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Heritability of growth traits and correlation with hepatic gene expression among hybrid striped bass exhibiting extremes in performance

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Abstract: Hybrid striped bass is a major aquaculture species in the United States. Artificial breeding of this species can introduce large variation in growth during production to market size. To assess the genetic and nutrigenomic basis behind growth variability in these hybrids, fingerlings \((n = 5072)\) from 47 families were size-matched and communally grown in earthen ponds for 115 days. Families were then ranked by weight gain and individuals from the three fastest growing (mean 240.8 ± 9.75 g; 242.0 ± 11.52 mm) and three slowest growing families (mean 153.5 ± 52.38 g; 223.3 ± 21.31 mm) were collected for liver RNA sequencing. As expected, growth characteristics in hybrid striped bass are highly heritable \((p < 0.0001)\). Through differential gene expression analysis we identified 86 genes that were responsive between groups including 40 up-regulated \((1.89 < \text{fold-change} < 7.66)\) and 46 down-regulated \((-1.71 > \text{fold-change} > -4.59)\) genes in the largest fish. This included two somatic growth-related genes, growth factor receptor gene and a gene encoding an insulin-like growth factor binding protein, that may directly explain some of the genetic variation between families. Several additional genes involved in metabolic pathways such as glycolysis/gluconeogenesis and lipid metabolism were also up and down-regulated. This study provides insights into the underlying genetic and nutrigenomic mechanisms that contribute to growth variability in hybrid striped bass, which can inform future breeding and management strategies for this species.
biosynthesis were also revealed. The candidate gene list may also provide some evidence that both physiological and behavioral factors may be influencing growth differences in communally reared fish.

Subjects: Fisheries Science; Aquaculture; Bioinformatics; Genetics; Marine Biology

Keywords: bass; gene expression; RNA sequencing; moronid; liver; growth; heritability; aquaculture; breeding; hepatic

1. Introduction
Hybrid striped bass (HSB; “sunshine”; Morone chrysops × M. saxatilis), created by artificial crosses of white bass (WB; M. chrysops) and striped bass (SB; M. saxatilis) are a major commodity in US aquaculture production (Hallerman, 1994). As a high value finfish, HSB are typically sold whole and thus weight at harvest can significantly impact economic gain. In HSB culture, the major breeders utilize wild parental bass in their breeding programs and thus selection programs for growth characteristics remain in their infancy (Garber & Sullivan, 2006). This uncontrolled breeding schema can and often does lead to great variability in HSB size at harvest, even when fish are size-matched at stocking. For instance, in a study of growth characteristics among Morone crosses communally reared in 0.10 ha earthen ponds for over a year, a wide range of sizes occurred from 268 g to over 1 kg (mean 634 ± 6 g), even though the HSB were tightly graded at the beginning of the experiment (19.8 ± 0.4 g) (McEntire, Snyder, & Freeman, 2015). We set out to better understand the genetic basis behind this variation by determining whether gene expression changes could be detected between the largest and smallest HSB in a population. Using a global gene expression approach by RNA sequencing of liver, a major metabolic tissue, and de novo assembly of a transcriptome, genes that may contribute at least in part to the variation in somatic growth during HSB culture could be detected. We hypothesized that any observed changes would be related to genes involving growth factors which predominate the liver such as insulin-like growth factor (IGF) along with energy production pathways.

2. Methods
Reciprocal cross HSB (♀ white bass × ♂ striped bass) were created by random-matings from F₁ domesticated WB and F₂ domesticated SB originally from North Carolina State University (Garber, 2006; Garber & Sullivan, 2006), utilizing a series of 2 × 2 diallel crosses from 25 contributing females and 30 contributing males, resulting in 47 families (46 dam half-sib, 30 sire half-sib, and 24 full-sib families) and reared in replicate 35-L fiberglass tanks at approximately 40 fry·L⁻¹ according to Fuller, McEntire, and Freeman (2013). Fingerlings were then grown until 25.0 ± 0.41 g, 105 days post hatch in 280-L fiberglass tanks with flow-through well water and continuous aeration at which time 128 fingerlings from each family were tagged intramuscularly with a passive integrated transponder (PIT) tag for individual identification according to the methods of Fuller and McEntire (2013). Fingerlings were monitored for one week for PIT tag retention, and initial total length and weight were recorded. Fingerlings were assigned randomly to one of four earthen 0.04 hectare ponds (32 fingerlings·family⁻¹·pond⁻¹; 1268 fingerlings per pond) at 121 dph supplied with 3/4-hp aerators (Little John Inc) set to run 24 h per day. Ponds were fed to apparent satiation twice per day with a standard high fish protein commercial diet (Cargill Animal Nutrition; 45% crude protein, 12% crude fat) for 115 d, with the amount of food that each pond was fed not differing over the study period (p > 0.05).

