Multi-Tissue Transcriptome Profiling of North American Derived Atlantic Salmon

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The availability of a reference genome assembly for Atlantic salmon, Salmo salar, SNP genotyping platforms and low cost sequencing are enhancing the understanding of both life history and production-related traits in this important commercial species. We collected and analyzed transcriptomes from selected tissues of Atlantic salmon to inform future functional and comparative genomics studies. Messenger RNA (mRNA) was isolated from pituitary gland, brain, ovary, and liver before Illumina sequencing produced a total of 640 million 150-bp paired-end reads. Following read mapping, feature counting, and normalization, cluster analysis identified genes highly expressed in a tissue-specific manner. We identified a cluster of 508 tissue specific genes for pituitary gland, 3395 for brain, 2939 for ovary, and 539 for liver. Functional profiling identified gene clusters describing the unique functions of each tissue. Moreover, highly-expressed transcription factors (TFs) present in each tissue-specific gene cluster were identified. TFs belonging to homeobox and bhlh families were identified for pituitary gland, pou and zf-c2h2 families for brain, arid, and zf-c2h2 for ovary and rxr-like family for liver. The data and analysis presented are relevant to the emerging Functional Annotation of All Salmonid Genomes (FAASG) initiative that is seeking to develop a detailed understanding of both salmonid evolution and the genomic elements that drive gene expression and regulation.

Keywords: Atlantic salmon, RNA-Seq, transcriptome, gene expression, transcription factors, next-gen sequencing

INTRODUCTION

The Atlantic salmon (Salmo salar), a member of the family Salmonidae, is endemic to the northern Atlantic Ocean and cultivated worldwide including in Tasmania, Australia. The Australian salmon industry is founded on introduced North American wild stock originating from the River Philip in Nova Scotia (Jungalwalla, 1991). To enhance productivity using genetics, an applied breeding program has been in operation since 2004, and the Tasmanian salmon industry is now the highest valued commercial fishery-related industry in the state (Australian Bureau of Agricultural Resource Economics and Sciences, 2014). A number of production issues remain which are potentially tractable using genomic approaches, including unwanted early maturation, disease susceptibility, product quality traits, and imperfect DNA based diagnostics for sex determination.

The ability to elucidate the basis of complex salmonid traits has been dramatically improved by the availability of reference genomes for both rainbow trout (Oncorhynchus mykiss) (Berthelot et al., 2014) and Atlantic salmon (Lien et al., 2016). To date, their major impact has been through...
enabling the development and application of high density SNP genotyping platforms and GWAS. The availability of annotated protein coding gene models supports the interpretation of GWAS to identify putatively causal variants contributing to trait variation (Ayllon et al., 2015; Barson et al., 2015). However, these reference genomes currently lack annotation of the sequences that regulate gene expression, including proximal, and distal promoters and enhancer elements. Functional annotation requires significant time and cost, and the animal science community has self-assembled into consortia to tackle the task for livestock genomes (FAANG consortium, Andersson et al., 2015) and salmonids inside the “Functional Annotation of All Salmonid Genomes” (FAASG, Macqueen et al., 2017). The experimental component involves collection of multiple data types to support functional annotation, including histone modification marks, DNA methylation status and gene expression. RNA-Seq has revolutionized transcriptomic research, previously based on testing expression of candidate genes using RT-qPCR or even thousands of genes using cDNA microarrays. RNA-Seq provides an efficient and unbiased tool for largescale gene expression analyses in non-model organisms (for example Mohamed et al., 2016, 2018) as well as in salmonids (Salem et al., 2015; Kim et al., 2016; Robinson et al., 2017).

Additional background information about available genomic tools for salmonid research, in the broader context of aquaculture genomics, is captured in a recent review (Abdelrahman et al., 2017).

The objective of this study was to begin contributions to FAASG by transcriptomic profiling selected tissues. We prioritized the brain-pituitary-gonad (BPG) axis, which plays a critical role in the development and regulation of the reproductive and immune systems in vertebrates including teleosts (Weltzien et al., 2004; Cowan et al., 2017). Fluctuations in this axis regulate hormone production, which exert local and systemic effects on the animal relating to sexual development, maturation, and other traits. The liver is an essential metabolic organ as it regulates most chemical levels in the blood, detoxifies various metabolites, synthesizes proteins, and produces energy.

