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Brian C. Peterson
United States Department of Agriculture, bpeterson@ars.usda.gov

Brian C. Small
United States Department of Agriculture

Geoffrey C. Waldbieser
United States Department of Agriculture

Brian G. Bosworth
United States Department of Agriculture

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[Article]

Endocrine Responses of Fast- and Slow-Growing Families of Channel Catfish

BRIAN C. PETERSON,* BRIAN C. SMALL, GEOFFREY C. WALDBIESER, AND BRIAN G. BOSWORTH

U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi 38776, USA

Abstract.—Sixty-eight families of USDA303 channel catfish *Ictalurus punctatus* were evaluated for growth performance for 30 d. The fastest- and slowest-growing catfish families were further evaluated to examine the hypothesis that genes or gene products associated with the growth regulatory and stress axes can be used to describe differences in growth performance. Research examined mRNA levels of genes involved in the growth hormone–insulin-like growth factor (GH–IGF) network in fast- (family A) and slow-growing (family H) USDA303 catfish. Fish (59.0 ± 2.4 g) were fed for 7 weeks, weighed, and had tissues for RNA extraction. The remaining fish were subjected to an acute 10-min dewatering stress. Insulin-like growth factor-II mRNA was higher in the muscle of fast-growing fish, while the levels of IGF-I receptor (IGF-IR) and IGF-II receptor (IGF-II) were similar. Muscle IGF-IIR mRNA was two-fold higher than muscle IGF-IR mRNA. There were no differences in liver and muscle IGF-I and GH receptor mRNA or pituitary GH mRNA between the fast- and slow-growing fish. Fast-growing fish consumed 135% more feed than slow-growing fish, though the abundances of ghrelin mRNA in the gut and neuropeptide Y mRNA in the hypothalamus were similar. Cortisol levels were negatively correlated to weight gain. These results suggest that the variation in growth between fast- and slow-growing USDA303 catfish is explained, in part, by the variation in the GH–IGF and stress axes. The relationship between cortisol and weight gain warrants further investigation for possible exploitation in our selective breeding program.

The USDA103 research strain of channel catfish *Ictalurus punctatus* was developed by family selection for improved growth rate. Continued selection within this line is focused on growth, fillet yield, and resistance to *Edwardsiella ictaluri*, the bacterium that causes enteric septicemia of catfish. The current method of identifying superior broodstock for growth is to conduct growth studies with large numbers of catfish families. This method is expensive and labor intensive owing to the numbers of families that are evaluated each year (approximately 100). The impetus to the current research is the desire to understand the physiological and genetic basis for the variation in growth phenotypes that is observed in our catfish families. We are interested in identifying markers that can be used to identify individuals with superior growth characteristics.

Genetic variation that affects growth will probably be reflected in the growth hormone–insulin-like growth factor (GH–IGF) regulatory axis. Pérez-Sánchez and Le Bail (1999) first suggested the use of the GH–IGF axis as a marker for growth performance in fish. In support of their hypothesis, GH levels were shown to be higher in faster-growing domestic Atlantic salmon *Salmo salar* than in a wild population (Fleming et al. 2002). Variations in growth performance among broodstock rainbow trout *Oncorhynchus mykiss* families were explained by variations in the resting levels of GH and IGF-I (Lankford and Weber 2006). In channel catfish, IGF-II mRNA levels were higher in both muscle and liver of faster-growing fish (Peterson et al. 2004b). These results suggest a genetic basis for the variation in the growth performance of the broodstock and families of fish. In addition, genes and gene products related to the GH–IGF axis partially explained differences in superior growth performance. Thus, these molecules could be used as markers for selective breeding.

The neuroendocrine control of GH is multifactorial, multiple stimulatory and inhibitory factors controlling GH secretion (Peter and Chang 1999). Ghrelin and neuropeptide Y (NPY) are two neuropeptides important in the regulation of GH in relation to food intake (Canosa et al., 2007). Two ghrelin genes were recently characterized in channel catfish, the highest abundance being reported in the stomach (Kaiya et al. 2005). The results also showed that ghrelin stimulates GH gene expression and GH release in channel catfish (Kaiya et al. 2005). Neuropeptide Y is a powerful stimulus of eating behavior in species of fish such as goldfish *Carassius auratus* (Lopez-Patino et al. 1999; Narnaware et al. 2000) and channel catfish (Silverstein and

* Corresponding author: bpeterson@ars.usda.gov

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Plisetskaya 2000). In channel catfish, NPY is found in the immature ovary, hypothalamus, myelencephalon, telencephalon, and optic tectum of the brain (Leonard et al. 2001). Regulation of gene expression in these tissues has not been examined.

It is clear that the USDA103 line of catfish exhibits superior growth characteristics (Wolters et al. 2000; Jackson et al. 2003; Peterson and Small 2006) compared with other strains of channel catfish. The faster growth of the USDA103 line is often attributed to its ability to consume more feed (Li et al. 2001). The stimulus that causes the USDA103 line to consume more feed is unknown but may include ghrelin, NPY, or both.

