

Memorandum

TO: Pat Brandes, Stockton FWO

DATE: November 8, 2007

FROM: J. Scott Foott, and Ron Stone, CA-NV FHC

SUBJECT: Health monitoring of 2006 VAMP marked MRH Chinook salmon

In support of the 2006 Vernalis Adaptive Management Plan (VAMP) out-migrant juvenile Chinook salmon study, the CA-NV FHC conducted a pre-release health inspection at Merced River Hatchery (MRH) on 25 April 2006 as well as examining kidney imprints of VAMP study salmon collected in the Chipp's Island trawl for the parasite *Tetracapsuloides bryosalmonae*. This myxosporean parasite causes Proliferative Kidney Disease (PKD) in salmonid fishes and has freshwater bryozoans as alternate hosts. Proliferative Kidney Disease has been diagnosed in MRH salmon for several decades with the incidence of infection in MRH salmon, inspected prior to and shortly after release, ranging from 4 – 100%. The vast majority of these infections have been deemed early and the fish were asymptomatic. In 2005, a cohort population of MRH Chinook smolts monitored for 50 days during the out-migration period progressed from the asymptomatic “early” infection state to one of clinical disease and mortality.

Methods

Pre-release inspection: On 25 April 2006, a 60 fish sample was obtained proportionally from the rearing units containing the VAMP Coded Wire Tagged (CWT) groups. Kidney was collected aseptically for viral assay, culture of systemic bacteria, and imprint smears later examined for *Renibacterium salmoninarum* by a direct fluorescent antibody test (DFAT). Archival kidney tissue, from several fish that tested positive for *R.salmoninarum* by DFAT, was later used in a confirmatory PCR assay for bacterial DNA. Posterior kidney from 20 salmon was processed for histological sections to evaluate *T. bryosalmonae* infection and kidney inflammation.

Chipp's Island trawl kidney imprints – On 10 April, a brief workshop on kidney imprint collection was performed for Stockton FWO biologists. A sub-sample of 407 adipose fin marked Chinook juveniles collected in the Chipp's Island trawl between 5 May and 18 June 2006 were sampled for kidney tissue by Stockton Fish & Wildlife Office biologists. Imprints of the kidney were made on numbered slides, fixed with a spray fixative solution of isopropyl alcohol and polyethylene glycol, and both the head tag and kidney sample number recorded. Imprints of 66 salmon with VAMP tag codes were later screened by an indirect fluorescent lectin assay utilizing biotin-labeled *Griffonia simplicifolia* agglutinin I lectin (GS-I) and fluorescein-labeled avidin stain (Vector Laboratories). The GS-1 stain reacts with carbohydrate moieties of *T. byosalmonae*. The imprints were examined at 200 - 400x magnification with an Olympus BHS fluorescent microscope. As a secondary screening method, a subsample of 22 of the 66 GS-1

stained imprints were later stained with Diff-Quick hematology stain and examined at 1000x magnification for *T. byrosalmonae*.

Results and Discussion: The mean fork length and condition factor of MRH salmon collected on 25 April was 73 mm (std. dev. = 5) and 1.208 (std. dev. = 0.093), respectively. Salmon had been fed prior to sampling and stomach contents artificially increased weight and condition factor. The mean gill ATPase activity of 12 MRH salmon was 9.9 μ moles ADP / mg protein / hr (std. dev. = 2.2) indicating advanced smolt development. Low numbers of motile aeromonid bacteria were isolated from 3 of 30 kidney samples and were considered biologically insignificant to the fish. Fourteen of 60 kidney imprints contained low numbers of bacteria that morphologically resembled *Renibacterium salmoninarum* and reacted positively in the DFAT screening assay. Low copy number of *R. salmoninarum* DNA was detected in 2 archival kidney samples from DFAT positive fish. While the fish were asymptomatic for Bacterial Kidney Disease, the 23% detection rate by the relatively insensitive DFAT method indicates the population contained a high number of *R. salmoninarum*-infected fish. Similar findings of low-level *R. salmoninarum* infections have been documented for MRH Chinook juveniles in previous years. It is unclear whether such infection later develops into clinical disease and is a health problem for the population. No viral agents were detected in the MRH salmon.

Tetracapsuloides byrosalmonae parasites were not observed in MRH kidney sections. Similarly, no *T. byrosalmonae* were detected in Chipps Island imprints by either staining method. A number of imprints were observed to have been improperly fixed as they contained high numbers of *Bacillus* bacteria (post-collection growth) and showed poor kidney cell morphology as well as poor staining characteristics. If kidney imprints are collected in the future, it may be necessary to use rapid methanol fixation. Given the limited sensitivities of microscopic methods we cannot conclusively rule out infection, however, our inability to detect *T. byrosalmonae* in both histological and cytological sample types strongly suggests that the MRH population was not infected in 2006.

Acknowledgements: Funding for this effort was supplied by VAMP through the Stockton FWO (1933-PY01). We thank Mike Cozart (MRH) and the FWS Chipps Island trawl crew for their efforts.

Data for 25April inspection.

