish were not readable and those fish were not used in the study. Of the 240 identifiable fish, low DO stress test designated 110 fish as LR and 130 fish as HR. Mean cortisol response of LR channel catfish was lower than that of HR catfish ($F = 50.5$, df = 1, 12, $P = 0.01$; Table 1). However, mean weight or length did not differ between LR and HR fish (F range 0.75 to 2.8, $P > 0.40$). Mean plasma concentration of cortisol of channel catfish was neither related to weight ($r^2 = 0.09$, $P > 0.05$) nor length ($r^2 = 0.003$, $P > 0.05$) of channel catfish. At the end of the 21-day ESC disease challenge, mean mortality of LR and HR groups held either separately or communally did not differ ($F = 0.99$, $df = 1,12$, $P = 0.34$), therefore all data for LR and HR fish were pooled regardless of how they were cultured. Percent mean mortality of LR channel catfish (mean plasma cortisol concentration = 33.1 ± 8.5 ng mL^{-1}) ranged from 25% to 45%, whereas percent mean mortality of HR fish (mean plasma concentration = 68.3 ± 11.8 ng mL^{-1}) ranged from 50% to 70%. No mortalities or evidence of ESC were observed in the control group during the disease challenge, but all four of the dead fish in the ESC challenge groups tested positive for the disease. Mean mortality of channel catfish was related to their mean plasma concentration of cortisol (Mean mortality = 0.89*mean plasma cortisol – 8.71, $P < 0.05$; Figure 1). Percent mean cumulative mortality of LR fish and HR fish were similar in the first five days of ESC disease challenge; however, on days 6–21 mean cumulative morality of HR fish (59%) was significantly higher than mean cumulative mortality of LR fish (39%; $F = 50.5$, df = 1, 12, $P = 0.01$; Figure 2).

Table 1. Mean (±SD) weight, length, cortisol concentration, and mortality of low responder (LR) and higher responder (HR) channel catfish used in low-DO stress trials. Means with the same superscript were similar (ANOVA, $P > 0.05$).

Phenotype	*n*	Weight (g)	Length (cm)	Cortisol (ng/mL)	Mortality (%)
LR	110	$44.5^a \pm 8.6$	$18.5^a \pm 2.7$	$33.1^a \pm 8.5$	$38.1^a \pm 2.1$
HR	130	$41.2^a \pm 6.5$	$17.4^a \pm 3.1$	$68.3^b \pm 11.8$	$59.0^b \pm 1.8$

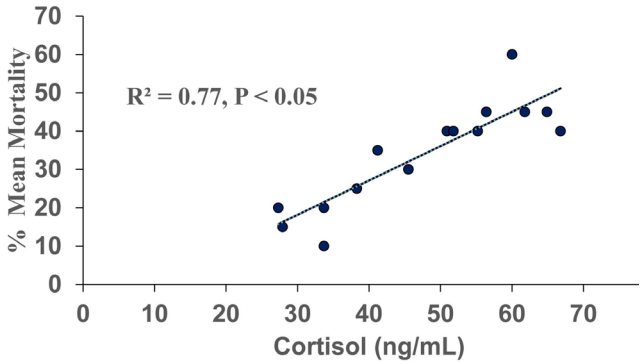

Figure 1. Relationship between mean plasma concentration of cortisol and mean percent mortality of channel catfish subjected to *Edwardsiella ictaluri* disease under controlled conditions.

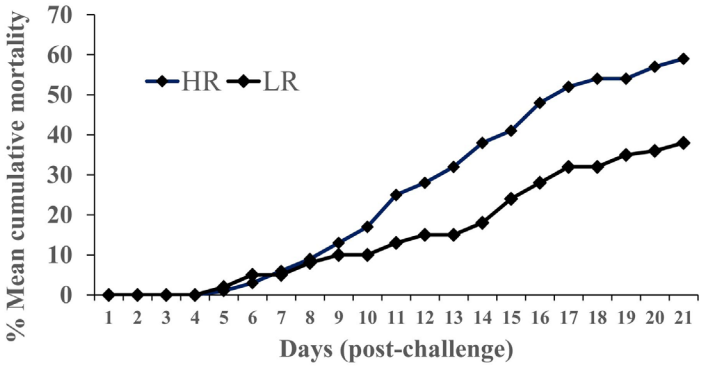

Figure 2. Mean percent cumulative mortality of LR (low responders) and HR (high responders) channel catfish fingerlings in replicated aquaria subject to *Edwardsiella ictaluri* challenge during the 21-day post-disease challenge. There was no mortality in the control group.