Cortisol responses may also be associated with growth performance. For example, rainbow trout with a consistently higher plasma cortisol response to stress were significantly larger than fish selected as low-plasma-cortisol responders (Pottinger and Carrick 1999). In two lines of rainbow trout broodstock, Lankford and Weber (2006) demonstrated a positive correlation between cortisol responsiveness and body weight. A clear understanding of the association between cortisol responsiveness and body growth has not been achieved to date. Furthermore, this association has not been examined among channel catfish families.

The present study was conducted to help better understand the mechanisms that regulate growth in channel catfish and test the hypothesis that differences in levels of components of the GH–IGF axis are correlated to differences in growth rates among fast- and slow-growing families of catfish. Further, the association between body weight and cortisol response was examined.

Methods

Catfish families.—The catfish used in this study were derived from the USDA103 line (described by Wolters et al. 2000). This line has been further selected for growth, fillet yield, and resistance to *E. ictaluri* for an additional two generations to produce the USDA303 line. The USDA303 fish used in the current research were obtained from natural pond spawns and reared in indoor tanks in a common environment at the U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, in Stoneville, Mississippi. The families used in the present study were selected from 68 full-sib families of the USDA303 strain that had undergone a 30-d growth study; the four fastest-growing and the four slowest-growing families were selected. The previous 30-d study only had one replicate per family, and the current study was conducted to increase the number of replicates between families.

Prior to randomization into experimental tanks, approximately 50 fish from each of the eight selected families were placed into separate 120-L holding tanks for 1 d. The slower-growing population averaged 51.0 ± 5.2 g (mean ± SE) and the faster-growing population averaged 58.8 ± 3.4 g. Forty catfish averaging 59.0 ± 2.4 g (average of all eight families, total $n = 320$) were then randomly assigned to four 151-L tanks (10 fish/tank) and allowed to acclimate for 11 d. The fish were fed once per day to apparent satiation and reared in 26.7 ± 0.3°C flow-through well water with a photoperiod of 14 h light : 10 h dark. A commercial 36% crude protein floating catfish feed (Melick Aquafeed, Catawissa, Pennsylvania) was used throughout the study. Water quality (pH ~8.5 and dissolved oxygen level >5.0 mg/L) and flow rates were similar between tanks. The fish were maintained for 7 weeks and the amount of feed was recorded weekly. No mortalities were observed throughout the study. Feed conversion ratios (FCRs) were calculated as ingested food (g)/weight increase (g). Specific growth rates (SGRs) were calculated as $[\log_e(\mathrm{WT}) - \log_e(\mathrm{wt})]/T \times 100$, where WT and wt are the initial and final weights, respectively, and T is time in days. The studies were conducted in accordance with the principles of and procedures approved by the Institutional Animal Care and Use Committee of the Catfish Genetics Research Unit.

Sample collection and RNA purification.—After the 7-week growth study, fish were anesthetized with 0.1 g tricaine methanesulfonate (MS-222; Western Chemical, Ferndale, Washington) per L of water and individually weighed and measured. Three fish from each tank (12/family) were euthanized with an overdose of MS-222 (0.3 g/L) and their tissues were taken for analysis. A transverse slice of fast muscle (approximately 100 mg) located beneath the dorsal fin was taken for RNA extraction. Liver, pituitary, hypothalamus, and stomach (~100 mg) were also taken for RNA extraction. Samples were immediately placed in 1 mL TRI-Reagent (Molecular Research Center, Cincinnati, Ohio) and then flash-frozen in liquid nitrogen. Total RNA was isolated and quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, Delaware), and the integrity of the RNA was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 2.0% agarose gels.

Stress challenge.—One week after the eight families of fish were sampled, the remaining fish in each tank (7 fish) were subjected to an acute confinement stressor. The stress was accomplished by total removal of the water from each aquarium. The volume of water was

TABLE 1.—Nucleotide sequences of the polymerase chain reaction (PCR) primers and probes used to assay gene expression by real-time quantitative PCR for neuropeptide Y (NPY), insulin-like growth factor I receptor (IGF-IR), and insulin-like growth factor II receptor (IGF-IIR).

Gene	Primer[a]	Sequence	PCR product length (base pairs)
NPY	Sense	CAT CAA CTA ACT ATG CGT CCT CG	118
	Antisense	CTG GGT TCT CCG GCT TTG T	
	Probe	TCA GCC AGC ACG CAC AAA CAC ACA AC	118
IGF-IR	Sense	AGT GGT GAA GTG GTC TCC TC	148
	Antisense	TGT GGC TGA GAT GCG GAT TG	
	Probe	AAC TAC TGC TCG AAA GAA CTG AAG ATC CC	
IGF-IIR	Sense	CTG CCA CAG AAT TTA TCA GCG	126
	Antisense	GCA GTG CGC CAT TCA AAC AAG	
	Probe	ACT GGC TTC CCC GGA TTC TTG CT	

[a] The probes for NPY (GenBank accession number AF267164), IGF-IR (EF470421), and IGF-IIR (EF470420) were dual-labeled with a reporter dye (FAM; 6-carboxyfluorescein) at the 5′ end and a quencher dye (Black Hole quencher-1) at the 3′ end (Biosearch Technologies, Novato, California).

drained in approximately 3 min and the fish exposed to air for 10 min. Four fish per tank were anesthetized with 6 mg metomidate hydrochloride/L and bled from the caudal vasculature into syringes coated with heparin. Metomidate hydrochloride blocks the handling-related release of cortisol into circulation, minimizing endogenous plasma cortisol variability due to sampling (Small 2003). The plasma was separated and frozen at −20°C.