At the end of the communal rearing period, each pond was seined, fingerlings were individually identified and measured for growth characteristics, where mean weight was 235.3 ± 17.8 g and mean length was 192.1 ± 48.7 mm with a mean survival of 90.7 ± 0.05% (range 87.8–97.5%). Statistically significant differences (p < 0.05) in sire and dam components of variance were then determined by analysis of variance using PROC GLM, and least square mean total length and weight, with initial total length and initial weight as covariates, was determined by analysis of covariance using PROC MIXED in SAS ver. 9.2 (SAS Institute). Genetic correlations for each trait were based on
sire and dam (co)variance components obtained using PROC MIXED, and standard errors were calculated using PROC IML in SAS ver. 9.2 (Xiang & Li, 2001). Pairwise phenotypic correlations among traits were estimated via Pearson’s product-moment correlation coefficient using PROC CORR in SAS ver. 9.2. We found significant dam and sire effects on least square weight (LS-weight) and least square total length (LS-length) \((p < 0.0001)\), and dam × sire interaction \((p < 0.0001)\) for both traits. Estimates of heritability were high for both traits. Values for LS-weight and LS-length were \(0.33 ± 0.02\) and \(0.75 ± 0.02\), respectively, for dams, and \(0.83 ± 0.09\) and \(0.56 ± 0.04\), respectively, for sires. Genetic correlation between LS-weight and LS-length based on the dam and sire components of variance were 0.92 and 0.95, respectively; the phenotypic correlation was 0.88.

After inspection of all HSB, families were ranked by weight gain and six individuals from the three fastest growing families (mean 240.8 ± 9.75 g; 229.2 to 241.9 g range; 242.0 ± 11.52 mm) and six individuals from the three slowest growing families (mean 153.5 ± 52.38 g; 105.1 to 140.2 g range; 223.3 ± 21.31 mm) were collected and euthanized. Liver tissue was harvested from each individual and total RNA was extracted using the RNeasy Plus Mini Kit (Qiagen). Equimolar amounts of total RNA were pooled in multiples of six to create three biological replicate pools for the largest HSB and smallest HSB. The six RNA pools were sent to a commercial service provider (Data2Bio, Ames, IA, USA) for library construction and high-throughput sequencing, using the TruSeq RNA Sample Preparation Kit (Illumina) and 100-bp, paired-end RNA sequencing (RNAseq) on an Illumina HiSeq2000.

The bioinformatics workflow for the RNAseq data is illustrated in Figure 1. Raw sequencing reads from each sample were processed for quality control (QC) using the TrimGalore! software with the default parameters. Since genomic and transcriptomic information on HSB is lacking, a de novo transcriptome was built by the Trinity (Grabherr et al., 2011) software using all QC reads. Transcripts were subjected to a preliminary functional characterization using Trinotate (Haas et al., 2013), where those with a significant \(10^{-5}\) BLASTx hit to the UniProt database were selected. These transcripts with putative protein-coding ability were then analyzed by CD-HIT (Fu, Niu, Zhu, Wu, & Li, 2012) to help remove redundancy in the transcriptome by collapsing similar sequences. Clustering was performed from 85 to 100% identity, in increments of 1%. Each set of contigs were analyzed with BUSCO (Simao, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) to help determine completeness of the protein-coding transcriptome by comparing gene content from orthologs in the “actinopterygii_odb9” subset of the OrthoDB v9 database. Based on these analyses, we selected 90% clustering, a level at which transcript redundancy was best reduced without a reduction in gene diversity. The workflow to this point (Figure 1) produced 38,047 putative protein-coding transcripts (herein HSB

![Figure 1. Bioinformatic pipeline for identifying and characterizing differentially expressed genes between hybrid striped bass presenting extremes in growth.](image-url)
transcriptome) in which to align our reads for gene expression analysis. Raw RNAseq data sets along with HSB transcriptome sequences and normalized expression counts from which conclusions were drawn have been submitted to the NCBI Gene Expression Omnibus (GEO) and can be retrieved under the accession number GSE97547.

