水 研 機 構 研 報, 第 45 号, 89 − 100, 平 成 29 年
Bull. Jap. Fish. Res. Edu. Agen. No. 45, 89 − 100, 2017

Hybrid Striped Bass National Breeding Program: Research Towards Genetic Improvement of a Non-Model Species

S. Adam FULLER[*1], Benjamin H. BECK[*1,5], Steven D. RAWLES[*1], Bartholomew W. GREEN[*1], Chao LI[*2], Eric PEATMAN[*2], Catherine J. CHILDRESS[*1], T. Gibson GAYLORD[*3], Frederick T. BARROWS[*4], and Matthew E. McENTIRE[*1]

Abstract: The hybrid striped bass (HSB) farming industry at present relies almost totally on wild broodstock for annual production of larvae and fingerlings, and industry efforts to domesticate the parent species of the HSB (white bass: WB, *Morone chrysops*; striped bass: SB, *M. saxatilis*) have been fairly limited in scope. At the USDA-ARS HKD Stuttgart National Aquaculture Research Center (HKDSNARC), multiple areas of research are being pursued, with the end result being to provide HSB producers with a better performing line of broodfish. Among the areas of research that are currently being pursued at HKDSNARC are: 1) the development of genomic resources for WB and SB; 2) the molecular and physiological consequences of alternative production and broodstock diets on HSB and parental species; and 3) the molecular and physiological consequences of exposure to different production environments. An overview of these findings will be discussed.

Key words: *Morone*, selective improvement, transcriptome, RNA-seq, performance testing

Introduction and background

The white bass (*Morone chrysops*) and the striped bass (*M. saxatilis*) are temperate basses with high ecological, recreational, and commercial value in North America. The hybrid striped bass (HSB) was found to exhibit improved performance in captivity with regard to growth, survival, hardiness and disease resistance, presumably through hybrid vigor (heterosis) resulting from the crossing of the two parent species, white bass and striped bass (Kerby and Harrell, 1990; Harrell, 1997). Commercial production of HSB began in the early 1980s with the original HSB cross, or palmetto bass (striped bass female × white bass male), which has been mostly replaced by the more easily spawned reciprocal cross, or sunshine bass (white bass female × striped bass male) (Garber and Sullivan, 2006).

In general, the HSB commercial production cycle is composed of four distinct phases (Hodson, 1995; Harrell and Webster, 1997). Following the hatchery phase (Phase 0), 3 - 5 day-old larvae (fry) are stocked into fertilized, outdoor ponds where they feed on natural zooplankton. Approximately 30 - 45 days after stocking, the fry are recovered as Phase I fingerlings, graded, trained to feed on prepared diets and restocked at approximately 50 mm length and1g body weight. Phase I is the interval from stocking of larvae to harvest of the juvenile fingerlings, which typically extends from the spawning season in April-

2017年 2 月28日受理（Received on February 28, 2017）
*1 United States Department of Agriculture (USDA), Agricultural Research Service (ARS) - Harry K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC), 2955 Highway 130 East, Stuttgart, Arkansas, 72160-1050, U.S.A.
*2 School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL, 36849, U.S.A.
*3 U.S. Department of the Interior, Fish and Wildlife Service, Bozeman Fish Technology Center, 4050 Bridger Canyon Road, Bozeman, MT 59715, U. S. A.
*4 USDA/ARS - Small Grains and Potato Germplasm Research Unit, Hagerman Fish Culture Experiment Station, 3059F National Fish Hatchery Road, Hagerman, ID 83332, U. S. A.
*5 Present address; United States Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, Auburn, AL, 36832, U. S. A.
E-mail: adam.fuller@ars.usda.gov

May until harvest of the Phase I fingerlings. Production Phase II begins when the Phase I fingerlings are restocked into ponds for growout until late winter or early spring, typically February or March, when the fish are harvested as Phase II fingerlings, usually 90 - 225 g in body weight. Phase II fingerlings are harvested, graded and restocked into ponds for growout until they weigh 0.68 kg or more, when they are ready for harvest. At harvest size, the fish are considered to be Phase III HSB. Typically, the entire growout time from hatching to harvest is from 18 - 24 months for most HSB producers. In intensive tank culture systems, as opposed to pond systems, seasonal influences are largely irrelevant, and the HSB production phases are defined by mesh size as opposed to the length of time in or season of production. In general, at the end of each production phase, the HSB must be enumerated, measured, graded and restocked or sold to alter total densities, make feed adjustments and reduce variability in fish size.

While HSB is among the most important aquacultured species in the United States, most producers are still reliant on wild-origin broodstock, meaning they are not able to take advantage of genetic gains available from a selectively improved broodstock (Garber and Sullivan, 2006). Given the importance of HSB to American aquaculture, it is critical to continue to develop research results and genetic resources that can be incorporated into a selectively improved broodstock available to American farmers. Following is a brief synopsis of several studies conducted by my lab.

Effect of amino acid supplementation on gene expression in hybrid striped bass lean muscle

Fishmeal represents one of the largest influences on the price of feed, leading to an increase in operating expenses for producers that affects the long-term sustainability of the industry. This is because ocean supplies of fishmeal are finite and at the maximum sustainable yield, which limits market availability and results in intense competition among all animal feed industries for this commodity (Naylor *et al*., 2000; Tacon and Metian, 2008; Naylor *et al*., 2009). As research continues to explore minimizing or eliminating fishmeal in commercial aquaculture diets, many of these alternative "replacement" formulations have resulted in poorer fish performance due to an array of reasons, including limited amounts of essential amino acids (EAA) needed for optimum growth (Small and Soares, 1998; Francis *et al*., 2001; Cheng *et al*., 2003; Glencross *et al*., 2007). Muscle is the largest tissue compartment in fish, comprising ~ 60% of the total fish mass and is the desired consumer end product. Hence, it is reasonable to postulate that the muscle amino acid profile provides an ideal model for practical diet formulation and for optimizing muscle growth. In fishmeal replacement diets, three EAAs —lysine, methionine, and threonine — are typically identified as first limiting (Keembiyehetty and Gatlin, 1992; Webster, 2002; Encarnação *et al*., 2004; Gatlin *et al*., 2007).