Cortisol determination.—Cortisol was measured using a DELFIA time-resolved fluroimmunoassay kit (Perkin-Elmer Life Sciences, Boston, Massachusetts). This kit has been validated for the quantification of plasma cortisol in channel catfish (Small and Davis 2003).

Real-time PCR.—One microgram of total RNA from each tissue was reverse-transcribed using the iScript cDNA Synthesis Kit (BioRad, Hercules, California) according to the protocol provided by the manufacturer. Quantitative real-time polymerase chain reaction (PCR) was performed using the iCycler iQ real-time PCR detection system (BioRad) to quantify IGF-I, IGF-II, alpha tubulin, ghrelin, GH, and growth hormone receptor (GHR) mRNA as previously described (Peterson et al. 2004b; Bilodeau and Waldbieser 2005; Kaiya et al. 2005; Peterson and Small 2005; Small et al. 2006). Primer and probe sequences and GenBank accession numbers for NPY, insulin-like growth factor-I receptor (IGF-IR), and insulin-like growth factor-II receptor (IGF-IIR) are listed in Table 1. The PCR fragments for these three genes were cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, California) and introduced into One Shot TOP10 Chemically Competent *Esherichia coli* (Invitrogen) cells, and the identity of the cloned inserts was confirmed by using an automated capillary DNA sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Foster City, California). The DNA concentration of each resulting recombinant plasmid was measured using the NanoDrop ND-1000 spectrophotometer. Each amplification reaction mixture (12.5 μL) contained 400 ng of cDNA; 1× iQ Supermix (Bio-Rad Laboratories), 2.5 μM dual-labeled probe, and 10 μM (IGF-IIR) or 20 μM (IGF-IR, NPY) of each primer. The real-time PCR protocol for NPY, IGF-IR, and IGF-IIR was 3 min at 95°C followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. All amplifications were performed in triplicate. The standard curve showed a linear relationship between cycle threshold values and the logarithm of the input gene copy number. All specific quantities were normalized against the amount of alpha tubulin amplified because there was no significant family effect on the alpha tubulin expression levels.

Statistical analysis.—Statistical analyses were conducted using the mixed procedure of the Statistical Analysis System (SAS version 9.1) followed by a Duncan's multiple-range test. Normalized gene expression data passed Levene's test for homogeneity of variance. Tissue gene expression levels, weight gain, SGR, FCR, food intake, and condition factor (K; ratio of body weight to cube of body length) were subjected to one-way analysis of variance (ANOVA) with family as a fixed effect and tank within family as a random effect. Tank served as the experimental unit for each variable measured. Differences among families were considered significantly different at $P < 0.05$. A Pearson product-moment correlation coefficient was used to determine the relationship between weight gain and level of cortisol. A critical value table was used to test for significance.

TABLE 2.—Weight gain, specific growth rate (SGR), food intake per tank, food conversion ratio (FCR), and condition factor (K) of fast- and slow-growing families of USDA303 channel catfish. Means are least-squares means and SEs are the pooled standard errors of the means. See text for variable definitions. Within columns, values with different letters are significantly different ($P < 0.05$).

Family[a]	Weight gain (g)	SGR	Food intake (g)	FCR	K
A	142.1 z	2.00 zy	169.2 z	1.19 z	1.01 z
B	123.1 zy	2.21 zy	154.7 zy	1.26 z	1.00 zy
C	114.3 zy	2.32 zy	140.3 zyx	1.23 z	0.94 zy
D	113.8 zy	2.24 z	121.9 zyx	1.08 y	0.94 zyx
E	98.7 xw	2.18 zy	119.8 yxw	1.21 z	0.97 zyx
F	93.9 xw	1.86 yx	113.5 xw	1.21 z	0.93 xw
G	78.9 wu	1.88 yx	91.3 wu	1.16 z	0.88 xy
H	61.4 u	1.67 x	72.1 u	1.17 z	0.86 w
SE	3.8	0.05	4.6	0.01	0.11

[a] Families A–D were designated as fast growing and families E–H as slow growing based on a previous 30-d study.

