GENETIC RESOURCES OF FISH AND SHELLFISH POPULATIONS IN SOUTHERN BALTIC SEA

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Populations of sea trout *Salmo trutta* m. trutta L., cod *Gadus morhua* L. and mussels *Mytilus* spp. from the Baltic Sea were studied using SNPs (single nucleotide polymorphisms) genotyping methods, next generations sequencing, gene expression using HRM (RT-PCR) and 44K-oligo microarray.

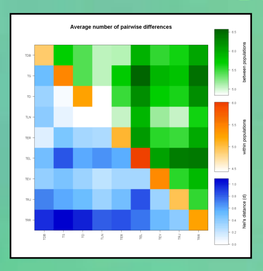
SEA TROUT

Sea trout, a migratory form of brown trout is represented by numerous populations in the Baltic Sea. Many populations are enhanced by releases of artificially reared alevins and smolts (supportive breeding).

Genotyping of nine Baltic populations of sea trout

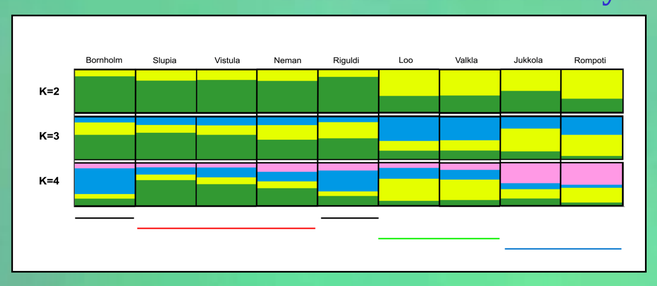
South East Baltic populations (from Poland, Lithuania, Denmark: Bornholm, Estonia and Russia) were genotyped with iPLEX Gold Sequenom method using a diagnostic panel of 23 SNPs. The highest level of pairwise Fst differences was observed between Russian population from East Gulf of Finland and Polish populations from the Baltic Main Basin and the lowest differences were between the both Polish, and Polish and Lithuanian populations.

Genetic differentiation

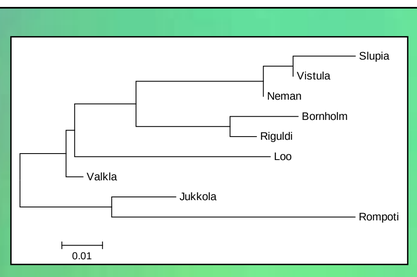


Above diagonal: pairwise comparisons of 9 sea trout populations. Below diagonal: comparisons using Nei's distance. On diagonal - average number of pairwise difference within population.

Genetic structure analysis



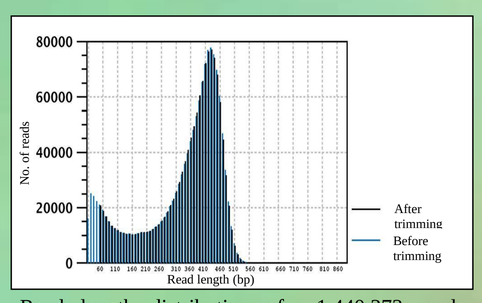
Results of genetic structure analysis indicate that individuals from 9 populations were grouped into four clusters.



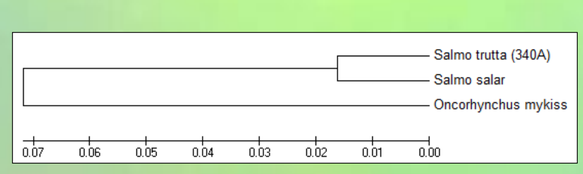
The NJ tree constructed using the Nei's distances among the nine sea trouts populations.