Two growth differentiation factors that are secreted as part of the regulatory process of myogenesis are myostatin and myogenin. In cattle, myostatin has been shown to act primarily on the muscles by inhibiting myoblast proliferation and differentiation; however, when myostatin is mutated and non-functional, a double muscling effect results (Bass *et al*., 1999; Thomas *et al*., 2000; Langley *et al*., 2002). On the other hand, myogenin is required for the differentiation of myoblasts and fusion of myogenic precursor cells to existing fibers, or to create new fibers during myogenesis (Johansen and Overturf, 2005).

Previously, we conducted a growth trial to test the hypothesis that ideal protein theory accurately predicts first-limiting amino acids and optimum lysine level for a fishmeal-free, commercial-grade diet formulated for hybrid striped bass (Rawles *et al*., 2013). In that work we concluded that even though lysine was not first-limiting in our replacement test diets, lysine concentration in the muscle of hybrid striped bass accurately predicted the appropriate dietary levels (between 2.7 and 4.1 g Lys / 100g) needed for improved fish performance with respect to a number of response variables, including protein accretion and muscle ratio (Rawles *et al*., 2013). The mechanisms by which dietary amino acid supplementation affect nutrient utilization and muscling in fish is unclear, but post-prandial elevations in plasma amino acids have been shown to stimulate protein synthesis in muscle fibers (Frank *et*

al., 2006). Moreover, cell culture studies suggest that there are multiple possible levels of crosstalk in the salient signaling pathways mediated by mammalian target of rapamycin (mTOR) – (Proud, 2004; Sarbassov *et al*., 2005; Wullschleger *et al*., 2006). While a significant body of research has focused on establishing the role of amino acids in regulating protein synthesis in livestock (Suryawan and Davis, 2003, 2005; Suryawan *et al*., 2001, 2003, 2004, 2006), very little information exists for agriculturally important finfish. Therefore, our goal in this follow-up study was to elucidate how differential dietary lysine supplementation of a fishmeal-free diet influences the expression of two muscle genes, myostatin and myogenin, in HSB with critical roles in myogenesis.

A practical diet for HSB was formulated without fishmeal and supplemented with Met and Thr and varying levels of Lys to form a series of dose–response diets that were fed to triplicate tanks of juvenile HSB for 12 weeks as described in Rawles *et al*. (2013). The lysine supplementation feeding trial was executed in tanks at the Harry K. Dupree Stuttgart National Aquaculture Research Center (HKDSNARC) using juvenile HSB over an 84 - day period according to Rawles *et al*. (2013). Briefly, the test diets were fed to triplicate tanks of juvenile HSB for 12 weeks. During acclimation, fish were fed a standard commercial diet for maintaining condition with minimal growth. Fish were then pooled, individually weighed (118.4 ± 0.9 g; average initial weight ± SE) and randomly stocked (35 fish/tank) into 27 circular fiberglass tanks (0.63 m^3) supplied with flow-through well-water (24 °C; 4 L / min/ tank) and ample aeration from a regenerative blower. HSB were fed to satiation once daily and feed intake was determined as previously described (Rawles *et al*. 2013). On the last day of the trial, feed was withheld and all fish were individually weighed and separated into three size categories within each treatment diet based on final weight – small, medium, and large. Randomly selected fish from each tank were used for determination of whole body composition, nutrient retention and body composition indices that included hepatosomatic index (HSI), intraperitoneal fat (IPF) ratio, and muscle ratio (MR). Muscle tissues were isolated from matching locations on each fish and stored in RNAlater solution (Ambion, Inc, Foster City, CA, USA) at -80 °C until RNA extraction. Real Time RT-PCR methods are described in Childress *et al*. (2015).

Our goal in the current study was to examine how dietary lysine supplementation of a fishmeal-free diet influenced the interplay of two muscle genes, myostatin and myogenin, with critical roles in myogenesis in HSB and to explore the possibility of being able to differentiate growth performance based on the genetic profile of HSB in the study. Based on the criteria we used for genetic variation, i.e., small vs. large growers, we were able to statistically separate differences in myogenin, but not myostatin, expression based on size. Our work showed that with minimal lysine supplementation, myogenin expression was significantly reduced in all fish ($P = 0.010$) as well as in the small growers ($P = 0.042$) as compared to an unsupplemented diet until the "ideal protein theory level" of 3.51 g Lys / 100g diet considered by Rawles *et al*. (2013) was achieved, then myogenin expression increased. Additionally, in all fish ($P = 0.003$) as well the larger growers ($P = 0.010$), when lysine was supplemented over what was considered by Rawles *et al*. (2013) as "ideal" at 3.51 g lysine / 100 g diet, myogenin expression was significantly reduced.

The interplay of myostatin in relation to myogenin and the effect of balanced diets in HSB found in this study contributes towards the goal of achieving marker-assisted selection, which has not yet been widely applied in HSB strains, in order to improve fish performance. To a large extent, this is due to the limited availability of molecular markers for genomic analysis in HSB strains. Compared to other farm animals, including rainbow trout, HSB is still a relatively new commercially produced taxon and has not been thoroughly investigated with respect to breeds or strains with distinct genotypic or phenotypic traits. Hence, the current work contributes towards development of fish that have improved growth performance when fed fishmeal-free diets by showing that dietary limiting amino acid supplementation can influence myogenesis growth factors in HSB.

