Genetic Response to Acute Hypoxia in Channel Catfish (*Ictaluruspunctatus*), Blue Catfish (*Ictalurusfurcatus*) and Hybrid Catfish

by

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Abstract

The catfish industry is one of the largestdomestic aquaculture markets in the United States. In aggregate, the industry was valued at $423 million dollars in 2011 and is dominated by the production of channel catfish (*Ictaluruspunctatus*) and, to a lesser extant, hybrid catfish, a cross between channel female and blue male catfish (*Ictalurusfurcatus).* Dissolved oxygen (DO) levels are a critical component governing the success and profitability of catfish pond aquaculture. Low DO levels are known to negatively impact feed utilization/growth, health/stress levels, and ultimately survival. However, major gaps remain in our understanding of differential susceptibilities of channel, blue, and hybrid catfish to low DO and the molecular consequences of these events on critical genes governing metabolism/growth, stress/immunity, and overall physiological functions. Here, therefore, we examined both phenotypic and genotypic responses to acute hypoxia in the three catfish groups. It was determined that genotypic reaction to hypoxia is highly variable between the different catfish families, the various tissues and at between time intervals. Six different known catfish genes were investigated at time points of 2, 4, and 8 hr at 2mg/l dissolved oxygen and at 2 and 4 hr at 1.5mg/L in liver and gill tissue. The observed genes HIF-1, HIF-2, BPI, Ferritin, Myostatin and NKEF showed highly variable regulation changes at different time points and oxygen levels.

Channel and hybrid catfish showed almost identical phenotypic stress response times while blue catfish were significantly quicker to show observable stress. This pattern of hybrid, channel similarity held true across the majority of treatments tested with this pairing showing

much greater sensitivity to the HIF family of genes than their blue counterparts. Hybrid and channel catfish also showed similar genotypic response for BPI genes with multiple significant down regulated time points for BPI genes in gill and liver tissue. Both hybrid and channel catfish recorded their largest fold change of any gene at the 8 hr at 2mg/L time point in the ferritin liver trial reporting an up regulation of 26.9 fold and 75 fold respectively. The only tested gene that showed any similarity between blue and hybrid catfish was myostatin. Blue catfish showed a 24.1 fold up regulation in liver at 4 hr and 1.5mg/L oxygen level while hybrid catfish showed a 21 fold increase in liver tissue at 8 hr at 2mg/L oxygen. Outside of the myostatin gene blue catfish showed muted sensitivity to the treatments compared to channel and hybrid catfish.

This study is one of the few investigating acute hypoxia as it relates to genetic change and so there are few other results to compare to. Our findings provide an early foundation of understanding of the consequences of low oxygen events and should provide a scientific basis upon which to set minimum DO thresholds for catfish aquaculture.

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List of Abbreviations

MS222- TricaineMethanesulfonate

HIF-1- Hypoxia Inducible Factor α subunit

HIF-2- Hypoxia Inducible Factor 2 α subunit

BPI- Bactericidal Permeability-Increasing

NKEF- Natural Killer Enhancing Factor

ESC- Enteric Septicemia of Catfish

LPS- Lipopolysaccharide

m-RNA- messenger Ribonucleic acid

q-RT PCR- Quantitative Real Time Polymerase Chain Reaction

TGFβ- Transforming Growth Factor Beta

ROS- Reactive Oxygen Species

MG/L-Milligrams per Liter

Introduction

Catfish aquaculture is one of the oldest and nationally important forms of aquaculture in North America. Commercial culture of the channel catfish, *Ictaluruspunctatus*, began in the 1960's in Arkansas and Alabama and quickly spread throughout the region with Mississippi becoming the largest producer. This trend continues through today with Mississippi still accounting for the highest percentage of farmed catfish, followed by Alabama, Arkansas, and South Carolina respectively (Hanson and Sites 2011). Domestic production steadily increased to a peak in 2003 of 662 million pounds processed and has since dropped precipitously in 2010 by 327 million pounds, a decrease of 49% and a level of domestic production not seen since the late 1980's (Hanson and Sites 2011). A combination of events have contributed to the decline of the domestic catfish industry include rise in feed costs, increasing labor and land costs, and perhaps most importantly a massive increase in the amount of imported catfish and tilapia from Asian producers.

Imported frozen fillets of Asian catfish were of minimal impact to the industry until 2005 when import quantity reached 30 million pounds of frozen fillet. Since 2005 imported catfish has increased every year to its current total of 204 million pounds (Hanson and Sites 2011). This has represented an almost complete reversal of frozen fillet percentages which in 2005 stood at 80 percent domestic and 20 percent imported for total frozen fillets to the current 76 percent imported and 26 percent domestically produced frozen catfish fillets. Even with this decline, the sales value of domestic catfish products (food fish, brood fish, stockers, fry and fingerlings) was $423 million dollars and 2011 showed profitability in the industry for the first time in

years(Hanson and Sites 2011). Pressure from overseas competitors combined with volatile feed and fuel prices have required domestic producers toimprove their farming practices and efficiency or leave the industry altogether. The only viable ways to accomplish this is through better and different farming practices, such as in-pond raceways, or through genetic improvements that lead to increased yields with minimal additional input costs.

Genetic enhancement has been occurring in aquaculture for as long as farmers have been able to control the conditions under which fish spawn. From the beginning of the industry farmers have chosen the largest and hardiest fish as the parents for future generations. The production of channel catfish initially dominated the industry due to its superior growth to market size of all ictalurid species studied(Dunham et al. 1993). However, it does not necessarily contain a superior genotype for all aspects of aquaculture.

Other species of ictalurids have traits that, taken independent of other aspects, would make them appropriate for culture systems andhave been considered as alternatives for culture or viacross breedingintroduced into existing channel catfish genomes. These species include the bullhead catfishes (genus *Ameiurus*), which tolerate low dissolved oxygen levels but have extremely slow growth and poor resistance to diseases, the white catfish (*Ameiuruscatus*), which has accelerated initial growth, relatively good growth at cold temperatures, and have shown resistance against low dissolved oxygen concentrations, but have slow growth during the adult grow out phase, low dress-out percentage, and poor survival. The flathead catfish (*Pylodictisolivaris*), which exhibitfast growth to market, but are cannibalistic and difficult to harvest. The blue catfish (*Ictalurusfurcatus*) exhibit relatively fast growth, high dress-out percentage, good resistance to enteric septicemia or (ESC), and are easy to harvest via seining, but are considered to have relatively poor resistance to pathogens (Dunham et al. 1993). Taken

in aggregate no other species of ictalurid shows as many characteristics amenable to commercial production as the mainstay of the industry, the channel catfish. After the channelcatfish is the blue catfish in terms of desirable traits for the industry and thus research to increase the production of these two species continues (Dunham et al. 1993). Both of these species show behavior and physical characteristics that, if specifically chosen independent of other deleterious traits and combined, would make the optimal aquaculture catfish.

Traits inwhich channel catfish are superior to the blue catfish are growth, tolerance to handling stress, tolerance of high ammonia, ability to withstand high nitrite, resistance to pathogens, particularly*Flavobacterium columnaris*and the parasite *Ichthyophthiriusmultfiliis*, and earlier sexual maturity (Dunham et al. 1993; Dunham and Argue 2000). Traits for which the blue catfish displays superiority are uniformity of growth, reduced susceptibility to channel catfish virus and *Edwardsiellaictaluri* (ESC), increased seinabilityover channel catfish, and increased dress-out percentage (Dunham et al. 1993; Dunham and Argue 2000). With each species showing different strengths and weaknesses neither can be considered optimal for every culture situation (Dunham et al. 1993).To further cloud the choice of species to culture, both exhibit a high degree of variability in culture traits that arises due to strain variation within each species (Dunham et al. 1993).

With both species showing superiority for various traits culturists have turned to genetic enhancement as a means of improvement**.** Channel catfish, being the dominant culture species, have received the majority of attention for enhancement research. Genetic research to improve the culture traits of catfish officially began in the 1960's although less rigorous selection for growth and size has likely occurred from the moment captive spawning was achieved. Multiple techniques have been employed in order to increase desirable production characteristics, most

notably mass selection of channel catfish for faster growth to market size (Dunham et al. 1987;Dunham and Brummett 1999; Dunham et al. 1999, Rezk et al. 2003), intraspecific breeding programs to isolate and combine preferred characteristics of different strains of channel catfish (Dunham et al. 1983; Dunham et al. 1987), creation of sterile triploid channel catfish (Lilyestrom et al. 1999), and interspecific hybridization (Dunham et al. 1987; Dunham and Brummett 1999; Dunham et al. 1999; Argue et al. 2003). These methods, with the exception of the development of triploid channel catfish (Lilyestrom et al. 1999), have resulted in significantly improved culture traits for the species examined. Mass selection has shown to be one of the most powerful methods for improvements in the growth of channel catfish (Bondari 1983; Dunham and Smitherman 1983b; Dunham et al. 1987; Dunham and Smitherman 1987; Dunham and Brummett 1999; Dunham et al. 1999).Studies have reported up to 50% increase in body weight after four generations of mass selection (Padi 1995). This impressive increase in growth as a response to selection for body weight also resulted in increased survival, feed conversion ratios, and disease resistance (Dunham and Smitherman 1983). While these improvements are substantial, a comparison of two channel catfish lines selected for faster growth for two generations compared to the interspecific cross of channel catfish female X blue catfish male hybrid (CB hybrid) indicated that the hybrid exhibited faster growth than either of the two select lines (Dunham and Brummet 1999).

Intraspecific breeding of various strains of channel catfish have also shown significant improvements for the species. Studies have reported that the intraspecific crossbreed from the pairing of a Marion strain female channel catfish with a Kansas strain male channel catfish (MK) exhibited faster growth to 100g than the CB hybrid. However these gains were mitigated once both fish reached 500g in the same time frame(Dunham et al. 1987). Other studies have

shown67% of intraspecific crossbreeds examined exhibited improved growth compared to parental controls, but reciprocal intraspecific hybrids did not grow at the same rates (Dunham and Smitherman 1983).

From the beginning of controlled selection in the 1960's, a total of fifty different types of ictalurid hybrids havebeencreated (Dupree and Green 1969; Dupree et al. 1969; Dunham et al. 1987; Goudieet al. 1993; Dunham et al. 2000). These hybrids were created as a result of various crosses of channel catfish with other members of the ictalurid family, including the following species: white catfish,brown bullhead (*Ameiurusnebulosus*), yellow bullhead (*A.natalis*),black bullhead (*Ameiurusmelas)*, flathead catfish, and blue catfish (Goudie et al. 1993). Offspring from these combinations produced organisms with characteristics of each of the parents but not always of the desirable trait (Goudie et al. 1993). The majority of these crosses resulted in inferior offspring in opposition to the desired result of an improved organism for culture in a commercial food production setting. The notable exception to these breeding outcomes being the cross between a female channel catfish and a male blue catfish which exhibits over dominance for traits desirable for intensive aquaculture (Dunham et al. 1982; Dunham and Smitherman 1983;Giudice 1966; Dunham et al. 2000).

This hybrid cross of the blue catfish male and channel catfish female has shown significant improvements in a variety of traits.Improvements include growth uniformity (Dunham et al. 1982; Smitherman et al.1983; Argue et al. 2003), accelerated grow out (Giudice 1966; Dunham and Smitherman1981; Dunham et al. 1987; Dunham et al. 1990; Dunham and Brummett1999), enhanced tolerance to lower dissolved oxygen concentrations (Dunham et al. 1983), greater resistance to some diseases (Dunham et al.1990),in particular the major bacterial disease of catfish ESC(Wolters et al. 1996), higher dress-out percentage (Smitherman et al. 1983;

Argue et al.2003), higher catchability or seinability (Tave et al. 1981; Dunham et al. 1982;Smitherman et al. 1983; Dunham et al. 1986), greater feed efficiency (Li et al. 2004), and lower mortality rates (Dunham et al. 1987). Studies have also shown the hybrid to exhibit increased body weight yields of 18-100% over channel catfish (Smitherman et al. 1983; Dunham et al.1987; Dunham et al. 1990; Dunham and Brummett 1999).

One of, if not, the most important aspects in the rearing of aquatic organisms is dissolved oxygen. Low dissolved oxygen levels in water and the resultant physiological stress on almost all fish is well documented. In catfish, hypoxia has been linked to increased susceptibility to *Edwardsiellaictaluri*, *Aeromonus hydrophila* and*Edwardsiellatarda*(Welker et al. 2007),in addition to being implicated as the stress stimulus resulting inhistopathological lesions in the gills, liver, spleen, trunk and head kidneys (Walters and Plumb,1980). In addition to being a causative agent for a wide variety of diseases, hypoxic conditions have been linked to reduced feed consumption and metabolic rate in a range of fish. For any aquacultured species the ultimate goal is growth of that organism. Hypoxia has been linked to suppressed growth in largemouth bass (Stewart et al. 1967), common carp (Chiba 1966), Coho salmon (Hermann et al. 1962; Fisher 1963), northern pike (Adelman and Smith 1970) brook trout (Whitworth 1968), yellow perch (Carlson et al. 1980), and most importantly for this study catfish (Andrews et al. 1973; Buentello et al. 1999 ; Carlson et al. 1980; Green et al. 2012). The list of species impacted by reduced feed intake, feed efficiency, overall metabolic rate and therefore growth as a result of these factors pursuant to hypoxic conditions could extend to nearly every aquatic species ever investigated. However, there are some species that exhibit a high degree of tolerance to hypoxic conditions.

Fish have evolved the ability to cope with a wide variety of physiological stressors present in aquatic environments and, based on the organism's native habitat, have adapted varying degrees of sensitivity to hypoxic stress. Bottom dwelling fish such as flounder often show good hypoxia tolerance (Weber and Dewilde 1975), compared to fish that live in moving, more oxygenated, water such as Chinese sucker (*Myxocyprinusasiaticus*) which exhibits poor hypoxia tolerance (Pan et al. 2007). Hypoxia sensitivity can also be variable within related fish, for example, Grayling is a salmonid with high-oxygen requirements, whereas the related pike (both species belong to Protacanthopterygii) is hypoxia tolerant (Cameron 1973). Some species have evolved highly specialized mechanisms for dealing with hypoxic stress. The crucian carp (*Carassiuscarassius)* possesses blood with extreme affinity for oxygen allowing it to maintain aroutine rate of oxygen consumption down toa water oxygen level of 5–10% of air saturation (Sollid et al.2003). Other species such as the epaulette shark *(Hemiscylliumocellatum)* employ a strategy of extreme metabolic depression (Renshaw et al. 2002). Whatever strategy an organism employs for hypoxia tolerance there is likely an accompanying array of internal genetic events that coincide with the response.

There are a variety of techniques for creating hypoxic conditions in a controlled manner for experimentation. These include the bubbling of nitrogen into water to strip it of oxygen (Nilson 1990; Gracey et al. 2001; Burleson et al. 2002), placing fish in ponds with known hypoxic conditions (Green et al. 2012), removing artificially supplied oxygen from a closed system (Rahman and Thomas 2007; Chen et al. 2012) andthe addition of anhydrous sodium sulfite to water to reduce oxygen (Kramer and McClure 1982; Melnychuck and Chapman 2002). Each technique carries with it pros and cons. Long running experiments with durations of weeks or months generally opt to use ponds due to the cost and complication of continuously adding

supplemental chemicals to the water. However, this technique does not allow tight control of the environment and oxygen levels may fluctuate greatly, both spatially and temporally. Medium duration experiments with high specificity of conditions are most easily achieved via nitrogen bubbling and, with a lesser degree of control, removal of supplemental oxygen. For an acute hypoxic experiment with a high level of control of oxygen levels the simplest method is the addition of anhydrous sodium sulfite to water. The drawback of this technique is that the desired dissolved oxygen level cannot be maintained indefinitely without the continued addition of more sulfite. Our study of genetic response to relatively brief, acute, hypoxic conditions used anhydrous sodium sulfite due to the short duration of the stress event and the necessity of highly controlled oxygen levels.

Genetic research in aquatic species is relatively new compared to mammalian studies and thus there is a less comprehensive understanding of genetic phenomenon. Terrestrial and aquatic environments are extremely different with highly variable selective pressures between the two suggesting that an aquatic organism's genetic response may not be identical to similar stress events encountered by terrestrial animals. This experiment sought to investigate the relative change in mRNA transcripts in six known genes in the three most highly represented species in catfish aquaculture. Channel, blue and hybrid catfish were subjected to acute hypoxic conditions at the end of which liver and gill tissue were harvested for molecular genetic testing. RNA was extracted and subjected to analysis by qRT-PCR to determine relative changes in gene expression between different tissues, time points and oxygen levels. The genes selected for this experiment, HIF-1, HIF-2, myostatin, ferritin, NKEF and BPI, have all been sequenced in the catfish genome, however there is little to no research on the effects of acute hypoxic stress and if regulation and function of these genes in catfish is similar to orthologs in other species. This

study seeks to investigate the relative change in these genes across multiple time points at two different levels of hypoxic stress in order to improve our understanding of the catfish genome and potentially lead to improvements in future selective breeding in aquacultured catfish.

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MATERIALS AND METHODS:

Experimental fish and design

Fingerling size fish for this experiment were of the obtained from existing brood stock at North Auburn Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University. Fish had initially been raised in PAS systems at the Clemson University Fisheries unit and subsequently moved to and maintained in the S-6 indoor recirculating system and had been held at uniform temperature and water quality for the entirety of the fish's lives. All fish were raised in extremely similar environments for the entirety of their lives.

Channel catfish used in this experiment were of mixed sex and ranged in size from 12 to 22g with a mean size of 14.4g . All were from a single family of the Marion strain catfish and any fish showing signs of deformities and or erratic behavior were discarded before trials began. Blue catfish were of mixed sex and ranged in size between 13g and 24g with a mean size of 16.2g. All blue catfish were from a single family of the Rio Grande strain and were checked for physical and behavioral abnormalities pre-trial. The hybrid catfish used in this trial were of mixed sex and ranged in size from 10.5g to 21g with and average size of 13.6g.The hybrids were from a single family and a cross of Marion strain channel catfish female with Rio Grande strain blue male. Hybrid catfishwere also checked for abnormalities before trials began.

Table 1. Raw fish weight data**.**

Family, Duration, Oxygen level. Trial#	**Fish1 weight (g)**	**Fish2 weight (g)**	**Fish3 weight (g)**	**Fish4 weight (g)**	**Fish5 weight (g)**
Blue 2hr 2mg/L 1	12.4	16	14.3	20.1	17
Blue 2hr 2mg/L 2	13.2	19	17.7	16.2	14.4
Blue 2hr 2mg/L 3	16	17.2	13	14	14.3
Blue 4 hr 2mg/L 1	24.2	14	16	17.7	17
Blue 4 hr 2mg/L 2	16	15.1	19	17	14.6

Blue 4 hr 2mg/L 3	16	20.2	16	13.2	15
Blue 8 hr 2mg/L 1	14	24	14.4	16.2	17
Blue 8 hr 2mg/L 2	17.3	14	15	13	14.3
Blue 8 hr 2mg/L 3	15	16.4	19.2	16	15
Blue 2hr 1.5mg/L 1	17.2	14.3	13	21.2	14
Blue 2hr 1.5mg/L 2	16	20.3	16	14	17.3
Blue 2hr 1.5mg/L 3	16.7	14	20	20.4	14
Blue 4hr 1.5mg/L 1	17	15	14.3	17	15
Blue 4hr 1.5mg/L 2	15.6	14	21.3	14	20.9
Blue 4hr 1.5mg/L 3	16	14.1	17.4	15.2	14
Channel 2hr 2mg/L 1	16.3	12.3	12.3	13.2	13.1
Channel 2hr 2mg/L 2	15	14.5	14.6	15.6	14
Channel 2hr 2mg/L 3	14.5	13	13.1	16.4	14
Channel 4hr 2mg/L 1	16.4	14.7	12	14.2	14.4
Channel 4hr 2mg/L 2	14.5	13.2	16.3	12.1	14.7
Channel 4hr 2mg/L 3	15.2	13	12.1	13.3	15.7
Channel 8hr 2mg/L 1	13.3	14.6	13.4	16.2	13
Channel 8hr 2mg/L 2	14.3	22.1	16.3	13.3	14
Channel 8hr 2mg/L 3	13.8	13.3	15.1	16.2	15.8
Channel 2hr 1.5mg/L 1	14	15	14.2	13.8	14
Channel 2hr 1.5mg/L 2	14.4	14.3	15	13.6	17
Channel 2hr 1.5mg/L 3	12.4	14.1	14.3	16.7	14.2
Channel 4hr 1.5mg/L 1	17.2	13.6	14.1	14.3	12.2
Channel 4hr 1.5mg/L 2	15.5	13.2	15.1	12	15.2
Channel 4hr 1.5mg/L 3	13	17.1	16.1	14.3	14.5
Hybrid 2hr 2mg/L 1	11.2	15.3	12	13.6	15.2
Hybrid 2hr 2mg/L 2	15	14.8	13.2	11	14.3
Hybrid 2hr 2mg/L 3	14.1	12.9	15.3	13.4	13
Hybrid 4hr 2mg/L 1	13.2	14	12.2	14.3	16.2
Hybrid 4hr 2mg/L 2	14.4	15.1	11.5	12.5	14.3
Hybrid 4hr 2mg/L 3	15.2	12.4	12.2	13	15
Hybrid 8 hr 2mg/L 1	15.6	14.2	12.1	12.3	13.6
Hybrid 8 hr 2mg/L 2	11.8	13.2	13.1	13.5	14
Hybrid 8 hr 2mg/L 3	18.5	13.1	13.9	12.2	12.1
Hybrid 2hr 1.5mg/L 1	14.6	15.3	14	10.5	14.2
Hybrid 2hr 1.5mg/L 2	14.2	12.3	13.1	15.2	11.4
Hybrid 2hr 1.5mg/L 3	12.3	12.1	15.2	14	14.2
Hybrid 4hr 1.5mg/L 1	15.1	11.1	14.2	14	12.3
Hybrid 4hr 1.5mg/L 2	13.2	13.1	14	11.3	21.2
Hybrid 4hr 1.5mg/L 3	13.4	12.1	12.6	11.9	14

Table 2. Summary statistics for fish weight data.

	Blue (g)	Channel (g)	Hybrid (g)	All Fish (g)
Mean	16.21	14.44	13.58	14.74
SD	2.54±	1.59±	1.67±	2.25±
Variance	6.44	2.55	2.8	5.096
Min	12.4	12	10.5	10.5
Max	24.2	22.1	21.2	24.2

A 2-way ANOVA analysis of fish weights determined that there was a significant difference between aggregate fish weights for each family tested but size disparities were spread evenly enough between trials so there was no statistical difference between treatments among fish family weights. (F, Fcrit, and tables in appendix 1)

Two 1,135 L tanks were placed on constructed support tables of 1.2meters in height in order to give each tank enough head pressure to gravity flow water through smaller holding units for the duration of each trial. Fivecm diameter PVC pipe was plumbed into each tank and equipped with a ball valve for flow regulation. Both tanks piping led to a centralized 10cm Y joint that allowed for both to flow to the same end point yetbe operated independently of one another. From this the Y joint extended to a line of 3 1.9cm ball valves with brass nipples fitted to each. Attached to each brass nipple was ½ inch flexible plastic tubing which led into isolated 19 liter plastic buckets. Water could then be fed into each individual tank at the set rate of 1.25 liters per minute per tank to maintain uniform dissolved oxygen levels. All dissolved oxygen levels were checked every 10 minutes using a YSI Pro20 dissolved oxygen sensor with galvanic probe and 4m cable to determine oxygen levels were held at desired levels.(Figure 1).

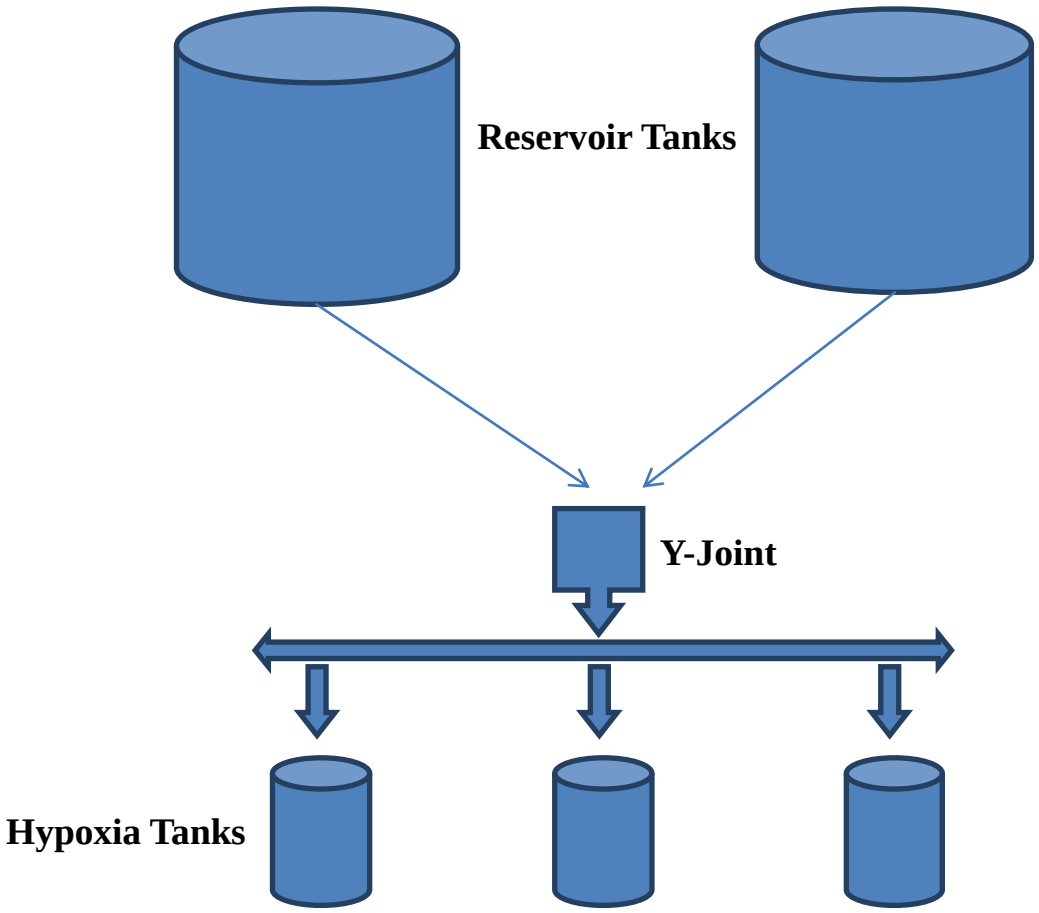


Figure 1. Diagram of tank set up and water flows. Two independent tanks were necessary due to the fact that one reservoir tank did not have enough capacity for the 8 hr trials.