**FIGURE 1 |** DEGs hierarchical clustering. Heat map showing genes (rows) with differential expression (fold ≥ 4, FDR ≤ 0.001) among the four replicates of the pituitary gland, brain, ovary, and liver samples. Expression values are log₂-transformed and median-centered by gene.
Hence, analyzing the complement of expressed genes within and across these four tissues will provide a basis for understanding key production and life history traits.

**MATERIALS AND METHODS**

**Ethics Statement**

The animals used are from the SALTAS selective breeding program which has been described previously (Dominik et al., 2010; Eisbrenner et al., 2014; Kijas et al., 2017). The four animals used were sexually immature 3 year old female fish from the 2014 year class. The fish were reared and euthanized in compliance with the CSIRO Animal Ethics Committee, under approval number 2017–02.

**Tissue Collection and Total RNA Isolation**

Samples from mid-brain, pituitary gland, ovary and liver were collected and stored in RNA-later at −80°C until RNA isolation. Total RNA was isolated from each tissue using RNeasy Kit (QIAGEN), eluted in 40 µL RNase-Free Water and stored at −80°C. RNA quantity were assessed using a Nanodrop™ ND-1000 spectrophotometer (Thermo Scientific), and integrity checked by electrophoretic profiling with Agilent Bioanalyzer 2100 (Agilent, CA).

**High-Throughput mRNA Sequencing (Illumina RNA-Seq)**

Messenger RNA (mRNA) was isolated from 500 ng total RNA and a total of 16 RNA-Seq libraries (4 tissues × 4 biological replicates per tissue) were prepared using the Illumina TruSeq stranded library Preparation Kit. Libraries were sequenced on an Illumina NovaSeq 6,000 sequencing platform producing 640.3 million individual 150-bp paired-end reads.

**Read Mapping, Differential Gene Expression, and Clustering Analyses**

Illumina reads were checked for quality using FastQC software. High quality reads (Q > 30) were mapped to Atlantic salmon genome ICSASG_v2 (Lien et al., 2016) using TopHat2 version 2.1.1 (Kim et al., 2009) with default parameters. Alignment BAM files were sorted by read name and converted into SAM files using SAMtools version 1.4 (Li et al., 2009). The Python package HTSeq version 0.7.2 (Anders et al., 2015) was applied to count unique reads mapped to exons, which is suitable for RNA-Seq analysis. Raw counts were analyzed using the edgeR package (Robinson et al., 2010) in the R statistical computing environment to infer differential gene expression among tissues. To identify differential expression between samples and tissues, we applied both fold-change and false discovery rate (FDR) thresholds. Specifically, we set FDR to ≤0.001 and log2 (fold change) ≥2 to declare significance in line with previous recommendations (Haas et al., 2013). It is important to note a log2 (fold change) > 2 equates with a minimum 4-fold change. To compare multiple expression profiles, we used the value of log2 (FPKM+1) centered by subtracting the mean across profiles (Haas et al., 2013), i.e. the mean-centered log2 (FPKM+1) value in which the FPKM+1 circumvents the non-definable instances of log2(0) if FPKM = 0. Tissue-specific gene clusters were identified as those exhibiting high expression in one tissue versus all others.

**Gene Ontology (GO) Enrichment Analysis of the Tissue-Specific Gene Clusters**

To infer function for identified tissue-specific clusters, GO enrichment analyses were performed using the Database For Annotation, Visualization And Integrated Discovery (DAVID) (Huang et al., 2009). The ENTREZ_GENE_ID of the gene clusters was used as identifier and Salmo salar was selected as the background dataset for the enrichment analysis. DAVID uses Fisher’s exact test to ascertain statistically significant enrichment of pathways amongst differentially expressed genes (DEGs) relative to the background transcriptome. For the purpose of the enrichment analysis, GO categories with a Benjamini-corrected $P \leq 0.05$ were considered significant (Huang et al., 2009). The RNA-Seq reads used in this study have been submitted to the NCBI Gene Expression Omnibus (GEO) database under Accession no. GSE114247.

