Abstract Book

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2015

ISGA XII

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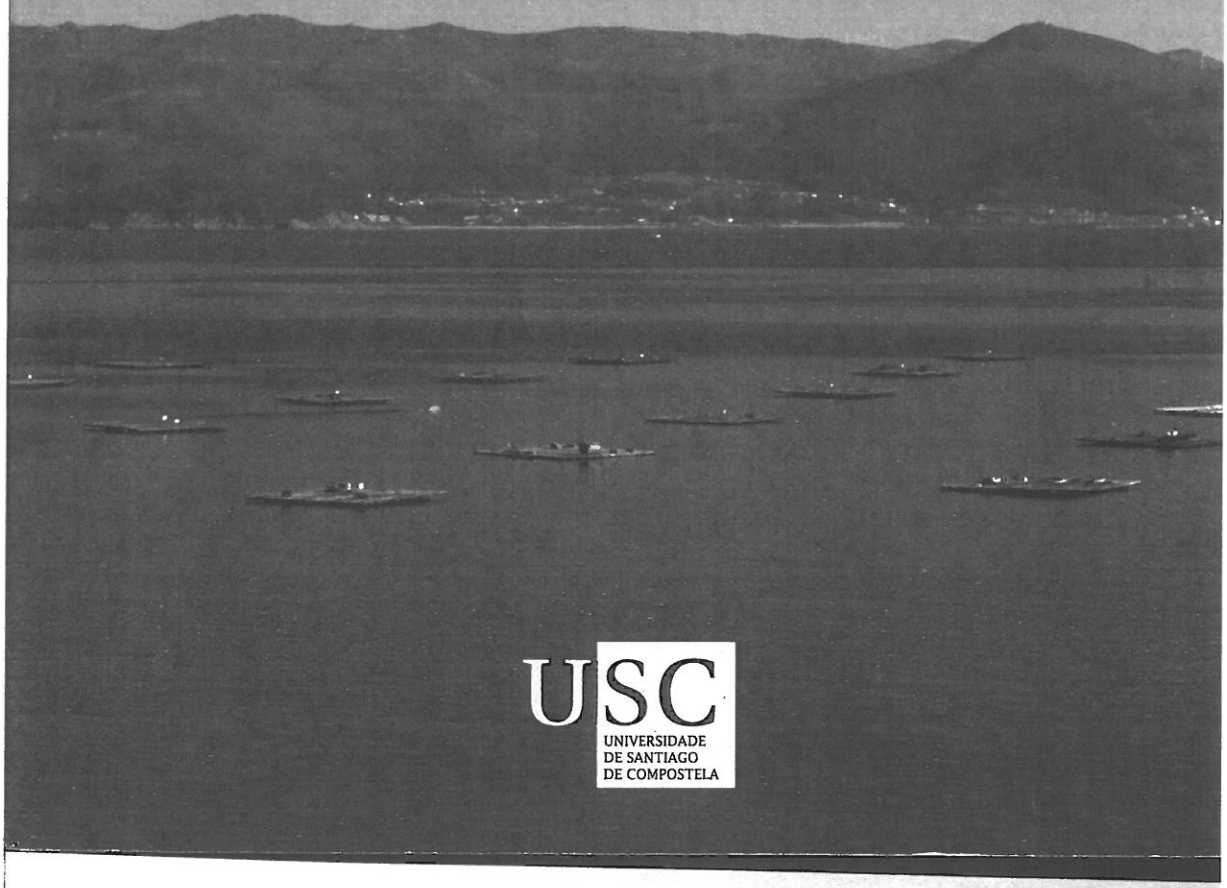
Santiago de Compostela, Spain
June 21st-27th, 2015



