U.S. Fish & Wildlife Service

California Nevada Fish Health Center
FY2012 Technical Report:

Ceratomyxa shasta myxospore survey of Fall-run Chinook salmon carcasses in the Klamath and Shasta Rivers, and comparison of Trinity River Spring-run to Fall-run carcasses, October-November 2012.

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Summary: Fall-run Chinook carcass intestines were collected from the Klamath River (Iron Gate Hatchery to Shasta R. mouth) and Shasta R. weir (Rm 1) in October and November 2012. Similar samples were collected from Trinity R. (Lewiston reach) spring and fall –run Chinook carcasses. An estimated 88% or greater of the Klamath and Shasta intestines were infected (DNA positive) with some life stage of C*eratomyxa.shasta.* Myxospores were detected in 22 – 36% of the surveyed carcasses with only 3- 9% producing high spore numbers (>500,000). This data is similar to previous surveys in 2008 - 2011. Mean percentage of intact myxospores (excludes methylene blue dye) in Shasta R. carcasses was 75 ± 8% and there was an inverse relationship between myxospore quantitiy and percent intact spores. Percent intact myxospores declined considerably over 32 – 53 days post-collection in refrigerated samples that were < 85% intact at the initial examination. Trinity R. spring -run carcasses contained higher myxospore concentrations than fall-run. This data highlights the influence of time post-infection in cold temperatures on myxospore input from adult salmon carcasses. Quantity of *C. shasta* myxospores in the sampled populations was as follows: Trinity Spring-run> Trinity Fall-run >Shasta Fall-run > Klamath Fall-run. The question of myxospore viability as well as quantity must be better understood in order to inform life cycle models.

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Objectives:

1. Determine prevalence of infection (POI) and concentration of *C.shasta* myxospores in adult fall-run Chinook carcass intestines from 2 regions immediately above the Klamath River *C.shasta* "infectious zone" for use in disease modeling.
2. Compare prevalence of infection (POI) and concentration of *C.shasta* myxospores in Trinity R. spring-run to fall-run Chinook carcass intestines

Methods:

Carcass myxospore – Trinity R. Spring-run and Fall-run Chinook carcass sampling occurred over consecutive weeks (12 and 18 Oct for spring-run, and 8 and 15 Nov for fall-run). All Trinity R. samples were collected between Old bridge (Rm 110.1) and Rush Creek (107.6 Rm). All Shasta R. samples were obtained at the California Department of Wildlife (previously Fish and Game) counting weir (Rm 1). Carcasses that washed onto the weir overnight were placed into a metal cage held within the river on Monday and sampled 3 d later. This procedure provided a minimum carcass age of 4d and was performed for 4 consecutive weeks (25 Oct – 15Nov). Klamath River carcasses (Rm 109.9 - 118.2, Iron Gate hatchery to confluence of Shasta R.) were collected between 10Oct and 6 Nov by USFWS Arcata FWO and Yurok Tribal Fisheries staff. An estimated 80% or more of the carcasses were deemed to be in stage 2 or 3 state of advanced decomposition (Baumsteiger and Kerby 2009). Fork length and sex were recorded for each sample, intestine (small intestine to rectum) was dissected from the carcass, placed into individually numbered plastic bags, and either refrigerated for 24h or frozen prior to processing. The sample was weighed, cut into 8 – 12 cm pieces, and an intestinal content sample (scraping) obtained by grasping the intestine with forceps and pushing the backside of a #21 scalpel blade, held at 45° angle, along the outside of the intestine. The process was repeated several times until only the serosa to stratum compactum layers remained. The scraping subsample was weighed, diluted 4x with PBS, poured into tubes, vortex mixed, and allowed to settle for 30s prior to microscopic examination. Any scrape weight <1.0 gram was diluted with 1.0 mL of PBS to provide enough sample material. A 100 µL aliquot was frozen for later QPCR analysis. Duplicate 20 µL samples of this suspension examined for the presence of *C.shasta* myxospores by 20x phase microscopy. Four hemocytometer counts on wet mount positive samples quantified myxospore concentration per gram of sample. This value multiplied by the scraping weight (g) provided the ***myxospore per scraping*** estimate. Given our limited detection sensitivity and other potentially infected tissues within a fish, we consider the myxospore / scraping value to represent the minimum spore load for a given fish. A serial dilution of a high spore concentration sample was examined by the wet mount method. The limit of screening detection for this sample was approximately 394 spores/scraping. In previous surveys we have observed a screening sensitivity of between 500 to 1000 spores/ scraping. The proportion of intact myxospores was evaluated in 19 "fresh" samples by diluting the hemocytometer sample in

0.25% (PBS) methylene blue dye solution (Hoffman and Markiw 1977). These samples were collected the day prior to processing and had not been frozen. Intact spores are refractive while a spore with an incomplete cell membrane and / or broken valve appears blue (Fig. 1).

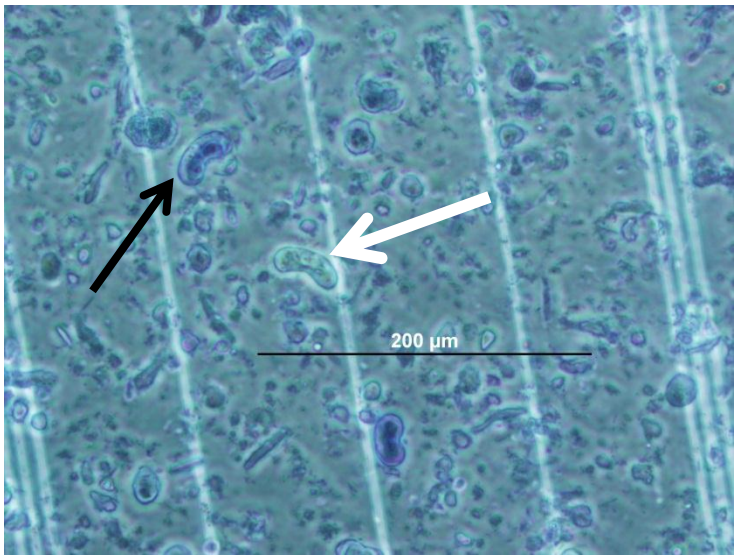

Figure 1. Photomicrograph (400x magnification) of *C.shasta* myxospores in 0.25% methylene blue dye. Intact spore (white arrow) is refractive while myxospore with disrupted valve or membrane absorbs the dye and is blue (black arrow).