**Identification of Highly Expressed Transcription Factors (TFs)**

In order to determine transcription factors (TFs) in the tissue-specific gene clusters in Salmon, BLASTX analysis were performed (E-value < 10−5) against the Cod annotated transcription factors database downloaded from the AnimalTFDB database (http://bioinfo.life.hust.edu.cn/AnimalTFDB/#/tf_summary?species=Gadus_morhua) for Gadus morhua. The retrieved transcription factors were ranked based on their expression in each of the tissue-specific clusters.
RESULTS

Expression Within and Between Four Selected Tissues

Transcriptomic data was collected from 16 RNA-Seq libraries constructed in a 4 (tissues) × 4 (replicates) experimental design. Sequencing yielded a total of 640 M raw reads, which after mapping to the Atlantic salmon reference assembly ICSASG_v2 yielded an average of 28 M reads per library (Table S1). To begin assessment between libraries, multidimensional scaling (based on the top 500 genes that best differentiate the samples) was performed (Figure S1). This confirmed biological replicates within tissue clustered together, and libraries from different tissues took non-overlapping positions. We performed hierarchical clustering using Spearman correlations of pairwise normalized expression for all 16 samples to explore the relative amount of variation between replicates within a tissue compared to that between the four tissues. This revealed that brain displayed the highest variation between the four biological replicates (animals) while the lowest was observed for ovary tissue. Further, clustering grouped the brain and pituitary as the two tissues with the most similar patterns of gene expression (Figure S2). Together these assessments indicated gene expression variability was much lower between replicates of the same tissue as compared with variation between tissues, as expected for a high quality dataset.

Gene Clusters With Tissue Specific Expression

Genes exhibiting significant differential expression (DEG, ≥4-fold change with false discovery rate FDR ≤ 0.001) were identified and used to search for gene clusters exhibiting tissue specific expression. Hierarchical clustering using only significant DEGs revealed distinctive expression profiles for each tissue (Figure 1). Brain appeared to have the highest occurrence of tissue specific expression (3395 genes) followed by ovary (2939), liver (539), and the pituitary gland (508) (Figure 2). Apart from 17542 genes that were shared among the four tissues, the highest number of shared genes were 8819 between pituitary gland and brain followed by 5547 between brain and ovary, and interestingly 1839 among pituitary gland, brain and ovary (Figure 2). This can be expected given the important role of the brain-pituitary-gonad (BPG) axis in regulating sexual development in vertebrates including fish.
TABLE 1 | Gene Ontology (GO) categories enriched (corrected P ≤ 0.05) among tissue-specific gene clusters.

| Tissue | Category | GO term ID | GO term description                     | Gene count | Fold enrichment |
|--------|----------|------------|-----------------------------------------|------------|----------------|
| Brain  | GO_CC    | GO:0005576 | Extracellular region                     | 21         | 3.56           |
|        | GO_MF    | GO:0005509 | Calcium ion binding                      | 17         | 2.86           |
| Pituitary | GO_BP    | GO:0051023 | Regulation of Immunoglobulin secretion   | 3          | 149.66         |
|        |          | GO:0050766 | Positive regulation of phagocytosis      | 3          | 112.25         |
|        |          | GO:0032930 | Positive regulation of superoxide anion generation | 3          | 74.83         |
|        | GO_CC    | GO:0005576 | Extracellular region                     | 11         | 7.18           |
|        | GO_MF    | GO:0043565 | Sequence-specific DNA binding            | 7          | 4.13           |
| Ovary  | GO_CC    | GO:0005730 | Nucleolus                                | 9          | 5.39           |
|        | GO_MF    | GO:0016502 | Integral component of membrane           | 6          | 9.05           |
| Liver  | GO_BP    | GO:0006889 | Lipid transport                          | 4          | 51.31          |
|        | GO_CC    | GO:0042157 | Lipoprotein metabolic process            | 4          | 44.9           |
|        | GO_MF    | GO:0055576 | Extracellular region                     | 11         | 6.47           |
|        | GO_MF    | GO:0005615 | Extracellular space                      | 6          | 7.03           |
|        | GO_MF    | GO:0008289 | Lipid binding                            | 6          | 11.11          |

Significant enrichment for biological process (GO_BP), cellular component (GO_CC) and molecular function (GO_MF) are indicated along with their term descriptors. The identity of the genes driving each enrichment are provided in the Supplementary material.

