

U.S. Fish & Wildlife Service

California Nevada Fish Health Center FY2011 Technical Report:
***Ceratomyxa shasta* myxospore survey of Fall-run Chinook
salmon carcasses in the Klamath and Shasta Rivers, and
Bogus Creek, 2011**



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Summary:

Intestines of 175 Fall-run Chinok carcasses, collected from the Klamath River (Iron Gate hatchery to Shasta River reach) and the lower sections of both Bogus creek and Shasta river between 25-October and 22-November 2011, were surveyed for the myxospores stage of *Ceratomyxa shasta*. Both carcass age and sex ratios varied greatly between sample sites. Myxospores were detected in 22 – 38% of the sample groups. QPCR analysis of “undetected” scraping samples suggests that ~92% of the carcasses were infected with either low myxospores numbers and/or other stages of the parasite. The range of myxospore per scraping varied from 1,131 to 13.2 million. Only 2-12% of the samples are classified as high spore contributors (>500K spores). These carcasses likely input 91% of the myxospores into the water. While only intact myxospores were counted, their viability is unknown which limits our ability to forecast transmission rates to the polychaete host. These findings are similar to the 2008 and 2009 carcass surveys at these sites.

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Objective:

1. Determine prevalence of infection (POI) and concentration of *C.shasta* myxospores in adult fall-run Chinook carcass intestine from three general locations above the Klamath River *C.shasta* “infectious zone” for use in modeling.

Methods:

Carcass myxospore – Fall-run Chinook salmon (carcass) intestine sampling occurred over 5 weeks (10/23-29, 10/30-11/5, 11/6-12, 11/13-19, and 11/20-26) in 2011 (Table 1). Sixty samples were collected by CA-NV FHC staff from both Bogus creek (weir and reach1) and from the Shasta River weir, while 55 samples from Klamath River carcasses (RM 109.9 -118.2, Iron Gate hatchery to confluence of Shasta R.) by USFWS Arcata FWO staff. Carcass age is unknown for the majority of intestine samples and varies greatly. Due to CDFG carcass survey schedule, Bogus creek (except the 10/25 sample where over half of the samples were stage 2 and 3 decomposed fish, Baumsteiger and Kerby 2009) and Shasta River samples tended to be from salmon that were less than 48h post-death. It is likely that the Klamath R. carcasses were over 48h old given the weekly survey schedule. Intestine (small intestine to rectum) was dissected from the carcass, placed into individually numbered plastic bags, and either refrigerated for 24-48h 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 1x with PBS (2x final dilution), poured into tubes, vortex mixed, and allowed to settle for 1 -3 min. Any scrape weights <1.0 grams were diluted with 1.0 mL’s of PBS diluent to provide enough sample material. A 100 µL aliquot was frozen for later QPCR analysis. Duplicate 10 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. No evaluation for spore viability was performed (i.e. detect broken spores with methylene blue dye incubation).

In order to estimate the prevalence of infection by all *C.shasta* stages in the undetected samples, 9 or 10 scrapings, classified as myxospore “undetected”, from each sample site were randomly selected and 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 Processor). Extracted DNA was stored at -20°C 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 5L DNA template, were used for both assays under the following amplification conditions: 50°C for 2 min.; 95°C for 10 min; 40 cycles of 95°C for 15s and 60°C 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:

Prevalence -Prevalence of myxospore detection ranged from 22% for Bogus creek samples to 38% in the Shasta River samples (Table 1, Figure 1). No obvious trend in weekly prevalence was observed in the Shasta and Klamath R. collections. It is likely that the higher prevalence seen in the 25October Bogus creek sample was influenced by the higher proportion of decomposed carcasses obtained on this date.

Table 1. Prevalence of *C. shasta* myxospore detection in intestine samples from Fall-run Chinook Salmon carcasses collected over the 5 week study period in Bogus Creek, Shasta River, and the Klamath River (RM 109.9 - 118.2). Data recorded as number positive/total samples examined.