Each 1,135 L tank was filled to capacity with well water and heated to 24° C to match the temperature at which fish were being held. Anhydrous sodium sulfite was added to the filled reserve tanks until desired dissolved oxygen levels of 2 mg/L or 1.5 mg/L were achieved, dependant on the treatment desired. These relative oxygen levels were determined by observing multiple test runs comparing time to show visible stress against a range of oxygen

levels.Phenotypic stress was defined as fish leaving a resting state on the tank bottom and actively swimming on the surface. Oxygen levels above 2 mg/L did not achieve visible stress in channel and hybrid catfish while values below 1.5 mg/L produced mortality too quickly in blue catfish. At the selected time intervals and dissolved oxygen levels trials could achieve phenotypic stress in the majority of fish but not induce unwanted mortality. Fish were selected at random in groups of 5 from each of the aforementioned families and placed into the treatment tanks at normal oxygen levels. The valves were fully opened for 10 minutes allowing oxygen levels to quickly drop from 5.5-6.0 mg/L holding levels to the desired 1.5 or 2.0 mg/L level desired. After individual tank oxygen levels had stabilized at desired levels, flow was returned to the set rate of 1.25 liters per minute for the duration of the trial.2,4, and 8 hr trials were done at 2mg/L, at 1.5 mg/L only two and four hr trials were done as eight hr trials induced mortality in an unacceptable amount of blue catfish. Trials occurred over a three month period from October to December. All trials were done with 5 fish per family per tank and oxygen levels were monitored at 10 minute intervals. Control values were determine using the same protocols but under normal oxygen levels of 5.5 to 6.0 mg/L for a period of four hr. All treatments were repeated 3 times.

Tissue removal and RNA extraction

After the predetermined time point had been reached all valves were closed and MS-222 was added to each individual tank to anesthetize fish. Fish were then weighed and had their liver and gills removed to be placed into separateten ml test tubes containing 2ml of RNAlater (RNA stabilizing buffer) for storage. Each group of 5 fish per treatment had their organs pooled in to one communal vial per organ per treatment. These tubes were placed in a refrigerator at 4°C for 24 hr. After temporary storage at 4°C samples they were moved to a -80 C° freezer for long term

storage. Samples were prepared for RNA extraction by submersion in liquid nitrogen and then ground to a fine powder using a mortar and pestle. Once samples were ground, RNA was extracted using the RNAeasy mini kit (Qiagen, Valencia, CA). RNA concentrations were checked using an Amersham Bio Sciences ultrospec 1100 pro spectrophotometer. Any RNA with wavelength ratios outisde the accepted OD260/OD280 of 1.8-2.0 or with concentrations lower than 200µg/µl were discarded and the extraction process was run again.

After RNA was obtained in sufficient quantity and quality samples were converted to cDNA using the BioRadiscriptcDNA synthesis kit via reverse transcription. Samples were then amplified using PCR in a BioRad thermal cycler and checked for cDNA quantity. Next, samples were diluted with RNAase free water to a uniform 250 µg/µl ±30µg/µl.

Primers for six different genes HIF-1, HIF-2, Ferritin, Myostatin, NKEF, and BPI genes were designed and obtained from Invitrogen Custom Primers and were tested for specific amplification prior to use in qRT-PCR (Table 3).

Table 3. Primers used in this study.

Gene Name	Primer Sequence (5' to 3')
HIF-1	Upper ACCACCTCAGCAAGACACAT
	Lower TCCTCCTCCACAATACCACTG
HIF-2	Upper TCACCAGAAGCCACCAGAAT
	Lower CACTCAGGACATAGTTGACACA

Myostatin	Upper AGTATTGTGAGGAGTGTGAGAC
	Lower GACTCGCCTTCCTTATTCTTCT
Ferritin	Upper AAAGTCCAGAACCAGAGAGGA
	Lower ACCCAGTCAGAAAGCTCCTTA
NKEF	Upper ACAGATTTTGTAACGCACGTT
	Lower TGTTTCTCTGGATGAAATGCAG
BPI	Upper AGAAGCAGAGACAGAGACCAA
	Lower GCCAATCTGACGACCATACTC

qRT-PCR was performed on a BioRad CFX 96 Touch™ real time PCR detection machine using a qRT-PCR so fast EvaGreenSuppermix kit. CFX Manager Software version 1.6 was used for data collection and results were then exported unto Microsoft Excel spreadsheets for graphing and analysis. The Relative Expression Software Tool or R.E.S.T was used for statistical analysisand significance testing of the genetic data. Control samples were obtained after 3 replicates of 5 fish per family were placed in the test tanks for a duration of 4 hr at normal dissolved oxygen levels of 5.5-6.0 mg/L and had their organs extracted and pooled according to the protocol. These samples provided the base like C/T value to determine future fold regulation changes post hypoxic treatment. The housekeeping gene 18s was used for standardization of transcript expression during the statistical analysis.

Hypoxia trials and phenotypic measurements

Prior to any trials documented in this experiment the groups of catfish were subjected to a range of dissolved oxygen levels and time periods to determine differences among the groups in terms of phenotypic stress. Normal behavior for all groups in this experiment, post placement in tanks, was to maintain an upright orientation, displaying little or no activity on the tank bottom. Phenotypic stress was defined as leaving this position on the tank bottom and swimmingerratically at the water's surface. Fish that left the tank bottom never returned to this resting state in any trial. It was determined that dissolved oxygen levels greater than 2 mg/L was insufficiently stressful to induce behavioral changes consistently in all groups of fish tested. At the 2mg/L threshold a large degree of phenotypic variability was observed in the three families tested but there was observable responses in all families. In Table 2 we can see a marked difference in behavior between our groups at 2 mg/Lwith blue catfish showing phenotypic stress much sooner than their channel and hybrid counter parts. Oxygen levels of 2 mg/L elicited a range of responses for channel and hybrid catfish that were between no visible stress for 8 hr to showing clear agitation after only 105 minutes. An oxygen level of 1.5 mg/L was determined to be sufficiently stressful to induce phenotypic change in all fish within two hr of introduction. Oxygen levels below 1.5 mg/L induced mortality quickly, often within two hr, across all tested fish especially blue catfish.

Table 5 shows mean time to stress at 2.0 mg/L for channel and hybrid catfish to be more than double that of the blue. Mean time to stress of channel and hybrid catfish was nearly quadrupled compared to blue's at 1.5 mg/L. Based on these observations it was determinedthat time points of two, four and eight hrwere selected as bench marks for differences in phenotypic stress and used these set periods as intervals for tissue collection to determine molecular

changes. No 8 hr 1.5 mg/L time point was used as it caused a high degree of mortality in blue catfish.

Genes examined in the study consisted of two known oxygen sensitive factors (HIF-1, HIF-2), two primarily metabolic effectors (ferritin, myostatin) and two immune related factors (NKEF, BPI) gill and liver tissue were harvested for molecular testing in this experiment due to their central role in all functions the selected genes are known to effect. Gill was selected due to its role as the major respiratory organ in fish. Oxygen is integrated into the fish body through the gills and therefore it is reasonable to believe that drastic changes in ambient oxygen would alter the molecular patterns in this organ. Gills can also be an induction point for diseases and catfish which may enhance the organs molecular sensitivity among immune related genes. Liver tissue was selected due to its major role in metabolism for all vertebrates, the process of which is highly oxygen dependent.

Table 4. First signs of phenotypic stress in minutes, determined by time for fish behavior to change from lying on the bottom of the tank respiring to swimming at the surface apparently agitated.

Species, Duration, Oxygen Level, Trial #	Time to surface-- 1st fish	Time to surface-- 2nd fish	Time to surface-- 3rd fish	Time to surface-- 4th fish	Time to surface--last fish
Blue 2hr 2mg/L 1	74	78	81	82	none
Blue 2hr 2mg/L 2	62	74	76	none	none
Blue 2hr 2mg/L 3	65	69	73	81	98
Blue 4 hr 2mg/L 1	73	75	79	82	126
Blue 4 hr 2mg/L 2	52	58	76	96	131
Blue 4 hr 2mg/L 3	61	74	83	102	128
Blue 8 hr 2mg/L 1	62	69	74	98	105
Blue 8 hr 2mg/L 2	71	81	98	122	125
Blue 8 hr 2mg/L 3	74	87	92	101	142

Blue 2hr 1.5mg/L 1	12	15	17	18	20
Blue 2hr 1.5mg/L 2	9	11	11	14	16
Blue 2hr 1.5mg/L 3	15	17	17	18	21
Blue 4hr 1.5mg/L 1	6	10	10	12	14
Blue 4hr 1.5mg/L 2	12	13	13	13	13
Blue 4hr 1.5mg/L 3	9	12	15	16	19
Channel 2hr 2mg/L 1	107	none	none	none	none
Channel 2hr 2mg/L 2	111	118	none	none	none
Channel 2hr 2mg/L 3	none	none	none	none	none
Channel 4hr 2mg/L 1	121	145	none	none	none
Channel 4hr 2mg/L 2	155	178	193	none	none
Channel 4hr 2mg/L 3	137	none	none	none	none
Channel 8hr 2mg/L 1	143	237	329	none	none
Channel 8hr 2mg/L 2	225	343	422	none	none
Channel 8hr 2mg/L 3	157	417	none	none	none
Channel 2hr 1.5mg/L 1	45	49	57	68	76
Channel 2hr 1.5mg/L 2	51	53	55	71	73
Channel 2hr 1.5mg/L 3	49	62	74	77	77
Channel 4hr 1.5mg/L 1	52	62	65	66	74
Channel 4hr 1.5mg/L 2	31	45	61	62	68
Channel 4hr 1.5mg/L 3	42	52	56	62	77
Hybrid 2hr 2mg/L 1	111	none	none	none	none
Hybrid 2hr 2mg/L 2	none	none	none	none	none
Hybrid 2hr 2mg/L 3	105	116	none	none	none
Hybrid 4hr 2mg/L 1	123	168	none	none	none
Hybrid 4hr 2mg/L 2	113	212	none	none	none
Hybrid 4hr 2mg/L 3	137	178	none	none	none
Hybrid 8 hr 2mg/L 1	111	214	400	none	none
Hybrid 8 hr 2mg/L 2	200	315	none	none	none
Hybrid 8 hr 2mg/L 3	157	235	410	none	none
Hybrid 2hr 1.5mg/L 1	47	52	56	62	68
Hybrid 2hr 1.5mg/L 2	52	56	62	67	73
Hybrid 2hr 1.5mg/L 3	23	45	53	58	64
Hybrid 4hr 1.5mg/L 1	37	50	54	61	69
Hybrid 4hr 1.5mg/L 2	55	57	57	67	73

Table 5.Summary statistics of time to distress for each group of catfish. Fish showing no signs of stress were not included in these measures.

	Blue	**SD**	**Channel**	**SD**	**Hybrid**	**SD**
Mean—2 mg/L	89.7 min	±21.7min	188.9 min	±105.6min	206.5 min	±97.2min
Mean—1.5 mg/L	13.95 min	±3.54min	60.4 min	±12 min	57.5 min	±11.2min
Min time—2 mg/L	52min		107 min		105 min	
Max time—2 mg/L	142 min		422 min		410 min	
Min time--1.5 mg/L	6 min		31 min		23 min	
Max time--1.5 mg/L	15 min		77 min		78 min	

One way ANOVA analysis determined time to stress was significantly different between blue catfish and both channel and hybrid catfish at 2mg/L and 1.5mg/L dissolved oxygen content. ANOVA analysis also determined no significant difference between channel and hybrid catfish time to stress at both oxygen concentrations. (F and Fcritical values in appendix 2)

RESULTS:

Molecular Expression Measurements

The following graphs the represent the relative fold changes of the genes HIF-1, HIF-2, BPI, Ferritin, Myostatin and NKEF in gill and liver tissue at pre-determined oxygen levels and time intervals. Significance was determined using the REST qRT-PCR statistical analysis tool. Significant fold changes are denoted by stars.

Hypoxia-inducible factor-1 (HIF-1)

HIF-1 gene gill expression showed significant fold changes only in hybrid catfish at treatments of 2 hr at 2 mg/L oxygen, 4 hr at 2 mg/L oxygen, and 4 hr at 1.5 mg/L oxygen levels. The greatest fold change of an approximately 5-fold increase in expression came at 4 hr 1.5 mg/L oxygen in hybrid catfish. None of the results for channel or blue catfish were deemed to be significant for HIF-1 gene regulation in gill tissue. Notably, while not significant due to individual variation, blue catfish manifested an overall pattern of down-regulation of HIF-1 at all examined time points. HIF-1 regulation for channel catfish at these time points and oxygen levels did not show significant change. However, in general they showed a pattern of up-regulation, the generally expected response to hypoxic conditions (Figure 2)

HIF-1 in liver tissue showed significant up and down- regulation in blue catfish.We see very erratic significant movement as well as the majority of blue time points showing down-regulation. Channel catfish showed the largest change with a significant up-regulation of 13.669 fold at 4 hr and 1.5 mg/L as well as a general overall trend of up-regulation although to a lesser

and not always significant degree. Hybrid catfish showed one significant result for HIF-1of down- regulation at 2 hr2 mg/Lin liver tissue however the overall trend was that of up-regulation.

Figure 2 – Fold change in HIF-1 gene in gill tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.

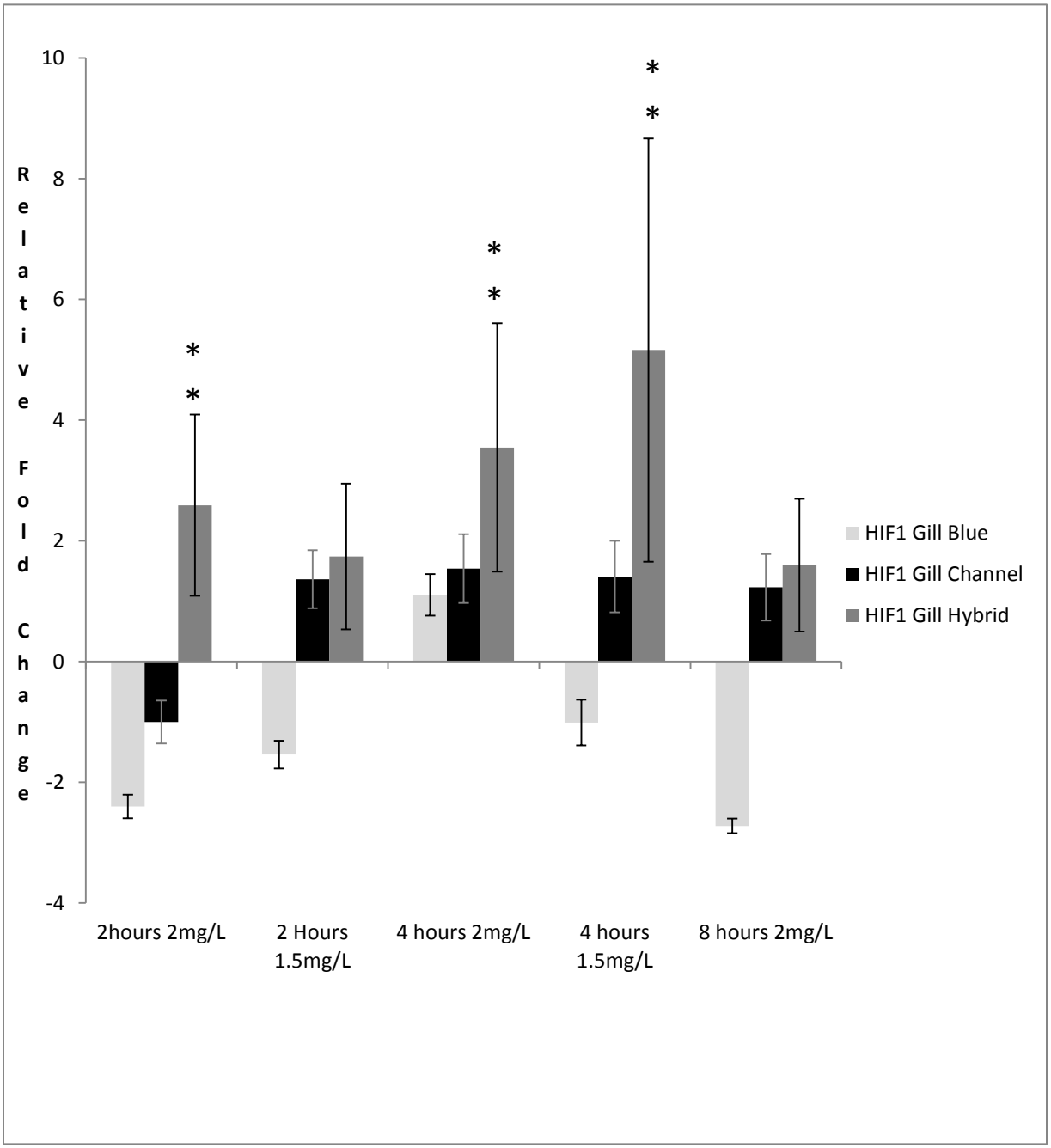
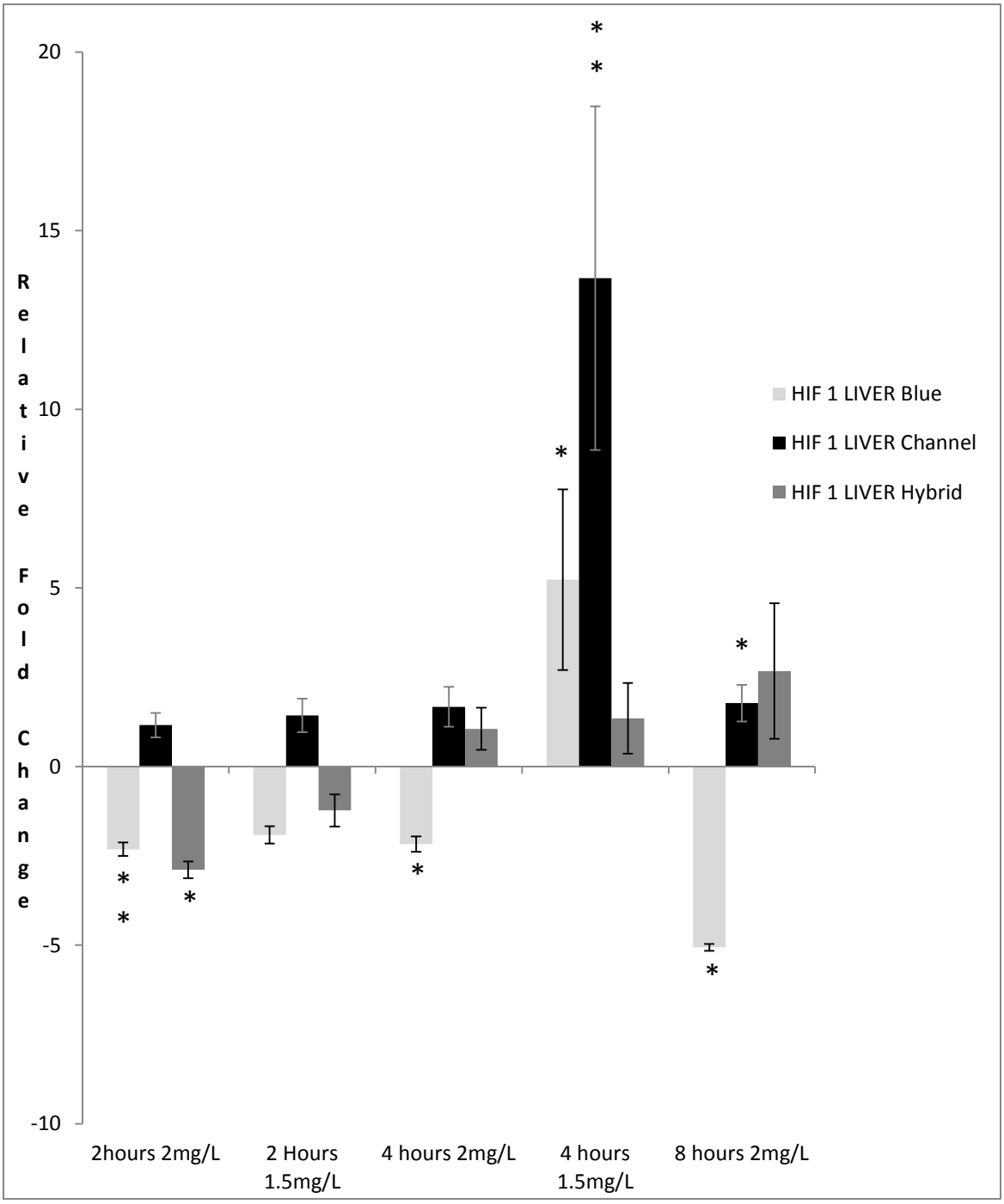


Figure 3 – Fold change in HIF-1 gene in Liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.



Hypoxia inducible factor-2 (HIF-2)

HIF-2 is believed to have similar affects to HIF-1 in regulation of internal cellular signaling cascades in response to hypoxia. HIF-2 has been shown to have differential sensitivity when compared across species, even those of similar evolutionary origin. Blue catfish showed significant up and down regulation while hybrid catfish's only significant change was down regulation at 2 hr 2 mg/L . All other time points did not show significant change for HIF-2 in gill when analyzed with the REST program. Blue catfish show up and down-regulation with the majority being down. Channel and hybrid catfish showed an overall trend of up-regulation but not to any significant degree.

Channel catfish showed significant up-regulation for HIF-2 in liver at 4 hr 1.5 mg/L and 8 hr2 mg/L when analyzed with the REST program. Blue catfish show up and down-regulation with the majority being down. Hybrid catfish showed an overall trend of up-regulation but not to any significant degree. Channel catfish showed initial down-regulation but to a small degree and then change to up-regulation that was significant. Only channel catfish showed significant molecular change for HIF-2 in either tissue examined.

Figure 4 – Fold change in HIF-2 gene in gill tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.

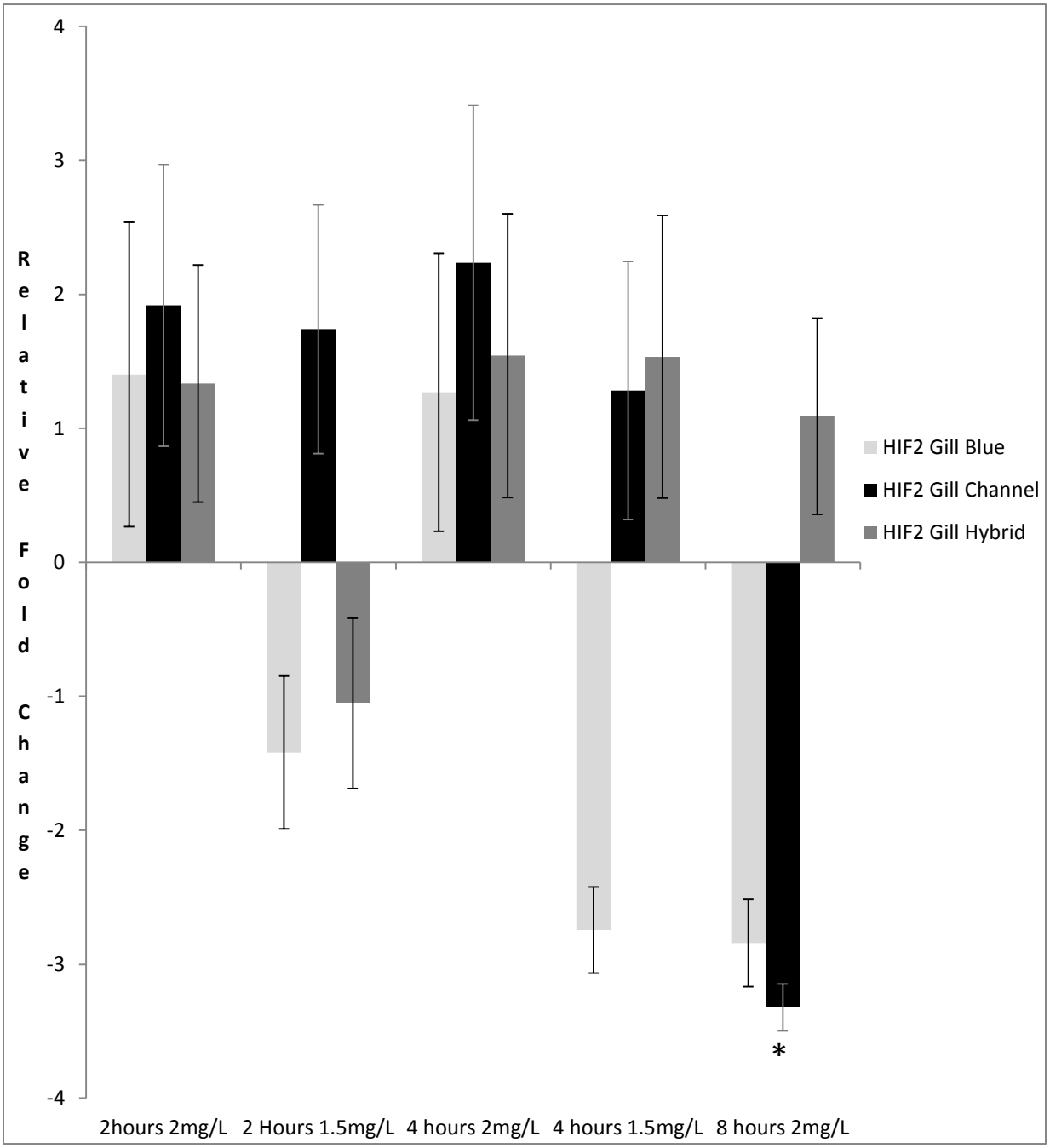
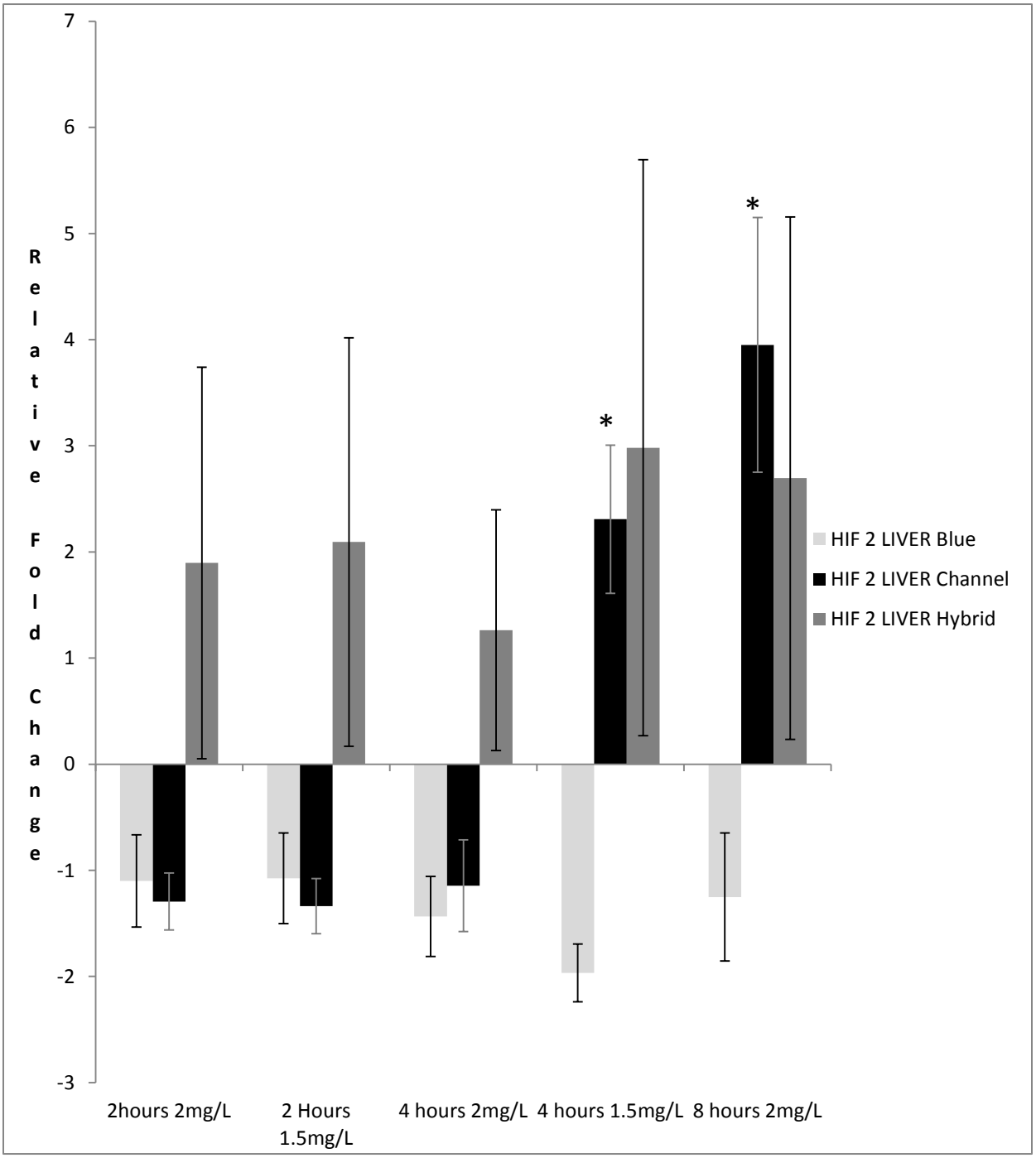


Figure 5 – Fold change in HIF-2 gene in liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.