The relative expression of the identified tissue specific clusters is compared across tissues in Figure 3. We sought to explore if tissue specific gene clusters were enriched for gene ontologies (GO) relating to molecular function (GO-MF), cellular component (GO-CC), or biological process (GO-BP). Pituitary gland-specific genes contained significant enrichment for all three gene ontology classes (GO-BP, GO-CC, GO-MF) terms at corrected P ≤ 0.05, Table 1. Despite being comprised of only three genes (growth hormone pre-peptide, prolactin, somatotropin-2), the most highly over-represented term (149 fold) was regulation of Immunoglobulin secretion that includes genes coding for growth hormones, prolactin, and somatotropin. The GO-CC extracellular region was identified via pituitary gland hormones such as somatotactin, thyroid stimulating hormone, pro-opiomelanocortin, and gonadotropin which is consistent with the expected gene expression profile of the pituitary (Table 1, Table S3). The list of highly expressed genes in the pituitary gland included genes with function related to hormone activity such as somatotactin beta, pro-opiomelanocortin A1 and B (Figure 4; Table S2). The list of highly expressed transcription factors (TFs) identified in the pituitary gland (Table 2) included lhx3, six1b, other homeodomain proteins, ascl1a, bhila15, zf-c2h2, and zbtb. Brain-specific genes were enriched for the GO-CC extracellular region term due to expression of neuropeptides including proenkephalin, myostatins, cholecystokinin, and thyrotropin-releasing hormone (Table 1, Table S4). Further, GO-MF term calcium ion binding was enriched based on expressed neuronal calcium-sensor/binding proteins such as visinin-like proteins, calretinin-like proteins and calmodulins (Table 1; Table S4). The list of highly expressed genes in the brain (Figure 4; Table S2) included Vesicular glutamate transporter 2.1 (gene ID= gene48716:106587477) and several genes coding for neuropeptides such as Glucagon family neuropeptides-like (gene ID= gene39762:106578701). The list of highly expressed TFs identified in the brain included zic2a, zic5, pou3f2a, pou3f3b, esrrb (Table 2).

Ovary-specific genes were enriched for two GO-CC terms nucleolus and integral component of membrane (Table S5) and one GO-MF term isomerase activity (Table 1). The drivers of enrichment included hormone receptors such as follicle stimulating hormone receptor, growth hormone receptor, transmembrane proteins members of solute carrier family and 3-oxo-5-alpha-steroid 4-dehydrogenase. The list of highly expressed genes in the ovary is shown in Figure 4 and Table S2. The list of highly expressed TFs identified in the ovary (Table 2) included e2f8, fos, kdm5ba, arid3b, two Homeodomain proteins (lhx1a and mkxa) and two zinc finger proteins (snail1b and si:dekey-208k4.2).

The final tissue investigated was the liver, which had enriched GO terms in all three categories that included lipid transport, lipoprotein metabolic process, extracellular region, extracellular space, and lipid binding (Table 1, Table S6). The lipid transport term was the most highly over-represented (51-fold) based on genes coding for lipid transport-proteins (apolipoproteins). Other notable genes expressed primarily in the liver included coagulation factor, insulin-like growth factor-binding protein, peptide hormones, and genes with immune-related functions. The list of highly expressed genes in the liver (Figure 4; Table S2) included serum albumins (gene ID= gene1886:100136575, gene38403:100136922), eggshell protein (gene ID= gene22108:100136930), and saxtoxins and tetrodotoxin-binding protein 1-like (gene ID= gene18707:106611802).
FIGURE 4 | Top 10 differential expressed gene expression (DEGs). Heat map and dendrogram of the 10 most highly expressed genes in each tissue-specific cluster. The red-blue spectrum represents the scaled expression values.