Results

Growth Performance

Of the eight families tested, family A gained more weight, consumed more feed, and had a higher K and SGR than family H (Table 2); the food conversion ratio was similar between the two families. Because family A had the highest growth rate during a previous 30-d growth study as well as the highest SGR in the present 7-week study, it was designated as the "fast"-growing family. Similarly, because family H had one of the lowest growth rates during a previous 30-d growth study as well as the lowest SGR in the present 7-week study, it was designated as the "slow"-growing family. All gene expression comparisons were made against these two families. These results confirmed genetic differences in fingerling growth among USDA303 channel catfish families.

Abundance of IGF-I, IGF-IR, IGF-II, and IGF-IIR mRNA

Partial cDNA sequences of the IGF-IR and IGF-IIR genes were identified by sequencing plasmid clones in a library produced from whole-fry cDNA. The similarity of catfish clones to known peptides was determined by means of BlastX similarity searches against the GenBank nonredundant database. Two clones contained partial-length inserts that provided an 828-amino-acid open reading frame closely matching the IGF-Ia receptor of the zebrafish *Danio rerio* (amino acid identity, 79%; probability of sequence match by chance, $<1 \times 10^{-178}$). A third clone provided a 226-amino-acid open reading frame most similar to the zebrafish mannose-6-phosphate IGF-II receptor (amino acid identity, 79%; probability of sequence match by chance, $<1 \times 10^{-106}$). These sequences also matched the IGF-I and IGF-II receptor sequences, respectively, of other teleosts and mammals at high levels of sequence identity.

The levels of IGF-I mRNA were similar in the muscle and liver of the fast- and slow-growing fish, respectively (Figure 1A, B). The abundance of IGF-II mRNA was greater in the muscle of fast-growing fish than in that of slow-growing fish (Figure 2A, B), while there was no difference in the abundance of IGF-II mRNA in the liver. The levels of IGF-IR and IGF-IIR in the muscle were similar between the two groups of fish (Figures 1C and 2C). However, the abundance of IGF-IIR was 2.5 times higher than that of IGF-IR mRNA in the muscle.

Abundance of GH and GHR, Ghrelin, and NPY mRNA

Growth hormone receptor mRNA in muscle and liver was similar between fast- and slow-growing channel catfish (Figure 3A, B). Pituitary GH mRNA was also similar between fast- and slow-growing fish (Figure 3C). Although the fast-growing fish consumed over twice as much feed as the slow-growing fish, there was no significant difference in the abundance of ghrelin or NPY mRNA (Figure 4).

Stress Challenge

There was a negative correlation (–0.4907) between weight gain and plasma cortisol level among the four fast-growing and four slow-growing families of channel catfish. Fish that gained more weight during the 7-week growth study tended to have a lower level of cortisol after they were subjected to an acute 10-min confinement stressor.

Discussion

The objectives of the present study were to compare the components of the GH–IGF regulatory axis between fast-growing and slow-growing channel catfish and to gain a better understanding of the connection between stress responsiveness and growth performance. Toward this goal, we utilized real-time

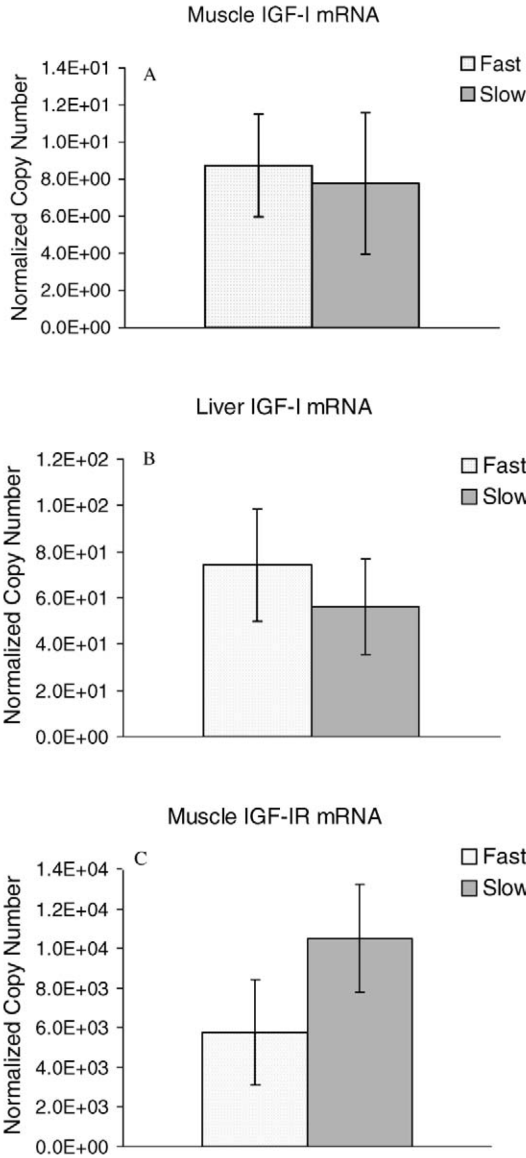

FIGURE 1.—Mean ± SE (**A**) muscle IGF-I, (**B**) liver IGF-I, and (**C**) muscle IGF-I receptor (IGF-IR) mRNA levels in fast- and slow-growing families of channel catfish ($N = 4$ fish per family). The IGF-I copy numbers were normalized as ratios of the amount of alpha tubulin. The means were not significantly different ($P > 0.05$).