Bactericidal permeability-increasing protein (BPI)

BPI genes are believed to enhance an organisms response to Gram negative bacteria such as the common catfish pathogens *Flavobacterium columnaris* and *Edwardsiella ictaluri.* This response is mediated by the presence of LPS in the Gram negative bacterial cell wall. Hypoxia has been shown to increase catfish susceptibility to these pathogens after exposure. Blue catfish did not show any significant change in regulation and showed no real trend with 2 time points showing up-regulation with a large degree of error. Channel and hybrid catfish each showed multiple significant points of down- regulation. Both channel and hybrid catfish also showed a very large degree of down-regulation at the 8 hr2 mg/L time point with Channel showing a 9.9 negative fold change from the control and hybrid catfish showing an negative 8 fold change. These extremely large changes at the final time point suggest that there is a cumulative effect occurring that induces this large change overperiods prolonged stress. Blue catfish it should be noted while not having any significant regulation changes also had much lower levels of this gene present in the tissue at all time points.

Blue catfish again showed no significant differences across any of the time points but did show an overall downward trend that had a closer resemblance to the other fish's genetic profiles. Channel catfish with exclusion of the first time point showed an overall trend of down-regulation with one time point at 4 hr and 2 mg/L oxygen showing significance. Hybrid catfish showed significant down-regulation at 4 hr2 mg/L and 1.5 mg/L as well as the largest down-regulation of negative 8.4 fold at 8 hr and 2 mg/L. Just as with gill tissue blue catfish had a much lower initial expression quantity of the BPI genes.

Figure 6 – Fold change in BPI gene in gill tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.

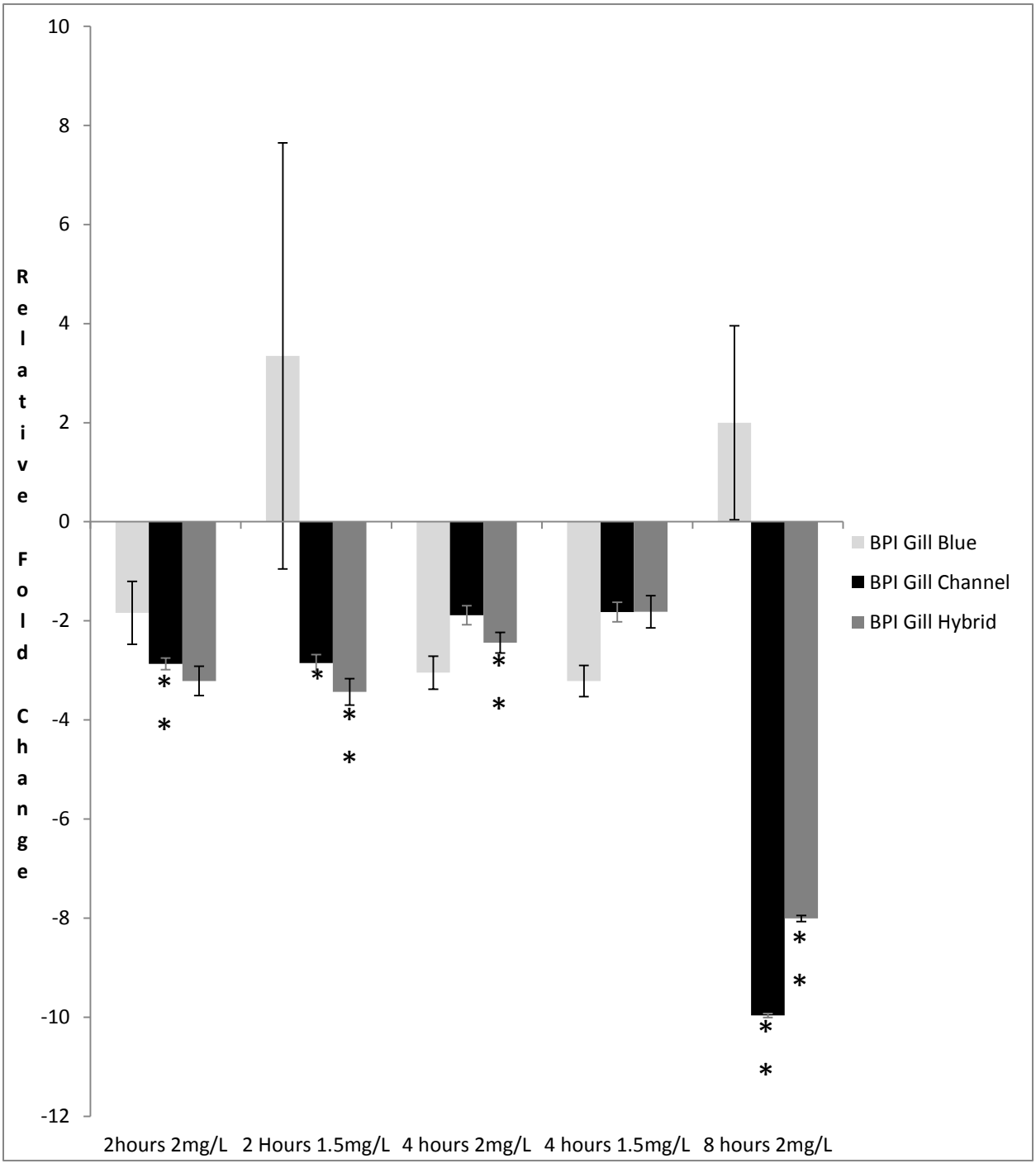
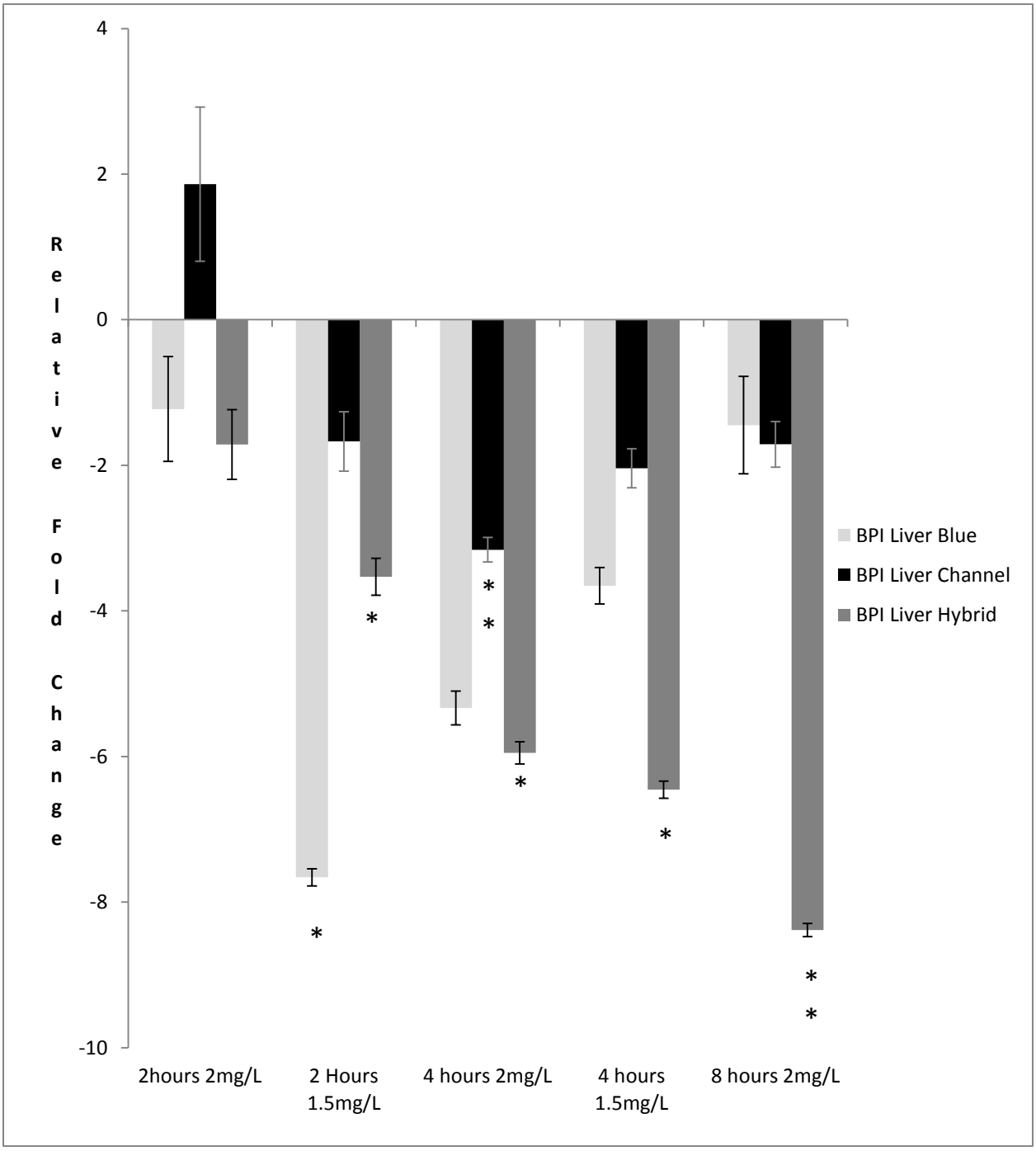


Figure 7 – Fold change in BPI gene in liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.



Ferritin

Ferritin is a key factor in iron regulation in organisms. Ferritins iron binding activity prevents excessive free iron accumulation in cells and aids in preventing oxidative damage to cells from ROS. No significant changes were seen in gill trials until the final time point at 8 hr and 2 mg/L oxygen. At the 8 hr time point we see a large and significant up-regulation in channel and hybrid catfish of 8.2 times and 10.9 times respectively. The small and insignificant changes up until the 8 hr point suggest that up-regulation of the ferritin gene is the result of a cumulative effect of genetic change in the fish that does not manifest until the duration of the stress event crosses a certain threshold, in this instance at some time greater than 4 hr of hypoxia. Blue catfish only showed minor and insignificant regulation changes throughout the trial.

Hybrid catfish showed significant down-regulation of about 4 fold at 2 and 4 hr at 2 mg/L oxygen and then significant and large up-regulation of ferritin at 8 hr and 2 mg/L oxygen. The large up-regulation of 26.9 fold shows a similar pattern of delayed large up-regulation in the gill tissue for hybrids. Blue catfish showed significant down-regulation of 2.7 fold at 4 hr and 2 mg/L oxygen and also significant up-regulation of 9.1 times at 4 hr and 1.5 mg/L oxygen levels suggesting that not only the duration of the stress even but the intensity plays a large role for blue catfish ferritin regulation. Channel catfish did not show significant gene regulation changes until a 4.4 fold up-regulation at the 4 hr 1.5 mg/L time point and then a massive 75 fold increase at the 8 hr2 mg/L time point. This is the largest fold change for any gene in this experiment and follows the pattern seen in channel gill tissue of initial small insignificant change followed by large up-regulation after a certain threshold has been reached.

Figure 8 – Fold change in ferritin gene in gill tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.

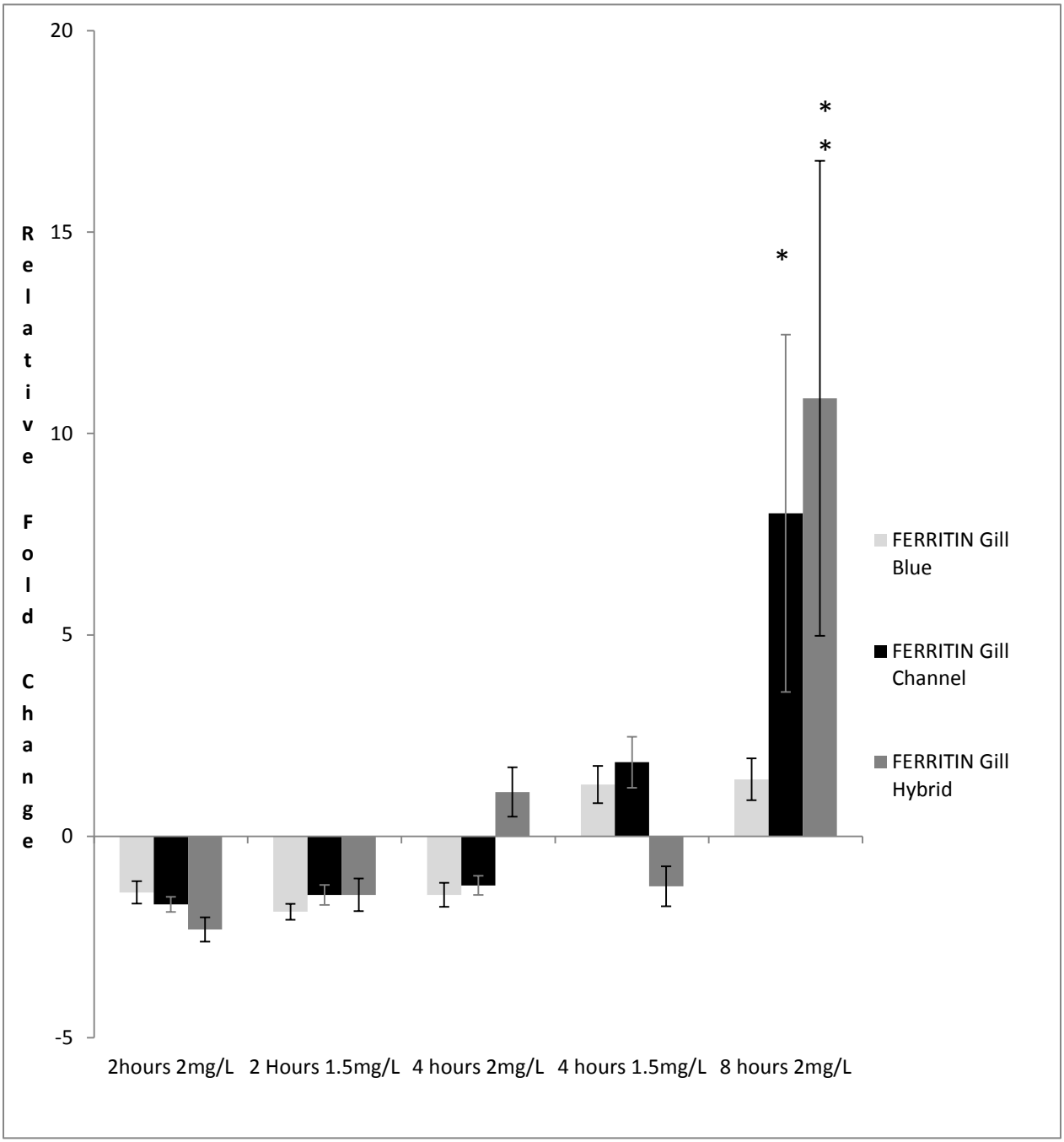
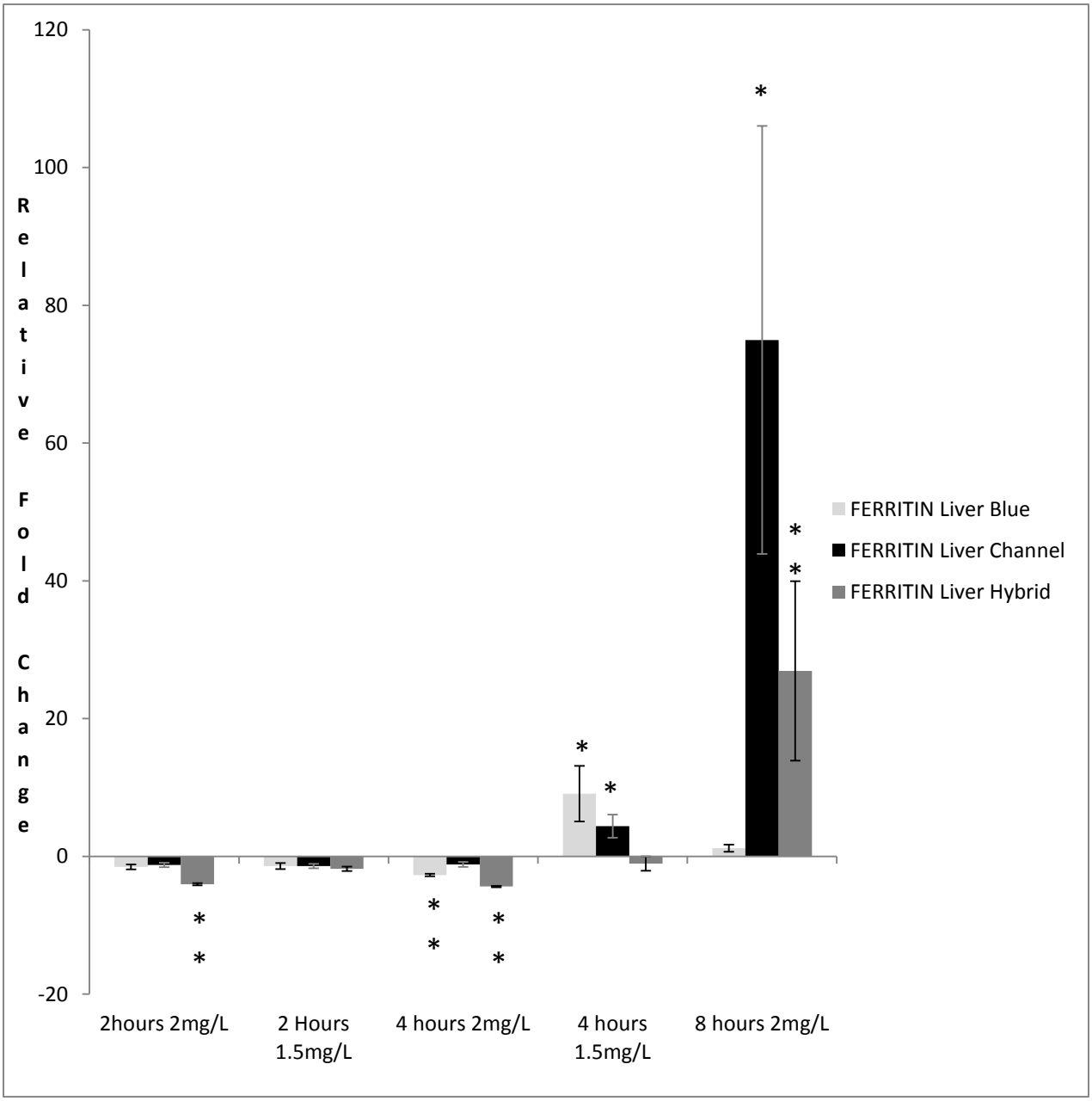


Figure 9 – Fold change in ferritin gene in liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.



Myostatin

Myostatin is a major regulator of skeletal muscle growth directly effecting metabolism and recent evidence suggests it may possess other unknown regulatory functions. In gill tissue myostatin shows a general trend of down-regulation across all fish and time points with the exception of channels at 2 hr 1.5 mg/L and hybrids at 4 hr2 mg/L but both of these time points are not statistically significant. Channel catfish did show a significant down-regulation of 2.55 fold at 2hr and 2 mg/L oxygen. Hybrid catfish had the only other significant difference with down-regulation of 1.68 fold at 8 hr and 2 mg/L. Blue catfish while showing overall down-regulation of myostatin did not have any significant results in these trials.

Myostatin showed significant up-regulation in liver tissue at the 4 hr 1.5 mg/L time point for both channel and blue catfish. It was generally up regulated in all time points and all fish except for the 8 hr2 mg/L time point in channel catfish. Blue catfish showed in general large up-regulation at both 2 hr, and the 4 hr 1.5 mg/L time points however, there is a very large amount of error at all 3 time points and only one was deemed to be significant. Hybrid catfish also showed a large degree of up-regulation at the 8 hr time point but it also has a large amount of error so as not to be considered significant.Myostatin in gill tissue moved opposite among all fish to liver samples, where we saw significant down-regulation in gill tissue at some time points, conversely significant up-regulation was observed in liver tissue.

Figure 10 – Fold change in myostatin gene in gill tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.

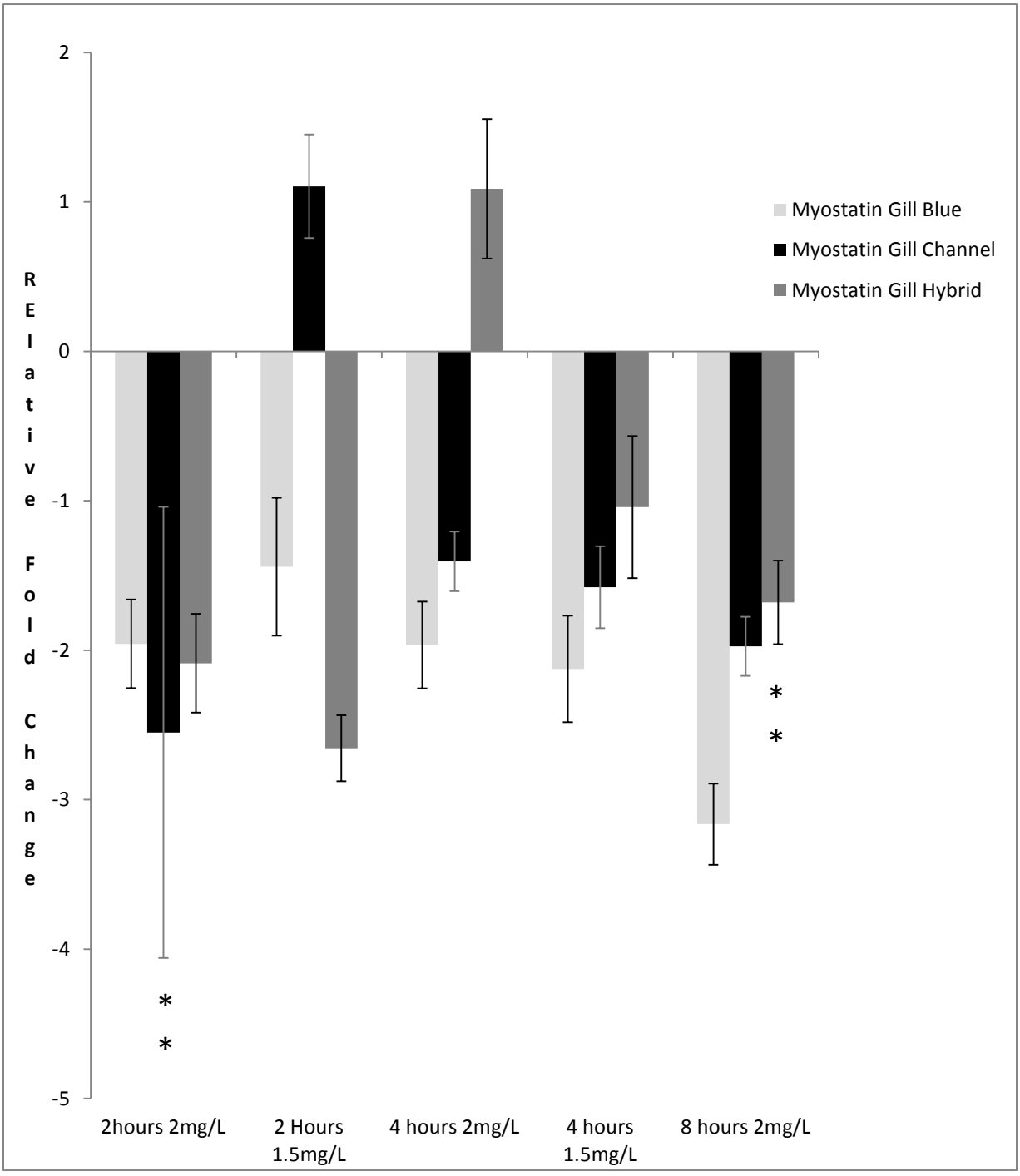
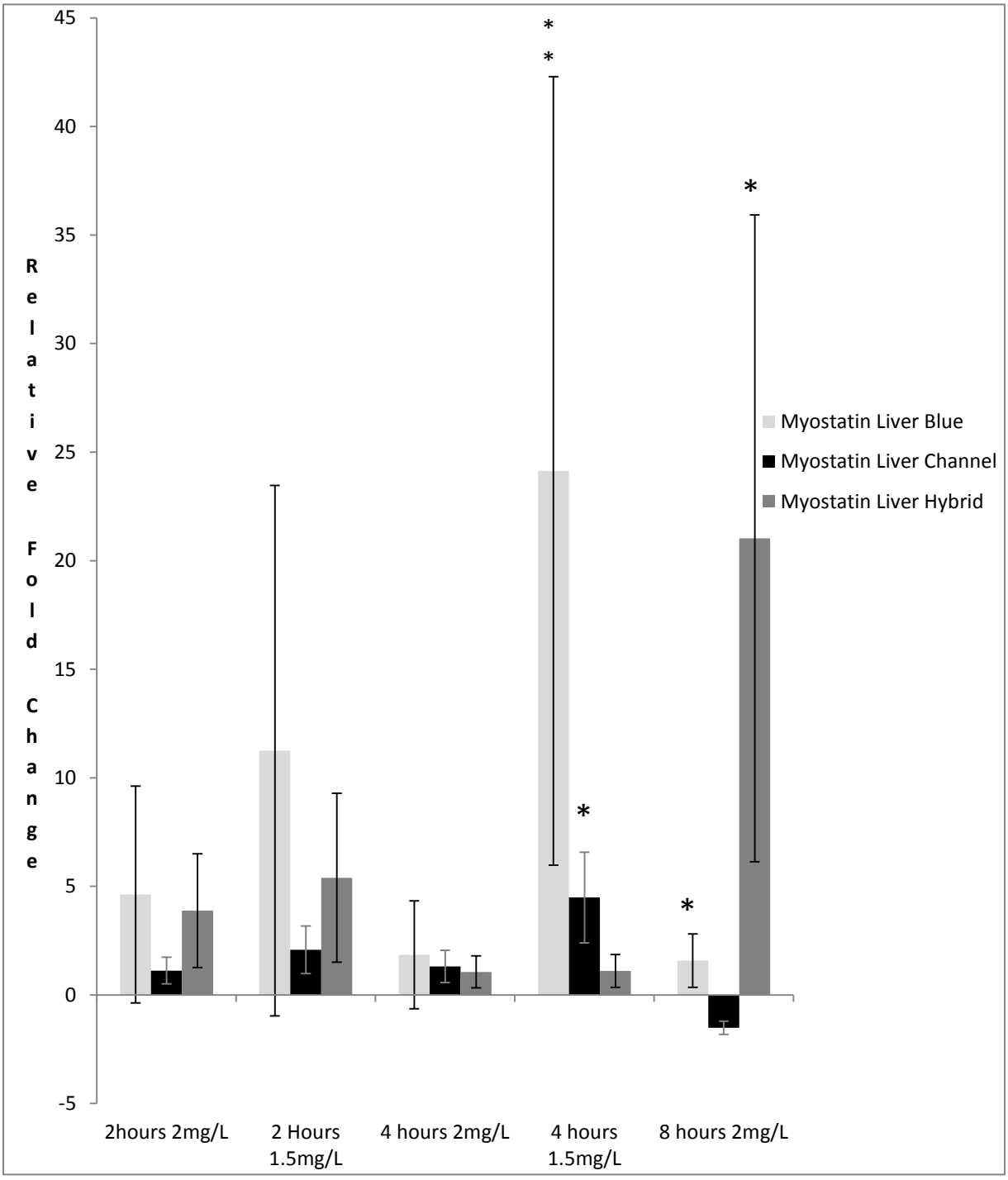


Figure 11 – Fold change in myostatin gene in liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.