DISCUSSION

Identifying the genes expressed in cells of a given tissue represents important baseline information essential for understanding tissue function and physiology more broadly. This study cataloged the collection of expressed genes in four salmon tissues, and documented significant changes that characterized tissues from each other. We identified the most abundant transcripts and the collection of genes and transcription factors (TFs) exhibiting tissue specific expression, and our findings were in broad agreement with expectation about the specific role of each organ. It is worthwhile noting that tissue specificity was considered only within the context of the tissues tested, and that examination of a more diverse collection of tissues may alter these findings. Nonetheless, the profile of each transcriptome captured GO enrichment terms consistent with expectation, and provided confidence that the dataset is of both high quality and useful for subsequent analysis. For example the enrichment of the GO term regulation of immunoglobulin secretion and extracellular region derived from the presence of genes coding for pituitary hormones. Similarly in the ovary the enrichment of the GO term integral component of membrane derived from the presence of many hormone receptors such as follicle stimulating hormone receptor and growth hormone receptor which aligns with functions of the ovary. The identified signals with high expression reflect the physiological function of each tissue. For example, the high expression of the pituitary hormone somatolactin beta and pro-opiomelanocortin (pomc) was observed in the pituitary gland. Similarly the pomc gene is expressed in both the anterior and intermediate lobes of the pituitary gland. The same is true for other tissues as expression of genes encoding neuropeptides in the brain and genes encoding liver proteins such as albumin and eggshell proteins in the liver.

The dataset adds to the increasing volume of RNA-Seq information being made available by the research community. The majority of existing Atlantic salmon RNA-Seq datasets have been generated to address specific biological questions, including host response to disease challenge (Dettleff et al., 2017; Valenzuela-Muñoz et al., 2017; Rozas-Serri et al., 2018), developmental progression (Wang et al., 2014) or nutritional requirements (Jin et al., 2018). The consequence is a paucity of transcriptomic profiling of animals not subject to an experimental regime designed to elicit specific responses or that characterize a biological extreme. The survey sequencing performed here can therefore be considered baseline data, making it applicable to general studies seeking to understand normal state expression levels across tissues. Importantly, when co-analyzed with the other datatypes identifying gene regulatory elements inside FAASG, we anticipate utility for at least three experimental applications. Firstly, for interpretation of GWAS to prioritize candidate genes on the basis of their tissue specific expression in relation to the biology of the trait under investigation. Secondly, it will assist assigning putative function to salmonid specific genes without annotation using...
### TABLE 2 | Transcription factors highly expressed (top 10 ranked) in each tissue-specific gene clusters along with their expression.

| Salmon gene ID | ENSB ID          | Gene symbol | Family        | Description                                      | Pituitary   | Brain    | Ovary     | Liver     |
|----------------|------------------|-------------|---------------|--------------------------------------------------|-------------|----------|-----------|-----------|
| gene296567     | 106568496        | z-f-c2h2    | Zinc Finger Protein 532 | 7.076638 | -1.18989 | -1.75861 | -3.4991  |
| gene18311      | 106690474        | bhh         | Achaete-scute homolog 1 | 6.812102 | -1.07506 | -2.32828 | -3.40876 |
| gene15527      | 106608860        | lim3        | Homeodomain protein | 6.511932 | -1.68275 | -1.85147 | -2.99771 |
| gene24086      | 106583263        | inox3       | LIM homeodomain 3 | 6.489042 | -2.28645 | -0.13718 | -0.0677  |
| gene38047      | 106576936        | bhh         | Achaete-scute homolog 1 | 6.357436 | -1.26608 | -0.20003 | -4.89135 |
| gene37839      | 106576285        | bhh         | Basic Helix-Loop-Helix Family Member A15 | 6.204624 | -0.17073 | -2.43396 | -3.59994 |
| gene5125       | 106589580        | bhh         | Homeodomain protein | 6.181782 | -1.24124 | -2.72763 | -2.12911 |
| gene1631       | 106610965        | homeobox    | 6.188265 | 0.592351 | -2.62659 | -4.14304 |
| gene32739      | 106671735        | homeobox    | 6.054175 | 0.013972 | -2.49383 | -3.57431 |
| gene598       | 106598867        | homeobox    | 5.780814 | 0.274847 | -2.0676 | -3.98906 |
| gene14966      | 106608144        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene48129      | 106568884        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene14733      | 106607949        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene42796      | 106581638        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene47289      | 106586041        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene34340      | 106573496        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene35702      | 106574614        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene17894      | 10661051         | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene4377      | 106584047        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene11670      | 106605033        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene50315      | 106588731        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene31170      | 106569971        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene45968      | 106584708        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene22786      | 106561922        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene76560      | 106566562        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene14139      | 106607399        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene269784     | 106588797        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene86444      | 106575359        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene32174      | 106571028        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene11227      | 106604581        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene20791      | 106613765        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene48589      | 106587347        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene22207      | 106561422        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene6072       | 106599177        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene22802      | 106561958        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene31376      | 106570251        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene48566      | 106587325        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene52087      | 106590549        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene33755      | 106572609        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |
| gene27953      | 106566843        | homeobox    | 4.910814 | 0.274847 | -2.0676 | -3.98906 |