Week	1 25-Oct	2 1-Nov	3 8-Nov	4 15-Nov	5 22-Nov	Incidence
Bogus	8/20	2/20	3/20	NC	NC	13/60 21.6%
Shasta	7/20	9/20	7/20	NC	NC	23/60 38.3%
Klamath	5/12	5/12	4/12	4/12	2/7	20/55 36.4%

NC = No carcasses collected

The sex ratio of spore positive carcasses was 1.6 male to female for Bogus creek and 1.3 female to male in the Klamath R. (Table 2). Only males were obtained at the Shasta weir. If single outliers are ignored, the average myxospores / scraping value is relatively similar between the sexes in the Klamath but 2.7x higher in male Bogus creek carcasses. Sampling bias prevents any in-depth

analysis of trends by sex. No obvious temporal trend in myxospores / scraping was observed in the Bogus and Klamath collections (Figure 1), however 3 of the 4 highest spore samples from the Shasta weir occurred in the last week of collection (22 November).

Table 2. Number of female and male carcasses with detected myxospores and the mean spore/ scraping from Bogus creek, Shasta River, and Klamath River.

	Male	Female
<u>Bogus</u>		
no. spore detected	5	8
mean spore/scraping**	224,214	81,815
<u>Shasta</u>		
no. spore detected	23	0
mean spore/scraping	402,298	na
<u>Klamath</u>		
no. spore detected	13	7
mean spore/scraping**	532,820	457,760

** High spore outliers (13 million spore Klamath carcass and 4.6 million spore Bogus carcass not included in calculation of mean value.

na not applicable

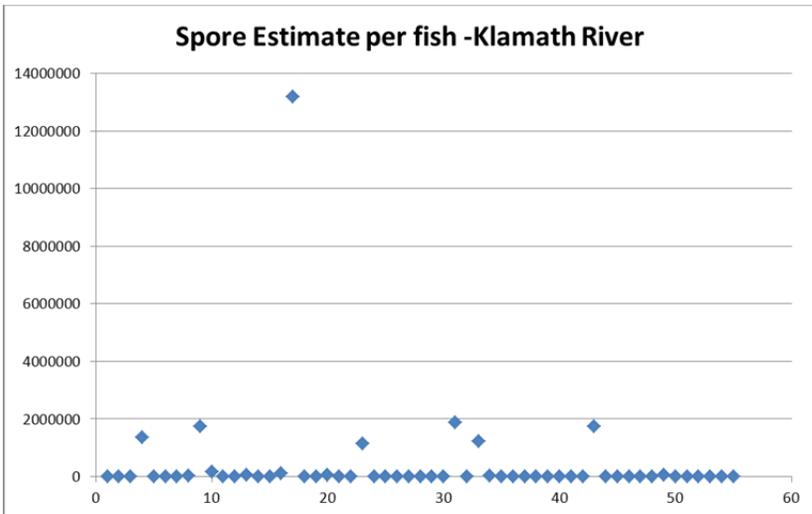
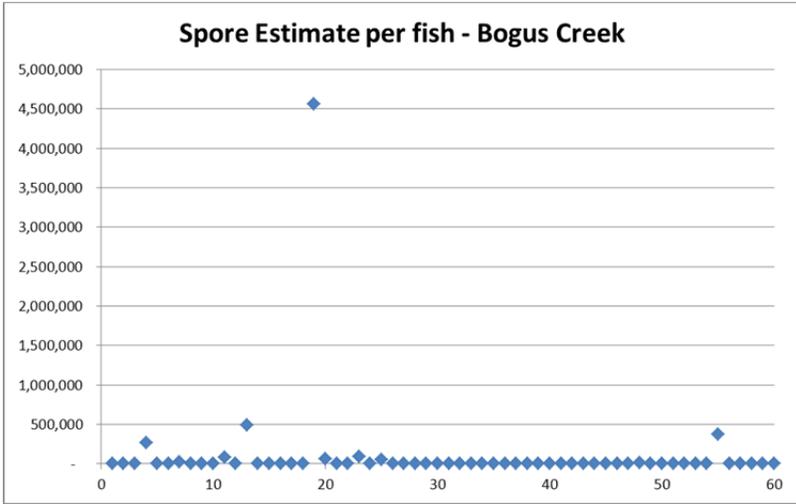
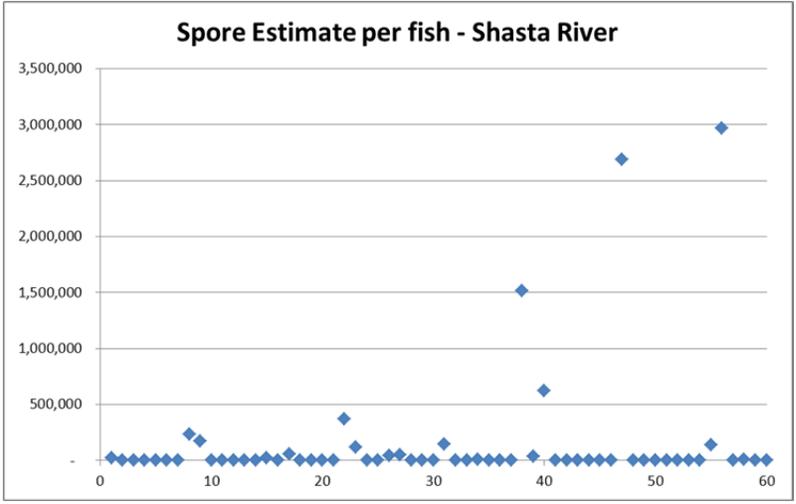