	FL(mm)	Wt(g)	KFL
	72	4.6	1.232
	75	4.9	1.161
	76	5.5	1.253
	70	4.0	1.166
	74	5.0	1.234
	74	5.2	1.283
	63	2.5	1.000
	64	2.7	1.030
	77	5.5	1.205
	77	5.0	1.095
	85	7.2	1.172
	74	4.6	1.135
	70	4.3	1.254
	71	4.4	1.229
	72	4.4	1.179
	75	5.3	1.256
	70	4.5	1.312
	71	4.9	1.369
	75	5.4	1.280
	68	4.1	1.304
mean	73	4.7	1.208
std	5	1.0	0.093
cv	6.63%	21.27%	7.70%

Table *T. byrosalmonae* infection (# parasite / 60 fields) in either GS1 DFAT or Diffquick samples of VAMP coded –wire tagged juvenile Chinook salmon collected in the Chippis Island trawl. Poor fixation (pf) and *Bacillus* (b) contamination are noted.

Slide #	Head #	Date	Time	Station Code	FL	Tag Code	Rdate	Rsite	dal	20x	100x	Slide #	note
										GS1 DFAT	DiffQuick		
61	20161	5/8/2006	1913	SB018M	92	06-47-13	5/4/2006	MD	4	"0 / 60f		61	
74	20457	5/9/2006	1550	SB018N	91	06-47-13	5/4/2006	MD	5	"0 / 60f	"0 / 60f	74	pfb
106	20920	5/10/2006	1856	SB018N	96	06-47-13	5/4/2006	MD	6	"0 / 60f		106	
159	20782	5/12/2006	1852	SB018N	91	06-47-13	5/4/2006	MD	8	"0 / 60f		159	
118	20928	5/11/2006	815	SB018S	75	06-47-14	5/4/2006	MD	7	"0 / 60f	"0 / 60f	118	pfb
73	20498	5/9/2006	1550	SB018N	85	06-47-15	5/8/2006	JP	1	"0 / 60f		73	
75	20471	5/9/2006	1615	SB018N	81	06-47-15	5/8/2006	JP	1	"0 / 60f		75	
77	20499	5/9/2006	1824	SB018S	82	06-47-15	5/8/2006	JP	1	"0 / 60f		77	
80	20222	5/10/2006	611	SB018N	82	06-47-15	5/8/2006	JP	2	"0 / 60f	"0 / 60f	80	
81	20236	5/10/2006	635	SB018N	91	06-47-15	5/8/2006	JP	2	"0 / 60f		81	
82	20195	5/10/2006	726	SB018M	86	06-47-15	5/8/2006	JP	2	"0 / 60f		82	pfb
83	20209	5/10/2006	751	SB018M	84	06-47-15	5/8/2006	JP	2	"0 / 60f	"0 / 60f	83	
84	20208	5/10/2006	751	SB018M	87	06-47-15	5/8/2006	JP	2	"0 / 60f		84	
85	20223	5/10/2006	751	SB018M	89	06-47-15	5/8/2006	JP	2	"0 / 60f		85	
86	20231	5/10/2006	840	SB018S	80	06-47-15	5/8/2006	JP	2	"0 / 60f	"0 / 60f	86	
91	20191	5/10/2006	929	SB018M	89	06-47-15	5/8/2006	JP	2	"0 / 60f		91	
92	20109	5/10/2006	1627	SB018S	87	06-47-15	5/8/2006	JP	2	"0 / 60f		92	
96	20096	5/10/2006	1627	SB018S	85	06-47-15	5/8/2006	JP	2	"0 / 60f		96	
99	20135	5/10/2006	1652	SB018S	91	06-47-15	5/8/2006	JP	2	"0 / 60f	"0 / 60f	99	
101	20889	5/10/2006	1806	SB018M	85	06-47-15	5/8/2006	JP	2	"0 / 60f		101	
103	20891	5/10/2006	1806	SB018M	81	06-47-15	5/8/2006	JP	2	"0 / 60f		103	
105	20922	5/10/2006	1806	SB018M	84	06-47-15	5/8/2006	JP	2	"0 / 60f		105	pfb
107	20918	5/10/2006	1856	SB018N	87	06-47-15	5/8/2006	JP	2	"0 / 60f		107	pfb
109	20917	5/10/2006	1856	SB018N	81	06-47-15	5/8/2006	JP	2	"0 / 60f		109	
111	20902	5/10/2006	1922	SB018N	90	06-47-15	5/8/2006	JP	2	"0 / 60f		111	
114	20926	5/11/2006	634	SB018N	92	06-47-15	5/8/2006	JP	3	"0 / 60f	"0 / 60f	114	
115	20916	5/11/2006	659	SB018N	90	06-47-15	5/8/2006	JP	3	"0 / 60f		115	
119	20929	5/11/2006	815	SB018S	76	06-47-15	5/8/2006	JP	3	"0 / 60f		119	
120	20930	5/11/2006	841	SB018S	84	06-47-15	5/8/2006	JP	3	"0 / 60f	"0 / 60f	120	pfb
121	20777	5/11/2006	841	SB018S	75	06-47-15	5/8/2006	JP	3	"0 / 60f		121	