The red-blue spectrum represents the scaled mean expression values.
the “guilt by association” principal. Specifically, the function of uncharacterized genes can be deduced from their tissue specific co-expression with genes of known function. Finally, we anticipate the data to contribute to characterization of transcriptomic complexity that includes expression of transcript isoforms and temporal variation in expression that can only be addressed by extensive tissue sampling across multiple life history time points.

**AUTHOR CONTRIBUTIONS**

AM, HK, JK, and BE conceived the study and contributed to the experimental design. HK and BE performed animal management and AM and JK contributed to sample acquisition. AM generated and analyzed the RNA-Seq data. TR contributed to the analysis. AM and JK drafted the manuscript which all authors contributed to.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2018.00369/full#supplementary-material

**REFERENCES**

Abdelrahman, H., ElHady, M., Alcivar-Warren, A., Allen, S., Al-Tobasei, R., and Bao, L. (2017). Aquaculture genomics, genetics and breeding in the United States: current status, challenges, and priorities for future research. *BMC Genomics* 18:191. doi: 10.1186/s12864-017-3557-1

Anders, S., Pyl, P. T., and Huber, W. (2015). HTseq - a Python framework to work with high-throughput sequencing data. *Bioinformatics* 31, 166–169. doi: 10.1093/bioinformatics/btu638

Andersson, L., Archibald, A. L., Bottema, C. D., Brauning, R., Burgess, S. C., Burt, D. W., et al. (2015). Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. *Genome Biol.* 16:57. doi: 10.1186/s13059-015-0622-4

Australian Bureau of Agricultural Resources Economics (2014). *Australian Fisheries Statistics*. Canberra: ABARES.

Ayllon, F., Kjærnær-Semb, E., Furmanek, T., Wennevik, V., Solberg, M. F., Dahle, G., et al. (2015). The vgfl3 locus controls age at maturity in wild and domesticated Atlantic salmon (*Salmo salar*) males. *PLoS Genet.* 11:e1005628. doi: 10.1371/journal.pgen.1005628

Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., et al. (2015). Sex dependent dominance at a single locus maintains variation in age at maturity in salmon. *Nature* 528, 405–408. doi: 10.1038/nature16062

Berthelot, C., Brunet, F., Chalopin, D., Juanchich, A., Bernard, M., Noil, B., et al. (2014). The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. *Nat. Commun.* 5:5657. doi: 10.1038/ncomms5657

Cowan, M., Arpeleta, C., and Lópex-Olmeda, J. F. (2017). Rhythms in the endocrine system of fish: a review. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 187, 1057–1089. doi: 10.1007/s00360-017-1094-5

Dettlaff, P., Moen, T., Santi, N., and Martinez, V. (2017). Transcriptomic analysis of spleen infected with infectious salmon anemia virus reveals distinct pattern of viral replication on resistant and susceptible Atlantic salmon (*Salmo salar*). *Fish Shellfish Immunol.* 61, 187–193. doi: 10.1016/j.fsi.2017.01.005

Dominik, S., Henshall, J., Kube, P., King, H., Lien, S., Kent, M., et al. (2010). Evaluation of an Atlantic salmon SNP chip as a genomic tool for the swimming performance and selection on gene expression in Atlantic salmon. *BMC Genomics* 11:e191. doi: 10.1186/1471-2164-11-191