Natural Killer enhancing factor (NKEF)

Natural Killer enhancing factor (NKEF) is believed to be involved in the innate immune response of organisms and has been shown to up-regulate in response to challenge by pathogens. NKEF is also believed assist in the clearance of ROS which result as a byproduct of phagocytosis of Natural Killer cells. The only significant change in the NKEF gene occurred at the 8 hr2 mg/L time point in hybrid catfish. Overall movement for NKEF in gill tissue was very limited never up regulating over 1.5 fold and only down regulating slightly over 2 fold for any fish. It is interesting to note that channel and blue catfish moved together and opposite of hybrids in all but 1 time point. While these moves were not significant it is unusual the hybrid which is a product of channel and blue moved in a opposite direction at nearly all time points.

NKEF showed significant down-regulation in blue catfish at 4 hr and 2 mg/L oxygen level. Channel catfish showed significant up-regulation at 4 hr1.5 mg/L time point. No other time points showed significant changes in regulation. It is interesting to note that channel catfish showed an overall trend of down-regulation in gill tissue and small and insignificant regulation changes at all time points for NKEF regulation in liver tissue with the one exception of up-regulation at the 4 hr1.5 mg/L time point in both trials.

Figure 12 – Fold change in myostatin gene in liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.

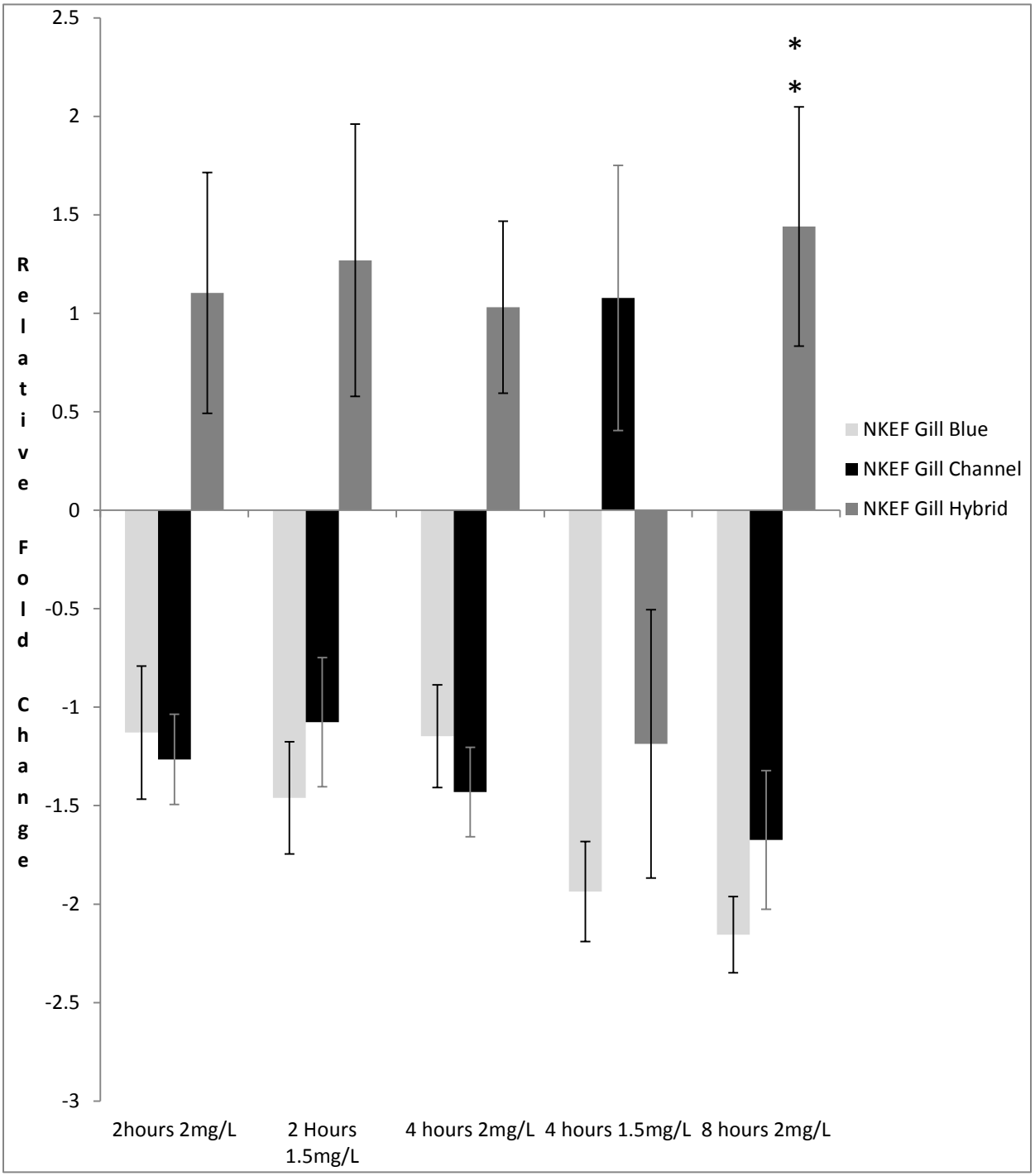
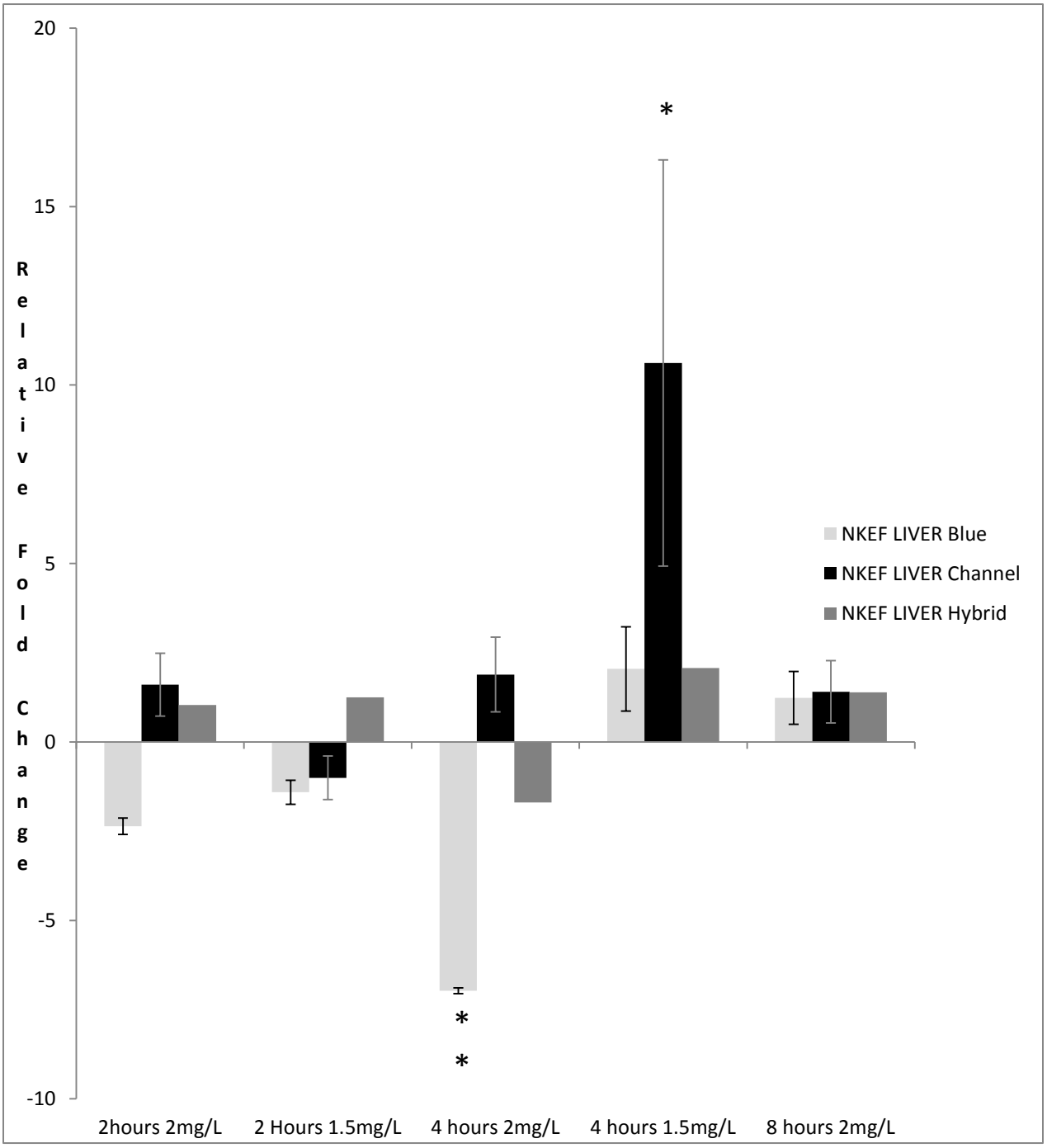


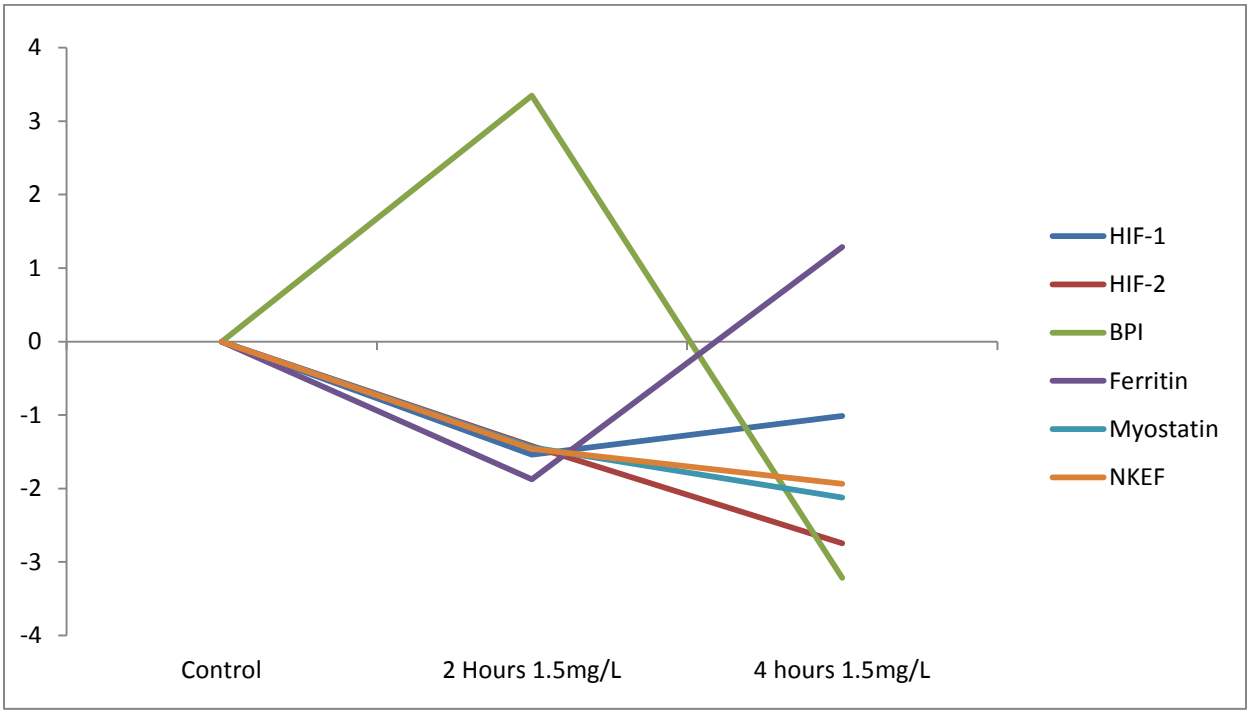
Figure 13 – Fold change in myostatin gene in liver tissue (y axis) at different time points and oxygen levels (x axis). Asterisks denote significant difference (p value<0.10) double asterisk denotes (p value<0.05) in fold change using REST software for gene quantification analysis relative to control, normalized to changes in 18S rRNA values among the same samples.



General Pattern of Hypoxia-Induced Regulation

Research has shown that the level of intensity of a stress event can have profound effects on the relative gene expression pursuant to that event. Catfish are a relatively hypoxia tolerant family of fishes andan event that may induce large degrees of molecular change in other species of fish may not meet the necessary threshold to cause significant molecular change in catfish. The oxygen parameters in this experiment were specifically chosen because they caused varying degrees of observable phenotypic stress and as result produced measurable fluctuations in gene expression patterns. The following graphs show a side by side comparison of each group and tissues relative fold changes in all genes at the two different dissolved oxygen levels tested in this experiment.

Figure 14 Side by side comparison relative fold changes1.5 mg/L time points vs.2 mg/L time points in all genes over all time points blue catfish gill tissue.



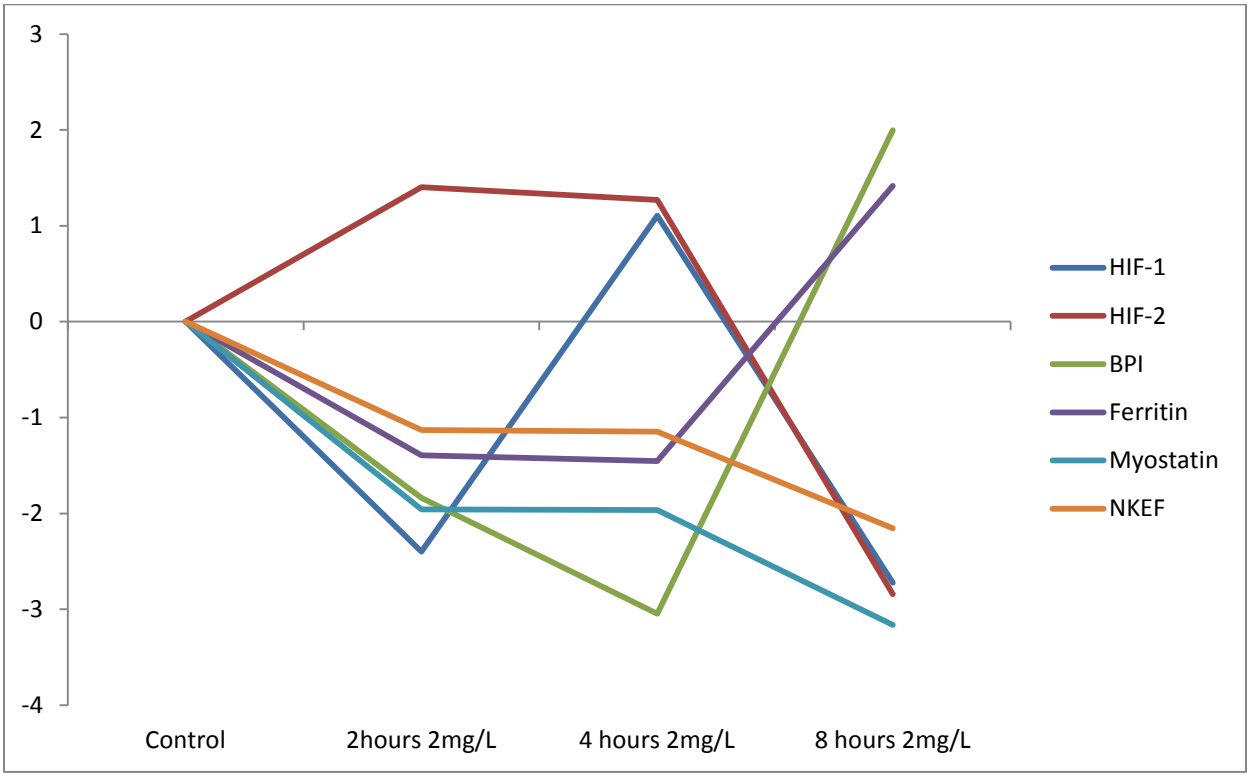
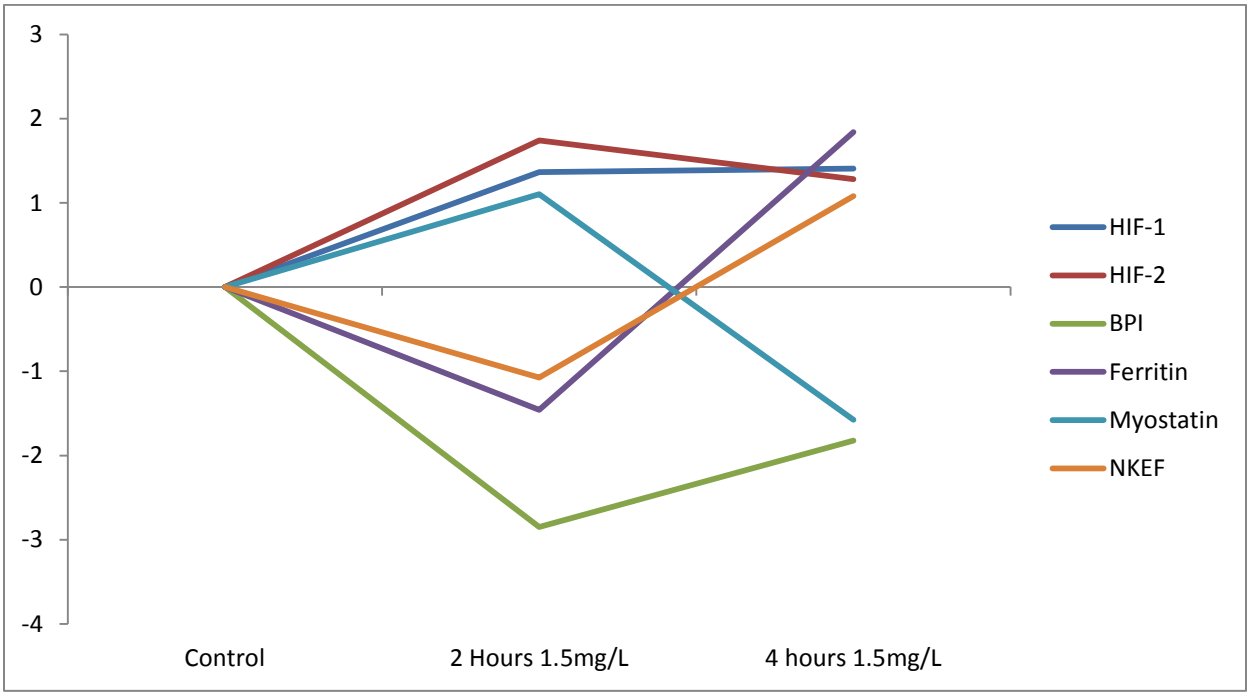


Figure 15. Side by side comparison relative fold changes1.5 mg/L time points vs.2 mg/L time points in all genes over all time points channel catfish gill tissue.



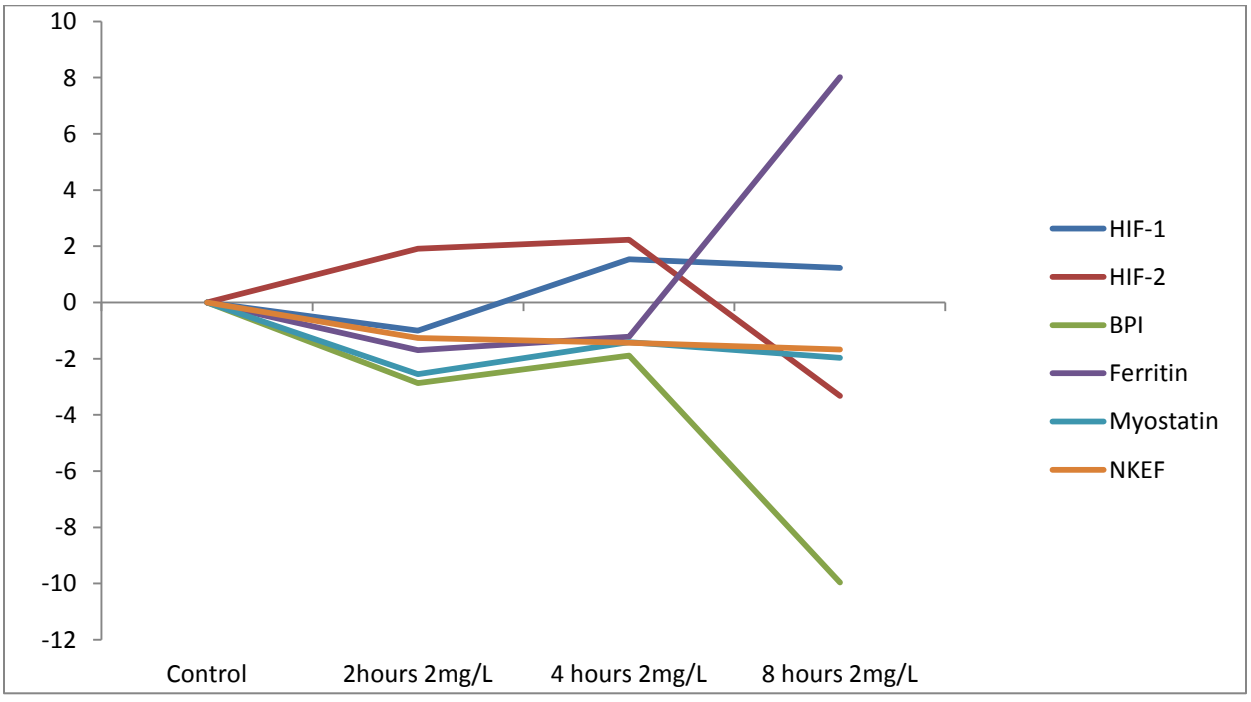
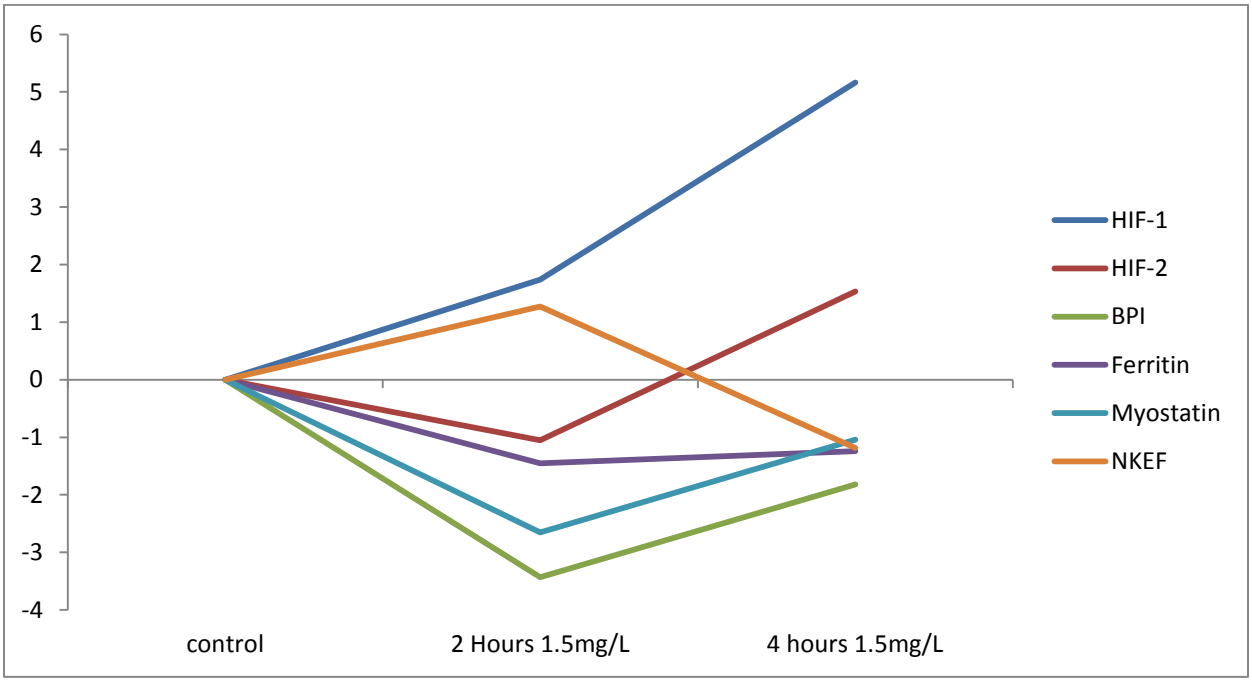


Figure 16. Side by side comparison relative fold changes1.5 mg/L time points vs.2 mg/L time points in all genes over all time points hybrid catfish gill tissue