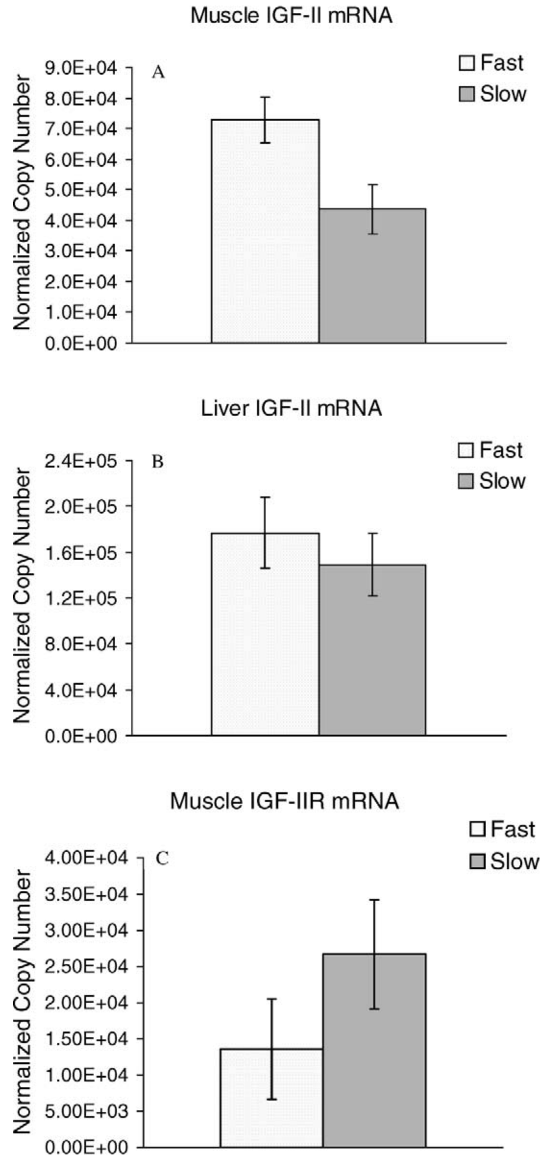

FIGURE 2.—Mean ± SE (**A**) muscle IGF-II, (**B**) liver IGF-II, and (**C**) muscle IGF-II receptor (IGF-IIR) mRNA levels in fast- and slow-growing families of channel catfish ($N = 4$ fish per family). The IGF-II copy numbers were normalized as ratios of the amount of alpha tubulin. Significant differences ($P < 0.05$) are denoted by asterisks.

PCR assays to measure the levels of GH, GHR, IGF-I, IGF-IR, IGF-II, IGF-IIR, ghrelin, and NPY mRNA. The fast- and slow-growing fish used in this study were selected from 68 full-sib families that had previously undergone a 30-d growth study. The results of the prior study were similar to those of the present study. These experiments demonstrated genetic growth differences among USDA303 channel catfish families.

The relationship between growth and the GH–IGF axis is complex. Studies in fish have suggested that ideal growth performance is achieved with low plasma GH in combination with a high concentration of hepatic GHRs and high circulating levels of IGFs