Eisbremer, W. D., Botwright, N., Cook, M., Davidson, E. A., Dominik, S., Elliott, N. G., et al. (2014). Evidence for multiple sex-determining loci in Tasmanian Atlantic salmon (*Salmo salar*). *Heredity* 113, 86–92. doi: 10.1038/hdy.2013.55

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., et al. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat. Protoc.* 8, 1494–1512. doi: 10.1038/nprot.2013.084

Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4, 44–57. doi: 10.1038/nprot.2008.211

Jin, Y., Olsen, R. E., Østensen, M. A., Gillard, G. B., Korsvoll, S. A., Santi, N., et al. (2018). Transcriptional development of phospholipid and lipoprotein metabolism in different intestinal regions of Atlantic salmon (*Salmo salar*) fry. *BMC Genomics* 19:253. doi: 10.1186/s12864-018-4651-8

Jungwaltalla, P. J. (1991). The development of an integrated saltwater salmonid farming industry in Tasmania, Australia. *Can. Tech. Rep. Fish. Aquat. Sci.* 1831, 65–73.

Kijas, J., Elliot, N., Kube, P., Evans, B., Botwright, N., and King, H. (2017). Diversity and linkage disequilibrium in farmed Tasmanian Atlantic salmon. *Anim. Genet.* 48, 237–241. doi: 10.1111/age.12513

Kim, D., Pertea, G., Trappell, C., Pimentel, H., Kelley, R., Salzberg, S. L., et al. (2009). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* 14:R36. doi: 10.1186/gb-2013-14-4-36

Kim, J. H., Leong, J. S., Koop Ben, F., and Devin, R. H. (2016). Multi-tissue transcriptome profiles for coho salmon (Oncorhynchus kisutch), a species undergoing rediploidization following whole-genome duplication. *Mar. Genomics* 25, 33–37. doi: 10.1016/j.margen.2015.11.008

Li, H., Hsudaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The sequence alignment/map format and SAMtools. *Nat. Protoc.* 4, 44–57. doi: 10.1038/nprot.2008.211

Mohamed, A. R., Cumbo, V., Harii, S., Shinzato, C., Chan, C. X., Ragan, M. A., et al. (2016). Deciphering the nature of the coral-Chromera association. *ISME J.* 12, 776–790. doi: 10.1038/ismej.2015.257

Mohamed, A. R., Cumbo, V., Harii, S., Shinzato, C., Chan, C. X., Ragan, M. A., et al. (2018). The transcriptomic response of the coral Acrropora digitifera to a competent Symbiodinium strain: the symbiosome as an arrested early strategy to evade the adaptive immune response and modify cell-autonomous immunity. *Dev. Comp. Immunol.* 81, 348–362. doi: 10.1016/j.devimm.2017.12.023
Salem, M., Paneru, B., Al-Tobasei, R., Abdouni, F., Thorgaard, G. H., Rexroad, C. E., et al. (2015). Transcriptome assembly, gene annotation and tissue gene expression atlas of the rainbow trout. *PLoS ONE* 10:e0121778. doi: 10.1371/journal.pone.0121778

Valenzuela-Muñoz, V., Novoa, B., Figueras, A., and Gallardo-Escárate, C. (2017). Modulation of Atlantic salmon miRNome response to sea louse infestation. *Dev. Comp. Immunol.* 76, 380–391. doi: 10.1016/j.dci.2017.07.009

Wang, S., Furmanek, T., Kryvi, H., Krossøy, C., Totland, G. K., Grotmol, S., et al. (2014). Transcriptome sequencing of Atlantic salmon (*Salmo salar* L.) notochord prior to development of the vertebrae provides clues to regulation of positional fate, chordoblast lineage and mineralisation. *BMC Genomics.* 15:141. doi: 10.1186/1471-2164-15-141

Weltzien, F. A., Andersson, E., Andersen, Ø., Shalchian-Tabrizi, K., and Norberg, B. (2004). The brain–pituitary–gonad axis in male teleosts, with special emphasis on flatfish (Pleuronectiformes). *Comp. Biochem. Physiol. A Mol Integr. Physiol.* 137, 447–477. doi: 10.1016/j.cbpb.2003.11.007

**Conflict of Interest Statement:** BE was employed by company Tassal.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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