In order to estimate the prevalence of infection by all *C.shasta* stages (trophozoite, pansporoblast, myxospore) in ***myxospore undetected*** samples, a subsample of the "undetected" scrapings from each sample site were assayed for *C.shasta* DNA. Scrapings were digested in 100ul MagMax Proteinase K Buffer containing 100 mg/ml proteinase K at 55°C with constant shaking for 2 hours. A subsample of digested tissue homogenate was diluted 1:10 in molecular grade water, then 1:10 in MagMAX Multi-Sample DNA Lysis Buffer (Applied Biosystems, Foster City, CA) for a final dilution of 1:100. The diluted tissue homogenate was extracted in a 96 well magnetic bead sample processing system (Applied Biosystems MagMAX Express-96 Magnetic Particle Processer). Extracted DNA was stored at -20ºC until the QPCR assays were performed. Samples were assayed in Real Time PCR Sequence Detection Systems (SDS) using 18S rDNA using TaqMan Fam-Tamra probe and primers (Hallett and Bartholomew 2006) specific to the parasite. Reaction volumes of 30L, containing 5µL DNA template, were used for both assays under the following amplification conditions: 50ºC for 2 min.; 95ºC for 10 min; 40 cycles of 95ºC for 15s and 60ºC for 1 min. Plasmid standards, extraction control and no template control (NTC) wells were included on each assay plate. Cycle threshold (CT) values were calculated using SDS software (7300 SDS v 1.3.1, StepOne SDS v. 2.0 Applied Biosystems) and a standard curve to transformed CT values to parasite DNA copy number.

Results:

Klamath and Shasta R. - Prevalence of myxospore detection in Klamath carcasses was 22% and 36% in the Shasta R. (Table 1). Only 3 and 9% of these carcass groups had 500,000 or greater myxospores / scraping (Fig 2). The sex ratio was skewed in both groups. Shasta carcasses were mostly male (71 of 80 samples) while Klamath were predominately female (46 of 65). Prevalence of myxospore detection was higher in males from both sites (Table 2). Total quantity of myxospores (summation of estimated myxospore / scraping) was 8.5x higher (21,288,185) in Shasta R. carcasses than Klamath carcasses (2,495,059). This trend for Shasta R. carcasses to produce more myxospores (i.e. prevalence of samples producing greater than 500,000 spores) than Klamath R. carcasses was also observed in 2009 and 2011 (Fig. 3).

QPCR analysis of undetected scraping samples of Shasta and Klamath carcasses showed that 81% and 87%, respectively, contained *C.shasta* DNA (Table 1). It is likely that trophozoite and pansporoblast stages were associated with this finding. When the prevalence of DNA detection is applied to the undetected sample numbers, estimated prevalence of *C.shasta* (all parasite life stages) infection is greater than 88% for both groups (Table 3).

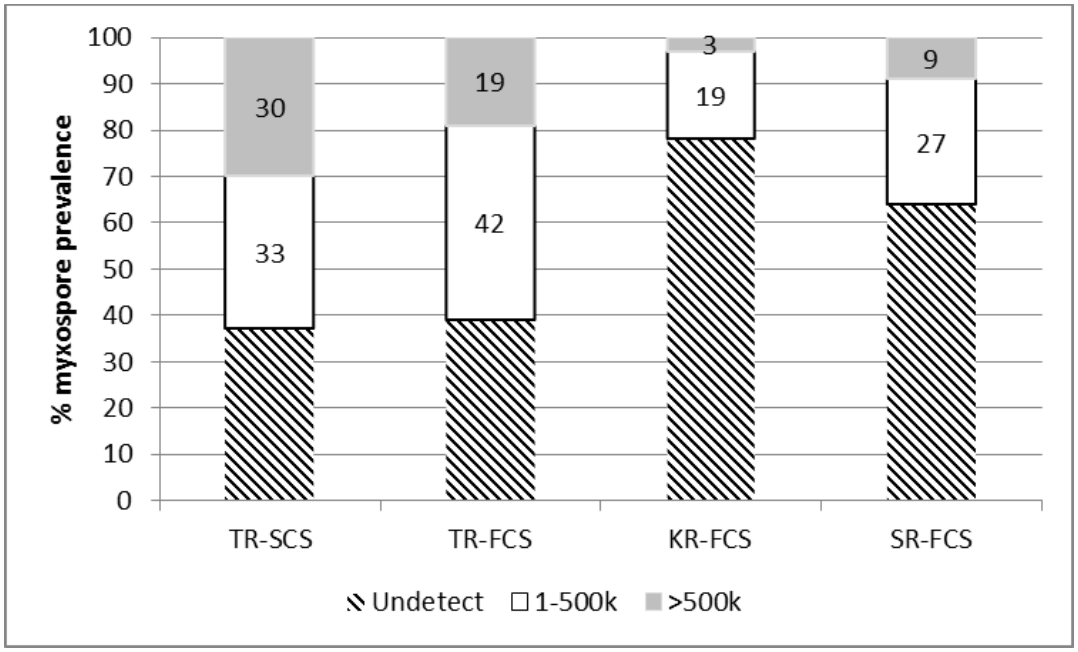

Figure 2. Prevalence of myxospore concentration categories of intestine samples collected from Trinity R (TR) spring-run Chinook (SCS) and fall-run (FCS), and fall-run Chinook carcasses from the Klamath R. (KR) and Shasta R. weir (SR). Categories are: Undetected by wet mount, 1 – 500,000 spores, and greater than 500,000 spores.

Table 1. Prevalence of *C. shasta* myxospore (POI = number positive / total collection) detection in intestine samples from Spring-run (SCS) and Fall-run Chinook (FCS) salmon carcasses collected in the Trinity, Shasta, and Klamath Rivers between 12October and 15November. Data includes prevalence of carcasses yielding ≥ 500,000 myxospores/ scraping and *C.shasta* DNA detection prevalence of myxospore undetected samples.

Carcass group	Sample date	POI myxospore	POI > 500,000 spores	myxospore sum	POI Cs DNA in undetects
TrinityR-SCS	12-Oct	21 / 30 (70%)			
	18-Oct	17 / 30 (57%)			
	totals	38 / 60 (63%)	18/60 (30%)	81,268,525	8/10 (80%)
TrinityR-FCS	8-Nov	17 / 30 (57%)			
	15-Nov	19 / 29 (65%)			
	totals	36 / 59 (61%)	11 /59 (19%)	95,821,713	10 /10 (100%)
				(33,871,713**)	
ShastaR-FCS	25-Oct	10 / 20 (50%)			
	1-Nov	7 / 20 (35%)			
	8-Nov	7 / 20 (35%)			
	15-Nov	5 / 20 (25%)			
	totals	29 / 80 (36%)	7 / 80 (9%)	21,288,185	22 / 27 (81%)
KlamathR-FCS	10-Oct	0 / 2 (0%)			
	16-Oct	1 / 4 (25%			
	23-Oct*	4 / 21 (19%)			
	30-Oct	2 / 20 (10%)			
	6-Nov	7 / 18 (39%)			
	totals	14 / 65 (22%)	2 / 65 (3%)	2,495,069	20 / 23 (87%)

*includes 3 carcasses collected on 24October
**One TR-FCS carcass contain 61.95million spore and skewed the stock spore total. When this individual outlier is removed, the sum is 33.87 million for TR-FCS.

