Transcriptome of the Atlantic halibut (*Hippoglossus hippoglossus*)

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The Atlantic halibut (*Hippoglossus hippoglossus*) is a commercially important species, which due to historic overfishing and a high value is being developed as an aquaculture species. However there are currently issues in the efficient and successful supply of healthy juveniles for aquaculture production due to difficulties particularly in the first feeding stages and abnormal development during metamorphosis. Examples of such developmental problems include abnormal pigmentation (albinism, ambicoloration or mosaicism), failed migration of the left eye and skeletal deformities (reviewed in Power et al. 2008). Although the Atlantic halibut has been the subject of several traditional EST projects (Bai et al. 2007; Douglas et al. 2007) and more recently Next Generation analyses into microRNAs (Bizuayehu et al. 2012, 2013), there is still a deficit with regard to the number of transcripts in the databases, which can be accessed and exploited for targeted candidate gene and pathway studies. In an effort to increase the genomic resources and underpin future molecular investigations into this species, we have generated a transcriptome drawing on RNA from the head, skin and gastrointestinal (GI-) tract using 454 pyrosequencing.

Atlantic halibut larvae were obtained from the aquaculture company Fiskeldi Eyjafjarðar Ltd. (Iceland) in December 2009. Larvae were reared in full-sea water using standard commercial procedures and normal metamorphosis was observed (Einarsdottir et al., 2005). In brief, fertilised eggs from several spawning batches were hatched in an open system of egg incubators. Yolk sack larvae were transferred to silo-shaped (10 m^3) through-flow systems in complete darkness at 5°C until absorption of the yolk sack when they were moved to 100 l, round polyethylene start-feeding tanks containing sea-water at 10-11°C under constant light conditions. The larvae were fed live artemia (Olsen et al., 1999) twice daily. Dead larvae were siphoned from the tanks each day and the mortality in each tank registered. The larvae were euthanized with a lethal dose of MS-222 (50 mg.l^{-1}, ethyl 3-aminobenzoate methanesulfonate salt, Sigma-Aldrich, St. Louis, USA). Photographs were taken of each larvae and developmental staging was performed using mytome height and standard length (defined in Sæle et al., 2004) and then stored individually in RNAlater (Life Technologies, Carlsbad, USA) at −20ºC. All handling procedures followed European guidelines (86/609/EU). Larvae were dissected into head, GI-tract and skin at standard development stages before, during and after metamorphic climax (n=6 per stage). Total RNA was extracted from all tissue/stages using a Maxwell®16 System (Promega, Madison, USA) and following the manufacturer's instructions. Total RNA integrity was verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) and only the samples with RIN values equal to, or above 8 were used.

cDNA libraries were prepared and sequenced at the Max Planck Genome Centre (Cologne, Germany), using 5 μg of total RNA obtained from a pool of 6 samples for each tissue/stage. First, the whole transcriptome was enriched by depletion of the ribosomal RNA (rRNA, 28S, 18S, 5.8S and 5S) using a RiboMinusTM Eukaryote Kit (Life Technologies, Carlsbad, USA) following the manufacturer's instructions. Total RNA (after rRNA depletion), was used to construct sixteen cDNA libraries (head from stage 5; head, skin and GI-tract from stages 7, 8, and 9, Sæle et al., 2004; stage 9 samples were split into 3 groups, 9A, 9B and 9C to differentiate by eye position) using a cDNA Rapid Library Preparation Kit (Roche 454 Life Sciences, Branford, USA) following the manufacturer's instructions. Each library had a unique barcode and was amplified by emulsion PCR and sequenced on the GS-FLX platform (Roche 454 Life Sciences, Branford, USA). 6,091,832 raw sequence reads (.sff format) from the sixteen libraries were extracted and quality clipped with the additional removal of sequencing adapters, primers and also poly-A tails. Only sequences above 100bp were retained for assembly (Table 1). The resulting reads were then screened against all available *Artemia* species in NCBI (38,287 sequences at 04.2012) to remove food source contamination and also *H. hippoglossus* mitochondrial DNA (27 sequences at 04.2012) using BLASTn (settings: score > 100; e-value <1e-25) and contaminating sequences removed. The remaining reads were used in the Newbler (www.454.com) assembly, using default parameters. 36% of the reads were assembled into the contigs, with, as expected, read density increasing with contig length (Figure 1), the remaining were either repeats, singletons, outliers or too short after being trimmed in Newbler. 22,272 contigs of 500 base pairs or greater, with a median length of 937 were assembled (Figure2), with an annotation rate of 85% against the NCBI nr database at an e-value threshold of 1e-10 using Blast sequence similarity searching.

The present molecular resources were generated for a critical production stage that underpins the sustainability of the aquaculture industry. The resource should be of interest for Atlantic halibut producers and for fish stock management of the endangered wild fish. From a research perspective the molecular dataset can be used to understand the molecular changes accompanying flatfish metamorphosis.

Data deposition

The sequences for Atlantic halibut obtained in this study are available at the NCBI Short Read Archive (Accession number: SRP044664) and the consensus sequences of the contigs is available at http://ramadda.nerc-bas.ac.uk/repository in the folder: NERC-BAS datasets/ Genomics/ Transcriptomes/ Hippoglossus_hippoglossus.

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Figure Legends

Figure 1: Log_{10} plot of reads to contig lengths

Figure 2: Distribution of the contig lengths of those contigs up to 4000bp. 225 contigs bigger than 4,000bp are not shown.

Table Legend

Table 1: Contribution of each library to the final transcriptome assembly.

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