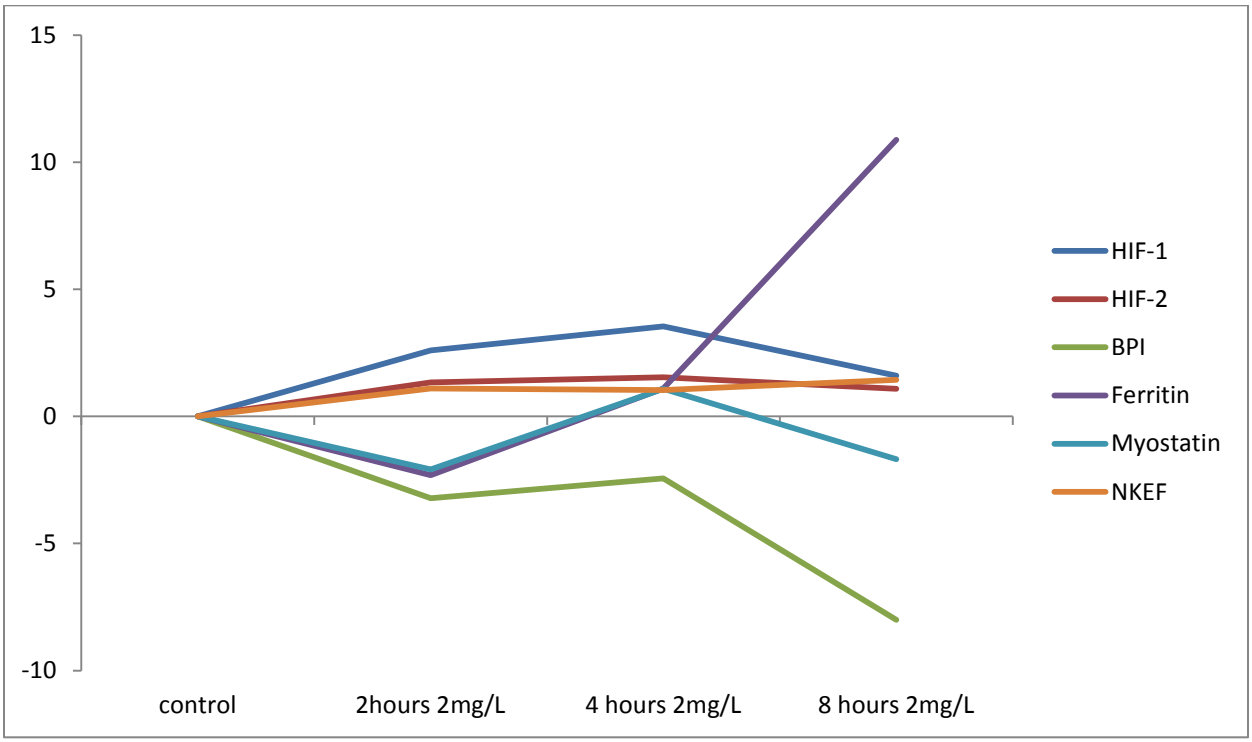
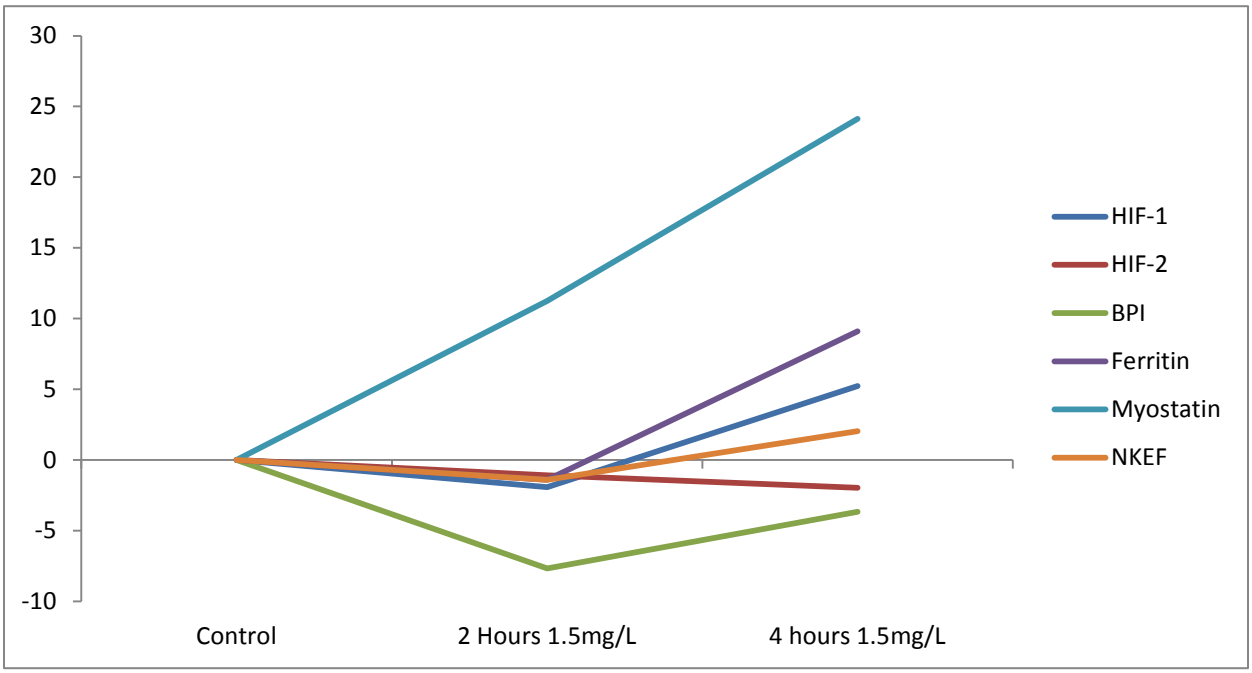


Figure 17. Side by side comparison relative fold changes1.5 mg/L time points vs.2 mg/L time points in all genes over all time points blue catfish liver tissue



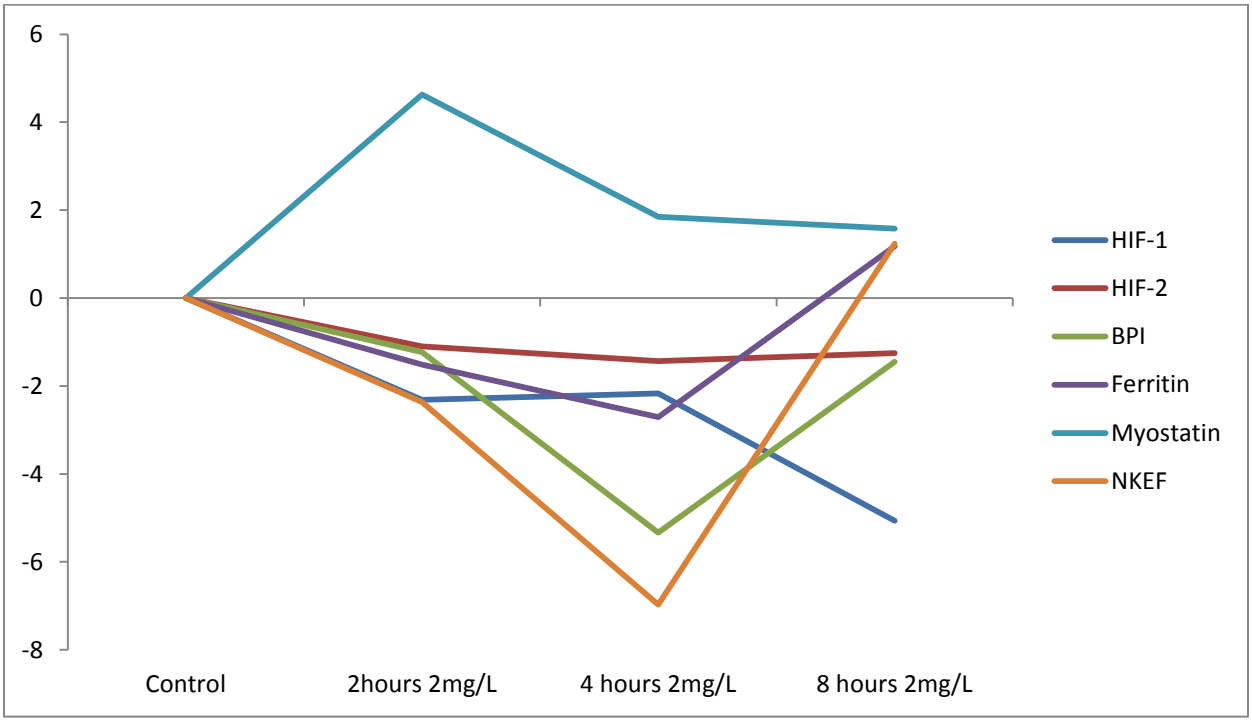
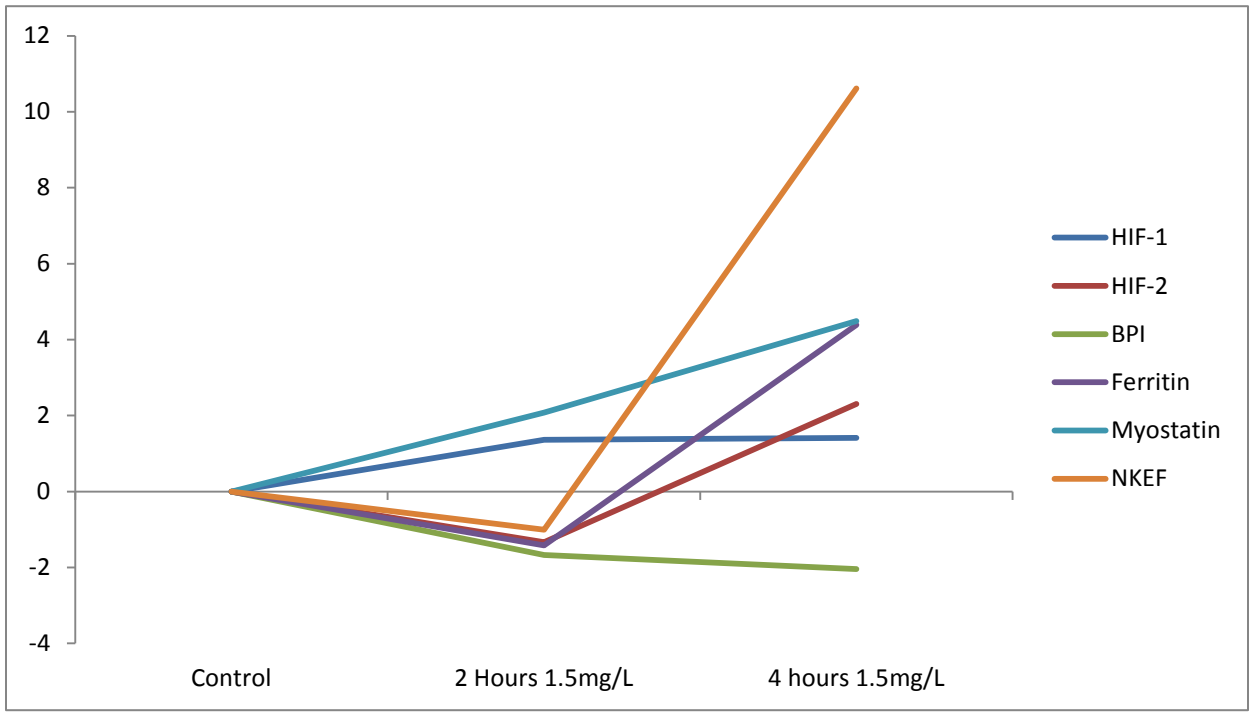


Figure 18. Side by side comparison relative fold changes1.5 mg/L time points vs.2 mg/L time points in all genes over all time points channel catfish liver tissue



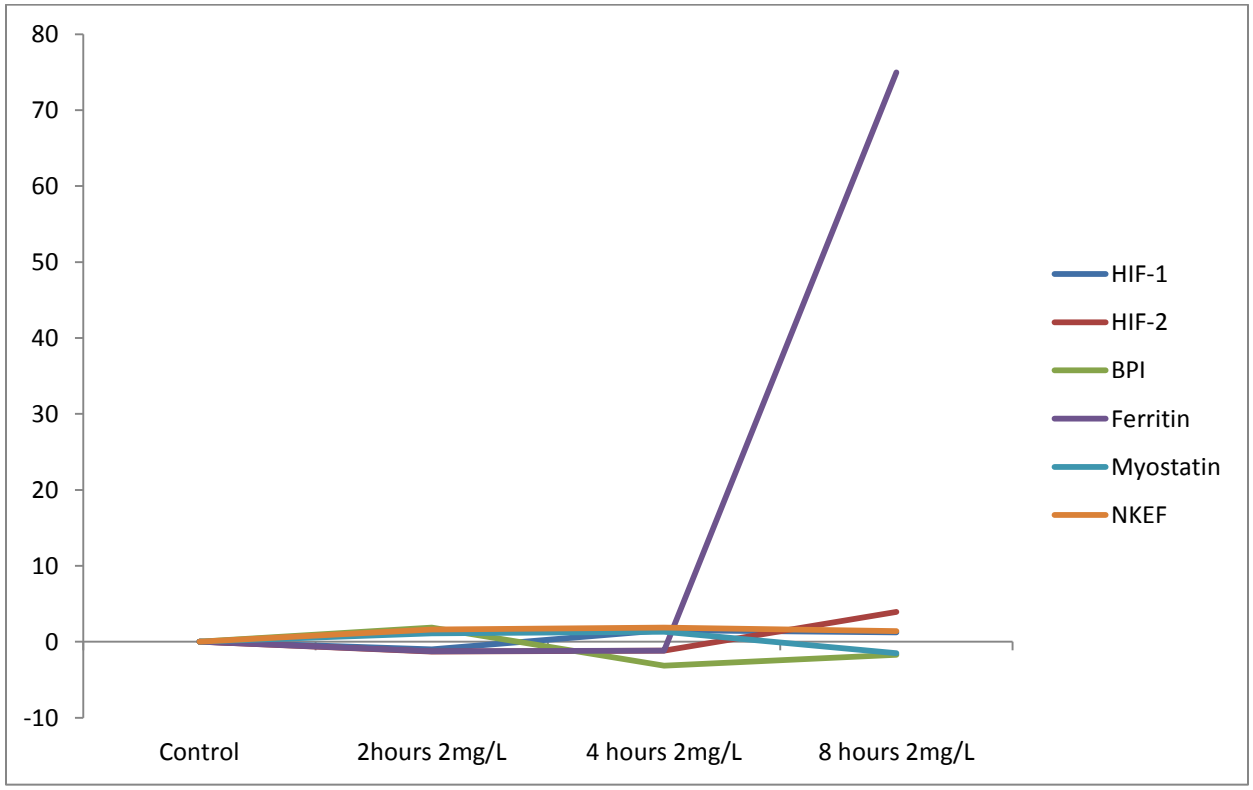
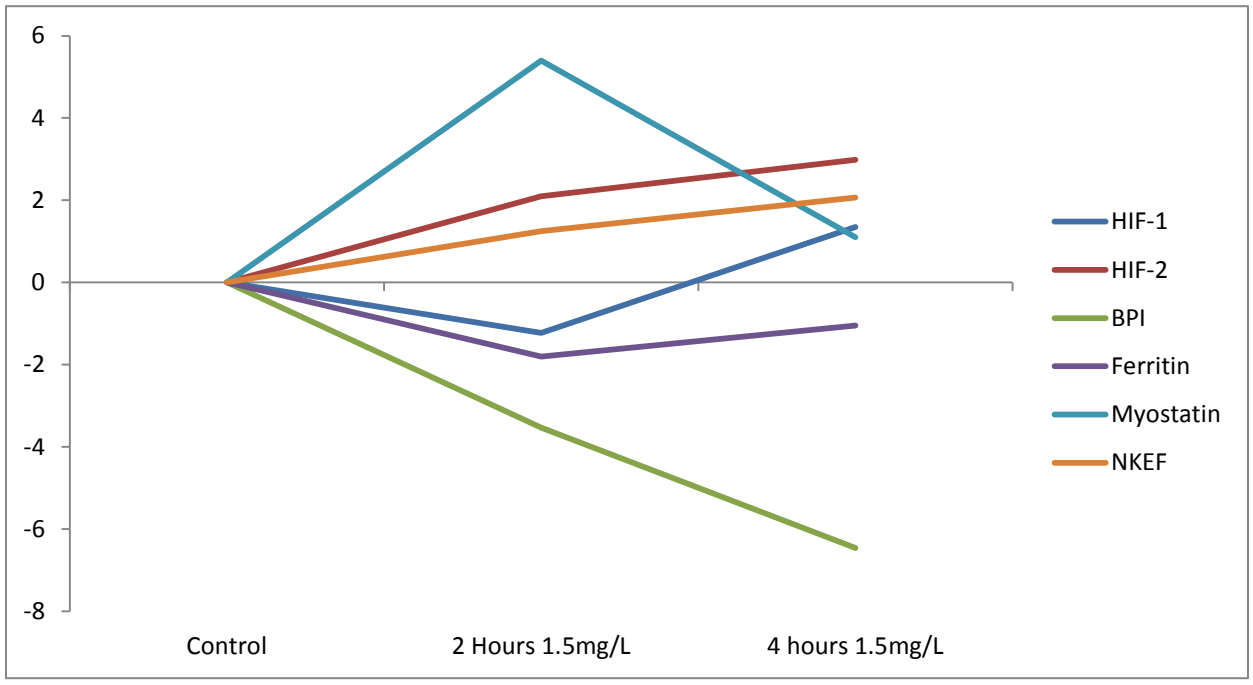
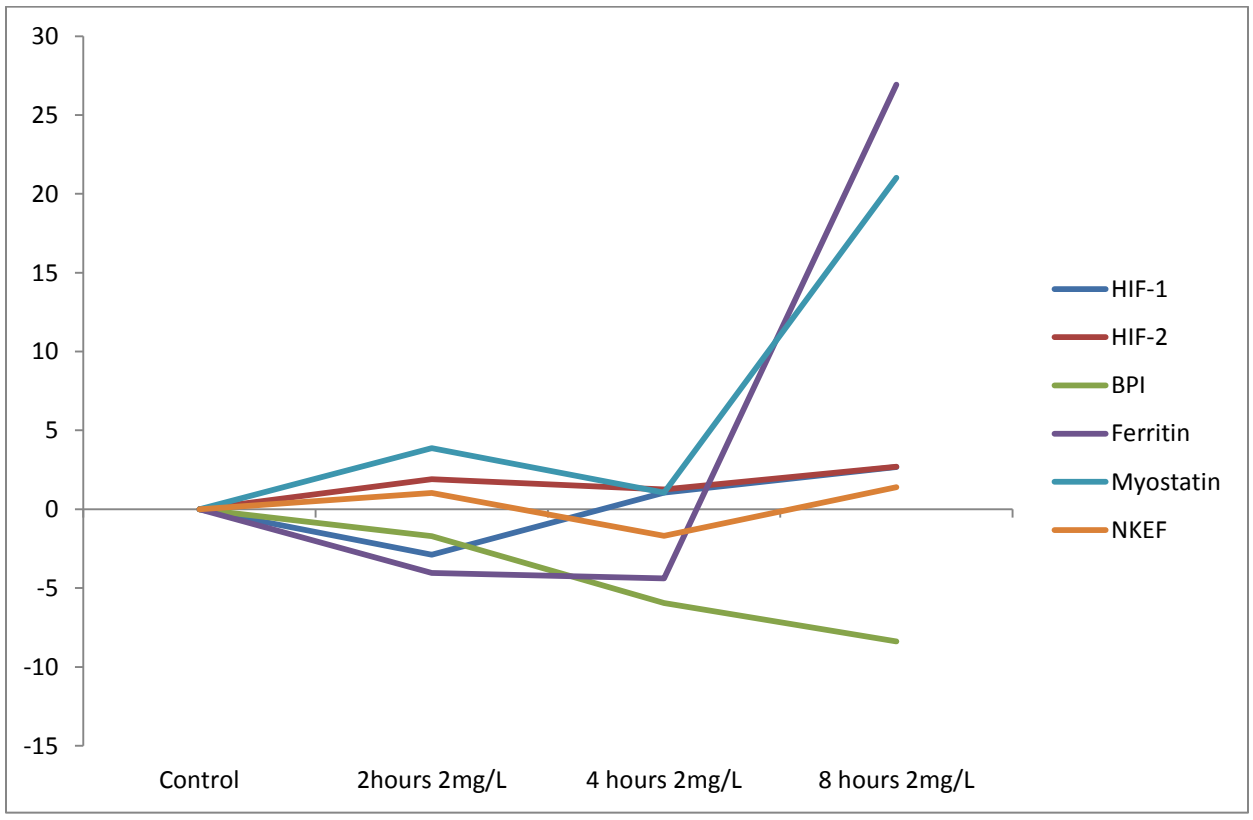


Figure 19. Side by side comparison relative fold changes1.5 mg/L time points vs.2 mg/L time points in all genes over all time points hybrid catfish liver tissue





DISCUSSION

Oxygen levels are a critical control point in aquaculture production upon which feed conversion, fish health, and production capacity often hinge. In spite of the importance of this area, relatively little research has been devoted to identification of phenotypic differences among strains/species of aquacultured organisms, to selection for superior oxygen tolerance, or to a greater understanding of the molecular regulation of these phenomena. In this study, comparisons of the tolerance capacities of channel catfish, blue catfish, and their strain-matched hybrid to acute hypoxic conditions. These conditions were designed to model low DO episodes these fish may experience in industry settings during a pond turnover event, phytoplankton bloom die-off, or failure of aerators to turn on. After initial characterization of the phenotypic differences between the catfish groups, we chose key DO levels and durations to examine expression levels of six genes chosen *a priori*due to known roles in metabolism, immunity, and growth. This study represents, to our knowledge, the first molecular characterization of physiological events associated with acute hypoxia in catfish.

The three groups of organisms (two ictalurid catfish species and their hybrid) in this trial showed a high phenotypic variability among individuals upon induction of acute hypoxic conditions with blue catfish across all time points and oxygen levels being the first to show signs of physical stress. Previous studies comparing differing oxygen tolerances between catfish species and strains (Dunham et al. 1983; Green and Rawles 2011; Green et al. 2012), have been pond-based and have examined the impacts of chronic hypoxic conditions. These studies, while important, introduce a myriad of opportunities for environmental variability to mask genetic differences. Additionally, they do not model responses to dramatic swings in DO levels that can occur over the course of a production cycle. Channel and hybrid catfish both showed a very low

degree of visible stress during 2 mg/L oxygen trials only showing phenotypic stress 35.5 and 37.7% respectively compared to blue catfish which showed stress in 93.3% of 2mg/L trials. Channel and hybrid catfish showed a similar pattern of significantly delayed stressresponse compared to blue catfish during 1.5 mg/L trials taking on average 4 times longer to manifest phenotypic stress. While channel and hybrid catfish commonly could tolerate 8 hrs at 2 mg/L with no visible signs of stress, no blue catfish tolerated greater than 2.5 hrs at this dissolved oxygen level. Some variability, however, was observed between blue catfish, raising the possibility that selection could improve this trait in blues. It should be noted that only a single strain of channel catfish (Marion) and blue catfish (Rio Grande) and their matched hybrid were used in this study. Further work would be needed to determine if these strains are good representatives of the phenotypic performance of their species. This study may serve as starting point for development of methods and baseline measurements with which to assess the range of cultured catfish oxygen tolerances.

The six genes profiled in this study showed a large degree of variability in response to the stimulus encountered across the different species, time points, and oxygen levels. Some of the most studied genes in response to hypoxia are the appropriately named hypoxia inducible factor genes or HIF family of genes. These genes have been studied extensively in mammalian models and tissues (Wieneret al. 1996; Rossignol et al. 2002; Zhao et al. 2004; Wang et al. 2006) but have only been studied to a limited extent in teleost fishes (Soitamo et al. 2001; Lawet al. 2006; Rahmanand Thomas 2007) and to a less extensive degree in siluriforms.There is also limited study on rapid and acute changes in oxygen concentrations more akin to what is encountered in a commercial aquaculture setting in which a system contains an unnaturally high amount of respiring organism opposed to that of a natural system.

HIF-1 is an oxygen sensitive gene believed to initiate a cascade of cellular events upon exposure to hypoxia in all obligate aerobes. HIF-1 genes for hybrid catfish showed significant up- regulation in gill at 2 hr2 mg/L trial 4 hr2 mg/L and 4 hr1.5 mg/L. Channel and blue catfish did not show significant up-regulationat any points in gill tissue. It is interesting to note that that while none of the time points were deemed to be significant, HIF-1 appears to show an overall trend of down- regulation to hypoxic stress in blue catfish which is the opposite of the expected response observed in HIF-1 studies across most animals. The three groups also showed significant response differences in liver compared to gill with channel catfish showing the only significant movement in liver tissue. Channel catfish showed the only significant increase of HIF-1 in liver tissue at the 4 hr 1.5 mg/L time point with hybrids showing an overall trend of up-regulation but not to a significant degree. Blue catfish again showed an overall trend of down-regulation with significant down-regulation at 2 and 8 hr at 2 mg/L. Duration and intensity of stimulus have shown in other studies to be extremely important factors in determining genetic response.The Terova et al. (2008) study of sea bass showed significant up-regulation of HIF-1 at 2 days, 5 days and 15 days but not after 24 hr at4.3 ± .8 mg/L oxygen levels in liver tissue they also reported significant up-regulation at 4 hr under 1.9 ±.2 mg/L oxygen levels. A similar response was found in this study where the largest fold change at both oxygen concentrations occurred at the 4 hr 1.5 mg/L trial which was the most visibly stressful for all fish.

HIF- 2 is even less studied than HIF-1 in fish, and so there is even less data to compare our results with(Raman and Thomas 2007; Shen et al 2010). In this study, HIF-2 only showed significant up regulation differences in channel catfish and in no other group. However the general trends in regulation of HIF-2 compared to HIF-1 were similar. Hybrid catfish showed up-regulation across all time points except for 2 hr 1.5 mg/L in gill tissue. Channel catfish also

showed an overall trend of up-regulation particularly in the gills as opposed to up and down-regulation in liver tissue leading to significant up-regulation after the two most stressful trials. Blue catfish again showed an overall trend of down-regulation across both tissues oxygen levels and time points although not to a significant degree.

Other studies agree that HIF-1 and HIF-2 can move differentially across tissues and species. Soitamo et al. (2001) showed no significant changes in rainbow trout HIF-1 mRNA transcripts as a response to hypoxia. Shen et al. (2010) reported no significant up-regulation and even some insignificant down-regulation of HIF-1 across liver brain and kidney of the Wuchang bream under hypoxic conditions. They also reported significant up-regulation of HIF-2 in liver and kidney but not in brain. Remoldiet al. (2011) reported no change in HIF-1 in liver and kidney of Eurasian perch under hypoxic conditions but significant up-regulation for HIF-2 in these tissues. HIF gene family regulation has been observed to behave uniquely between different species and under different levels of dissolved oxygen, our results suggest that HIF-1 may be more important to hybrid catfish oxygen tolerance while HIF-2 may be more impactful to channel catfish. Hybrid catfish HIF-1 genes show the greatest degree of sensitivity to ambient conditions followed by channel catfish while channel catfish were the only group to show HIF-2 sensitivity.Based on the relative observable stress performance of the three catfish groups tested and their corresponding HIF gene reaction it is a reasonable assumption that HIF gene family sensitivity is beneficial to survival of catfish.

Blue catfish showed the least degree of significant movement in HIF genesnever reporting larger than a 5 fold up or down change and consistently were the first to show observable phenotypic stress during trials. It is possible that the low degree of HIF gene family sensitivity in blue catfish contributes to the fish's inability to withstand acute hypoxic

conditions.When discussing the lack of significant HIF movement across all time points and species it should also note the large amount of variability between manifestations of observable stress. Channel and hybrid catfish showed a wide range in time to surface between individual fish in the same trial. This high degree of variability in time to stress between trials and individual fish could explain the lack of significant movement at similar stress intensity but different time points.

BPI genesorbactericidal permeability increasing protein (BPI) is an antimicrobial peptide belonging to the lipid transfer/LPS-binding protein family. It serves important roles in early protection against Gram-negative bacteria in the innate immune system. BPI genes showed highly differential expression levels between the three groups of fish tested. Channel and hybrid catfish showed similar levels of expression in both tested tissue whereas blue catfish had significantly less expression levels inall control samples of all tissues. BPI genes in catfish have been shown to up-regulate in response to challenge from the Gram-negative bacteria, *Edwardsiellaictaluri*,the causative agent of ESC in catfish (Xu et al. 2005) and have been linked to innate immune responses of other organisms.

BPI genes were significantly down-regulated in both channel and hybrid catfish at multiple time points and in both tissues observed. In gill tissue, both channel and hybrid catfish showed by far the largest down-regulationwhich occurred at the 8 hr2 mg/L time point suggesting that duration of the stress event plays a large role in determining the magnitude of movement for BPI genes in gill tissue. In liver tissue channel catfish showed the largest down-regulation at 4 hrand1.5 mg/L and no significant movement for all other observed trials. Hybrid catfish showed steadily decreasing expression profiles with duration and intensity of the trials. The first significant down-regulation for BPI genes in hybrid liver occurred at 4 hr for both 2

mg/L and 1.5 mg/L and fell even lower at the 8 hr2 mg/L time point. These significant drops in BPI gene expression under hypoxic conditions could help explain the increased susceptibility to disease encountered by all fish that experience similar stress events. Blue catfish also showed an overall pattern of down-regulation however none of the time points across both tissues were deemed significant when contrasted against the low initial expression levels of the BPI gene. It cannot determine from this experiment if the movement in BPI genes exhibited by each class of fish is the optimal genotype for culture. Previous studies have shown blue catfish with superior resistance to ESC (Bosworth et al. 2003) and channel catfish with increased resistance to columnaris (Dunham et al. 1993; Dunham and Argue 2000),both Gram negative bacteria,and therefore should be affected by BPI production, because of this apparent difference between the species in both susceptibility and response to these bacterial infections there are likely other factors at work in determining optimal genetic response and genotype for disease resistance pursuant to hypoxia in all species of catfish.