122	20833	5/11/2006	841	SB018S	90	06-47-15		5/8/2006	JP	3	"0 / 60f		122	
123	20805	5/11/2006	841	SB018S	81	06-47-15		5/8/2006	JP	3	"0 / 60f	"0 / 60f	123	pfb
124	20791	5/11/2006	905	SB018S	84	06-47-15		5/8/2006	JP	3	"0 / 60f		124	
127	20193	5/11/2006	1722	SB018S	83	06-47-15		5/8/2006	JP	3	"0 / 60f		127	
128	20772	5/11/2006	1722	SB018S	89	06-47-15		5/8/2006	JP	3	"0 / 60f	"0 / 60f	128	
130	20733	5/11/2006	1722	SB018S	85	06-47-15		5/8/2006	JP	3	"0 / 60f		130	
131	20775	5/11/2006	1722	SB018S	87	06-47-15		5/8/2006	JP	3	"0 / 60f		131	
133	20207	5/11/2006	1722	SB018S	94	06-47-15		5/8/2006	JP	3	"0 / 60f	"0 / 60f	133	pfb
136	20729	5/11/2006	1812	SB018M	86	06-47-15		5/8/2006	JP	3	"0 / 60f		136	
139	20730	5/11/2006	1903	SB018N	90	06-47-15		5/8/2006	JP	3	"0 / 60f		139	
142	20731	5/11/2006	1927	SB018N	81	06-47-15		5/8/2006	JP	3	"0 / 60f	"0 / 60f	142	
144	20759	5/12/2006	542	SB018M	80	06-47-15		5/8/2006	JP	4	"0 / 60f		144	
154	20864	5/12/2006	1737	SB018M	71	06-47-15		5/8/2006	JP	4	"0 / 60f	"0 / 60f	154	
89	20218	5/10/2006	903	SB018M	79	06-47-16		5/5/2006	DR	5	"0 / 60f		89	
110	20901	5/10/2006	1856	SB018N	74	06-47-16		5/5/2006	DR	5	"0 / 60f		110	
303	21510	5/20/2006	1901	SB018N	92	06-47-21		5/19/2006	MD2	1	"0 / 60f	"0 / 60f	303	
307	21455	5/20/2006	1901	SB018N	93	06-47-21		5/19/2006	MD2	1	"0 / 60f		307	
330	21243	5/23/2006	1813	SB018N	90	06-47-23		5/19/2006	MD2	4	"0 / 60f	"0 / 60f	330	
333	21039	5/24/2006	543	SB018M	94	06-47-23		5/19/2006	MD2	5	"0 / 60f		333	
338	21005	5/24/2006	1605	SB018M	90	06-47-23		5/19/2006	MD2	5	"0 / 60f	"0 / 60f	338	
345	21300	5/24/2006	1807	SB018N	87	06-47-23		5/19/2006	MD2	5	"0 / 60f		345	PF
327	21264	5/23/2006	831	SB018N	94	06-47-24	JP2=15	5/22/2006	JP2	1	"0 / 60f		327	
329	21211	5/23/2006	1635	SB018M	88	06-47-24		5/22/2006	JP2	1	"0 / 60f		329	
331	21229	5/23/2006	1837	SB018N	83	06-47-24		5/22/2006	JP2	1	"0 / 60f	"0 / 60f	331	
332	21339	5/23/2006	1927	SB018S	105	06-47-24		5/22/2006	JP2	1	"0 / 60f		332	
341	21011	5/24/2006	1719	SB018N	89	06-47-24		5/22/2006	JP2	2	"0 / 60f		341	
343	21293	5/24/2006	1743	SB018N	95	06-47-24		5/22/2006	JP2	2	"0 / 60f	"0 / 60f	343	
346	21335	5/24/2006	1807	SB018N	102	06-47-24		5/22/2006	JP2	2	"0 / 60f	"0 / 60f	346	
347	21286	5/24/2006	1832	SB018M	96	06-47-24		5/22/2006	JP2	2	"0 / 60f		347	
350	21294	5/24/2006	1923	SB018S	83	06-47-24		5/22/2006	JP2	2	"0 / 60f		350	
351	21349	5/25/2006	538	SB018M	86	06-47-24		5/22/2006	JP2	3	"0 / 60f	"0 / 60f	351	
353	21342	5/25/2006	836	SB018S	90	06-47-24		5/22/2006	JP2	3	"0 / 60f		353	
358	21277	5/26/2006	604	SB018S	85	06-47-24		5/22/2006	JP2	4	"0 / 60f	"0 / 60f	358	
364	21242	5/26/2006	1825	SB018M	88	06-47-24		5/22/2006	JP2	4	"0 / 60f		364	
365	21270	5/26/2006	1917	SB018S	95	06-47-24		5/22/2006	JP2	4	"0 / 60f		365	
371	21254	5/27/2006	1752	SB018S	94	06-47-24		5/22/2006	JP2	5	"0 / 60f	"0 / 60f	371	