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UNDERSTANDING THE TRANSCRIPTIONAL CHANGES ASSOCIATED WITH ONSET OF MATURATION IN ATLANTIC SALMON

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SUMMARY

Sexual maturation of Atlantic salmon, *Salmo salar*, is a complicated process that involves many variables that can act to activate and/or inhibit sexual development. Unwanted early onset of sexual maturation of Atlantic salmon is a challenge for the salmon aquaculture industry, as it has negative impacts on growth rate and product quality. Consequently, there has been a significant amount of research aiming to understand the biological mechanisms driving early salmon maturation. We present the description of a proposed animal trial, coupled with RNA-Seq based transcriptomics, designed to elucidate the earliest triggers which commit animals to sexual maturation. Our approach has two major components. First, animals will be photoperiod manipulated to artificially narrow the time window during which maturation is initiated. Tissue samples will be collected before, during and after the initiation event. The second component involves next generation sequencing to obtain detailed gene expression profiles. We will target the brain, pituitary and gonad tissues as the brain-pituitary-gonad (BPG) axis is central to regulating sexual maturation. We anticipate our approach has the potential to both identify the genes involved, and open new approaches to control the timing of maturation in this important production species.

INTRODUCTION

Sexual maturation is the process by which organisms become mature and are capable of reproducing. In Atlantic salmon, the development of sexual maturation is complex, with extreme variability in age and size at maturation (Good and Davidson 2015). Moreover, the variability in timing of maturation is considered a significant problem to Atlantic salmon aquaculture, specifically Atlantic salmon that mature at an early age are more susceptible to opportunistic microbes (St-Hilaire *et al*. 1998), exhibit decreased feed conversion efficiency and lower than normal growth rate (McClure *et al*. 2007), and have reduced product quality (Aksnes *et al.* 1986). In salmon industry, photoperiod management is the general practice to control animal maturation. The brain-pituitary-gonad (BPG) axis is a key regulator of sexual development in vertebrates. Activation of neurons in the hypothalamus leads to production of gonadotropin releasing hormones (GnRH), which stimulate the release of gonadotropins such as follicle stimulating hormone (FSH) and luteinising hormone (LH) from the pituitary gland. In the gonads, gonadotropins induce the production of gonadal steroids (e.g., testosterone, estrogen and progesterone), which in turn affect various aspects of sex-related physiology, secondary sexual characteristics and behaviour. Consequently, analysing transcriptomic changes in the BPG axis during the early stages of sexual maturation in Atlantic salmon could identify differentially expressed genes and gene co-expression networks operating to control the process.

It is possible maturation is inhibited during the juvenile life stages until specific physiological/biochemical thresholds are attained. The thresholds include, for example, levels of adipose tissue (Rowe *et al.* 1991) and energy reserves (Kadri *et al.* 1996), which provide information about the optimal fitness and triggers a developmental switch towards maturation. These thresholds are influenced by environmental factors (Taranger *et al.* 2010) of which photoperiod is considered an essential determinant for initiating sexual maturation in teleosts including Atlantic salmon

(Bromage *et al*. 2001). Photoperiod effects facilitate optimal timing of conditions that favour growth and survival of young animals. Moreover, those physiological thresholds are genetically determined to some extent. For example, Barson *et al*. (2015) identified a single locus in the Atlantic salmon genome that is associated with age at maturity through a genome wide association study. The causal gene is likely to be the vestigial-like family member 3 gene (*VGLL3*), which has a role in adiposity, however its precise role is yet to be determined.

The mechanisms underlying the onset of maturation are not understood in Atlantic salmon. This is primarily because it is difficult to sample animals as they commit to the maturation pathway. This project describes an animal experiment designed to identify the genes, gene expression differences and gene networks driving initiation of sexual maturation in Atlantic salmon.