Table 2. Prevalence of *C. shasta* myxospore (POI = number positive / total collection) detection in male and female intestine samples from Spring-run (SCS) and Fall-run Chinook (FCS) salmon carcasses collected in the Trinity, Shasta, and Klamath Rivers between 12October and 15November. Data includes estimated myxospore / scraping range for positive samples.

Carcass group	Sample date	Male	Female	Myxospore/scraping
TrinityR-SCS	12-Oct	11 / 15 (73%)	10 / 15 (67%)	
	18-Oct	9 / 12 (75%)	8 / 18 (44%)	
	totals	20 /27 (74%)	18 / 33 (55%)	1175 - 17,760,000
TrinityR-FCS	8-Nov	11 / 16 (69%)	7 / 14 (50%)	
	15-Nov	7 / 15 (47%)	8 / 14 (57%)	
	totals	18 / 31 (58%)	15 / 28 (52%)	1750 - 61,950,000
ShastaR-FCS	25-Oct	9 /16 (56%)	1 / 4 (25%)	
	1-Nov	7 / 19 (37%)	0 / 1 (0%)	
	8-Nov	7 / 18 (39%)	0 / 2 (0%)	
	15-Nov	5 / 18 (28%)	0 / 2 (0%)	
	totals	28 / 71 (39%)	1 / 9 (11%)	375 - 7,280,000
KlamathR-FCS	10-Oct	0 / 1 (0%)	0 / 1 (0%)	
	16-Oct	1 / 3 (33%)	0 / 1 (0%)	
	23-Oct*	2 / 8 (25%)	2 / 13 (15%)	
	30-Oct	1 / 5 (20%)	1 / 15 (7%)	
	6-Nov	2 / 2 (100%)	5 /16 (31%)	
	totals	6 / 19 (32%)	8 / 46 (17%)	312 - 812,270

Table 3. Estimated *C.shasta* infection (all parasite stages) in carcass intestines from Spring-run (SCS) and Fall-run Chinook (FCS) salmon collected in the Trinity (TR), Shasta (SR), and Klamath Rivers (KR) between 12October and 15November. POI of DNA positive samples was multiplied by the number of myxospore undetected samples and the product added to the myxospore positive sample number.

			POI	Est. infect	Estimate	Estimate
Stock	Undetect	Myxo+	DNA+	Undetect	Cs infected	POI
TR-SCS	22	38	0.8	22x 08 = 18	38+18 = 56	56 / 60= 93%
TR-FCS	23	36	1.0	23x 1.0 = 23	23+36 = 59	59 / 59 = 100%
SR-FCS	51	29	0.81	51x0.81 = 41	41+29 = 70	70 / 80 = 88%
KR-FCS	51	14	0.87	51x 0.87 = 44	44+14 = 58	58 / 65 = 89%

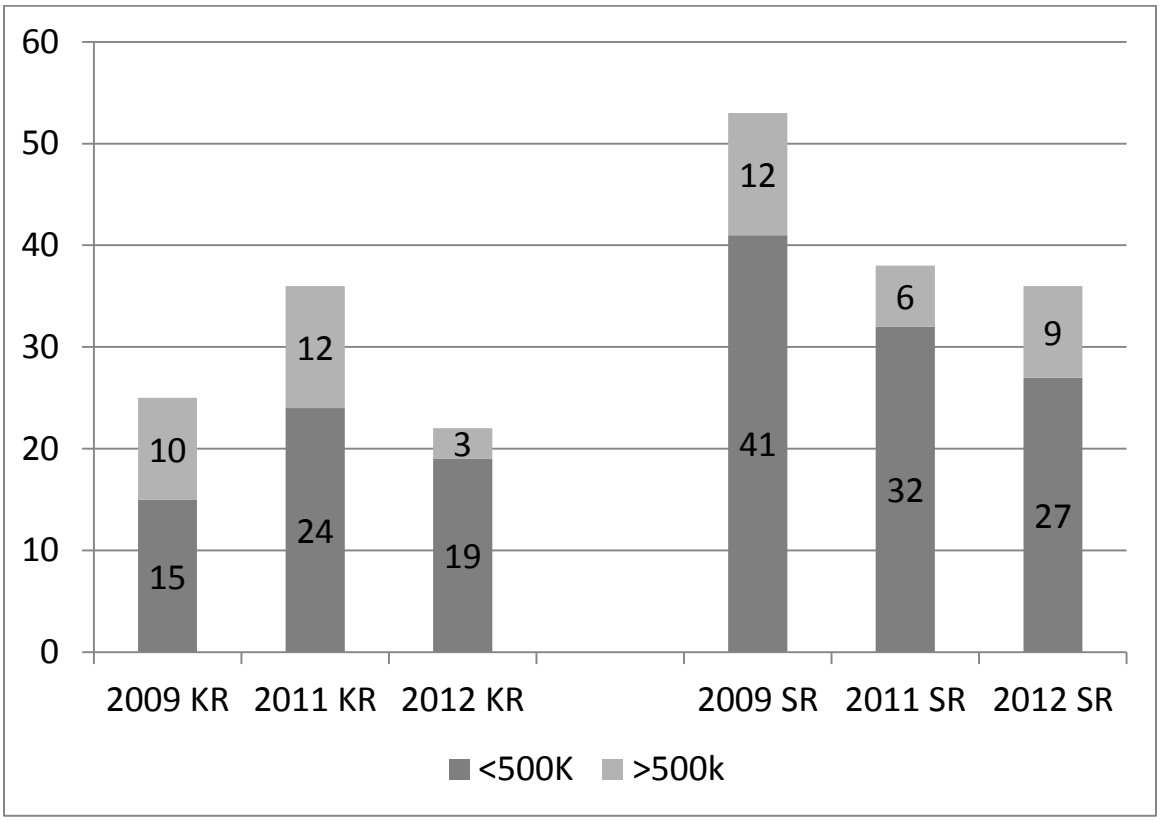

Figure 3. Prevalence of *C.shasta* myxospore detection in Fall-run Chinook salmon carcass intestines from Klamath (KR) and Shasta R. (SR) collected in 2009, 2011, and 2012. Bars are divided between low spore (<500,000/scraping) and high spore (≥ 500,000 / scraping) samples.