3. Results and discussion

After construction of a reference HSB transcriptome, QC data were aligned to the reference using bowtie2 (Langmead & Salzberg, 2012) and then effective read counts were assigned using eXpress (Roberts & Pachter, 2013) software (Figure 1). Using this read count information, statistical comparisons between groups (low growing HSB vs. high growing HSB) were performed using the DESeq2 (Love, Huber, & Anders, 2014) package of R-bioconductor. Significant (padj < 0.05; p-value adjusting for multiple testing) differentially expressed genes (DEGs) between low and high growth HSB were collected and identified by BLASTx searches (1e−3) to the non-redundant (nr) protein database at the NCBI. After manual screening for duplicates, unknown and uncharacterized proteins as well as genes with low mapping rates (median FPKM < 1) for highest confidence of expression (Yendrek, Ainsworth, & Thimmapuram, 2012), we identified 86 DEGs between groups (Table 1). This included 40 up-regulated (1.89 < fold-change < 7.66) and 46 down-regulated (−1.71 > fold-change > −4.59) genes in large HSB, as we are using small HSB as the baseline for comparison.

Upon initial inspection, somatic growth-related genes were identified from our candidate gene list (Table 1) that may directly explain some of the genetic differences observed between the largest and smallest HSB families. A growth factor receptor gene was expressed greater than two-fold higher in the largest HSB. Endocrine control of growth in fish is regulated primarily through the growth hormone (GH)/insulin-like growth factor (IGF) axis (Wood, Duan, & Bern, 2005). An increase in hepatic expression of growth hormone receptors has been shown to signal IGF-1 production and somatic growth in carnivorous fish (Norbeck, Kittilson, & Sheridan, 2007; Picha et al., 2009; Picha, Turano, Tipsmark, & Borski, 2008; Won & Borski, 2013). Further, the gene for insulin-like growth factor binding protein 2a was highly down-regulated in the largest HSB. Insulin growth factor binding proteins are reported to bind to insulin-like growth factors thus preventing binding between IGFs and their associated receptors, which inhibits the activities of IGFs (Chauvigné, Gabillard, Well, & Rescan, 2003; Clemmons, 2001; Li et al., 2009; Picha et al., 2014). Activity of ILGFBP2a as a growth inhibitory protein dependent upon nutritional status appears to be conserved, as observed from fish to mammals (Duan, Ding, Li, Tsai, & Pizios, 1999; Kang et al., 2015). Growth factor binding proteins have also been indicated in the nutritional status of carp, where up-regulation was observed in fish under fasting conditions (He et al., 2015). Thus, up-regulated ILGFBP2a in the smallest HSB as observed in this study may also be a contributing factor for their decreased weight.

DEGs were separated by regulation (+ or −) and functionally categorized by gene ontology (GO) using the Blast2GO (Gotz et al., 2008) software. This classified genes based on the major GO categories of cellular component (CC), molecular function (MF) and biological processes (BP). For the positively regulated DEGs, all three GO categories are represented, with metabolic processes being the top represented (Figure 2). Cellular processes, catalytic activity, binding, and cell membrane terms are also among the most represented in the positively regulated DEGs (Figure 2). For the negatively regulated DEGs, again all three GO categories are represented and as well the most-represented terms are similar to the down-regulated DEGs but the top represented category by number of sequences is single-organism processes (Figure 3).

As GO was similar for both up and down-regulated DEGs, and as positive and negative perturbation of a biological pathway can and often does occur in concert, we were interested in a systems approach where all 86 DEGs (Table 1) were grouped for enrichment analysis. For this analysis, first BLASTx searches (1e−3) to the nr database were performed on the complete HSB transcriptome. These data were supplied to the Blast2GO software, where the transcriptome was functionally characterized with gene ontology, protein structure, enzyme commission and gene pathway information. Blast2GO was then used to perform functional enrichment analysis comparing all DEGs (Table
## Table 1. List of genes significantly differentially regulated between high and low growth HSB