Discussion

The cortisol responsiveness to stress in channel catfish and their associated mortalities subject to ESC disease challenge were demonstrated in the present study. Cortisol response in channel catfish exposed to sub-lethal hypoxia has been shown to activate the stress response (Tomasso et al. 1981). Welker et al. (2007) and Small (2004) also suggested that cortisol concentrations provide an adequate measure of stress response to hypoxia in channel catfish. Episodes of stress often invoke disease outbreaks in cultured fish, and stress is typically characterized by an increase in concentration of cortisol.

In the present study, channel catfish were classified as LR and HR based on their ranks of cortisol concentration criterion suggested previously by Weil et al. (2001). Previously, Hori et al. (2011) categorized Atlantic cod (*Gadus morhua*) either as high or low cortisol responders in 10 families based on their plasma cortisol levels subject to 1-h post-handling stress; cortisol response of these fish was 2.5-fold higher in HR fish. Similar results were also found in our study, but the difference in cortisol concentrations between

groups was only 1.5-fold, which may be attributed to differences in species, life stages, and number of families available from which to choose, type of stressor chosen, and duration of stress.

Results of this study suggested that cortisol responsiveness in channel catfish was correlated with mortalities associated with ESC. Also, LR and HR catfish that were either co-cultured or mono-cultured did not affect their susceptibility to ESC under controlled conditions. Previously, Sink and Strange (2004) observed ESC-related mortalities were 22.5% for non-stressed channel fish and 81.7% for stressed fish. Small and Bilodeau (2005) also observed higher ESC related mortalities of stressed channel catfish compared to non-stressed fish. An increase in cortisol concentration of confinement-stressed channel catfish fingerlings positively correlated with susceptibility to ESC (Klesius et al. 2003, Sink and Strange 2004). Cortisol responsiveness of channel catfish to a standardized stress was positively correlated to ESC mortality in our study. Sink and Strange (2004) concluded that an increase in ESC mortality in stressed fish may be a result of increased cortisol concentration to arbitrate reduction in immunity, thus supporting our hypothesis.

Many mechanisms are involved in the transduction of a stress response into increases in plasma cortisol and plasma glucose (Mommsen et al. 1999). The extent to which cortisol and glucose responses are correlated likely depends on where genetic variation in these mechanisms exists. Ellsasser and Clem (1987) found that cortisol injection reduced the densities of circulating lymphocytes, while increasing the densities of neutrophils in channel catfish. Similar increases in cortisol were associated with decreases in leukocytes in the blood and spleen of juvenile coho salmon (*Oncorhynchus kisutch*) after stress treatments, and a reduction in the distribution of leukocytes in bloodstream were observed (Maule and Schreck 1990). A reduction in circulating leukocytes in the bloodstream of a fish will likely make it more susceptible to septicemia, providing a direct link between cortisol response and increased disease mortality (Kaattari and Tripp 1987).

Bilodeau et al. (2003) determined plasma cortisol concentrations in susceptible and resistant families of channel catfish exposed to ESC disease challenge. Mean plasma cortisol concentrations were lower in susceptible families compared to resistant families. Peak cortisol concentration in susceptible families was evident until day 5 after pathogen exposure. However, the peak cortisol in resistant families was observed until the second day of pathogen exposure. Chronic elevation in circulating cortisol levels were considered to have deleterious effect in susceptible families to ESC. In the present study, similar such elevations in cortisol levels may have occurred in HR fish that would have resulted in higher ESC mortalities.

Pottinger and Carrick (2001) observed that LR rainbow trout had higher growth rates compared to HR trout when reared together; but this difference was not evident when the groups were reared separately. Performance of LR and HR channel catfish in our study did not differ when reared either in mono or co-culture. This discrepancy between the two studies may be attributed to differences in species and type of stressors. Peterson et al. (2008) selected four high and four low-growth families from 64 families of a select strain of channel catfish and observed an inverse relationship between cortisol stress response and body weight of channel catfish fingerlings. Stressors can affect growth through the actions of cortisol; exposure of stressful events, such as oxygen-depleted waters elevate plasma cortisol concentration in channel catfish (Tomasso et al. 1981, Small 2004). Further, Davis et al. (1995) suggested that stressful conditions trigger the release of cortisol release in circulation to promote protein catabolism, lipolysis, and inefficient feed conversion to retard growth.