Comparison of Spring-run to Fall-run: Prevalence of myxospore detection in Trinity R. spring-run (63%) was similar to fall-run (61%) Chinook carcasses, however spring-run had a higher prevalence of carcasses (30% compared to 19% for fall-run) containing ≥ 500,000 myxospores (Table 1, Fig. 2). A fall-run carcass, collected on 15Nov, contained an estimated 61.5 million myxospores / scraping. If this outlier is removed from the fall-run dataset, total myxospore estimate from spring-run (81.3 million) is 2.4x greater than fall-run (33.9 million). One Coho carcass was sampled on 15Nov and contained 45 million myxospores.

Similar numbers of male and female carcasses were collected from both runs (Table 2). No difference by sex was detected in myxospore quantity for either run (Mann-Whitney rank sum test, P=3.85 and P= 0.088). No obvious relationship between fork length (surrogate for age) and myxospore yield was seen for either run or sex in linear regression equations (R-square ≤ 0.055). Greater than 93% of the carcasses were estimated to be infected by some stage of *C.shasta* ([POI DNA positive x undetected]+myxospore positive/ total carcass).

The higher myxospore load observed in Spring-run could bep a result of 3 factors: 1) longer period for parasite development (time post-infection), 2) higher cumulative temperature history and, 3) potentially a different infectious dose upon entry into the Klamath River. An estimate of freshwater residency and temperature history is presented in Table 4. Peak entry timing and carcass recovery (Lewiston reach) of the 2 runs was estimated from California Department of Wildlife (previously named Fish and Game) 2012 Klamath estuary angler harvest and carcass data (S. Borok and A. Hill, CDW, pers. comm.). Mean weekly water temperature in the estuary (USGS 11530500 KLAMATH R NR KLAMATH CA), lower Trinity R. (USGS 11530000 TRINITY R A HOOPA CA), and spawning reach (Trinity River at Douglas City (DGC), Douglas City – Lewiston) was obtained from USGS and CDWR gauges (KLAMATH CA http://waterdata.usgs.gov/usa/nwis/uv?11530500, HOOPA CA http://waterdata.usgs.gov/ca/nwis/uv/?site_no=11530000&PARAmeter_cd=00065,00060, Douglas City http://cdec.water.ca.gov/cgi-progs/selectQuery?station_id=DGC&sensor).

A two week period, before and after the peak, was selected for the calculation of residency and temperature exposure range. We made the following assumptions in calculating accumulated temperature units (ATU= summation of mean daily temperature ºC); an adult salmon would spend 7d in the estuary / Klamath river, another 7d in the lower Trinity, and then hold in the lower temperature zone above Douglas city until spawning and death (carcass recovery). Mean weekly water temperatures of these 3 sites were multiplied by 7 d and the sum of weekly temperature exposure summed to obtain the ATU (Appendix 3).

Spring-run spend almost twice as long in freshwater (mean 119d, 105-123 d range) as fall-run (63d, 49 – 77d range). Primarily due to a longer holding period in the spawning reach, spring-run experienced 46 % to 59% higher ATUs (1581 ºC ATU for peak entry, 1466 – 1716 range) than fall-run (846 ºC ATU, 675 –

1012 range). Wagner et al. (2005) report the positive influence of ATU > 450ºC on the myxosporean parasite *Parvicapsula minibicornis* infection severity on adult Fraser R. sockeye but did not discuss myxospore production.

The hypothesis that spring-run experienced a higher infectious actinospore challenge upon entry into the Klamath R is not supported by 2012 water sample data (S. Hallett, Oregon State University, http://microbiology.science.oregonstate.edu/files/micro/BOR_Update_Sept_2012.pdf). *C.shasta* eDNA quantity at Orleans (Rm 56, KOR) and Tully Creek (Rm 39, KTC) was similar in mid-June and early September 2012 in the lower Klamath River (Fig. 4).

Table 4. Estimated peak freshwater entry timing and carcass recovery for Trinity R. Spring and Fall-run Chinook adults by Julian week (first day of this week),

	FW entry	Carcass Recovery
Spring-run	Julian week 25 (17June)	Julian week 42 (14October)
Fall-run	Julian week 37 (9September)	Julian week 46 (11November)

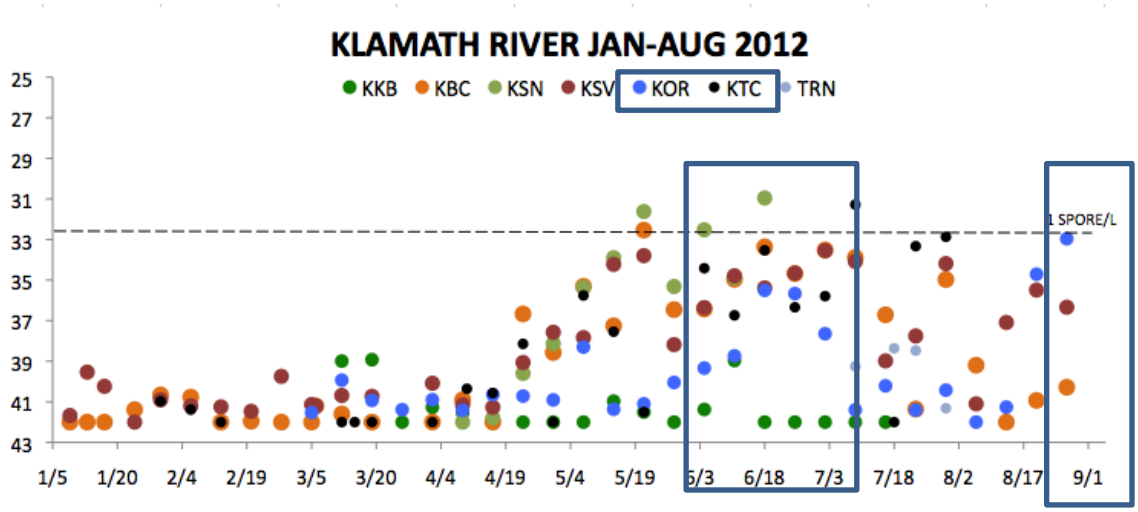

Figure 4. Density of *Ceratomyxa shasta* in 1L water samples collected at Klamath River mainstem index sites in 2012**.** Each data point is the average Cq of 3 x 1L water samples collected weekly. A lower Cq value indicates more parasite is present. Boxes highlight the mid-June to early July (spring-run) and early September (fall-run) freshwater entry times for *C.shasta* DNA collected at Orleans (KOR) and Tully Creek (KTC). Figure was modified from that produced by S. Hallett, Oregon State University.

Despite a shorter migration (exposure) in the Klamath River, Trinity R. Fall-run had a higher prevalence of myxospore infection (61%) than either Shasta (36%) or Klamath R. (22%) carcasses. Trinity fall-run carcasses also yielded more myxospores than the Klamath or Shasta carcasses. This difference in myxospore infection prevalence and severity may be related to lower resistance to *C.shasta* infection in Trinity R salmon given the reduced ceratomyxosis selective pressure on this population.