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DE COMPOSTELA

SESSION GENOMIC SELECTION AND MAS **(SPONSORED BY ILLUMINA)** MODERATOR Prof. Laszlo Orban	14:50-15:05	**[052]** QTL-SELECTION CONTRIBUTES TO INCREASED RESISTANCE TO CARDIOMYOPATHY SYNDROME (CMS) IN ATLANTIC SALMON *(Salmo salar* L.). **Kjøglum S.**, Moen T., Korsvoll S.A., Ødegård J., Santi N.
	15:05-15:20	**[053]** GENOTYPING-BY-SEQUENCING USING CUSTOM ION AMPLISEQ™ TECHNOLOGY AS A TOOL FOR GENOMIC SELECTION IN ATLANTIC SALMON *(Salmo salar).* **Baranski M.**, Jowdy C., Moghadam H., Norris A., Bakke H., Sonesson A., Meuwissen T., Lillehammer M., Lund V.
	15:20-15:35	**[054]** EFFECT OF IMPUTED MARKER GENOTYPES ON ACCURACY OF GENOMIC SELECTION IN AQUACULTURE POPULATIONS. **Vela-Avitúa S.**, Ødegård J.
	15:35-15:50	**[055]** CANDIDATE GROWTH GENES IDENTIFIED BY QTL FINE MAPPING IN BIGHEAD CARP *Aristichthys nobilis.* Sun Y.H., Liu H.Y., Feng X., Yu X., Fu B.D., **Tong J.**
	15:50-16:05	**[056]** ESTIMATES OF HERITABILITY FOR DISEASE RESISTANCE TO SRS USING GENOMIC RELATIONSHIPS PREDICTED USING HIGH DENSITY SNP DATA IN ATLANTIC SALMON AND RAINBOW TROUT. **Martínez V.**, Santi N., Odegard J., Moen T.
	16:05-16:20	**[057]** A GENOME-WIDE ASSOCIATION STUDY FOR SEX DETERMINATION IN ATLANTIC SALMON. **Covelo-Soto L.**, Morán P., Kent M.P., Saura M.
16:20-16:40	**COFFEE BREAK**	
16:40-17:45	**BROKERAGE/POSTER SESSION**	
17:45-19:15	**ROUND TABLE (TECHNOLOGICAL TRANSFER). MODERATOR: MS. ROSA FERNÁNDEZ (CETMAR)**	

Ms. Ana Riaza, Stolt Sea Farm S.A. (ES)
Dr. Marine Herlin, Culmarex S.A. (ES)
Dr. Pierrick Haffray, SYSAAF, Syndicat des Sélectionneurs Avicoles et Aquacoles (FR)
Dr. Anna Kristina Sonesson, Nofima (NO)
Mr. Courtney Hough, Federation of European Aquaculture Producers and European Aquaculture Technology Platform (EU)

NEXT GENERATION SEQUENCING, *DE NOVO* ASSEMBLY, AND EXPRESSION ANALYSIS OF GONADAL TRANSCRIPTOMES IN *Acipenser naccarii*

Robles F., Bakkali M., Molina-Luzón M.J., García Zea A.J., Martín-Blázquez R., Navajas-Pérez R., De la Herrán R., Ruiz Rejón C.

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We carried out Illumina sequencing of two cDNA libraries from the gonads of two males and two females of the sturgeon *Acipenser naccarii* —both belonging to 4-5 years old specimens. Proof of the quality of the resulting 73,927,492 testis and 72,170,178 ovary library reads are the facts that only 0.039% of their 147,558,646,70 bases were undetermined (N), the average GC content (46.71%) was similar for both testis and ovary libraries (46.17% and 47.24% respectively), and over 75% of the bases did satisfy the Q30 (78.34% testis and 86.24% ovary).
The reads were assembled into 652,358 sequences with lengths ranging from 35 to 15,730 bases (N50=670). The average GC content of the assembled sequences was 42.4% and BLASTX against the NCBI non-redundant protein database (nr) resulted in 586,017 annotated sequences.
Of the assembled reference transcriptome, 9,238 gene transcripts and undetermined sequences were 'sex-specific', of which 89.4% were found only in the testis transcriptome and the remaining 10.6% only in the ovary. Analysis by GO terms assigned biological processes to 6,836 of these sequences, 6,098 of which were 'testis-specific' and the remaining 738 'ovary-specific'.
In this preliminary effort aimed at identifying potential sturgeon sex-specific markers, we screened the 'sex-specific' sequences for genes known to be involved in sex determination and sexual development in vertebrates. After primer design and quantitative PCR analysis of the expression levels of the selected potential sturgeon sex-specific markers, we found the expression of the *Dmrt1* and the *steroidogenic factor 1* gene to apparently be exclusive, thus specific markers, of at least the 4-5 years old male specimens of *A. naccarii*.
We also looked for microsatellite repeats within the set of assembled sequences that had sex-related annotations and obtained 780 microsatellite-containing sequences; 257 of which had dinucleotide motives, 288 had trinucleotide motives and 235 had tetranucleotide motives (the average length of the microsatellites was 15 to 16 bases). Of all of them, microsatellite-flanking primers were designed on 30 sequences. The levels of polymorphism were then analyzed in a sample of 26 *A. naccarii* specimens (10 males, 10 females and 6 sex-undifferentiated).