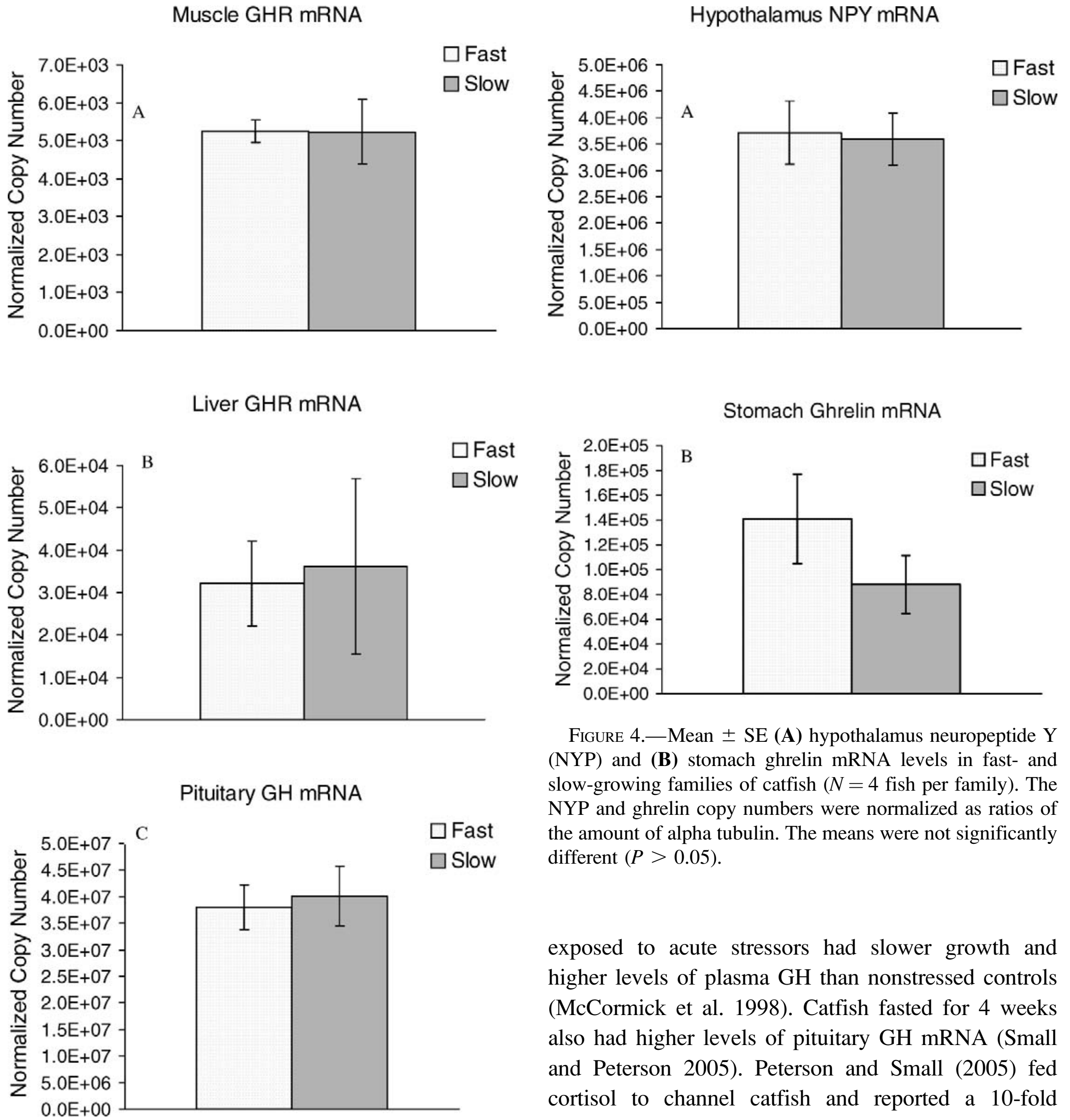

FIGURE 3.—Mean ± SE **(A)** muscle GH receptor (GHR), **(B)** liver GH receptor, and **(C)** pituitary GH mRNA levels in fast- and slow-growing families of channel catfish ($N = 4$ fish per family). The GH receptor and GH copy numbers were normalized as ratios of the amount of alpha tubulin. The means were not significantly different ($P > 0.05$).

FIGURE 4.—Mean ± SE **(A)** hypothalamus neuropeptide Y (NYP) and **(B)** stomach ghrelin mRNA levels in fast- and slow-growing families of catfish ($N = 4$ fish per family). The NYP and ghrelin copy numbers were normalized as ratios of the amount of alpha tubulin. The means were not significantly different ($P > 0.05$).

(Pérez-Sánchez and Le Bail 1999). In general, plasma GH levels follow physiological and nutritional status, increased levels being correlated with reduced growth and nutritional status. In support of this hypothesis, Lankford and Weber (2006) reported a negative correlation between body weight and resting plasma GH in rainbow trout. Conversely, Atlantic salmon parr exposed to acute stressors had slower growth and higher levels of plasma GH than nonstressed controls (McCormick et al. 1998). Catfish fasted for 4 weeks also had higher levels of pituitary GH mRNA (Small and Peterson 2005). Peterson and Small (2005) fed cortisol to channel catfish and reported a 10-fold reduction in pituitary GH mRNA. The results of the catfish studies suggest that GH may also track the physiological and nutritional status of the fish. In the current study, we did not measure the abundance of pituitary GH mRNA in all eight catfish families to see if there was an association with weight gain. However, there was no difference in the levels of pituitary GH mRNA between fast- and slow-growing fish.

Growth hormone treatment has been found to improve the FCR in catfish studies (Wilson et al. 1988; Peterson et al. 2004a). We found that the FCR was similar between the fast- and slow-growing catfish. Given this, it is not surprising that pituitary GH mRNA was also similar. However, the lack of difference in

pituitary GH mRNA may simply reflect the fact the both groups of fish were in an anabolic growing state.

We found no statistical difference in levels of GHR mRNA in the liver and muscle of fast- and slow-growing catfish. The role of GHR in growth is complex and probably involves the coordination of many components of the GH–IGF axis. A recent study reported that GHR mRNA abundance in the liver of cortisol-fed and fasted catfish was significantly reduced, supporting the role of GHR under catabolic conditions (Small et al. 2006). The lack of difference between muscle and liver GHR mRNA between the fast- and slow-growing catfish may also reflect the anabolic status of the fish.