MATERIALS AND METHODS

Experimental design. In order to maximise the probability of sampling animals during the earliest stages of the maturation process, well before the appearance of the phenotypic changes associated with maturing fish, the decision window for animals to initiate maturation should be as short as possible. Consequently animals will be managed via photoperiod manipulation to synchronise the timing of commitment into maturation. We will study a population of female broodstock that will be approximately 36 months post fertilization in April 2017 (~3.1 to 3.4 yrs at sampling). The proposed management of the animals and associated timeline is given in Fig.1.

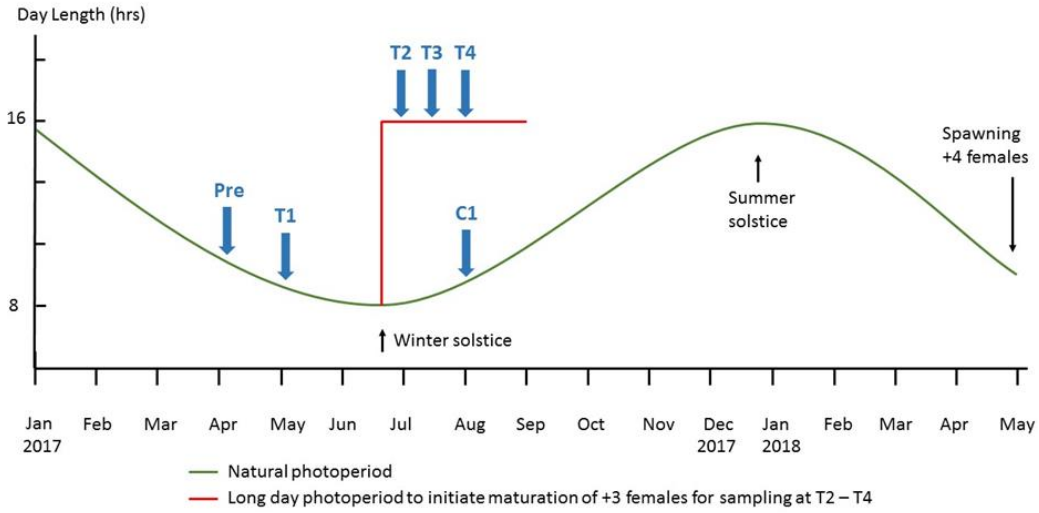

Figure 1. Induction of maturation through photoperiod manipulation and suggested time points for tissue sampling and RNA isolation.

Sampling. Activation of the brain-pituitary-gonad axis is central to reproductive development and prioritises the three target tissues for examination in the experiment. An expanded set of tissues (liver, spleen and muscle) may be harvested at sampling, however these three are the focus for RNA-Seq data generation. In order to measure and control for variation between individuals, we propose to sample 4 fish at each of the T1 – T4 and the C1 time point (total of 20 fish). This will enable variation within tissues and time points to be evaluated. The maturation status of animals (leading up to the long day photoperiod initiation) is currently being monitored by ultrasound. Ultrasound data and update on the T1 samples will be presented at the conference.

Transcriptomic data generation and high-throughput sequencing. A total of 60 RNA libraries will be generated arising from 5 (time points) x 3 (tissues; brain, pituitary, ovary) x 4 (biological replicates). RNA-Seq libraries will be prepared using the Illumina TruSeq RNA Sample Preparation Kit. RNA-Seq libraries will be sequenced on four lanes on an Illumina HiSeq platform. Sequencing should produce (at least) 25 million individual 100-bp paired-end reads per library. RNA-Seq data will be processed and analysed for differential expression in response to the onset of maturation Fig.2.