Myxospore quality (intact valve) –Nineteen non-frozen samples were examined with 0.25% methylene blue twenty-four hours after collection (Appendix 2). The percent intact myxospore values ranged from 7 to 100% at this time point (Fig. 5) with a trend towards lower intact spores in higher concentration samples (Linear regression equation %intact=83.180-(12.872*million myxospore /scraping, R-squared = 0.425). When the refrigerated samples were read a second time at 32 to 53d post-collection (dpc), the percent intact spore declined sharply in samples where the 1 dpc value was < 85% (Fig . 6).

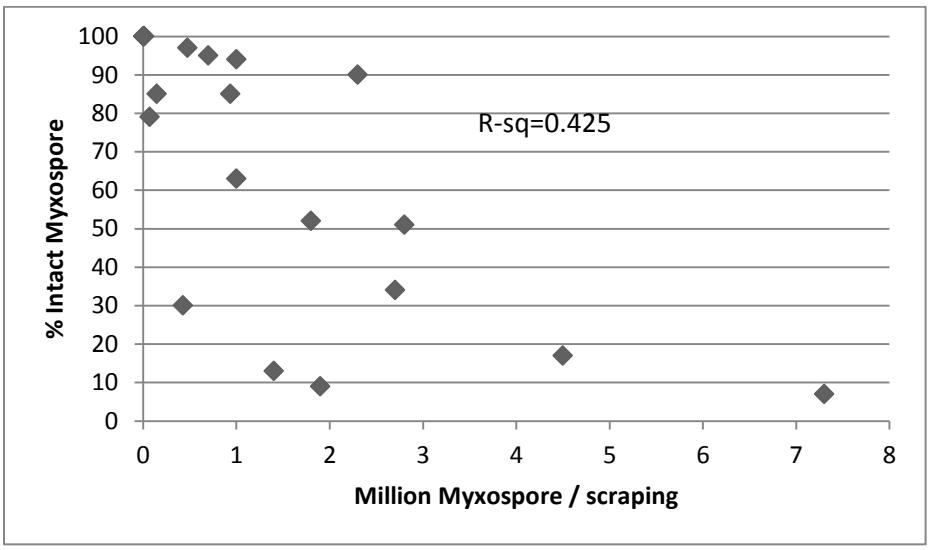

Figure 5. Relationship between myxospore/scraping quantity and percent intact myxospore.

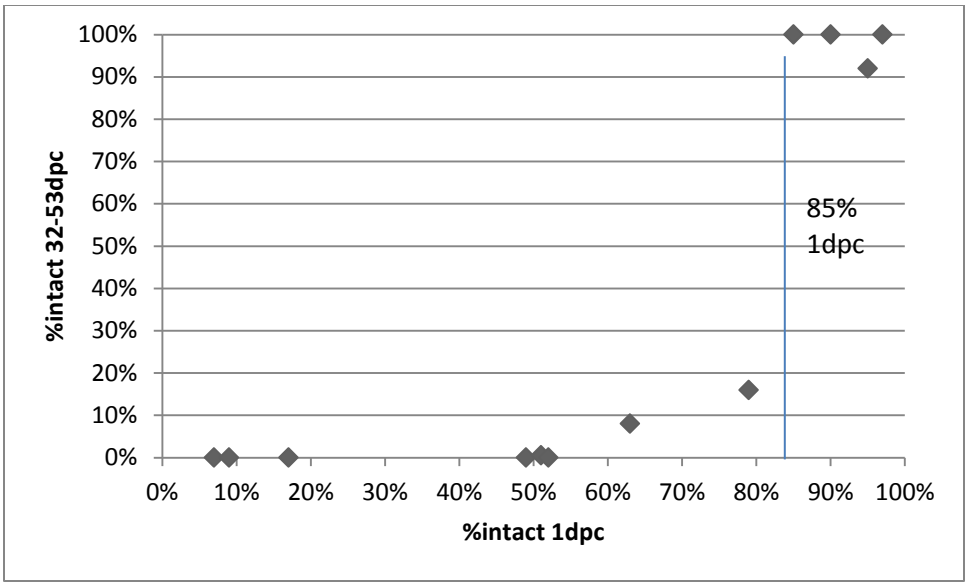

Figure 6 . Relationship between %intact myxospore at 1 day post collection (dpc) and 32-53 dpc.

In summary, an estimated ≥ 88% of the Klamath and Shasta R. carcasses were infected (DNA positive) with some stage of *C.shasta.* Myxospores were detected in 22 – 36% of the surveyed carcasses with only 3- 9% of these carcasses considered to be high spore contributors (>500,000). This data is similar to previous surveys. Mean percentage of intact myxospores in Shasta R. carcasses was 75 ± 8% and there was an inverse relationship between myxospore number and % intact spores. When the refrigerated myxspores were examined after 32-53 dpc, only those samples with ≥ 85% intact at 1 dpc remained intact. While POI of myxospore infection was similar, Trinity spring-run produced higher myxospore concentrations than fall-run carcasses. It is likely that this difference in myxospore production is related to longer time post-infection experienced by spring-run.

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Morgan Knechtle and the CDW carcass crew for access to Shasta weir samples and Arcata FWO and Yurok fisheries carcass crew for collection of Klamath carcasses. Andy Hill (CDW) for providing rafting assistance for Trinity R. carcass collection and carcass data. Wade Sinnen and Sarah Borok (CDW) for Klamath R. angler harvest and Trinity R. weir data.

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Appendix 1.

Site, subsite (reach), collection date, carcass forklength (cm) and sex, decomposed (D) or fresh (R), and myxospores/scraping data for Shasta R., Klamath R., and Trinity R. collections in 2012.