Influence of diet on white bass egg fatty acid profile

Nutrition has significant effects on ovarian growth, fecundity, and progeny robustness (Mourente and Odriozola, 1990; Harel *et al*., 1994; Mazorra *et al*., 2003). Among the dietary constituents of prepared feeds, dietary lipids and their fatty acids are critical for the overall reproductive performance of the female as well as progeny development and survival during yolk sac resorption (Fernandez-Palacios *et al*., 1995, 1997; Navas *et al*., 1997; Bruce *et al*., 1999; Mazorra *et al*., 2003). Although some organisms can synthesize EPA and DHA de novo to satisfy their EFA requirements, many fish lack adequate enzymatic function to produce these fatty acids at a rate sufficient to meet nutrient requirements (Lane and Kohler, 2007). In the case of broodstock, the requirement for dietary EFAs is most likely greatest from a previtellogenic period to ovulation. Therefore, EPA and DHA are often supplemented in diets of broodstock for normal growth and development of progeny (March, 1993; Sargent *et al*., 1995).

Feeding live food sources has proven to be an effective strategy to satisfy the EFA requirements of broodfish. However, an effective formulated feed for broodfish would reduce production costs, simplify feed management, and eliminate a potential pathogen source in culture facilities. These feeds may potentially outperform live foods (nutritionally), ensuring the supply and continuity of viable progeny to the aquaculture industry. Further knowledge of nutritional factors responsible for reproductive success is needed to develop a broodstock diet and improve fingerling production for aquaculture producers. Therefore, the objective of this study was to evaluate the reproductive performance of white bass broodstock fed one of six commercial diets and evaluate the relative fatty acid compositions of their eggs.

Female white bass were held in earthen ponds during the winter and were seined the first week of April when water temperatures reached 15 degrees Celsius. Ninety white bass were evenly stocked into six 600 L fiberglass tanks. The temperature in all six tanks was maintained at 16 degrees Celsius with a water chiller. Lids were placed over the tanks and artificial lights and a timer were used to mimic natural photoperiod. All six tanks of fish were given three weeks to acclimate and were fed Cargill AquaFeed 48-18 (protein-lipid) during this time. Fish were weighed on April 22 to determine initial weight and each tank was then randomly assigned a diet. All tanks were fed to satiation once daily. Each tank was fed its specific diet for 30 days and five fish were randomly selected for samples and weighed on day 31. The remaining fish were fed an additional 28 days and five fish were randomly selected for samples and weighed on day 60. The six diets used included: Zeigler Bass Brood 45-15, Bio-Oregon BioBrood 48-20, Cargill AquaFeed 45-12, Cargill AquaFeed 48-18, Skretting Extruded Steelhead 45-16, Skretting Classic Brood 46-12.

Step-wise discriminant analysis (SDA) was conducted on the fatty acid profiles of eggs from white bass fed six different commercial diets using the SAS 9.3 program STEPDISC (SAS Institute, Cary, North Carolina). Overall, time had an influence in fatty acid content in white bass eggs, with profile of fatty acids changing from the 4 week sampling to the 8 week sampling. Overall, diets grouped by manufacturer regardless of protein or lipid level. A few of the heavy hitters (known to be important in larvae) were different among these diets, including DHA, and EPA. Our next step is to get larval performance data for comparison. We anticipate full results to be completed and published soon.

Genomic consequences of rearing hybrid striped bass under hypoxic conditions

Hypoxia is a state of oxygen deficiency that is sufficient to cause impairment of organismal function or in extreme cases, death. Hypoxia is becoming an increasingly important environmental concern (Diaz 2001; Wu *et al*., 2003) and has received extensive attention in the fisheries sciences, particularly aquaculture. Understanding the tissue-specific and temporal changes in gene expression in fishes exposed to hypoxia could reveal new mechanisms of hypoxia tolerance and shed light on this adaptive response in vertebrates. In settings of aquaculture, the intensity and duration of hypoxia depends on a variety of factors including fish biomass, feeding rate, phytoplankton blooms, and installed aeration capacity (Green *et al*., 2015). Previously, we characterized the effects of hypoxia on performance metrics of hybrid

striped bass and found that hypoxia led to reduced feed intake, which resulted in lower nutrient retention and growth (Green *et al*., 2015). However, the precise molecular mechanisms contributing to these disparate phenotypes were unknown. However, recent advancement in genomic resources for striped bass (Li *et al*., 2014; Reading *et al*., 2012) and white bass (Li *et al*., 2014) offer researchers the ability to take a closer look directly at many *Morone* functional pathways that were previously studied via model species. Given the importance of hypoxia on the management and captive rearing of *Morone*, we examine here the transcriptional responses of hybrid striped bass to acute and chronic hypoxia. We highlight unique and shared signatures between hypoxic treatments and the bioenergetic consequences of chronic oxygen deprivation on hepatocellular function. Our findings offer a more comprehensive view of the cellular and molecular consequences of hypoxia and reveal new mechanisms of hypoxia tolerance in teleosts.

The fish used in this study were cohorts of those subjected to hypoxia as previously described by Green *et al*. (2015). Fish were held at DO25 and DO100 levels for 90 d after which nine fish from each treatment were sacrificed and liver samples were collected for RNA-seq. Remaining fish from each tank were removed from their respective treatments using a dip net and immediately placed in the opposite DO saturation DO25 → DO100 (restoration of normoxia) and DO100→DO25 (acute hypoxia) for 6 h, after which, nine fish per treatment were sacrificed and livers collected for RNA-seq. For RNA-seq methods, see Beck *et al*. (2016).