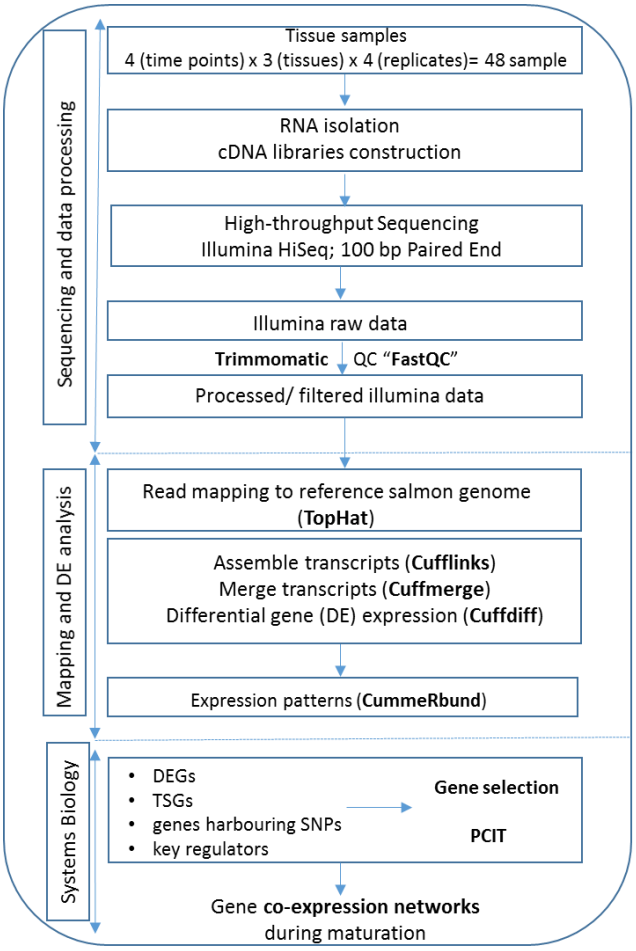

Figure 2. Flow chart of the pipeline for RNA-Seq (transcriptomic) data generation, identification of differentially expressed genes and subsequent gene co-expression networks analyses.

RNA-Seq data processing and differential expression analysis. Illumina raw reads will be checked using FastQC, a quality control tool for NGS data. Illumina universal and indexed adapters will be removed and data will be filtered based on quality using Trimmomatic software (Bolger *et al.* 2014). Illumina reads will be analysed according to the Tuxedo protocol (Trapnell *et al.* 2012). Briefly, the processed Illumina reads will be mapped separately against the salmon reference genome (Lien *et al.* 2016) using TopHat, a gapped/ spliced mapper, in order to generate alignment

(accepted_hits.bam) files. Then the Cufflinks suite will be used for differential expression. First transcripts will be assembled and quantified using cufflinks, then transcripts will be merged into a single transcriptome using cuffmerge and differential expression will be calculated using cuffdiff. The R package CummeRbund will be then used to explore the gene expression data and create volcano plots and heatmaps to visualise the differential expression. The list of differentially expressed genes (DEGs) will be analysed using GO and KEGG databases for pathway enrichment among the gene lists.

Gene co-expression network analysis. Gene co-expression networks will be analysed as described in (Canovas *et al.* 2014). Briefly, in addition to the list of DEGs, tissue-specific genes (TSGs), genes harbouring SNPs reported to be associated with maturation traits and key regulators such as transcription factors (TF) will be used to generate and analyse gene co-expression networks. The DEGs, TSGs, key TF and SNP harbouring genes will be used as nodes and significant connections will be identified using the partial correlation and information theory (PCIT) algorithm (Watson-Haigh *et al.* 2010) in the R environment. The PCIT ascertains the correlation between genes and network nodes after taking into account all other genes present in the dataset. The PCIT output will be viewed with Cytoscape, a software for analysis and visualisation of gene co-expression networks (Shannon *et al.* 2003). The highly interconnected gene clusters and significantly overrepresented Gene Ontology terms will be identified. Those clusters may be of biological significance to maturation in Atlantic salmon. The analysis flowchart is summarized in Fig.2.

CONCLUSIONS

Execution of the proposed experiment will generate a tissue collection and a large transcriptomic dataset that has not yet been obtained by the research community. The project is focused to investigate the biological mechanisms driving the onset of sexual development, with a view to developing novel approaches to assist management of unwanted early maturation within the Atlantic salmon industry.

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