site	subsite	presumpRUN	col-date	FLcm	SEX	Decomp	myxo/scrape
TRINITY	LEWISTON	SCS	10/12/2012	61	F	D	0
TRINITY	LEWISTON	SCS	10/12/2012	64	M	D	0
TRINITY	LEWISTON	SCS	10/12/2012	76	F	D	0
TRINITY	LEWISTON	SCS	10/12/2012	78	M	D	0
TRINITY	LEWISTON	SCS	10/12/2012	65	F	D	5915000
TRINITY	LEWISTON	SCS	10/12/2012	61	F	D	0
TRINITY	LEWISTON	SCS	10/12/2012	65	F	D	0
TRINITY	LEWISTON	SCS	10/12/2012	75	M	D	77500
TRINITY	LEWISTON	SCS	10/12/2012	75	M	D	0
TRINITY	LEWISTON	SCS	10/12/2012	68	M	D	1848000
TRINITY	LEWISTON	SCS	10/12/2012	62	M	D	532000
TRINITY	LEWISTON	SCS	10/12/2012	67	F	D	43500
TRINITY	LEWISTON	SCS	10/12/2012	66	F	D	16000
TRINITY	LEWISTON	SCS	10/12/2012	58	F	D	990000
TRINITY	LEWISTON	SCS	10/12/2012	63	F	D	22000
TRINITY	LEWISTON	SCS	10/12/2012	85	M	D	210000
TRINITY	LEWISTON	SCS	10/12/2012	80	M	D	17760000
TRINITY	LEWISTON	SCS	10/12/2012	67	M	D	7500
TRINITY	LEWISTON	SCS	10/12/2012	65	M	D	477000
TRINITY	LEWISTON	SCS	10/12/2012	68	F	D	6500
TRINITY	LEWISTON	SCS	10/12/2012	60	F	D	13500
TRINITY	LEWISTON	SCS	10/12/2012	75	M	D	5940000
TRINITY	LEWISTON	SCS	10/12/2012	84	M	D	6080000
TRINITY	LEWISTON	SCS	10/12/2012	70	M	D	0
TRINITY	LEWISTON	SCS	10/12/2012	63	F	D	3500
TRINITY	LEWISTON	SCS	10/12/2012	65	F	D	6545000
TRINITY	LEWISTON	SCS	10/12/2012	67	M	D	880000
TRINITY	LEWISTON	SCS	10/12/2012	75	M	D	17752500
TRINITY	LEWISTON	SCS	10/12/2012	63	F	D	0
TRINITY	LEWISTON	SCS	10/12/2012	70	F	D	164500
TRINITY	LEWISTON	SCS	10/18/2012	61	F	D	1168000
TRINITY	LEWISTON	SCS	10/18/2012	73	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	89	m	D	1032750

TRINITY	LEWISTON	SCS	10/18/2012	73	m	D	0
TRINITY	LEWISTON	SCS	10/18/2012	63	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	63	f	D	454725
TRINITY	LEWISTON	SCS	10/18/2012	72	m	D	271350
TRINITY	LEWISTON	SCS	10/18/2012	67	m	D	1175
TRINITY	LEWISTON	SCS	10/18/2012	68	f	D	838750
TRINITY	LEWISTON	SCS	10/18/2012	68	f	D	1926000
TRINITY	LEWISTON	SCS	10/18/2012	71	m	D	476000
TRINITY	LEWISTON	SCS	10/18/2012	63	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	79	m	D	12000
TRINITY	LEWISTON	SCS	10/18/2012	63	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	64	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	64	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	73	m	D	2250
TRINITY	LEWISTON	SCS	10/18/2012	63	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	68	m	D	1416525
TRINITY	LEWISTON	SCS	10/18/2012	72	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	59	m	D	0
TRINITY	LEWISTON	SCS	10/18/2012	84	f	D	72000
TRINITY	LEWISTON	SCS	10/18/2012	73	m	D	0
TRINITY	LEWISTON	SCS	10/18/2012	69	f	D	429000
TRINITY	LEWISTON	SCS	10/18/2012	61	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	61	f	D	4462500
TRINITY	LEWISTON	SCS	10/18/2012	55	f	D	0
TRINITY	LEWISTON	SCS	10/18/2012	85	m	D	1500
TRINITY	LEWISTON	SCS	10/18/2012	77	m	D	690000
TRINITY	LEWISTON	SCS	10/18/2012	70	f	D	2730000
TRINITY	LEWISTON	FCS	11/8/2012	67	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	68	M	D	0
TRINITY	LEWISTON	FCS	11/8/2012	74	M	D	15000
TRINITY	LEWISTON	FCS	11/8/2012	62	M	D	70788
TRINITY	LEWISTON	FCS	11/8/2012	60	F	D	9000
TRINITY	LEWISTON	FCS	11/8/2012	54	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	77	M	D	66500
TRINITY	LEWISTON	FCS	11/8/2012	70	M	D	802438
TRINITY	LEWISTON	FCS	11/8/2012	77	M	D	0
TRINITY	LEWISTON	FCS	11/8/2012	74	M	D	0
TRINITY	LEWISTON	FCS	11/8/2012	70	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	68	M	D	271863
TRINITY	LEWISTON	FCS	11/8/2012	77	M	D	0
TRINITY	LEWISTON	FCS	11/8/2012	82	M	D	6750
TRINITY	LEWISTON	FCS	11/8/2012	77	M	D	0
TRINITY	LEWISTON	FCS	11/8/2012	78	M	D	5002250

TRINITY	LEWISTON	FCS	11/8/2012	60	F	D	30000
TRINITY	LEWISTON	FCS	11/8/2012	54	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	45	M	D	0
TRINITY	LEWISTON	FCS	11/8/2012	68	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	68	F	D	3350
TRINITY	LEWISTON	FCS	11/8/2012	72	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	59	F	D	21088
TRINITY	LEWISTON	FCS	11/8/2012	84	F	D	0
TRINITY	LEWISTON	FCS	11/8/2012	70	F	D	1750
TRINITY	LEWISTON	FCS	11/8/2012	77	F	D	193938
TRINITY	LEWISTON	FCS	11/8/2012	71	F	D	687500
TRINITY	LEWISTON	FCS	11/8/2012	62	M	D	162500
TRINITY	LEWISTON	FCS	11/8/2012	64	M	D	6720000
TRINITY	LEWISTON	FCS	11/8/2012	62	M	D	300000
TRINITY	LEWISTON	FCS	11/15/2012	72	M	D	190000
TRINITY	LEWISTON	coho	11/15/2012	63	F	D	44950000
TRINITY	LEWISTON	FCS	11/15/2012	58	M	D	656250
TRINITY	LEWISTON	FCS	11/15/2012	64	F	D	6000
TRINITY	LEWISTON	FCS	11/15/2012	68	M	D	1202500
TRINITY	LEWISTON	FCS	11/15/2012	71	M	D	25000
TRINITY	LEWISTON	FCS	11/15/2012	63	F	D	5513
TRINITY	LEWISTON	FCS	11/15/2012	67	M	D	29750
TRINITY	LEWISTON	FCS	11/15/2012	50	M	D	45000
TRINITY	LEWISTON	FCS	11/15/2012	78	M	D	0
TRINITY	LEWISTON	FCS	11/15/2012	87	M	D	33000
TRINITY	LEWISTON	FCS	11/15/2012	73	F	D	181688
TRINITY	LEWISTON	FCS	11/15/2012	65	F	D	324000
TRINITY	LEWISTON	FCS	11/15/2012	73	M	D	9546500
TRINITY	LEWISTON	FCS	11/15/2012	68	M	D	4000
TRINITY	LEWISTON	FCS	11/15/2012	77	F	D	0
TRINITY	LEWISTON	FCS	11/15/2012	62	F	D	61950000
TRINITY	LEWISTON	FCS	11/15/2012	66	F	D	746550
TRINITY	LEWISTON	FCS	11/15/2012	72	M	D	0
TRINITY	LEWISTON	FCS	11/15/2012	69	M	D	0
TRINITY	LEWISTON	FCS	11/15/2012	75	F	D	4500
TRINITY	LEWISTON	FCS	11/15/2012	71	F	D	60000
TRINITY	LEWISTON	FCS	11/15/2012	66	M	D	2070000
TRINITY	LEWISTON	FCS	11/15/2012	66	F	D	0
TRINITY	LEWISTON	FCS	11/15/2012	78	M	D	0
TRINITY	LEWISTON	FCS	11/15/2012	84	F	D	4376750
TRINITY	LEWISTON	FCS	11/15/2012	98	M	D	0
TRINITY	LEWISTON	FCS	11/15/2012	71	F	D	0
TRINITY	LEWISTON	FCS	11/15/2012	78	F	D	0