Differential expression analysis was performed between 100% DO saturation (DO100 or normoxia) and 25% DO saturation groups (DO25 or chronic hypoxia), between DO100 and DO100 → DO25 (acute hypoxia), and between DO25 and DO25 → DO100 groups. A total of 1403 unique genes (based on assigned identifies from the NR database) showed significant differential expression in liver (Fig. 1). In detail, there were 91 differentially expressed genes between DO100 and DO25 saturation groups, a meager 39 genes differently expressed between DO25 saturation and DO25 → DO100 saturation groups, and the greatest degree of differential

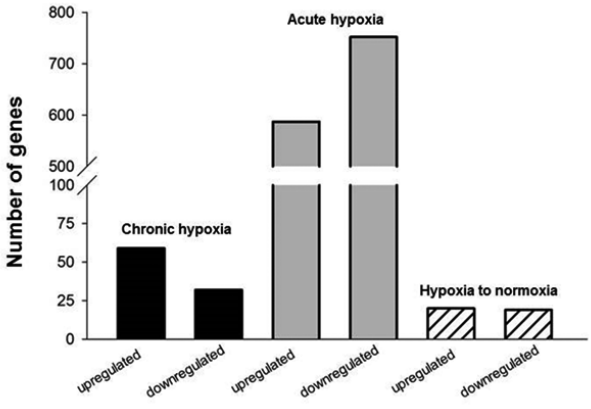

Fig. 1. The number of genes that were statistically differentially expressed under each condition and the direction of their expression. Chronic hypoxia (25% oxygen saturation for 90 days) resulted in transcriptional changes to 91 genes (59 upregulated, 32 downregulated); acute hypoxia (25% oxygen saturation for 6 h) altered 1339 genes (587 upregulated, 752 downregulated), and the movement of fish from the chronic hypoxia treatment to normoxia treatment (100% oxygen saturation) changed 39 genes (20 upregulated, 19 downregulated).

expression was observed in DO100 and DO100 → DO25 saturation comparison, with 587 up-regulated genes and 752 down-regulated genes (total 1339 genes). Extracellular flux analysis of liver cells obtained from DO100 and DO25 fish revealed significant differences in oxygen consumption rates (OCR), with DO25 liver possessing around 25% higher OCR (Fig. 2A). Mitotracker Red staining of liver cell suspensions from DO25 and DO100 fish showed that DO25 liver cells showed heightened fluorescence levels, indicative of a greater mitochondrial content (Fig. 2B).

Next-generation sequencing platforms have propelled rates of discovery, particularly in non-model species where few genomic and transcriptomic resources are available. Work by our group showed previously the phenotypic impacts of chronic hypoxia on hybrid striped bass feeding behavior and growth traits (Green *et al*., 2015). Here, we delve further into this area to examine the molecular consequences of oxygen deprivation in an effort to reveal the molecular underpinnings responsible for the overall poorer performance under hypoxemia.

Previously (using the same experimental cohorts of

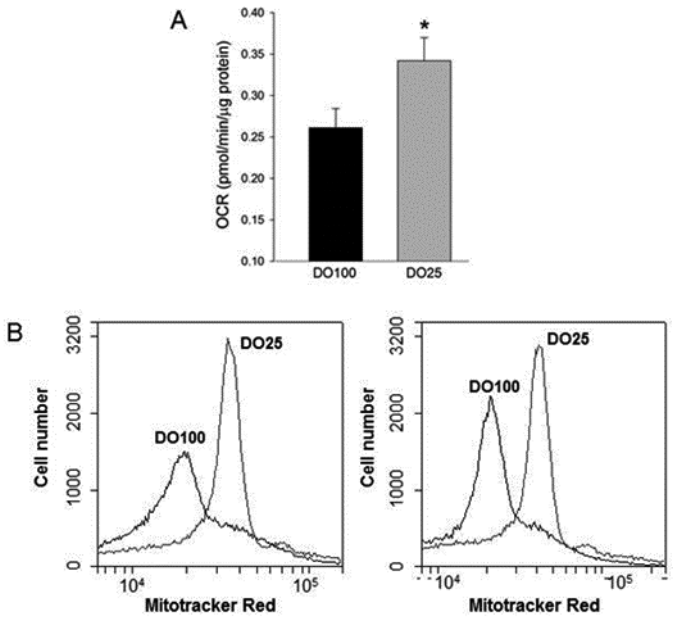

Fig. 2. A) Liver tissue isolated from fish subjected to chronic hypoxia show greater oxygen consumption rates (OCR; mean ± SEM; five fish per treatment) and B) increased mitochondrial content (two representative fish from each treatment) as indicated by the fluorescence intensity derived from staining with the mitochondrion-selective dye Mitotracker Red.

the fish employed here), we observed significantly larger livers (as indicated by the hepatosomatic index) in HSB reared in normoxia versus hypoxia and reported significantly higher levels of whole body lipid (Green *et al*., 2015). Qualitatively, the enlarged livers were noticeably pale and soft in texture, while fish exposed to hypoxia exhibited livers that were darkened in appearance, firm, and friable (Green *et al*., 2015). These observations led us to focus on examining the liver transcriptome in the present study. Indeed, the liver plays a central role in metabolic homeostasis and is a major site for the synthesis, metabolism, storage and redistribution of processed carbohydrates, proteins and lipids (Bechmann *et al*., 2012). In tissues such as the heart and liver, lipids provide a rich source of energy via oxidative phosphorylation by mitochondria (Jungermann, 1988; Shohet and Garcia, 2007). In mammals, where hypoxic stress is typically viewed as a pathological state (e.g., fatty liver disease, ischemia), lipid metabolism under low oxygen conditions is reprogrammed to suppress mitochondrial oxidation of lipids as a protective measure against toxic metabolites and oxidative stress. Broadly, hypoxia promotes lipid storage and inhibits lipid catabolism through β oxidation (Whitmer *et al*., 1978; Bostrom *et al*., 2006). In contrast, in aquatic animals, environmental hypoxia is a common challenge that many aquatic organisms experience in their habitat and responding to hypoxia requires metabolic reprogramming so that energy-demanding processes are regulated to match available energy reserves (Gracey *et al*., 2011). Here, we observed changes — with similarities and differences to work in mammals and other teleosts — in multiple genes involved in lipid catabolism and β oxidation, particularly following acute hypoxia. Enrichment analysis pointed us towards a deeper examination of cell death processes. Two pathways related to cellular preservation, apoptosis and autophagy, were clearly influenced by hypoxic insult. In taking together the gross differences in liver size, the alterations in lipid utilization, the suppression of apoptosis combined with the induction of autophagy, our findings suggest that the hepatic tissue of hybrid striped bass may have entered a state of senescence. This may have allowed the animals to liberate, redirect, or shunt resources to other vital tissues to survive hypoxia.