Keywords: Acipenser naccarii, transcriptome sequencing, gonad, differential gene expression, microsatellites

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NEXT-GENERATION SEQUENCING, *DE NOVO* ASSEMBLY AND EXPRESSION ANALYSIS OF GONADAL TRANSCRIPTOMES IN *Acipenser naccarii*

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Figure 1: Adriatic sturgeon, *A. naccarii*

INTRODUCTION

Sturgeons are a group of fish with great evolutionary interest because they are considered true living fossils, dating from 200 million years ago (Gardiner, 1984). They also have high economic value because the legal sale of their eggs (caviar) moves millions of euros per year (CITES 2001). In this sense, the main problem for harvesting sturgeon caviar is the inability to determine the sex of these fish until they have reached sexual maturity (between 5 and 16 years depending on the species). This implies increased costs during culture, because the entire stock must be kept to determine the sexes and thus obtain the caviar from the female. It would be of great interest to the aquaculture industry to develop molecular markers for early identification of the sex of the sturgeon.

RESULTS AND DISCUSSION

RNA was extracted from the gonadal tissue *Acipenser naccarii* (**Figure 1**) individuals of 4-5 years old, two females and two males, and Illumina (Hiseq2000) sequencing was carried out separately for the two sexes.
Proof of the quality of the resulting in the 72.17.178 ovary library and 73.927.492 testis library reads are the facts that only 0.039% of their 147,558,646,70 bases were undetermined (N), the average GC content (46.71%) was similar for both testis and ovary libraries, and over 75% of the bases satisfied the Q30 (1 error per 1000 bases) **Table 1**.
The reads were assembled into 652,358 contigs with lengths ranging from 35 to 15,730 bases (N50=670). The average GC content of the assembled sequences was 42.4% and BLASTX against the NCBI non-redundant protein database (nr) resulted in 586,017 annotated sequences.
Of the assembled reference transcriptome, 9,238 gene transcripts and undetermined sequences were "sex-specific", of which 89.4% were found only in the testis transcriptome and the remaining 10.6% only in the ovary. Analysis by GO terms assigned biological processes to 6,836 of these sequences, 6,098 of which were 'testis-specific' and the remaining 738 'ovary-specific' **Table 2**

	Reads	GC %	Q30	N%
Female	73.927.492	47,24	86,24	0.003
Male	72.170.178	46,17	78,34	0.036

Table 1: Number of reads, GC %, Q30 and N % bases undetermined value to both sexes

	Sequences	%	GO
Female	980	10,6	738
Male	8.258	89,4	6098

Table 2: Number of sequences and percentage "sex-specific". GO terms assigned biological process to 6836 sequences

Figure 2: Sequence distribution of the biological processes in (A) female and (B) males. Red box shows response to stress and blue box shows chemotaxis

The sequences from female library (**Figure 2A**) showed processes related to the cell cycle, cell differentiation, embryonic development, and morphogenesis, whereas the sequences from male library (**Figure 2B**) were more diverse and included most of the processes identified in the former library. Among these, here we highlight chemotaxis-related processes in males and, stress-related processes in the libraries of both sexes.

The male library contains about 10 times the number of sequences not present in the other sex's library than the female one, but even the differential expression levels of the male-only-library sequences are higher than those of the female-only library (**Figure 3**). It might thus be speculated that the development towards males in the sturgeon might, as in mammals, be an active process (i.e. the default development might be towards the female unless the male-specifying genes are activated)

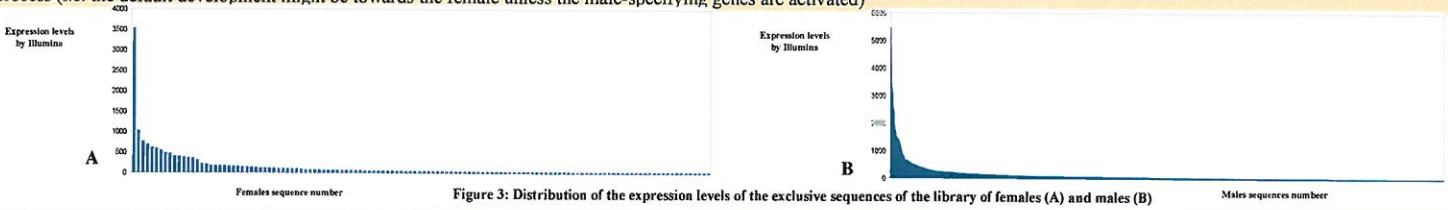

Figure 3: Distribution of the expression levels of the exclusive sequences of the library of females (A) and males (B)