| Gene                                | FC    | p-adj    | Accession | Gene description                                                                 |
|--------------------------------------|-------|----------|-----------|----------------------------------------------------------------------------------|
| HSB_liver_CDS_3407                   | 7.66  | 8.22E-12 | KKF17585.1 | 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2                           |
| HSB_liver_CDS_25458                  | 4.91  | 2.73E-09 | CBN80677.1 | GTP-binding protein REM 1                                                         |
| HSB_liver_CDS_26173                  | 3.43  | 2.58E-05 | KKF10972.1 | Farnesyl pyrophosphate synthase                                                   |
| HSB_liver_CDS_29463                  | 3.40  | 4.15E-05 | XP_003439749.1 | 3-Keto-steroid reductase isoform X1      |
| HSB_liver_CDS_8509                   | 3.17  | 6.97E-06 | XP_007550766.1 | Beta-sarcoglycan                  |
| HSB_liver_CDS_15540                  | 2.96  | 1.03E-03 | KKF28278.1 | OX-2 membrane glycoprotein                                                        |
| HSB_liver_CDS_12535                  | 2.93  | 1.27E-03 | XP_003964007.1 | Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase/dual-specificity PTEN   |
| HSB_liver_CDS_16126                  | 2.86  | 1.85E-03 | KKF17856.1 | Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating                      |
| HSB_liver_CDS_17655                  | 2.76  | 1.85E-03 | NP_001133968.1 | Diphosphoehavalonate decarboxylase                                              |
| HSB_liver_CDS_17931                  | 2.74  | 2.94E-03 | XP_003972472.1 | Methylsterol monoxygenase 1                                                     |
| HSB_liver_CDS_22701                  | 2.74  | 3.56E-03 | XP_005812741.1 | Inhibitor of growth protein 3                                                   |
| HSB_liver_CDS_29195                  | 2.66  | 6.17E-03 | XP_009289469.1 | Disks large homolog 3 isoform X1      |
| HSB_liver_CDS_25813                  | 2.57  | 9.27E-03 | XP_005462201.1 | Glucose-6-phosphatase-exchanger SLC37A2                                        |
| HSB_liver_CDS_27639                  | 2.55  | 6.90E-03 | XP_003971109.1 | Delta(14)-sterol reductase                                                      |
| HSB_liver_CDS_13734                  | 2.54  | 9.89E-03 | XP_019218129.1 | 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase epsilon-1           |
| HSB_liver_CDS_17852                  | 2.51  | 1.12E-02 | NP_001293026.1 | Mitochondrial carrier homolog 2                                                  |
| HSB_liver_CDS_23759                  | 2.47  | 1.55E-02 | XP_00347658.1 | 3-Hydroxy-3-methylglutaryl-coenzyme A reductase                                  |
| HSB_liver_CDS_24341                  | 2.46  | 1.51E-02 | AA68786.1 | MHC class II alpha                                                              |
| HSB_liver_CDS_17284                  | 2.43  | 2.09E-02 | XP_019131981.1 | N-acetylg glucosamine-6-phosphate deacetylase                                  |
| HSB_liver_CDS_19643                  | 2.39  | 8.34E-03 | XP_003974861.1 | Protein MAK16 homolog                                                           |
| HSB_liver_CDS_29445                  | 2.38  | 2.51E-02 | XP_005447809.1 | 7-Dehydrocholesterol reductase isoform X2                                       |