The levels of IGF-I and IGF-II mRNA were measured in both the muscle and liver of experimental fish, while IGF-IR and IGF-IIR mRNAs were quantified in the muscle. In all fish (fast and slow growing), IGF-II mRNA was more abundant in the muscle and liver than IGF-I mRNA and IGF-IIR mRNA levels were two-fold higher than IGF-IR mRNA levels. These results confirmed prior research showing that the levels of IGF-II mRNA levels in muscle and liver were higher than those of IGF-I mRNA (Peterson et al. 2004b). Similarly, the present research confirmed that the levels of IGF-II mRNA are greater in the muscle and liver of fast-growing fish (Peterson et al. 2004b). However, the levels of IGF-II mRNA in the liver were similar between the two phenotypes in the present study. The reason for the disparity between the two studies is not clear, but the results of both studies suggest a role for IGF-II in channel catfish growth.

The physiological role of IGF-II in fish is slowly evolving. In mammals, IGF-II mRNA is detected in fetal tissues but decreases quickly during postnatal development (Daughaday and Rotwein 1989). In contrast, teleostean tissues express substantial amounts of IGF-II later in life (Gabillard et al. 2003; Chauvigné et al. 2003; Radaelli et al. 2003; Vong et al. 2003; Caelers et al. 2004). Growth hormone dependent expression of IGF-II mRNA has been demonstrated in fish (Shamblott et al. 1995; Greene et al. 1999; Vong et al. 2003; Peterson et al. 2005). Using Mozambique tilapia *Oreochromis mossambicus* as a model, Kajimura et al. (2001) have shown that IGF-II also negatively regulates pituitary GH expression in vitro. In a recent study, handling and confinement stress resulted in decreases in plasma levels of IGF-II in Atlantic salmon and rainbow trout (Wilkinson et al. 2006). Starvation causes a reduction in the circulating levels of IGF-II (Gentil et al. 1996; Wilkinson et al. 2006). The lack of commercially available channel catfish IGF-II and antibody has hampered our efforts to understand the role of circulating IGF-II under anabolic and catabolic conditions. The production of recombinant catfish IGF-II is currently under way.

The levels of IGF-I mRNA were similar in the muscle and liver of the fast- and slow-growing fish, confirming previous research (Peterson et al. 2004b). The lack of difference in hepatic expression of IGF-I was surprising in both studies, since faster growth was presumably being mediated by IGF-I. However, this does not imply that IGF-I has no function in the growth of channel catfish. The fact that muscle and liver IGF-I mRNA levels were not related to the growth rate of the faster-growing fish only means that muscle and liver IGF-I mRNA cannot explain differences in growth between the two families (Peterson et al. 2004b). It is possible that IGF-I produced by other tissues plays a significant role in the growth of channel catfish. The IGF-I gene is expressed in several teleostean tissues, including the brain, heart, gill, intestine, kidney, spleen, and testes (Vong et al. 2003; Biga et al. 2004; Caelers et al. 2004; Clay et al. 2005). Perhaps an autocrine/paracrine role for IGF-I is important in catfish and other species of fish. In mice that have had the IGF-I gene deleted in the liver, liver IGF-I was not essential for normal growth and development (Yakar et al. 1999; Sjogren et al. 1999). These studies provide evidence that autocrine/paracrine IGF-I can support normal postnatal growth and development.

The abundance of muscle IGF-IR mRNA was not different in fast- and slow-growing channel catfish. Similarly, in chickens selected for fast and slow growth, IGF-IR mRNA were similar in the brain, heart, muscle, and liver (Armstrong and Hogg 1994). In rainbow trout, a significant decrease in IGF-IR (types a and b) mRNA abundance was found in the gill of a rapidly growing juvenile rainbow trout relative to that in slower-growing adults (Greene and Chen 1999). In the same study, significant increases were found in the heart for IGF-IRa and the heart, kidney, pyloric caeca, pancreas, adipose, and spleen for IGF-IRb for the faster-growing trout (Greene and Chen 1999). The role of IGF-IR in the growth of channel catfish is currently not known.

The IGF-II receptor has been identified in embryos of the brown trout *Salmo trutta* (Mendez et al. 2001). In mammals, the IGF-II cation-independent mannose-6-phosphate (IGF-II/Man-6-P) receptor is a monomeric receptor that binds to mannose-6-phosphate residues on lysosomal enzymes (Jones and Clemmons 1995). The IGF-II/Man-6-P receptor is thought to function primarily as a degradative pathway to remove IGF-II from the extracellular environment (Jones and Clemmons 1995). The physiological role of the IGF-II/Man-6-P receptor in fish in currently not known. Insulin-like growth

factor-II receptor mRNA was approximately two-fold higher than IGF-IR mRNA in the muscle in the present study. This study is the first to compare IGF-IR abundance with IGF-IIR abundance in any fish species. The higher levels of IGF-IIR and the high levels of IGF-II mRNA in the muscle suggest a role for IGF-II in muscle growth in channel catfish. An IGF-II mutation was identified as a quantitative trait locus affecting postnatal muscle growth in swine (Van Laere et al. 2003), and allelic polymorphism at the IGF-II locus may prove useful in selecting pigs for faster growth. As such, polymorphism typing of IGF-II in channel catfish merits investigation.