Iron regulation is critical in many physiological and biochemical processes such as oxygen transportation, electron transfer, DNA replication and photosynthesis (Theil1987). The concentration level of iron within an organism is vitally important for both cell growth and metabolism. High levels of iron in cells will lead to oxidative damage of proteins, lipids and DNA (Reif 1992; Linn 1998). Ferritin plays a key role in maintaining normal iron levels (Theil 1987). Its main functions are iron storage and detoxification (Harrison and Arosio 1996; Connolly and Guerinot 2002). Ferritin has also been suggested as an acute phase protein responding to a nonlethal injury to the organism (Beck et al. 2002).

Ferritin regulation in the fish tested was variable and dramatic with channel catfish showing an over 70-fold up-regulation in liver tissue at the 8 hr2 mg/L time point. This was by

far the largest fold change for any gene, tissue or time point in the entire study. We also observed a slightly smaller but significant fold change in liver tissue for hybrid catfish with a 27-fold up-regulation at the 8 hr at 2 mg/L time point. Blue catfish also showed a significant 9-fold up-regulation of ferritin at the 4 hr at 1.5 mg/L time point. Interestingly both blue and hybrid catfish showed small but significant down-regulation of expression at the 2 hr and 4 hr2 mg/L trials.

Similar results were observed for the ferritin gene in gill tissue as well. In channel and hybrid catfish no significant changes occurred in the early time points of the trials, and even showed small down-regulation, then at the 8 hr time point both species showed large and significant increases in ferritin regulation. No such increases were observed in blue catfish across all time points. These delayed increases in the ferritin gene suggest that the fish are over time accumulating iron, andas a result, reactive oxygen species (ROS)in their organs. Our results suggest that it takes a minimum of fourhr of hypoxic stress to induce significant changes in transcript levels and as the stress event continues ferritin up-regulation increases with it. It would have been interesting to see, if returned to normoxic conditions, how long ferritin levels would remain elevated in the affected fish. It should also be noted that the blue catfish showed a much lower degree of ferritin regulation than the other fish and while blue catfish showed outward stress signals long before any changes to ferritin regulation were observed, the inability to clear iron and ROS buildup in this species may be a major component of its poor hypoxia tolerance in general. Had recovery trials been observed it is likely that blue catfish would have shown much poorer ability to return to homeostasis than its channel and hybrid counterparts.

Myostatin, a member of the TGFβsuper family of ligands,has been shown to be a negative regulator of skeletal musclemass during embryogenesis and early postnatal muscle

growth(Kambadur et al. 1997; McPherron et al. 1997).Myostatin has only recently been studied in aquatic species with much of our knowledge coming from mammalianmodels. However, its physical structure is highly conserved across all animals suggesting high levels of evolutionary constraint and the importance of its function (Kocabas et al. 2002). It was initially believed that myostatin was only expressed in skeletal muscle but other studies (Kocabas et al. 2002) and our study have shown myostatin to be expressed in other tissues as well.

Myostatin in gill tissue showed significant down-regulation in channel catfish at the 2 hr at2 mg/L time point and in hybrid catfish at 8 hr and 2 mg/L. While there were no other significant time points, the overall trend in gill tissue was one of down-regulation across all time points and species. While none of the trials showed extreme changes in down-regulation,any decrease is the opposite effect of the typical results (Hayot et al. 2010) found for myostatin in other organisms subjected to hypoxia. Gills are the major respiratory organ in fish and lacking other acute hypoxic studies with which to make comparisons, we expected similar results in other animals. Contrarily, mammalian studies of mice and humans show up-regulation in lung tissue under conditions of hypoxic stress (Bartman and Speer 2004;Hayot et al. 2010).

Myostatin showed significant increases in both channel and blue catfishliverat the 4 hr1.5 mg/L time point. While no other time points were deemed significant, it should be noted that blue catfish also showed a high degree of up-regulation at the 2 hr 1.5 mg/L time point with an 11-fold increase and a nearly linear progression to its peak expression at 4 hr 1.5 mg/Lwith a 24-fold increase. Hybrid catfish did not show significant change in myostatin,due to high variability,but showed a large 21 fold increase at the 8 hr at2 mg/L time point. Both hybrid and blue catfish showed very large degrees of myostatin change under different conditions with blue catfish changes being stronger at lower dissolved oxygen levels and hybrid changes occurring

after longer duration. All results for myostatin show a very high degree of error preventing more time points from achieving significance. Channel catfish myostatin also appears to be much less sensitive than that of the other species, never showing a greater than 4.5 times fold change in either direction across all treatmentsand tissues. Blue catfish showed the highest degree of change in myostatin as a response to hypoxia followed by the hybrid. Of all the genes examined, myostatin was the only gene in which hybrid expression patterns showed greater similarity to blue catfish than channel catfish.

It is interesting to note that regulation patterns were reversed for liver and gill tissue suggesting that myostatin may have tissue specific function as well as regulation. Recent studies on myostatin in barramundi also showeddown-regulation in gill tissue with corresponding up-regulation in liver tissue as well as greater magnitude changes occurring in liver in response to fasting (De Santis and Jerry 2010). Studies such De Santis and Jerry (2010) and this study that show opposing regulation direction between tissues bolster the hypothesis that myostatin may be responsible for other factors of fish homeostasis, such as osmoregulation and not just an arbiter of muscle growth. De Santis and Jerry (2010)also determined that myostatin in barramundi contained two paralogs with 90% similarity which could show differential and often opposite expression between tissues. It is unknown if catfish contain these two paralogs or if the primers used in this experiment were specific enough to differentiate one from the other.

NKEF or natural killer enhancing factor effects NK cells in mammals and natural killer-likecells in fish (also called non-specific cytotoxic cells). NK cells are important early effectors of the innate immune response. They may be essential for priming the adaptive immune response that plays an important role in defense against pathogens (Kuznetsov 1996). Catfish NKEF shares high levels of sequence identity with other teleost NKEFs and are expressed in all major

tissues of catfish (Li and Waldbieser 2004).NKEF has also been confirmed and shows a highly conserved sequence in rainbow trout, common carp, and puffer fish(*Tetraodonnigroviridis)*(Zhang et al. 2001; Shin et al. 2001;Dong et al. 2006). NKEF's response to hypoxia has not been studied with the majority of research determining its regulation after challenge with disease of LPS (Shin et al.2001; Kim et al. 2011).

In gill tissue, only hybrid catfish showed any significant change in NKEF which occurred at the 8 hr at2 mg/L time point. No other fish showed significant change in gill across all time points. It is interesting to note that hybrid catfish were the only fish to show an overall pattern of up-regulation in gill tissue. Channel and blue catfish showed general trends of down-regulation with channels only showing insignificant up-regulation at the 4 hrat 1.5 mg/L time point. This finding is unusual due to the fact that hybrids, being a combination of channel and blue catfish, showed a completely different expression pattern compared to their genetic source material. In this study hybrid expression patterns in general were relatively close to one of the other species challenged, NKEF is the only gene in this study in which hybrid catfish showed a total divergence in the general regulation trend of the other two species.

In liver tissue only, blue catfish showed significant NKEF down-regulation at the 4 hr 1.5 mg/L time point. Channel catfish while not showing significant change did show an overall trend of up-regulation for NKEF in liver tissue including a large upward fold change of 10.6 times at the 4 hr 1.5 mg/L time point. In liver tissue again divergent trends were observed in overall regulation between the species with hybrid and channel catfish showing general trends of up-regulation and blue catfish showing the opposite. Kim et al. (2011), reported up-regulation in response to challenge from various pathogens and also suggested that NKEF regulation responds to ROS accumulation as a result of phagocytic activity of the immune system. If NKEF indeed

responds to ROS build up we would expect similar expression patterns to that of ferritin in catfish, but this was not the case. NKEF may indeed be involved in ROS mitigation, only more specifically targeted to byproducts of phagocytosis and not hypoxic stress-related buildup of metabolites that caused the aforementioned ferritin spike.

The results of this study show the three genotypes of catfish tested exhibited a large degree of genetic variation in response to acute hypoxic stress, dependent on duration of the event, as well as the intensity. Directly opposite molecular responses were observed to the same stimulus which was interesting considering the high degree of genetic similarity between the fish challenged. The lack of consistently significant changes in regulation for genes, particularly in the HIF family, over similar stress levels also requires further investigation. When time to show visible stress, classified in this study as agitated swimming at the surface, a very large degree of variability was observed for individual fish at different time points and oxygen levels. This extreme amount of difference between individual fish could represent significantdifferences of genetic sensitivitythat become masked when tissues are taken in aggregate for a trial, introducing the large degree of error observed for some of the genes in this study. For future studies it may be beneficial to challenge fish individually noting the time to stress and investigating the resultant fold change.

The catfish is also unique when compared to other species of teleosts in the fact that it has been an aquaculture species for decades and, therefore, faced an extremely different set of artificial selection pressures than other less domesticated species. Farmers selecting for growth, disease resistance, or dissolved oxygen tolerance based on the ambient culture conditions could have inadvertently changed the sensitivity of any of the investigated genes to hypoxic conditions. Future studies might benefit from obtaining wild caught fish for a control against unknown

selection bias introduced by aquaculturalists in the pre-genetics era. Future studies would also benefit from a subsequent disease challenge post hypoxia to determine if the fold changes of the immune specific genes tested were beneficial or harmful from a disease resistance standpoint.

A genetic optimum is difficult to ascertain for aquaculture species due to the fact that no two sites have identical conditions. Therefore, the optimum genetic signature can change from farm to farm or even pond to pond. In this study, channel and hybrid catfish showed significantly less phenotypic stress than their blue counterparts measured in time to surface. Furthermore the genetic expression profiles of channel and hybrid catfish showed much higher similarity when compared to the blue catfish. This study gives us a basis for future marker assisted selection in catfish. As technology continues to improve making it easier and cheaper to examine the genetic makeup of an organism we will hone the ability to view large arrays of genes and their changes in sensitivity over generations helping to determine our optimal fish. Currently, it would appear that any immediate improvements to hybrid catfish hypoxia tolerance would come as a result of improvements in the channel mother in a hybrid cross. Blue catfish did not appear to contribute significantly to hybrid catfish hypoxia tolerance as evidenced by their rapid time to surface and erratic gene regulation profiles.

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Appendix 1.Two Way ANOVA Tables.

Anova: Two-Factor With Replication

SUMMARY	Blue	Hybrid	Total
2hr 2mg/L			
Count	15	15	30
Sum	234.8	204.3	439.1
Average	15.65333	13.62	14.63667
Variance	5.108381	2.043143	4.521713
4hr 2mg/L			
Count	15	15	30
Sum	251	205.5	456.5
Average	16.73333	13.7	15.21667
Variance	7.59381	1.904286	6.964885
8hr 2mg/L			
Count	15	15	30
Sum	240.8	203.2	444
Average	16.05333	13.54667	14.8
Variance	7.324095	2.902667	6.562069
2hr 1.5mg/L			
Count	15	15	30
Sum	248.4	202.6	451
Average	16.56	13.50667	15.03333
Variance	7.692571	2.186381	7.18023
4hr 1.5 mg/L			
Count	15	15	30
Sum	240.8	203.5	444.3
Average	16.05333	13.56667	14.81
Variance	5.522667	5.760952	7.046448
Total			
Count	75	75	
Sum	1215.8	1019.1	
Average	16.21067	13.588	
Variance	6.442047	2.804043	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.164933	4	1.541233	0.32083	0.863652	2.436317
Columns	257.9393	1	257.9393	53.69377	1.7E-11	3.908741
Interaction	5.5004	4	1.3751	0.286247	0.886512	2.436317
Within	672.5453	140	4.803895			
Total	942.1499	149				

Appendix 2. One and Two way ANOVA tables for time to phenotypic stress.

Anova: Single Factor **2mg/L Channel vs.Hybrid catfish that surfaced**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Channel	17	3538	208.1176	11157.61
Hybrid	17	3305	194.4118	9444.132

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1596.735	1	1596.735	0.15501	0.696404	4.149097
Within Groups	329627.9	32	10300.87			
Total	331224.6	33				

Anova: Single Factor **1.5mg/L Channel vs Hybrid**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Channel	30	1812	60.4	143.8345
Hybrid	30	1724	57.46667	125.5678

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	129.0667	1	129.0667	0.95817	0.331714	4.006873
Within Groups	7812.667	58	134.7011			
Total	7941.733	59				

Anova: Two-Factor With Replication **1.5mg/L All fish**

SUMMARY	Blue	Channel	Hybrid	Total
2hr 1.5mg/L				
Count	15	15	15	45
Sum	231	937	838	2006
Average	15.4	62.46667	55.86667	44.57778
Variance	11.97143	137.6952	144.6952	536.4313
4hr 1.5 mg/L				
Count	15	15	15	45
Sum	187	875	886	1948
Average	12.46667	58.33333	59.06667	43.28889
Variance	9.409524	151.0952	109.9238	571.9374
Total				
Count	30	30	30	
Sum	418	1812	1724	
Average	13.93333	60.4	57.46667	
Variance	12.54713	143.8345	125.5678	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	37.37778	1	37.37778	0.397079	0.530312	3.954568
Columns	40629.07	2	20314.53	215.8096	7.98E-34	3.105157
Interaction	232.0889	2	116.0444	1.232788	0.296698	3.105157
Within	7907.067	84	94.13175			
Total	48805.6	89				

Appendix 3.Raw Data with additional REST statistical data

Well	Fluor	Threshold Cycle (C(t)	C(t) Mean	Gene	Fish, Duration, Intensity Tissue	Direction of regulation, P value, Standard Error
A01	FAM	24.42		HIF-1	blue 4 hr control gill	
A02	FAM	24.82	24.42	HIF-1	blue 4 hr control gill	
A03	FAM	24.01		HIF-1	blue 4 hr control gill	
B07	FAM	25.43		HIF-1	blue 2hr 2 mg/L gill	down 2.399
B08	FAM	24.07	24.40	HIF-1	blue 2hr 2 mg/L gill	p .512
B09	FAM	23.71		HIF-1	blue 2hr 2 mg/L gill	0.197
D01	FAM	23.24		HIF-1	blue 2hr 1.5 mg/L gill	down 1.541
D02	FAM	24.15	23.76	HIF-1	blue 2hr 1.5 mg/L gill	p.892
D03	FAM	23.90		HIF-1	blue 2hr 1.5 mg/L gill	0.22967
E07	FAM	23.23		HIF-1	blue 4hr 2 mg/L gill	up 1.105
E08	FAM	22.83	22.99	HIF-1	blue 4hr 2 mg/L gill	p.946
E09	FAM	22.92		HIF-1	blue 4hr 2 mg/L gill	0.37756
G01	FAM	23.68		HIF-1	blue 4hr 1.5 mg/L gill	down 1.012
G02	FAM	22.52	23.16	HIF-1	blue 4hr 1.5 mg/L gill	p. 946
G03	FAM	23.27		HIF-1	blue 4hr 1.5 mg/L gill	3.776
H07	FAM	24.53		HIF-1	blue 8hr 2 mg/L gill	down 2.721
H08	FAM	24.95	24.58	HIF-1	blue 8hr 2 mg/L gill	p.31
H09	FAM	24.28		HIF-1	blue 8hr 2 mg/L gill	0.1211
A04	FAM	26.03		HIF-1	blue 4 hr control liver	
A05	FAM	26.39	26.20	HIF-1	blue 4 hr control liver	
A06	FAM	26.19		HIF-1	blue 4 hr control liver	
B10	FAM	26.76		HIF-1	blue 2hr 2 mg/L liver	down 2.37
B11	FAM	26.48	26.76	HIF-1	blue 2hr 2 mg/L liver	p .001
B12	FAM	27.03		HIF-1	blue 2hr 2 mg/L liver	1.88
D04	FAM	26.14		HIF-1	blue2hr 1.5 mg/L liver	down 1.916
D05	FAM	26.17	26.48	HIF-1	blue2hr 1.5 mg/L liver	p .279
D06	FAM	27.13		HIF-1	blue2hr 1.5 mg/L liver	0.245

E10	FAM	26.06		HIF-1	blue4hr 2 mg/L liver	down 2.171
E11	FAM	26.94	26.66	HIF-1	blue4hr 2 mg/L liver	p .093
E12	FAM	26.99		HIF-1	blue4hr 2 mg/L liver	0.2166
G01	FAM	23.68		HIF-1	blue4hr 1.5 mg/L liver	up 5.229
G02	FAM	22.52	23.16	HIF-1	blue4hr 1.5 mg/L liver	p.062
G03	FAM	23.27		HIF-1	blue4hr 1.5 mg/L liver	2.53
H10	FAM	27.40		HIF-1	blue8hr 2 mg/L liver	down 5.663
H11	FAM	27.67	27.88	HIF-1	blue8hr 2 mg/L liver	p .001
H12	FAM	28.58		HIF-1	blue8hr 2 mg/L liver	0.09645
A07	FAM	25.35		HIF-1	channel 4hr control gill	
A08	FAM	24.24	24.83	HIF-1	channel 4hr control gill	
A09	FAM	24.89		HIF-1	channel 4hr control gill	
C01	FAM	24.42		HIF-1	channel 2hr 2 mg/L gill	down 1.001
C02	FAM	24.19	24.25	HIF-1	channel 2hr 2 mg/L gill	p .944
C03	FAM	24.15		HIF-1	channel 2hr 2 mg/L gill	0.351
D07	FAM	23.68		HIF-1	channel 2hr 1.5 mg/L gill	up 1.364
D08	FAM	23.76	23.80	HIF-1	channel 2hr 1.5 mg/L gill	p .653
D09	FAM	23.98		HIF-1	channel 2hr 1.5 mg/L gill	0.48
F01	FAM	23.99		HIF-1	channel 4hr 2 mg/L gill	up 1.540
F02	FAM	23.52	23.63	HIF-1	channel 4hr 2 mg/L gill	p .451
F03	FAM	23.38		HIF-1	channel 4hr 2 mg/L gill	0.568
G07	FAM	24.20		HIF-1	channel 4hr 1.5 mg/L gill	up 1.408
G08	FAM	23.08	23.76	HIF-1	channel 4hr 1.5 mg/L gill	p .527
G09	FAM	23.99		HIF-1	channel 4hr 1.5 mg/L gill	0.593
C01	FAM	24.22		HIF-1	channel 8hr 2 mg/L gill	up 1.230
C02	FAM	24.50	23.95	HIF-1	channel 8hr 2 mg/L gill	p .65
C03	FAM	23.15		HIF-1	channel 8hr 2 mg/L gill	0.551
A10	FAM	27.11		HIF-1	channel 4hr control liver	
A11	FAM	27.23	27.19	HIF-1	channel 4hr control liver	
A12	FAM	27.23		HIF-1	channel 4hr control liver	
C04	FAM	27.19		HIF-1	channel 2hr 2 mg/L liver	up 1.158
C05	FAM	26.80	26.88	HIF-1	channel 2hr 2 mg/L liver	p .243

C06	FAM	26.65		HIF-1	channel 2hr 2 mg/L liver	0.342
D10	FAM	26.19		HIF-1	channel 2hr 1.5 mg/L liver	up 1.427
D11	FAM	26.45	26.58	HIF-1	channel 2hr 1.5 mg/L liver	p .19
D12	FAM	27.08		HIF-1	channel 2hr 1.5 mg/L liver	0.47
F04	FAM	26.13		HIF-1	channel 4hr 2 mg/L liver	up 1.67
F05	FAM	26.90	26.35	HIF-1	channel 4hr 2 mg/L liver	p .104
F06	FAM	26.03		HIF-1	channel 4hr 2 mg/L liver	0.557
G10	FAM	23.34		HIF-1	channel 4hr 1.5 mg/L liver	up 13.669
G11	FAM	23.86	23.32	HIF-1	channel 4hr 1.5 mg/L liver	p.048
G12	FAM	22.75		HIF-1	channel 4hr 1.5 mg/L liver	4.814
C04	FAM	26.45		HIF-1	channel 8 hr2 mg/L liver	up 1.772
C05	FAM	25.97	26.31	HIF-1	channel 8 hr2 mg/L liver	p .056
C06	FAM	26.51		HIF-1	channel 8 hr2 mg/L liver	0.513
B01	FAM	24.14		HIF-1	hybrid 4hr control gill	
B02	FAM	26.20	25.32	HIF-1	hybrid 4hr control gill	
B03	FAM	25.61		HIF-1	hybrid 4hr control gill	
C07	FAM	23.97		HIF-1	hybrid 2hr 2 mg/L gill	up 2.59
C08	FAM	23.84	23.95	HIF-1	hybrid 2hr 2 mg/L gill	p .034
C09	FAM	24.04		HIF-1	hybrid 2hr 2 mg/L gill	1.502
E01	FAM	24.83		HIF-1	hybrid 2hr 1.5 mg/L gill	up 1.739
E02	FAM	23.45	24.52	HIF-1	hybrid 2hr 1.5 mg/L gill	p .349
E03	FAM	25.29		HIF-1	hybrid 2hr 1.5 mg/L gill	1.206
F07	FAM	23.76		HIF-1	hybrid 4hr 2 mg/L gill	up 3.459
F08	FAM	23.14	23.53	HIF-1	hybrid 4hr 2 mg/L gill	p .034
F09	FAM	23.70		HIF-1	hybrid 4hr 2 mg/L gill	2.057
H01	FAM	22.36		HIF-1	hybrid 4hr 1.5 mg/L gill	up 5.161
H02	FAM	22.52	22.95	HIF-1	hybrid 4hr 1.5 mg/L gill	p .034
H03	FAM	23.98		HIF-1	hybrid 4hr 1.5 mg/L gill	3.505
C07	FAM	24.34		HIF-1	hybrid 8hr 2 mg/L gill	up 1.596
C08	FAM	23.90	24.65	HIF-1	hybrid 8hr 2 mg/L gill	p .424
C09	FAM	25.70		HIF-1	hybrid 8hr 2 mg/L gill	1.101
B04	FAM	26.29		HIF-1	hybrid 4hr control liver	

B05	FAM	26.26	26.77	HIF-1	hybrid 4hr control liver	
B06	FAM	27.74		HIF-1	hybrid 4hr control liver	
C10	FAM	26.69		HIF-1	hybrid 2hr 2 mg/L liver	down 2.891
C11	FAM	28.84	27.71	HIF-1	hybrid 2hr 2 mg/L liver	p.089
C12	FAM	27.60		HIF-1	hybrid 2hr 2 mg/L liver	0.236
E04	FAM	26.87		HIF-1	hybrid 2hr 1.5 mg/L liver	down 1.229
E05	FAM	26.05	26.48	HIF-1	hybrid 2hr 1.5 mg/L liver	p .664
E06	FAM	26.51		HIF-1	hybrid 2hr 1.5 mg/L liver	0.451
F10	FAM	26.41		HIF-1	hybrid 4hr 2 mg/L liver	up 1.054
F11	FAM	26.33	26.10	HIF-1	hybrid 4hr 2 mg/L liver	p.988
F12	FAM	25.57		HIF-1	hybrid 4hr 2 mg/L liver	0.5903
H04	FAM	26.51		HIF-1	hybrid 4hr 1.5 mg/L liver	up 1.346
H05	FAM	26.46	25.75	HIF-1	hybrid 4hr 1.5 mg/L liver	p .772
H06	FAM	24.27		HIF-1	hybrid 4hr 1.5 mg/L liver	0.99
C10	FAM	26.12		HIF-1	hybrid 8hr 2 mg/L liver	up 2.672
C11	FAM	23.97	24.76	HIF-1	hybrid 8hr 2 mg/L liver	p .215
C12	FAM	24.19		HIF-1	hybrid 8hr 2 mg/L liver	1.897
A01	FAM	23.99		HIF-2	blue 4 hr control gill	
A02	FAM	24.82	24.28	HIF-2	blue 4 hr control gill	
A03	FAM	24.01		HIF-2	blue 4 hr control gill	
B07	FAM	25.43		HIF-2	blue 2hr 2 mg/L gill	up 1.402
B08	FAM	24.07	24.40	HIF-2	blue 2hr 2 mg/L gill	p .622
B09	FAM	23.71		HIF-2	blue 2hr 2 mg/L gill	1.136
D01	FAM	23.24		HIF-2	blue 2hr 1.5 mg/L gill	down 1.420
D02	FAM	24.15	23.76	HIF-2	blue 2hr 1.5 mg/L gill	p .674
D03	FAM	23.90		HIF-2	blue 2hr 1.5 mg/L gill	0.571
E07	FAM	23.23		HIF-2	blue 4hr 2 mg/L gill	up 1.269
E08	FAM	22.83	22.99	HIF-2	blue 4hr 2 mg/L gill	p .479
E09	FAM	22.92		HIF-2	blue 4hr 2 mg/L gill	1.037
G01	FAM	23.68		HIF-2	blue 4hr 1.5 mg/L gill	down 2.745
G02	FAM	22.52	23.16	HIF-2	blue 4hr 1.5 mg/L gill	p .815
G03	FAM	23.27		HIF-2	blue 4hr 1.5 mg/L gill	0.313

H07	FAM	24.53		HIF-2	blue 8hr 2 mg/L gill	down 2.842
H08	FAM	28.95	27.58	HIF-2	blue 8hr 2 mg/L gill	p .418
H09	FAM	29.28		HIF-2	blue 8hr 2 mg/L gill	0.3251
A04	FAM	26.03		HIF-2	blue 4 hr control liver	
A05	FAM	26.39	26.20	HIF-2	blue 4 hr control liver	
A06	FAM	26.19		HIF-2	blue 4 hr control liver	
B10	FAM	26.76		HIF-2	blue 2hr 2 mg/L liver	down 1.10
B11	FAM	26.48	26.76	HIF-2	blue 2hr 2 mg/L liver	p .845
B12	FAM	27.03		HIF-2	blue 2hr 2 mg/L liver	0.436
D04	FAM	27.14		HIF-2	blue2hr 1.5 mg/L liver	1.075
D05	FAM	26.17	26.81	HIF-2	blue2hr 1.5 mg/L liver	p.905
D06	FAM	27.13		HIF-2	blue2hr 1.5 mg/L liver	0.42641
E10	FAM	27.06		HIF-2	blue4hr 2 mg/L liver	down 1.435
E11	FAM	26.94	26.99	HIF-2	blue4hr 2 mg/L liver	p.554
E12	FAM	26.99		HIF-2	blue4hr 2 mg/L liver	0.37785
G01	FAM	23.68		HIF-2	blue4hr 1.5 mg/L liver	down 1.966
G02	FAM	22.52	23.16	HIF-2	blue4hr 1.5 mg/L liver	p.316
G03	FAM	23.27		HIF-2	blue4hr 1.5 mg/L liver	0.27226
H10	FAM	28.40		HIF-2	blue8hr 2 mg/L liver	down 1.252
H11	FAM	27.67	28.55	HIF-2	blue8hr 2 mg/L liver	p.759
H12	FAM	29.58		HIF-2	blue8hr 2 mg/L liver	0.60337
A07	FAM	25.35		HIF-2	channel 4hr control gill	
A08	FAM	24.24	24.49	HIF-2	channel 4hr control gill	
A09	FAM	23.89		HIF-2	channel 4hr control gill	
C01	FAM	24.42		HIF-2	channel 2hr 2 mg/L gill	up 1.917
C02	FAM	24.19	24.25	HIF-2	channel 2hr 2 mg/L gill	p.388
C03	FAM	24.15		HIF-2	channel 2hr 2 mg/L gill	1.051
D07	FAM	23.68		HIF-2	channel 2hr 1.5 mg/L gill	up 1.740
D08	FAM	23.76	23.80	HIF-2	channel 2hr 1.5 mg/L gill	p.312
D09	FAM	23.98		HIF-2	channel 2hr 1.5 mg/L gill	0.928
F01	FAM	23.99		HIF-2	channel 4hr 2 mg/L gill	up 2.236
F02	FAM	23.52	23.63	HIF-2	channel 4hr 2 mg/L gill	p.185
F03	FAM	23.38		HIF-2	channel 4hr 2 mg/L gill	1.175