The use of RNA-seq allows for the examination of global transcriptional changes in a tissue at a particular snapshot in time. However, it is widely understood that the level of transcriptional message does not always correlate with protein. With little to no resources (i.e., antibodies) available for HSB, we utilized extracellular flux analysis to quantify aerobic respiration in liver tissue obtained from DO100 and DO25 fish. Unexpectedly, under conditions of reduced oxygen availability, liver cells from DO25 fish showed significant increases in basal oxygen consumption rates, a surrogate measure of mitochondrial function. Curiously, as compared to our previous work with established fish cell lines (Beck and Fuller, 2012), total

aerobic respiration was lower and glycolysis was virtually non-detectable. By using a fluorophore that selectively stains mitochondria, we documented an increase in the mass (linked to number/size of mitochondria) of the mitochondrial compartment, which could have accounted for the increase in oxygen consumption by DO25 liver cells. An increase in mitochondrial number, a process termed mitochondrial biogenesis, is a common cellular response to hypoxia and is thought to improve the efficiency of oxygen consumption and ATP synthesis, and in parallel counteract potential cellular damage brought about by hypoxia (Kopp *et al*., 2014). Consistent with these observations is a greater than 400 fold increase in PGC-1β message in liver tissue from the acute hypoxia treatment. In contrast to mammals, where PGC-1*α* is regarded as the archetypal master regulator of mitochondrial biogenesis, recent evidence from teleosts suggests that PGC-1β may exert more control over mitochondrial gene expression (LeMoine *et al*., 2008). Intriguingly, and related to the above discussion on lipid metabolism, PGC-1β is also known to play a key regulatory role in hepatic lipid metabolism with PGC-1β knockout mice being more susceptible to hepatic steatosis (Sonoda *et al*., 2007) and the transduction of rodent liver with PGC-1β decreased hepatic lipid while plasma triglyceride and cholesterol levels were significantly elevated (Lin *et al*., 2005). Clearly, further research is needed to better understand the interactions between mitochondrial abundance, regulation, and lipid metabolism in hypoxic teleosts.

Development of moronid genomic resources

Genetic information has been restricted to a single tissue (ovary) transcriptome and a microsatellite linkage map from striped bass (Reading *et al*., 2012; Liu *et al*., 2012), limiting gene discovery and expression and functional studies in the two species and their hybrid. Here we set out to produce well-annotated transcriptomes for both species to advance future broad-based RNA-seq studies of gene expression as well as aid in more targeted studies of important genes and pathways.

Major tissues and organs (brain, liver, spleen, trunk kidney, ovary, testes, gill, and intestine) from 10 individuals from white bass and 10 individuals from striped bass were harvested, and equal amounts of tissue from each system were pooled prior to RNA extraction. The result was two master pools of RNA, one for each species. Each pool was used for library construction and sequencing in a lane of Illumina HiSeq2000 platform. A total of 262 x 10^6 total high quality reads were obtained with 135 x 10^6 reads from striped bass and 127 x 10^6 reads from white bass. Using the TRINITY de novo assembly software (Grabherr *et al*., 2011), reads were assembled into 203,587 striped bass unique contigs and 185,531 white bass unique contigs. N50 and average contig sizes were 2915 and 1263 bp respectively for striped bass, and 3132 and 1371 bp respectively for white bass (Table 1). These included 166,867 and 185,351 transcripts that were identified for the first time in striped bass and white bass, respectively. Annotation was carried out by BLAST against the UniProt and NR (NCBI non-redundant) databases for both species. At an E-value ≤ 1e-5, 21,186 and 29,624, unigene matches were obtained against the UniProt and NR databases respectively in striped bass, and 21,001 and 28,906 matches were returned in white bass against the same databases. Of these NR matches, 25,902 (87.4%) in striped bass and 25,484 (88.2%) in white bass were predicted to have full-length transcript coverage based on TRINITY analysis. Using more stringent criteria, similar results were obtained from both species, with 18,630 UniProt and 23,605 NR annotated unigenes in striped bass and 18,584 UniProt and 22,354 NR annotated unigenes in white bass (score ≥ 100, E-value ≤ 1e-20; Table 2).

In both species most valuably from a management standpoint, the transcriptomes yielded microsatellite and SNP markers valuable in future downstream

Table 1. Summary of de novo assembly results of Illumina RNA-seq data from striped bass and white bass using Trinity assembler

	Striped Bass	White Bass
Contigs	203,587	185,531
Largest contig (bp)	21,100	28,262
Large contigs (≥1000bp)	68,395	66,891
Large contigs (≥500bp)	98,864	94,485
N50 (bp)	2,915	3,132
Average contig length (bp)	1,263	1,371

Table 2. Summary of gene identification and annotation of assembled Striped bass and White bass contigs based on BLAST homology searches against various protein databases (UniProt and nr). Putative gene matches were at E-value ≤ 1e-5. Hypothetical gene matches denote those BLAST hits with uninformative annotation. Quality unigene hits denote more stringent parameters, including score ≥ 100, E-value ≤ 1e-20.

	Striped bass		White bass	
	UniProt	nr	UniProt	nr
Contigs with putative gene matches	69,134	79,062	68,312	76,884
Annotated contigs ≥1000bp	54,487	58,316	54,430	57,839
Annotated contigs ≥500bp	62,602	68,876	62,158	67,682
Unigene matches	21,186	29,624	21,001	28,906
Hypothetical gene matches	0	1,901	0	1,858
Quality Unigene matches	18,630	23,605	18,584	22,354

analyses. In striped bass, from a total of 32,111 microsatellites identified by MSATFINDER (Thurston and Field, 2005), 36.05% (n = 11,577) had sufficient flanking regions to allow design of primers. These 11,577 microsatellites were distributed across 10,055 contigs. Similarly, in white bass, from a total of 30,408 microsatellites, 34.53% (n = 10,500) had sufficient flanking regions to allow design of primers. These 10,500 microsatellites were distributed across 9054 contigs. A SNP analysis comparing between species yielded 2220 markers polymorphic in one species but not the other, including 1661 SNPs associated with genes. Additionally, in the future, the reference transcriptomes will serve as an important sequence anchor for short-read genotyping studies using techniques such as RAD-seq or genotyping by sequencing (GBS) (Davey *et al*., 2011).