454 pyrosequencing

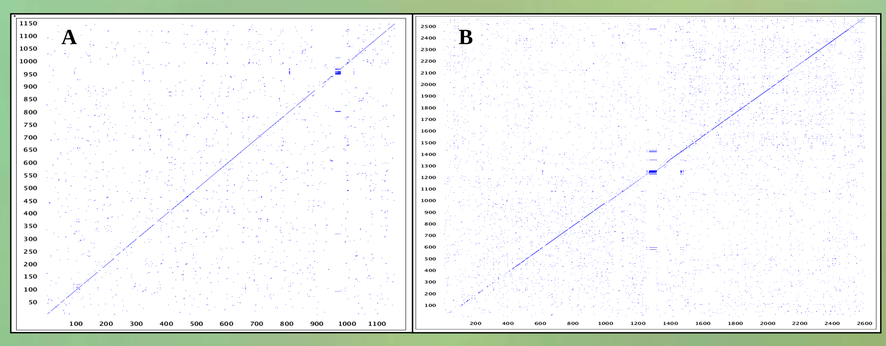
Selected transcripts putatively involved in immune response, by using 454 pyrosequencing technology were studied in Vistula river specimens. A total of 1,440,373 reads were obtained with the average read length 334 nucleotides. At present, 3 groups of genes were identified: Mx, C7 and MHC.



Read length distribution of 1,440,373 reads produced from the transcriptome of *Salmo trutta* generated by CLC GenomicWorkbench.



Phylogenetic tree for myxovirus resistance gene (Mx2). Identification of gene in sea trout genome.



DotPlot for: A- iso114 and reference sequence from *Salmo trutta* MHC I, *Satr-UBA**0601 allel (gi9937600); B- iso54 and reference sequence from *Salmo salar* MHC I, *UBA* *0301 allel (gi25573077).

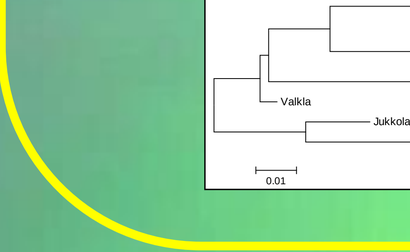
44K-oligo microarray

Name	PLFUR1 bin>157.diff	PLFUR2 bin>158.diff	PLFUR3 bin>159.diff	PLFUR4 bin>160.diff	PLFUR5 bin>161.diff	PLFUR6 bin>162.diff	PLFUR7 bin>163.diff	PLFUR8 bin>164.diff	mean
Immune system genes									
acute phase serum amyloid A (SAA)	-3,1	-3	-3,6	-3,4	-2,8	-0,8	-2,5	-2,6	-2,73
Interleukin-1 beta; Flags: Precursor	-4,7	-2,9	-7,1	-6,4	-3,9	-0,5	-4,8	-1,5	-3,99
interleukin 17 isoform D	2,55	1,09	2,07	2,07	1,69	1,15	0,21	0,97	1,48
Small inducible cytokine A13	3,34	1,21	4,51	2,94	1,49	1,91	2,79	0,6	2,35
Hepcidin-1 precursor	-4,7	-4	-4,5	-4,8	-4,7	0,74	-3,8		-3,67
cathelicidin antimicrobial peptide	-5,6	-4,3	-5,2	-5,1	-4,3	0,1	-4,1	-0,2	-3,58
cyclooxygenase-2	-2,9	-1	-4,2	-3,5	-2,1	1,39	-2,7	0,11	-1,86
Mx1 protein	1,65	1,87	1,51	1,83	2,98	-1,5	1,98	-0	1,29
C type lectin receptor B	-7	-3,7	-6,5	-6,8	-6,2	0,31	-3,9	-3,6	-4,66
Collagenase 3 precursor	-4	-4,1	-5,1	-4,2	-2,7	-0,7	-4,6	-1,3	-3,34
matrix metalloproteinase 9	-4,9	-4,3	-4,2	-4,2	-2,8	0,44	-4,4	-1,4	-3,22
Metalloreductase STEAP4	-4,1	-2,4	-3,8	-4,5	-3,1	1,27	-2,9	-2,7	-2,77

Name	PLFUR1 bin>157.diff	PLFUR2 bin>158.diff	PLFUR3 bin>159.diff	PLFUR4 bin>160.diff	PLFUR5 bin>161.diff	PLFUR6 bin>162.diff	PLFUR7 bin>163.diff	PLFUR8 bin>164.diff	mean
Other genes									
DNA replication licensing factor mcm5	1,2	3,58	2,43	1,85	2,76	-0,2	2,92		2,07
heat shock 70kDa protein 8 isoform b	1,92	2,64	-1,4	2,19	3	0,44	2,7		1,64
heat shock protein hsp90	6,13	5,92	3,48	2,32	-0,5	2,74	1,41	-1,1	2,55
Cathepsin L1	4,15	1,44	-1,8	4,22	3,65	4,03	3,66	-0,2	2,39
procathepsin B	5,86	0,92	-1,5	5,65	5,54	5,32	4,89	-0,1	3,32