TRINITY	LEWISTON	FCS	11/15/2012	69	F	D	0
SHASTA	WEIR	FCS	10/25/2012	80	m	D	0
SHASTA	WEIR	FCS	10/25/2012	53	f	D	0
SHASTA	WEIR	FCS	10/25/2012	55	f	D	697500
SHASTA	WEIR	FCS	10/25/2012	75	m	D	1807800
SHASTA	WEIR	FCS	10/25/2012	52	m	D	0
SHASTA	WEIR	FCS	10/25/2012	51	m	D	375
SHASTA	WEIR	FCS	10/25/2012	51	m	D	0
SHASTA	WEIR	FCS	10/25/2012	53	m	D	34746
SHASTA	WEIR	FCS	10/25/2012	51	m	D	476250
SHASTA	WEIR	FCS	10/25/2012	50	m	D	1003200
SHASTA	WEIR	FCS	10/25/2012	70	m	D	0
SHASTA	WEIR	FCS	10/25/2012	50	m	D	0
SHASTA	WEIR	FCS	10/25/2012	46	m	D	93750
SHASTA	WEIR	FCS	10/25/2012	73	m	D	0
SHASTA	WEIR	FCS	10/25/2012	75	f	D	0
SHASTA	WEIR	FCS	10/25/2012	64	m	D	0
SHASTA	WEIR	FCS	10/25/2012	79	m	D	2760000
SHASTA	WEIR	FCS	10/25/2012	69	f	D	0
SHASTA	WEIR	FCS	10/25/2012	40	m	D	0
SHASTA	WEIR	FCS	10/25/2012	41	m	D	6000
SHASTA	WEIR	FCS	11/1/2012	53	M	D	0
SHASTA	WEIR	FCS	11/1/2012	51	M	D	0
SHASTA	WEIR	FCS	11/1/2012	48	M	D	1375
SHASTA	WEIR	FCS	11/1/2012	53	M	D	0
SHASTA	WEIR	FCS	11/1/2012	61	M	D	0
SHASTA	WEIR	FCS	11/1/2012	81	M	D	0
SHASTA	WEIR	FCS	11/1/2012	53	M	D	0
SHASTA	WEIR	FCS	11/1/2012	79	M	D	0
SHASTA	WEIR	FCS	11/1/2012	51	M	D	7280000
SHASTA	WEIR	FCS	11/1/2012	46	M	D	6563
SHASTA	WEIR	FCS	11/1/2012	81	M	D	0
SHASTA	WEIR	FCS	11/1/2012	83	M	D	0
SHASTA	WEIR	FCS	11/1/2012	47	M	D	2365000
SHASTA	WEIR	FCS	11/1/2012	53	M	D	3025
SHASTA	WEIR	FCS	11/1/2012	70	M	D	0
SHASTA	WEIR	FCS	11/1/2012	64	F	D	0
SHASTA	WEIR	FCS	11/1/2012	57	M	D	0
SHASTA	WEIR	FCS	11/1/2012	48	M	D	11700
SHASTA	WEIR	FCS	11/1/2012	45	M	D	0
SHASTA	WEIR	FCS	11/1/2012	37	M	D	70225
SHASTA	WEIR	FCS	11/8/2012	50	M	D	3025
SHASTA	WEIR	FCS	11/8/2012	77	M	D	98000

SHASTA	WEIR	FCS	11/8/2012	52	M	D	0
SHASTA	WEIR	FCS	11/8/2012	53	M	D	11375
SHASTA	WEIR	FCS	11/8/2012	44	M	D	0
SHASTA	WEIR	FCS	11/8/2012	48	M	D	0
SHASTA	WEIR	FCS	11/8/2012	52	M	D	144788
SHASTA	WEIR	FCS	11/8/2012	63	F	D	0
SHASTA	WEIR	FCS	11/8/2012	68	M	D	0
SHASTA	WEIR	FCS	11/8/2012	42	M	D	0
SHASTA	WEIR	FCS	11/8/2012	78	M	D	303125
SHASTA	WEIR	FCS	11/8/2012	46	M	D	0
SHASTA	WEIR	FCS	11/8/2012	80	M	D	935175
SHASTA	WEIR	FCS	11/8/2012	71	F	D	0
SHASTA	WEIR	FCS	11/8/2012	49	M	D	0
SHASTA	WEIR	FCS	11/8/2012	67	M	D	30375
SHASTA	WEIR	FCS	11/8/2012	55	M	D	0
SHASTA	WEIR	FCS	11/8/2012	55	M	D	0
SHASTA	WEIR	FCS	11/8/2012	50	M	D	0
SHASTA	WEIR	FCS	11/15/2012	55	M	D	0
SHASTA	WEIR	FCS	11/15/2012	82	M	D	0
SHASTA	WEIR	FCS	11/15/2012	50	M	D	0
SHASTA	WEIR	FCS	11/15/2012	46	M	D	13750
SHASTA	WEIR	FCS	11/15/2012	56	M	D	377813
SHASTA	WEIR	FCS	11/15/2012	87	M	D	0
SHASTA	WEIR	FCS	11/15/2012	52	M	D	0
SHASTA	WEIR	FCS	11/15/2012	69	F	D	0
SHASTA	WEIR	FCS	11/15/2012	76	M	D	0
SHASTA	WEIR	FCS	11/15/2012	56	M	D	0
SHASTA	WEIR	FCS	11/15/2012	54	M	D	0
SHASTA	WEIR	FCS	11/15/2012	50	M	D	315000
SHASTA	WEIR	FCS	11/15/2012	83	M	D	0
SHASTA	WEIR	FCS	11/15/2012	48	M	D	0
SHASTA	WEIR	FCS	11/15/2012	54	M	D	2437500
SHASTA	WEIR	FCS	11/15/2012	59	M	D	750
SHASTA	WEIR	FCS	11/15/2012	59	F	D	0
SHASTA	WEIR	FCS	11/15/2012	52	M	D	0
SHASTA	WEIR	FCS	11/15/2012	52	M	D	0
SHASTA	WEIR	FCS	11/15/2012	53	M	D	0