| HSB_liver_CDS_25387                  | 2.36  | 3.13E-02 | AHY22365.1 | Growth factor receptor-1                                                        |
| HSB_liver_CDS_15819                  | 2.31  | 1.13E-02 | XP_007566227.1 | Acyl-CoA-binding domain-containing protein 7 isoform X1            |
| HSB_liver_CDS_7939                   | 2.30  | 4.34E-04 | ADX01347.1 | Rhamnose-binding lectin                                                         |
| HSB_liver_CDS_21813                  | 2.30  | 3.97E-02 | KKF23443.1 | E3 ubiquitin-protein ligase KCMF1                                                |
| HSB_liver_CDS_30838                  | 2.30  | 3.13E-02 | XP_010752877.2 | Cyclic AMP-responsive element-binding protein 3-like protein 4                 |
| HSB_liver_CDS_31012                  | 2.29  | 9.64E-03 | XP_003452513.1 | Lanosterol 14-alpha demethylase                                                  |
| HSB_liver_CDS_4795                   | 2.27  | 4.31E-02 | ACQ58692.1 | Transmembrane protein LOC124446                                                |
| HSB_liver_CDS_8725                   | 2.27  | 4.01E-02 | XP_005462617.1 | RNA-sphling endonuclease subunit Sen2 isoform X2                               |
| HSB_liver_CDS_37813                  | 2.25  | 4.31E-02 | XP_019119287.1 | Acetoacetyl-CoA synthetase                                                       |
| HSB_liver_CDS_26662                  | 2.23  | 3.84E-02 | XP_011610382.1 | Squalene synthase isoform X1                                                     |
| HSB_liver_CDS_24347                  | 2.23  | 2.68E-02 | CAQ13207.1 | MHC class I antigen, partial                                                    |
| HSB_liver_CDS_3261                   | 2.21  | 3.34E-02 | XP_003451814.1 | Ubiquitin-conjugating enzyme E2 D2 isoform X1                                  |
| HSB_liver_CDS_25360                  | 2.21  | 2.57E-02 | KKF27353.1 | GTPase IMAP family member 4                                                   |
| HSB_liver_CDS_31450                  | 2.17  | 9.34E-03 | AKM12675.1 | Complement component C7-1                                                    |
| HSB_liver_CDS_3968                   | 2.16  | 4.44E-02 | KKF21934.1 | Zinc finger protein RFP                                                        |
| HSB_liver_CDS_26056                  | 2.11  | 3.84E-02 | KFO28071.1 | Formimidoyltransferase-cyclodeaminase                                            |
| HSB_liver_CDS_15397                  | 2.09  | 8.83E-03 | XP_00345809.1 | Pyrroline-5-carboxylate reductase 1, mitochondrial                               |
| HSB_liver_CDS_7138                   | 2.00  | 1.85E-03 | XP_007559381.1 | Single-stranded DNA-binding protein 2 isoform X4                                |
| HSB_liver_CDS_18581                  | 1.89  | 4.62E-02 | XP_010739859.2 | Multiple inositol polyphosphate phosphatase 1-like                              |
| HSB_liver_CDS_36553                  | −1.71 | 3.89E-02 | XP_003450339.1 | Alkylglycerol monoxygenase isoform X1                                           |
| HSB_liver_CDS_20407                  | −1.80 | 4.31E-02 | KKF32204.1 | Lys protease                                                                   |
| HSB_liver_CDS_3635                   | −1.85 | 4.62E-02 | XP_019123002.1 | Tetraspanin-8-like                                                              |