The TRINITY-based assembly of the white bass and striped bass transcriptomes generated high-quality, gene-length transcripts, which will be of great utility in future expression and functional studies in moronid species. Microsatellite and SNP markers identified at the same time are expected to aid in aquaculture, conservation, and sportfish genetic management and improvement.

References

Bass J., Oldham J., Sharma M., and Kambadur R., 1999: Growth factors controlling muscle development. *Dom. Anim. Endocrin.* **17**, 191-197.

Bechmann L. P., Hannivoort R. A., Gerken G., Hotamisligil G. S., Trauner M., and A. Canbay A., 2012: The interaction of hepatic lipid and glucose metabolism in liver diseases. *J. Hepatol.* **56**, 952–964.

Beck B. H. and Fuller S. A., 2012: The impact of mitochondrial and thermal stress on the bioenergetics and reserve respiratory capacity of fish cell lines. *J. Aquat. Anim. Health* **24**, 244–250.

Bostrom P., Magnusson B., Svensson P., Wiklund O., Boren J., Carlsson L. M. S., Stahlman M., Olofsson S., and Hulten L. M., 2006: Hypoxia converts human macrophages into triglyceride-loaded foam cells. Arterioscler. Thromb. Vasc. *Biol.* **26**, 1871–1876.

Cheng Z. J., Hardy R. W., and Usry J. L., 2003: Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. *Aquaculture* **215**, 255-265.

Davey J. W., Hohenlohe P. A., Etter P. D., Boone J. Q., Catchen J. M., and Blaxter M. L., 2011: Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.* **12**, 499–510.

Diaz R. J., 2001: Overview of hypoxia around the world. *J. Environ. Qual.* **30**, 275-281.

Encarnação P., de Lange C., Rodehutscord M., Hoehler D., Bureau W., and Bureau D. P., 2004: Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. *Aquaculture* **235**, 569-586.

Fernandez-Palacios H., Izquierdo M. S., Robaina L.,

Valencia A., Salhi M., and Montero D., 1997: The effect of dietary protein and lipid from squid and fish meals on egg quality of broodstock for gilthead seabream (*Sparus aurata*). *Aquaculture* **148**, 233–246.

Fernandez-Palacios H., Izquierdo M. S., Robaina L., Valencia A., Salhi M., and Vergara J., 1995: Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead seabream (*Sparus aurata* L.). *Aquaculture* 132, 325–337.

Francis G., Makkar H. P. S., and Becker K., 2001: Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* **199**, 197-227.

Frank J. W., Escobar J., Suryawan A., Nguyen H. V., Kimball S. R., Jefferson L. S., and Davis T. A., 2006: Dietary protein and lactose increase translation initiation factor activation and tissue protein synthesis in neonatal pigs. *Am. J. Physiol-Endoc. M.* **290**, E225-E233.

Garber A. F. and Sullivan C. V., 2006: Selective breeding for the hybrid striped bass (*Morone chrysops,* Rafinesque ×*M. saxatilis,* Walbaum) industry: status and perspectives. *Aquacult. Res.* **37**, 319–338.

Gatlin D. M., Barrows F. T., Brown P., Dabrowski K., Gaylord T. G., Hardy R. W., Herman E., Hu G., Krogdahl A., Nelson R., Overturf K., Rust M., Sealey W., Skonberg D., Souza E. J., Stone D., Wilson R., and Wurtele E., 2007: Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.* **38**, 551-579.

Glencross B. D., Booth M., and Allan G. L., 2007: A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquacult. Nutr.* **13**, 17-34.

Grabherr M. G., Haas B. J., Yassour M., Levin J. Z., Thompson D. A., Amit I., Adiconis X., Fan L., Raychowdhury R., Zeng Q., Chen Z., Mauceli E., Hacohen N., Gnirke A., Rhind N., di Palma F., Birren B. W., Nusbaum C., Lindblad-Toh K., Friedman N., and Regev A., 2011: Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* **29**, 644–652.

Gracey A. Y., Lee T.-H, Higashi R. M., and Fan T., 2011: Hypoxia-induced mobilization of stored triglycerides in the euryoxic goby Gillichthys mirabilis. *J. Exp. Biol.* **214**, 3005–3012.

Harel M., Tandler A., Kissil G. W., and Applebaum S., 1994: The kinetics of nutrient incorporation into body tissues of gilthead sea bream, *Sparus aurata*, females and subsequent effects on egg composition and egg quality. *Br. J. Nutr.* **72**, 45–58.

Harrell R. M., 1997: Hybridization and genetics. Chapter 8. In: *Striped Bass and Other Morone Culture* (ed. by Harrell R. M.), Elsevier Science Press, Amsterdam, the Netherlands, pp.217-234.

Harrell R. M. and D. Webster D. W., 1997: An overview of *Morone* culture. Chapter1. In: *Striped Bass and Other Morone Culture* (ed. by Harrell R. M.), Elsevier Science Press, Amsterdam, the Netherlands, pp. 217-234.

Hodson R. G., 1995: Farming a New Fish: Hybrid Striped Bass. North Carolina Sea Grant Publication, UNC-SG-95-10, Raleigh, NC, 41pp,

Johansen K. and Overturf K., 2005: Quantitative expression analysis of genes affecting muscle growth during development of rainbow trout (*Oncorhynchus mykiss*). *Mar. Biotechnol.* **7**, 576-587.

Keembiyehetty C. N. and Gatlin III D. M., 1992: Dietary lysine requirement of juvenile hybrid striped bass (*Morone chrysops* X *M. saxatilis*). *Aquaculture* 104, 271-277.