KLAMATH	Igh-shasta	FCS	10/10/2012	73	f	D	0
KLAMATH	Igh-shasta	FCS	10/10/2012	72	m	D	0
KLAMATH	Igh-shasta	FCS	10/16/2012	75	f	D	0
KLAMATH	Igh-shasta	FCS	10/16/2012	66	m	D	313
KLAMATH	Igh-shasta	FCS	10/16/2012	92	m	D	0
KLAMATH	Igh-shasta	FCS	10/16/2012	74	m	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	64	f	D	188438
KLAMATH	Igh-shasta	FCS	10/23/2012	57	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	68	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	80	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	70	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	70	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	63	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	63	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	68	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	75	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	71	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	90	f	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	80	m	D	140250
KLAMATH	Igh-shasta	FCS	10/23/2012	76	m	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	90	m	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	85	m	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	81	m	D	0
KLAMATH	Igh-shasta	FCS	10/23/2012	88	m	D	0
KLAMATH	Igh-shasta	FCS	10/24/2012	82	f	D	2813
KLAMATH	Igh-shasta	FCS	10/24/2012	84	m	D	821250
KLAMATH	Igh-shasta	FCS	10/24/2012	78	m	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	75	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	71	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	73	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	81	F	D	451050
KLAMATH	Igh-shasta	FCS	11/6/2012	70	F	D	3125
KLAMATH	Igh-shasta	FCS	11/6/2012	60	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	73	F	D	2794
KLAMATH	Igh-shasta	FCS	11/6/2012	68	M	D	55025
KLAMATH	Igh-shasta	FCS	11/6/2012	73	M	D	189281
KLAMATH	Igh-shasta	FCS	11/6/2012	75	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	95	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	77	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	73	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	73	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	69	F	D	52500

KLAMATH	Igh-shasta	FCS	11/6/2012	72	F	D	0
KLAMATH	Igh-shasta	FCS	11/6/2012	70	F	D	813
KLAMATH	Igh-shasta	FCS	11/6/2012	69	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	70	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	67	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	68	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	69	f	D	20419
KLAMATH	Igh-shasta	FCS	10/30/2012	70	m	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	71	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	67	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	80	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	68	f	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	80	m	D	567000
KLAMATH	Igh-shasta	FCS	10/30/2012	71	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	47	m	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	72	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	88	m	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	73	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	74	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	78	m	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	68	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	74	F	D	0
KLAMATH	Igh-shasta	FCS	10/30/2012	70	F	D	0

Appendix 2 Intact myxospore data of scarping suspension read at 2 time points (1 day post collection (dpc) and 32 -53 dpc) in 0.25% methylene blue dye / PBS.

million spore	collectdate		MEAN%INTACT	READ DATE	DPC		MEAN%INTACT	READ DATE	DPC
0.697	25-Oct	SHASTA3	0.95	26-Oct	1.00		0.92	10-Dec	46.00
1.8	25-Oct	SHASTA 4	0.52	26-Oct	1.00		0	10-Dec	46.00
0.476	25-Oct	SHASTA 9	0.97	26-Oct	1.00		1	10-Dec	46.00
1	25-Oct	SHASTA10	0.63	26-Oct	1.00		0.08	10-Dec	46.00
2.8	25-Oct	SHASTA17	0.51	26-Oct	1.00		0.005	10-Dec	46.00
0.145	8-Nov	SHASTA 52	0.85	9-Nov	1.00		1	10-Dec	32.00
0.935	8-Nov	s54	0.85	9-Nov	1.00				
0.011	1-Nov	s38	1	2-Nov	1.00				
7.3	1-Nov	SHASTA29 (TUBE108)	0.07	2-Nov	1.00		0	10-Dec	39.00
2.3	1-Nov	SHASTA33 (112)	0.9	2-Nov	1.00		1	10-Dec	39.00
0.072	1-Nov	SHASTA40 (119)	0.79	2-Nov	1.00		0.16	10-Dec	39.00
0.007	1-Nov	s30	1	2-Nov	1.00				
0.003	1-Nov	s34	1	2-Nov	1.00				
1.4	23-Oct	KLAMATH4(frozen)	0.49	26-Oct	3.00		0	10-Dec	48.00
1.9	18-Oct	TRINTIY40	0.09	19-Oct	1.00		0	10-Dec	53.00
4.5	18-Oct	TRINITY 56	0.17	19-Oct	1.00		0	10-Dec	53.00
1	18-Oct	t33	0.94	19-Oct	1.00				
1.4	18-Oct	t49	0.13	19-Oct	1.00				
0.429	18-Oct	t54	0.3	19-Oct	1.00				
2.7	18-Oct	t60	0.34	19-Oct	1.00				

Appendix 3. Accumulated temperature unit (ATU) calculations based on entry timing and time spent in spawning reach.

									mean weekly temp		
day	julian week		ATU-KR	ATU-HPA	ATU-DC				Klam	HPA	DC
6-May	19	xx									
13-May	20	xx									
20-May	21	xx									
27-May	22	xx									
3-Jun	23	xx	105						15		
10-Jun	24	xx	117.6	110.6					16.8	15.8	
17-Jun	25	SCSpeak	126.7	114.1	77				18.1	16.3	11
24-Jun	26	xx	119	108.5	77				17	15.5	11
1-Jul	27	xx	134.4	126.7	77				19.2	18.1	11
8-Jul	28	xx		139.3	77					19.9	11
15-Jul	29	xx			91						13
22-Jul	30	xx			91						13
29-Jul	31	xx			91						13
5-Aug	32				91						13
12-Aug	33		161		91	xx			23		13
19-Aug	34		153.3	140	91	xx			21.9	20	13
26-Aug	35		142.8	126	91	xx			20.4	18	13
2-Sep	36		139.3	124.6	91	xx			19.9	17.8	13
9-Sep	37		136.5	123.9	77	fcs peak entry			19.5	17.7	11
16-Sep	38		133	119	77	xx			19	17	11
23-Sep	39		129.5	119	82.6	xx			18.5	17	11.8
30-Sep	40			118	80.5	xx				17	11.5
7-Oct	41				77						11
14-Oct	42	SCS carcass peak			70						10
21-Oct	43				70						10
28-Oct	44				70						10
4-Nov	45	peak-peak 1wk/KR hpa			70						10
11-Nov	46	1581			70	fcs caracass peak					10
18-Nov	47										
25-Nov	48	2wk before peak									
		1716				ATU					
		2wk after peak				peak-peak 1wk/KR hpa					
		1466				846					
						2wk before peak					
						1012					
						2wk after peak					
						675					