(Continued)
### Table 1. (Continued)

| Gene                  | FC    | p-adj     | Accession          | Gene description                                      |
|-----------------------|-------|-----------|--------------------|-------------------------------------------------------|
| HSB_liver_CDS_28368  | −1.99 | 9.00E-03  | XP_005809173.1    | Extracellular matrix protein 1                        |
| HSB_liver_CDS_22594  | −2.01 | 4.02E-02  | NP_001129616.1    | Type I iodothyronine deiodinase                        |
| HSB_liver_CDS_26296  | −2.14 | 6.59E-03  | XP_004068320.1    | Dimethyline monoxygenase                               |
| HSB_liver_CDS_21376  | −2.18 | 4.96E-02  | XP_014325892.1    | Kruempel-like factor 10                                |
| HSB_liver_CDS_25849  | −2.19 | 1.27E-02  | CBN81681.1        | Glucose-6-phosphatase                                  |
| HSB_liver_CDS_37502  | −2.20 | 2.45E-02  | AEK25827.1        | Diazepam-binding inhibitor                             |
| HSB_liver_CDS_37688  | −2.21 | 9.30E-03  | XP_007565477.1    | Bile salt export pump-like                             |
| HSB_liver_CDS_24400  | −2.22 | 4.31E-02  | XP_007561743.1    | Histone H1-like                                        |
| HSB_liver_CDS_12163  | −2.22 | 7.19E-03  | ACQ58208.1        | Cytochrome b-c1 complex subunit 10                    |
| HSB_liver_CDS_17141  | −2.24 | 3.29E-02  | KKF15775.1        | Next to BRCA1 1 protein                                |
| HSB_liver_CDS_25849  | −2.27 | 3.65E-02  | XP_005451949.1    | DNA excision repair protein ERCC-1                    |
| HSB_liver_CDS_18320  | −2.28 | 3.18E-02  | KKF13003.1        | Mortality factor 4-like protein 1                     |
| HSB_liver_CDS_20848  | −2.28 | 4.39E-02  | XP_014330388.1    | Phosphatidylcholine-sterol acyltransferase            |
| HSB_liver_CDS_15489  | −2.31 | 3.56E-02  | XP_011472633.1    | Zinc transporter 7 isoform X2                         |
| HSB_liver_CDS_9077   | −2.31 | 3.17E-02  | KKF21186.1        | Sodium/glucose cotransporter 4                        |
| HSB_liver_CDS_4527   | −2.33 | 3.13E-02  | XP_010748887.1    | Tumor necrosis factor ligand superfamily member 11   |
| HSB_liver_CDS_34927  | −2.34 | 2.69E-02  | XP_019113738.1    | Tyrosine aminotransferase                              |
| HSB_liver_CDS_12249  | −2.36 | 1.77E-02  | AC132417.1        | Pentraxin                                              |
| HSB_liver_CDS_32520  | −2.39 | 2.26E-02  | AIN76765.1        | Complement component 2                                 |
| HSB_liver_CDS_22713  | −2.43 | 9.65E-03  | XP_003451768.1    | Sodium- and chloride-dependent GABA transporter       |
| HSB_liver_CDS_37868  | −2.44 | 8.56E-03  | KKF21859.1        | Alpha-2-macroglobulin                                 |
| HSB_liver_CDS_9353   | −2.47 | 6.59E-03  | XP_003446783.1    | Multidrug and toxin extrusion protein 1               |
| HSB_liver_CDS_5697   | −2.48 | 1.57E-02  | AAP49009.1        | Transposase                                            |
| HSB_liver_CDS_14963  | −2.50 | 1.31E-02  | XP_005807612.1    | Phosphoenolpyruvate carboxykinase, cytosolic          |
| HSB_liver_CDS_7767   | −2.50 | 8.12E-03  | XP_003451846.1    | Sorting nexin-25                                     |
| HSB_liver_CDS_9522   | −2.50 | 2.93E-03  | XP_005799791.1    | Very long-chain acyl-CoA synthetase                   |
| HSB_liver_CDS_26221  | −2.54 | 1.50E-03  | ABH06553.1        | Proto-oncogene protein c-Fos                           |
| HSB_liver_CDS_21684  | −2.56 | 9.81E-03  | XP_019134360.1    | Lysine-specific demethylase 2B-like isoform X3        |
| HSB_liver_CDS_16964  | −2.59 | 2.58E-03  | XP_007552581.1    | NADH dehydrogenase                                    |
| HSB_liver_CDS_23535  | −2.62 | 5.82E-03  | AEB31271.1        | Hydraoxypenolpyruvate dioxygenase, partial             |
| HSB_liver_CDS_20770  | −2.67 | 2.86E-03  | XP_003458375.1    | Galectin-9                                            |
| HSB_liver_CDS_4201   | −2.68 | 3.09E-03  | EMP35734.1        | Solute carrier family 13 member 3                     |
| HSB_liver_CDS_16883  | −2.72 | 3.88E-03  | XP_019121404.1    | Nectin-4-like isoform X1                               |
| HSB_liver_CDS_25413  | −2.99 | 1.44E-04  | XP_005490943.1    | Gamma-glutamyl hydrolase                              |
| HSB_liver_CDS_23170  | −3.00 | 7.62E-04  | XP_010753892.2    | Protein-S-isoprenylcysteine-O-methyltransferase       |
| HSB_liver_CDS_7217   | −3.01 | 7.78E-04  | ACO07708.1        | Serine/arginine repetitive matrix protein 1           |
| HSB_liver_CDS_23211  | −3.09 | 9.94E-08  | AHH84195.1        | Insulin-like growth factor binding protein-2a         |
| HSB_liver_CDS_13489  | −3.43 | 5.19E-05  | KKF24190.1        | Phospholipid transfer protein                          |
| HSB_liver_CDS_2534   | −3.51 | 3.59E-06  | XP_004084485.1    | V-type proton ATPase subunit G 1-like                 |
| HSB_liver_CDS_30999  | −3.62 | 3.33E-06  | XP_003456777.1    | Cholesterol 7-alpha-monooxygenase                     |
| HSB_liver_CDS_31055  | −3.76 | 2.35E-06  | KKF10925.1        | Cytochrome P450 2K1                                   |
| HSB_liver_CDS_26126  | −4.59 | 2.40E-09  | ACN80998.1        | Ferritin heavy polypeptide                             |

