Report, 12th November 2011

Testing of gill samples from salmonids collected in British Colombia, Canada.

RNA from all gill samples was extracted as described by Devold et al 2000. The amount of RNA in each extraction sample was measured by NanoDrop ND-1000 (Spectrophotometer). For each tissue sample a negative control sample was included. An assay targeting the housekeeping gene, elongation factor alpha, was used as an internal control to test the quality of the RNA. This time we used the elf-alpha from rainbow trout/coho salmon (unpublished assay). Two different assays targeting know ISA viruses were used: a) Assay **ISAV7** targeting segment seven from European ISA viruses (Plarre et al 2005), and b) assay **ISAV8-Uni** targeting segment 8 from all known ISA viruses (Snow et al 2006). The results of the analysis of the tissues are presented in table 1.

None of the gill samples were positive for ISA virus.

We also decided to test for two other viruses that have been associated with mortality in western Canada, Infectious haematopoietic necrosis virus (IHNV), viral haemorrhagic septicaemia virus (VHSV). In addition we looked for microsporidians and Ichthyobodo spp. in some of the material.

All samples were negative for presence of VHS virus while the following samples were positive for presence of IHN virus: SL1 – SL14, CS1, and CS14 – 16. The gill samples from CS1 – CS16 and Q1 – Q4 were all positive for presence of *Ichthyobodo* spp.

The 16S (SSU) from two "new" species of microsporidia were sequenced from the gills of selected samples. The same species of microsporidia were present in fish from H, BQ and LQ. An additional species were present in material from LQ.

Conclusion

The RNA from the gill tissues seem to be of reasonable quality for real time RT PCR, and the amount of RNA obtained after extraction was substantial. Hence, the amount and quality of the RNA should not have influenced on the results.

I have no information about the origin of the tissues analysed or from which species the gill tissues originated. Nor do I have any information about the age or condition of the fish sampled. However, some of these were strongly positive for IHN virus and *Ichthyobodo* spp. which both may cause mortalities in infected fish populations. In addition, a substantial number of fish were positive for presence of microsporidia and two different, possibly new species, were identified by sequencing of the SSU.

If the material that I have received comes from moribund or dead salmonids then the cause for this mortality is not ISA viruses. Other pathogens could, however, have played a role.

Table 1. Results from the testing of gill tissues for presence of ISA viruses, VHS viruses and IHN viruses.

	Gills	Gills	Gills	Gills	Gills	Gills
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
C1	19,2	Neg	Neg	Neg	NT	NT
C2	21,8	Neg	Neg	Neg	NT	NT
C3	22,8	Neg	Neg	Neg	NT	NT
C26	21,5	Neg	Neg	Neg	NT	NT
C36	21,8	Neg	Neg	Neg	NT	NT
C4	21,1	Neg	Neg	Neg	NT	NT
C5	22,8	Neg	Neg	Neg	NT	NT
C6	21,8	Neg	Neg	Neg	NT	NT
C7	23,5	Neg	Neg	Neg	NT	NT
C26 b	22,8	Neg	Neg	Neg	NT	NT
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
Cow 1	13,7	Neg	Neg	Neg	Neg	Neg
Cow 2	13,8	Neg	Neg	Neg	Neg	Neg
Cow 3	13,9	Neg	Neg	Neg	Neg	Neg
Cow 4	23,8	Neg	Neg	Neg	Neg	Neg
Cow 5	20,3	Neg	Neg	Neg	Neg	Neg
Cow 6	19,2	Neg	Neg	Neg	Neg	Neg
Cow 7	15,5	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
BQ 1	16,9	Neg	Neg	Neg	Neg	Neg
BQ 2	19,8	Neg	Neg	Neg	Neg	Neg
BQ 3	20,0	Neg	Neg	Neg	Neg	Neg
BQ 4	23,7	Neg	Neg	Neg	Neg	Neg
BQ 5	19,9	Neg	Neg	Neg	Neg	Neg
BQ 6	20,2	Neg	Neg	Neg	Neg	Neg
BQ 7	34,0	Neg	Neg	Neg	Neg	Neg
BQ 8	19,9	Neg	Neg	Neg	Neg	Neg
BQ 9	18,9	Neg	Neg	Neg	Neg	Neg
BQ 10	36,3	Neg	Neg	Neg	Neg	Neg
BQ 11	Neg	Neg	Neg	Neg	Neg	Neg
BQ 12	22,2	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
EN1	18,5	Neg	Neg	Neg	Neg	Neg
EN2	Neg	Neg	Neg	Neg	Neg	Neg
EN3	22,6	Neg	Neg	Neg	Neg	Neg
EN4	21,1	Neg	Neg	Neg	Neg	Neg
EN5	18,0	Neg	Neg	Neg	Neg	37,1
EN6	31,9	Neg	Neg	Neg	Neg	Neg
EN7	15,7	Neg	Neg	Neg	Neg	Neg
EN8	18,5	Neg	Neg	Neg	Neg	Neg
EN9	17,8	Neg	Neg	Neg	Neg	Neg
EN10	21,4	Neg	Neg	Neg	Neg	Neg
EN12	19,5	Neg	Neg	Neg	Neg	Neg
EN13	17,8	Neg	Neg	Neg	Neg	Neg
EN14	17,0	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
NA1	18,0	Neg	Neg	Neg	Neg	Neg
NA2	21,0	Neg	Neg	Neg	Neg	Neg
NA3	21,9	Neg	Neg	Neg	Neg	Neg