G07	FAM	24.20		HIF-2	channel 4hr 1.5 mg/L gill	up 1.282
G08	FAM	23.08	25.76	HIF-2	channel 4hr 1.5 mg/L gill	p.80
G09	FAM	29.99		HIF-2	channel 4hr 1.5 mg/L gill	0.96343
D01	FAM	24.34		HIF-2	channel 8hr 2 mg/L gill	down 3.332
D02	FAM	24.53	24.07	HIF-2	channel 8hr 2 mg/L gill	p.054
D03	FAM	23.35		HIF-2	channel 8hr 2 mg/L gill	0.177
A10	FAM	27.11		HIF-2	channel 4hr control liver	
A11	FAM	27.23	27.19	HIF-2	channel 4hr control liver	
A12	FAM	27.23		HIF-2	channel 4hr control liver	
C04	FAM	27.19		HIF-2	channel 2hr 2 mg/L liver	down 1.294
C05	FAM	26.80	26.88	HIF-2	channel 2hr 2 mg/L liver	p.236
C06	FAM	26.65		HIF-2	channel 2hr 2 mg/L liver	0.436
D10	FAM	26.19		HIF-2	channel 2hr 1.5 mg/L liver	down 1.337
D11	FAM	26.45	26.91	HIF-2	channel 2hr 1.5 mg/L liver	p.236
D12	FAM	28.08		HIF-2	channel 2hr 1.5 mg/L liver	0.25917
F04	FAM	26.13		HIF-2	channel 4hr 2 mg/L liver	down 1.145
F05	FAM	26.90	26.35	HIF-2	channel 4hr 2 mg/L liver	p.74
F06	FAM	26.03		HIF-2	channel 4hr 2 mg/L liver	0.43194
G10	FAM	23.34		HIF-2	channel 4hr 1.5 mg/L liver	up 2.308
G11	FAM	23.86	23.32	HIF-2	channel 4hr 1.5 mg/L liver	p.055
G12	FAM	22.75		HIF-2	channel 4hr 1.5 mg/L liver	0.69906
D04	FAM	26.27		HIF-2	channel 8 hr2 mg/L liver	up 3.951
D05	FAM	26.10	26.16	HIF-2	channel 8 hr2 mg/L liver	p.068
D06	FAM	26.11		HIF-2	channel 8 hr2 mg/L liver	0.603
B01	FAM	24.14		HIF-2	hybrid 4hr control gill	
B02	FAM	26.20		HIF-2	hybrid 4hr control gill	
B03	FAM	25.61	25.32	HIF-2	hybrid 4hr control gill	
C07	FAM	23.97		HIF-2	hybrid 2hr 2 mg/L gill	up 1.334
C08	FAM	23.84		HIF-2	hybrid 2hr 2 mg/L gill	p.591
C09	FAM	24.04	23.95	HIF-2	hybrid 2hr 2 mg/L gill	1.051
E01	FAM	24.83		HIF-2	hybrid 2hr 1.5 mg/L gill	down 1.053
E02	FAM	23.45		HIF-2	hybrid 2hr 1.5 mg/L gill	p.952

E03	FAM	25.29	24.52	HIF-2	hybrid 2hr 1.5 mg/L gill	0.635
F07	FAM	23.76		HIF-2	hybrid 4hr 2 mg/L gill	up 1.543
F08	FAM	23.14		HIF-2	hybrid 4hr 2 mg/L gill	p.499
F09	FAM	23.70	23.53	HIF-2	hybrid 4hr 2 mg/L gill	1.058
H01	FAM	22.36		HIF-2	hybrid 4hr 1.5 mg/L gill	up 1.534
H02	FAM	22.52		HIF-2	hybrid 4hr 1.5 mg/L gill	p.478
H03	FAM	23.98	22.95	HIF-2	hybrid 4hr 1.5 mg/L gill	1.055
D07	FAM	23.58		HIF-2	hybrid 8hr 2 mg/L gill	up 1.090
D08	FAM	24.15		HIF-2	hybrid 8hr 2 mg/L gill	p.794
D09	FAM	23.71	23.82	HIF-2	hybrid 8hr 2 mg/L gill	0.732
B04	FAM	26.29		HIF-2	hybrid 4hr control liver	
B05	FAM	26.26		HIF-2	hybrid 4hr control liver	
B06	FAM	27.74	26.77	HIF-2	hybrid 4hr control liver	
C10	FAM	26.69		HIF-2	hybrid 2hr 2 mg/L liver	up 1.890
C11	FAM	28.84		HIF-2	hybrid 2hr 2 mg/L liver	p.609
C12	FAM	27.60	27.71	HIF-2	hybrid 2hr 2 mg/L liver	1.844
E04	FAM	26.87		HIF-2	hybrid 2hr 1.5 mg/L liver	up 2.093
E05	FAM	26.05		HIF-2	hybrid 2hr 1.5 mg/L liver	p.411
E06	FAM	26.51	26.48	HIF-2	hybrid 2hr 1.5 mg/L liver	1.927
F10	FAM	26.41		HIF-2	hybrid 4hr 2 mg/L liver	up 1.262
F11	FAM	26.33		HIF-2	hybrid 4hr 2 mg/L liver	p.751
F12	FAM	25.57	26.10	HIF-2	hybrid 4hr 2 mg/L liver	1.134
H04	FAM	26.51		HIF-2	hybrid 4hr 1.5 mg/L liver	up 2,982
H05	FAM	26.46		HIF-2	hybrid 4hr 1.5 mg/L liver	p.257
H06	FAM	24.27	25.75	HIF-2	hybrid 4hr 1.5 mg/L liver	2.713
D10	FAM	26.99		HIF-2	hybrid 8hr 2 mg/L liver	up 2.695
D11	FAM	23.97		HIF-2	hybrid 8hr 2 mg/L liver	p.308
D12	FAM	24.25	25.07	HIF-2	hybrid 8hr 2 mg/L liver	2.461
A01	FAM	35.52		BPI	blue 4 hr control gill	
A02	FAM	38.58	36.03	BPI	blue 4 hr control gill	
A03	FAM	34.00		BPI	blue 4 hr control gill	
B07	FAM	35.81		BPI	blue 2hr 2 mg/L gill	down 1.838

B08	FAM	37.16	35.63	BPI	blue 2hr 2 mg/L gill	p .66
B09	FAM	33.91		BPI	blue 2hr 2 mg/L gill	0.645
D01	FAM	31.63		BPI	blue 2hr 1.5 mg/L gill	up 3.346
D02	FAM	31.95	33.01	BPI	blue 2hr 1.5 mg/L gill	p .521
D03	FAM	35.45		BPI	blue 2hr 1.5 mg/L gill	4.3
E07	FAM	35.58		BPI	blue 4hr 2 mg/L gill	down 3.046
E08	FAM	36.49	36.36	BPI	blue 4hr 2 mg/L gill	p .356
E09	FAM	37.01		BPI	blue 4hr 2 mg/L gill	0.331
G01	FAM	37.17		BPI	blue 4hr 1.5 mg/L gill	down 3.214
G02	FAM	35.77	36.44	BPI	blue 4hr 1.5 mg/L gill	p.363
G03	FAM	36.37		BPI	blue 4hr 1.5 mg/L gill	0.313
H07	FAM	34.06		BPI	blue 8hr 2 mg/L gill	up 1.997
H08	FAM	33.30	33.75	BPI	blue 8hr 2 mg/L gill	p.553
H09	FAM	33.90		BPI	blue 8hr 2 mg/L gill	1.96
A04	FAM	35.23		BPI	blue 4 hr control liver	
A05	FAM	33.31	35.23	BPI	blue 4 hr control liver	
A06	FAM	37.15		BPI	blue 4 hr control liver	
B10	FAM	34.52		BPI	blue 2hr 2 mg/L liver	down 1.227
B11	FAM	34.91	34.86	BPI	blue 2hr 2 mg/L liver	p .944
B12	FAM	35.15		BPI	blue 2hr 2 mg/L liver	0.72
D04	FAM	37.66		BPI	blue2hr 1.5 mg/L liver	down 7.66
D05	FAM	36.98	37.50	BPI	blue2hr 1.5 mg/L liver	p.056
D06	FAM	37.87		BPI	blue2hr 1.5 mg/L liver	0.117
E10	FAM	39.48		BPI	blue4hr 2 mg/L liver	down 5.33
E11	FAM	35.42	36.98	BPI	blue4hr 2 mg/L liver	p.144
E12	FAM	36.04		BPI	blue4hr 2 mg/L liver	0.232
G01	FAM	37.17		BPI	blue4hr 1.5 mg/L liver	down 3.564
G02	FAM	35.77	36.44	BPI	blue4hr 1.5 mg/L liver	p.144
G03	FAM	36.37		BPI	blue4hr 1.5 mg/L liver	0.251
H10	FAM	34.61		BPI	blue8hr 2 mg/L liver	down 1.448
H11	FAM	34.39	35.10	BPI	blue8hr 2 mg/L liver	p .642
H12	FAM	36.31		BPI	blue8hr 2 mg/L liver	0.67

A07	FAM	24.92		BPI	channel 4hr control gill	
A08	FAM	24.39	24.39	BPI	channel 4hr control gill	
A09	FAM	23.87		BPI	channel 4hr control gill	
C01	FAM	25.39		BPI	channel 2hr 2 mg/L gill	down 2.866
C02	FAM	25.27	25.34	BPI	channel 2hr 2 mg/L gill	p .001
C03	FAM	25.34		BPI	channel 2hr 2 mg/L gill	0.118
D07	FAM	25.62		BPI	channel 2hr 1.5 mg/L gill	down 2.85
D08	FAM	26.00	25.33	BPI	channel 2hr 1.5 mg/L gill	p.095
D09	FAM	24.37		BPI	channel 2hr 1.5 mg/L gill	0.168
F01	FAM	24.55		BPI	channel 4hr 2 mg/L gill	down 1.887
F02	FAM	25.12	24.73	BPI	channel 4hr 2 mg/L gill	p 0.144
F03	FAM	24.53		BPI	channel 4hr 2 mg/L gill	0.192
G07	FAM	24.84		BPI	channel 4hr 1.5 mg/L gill	down 1.822
G08	FAM	24.35	24.68	BPI	channel 4hr 1.5 mg/L gill	p .307
G09	FAM	24.86		BPI	channel 4hr 1.5 mg/L gill	0.195
G01	FAM	27.23		BPI	channel 8hr 2 mg/L gill	down 9.964
G02	FAM	27.67	27.13	BPI	channel 8hr 2 mg/L gill	p.001
G03	FAM	26.50		BPI	channel 8hr 2 mg/L gill	0.041
A10	FAM	24.33		BPI	channel 4hr control liver	
A11	FAM	25.93	25.61	BPI	channel 4hr control liver	
A12	FAM	26.58		BPI	channel 4hr control liver	
C04	FAM	25.15		BPI	channel 2hr 2 mg/L liver	up 1.863
C05	FAM	24.39	24.61	BPI	channel 2hr 2 mg/L liver	p .313
C06	FAM	24.30		BPI	channel 2hr 2 mg/L liver	1.06
D10	FAM	25.15		BPI	channel 2hr 1.5 mg/L liver	down 1.672
D11	FAM	27.27	26.25	BPI	channel 2hr 1.5 mg/L liver	p .424
D12	FAM	26.33		BPI	channel 2hr 1.5 mg/L liver	0.409
F04	FAM	27.18		BPI	channel 4hr 2 mg/L liver	down 3.16
F05	FAM	27.12	27.17	BPI	channel 4hr 2 mg/L liver	p .015
F06	FAM	27.21		BPI	channel 4hr 2 mg/L liver	0.169
G10	FAM	26.24		BPI	channel 4hr 1.5 mg/L liver	down 2.041
G11	FAM	26.67	26.54	BPI	channel 4hr 1.5 mg/L liver	p.116
G12	FAM	26.71		BPI	channel 4hr 1.5 mg/L liver	0.268

G04	FAM	26.37		BPI	channel 8 hr2 mg/L liver	down 1.711
G05	FAM	26.21	26.28	BPI	channel 8 hr2 mg/L liver	p.297
G06	FAM	26.28		BPI	channel 8 hr2 mg/L liver	0.314
B01	FAM	25.67		BPI	hybrid 4hr control gill	
B02	FAM	24.20	25.03	BPI	hybrid 4hr control gill	
B03	FAM	25.21		BPI	hybrid 4hr control gill	
				BP		
C07	FAM	24.39		BPI	hybrid 2hr 2 mg/L gill	down 3.214
C08	FAM	27.67	26.72	BPI	hybrid 2hr 2 mg/L gill	p .279
C09	FAM	28.09		BPI	hybrid 2hr 2 mg/L gill	0.296
E01	FAM	26.00		BPI	hybrid 2hr 1.5 mg/L gill	down 3.433
E02	FAM	28.30	26.81	BPI	hybrid 2hr 1.5 mg/L gill	p .05
E03	FAM	26.13		BPI	hybrid 2hr 1.5 mg/L gill	0.269
F07	FAM	26.24		BPI	hybrid 4hr 2 mg/L gill	down 2.44
F08	FAM	26.57	26.32	BPI	hybrid 4hr 2 mg/L gill	p.05
F09	FAM	26.15		BPI	hybrid 4hr 2 mg/L gill	0.206
H01	FAM	25.60		BPI	hybrid 4hr 1.5 mg/L gill	down 1.817
H02	FAM	25.18	25.89	BPI	hybrid 4hr 1.5 mg/L gill	p.464
H03	FAM	26.90		BPI	hybrid 4hr 1.5 mg/L gill	0.3367
G07	FAM	28.09		BPI	hybrid 8hr 2 mg/L gill	down 8.006
G08	FAM	28.16	28.03	BPI	hybrid 8hr 2 mg/L gill	p.05
G09	FAM	27.85		BPI	hybrid 8hr 2 mg/L gill	0.062
B04	FAM	24.65		BPI	hybrid 4hr control liver	
B05	FAM	25.15	25.81	BPI	hybrid 4hr control liver	
B06	FAM	27.62		BPI	hybrid 4hr control liver	
C10	FAM	25.37		BPI	hybrid 2hr 2 mg/L liver	down 1.714
C11	FAM	26.89	26.00	BPI	hybrid 2hr 2 mg/L liver	p .504
C12	FAM	25.72		BPI	hybrid 2hr 2 mg/L liver	0.479
E04	FAM	26.11		BPI	hybrid 2hr 1.5 mg/L liver	down 3.53
E05	FAM	26.82	27.04	BPI	hybrid 2hr 1.5 mg/L liver	p.095
E06	FAM	28.19		BPI	hybrid 2hr 1.5 mg/L liver	0.245
F10	FAM	28.04		BPI	hybrid 4hr 2 mg/L liver	down 5.95
F11	FAM	28.90	27.79	BPI	hybrid 4hr 2 mg/L liver	p.051

F12	FAM	26.44		BPI	hybrid 4hr 2 mg/L liver	0.152
H04	FAM	27.53		BPI	hybrid 4hr 1.5 mg/L liver	down 6.456
H05	FAM	27.92	27.91	BPI	hybrid 4hr 1.5 mg/L liver	p.051
H06	FAM	28.27		BPI	hybrid 4hr 1.5 mg/L liver	0.119
G10	FAM	28.05		BPI	hybrid 8hr 2 mg/L liver	down 8.385
G11	FAM	28.37		BPI	hybrid 8hr 2 mg/L liver	p.001
G12	FAM	28.44		BPI	hybrid 8hr 2 mg/L liver	0.091
A01	FAM	26.62		Ferritin	blue 4 hr control gill	
A02	FAM	25.78	26.46	Ferritin	blue 4 hr control gill	
A03	FAM	26.96		Ferritin	blue 4 hr control gill	
B07	FAM	25.43		Ferritin	blue 2hr 2 mg/L gill	down 1.394
B08	FAM	25.41	25.65	Ferritin	blue 2hr 2 mg/L gill	p.921
B09	FAM	26.12		Ferritin	blue 2hr 2 mg/L gill	0.277
D01	FAM	25.79		Ferritin	blue 2hr 1.5 mg/L gill	down 1.875
D02	FAM	26.13	26.08	Ferritin	blue 2hr 1.5 mg/L gill	p.76
D03	FAM	26.33		Ferritin	blue 2hr 1.5 mg/L gill	0.196
E07	FAM	26.39		Ferritin	blue 4hr 2 mg/L gill	down 1.454
E08	FAM	25.15	25.71	Ferritin	blue 4hr 2 mg/L gill	p .92
E09	FAM	25.60		Ferritin	blue 4hr 2 mg/L gill	0.296
G01	FAM	24.78		Ferritin	blue 4hr 1.5 mg/L gill	up 1.287
G02	FAM	25.03	24.81	Ferritin	blue 4hr 1.5 mg/L gill	p.846
G03	FAM	24.63		Ferritin	blue 4hr 1.5 mg/L gill	0.462
H07	FAM	24.47		Ferritin	blue 8hr 2 mg/L gill	up 1.415
H08	FAM	24.97	24.67	Ferritin	blue 8hr 2 mg/L gill	p.788
H09	FAM	24.58		Ferritin	blue 8hr 2 mg/L gill	0.517
A04	FAM	29.04		Ferritin	blue 4 hr control liver	
A05	FAM	28.44	28.66	Ferritin	blue 4 hr control liver	
A06	FAM	28.50		Ferritin	blue 4 hr control liver	
B10	FAM	28.94		Ferritin	blue 2hr 2 mg/L liver	down 1.511
B11	FAM	27.66	28.59	Ferritin	blue 2hr 2 mg/L liver	p.538
B12	FAM	29.18		Ferritin	blue 2hr 2 mg/L liver	0.361
D04	FAM	27.26		Ferritin	blue2hr 1.5 mg/L liver	down 1.401

D05	FAM	29.16	28.48	Ferritin	blue2hr 1.5 mg/L liver	p 0.526
D06	FAM	29.04		Ferritin	blue2hr 1.5 mg/L liver	0.435
E10	FAM	29.46		Ferritin	blue4hr 2 mg/L liver	down 2.708
E11	FAM	29.69	29.44	Ferritin	blue4hr 2 mg/L liver	p.001
E12	FAM	29.16		Ferritin	blue4hr 2 mg/L liver	0.166
G01	FAM	24.78		Ferritin	blue4hr 1.5 mg/L liver	up 9.106
G02	FAM	25.03	24.81	Ferritin	blue4hr 1.5 mg/L liver	p.053
G03	FAM	24.63		Ferritin	blue4hr 1.5 mg/L liver	4.04
H10	FAM	27.85		Ferritin	blue8hr 2 mg/L liver	up 1.182
H11	FAM	27.70	27.76	Ferritin	blue8hr 2 mg/L liver	p.799
H12	FAM	27.72		Ferritin	blue8hr 2 mg/L liver	0.517
A07	FAM	26.03		Ferritin	channel 4hr control gill	
A08	FAM	25.75	25.95	Ferritin	channel 4hr control gill	
A09	FAM	26.07		Ferritin	channel 4hr control gill	
C01	FAM	26.37		Ferritin	channel 2hr 2 mg/L gill	down 1.692
C02	FAM	26.36	26.13	Ferritin	channel 2hr 2 mg/L gill	p.255
C03	FAM	25.67		Ferritin	channel 2hr 2 mg/L gill	0.187
D07	FAM	26.44		Ferritin	channel 2hr 1.5 mg/L gill	down 1.457
D08	FAM	26.04	25.92	Ferritin	channel 2hr 1.5 mg/L gill	p.577
D09	FAM	25.27		Ferritin	channel 2hr 1.5 mg/L gill	0.249
F01	FAM	25.40		Ferritin	channel 4hr 2 mg/L gill	down 1.219
F02	FAM	25.87	25.66	Ferritin	channel 4hr 2 mg/L gill	p.89
F03	FAM	25.71		Ferritin	channel 4hr 2 mg/L gill	0.237
G07	FAM	24.13		Ferritin	channel 4hr 1.5 mg/L gill	up 1.859
G08	FAM	24.25	24.48	Ferritin	channel 4hr 1.5 mg/L gill	p.293
G09	FAM	25.06		Ferritin	channel 4hr 1.5 mg/L gill	0.634
E01	FAM	22.99		Ferritin	channel 8hr 2 mg/L gill	up 8.21
E02	FAM	23.14	22.37	Ferritin	channel 8hr 2 mg/L gill	p.052
E03	FAM	20.98		Ferritin	channel 8hr 2 mg/L gill	4.437
A10	FAM	29.18		Ferritin	channel 4hr control liver	
A11	FAM	27.95	28.67	Ferritin	channel 4hr control liver	
A12	FAM	28.89		Ferritin	channel 4hr control liver	

C04	FAM	29.08		Ferritin	channel 2hr 2 mg/L liver	down 1.243
C05	FAM	29.13	28.88	Ferritin	channel 2hr 2 mg/L liver	p.497
C06	FAM	28.43		Ferritin	channel 2hr 2 mg/L liver	0.327
D10	FAM	29.74		Ferritin	channel 2hr 1.5 mg/L liver	down 1.423
D11	FAM	28.43	29.08	Ferritin	channel 2hr 1.5 mg/L liver	p.35
D12	FAM	29.05		Ferritin	channel 2hr 1.5 mg/L liver	0.321
F04	FAM	28.56		Ferritin	channel 4hr 2 mg/L liver	down 1.74
F05	FAM	29.30	28.80	Ferritin	channel 4hr 2 mg/L liver	p.674
F06	FAM	28.53		Ferritin	channel 4hr 2 mg/L liver	0.354
G10	FAM	26.30		Ferritin	channel 4hr 1.5 mg/L liver	up 4.387
G11	FAM	26.56	26.43	Ferritin	channel 4hr 1.5 mg/L liver	p.054
G12	FAM	26.45		Ferritin	channel 4hr 1.5 mg/L liver	1.66
E04	FAM	21.90		Ferritin	channel 8 hr2 mg/L liver	up 74.965
E05	FAM	22.33	22.34	Ferritin	channel 8 hr2 mg/L liver	p.054
E06	FAM	22.78		Ferritin	channel 8 hr2 mg/L liver	21.071
B01	FAM	24.51		Ferritin	hybrid 4hr control gill	
B02	FAM	25.55	25.33	Ferritin	hybrid 4hr control gill	
B03	FAM	25.93		Ferritin	hybrid 4hr control gill	
C07	FAM	27.91		Ferritin	hybrid 2hr 2 mg/L gill	down 2.314
C08	FAM	25.52	26.55	Ferritin	hybrid 2hr 2 mg/L gill	p.251
C09	FAM	26.20		Ferritin	hybrid 2hr 2 mg/L gill	0.301
E01	FAM	26.56		Ferritin	hybrid 2hr 1.5 mg/L gill	down 1.455
E02	FAM	26.11	25.88	Ferritin	hybrid 2hr 1.5 mg/L gill	p 0.35
E03	FAM	24.96		Ferritin	hybrid 2hr 1.5 mg/L gill	0.407
F07	FAM	25.79		Ferritin	hybrid 4hr 2 mg/L gill	up 1.1
F08	FAM	24.48	25.20	Ferritin	hybrid 4hr 2 mg/L gill	p .585
F09	FAM	25.33		Ferritin	hybrid 4hr 2 mg/L gill	0.614
H01	FAM	24.76		Ferritin	hybrid 4hr 1.5 mg/L gill	down 1.243
H02	FAM	25.59	25.65	Ferritin	hybrid 4hr 1.5 mg/L gill	p.678
H03	FAM	26.60		Ferritin	hybrid 4hr 1.5 mg/L gill	0.494
E07	FAM	21.51		Ferritin	hybrid 8hr 2 mg/L gill	up 10.875
E08	FAM	22.56	21.89	Ferritin	hybrid 8hr 2 mg/L gill	p.001
E09	FAM	21.61		Ferritin	hybrid 8hr 2 mg/L gill	5.896

B04	FAM	27.56		Ferritin	hybrid 4hr control liver	
B05	FAM	27.15	27.50	Ferritin	hybrid 4hr control liver	
B06	FAM	27.79		Ferritin	hybrid 4hr control liver	
C10	FAM	27.60		Ferritin	hybrid 2hr 2 mg/L liver	down 4.034
C11	FAM	29.75	28.93	Ferritin	hybrid 2hr 2 mg/L liver	p.04
C12	FAM	29.43		Ferritin	hybrid 2hr 2 mg/L liver	0.156
E04	FAM	28.31		Ferritin	hybrid 2hr 1.5 mg/L liver	down 1.8
E05	FAM	26.75	27.76	Ferritin	hybrid 2hr 1.5 mg/L liver	p.247
E06	FAM	28.22		Ferritin	hybrid 2hr 1.5 mg/L liver	0.307
F10	FAM	29.26		Ferritin	hybrid 4hr 2 mg/L liver	down 4.377
F11	FAM	28.85	29.04	Ferritin	hybrid 4hr 2 mg/L liver	p.001
F12	FAM	29.03		Ferritin	hybrid 4hr 2 mg/L liver	0.099
H04	FAM	28.54		Ferritin	hybrid 4hr 1.5 mg/L liver	down 1.046
H05	FAM	28.32	26.98	Ferritin	hybrid 4hr 1.5 mg/L liver	p 0.968
H06	FAM	24.07		Ferritin	hybrid 4hr 1.5 mg/L liver	1.046
E10	FAM	22.82		Ferritin	hybrid 8hr 2 mg/L liver	up 26.902
E11	FAM	21.83	22.17	Ferritin	hybrid 8hr 2 mg/L liver	p.05
E12	FAM	21.85		Ferritin	hybrid 8hr 2 mg/L liver	13.017
A01	FAM	31.67		Myostatin	blue 4 hr control gill	
A02	FAM	32.77	32.76	Myostatin	blue 4 hr control gill	
A03	FAM	33.85		Myostatin	blue 4 hr control gill	
B07	FAM	33.28		Myostatin	blue 2hr 2 mg/L gill	down 1.957
B08	FAM	32.05	32.45	Myostatin	blue 2hr 2 mg/L gill	p .698
B09	FAM	32.02		Myostatin	blue 2hr 2 mg/L gill	0.296
D01	FAM	30.94		Myostatin	blue 2hr 1.5 mg/L gill	down 1.441
D02	FAM	33.10	32.01	Myostatin	blue 2hr 1.5 mg/L gill	p .79
D03	FAM	31.99		Myostatin	blue 2hr 1.5 mg/L gill	0.461
E07	FAM	33.22		Myostatin	blue 4hr 2 mg/L gill	down 1.964
E08	FAM	31.98	32.46	Myostatin	blue 4hr 2 mg/L gill	p .687
E09	FAM	32.16		Myostatin	blue 4hr 2 mg/L gill	0.29
G01	FAM	31.96		Myostatin	blue 4hr 1.5 mg/L gill	down 2.125
G02	FAM	32.89	32.57	Myostatin	blue 4hr 1.5 mg/L gill	p .565