Figure 1. Scattergram of myxospores per scraping data from Shasta R., Bogus Creek, and the Klamath R. collections (note different higher scale for Klamath graph). Y-axis is myxospores/ scraping of individual carcasses in order of collection date (X-axis).

Myxospore quantity- As in previous surveys, no myxospores were detected in 62 – 78% of the intestine sample groups (Figure 2). Between 2 and 12 % of the sample groups were considered to be high spore contributors to the system ($\geq 500,000$ myxospores) while the remaining 20 – 32% of samples were categorized as low contributors ($> 1,000$ to 499,999 spores). The threshold value of 1000 spores/scraping was based on the observed sensitivity of 3 years of survey work. In previous surveys (2008 and 2009), the POI for high spore contributor ranged from 5 - 11% with a 3 year mean of 8%. The range of myxospore per scraping varied from 1,131 to 13.2 million (13 million from a Klamath River carcass). The spore total of all myxospores positive samples was 38 million. If the 13 million spore sample outlier is removed from the dataset, percent contribution of carcasses at each site to the reduced calculated 24.9 million spore total is 39% Klamath, 37% Shasta, and 24% Bogus. Biased sample collection of recent mortality verses decomposed carcasses likely influences this data to an unknown degree.

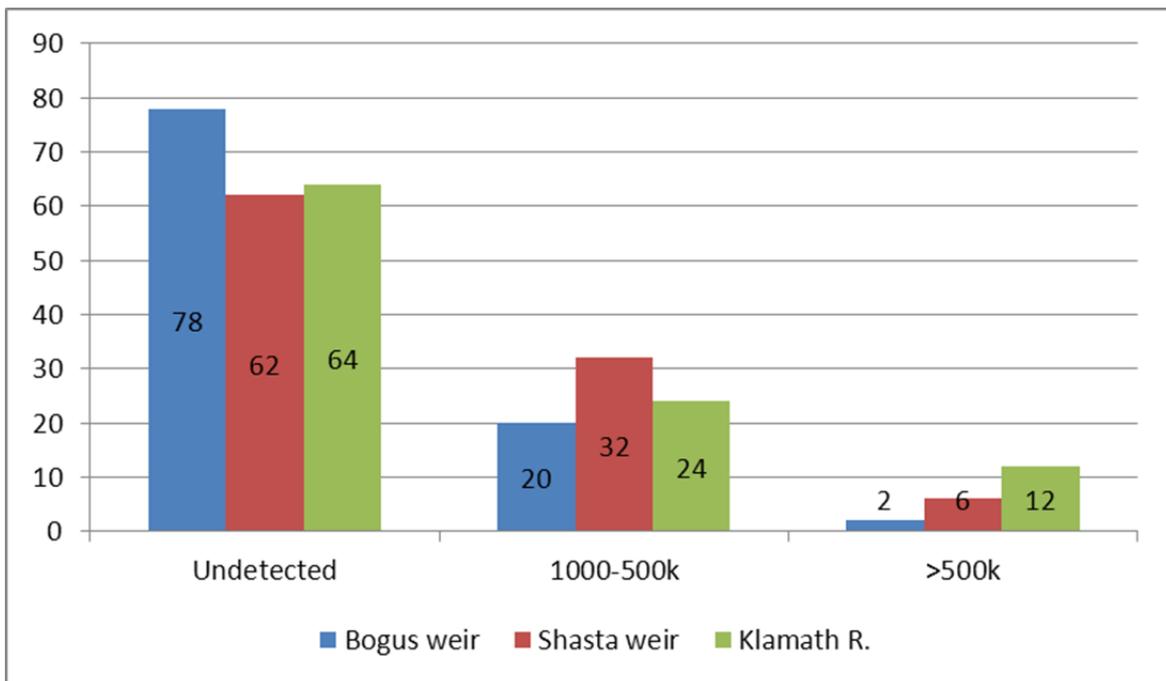


Figure 2. Prevalence of myxospores concentration categories of intestine samples collected at Bogus Weir, Shasta Weir, and Klamath River. Categories are: 1) Undetected by wet mount, 2) 1000-49,999 spores, 3) Greater or equal to 500,000 spores.