Mortality rates increase with cortisol concentrations when healthy fish are stressed before becoming infected with disease. In our study, LR fish had higher survival when exposed to *E. ictaluri* than HR fish, supporting our hypothesis that lower cortisol stress response reduced mortalities associated with ESC. Developing mitigating strategies to overcome stressful conditions in aquaculture are needed, and our results suggested that a possible strategy would be to develop LR fish for use in production facilities.

The aim of the study was to assess the relationship between cortisol stress response of channel catfish and susceptibility to ESC disease. It was also determined that co-culture of LR and HR fish did not affect their susceptibility to ESC, and cortisol responsiveness in channel catfish was not correlated to body weight. In general, readily measurable cortisol has been widely employed as a major index of stress in fish. Past studies suggest that magnitude of cortisol elevation in response to stress in fish is an individual characteristic (Pottinger et al. 1992). Crosses of parental fish selected for high and low cortisol responsiveness result in progeny with similar cortisol responsive traits. Cortisol responsiveness is a low to moderately heritable trait in fish (Fevolden et al. 2002).

Cortisol responsiveness to stress has been identified as a trait of interest for genetic improvement (Weber and Silverstein 2007). Stress has negative impacts on many aquaculture production traits, such as growth, feed efficiency, disease resistance and reproduction. To date, selection has been focused on characteristics associated with growth and disease resistance, both of which can be affected by stress. Hence it follows that selective breeding of fish with a lower (reduced) response to stressors would improve the performance of fish raised under aquaculture conditions as they can tolerate stressful conditions and have lower disease suscepti-

bility and death losses. Selecting and developing LR fish that are resilient and tolerable is a desirable trait for intensive aquaculture production. Our findings suggest that it may be possible to genetically select disease resistant fish based on a specific, easily measurable physiological parameter-cortisol responsiveness to stress. These fish should be more resilient and have reduced mortalities associated with ESC compared to other conspecifics and are projected to have superior growth and survival in intensive production systems.

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Literature Cited

Barton, B. A. and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1:3–26.

Bernier, N. J. 2006. The corticotropin-releasing factor system as a mediator of appetite-suppressing effects of stress in fish. General and Comparative Endocrinology 146:45–55.

Bilodeau, A. L., B. C. Small, and W. R. Wolters. 2003. Pathogen loads, clearance and plasma cortisol response in channel catfish *Ictalurus punctatus* (Rafinesque) following challenge with *Edwardsiella ictaluri*. Journal of Fish Diseases 26:433–437.

Booth, N. J. and A. L. Bilodeau-Bourgeois. 2009. Proteomic analysis of head kidney tissue from high and low susceptibility families of channel catfish following challenge with *Edwardsiella ictaluri.* Fish and Shellfish Immunology 26:193–196.

Crespel A., L. Bernatchez, D. Garant, and C. Audet. 2011. Quantitative genetic analyses of the physiological stress response in three strains of brook charr, *Salvelinus fontinalis* and their hybrids. Journal of Fish Biology 79:2019–2033.

Davis, K. B., B. A. Simco, and C. A. Goudie. 1995. Genetic and hormonal control of sex determination in channel catfish. Pages 244–246 *in* F. Goetz and P. Thomas, editors. Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish. The University of Texas, Austin.

Ellsasser, C. F. and L. W. Clem. 1987. Cortisol-induced hematologic and immunologic changes in channel catfish, *Ictalurus punctatus*. Comparative Biochemistry and Physiology A 87: 405–408.

Fevolden, S. E., K. H. Roed, and K. T. Fjalestad. 2002. Selection response of cortisol and lysozyme in rainbow trout and correlation to growth. Aquaculture 205:61–75

Hori, T. S., A. K. Gamperl, C. E. Hastings, G. E. Vander Voort, J. A. B. Robinson, S. C. Johnson, and L. O. B. Afonso. 2011. Inter-individual and -family differences in the cortisol responsiveness of Atlantic cod (*Gadus morhua*). Aquaculture 324–325:165–173.

Kaattari, S. L. and R. A. Tripp. 1987. Cellular mechanisms of glucocorticoid immunosuppression in salmon. Journal of Fish Biology 31(Supp A):129–132.

Klesius, P. H., C. A. Shoemaker, and J. J. Evans. 2003. The disease continuum model: bi-directional response between stress and infection linked by neuroimmune change. Pages 13–34 *in* C. S. Lee and P. J. O'Bryen, editors. Biosecurity in aquaculture production systems: exclusions of pathogens and other undesirables. The World Aquaculture Society, Baton Rouge, Louisiana.