The regulation of food intake in fish is achieved via a complex hypothalamic neural network that integrates multiple stimulatory and inhibitory neuroendocrine signals (Lin et al. 2000). Two stimulatory peptides in fish include ghrelin and NPY. The physiological functions of ghrelin in fish are the regulation of pituitary GH, regulation of food intake, and control of drinking behavior (Unniappan and Peter 2005). In channel catfish, ghrelin has also been shown to stimulate GH gene expression and GH release (Kaiya et al. 2005). Neuropeptide Y is a highly conserved peptide that has been shown to be a powerful stimulator of food intake in goldfish (Lopez-Patino et al. 1999; Narnaware et al. 2000) and channel catfish (Silverstein and Plisetskaya 2000). In the current study, it was hypothesized that the faster-growing fish would consume more feed. As expected, the fast-growing fish consumed more than twice as much feed as the slow-growing fish. However, there was no significant difference in the abundance of ghrelin or NPY mRNA between these two phenotypes.

Time course changes in the levels of NPY and ghrelin relative to pre- and postfeeding in channel catfish are not known. In the current study, tissue samples were taken during their normal scheduled feeding period, and tissue sampling continued for approximately 1 h. In goldfish and Atlantic cod *Gadus morhua*, NYP mRNA expression in the brain increases in anticipation of a meal (Narnaware et al. 2000; Kehoe and Volkoff 2007). Although NYP has been shown to a regulator of appetite in catfish (Silverstein and Plisetskaya 2000), it does not appear that NPY mRNA levels are a good modulator of differences in appetite.

Time course changes in ghrelin mRNA expression appear to be species specific. For example, ghrelin expression in the stomach increased after 35 d of fasting in sea bass *Dicentrarchus labrax* (Terova et al., in press) and in goldfish after 7 d (Unniappan et al. 2004), while in Nile tilapia *Oreochromis niloticus*, ghrelin levels did not change even after 7 d of fasting (Parhar et al. 2003). Ghrelin mRNA levels in the current study were higher in the faster-growing fish ($P = 0.09$). However, the observed higher levels of ghrelin mRNA in fast-growing fish may have little biological significance given the number of fish that were sampled from both phenotypes (12 from each group). As with NPY mRNA, ghrelin mRNA levels may not be a good modulator of differences in appetite.

Future studies should look at plasma peptide levels of ghrelin and NPY as well as other orexigenic neuropeptides such as cholecystokinin, peptide YY, pancreatic polypeptide, and peptide Y. It is currently not known what regulates the observed higher feed intake among some channel catfish families. An understanding of the neuropeptide(s) involved in regulating appetite in catfish could lead to methods of identifying fish with a higher propensity to consume more feed and thus to have higher growth.

There was a negative correlation between weight gain and plasma cortisol level among the four fast-growing and four slow-growing families of channel catfish. The results of this study suggest that fish that gained more weight during the study had lower levels of cortisol after they were subjected to an acute 10-min confinement stressor. Associations between cortisol response to a confinement stressor and growth performance have been reported in other species, but they have not been consistent. In a study with rainbow trout broodstock, Lankford and Weber (2006) found a positive correlation between body weight and cortisol response. However, that relationship disappeared when growth performance was quantified by the thermal growth coefficient. Pottinger and Carrick (1999) also found a positive association between cortisol responsiveness and body size in female rainbow trout. Among the progeny of these fish, those responding with low concentrations of cortisol grew faster than the others, but only when the two groups of fish were cocultured (Pottinger and Carrick 2001). In another study, low-cortisol-responding rainbow trout had higher specific growth rates at early life stages than high-cortisol-responding trout (Fevolden et al. 2002). However, the progeny groups in that study were hatched 1 month apart, which makes it difficult to compare specific growth rates. A clear understanding of the relationship between cortisol and growth rate has not evolved. It is likely that there are differences between species and strains and that the elucidation of the relationship between cortisol and growth will require characterization of hormones in addition to cortisol (Lankford and Weber 2006). The significant correlation between stress responsiveness and weight gain should be further investigated for possible exploitation in our selective breeding program.

In conclusion, we utilized quantitative real-time PCR

assays to measure eight genes in fast- and slow-growing USDA303 families of channel catfish to test the hypothesis that genes or gene products associated with the growth regulatory and stress axes can be used to describe differences in growth performance. We found that IGF-II mRNA levels in muscle were higher in fast-growing fish. We showed (for the first time in any fish species) that IGF-IIR mRNA levels were twice as high as those of IGF-IR mRNA in muscle. We also demonstrated a negative correlation between weight gain and cortisol. These results suggest that the variation in growth among families of USDA303 catfish is explained, in part, by variation in the GH–IGF and stress axes. The expression of the gene for IGF-II may reflect differences in muscle growth. The relationship between stress responsiveness and weight gain warrants further investigation.

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