Notes: Data includes gene name based on contig numbering, fold-change of expression (FC) and p-value adjusted for multiple comparisons (p-adj) along with top-hit BLAST accession number and description of the gene annotated from BLAST results. Fold-change direction and magnitude are representative of the largest change observed.
1) to the HSB transcriptome by Fisher’s Exact test, using the False Discover Rate (FDR) to assess significance (FDR < 0.05). The result of this enrichment analysis is shown in Table 2.

Assessment of both DEGs and GO terms indicated that differences in hepatic gene expression between large and small HSB after grow-out in a communal pond are linked to those genes involved in cellular and metabolic processes as well as immunity. There also appears to be a relationship of metabolic or nutritional status between HSB extremes and our DEG list and GO terms. For instance, in terms of the cellular and metabolic processes GO, the bifunctional enzyme 6PF-2K/F-2,6BPase is a key regulator of glycolysis/gluconeogenesis in the liver and is highly differentially regulated in this study (Table 1). This gene is in fact the largest by magnitude of our DEGs, displaying greater than a seven-fold up-regulation in the largest HSB (Table 1). This gene produces fructose 2,6-bisphosphate and was found in rainbow trout to be highly correlated with the nutritional status of the fish, where feeding induced expression (Panserat, Plagnes-Juan, & Kaushik, 2001). There is also evidence that hepatic 6PF-2K/F-2,6BPase is regulated by diet composition and ration size in another carnivorous fish, the gilthead sea bream, Sparus aurata (Metón, Caseras, Fernandez, & Baanante, 2000). Hepatic glucose-6-phosphatase (G6Pase), whose gene expression is hormonally and nutritionally regulated, is a positive DEG in small HSB (Table 1). Even though carnivorous finfish utilize carbohydrates differently than their mammalian counterparts (Metón, Fernández, & Baanante, 2003; Panserat et al., 2001), G6Pase was identified across the spectrum of playing an important role in providing glucose during starvation (van...
| GO ID       | GO name                  | Gene ID          | Gene description                                      | FC    |
|------------|--------------------------|------------------|-------------------------------------------------------|-------|
| GO:0006694 | Steroid biosynthetic process | HSB_liver_CDS_16126 | Sterol-4-alpha-carboxylate 3-dehydrogenase            | 2.86  |
|            | Steroid metabolic process | HSB_liver_CDS_17655 | Diphosphomevalonate decarboxylase                    | 2.76  |
|            |                          | HSB_liver_CDS_27639 | Delta(14)-sterol reductase                          | 2.55  |
|            |                          | HSB_liver_CDS_23759 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase       | 2.47  |
|            |                          | HSB_liver_CDS_26662 | Squalene synthase                                    | 2.23  |
|            |                          | HSB_liver_CDS_30999 | Cholesterol 7-alpha-monoxygenase                     | -3.62 |
| GO:0008610 | Lipid biosynthetic process | HSB_liver_CDS_26173 | Farnesyl pyrophosphate synthase                      | 3.43  |
| GO:0006629 | Lipid metabolic process   | HSB_liver_CDS_16126 | Sterol-4-alpha-carboxylate 3-dehydrogenase            | 2.86  |
|            |                          | HSB_liver_CDS_17655 | Diphosphomevalonate decarboxylase                    | 2.76  |
|            |                          | HSB_liver_CDS_17931 | Methylsterol monoxygenase 1                         | 2.74  |
|            |                          | HSB_liver_CDS_30999 | Cholesterol 7-alpha-monoxygenase                     | -3.62 |
|            |                          | HSB_liver_CDS_27639 | Delta(14)-sterol reductase                          | 2.55  |
|            |                          | HSB_liver_CDS_23759 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase       | 2.47  |
|            |                          | HSB_liver_CDS_26662 | Squalene synthase                                    | 2.23  |
|            |                          | HSB_liver_CDS_36553 | Alkylglycerol monoxygenase isoform X1                | -1.71 |
| GO:0006955 | Immune response           | HSB_liver_CDS_16126 | Sterol-4-alpha-carboxylate 3-dehydrogenase            | 2.86  |
|            |                          | HSB_liver_CDS_17655 | Diphosphomevalonate decarboxylase                    | 2.76  |
|            |                          | HSB_liver_CDS_17931 | Methylsterol monoxygenase 1                         | 2.74  |
|            |                          | HSB_liver_CDS_30999 | Cholesterol 7-alpha-monoxygenase                     | -3.62 |
|            |                          | HSB_liver_CDS_27639 | Delta(14)-sterol reductase                          | 2.55  |
|            |                          | HSB_liver_CDS_23759 | 3-hydroxy-3-methylglutaryl-coenzyme A reductase       | 2.47  |
|            |                          | HSB_liver_CDS_26662 | Squalene synthase                                    | 2.23  |