Kerby J. H. and Harrell R. M., 1990: Hybridization, genetic manipulation, and gene pool conservation of striped bass. Chapter 11. In: *Culture and Propagation of Striped Bass and its Hybrids* (eds. by Harrell R. M., Kerby J. H., and Minton R. V.), Striped Bass Committee, Southern Division, American Fisheries Society, Bethesda, MD, USA, pp.159-190.

Kopp R., Bauer I., Ramalingam A., Egg M., and Schwerte T., 2014: Prolonged hypoxia increases survival even in zebrafish (*Danio rerio*) showing cardiac arrhythmia. *PLoS One* **9 (2)**.

Langley B., Thomas M., Bishop A., Sharma M., Gilmour S., and Kambadur R., 2002: Myostatin inhibits myoblast differentiation by down-regulating MyoD expression. *J. Biol. Chem.* **277**, 49831-49840.

LeMoine C. M., Genge C. E., and Moyes C. D., 2008:

Role of the PGC-1 family in the metabolic adaptation of goldfish to diet and temperature. *J. Exp. Biol.* **211**, 1448–1455.

Li C., Beck B. H., Fuller S. A., and Peatman E., 2014: Transcriptome annotation and SNP discovery in white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*). *Anim. Genet.* **45**, 885-887.

Lin J., Yang R., Tarr P. T., Wu P.-H., Handschin C., Li S., Yang W., Pei L., Uldry M., Tontonoz P., Newgard C. B., and Spiegelman B. M., 2005: Hyperlipidemic effects ofdietary saturated fats mediated through PGC-1β coactivation of SREBP. *Cell* **120**, 261–273.

Liu S., Rexroad III C. E., Couch C. R., Cordes J. F., Reece K. S., and Sullivan C. V., 2012: A microsatellite linkage map of striped bass (*Morone saxatilis*) reveals conserved synteny with the threespined stickleback (*Gasterosteus aculeatus*). *Mar. Biotechnol.* **14**, 237–44.

March B. E., 1993: Essential fatty acids in fish physiology. *Can. J. Physiol. Pharmacol.* **71**, 684–689.

Mazorra C., Bruce M., Bell J. G., Davie A., Alorend E., Jordan N., Rees J., Papanikos N., Porter M., and Bromage N., 2003: Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture* 227, 21–33.

Mourente G. and Odriozola J. M., 1990: Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). *Fish Physiol. Biochem.* **8**, 93–101.

Naylor R. L., Goldburg R. J., Primavera J. H., Kautsky N., Beveridge M. C., Clay J., Folke C., Lubchenco J., Mooney H., and Troell M., 2000: Effect of aquaculture on world fish supplies. *Nature* **405**, 1017-1024.

Naylor R. L., Hardy R. W., Bureau D. P., Chiu A., Elliott M., Farrell A. P., Forster I., Gatlin D. M., Goldburg R. J., Hua K., and Nichols P. D., 2009: Feeding aquaculture in an era of finite resources. *P. Natl. Acad. Sci. USA*. **106**, 15103-15110.

Proud C. G., 2004: The multifaceted role of mTOR in cellular stress responses. *DNA Repair* **3**, 8–9, 927-934.

Rawles S. D., Fuller S. A., Beck B. H., Gaylord T. G., Barrows F. T., and McEntire M. E., 2013: Lysine optimization of a commercial fishmeal-free diet for hybrid striped bass (*Morone chrysops* X *M. saxatilis*). *Aquaculture* **396–399**, 89-101.

Reading B. J., Chapman R. W., Schaff J. E., Scholl E. H., Opperman C. H., and Sullivan C. V., 2012: An ovary transcriptome for all maturational stages of the striped bass (*Morone saxatilis*), a highly advanced perciform fish. *BMC Res. Notes* **5**, 111.

Sarbassov D. D., Ali S. M., and Sabatini D. M., 2005: Growing roles for the mTOR pathway. *Curr. Opin. Cell. Biol.* **17**, 596-603.

Sargent J. R., 1995: Origin and functions of egg lipids: nutritional implications. In *Broodstock management and egg and larval quality* (edds. By Bromage N. R. and Roberts J. R.), Blackwell Scientific Publications, London, pp.353–372

Small B. C. and Soares Jr. J. H., 1998: Estimating the quantitative essential amino acid requirements of striped bass *Morone saxatilis*, using fillet A/E ratios. *Aquacult. Nutr.* **4**, 225-232.

Sonoda J., Mehl I. R., Chong L.-W., Nofsinger R. R., and Evans R. M., 2007: PGC-1β controls mitochondrial metabolism to modulate circadian activity, adaptive thermogenesis, and hepatic steatosis. *Proc. Natl. Acad. Sci.* **104**, 5223–5228.

Suryawan A., Nguyen H. V., Bush J. A., and Davis T. A., 2001: Developmental changes in the feeding-induced activation of the insulin-signaling pathway in neonatal pigs. *Am. J. Physiol-Endoc. M.* **281**, e908-915.

Suryawan A., Nguyen H. V., Orellana R. A., Bush J. A., and Davis T. A., 2003: Insulin/insulin-like growth factor-I hybrid receptor abundance decreases with development in suckling pigs. *J. Nutr.* **133**, 2783-2787.

Suryawan A., O'Connor P. M., Kimball S. R., Bush J. A., Nguyen H. V., Jefferson L. S., and Davis T. A., 2004: Amino acids do not alter the insulin-induced activation of the insulin signaling pathway in neonatal pigs. *J. Nutr.* **134**, 24-30.

Suryawan A., Escobar J., Frank J. W., Nguyen H. V., and Davis T. A., 2006: Developmental regulation of the activation of signaling components leading to translation initiation in skeletal muscle of neonatal pigs. *Am. J. Physiol-Endoc. M.* **291**,

e849-859.

Suryawan A. and Davis T. A., 2003: Protein-tyrosine-phosphatase 1B activation is regulated developmentally in muscle of neonatal pigs. *Am. J. Physiol-Endoc. M.* **284**, e47-54.