G03	FAM	32.87		Myostatin	blue 4hr 1.5 mg/L gill	0.257
H07	FAM	31.35		Myostatin	blue 8hr 2 mg/L gill	down3.164
H08	FAM	34.83	33.14	Myostatin	blue 8hr 2 mg/L gill	p.395
H09	FAM	33.25		Myostatin	blue 8hr 2 mg/L gill	0.272
A04	FAM	36.43		Myostatin	blue 4 hr control liver	
A05	FAM	37.68	37.82	Myostatin	blue 4 hr control liver	
A06	FAM	39.37		Myostatin	blue 4 hr control liver	
B10	FAM	33.52		Myostatin	blue 2hr 2 mg/L liver	up 4.626
B11	FAM	37.24	34.95	Myostatin	blue 2hr 2 mg/L liver	p.209
B12	FAM	34.10		Myostatin	blue 2hr 2 mg/L liver	4.99
D04	FAM	35.98		Myostatin	blue2hr 1.5 mg/L liver	up 11.246
D05	FAM	32.20	33.67	Myostatin	blue2hr 1.5 mg/L liver	p.194
D06	FAM	32.83		Myostatin	blue2hr 1.5 mg/L liver	12.214
E10	FAM	39.54		Myostatin	blue4hr 2 mg/L liver	up 1.849
E11	FAM	34.73	36.28	Myostatin	blue4hr 2 mg/L liver	p 0.704
E12	FAM	34.56		Myostatin	blue4hr 2 mg/L liver	2.481
G01	FAM	31.96		Myostatin	blue4hr 1.5 mg/L liver	up 24.133
G02	FAM	32.89	32.57	Myostatin	blue4hr 1.5 mg/L liver	p.048
G03	FAM	32.87		Myostatin	blue4hr 1.5 mg/L liver	18.163
H10	FAM	36.83		Myostatin	blue8hr 2 mg/L liver	up 1.579
H11	FAM	37.03	36.50	Myostatin	blue8hr 2 mg/L liver	p.064
H12	FAM	35.65		Myostatin	blue8hr 2 mg/L liver	1.234
A07	FAM	26.22		Myostatin	channel 4hr control gill	
A08	FAM	26.37	26.31	Myostatin	channel 4hr control gill	
A09	FAM	26.35		Myostatin	channel 4hr control gill	
C01	FAM	27.89		Myostatin	channel 2hr 2 mg/L gill	down 2.55
C02	FAM	26.66	27.09	Myostatin	channel 2hr 2 mg/L gill	p.001
C03	FAM	26.72		Myostatin	channel 2hr 2 mg/L gill	1.51
D07	FAM	25.17		Myostatin	channel 2hr 1.5 mg/L gill	up 1.104
D08	FAM	25.62	25.60	Myostatin	channel 2hr 1.5 mg/L gill	p.791
D09	FAM	26.00		Myostatin	channel 2hr 1.5 mg/L gill	0.3466
F01	FAM	26.49		Myostatin	channel 4hr 2 mg/L gill	down 1.406

F02	FAM	26.10	26.23	Myostatin	channel 4hr 2 mg/L gill	p.71
F03	FAM	26.10		Myostatin	channel 4hr 2 mg/L gill	0.2
G07	FAM	26.03		Myostatin	channel 4hr 1.5 mg/L gill	down 1.406
G08	FAM	27.38	26.40	Myostatin	channel 4hr 1.5 mg/L gill	p.71
G09	FAM	25.79		Myostatin	channel 4hr 1.5 mg/L gill	0.275
B01	FAM	26.64		Myostatin	channel 8hr 2 mg/L gill	down 1.974
B02	FAM	26.05	26.72	Myostatin	channel 8hr 2 mg/L gill	p.22
B03	FAM	27.47		Myostatin	channel 8hr 2 mg/L gill	0.197
A10	FAM	29.33		Myostatin	channel 4hr control liver	
A11	FAM	29.87	30.10	Myostatin	channel 4hr control liver	
A12	FAM	31.11		Myostatin	channel 4hr control liver	
C04	FAM	30.11		Myostatin	channel 2hr 2 mg/L liver	up 1.22
C05	FAM	30.40	29.83	Myostatin	channel 2hr 2 mg/L liver	p .81
C06	FAM	28.99		Myostatin	channel 2hr 2 mg/L liver	0.612
D10	FAM	28.18		Myostatin	channel 2hr 1.5 mg/L liver	up 2.079
D11	FAM	29.38	28.94	Myostatin	channel 2hr 1.5 mg/L liver	p 0.185
D12	FAM	29.27		Myostatin	channel 2hr 1.5 mg/L liver	0.098
F04	FAM	30.57		Myostatin	channel 4hr 2 mg/L liver	up 1.314
F05	FAM	29.06	29.60	Myostatin	channel 4hr 2 mg/L liver	p.539
F06	FAM	29.19		Myostatin	channel 4hr 2 mg/L liver	0.743
G10	FAM	27.67		Myostatin	channel 4hr 1.5 mg/L liver	up 4.484
G11	FAM	27.75	27.83	Myostatin	channel 4hr 1.5 mg/L liver	p.044
G12	FAM	28.08		Myostatin	channel 4hr 1.5 mg/L liver	2.083
B04	FAM	30.52		Myostatin	channel 8 hr2 mg/L liver	down 1.204
B05	FAM	30.45	30.60	Myostatin	channel 8 hr2 mg/L liver	p.712
B06	FAM	30.83		Myostatin	channel 8 hr2 mg/L liver	0.305
B01	FAM	27.03		Myostatin	hybrid 4hr control gill	
B02	FAM	26.75	26.72	Myostatin	hybrid 4hr control gill	
B03	FAM	26.37		Myostatin	hybrid 4hr control gill	
C07	FAM	26.20		Myostatin	hybrid 2hr 2 mg/L gill	down 2.087
C08	FAM	28.62	27.78	Myostatin	hybrid 2hr 2 mg/L gill	p.345
C09	FAM	28.53		Myostatin	hybrid 2hr 2 mg/L gill	0.33

E01	FAM	28.31		Myostatin	hybrid 2hr 1.5 mg/L gill	down 2.653
E02	FAM	29.06	28.13	Myostatin	hybrid 2hr 1.5 mg/L gill	p.167
E03	FAM	27.02		Myostatin	hybrid 2hr 1.5 mg/L gill	0.221
F07	FAM	26.31		Myostatin	hybrid 4hr 2 mg/L gill	up 1.088
F08	FAM	26.65	26.60	Myostatin	hybrid 4hr 2 mg/L gill	p.622
F09	FAM	26.84		Myostatin	hybrid 4hr 2 mg/L gill	0.467
H01	FAM	27.07		Myostatin	hybrid 4hr 1.5 mg/L gill	down 1.043
H02	FAM	27.26	26.78	Myostatin	hybrid 4hr 1.5 mg/L gill	p.846
H03	FAM	26.01		Myostatin	hybrid 4hr 1.5 mg/L gill	0.475
B07	FAM	27.16		Myostatin	hybrid 8hr 2 mg/L gill	down 1.68
B08	FAM	27.15	27.47	Myostatin	hybrid 8hr 2 mg/L gill	p.043
B09	FAM	28.10		Myostatin	hybrid 8hr 2 mg/L gill	0.279
B04	FAM	31.02		Myostatin	hybrid 4hr control liver	
B05	FAM	32.67	32.31	Myostatin	hybrid 4hr control liver	
B06	FAM	33.25		Myostatin	hybrid 4hr control liver	
C10	FAM	30.30		Myostatin	hybrid 2hr 2 mg/L liver	up 3.878
C11	FAM	29.00	29.77	Myostatin	hybrid 2hr 2 mg/L liver	p.175
C12	FAM	30.00		Myostatin	hybrid 2hr 2 mg/L liver	2.62
E04	FAM	30.34		Myostatin	hybrid 2hr 1.5 mg/L liver	up 5.397
E05	FAM	28.56	29.29	Myostatin	hybrid 2hr 1.5 mg/L liver	p .122
E06	FAM	28.97		Myostatin	hybrid 2hr 1.5 mg/L liver	3.889
F10	FAM	32.22		Myostatin	hybrid 4hr 2 mg/L liver	up 1.059
F11	FAM	31.98	31.64	Myostatin	hybrid 4hr 2 mg/L liver	p.906
F12	FAM	30.73		Myostatin	hybrid 4hr 2 mg/L liver	0.736
H04	FAM	31.23		Myostatin	hybrid 4hr 1.5 mg/L liver	up 1.101
H05	FAM	31.07	31.59	Myostatin	hybrid 4hr 1.5 mg/L liver	p.817
H06	FAM	32.46		Myostatin	hybrid 4hr 1.5 mg/L liver	0.758
B10	FAM	28.32		Myostatin	hybrid 8hr 2 mg/L liver	up 21.025
B11	FAM	26.67	27.33	Myostatin	hybrid 8hr 2 mg/L liver	p.082
B12	FAM	27.00		Myostatin	hybrid 8hr 2 mg/L liver	14.894
A01	FAM	29.41		NKEF	blue 4 hr control gill	
A02	FAM	28.84	28.97	NKEF	blue 4 hr control gill	
A03	FAM	28.66		NKEF	blue 4 hr control gill	

B07	FAM	27.67		NKEF	blue 2hr 2 mg/L gill	down 1.128
B08	FAM	28.53	27.86	NKEF	blue 2hr 2 mg/L gill	p.968
B09	FAM	27.39		NKEF	blue 2hr 2 mg/L gill	0.338
D01	FAM	28.35		NKEF	blue 2hr 1.5 mg/L gill	down 1.46
D02	FAM	28.90	28.24	NKEF	blue 2hr 1.5 mg/L gill	p0.873
D03	FAM	27.46		NKEF	blue 2hr 1.5 mg/L gill	0.285
E07	FAM	27.92		NKEF	blue 4hr 2 mg/L gill	down 1.147
E08	FAM	27.84	27.89	NKEF	blue 4hr 2 mg/L gill	p.968
E09	FAM	27.91		NKEF	blue 4hr 2 mg/L gill	0.26
G01	FAM	29.77		NKEF	blue 4hr 1.5 mg/L gill	down 1.936
G02	FAM	28.09	28.64	NKEF	blue 4hr 1.5 mg/L gill	p.725
G03	FAM	28.07		NKEF	blue 4hr 1.5 mg/L gill	0.254
H07	FAM	27.97		NKEF	blue 8hr 2 mg/L gill	down 2.155
H08	FAM	29.07	28.80	NKEF	blue 8hr 2 mg/L gill	p0.609
H09	FAM	29.35		NKEF	blue 8hr 2 mg/L gill	0.194
A04	FAM	30.53		NKEF	blue 4 hr control liver	
A05	FAM	30.34	30.34	NKEF	blue 4 hr control liver	
A06	FAM	30.14		NKEF	blue 4 hr control liver	
B10	FAM	31.78		NKEF	blue 2hr 2 mg/L liver	down 2.362
B11	FAM	30.04	30.92	NKEF	blue 2hr 2 mg/L liver	p.16
B12	FAM	30.93		NKEF	blue 2hr 2 mg/L liver	0.232
D04	FAM	30.77		NKEF	blue2hr 1.5 mg/L liver	down 1.409
D05	FAM	29.74	30.17	NKEF	blue2hr 1.5 mg/L liver	p.659
D06	FAM	30.00		NKEF	blue2hr 1.5 mg/L liver	0.336
E10	FAM	32.13		NKEF	blue4hr 2 mg/L liver	down 6.971
E11	FAM	31.79	32.48	NKEF	blue4hr 2 mg/L liver	p.001
E12	FAM	33.51		NKEF	blue4hr 2 mg/L liver	0.08
G01	FAM	29.77		NKEF	blue4hr 1.5 mg/L liver	up 2.047
G02	FAM	28.09	28.64	NKEF	blue4hr 1.5 mg/L liver	p.233
G03	FAM	28.07		NKEF	blue4hr 1.5 mg/L liver	1.179
H10	FAM	28.28		NKEF	blue8hr 2 mg/L liver	up 1.237
H11	FAM	29.44	29.37	NKEF	blue8hr 2 mg/L liver	p.724

H12	FAM	30.40		NKEF	blue8hr 2 mg/L liver	0.741
A07	FAM	28.75		NKEF	channel 4hr control gill	
A08	FAM	28.38	28.57	NKEF	channel 4hr control gill	
A09	FAM	28.59		NKEF	channel 4hr control gill	
C01	FAM	28.53		NKEF	channel 2hr 2 mg/L gill	down 1.265
C02	FAM	28.41	28.34	NKEF	channel 2hr 2 mg/L gill	p.842
C03	FAM	28.07		NKEF	channel 2hr 2 mg/L gill	0.229
D07	FAM	28.45		NKEF	channel 2hr 1.5 mg/L gill	down 1.077
D08	FAM	28.40	28.10	NKEF	channel 2hr 1.5 mg/L gill	p0.842
D09	FAM	27.46		NKEF	channel 2hr 1.5 mg/L gill	0.328
F01	FAM	28.97		NKEF	channel 4hr 2 mg/L gill	down 1.431
F02	FAM	28.44	28.52	NKEF	channel 4hr 2 mg/L gill	p .55
F03	FAM	28.14		NKEF	channel 4hr 2 mg/L gill	0.224
G07	FAM	28.07		NKEF	channel 4hr 1.5 mg/L gill	up 1.076
G08	FAM	26.41	27.89	NKEF	channel 4hr 1.5 mg/L gill	p0.927
G09	FAM	29.20		NKEF	channel 4hr 1.5 mg/L gill	0.673
F01	FAM	29.36		NKEF	channel 8hr 2 mg/L gill	down 1.674
F02	FAM	29.62	28.74	NKEF	channel 8hr 2 mg/L gill	p.446
F03	FAM	27.24		NKEF	channel 8hr 2 mg/L gill	0.353
A10	FAM	31.13		NKEF	channel 4hr control liver	
A11	FAM	30.75	31.59	NKEF	channel 4hr control liver	
A12	FAM	32.89		NKEF	channel 4hr control liver	
C04	FAM	30.92		NKEF	channel 2hr 2 mg/L liver	up 1.606
C05	FAM	30.45	30.81	NKEF	channel 2hr 2 mg/L liver	p.53
C06	FAM	31.04		NKEF	channel 2hr 2 mg/L liver	0.878
D10	FAM	32.32		NKEF	channel 2hr 1.5 mg/L liver	down 1.005
D11	FAM	31.38	31.50	NKEF	channel 2hr 1.5 mg/L liver	p.949
D12	FAM	30.79		NKEF	channel 2hr 1.5 mg/L liver	0.612
F04	FAM	30.61		NKEF	channel 4hr 2 mg/L liver	up 1.882
F05	FAM	30.96	30.58	NKEF	channel 4hr 2 mg/L liver	p.31
F06	FAM	30.15		NKEF	channel 4hr 2 mg/L liver	1.048
G10	FAM	27.92		NKEF	channel 4hr 1.5 mg/L liver	up 10.618

G11	FAM	28.09	28.08	NKEF	channel 4hr 1.5 mg/L liver	p.061
G12	FAM	28.23		NKEF	channel 4hr 1.5 mg/L liver	5.69
F04	FAM	31.91		NKEF	channel 8 hr2 mg/L liver	up 1.407
F05	FAM	30.48	31.00	NKEF	channel 8 hr2 mg/L liver	p.586
F06	FAM	30.59		NKEF	channel 8 hr2 mg/L liver	0.873
B01	FAM	28.51		NKEF	hybrid 4hr control gill	
B02	FAM	28.91	28.65	NKEF	hybrid 4hr control gill	
B03	FAM	28.53		NKEF	hybrid 4hr control gill	
C07	FAM	29.58		NKEF	hybrid 2hr 2 mg/L gill	up 1.104
C08	FAM	27.78	28.51	NKEF	hybrid 2hr 2 mg/L gill	p.716
C09	FAM	28.17		NKEF	hybrid 2hr 2 mg/L gill	0.611
E01	FAM	28.39		NKEF	hybrid 2hr 1.5 mg/L gill	up 1.269
E02	FAM	29.18	28.31	NKEF	hybrid 2hr 1.5 mg/L gill	p.604
E03	FAM	27.35		NKEF	hybrid 2hr 1.5 mg/L gill	0.691
F07	FAM	28.34		NKEF	hybrid 4hr 2 mg/L gill	up 1.031
F08	FAM	28.70	28.61	NKEF	hybrid 4hr 2 mg/L gill	p.866
F09	FAM	28.78		NKEF	hybrid 4hr 2 mg/L gill	0.427
H01	FAM	30.58		NKEF	hybrid 4hr 1.5 mg/L gill	down 1.186
H02	FAM	27.09	28.90	NKEF	hybrid 4hr 1.5 mg/L gill	p.655
H03	FAM	29.03		NKEF	hybrid 4hr 1.5 mg/L gill	0.681
F07	FAM	28.29		NKEF	hybrid 8hr 2 mg/L gill	up 1.441
F08	FAM	28.31	28.13	NKEF	hybrid 8hr 2 mg/L gill	p.001
F09	FAM	27.78		NKEF	hybrid 8hr 2 mg/L gill	0.607
B04	FAM	31.57		NKEF	hybrid 4hr control liver	
B05	FAM	31.31	31.31	NKEF	hybrid 4hr control liver	
B06	FAM	31.07		NKEF	hybrid 4hr control liver	
C10	FAM	31.62		NKEF	hybrid 2hr 2 mg/L liver	up 1.032
C11	FAM	30.25	30.68	NKEF	hybrid 2hr 2 mg/L liver	p.94
C12	FAM	30.17		NKEF	hybrid 2hr 2 mg/L liver	0.549
E04	FAM	31.38		NKEF	hybrid 2hr 1.5 mg/L liver	up 1.247
E05	FAM	29.45	30.41	NKEF	hybrid 2hr 1.5 mg/L liver	p.695
E06	FAM	30.39		NKEF	hybrid 2hr 1.5 mg/L liver	0.711

F10	FAM	31.52		NKEF	hybrid 4hr 2 mg/L liver	down 1.697
F11	FAM	31.53	31.49	NKEF	hybrid 4hr 2 mg/L liver	p.151
F12	FAM	31.42		NKEF	hybrid 4hr 2 mg/L liver	0.248
H04	FAM	30.65		NKEF	hybrid 4hr 1.5 mg/L liver	up 2.068
H05	FAM	31.22	29.68	NKEF	hybrid 4hr 1.5 mg/L liver	p.562
H06	FAM	27.16		NKEF	hybrid 4hr 1.5 mg/L liver	2.015
F10	FAM	32.51		NKEF	hybrid 8hr 2 mg/L liver	up 1.393
F11	FAM	29.19	30.25	NKEF	hybrid 8hr 2 mg/L liver	p.657
F12	FAM	29.04		NKEF	hybrid 8hr 2 mg/L liver	1.24
A01	FAM	10.99		S18	blue 4 hr control gill	
A02	FAM	11.51	10.96	S18	blue 4 hr control gill	
A03	FAM	10.37		S18	blue 4 hr control gill	
B07	FAM	9.59		S18	blue 2hr 2 mg/L gill	
B08	FAM	9.85	9.86	S18	blue 2hr 2 mg/L gill	
B09	FAM	10.14		S18	blue 2hr 2 mg/L gill	
D01	FAM	9.90		S18	blue 2hr 1.5 mg/L gill	
D02	FAM	9.69	9.60	S18	blue 2hr 1.5 mg/L gill	
D03	FAM	9.21		S18	blue 2hr 1.5 mg/L gill	
E07	FAM	9.03		S18	blue 4hr 2 mg/L gill	
E08	FAM	9.96	9.70	S18	blue 4hr 2 mg/L gill	
E09	FAM	10.11		S18	blue 4hr 2 mg/L gill	
G01	FAM	9.92		S18	blue 4hr 1.5 mg/L gill	
G02	FAM	9.52	9.72	S18	blue 4hr 1.5 mg/L gill	
G03	FAM	9.73		S18	blue 4hr 1.5 mg/L gill	
H07	FAM	9.30		S18	blue 8hr 2 mg/L gill	
H08	FAM	9.51	9.50	S18	blue 8hr 2 mg/L gill	
H09	FAM	9.68		S18	blue 8hr 2 mg/L gill	
A04	FAM	10.53		S18	blue 4 hr control liver	
A05	FAM	11.62	10.62	S18	blue 4 hr control liver	
A06	FAM	9.71		S18	blue 4 hr control liver	
B10	FAM	10.07		S18	blue 2hr 2 mg/L liver	
B11	FAM	9.89	10.21	S18	blue 2hr 2 mg/L liver	
B12	FAM	10.65		S18	blue 2hr 2 mg/L liver	

D04	FAM	10.62		S18	blue2hr 1.5 mg/L liver
D05	FAM	8.52	9.49	S18	blue2hr 1.5 mg/L liver
D06	FAM	9.33		S18	blue2hr 1.5 mg/L liver
E10	FAM	10.09		S18	blue4hr 2 mg/L liver
E11	FAM	10.27	10.34	S18	blue4hr 2 mg/L liver
E12	FAM	10.66		S18	blue4hr 2 mg/L liver
G04	FAM	10.64		S18	blue4hr 1.5 mg/L liver
G05	FAM	10.14	10.06	S18	blue4hr 1.5 mg/L liver
G06	FAM	9.40		S18	blue4hr 1.5 mg/L liver
H10	FAM	9.03		S18	blue8hr 2 mg/L liver
H11	FAM	9.96	9.70	S18	blue8hr 2 mg/L liver
H12	FAM	10.11		S18	blue8hr 2 mg/L liver
A07	FAM	10.38		S18	channel 4hr control gill
A08	FAM	10.59	10.45	S18	channel 4hr control gill
A09	FAM	10.38		S18	channel 4hr control gill
C01	FAM	9.23		S18	channel 2hr 2 mg/L gill
C02	FAM	10.52	9.92	S18	channel 2hr 2 mg/L gill
C03	FAM	10.01		S18	channel 2hr 2 mg/L gill
D07	FAM	9.42		S18	channel 2hr 1.5 mg/L gill
D08	FAM	9.76	9.41	S18	channel 2hr 1.5 mg/L gill
D09	FAM	9.06		S18	channel 2hr 1.5 mg/L gill
F01	FAM	10.06		S18	channel 4hr 2 mg/L gill
F02	FAM	10.13	10.23	S18	channel 4hr 2 mg/L gill
F03	FAM	10.49		S18	channel 4hr 2 mg/L gill
G07	FAM	9.75		S18	channel 4hr 1.5 mg/L gill
G08	FAM	9.83	9.89	S18	channel 4hr 1.5 mg/L gill
G09	FAM	10.09		S18	channel 4hr 1.5 mg/L gill
A01	FAM	10.70		S18	channel 8hr 2 mg/L gill
A02	FAM	9.39	9.91	S18	channel 8hr 2 mg/L gill
A03	FAM	9.64		S18	channel 8hr 2 mg/L gill
A10	FAM	10.40		S18	channel 4hr control liver
A11	FAM	10.04	10.08	S18	channel 4hr control liver

A12	FAM	9.81		S18	channel 4hr control liver
C04	FAM	8.91		S18	channel 2hr 2 mg/L liver
C05	FAM	9.10	9.35	S18	channel 2hr 2 mg/L liver
C06	FAM	10.05		S18	channel 2hr 2 mg/L liver
D10	FAM	10.81		S18	channel 2hr 1.5 mg/L liver
D11	FAM	9.23	9.84	S18	channel 2hr 1.5 mg/L liver
D12	FAM	9.48		S18	channel 2hr 1.5 mg/L liver
F04	FAM	9.68		S18	channel 4hr 2 mg/L liver
F05	FAM	9.51	9.62	S18	channel 4hr 2 mg/L liver
F06	FAM	9.68		S18	channel 4hr 2 mg/L liver
G10	FAM	10.11		S18	channel 4hr 1.5 mg/L liver
G11	FAM	10.28	10.19	S18	channel 4hr 1.5 mg/L liver
G12	FAM	10.18		S18	channel 4hr 1.5 mg/L liver
A04	FAM	11.37		S18	channel 8 hr2 mg/L liver
A05	FAM	10.51	10.89	S18	channel 8 hr2 mg/L liver
A06	FAM	10.78		S18	channel 8 hr2 mg/L liver
B01	FAM	9.92		S18	hybrid 4hr control gill
B02	FAM	10.07	9.98	S18	hybrid 4hr control gill
B03	FAM	9.94		S18	hybrid 4hr control gill
C07	FAM	11.00		S18	hybrid 2hr 2 mg/L gill
C08	FAM	9.07	9.93	S18	hybrid 2hr 2 mg/L gill
C09	FAM	9.72		S18	hybrid 2hr 2 mg/L gill
E01	FAM	9.75		S18	hybrid 2hr 1.5 mg/L gill
E02	FAM	9.38	9.53	S18	hybrid 2hr 1.5 mg/L gill
E03	FAM	9.47		S18	hybrid 2hr 1.5 mg/L gill
F07	FAM	9.40		S18	hybrid 4hr 2 mg/L gill
F08	FAM	10.11	9.58	S18	hybrid 4hr 2 mg/L gill
F09	FAM	9.24		S18	hybrid 4hr 2 mg/L gill
H01	FAM	10.72		S18	hybrid 4hr 1.5 mg/L gill
H02	FAM	9.82	10.09	S18	hybrid 4hr 1.5 mg/L gill
H03	FAM	9.74		S18	hybrid 4hr 1.5 mg/L gill
A07	FAM	11.03		S18	hybrid 8hr 2 mg/L gill

A08	FAM	10.80	10.77	S18	hybrid 8hr 2 mg/L gill
A09	FAM	10.49		S18	hybrid 8hr 2 mg/L gill
B04	FAM	10.39		S18	hybrid 4hr control liver
B05	FAM	10.09	10.52	S18	hybrid 4hr control liver
B06	FAM	11.07		S18	hybrid 4hr control liver
C10	FAM	8.95		S18	hybrid 2hr 2 mg/L liver
C11	FAM	10.62	9.95	S18	hybrid 2hr 2 mg/L liver
C12	FAM	10.29		S18	hybrid 2hr 2 mg/L liver
E04	FAM	10.79		S18	hybrid 2hr 1.5 mg/L liver
E05	FAM	8.87	10.08	S18	hybrid 2hr 1.5 mg/L liver
E06	FAM	10.57		S18	hybrid 2hr 1.5 mg/L liver
F10	FAM	10.62		S18	hybrid 4hr 2 mg/L liver
F11	FAM	10.33	10.12	S18	hybrid 4hr 2 mg/L liver
F12	FAM	9.41		S18	hybrid 4hr 2 mg/L liver
H04	FAM	9.63		S18	hybrid 4hr 1.5 mg/L liver
H05	FAM	9.79	9.49	S18	hybrid 4hr 1.5 mg/L liver
H06	FAM	9.06		S18	hybrid 4hr 1.5 mg/L liver
A10	FAM	9.37		S18	hybrid 8hr 2 mg/L liver
A11	FAM	10.25	10.01	S18	hybrid 8hr 2 mg/L liver
A12	FAM	10.42		S18	hybrid 8hr 2 mg/L liver