Note: Data include GO ID and name along with the underlying gene information contributing to the GO together with the gene sequence ID, top-hit BLAST description and fold-change (FC) information. Note that no molecular function (MF) GO categories were significantly enriched and cellular component (CC) GO has been omitted for brevity.
Schaftingen & Gerin, 2002). Reports in carnivorous fish showed that long-term starvation and energy restriction increase hepatic G6Pase activity (Caseras et al., 2002; Salgado, Meton, Egea, & Baanante, 2004). Similarly, He et al. (2015) found that the most significant down-regulated genes involved glucose and fatty acid metabolism in starved carp. Growth hormone receptor type 1 genes also show reduced expression in fasted carnivorous fish (Norbeck et al., 2007). As immune response GO (Table 2) was also indicated in our DEG list (Table 1), it is well-established that there is a significant interplay between nutritional status and proper immune functioning in fish [reviewed in (Martin & Krol, 2017)].

Taken together, our candidate gene list may provide some evidence that both physiological and behavioral factors may be at work with regards to HSB growth when communally reared. That is, evidence from this study may have revealed that larger HSB could also be more efficient feeders than their smallest counterparts. This is demonstrated by the fact that several DEGs in this study are similar to those that would be revealed in finfish life-cycle and/or aquaculture studies where nutrition is restricted, such as those involving no/limited access to food during migration, while undergoing a period of fasting, or during diet replacement trials (e.g. Picha et al., 2014, 2009, 2008). This notion should supplement the original hypothesis that within these domesticated populations certain HSB have a genetic predisposition to processing a manufactured diet more efficiently toward somatic growth, while all other factors are even. Thus even though diet and feeding schedule were uniform throughout this study and HSB were communally reared, we cannot rule out that the largest HSB families may have had preferential feeding behavior or advantage within the population in addition to being genetically superior feed metabolizers, which would require further exploration.

4. Conclusions
Genetic effects for growth (weight and length) were observed in HSB. Differences in hepatic gene expression between large and small HSB after grow-out in communal ponds revealed that those genes involved in cellular and metabolic processes as well as immunity signify important differences. These differences may be at least in part explained by up-regulation of two growth-related genes. The differentially regulated genes might also suggest that these physiological processes as well as variation in feeding behavior may be at play, where fish from the largest HSB families may have been more active feeders or were better competitors for feed, even as fish were fed to apparent satiation twice daily. While this study was focused on mimicking typical stocking and grow-out conditions as would take place in an aquaculture production setting, future studies that focus on feeding behavior and/or specific feed rations in a controlled environment may explain even more of the variation observed in growth differences among HSB.

List of abbreviations

| Abbreviation | Description                          |
|--------------|--------------------------------------|
| BP           | biological processes                 |
| CC           | cellular component                   |
| DEGs         | differentially expressed genes       |
| FDR          | False Discover Rate                 |
| G6Pase       | glucose-6-phosphatase                |
| GEO          | NCBI Gene Expression Omnibus         |
| GH           | growth hormone                       |
| GO           | gene ontology                        |
| HSB          | hybrid striped bass                  |
| IGF          | insulin-like growth factor           |
| MF           | molecular function                   |
| nr           | non-redundant                        |
| QC           | quality control                      |
| WB           | white bass                           |
Ethics statement
Use of animals in this study was conducted under established policies and procedures at the Harry K. Dupree Stuttgart National Aquaculture Research Center. Fish were euthanized by an overdose of tricaine methane sulphonate prior to dissections.

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Competing interests
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