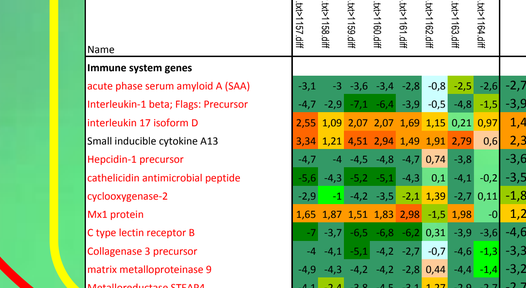
Differences in gene expression in hatchery *Salmo trutta* m. trutta and *Oncorhynchus mykiss* under stress caused by infection with a bacterial pathogen *Aeromonas salmonicida* spp *salmonicida* and non-infected were observed. Some genes were up-regulated (chaperones, mainly HSPs, Mx, interleukin IL17D a proinflammatory cytokine) and down-regulated (acute phase proteins, chemokines, cytokines, COX, lectins, lectin receptors and inflammation related proteases, TNF-related and apoptotic proteins and other) as found by the application of transcriptome hybridisations to 44K oligo-microarray (Agilent).

COD



Populations of Baltic cod were studied using 5 SNPs. According to the obtained HRM (high resolution melting) results populations of the cod were characterized by clinal variation of alleles at loci known to be linked with salinity gradient. The obtained results confirm that eastern population of cod is a separate one.

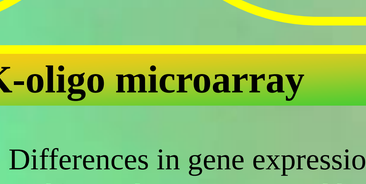
Exposure of cod from western (Kiel) and southern (Gdansk) Baltic to different salinity ranging from 3 to 33ppt. revealed its high tolerance to different environmental conditions. This tolerance is being studied at the level of gene expression and high number of SNPs.

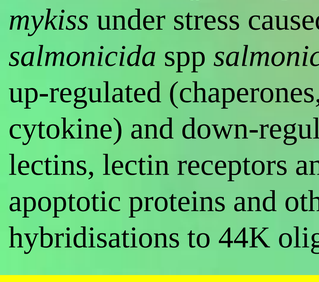


Linear graph of the percentage of individuals with homozygous (aa, bb) and heterozygous (ab) genotypes illustrating distribution of alleles of Aromatase 9 along latitudinal transect across the Baltic Sea.
Names of samples represents: NS - the North Sea, KIL - Kiel, G3 - South East Bornholm area, G2 - Słupsk (Stolpe) Bank, G1 - the Gulf of Gdańsk, LT - Lithuanian Coast.

MYTILUS SPP.

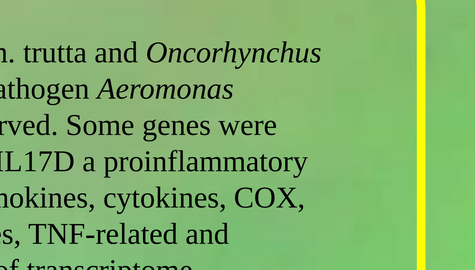
Correspondence analysis (CA) of *Mytilus* populations from Baltic Sea region and Canada. Each dot is a population. Populations from the North Sea (G1), North Danish Straits (G2), South Danish Straits (G3 and G4), inner Baltic Sea (G5), Canada (G6).





The locations and acronyms of 23 European *Mytilus* sampling sites. Structure results for the populations for $K = 3$ are presented in pie diagrams. These proportions are Q values.

Baltic populations of *Mytilus* spp. are locally adapted, with a unique composition of loci derived from *M. trossulus* and *M. edulis* genomes. To identify markers and genes associated with the hybrid zone in Danish Straits and to determine the uniqueness of Baltic populations, 60 polymorphic SNPs were used to genotype individual mussels from the Baltic, North Sea and Canada. In total 35 SNPs turned out to be significant in FST outlier analysis and therefore were clearly related to the interactions with environment. The majority of new SNPs show greater participation of *M. trossulus* than *M. edulis* genes in the nuclear DNA of Baltic *Mytilus*.



The geographic locations and acronyms of 27 Mytilus sampling sites in the Baltic and North Sea.