Suryawan A. and Davis T. A., 2005: Developmental regulation of protein kinase B activation is isoform specific in skeletal muscle of neonatal pigs. *Pediatr. Res.* **58**, 719-24.

Tacon A. G. J. and Metian M., 2008: Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* **285**, 146-158.

Thomas M., Langley B., Berry C., Sharma M., Kirk S., Bass J., and Kambadur R., 2000: Myostatin, a negative regulator of muscle growth, functions by inhibiting myoblast proliferation. *J. Biol. Chem.* **275**, 40235-40243.

Thurston M. I. and Field D. 2005: Msatfinder: detection and characterisation of microsatellites. Distributed by the authors at http://www.genomics.ceh.ac.uk/msatfinder/. CEH Oxford, Mansfield Road, Oxford OX1 3SR.

Webster C. D., 2002: Hybrid striped bass. In: Nutrient Requirements and Feeding of Finfish for Aquaculture(eds.by Webster,C. D. and Lim C. E.), CABI Publishing, New York, NY, USA, pp. 327-343.

Whitmer J. T., Idell-Wenger J. A., Rovetto M. J., and Neely J. R., 1978: Control of fatty acid metabolism in ischemic and hypoxic hearts. *J. Biol. Chem.* **253**, 4305–4309.

Wu R.-S., Zhou B.-S., Randall D. J., Woo N.-Y., and Lam P.-K., 2003: Aquatic hypoxia is an disrupter and impairs fish reproduction. *Environ. Sci. Technol.* **37**, 1137–1141.

Wullschleger S., Loewith R., and Hall M. N., 2006: TOR signaling in growth and metabolism. *Cell* **124**, 471-484.

Annotated bibliography

(1) Li C, Beck B. H., Fuller S. A. , and Peatman E., 2014: Transcriptome annotation and SNP discovery in white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*). *Anim. Genet.* **45**, 885-887.

The authors present the first ever multi-tissue reference transcriptomes for striped bass (*Morone saxatilis*) and white bass (*Morone chrysops*) which are the parental species of the hybrid striped bass, a major U.S. aquaculture species. Being non-model species, this was of critical importance, as prior to this there only existed a medium-density genetic linkage map and a well-annotated ovarian transcriptome. The assembled Moronid reference transcriptomes and identified simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) should advance ongoing studies of reproduction, physiology, and immunology in these species and provide markers for broodstock management and selection.

(2) Childress C. J., Fuller S. A., Rawles S. D., Beck B. H., Gaylord T. G., Barrows F. T., and McEntire M. E., 2015: Lysine supplementation of commercial fishmeal-free diet in hybrid striped bass *Morone chrysops* x *M. saxatilis* affects expression of growth-related genes. *Aquac. Nutr.* DOI: 10.1111/anu.12300

The authors present a follow-up study to a study (Rawles *et al*., 2013) where ideal protein theory accurately predicted first-limiting amino acids and optimum lysine level for a fishmeal-free, commercial-grade diet for hybrid striped bass (HSB). In the current study, authors sought to determine how dietary lysine supplementation of these same diets influences the expression of two genes, myostatin and myogenin, controlling myogenesis in differentially growing groups of HSB. Real-time rt-PCR results in HSB suggest that the levels of lysine added to the diet has an impact on myogenin relative to the unsupplemented diet, but no effect on myostatin. Moreover, presented data also suggests that the amount of dietary lysine supplementation influenced the ratio of myostatin/myogenin expression in HSB and that this pattern mimicked that of most of the growth, composition of growth and nutrient retention data from the authors' previous study and may therefore be a useful marker for selecting fish for improved growth performance.

(3) Fuller S. A., Farmer B. D. and Beck B. H., 2014: White bass *Morone chrysops* is less susceptible than its hybrid to experimental infection with *Flavobacterium columnare*. *Dis. Aquat. Org.* **109**, 15-

22.

The authors present research regarding *Flavobacterium columnare*, the causative agent of columnaris disease, susceptibility differences between hybrid striped bass (HSB) and white bass (WB) in a series of 3 fundamental studies. In the first experiment, the authors sought to determine whether columnaris disease could be developed using a low-water flow experimental challenge in HSB using 3 levels of *F. columnare* (60-, 30-, 10- ml). Each of these treatment groups exhibited significantly different survival rates: 0, 3.3, and 13.3%, with higher survival occurring in treatment groups exposed to less bacteria. In the second experiment (30 ml), both HSB and WB had a 0% survival rate, but the WB took significantly longer to reach 100% mortality. Finally in Expt 3 (10 ml), no HSB survived, whereas 33% of WB survived. Compared to controls, the authors observed extensive gill damage in HSB treated with 10 ml after 24 h, which they hypothesized could have contributed to the higher mortality observed in HSB; an observation not seen on the WB gills. From these series of experiments, it is clear that HSB are more sensitive to *F. columnare*, having lower survival and more extensive histological damage compared to WB following the bacterial challenge.

(4) Beck B. H., Fuller S. A., Peatman E., McEntire M. E., Darwish A. M., and Freeman D.W., 2012: Chronic exogenous kisspeptin administration accelerates gonadal development in basses of the genus Morone. *Comp. Biochem. Physiol. A Physiol.* **162**, 265-273.

The authors present the effects of chronic administration of kisspeptins to immature and mature white bass (WB), striped bass (SB), and hybrid striped bass (HSB) to determine its effects on gonadal development in these species. The authors determined that bi-weekly injections (over 7 weeks) differentially accelerate puberty, as evidenced by increases in the prevalence of spermatozoa in the testes of juvenile fish. Also, in sexually mature fish, kisspeptin treatment led to increased gonad weight, gonadosomatic index, and spermatocrit in some white and striped bass. Additionally, mature white bass treated with kisspeptins showed an advancement in oocyte development as determined by histological examination. Importantly, the gonadal changes occurred in the absence of any photothermal manipulation or hormone injections. This description was the first report of kisspeptin-mediated pubertal initiation in fish, and the first evidence that kisspeptins could modulate gonad maturation.