NA4	18,1	Neg	Neg	Neg	Neg	Neg
NA5	21,3	Neg	Neg	Neg	Neg	Neg
NA6	17,9	Neg	Neg	Neg	Neg	Neg
NA7	24,5	Neg	Neg	Neg	Neg	Neg
NA8	24,1	Neg	Neg	Neg	Neg	Neg
NA9	20,2	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
LQ1	16,2	Neg	Neg	Neg	Neg	Neg
LQ2	17,6	Neg	Neg	Neg	Neg	Neg
LQ3	Neg	Neg	Neg	Neg	Neg	Neg
LQ8	24,2	Neg	Neg	Neg	Neg	Neg
LQ9	16,3	Neg	Neg	Neg	Neg	Neg
LQ10	18,0	Neg	Neg	Neg	Neg	Neg
LQ11	27,0	Neg	Neg	Neg	Neg	Neg
LQ12	17,0	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
SL1	17,1	Neg	Neg	Neg	Neg	20,9
SL2	20,6	Neg	Neg	Neg	Neg	21,8
SL3	22,8	Neg	Neg	Neg	Neg	20,4
SL4	20,5	Neg	Neg	Neg	Neg	26,3
SL5	16,7	Neg	Neg	Neg	Neg	24,1
SL6	18,9	Neg	Neg	Neg	Neg	23,5
SL7	18,4	Neg	Neg	Neg	Neg	14,8
SL8	19,8	Neg	Neg	Neg	Neg	24,4
SL9	20,3	Neg	Neg	Neg	Neg	32,3
SL10	24,3	Neg	Neg	Neg	Neg	22,7
SL11	23,3	Neg	Neg	Neg	Neg	22,1
SL12	21,8	Neg	Neg	Neg	Neg	31,4
SL13	21,0	Neg	Neg	Neg	Neg	27,7
SL14	21,2	Neg	Neg	Neg	Neg	24,9
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
NSP1	17,9	Neg	Neg	Neg	Neg	22,7
NSP2	18,7	Neg	Neg	Neg	Neg	33,8
NSP3	16,7	Neg	Neg	Neg	Neg	28,1
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
CS1	19,0	Neg	Neg	Neg	Neg	36,0
CS2	Neg	Neg	Neg	Neg	Neg	Neg
CS3	Neg	Neg	Neg	Neg	Neg	Neg
CS4	18,0	Neg	Neg	Neg	Neg	Neg
CS5	30,3	Neg	Neg	Neg	Neg	Neg
CS6	18,0	Neg	Neg	Neg	Neg	Neg
CS7	24,3	Neg	Neg	Neg	Neg	Neg
CS8	17,3	Neg	Neg	Neg	Neg	Neg
CS9	18,6	Neg	Neg	Neg	Neg	Neg
CS10	22,4	Neg	Neg	Neg	Neg	Neg
CS11	Neg	Neg	Neg	Neg	Neg	Neg
CS12	15,5	Neg	Neg	Neg	Neg	Neg
CS13	31,8	Neg	Neg	Neg	Neg	Neg
CS14	17,7	Neg	Neg	Neg	Neg	16,2
CS15	15,8	Neg	Neg	Neg	Neg	21,8
CS16	13,8	Neg	Neg	Neg	Neg	34,9
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
Q1	15,1	Neg	Neg	Neg	Neg	Neg
Q2	20,4	Neg	Neg	Neg	Neg	Neg

Q3	16,8	Neg	Neg	Neg	Neg	Neg
Q4	15,4	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
JF1	16,5	Neg	Neg	Neg	Neg	Neg
JF2	14,5	Neg	Neg	Neg	Neg	Neg
JF3	17,1	Neg	Neg	Neg	Neg	Neg
JF4	15,9	Neg	Neg	Neg	Neg	Neg
JF5	15,6	Neg	Neg	Neg	Neg	Neg
JF6	15,6	Neg	Neg	Neg	Neg	Neg
JF7	17,3	Neg	Neg	Neg	Neg	Neg
JF8	16,2	Neg	Neg	Neg	Neg	Neg
JF9	15,3	Neg	Neg	Neg	Neg	Neg
JF10	15,2	Neg	Neg	Neg	Neg	Neg
JF11	16,0	Neg	Neg	Neg	Neg	Neg
JF12	17,1	Neg	Neg	Neg	Neg	Neg
JF13	19,1	Neg	Neg	Neg	Neg	Neg
JF14	17,2	Neg	Neg	Neg	Neg	Neg
JF15	16,1	Neg	Neg	Neg	Neg	Neg
Sample	**OMELF**	**ISAV7**	**UnilSAV8**	**Neg con**	**VHSV**	**IHNV**
Pos control	28,8	24,3	26,7			
Pos control	30,7	25,7	26,4			
Pos control	26,5	24,9	23,6			
Pos control	14,7	21,7	26,3			

Table 2. Results from the testing of gill tissues for presence of *Ichthyobodo* spp.

Sample	Ichthyo.spp.
CS1	24,8
CS2	22,2
CS3	24,9
CS4	15,5
CS5	22
CS6	19
CS7	21,6
CS8	18,6
CS9	23,4
CS10	14,2
CS11	25
CS12	20,5
CS13	13,8
CS14	17,4
CS15	13,6
CS16	12,8
Q1	17,6
Q2	17,3
Q3	14,5
Q4	21,2

Literature

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