Kime. 1977. Measurement of 1 alpha- hydroxycoticosterone and other corticosteroids in elasmobranch plasma by radioimmunoassay. General and Comparative Endocrinology 33:344–351

Martins, C. I. M., J. W. Schrama, and J. A. J. Verreth. 2006. The relationship between individual differences in feed efficiency and stress response in African catfish, *Clarias garipinus*. Aquaculture 256:588–595.

Maule, A. G. and C. B. Schreck. 1990. Changes in numbers of leukocytes in immune organs of juvenile coho salmon after acute stress or cortisol treatment. Journal of Aquatic Animal Health 2:298–304.

Mommsen, T. P., M. M. Vijayan, and T. M. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9:211–268.

Overli, O., C. Sorensen, A. Kiessling, T. G. Pottinger, and H. M. Gjoen. 2006. Selection for improved stress tolerance in rainbow trout *Oncorhynchus mykiss* leads to reduced feed waste. Aquaculture 261:776–781.

Pankhurst, N. W. and G. Van der Kraak. 2000. Evidence that acute stress inhibits ovarian steroidogenesis in rainbow trout *in vivo* through the action of cortisol. General and Comparative Endocrinology 117:225–237.

Peterson, B. C., B. C. Small, G. C. Waldbieser, and B. G. Bosworth. 2008. Endocrine responses of fast- and slow-growing families of channel catfish. North American Journal of Aquaculture 70:240–250.

Plumb, J. A. and S. Vinitnantharat. 1993. Vaccination of channel catfish, *Icatalurus punctatus* (*Rafinesque*) by immersion and oral booster against *Edwardsiella ictaluri*. Journal of Fish Diseases 16:65–71.

Pottinger, T. G. and T. R. Carrick. 1999. A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. Aquaculture 175:351–363.

——— and ——— 2001. Stress responsiveness affects dominant-subordinate relationships in rainbow trout. Hormones and Behavior 40:419–427.

———, A. D. Pickering, and M. A. Hurley. 1992. Consistency in the stress response of individuals of two strains of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 103:275–289.

SAS Institute. 2012. SAS system for linear models. Release 9.4. Cary, North Carolina.

Shoemaker, C. A., P. H. Klesius, J. J. Evans, and C. R. Arias. 2009. Use of modified live vaccines in aquaculture. Journal of the World Aquaculture Society 40:573–585.

Sink, T. D. and R. J. Strange. 2004. Linking stress to the increased susceptibility of channel catfish to enteric septicemia using cortisol. Journal of Aquatic Animal Health 16:93–98.

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

——— and A. L. Bilodeau. 2005. Effects of cortisol ad stress level on Channel catfish, *Ictalurus punctatus* pathogen susceptibility and lysozyme activity following exposure to *Edwardsiella ictaluri*. General and Comparative Endocrinology 142:256–262.

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

———, ———, and B. C. Peterson. 2008. Elucidating the effects of cortisol

and stress on economically important traits in channel catfish. North American Journal of Aquaculture 70:223–235.

Tomasso, J. R., K. B. Davis, and B. A. Simco. 1981. Plasma corticosteroid dynamics in channel catfish, *Ictalurus punctatus,* during and after oxygen depletion. Journal of Fish Biology 18:519–526.

Trenzado, C. E., T. R. Carrick, and T. G. Pottinger. 2003. Divergence of endocrine and metabolic responses to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress. General and Comparative Endocrinology 133:332–340.

Weil, L. S., T. P. Barry, and J. A. Malison. 2001. Fast growth in rainbow trout is correlated with a rapid decrease in post-stress cortisol concentrations. Aquaculture 193:373–380

Welker, T. L., S. T. Mcnulty, and P. H. Klesius. 2007. Effect of sublethal hypoxia on the immune response and susceptibility of channel catfish, *Ictalurus punctatus* to Enteric Septicemia of Catfish. Journal of the World Aquaculture Society 38(1):12–23.

Wendelaar-Bonga, S. E. 1997. The stress response of fish. Physiological Reviews 3:591–625.

Wise, D. J., T. E. Greenway, T. S. Byars, M J. Griffin, and L. H. Khoo. 2015. Oral vaccination of channel catfish against enteric septicemia of catfish using a live attenuated *Edwardsiella ictalrui* isolate. Journal of Aquatic Animal Health 27:135–143.