In this preliminary work, aimed at identifying potential sturgeon sex-specific markers and differential expression, we checked four 'sex-specific' sequences for genes known to be involved in sex determination and sexual development in vertebrates, such as *maternal protein*, *wnt9b protein*, *doublesex* (*Dmrt1)* and *steroidogenic factor 1* genes. After primer design and quantitative PCR analysis of the expression levels of the selected potential sturgeon sex-specific markers, we found the expression of the doublesex (*Dmrt1)* (**Figures 4 and 5**) and the *steroidogenic factor 1* genes (**Figures 6 and 7**) to be apparently exclusive, thus specific markers, of at least the 4- to 5-year-old male specimens of *A. naccarii*. The sex-dependent differential expression of the *Dmrt1* gene in the sturgeon appears to be age-dependent. In the species *A. baerii*, there is over-expression in testis compared to ovaries of 3-year-old specimens (Berbejillo et al. 2012), while in the gonads of 2-year-old *Scaphirhynchus platorynchus*, the differences are not significant. In 6-month-old *A. naccarii*, however, the expression of this gene is missing (Vidotto et al. 2013). Expression of the *steroidogenic factor 1* gene is larger in the testis than in the ovaries of the species *A. baerii* (Berbejillo et al. 2012); Vidotto et al. 2013 did not detect this gene in the transcriptome of *A. naccarii*, possibly due to much lower age range of the specimens used in their study.

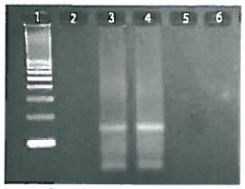

Figure 4: Conventional PCR amplification of the *Dmrt1* gene. Line 1: ladder, 2: negative control, 3-4: male samples and 5-6: female samples.

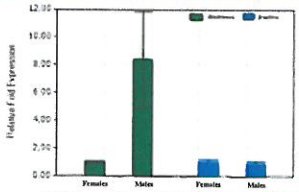

Figure 5: Quantitative PCR amplification of the *Dmrt1* gene. Green box *Dmrt1* gene and blue box housekeeping *beta-actin*.

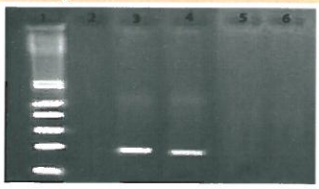

Figure 6: Conventional PCR amplification of the *Steroidogenic factor 1* gene. Line 1: ladder, 2: negative control, 3-4: male samples and 5-6: female samples.

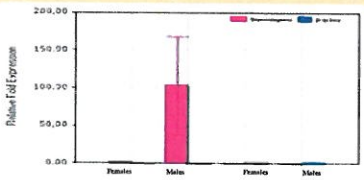

Figure 7: Quantitative PCR amplification of the *Steroidogenic factor 1* gene. Pink box *Steroidogenic factor 1* gene and blue box housekeeping *beta-actin*.

In addition, we looked for microsatellite repeats within the set of assembled sequences that had sex-related annotations. A total of 780 microsatellite were found. In a first analysis, we designed primers for 14 microsatellite-containing sequences present only in the library of one sex (sex, doublesex, testis-expresed1, testis expresed2, prostaglandin, sox, sperm, tex10, male-specific, spermatogenesis-associated genes from male library and oocyte-specific, estrogen, wnt10, wnt2, from female library). Ten of these were successful in amplifying by PCR (sex, doublesex, wnt2, prostaglandin, wnt10, sox, sperm, testis1, tex10, male-specific). In this work, we show the results for two microsatellite loci associated with *prostaglandin* and *wnt10* genes. The levels of polymorphism were then analyzed in 27 *A. naccarii* specimens (10 males, 10 females and 7 sex-undifferentiated). The results are shown in **Table 3**. The analyses performed to date through the study of microsatellites, show no evidence for differences between males and females.

Locus	k	N	Ho	He	PIC	F(null)	P-value
prostaglandin	5	27	0,78	0,685	0,623	-0,0667	0,1321
wnt10	4	21	0,62	0,628	0,558	-0,009	0,047

Table 3: Diversity statistic value per locus. k number of alleles, N number of individual genotyped, Ho and He observed and expected heterozygosities, PIC polimorphic information content, Fnull frequency of null alleles.

CONCLUSION AND PERSPECTIVES

We have developed a transcriptome database with sequences from gonads of females and males of the sturgeon *Acipenser naccarii*. This dababase is composed by high-quality sequences which were assembled into 652,358 contigs. Of all, 586,017 show homology with annotated sequences and 9,238 are gene transcripts and undetermined sequences 'sex-specific'. In our preliminary results, we detected differential expression genes between sex and microsatellite markers. All these data, *in silico* and *in vitro*, will be useful in the analysis of the sex determination and development in sturgeons and in the search for sex-specific molecular markers to improve the culture of this fish.

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