It is important to note that undetected samples are largely infected with either low myxospores number or other non-spore life stages of the parasite. QPCR analysis of undetected, low, and high spore samples demonstrates that approximately 90% of the “undetected” intestine samples had *C.shasta* DNA and that the quantity of DNA (greater the DNA the lower the C_t value) is proportional to the observed myxospores concentration (Table 3.)

Table 3. Incidence of *C. shasta* DNA detection in scrapings classified as myxospore detected or undetected by microscopy. Detected samples were placed into 2 categories (low = 1000-499,999 and high = $\geq 500,000$). Data is reported as positive / total for undetected samples and myxospore/scraping (sp) with corresponding CycleThreshold value for low and high sample types.

	Undetected by microscopy	Low	High
Bogus	8/9	1594sp=31.5 5062sp=34.2	4.6M sp=22.7
Shasta	8/9	1827sp=27.5 1396sp=29.5	Not Done
Klamath	9/10	2150sp=31.0 1131.3=35.7	13.2M sp=20.6

When the QPCR prevalence of infection of undetected sampled is multiplied to the number of undetected samples per site and added to the myxospores positive sample number, the estimated *C.shasta* infected prevalence ranged from 92 – 95% (Table 4).

Table 4. Estimated *C. shasta* infection (Cs+) using prevalence of QPCR positive results for 'undetected' scraping samples (Und.)

	Spore +	Undetected	Incidence Cs+	Incid. X Undetected	Spore(+) + est. Cs + Und.	Cs +
Bogus	13	47	0.89	42	13 + 42 = 55	55/60 92%
Shasta	23	37	0.89	33	23 + 33 = 56	56/60 93%
Klamath	20	35	0.90	32	20 + 32 = 52	52/55 95%

In 2011, estimated adult fall-run returns to Bogus creek, Shasta R, and Klamath R. reach 1 was 522, 11,400, and 4963 respectively (M.Knechtle, CDFG pers. comm.). We project that 4.3 billion myxospores could have been released from carcasses at these 3 locations in 2011 (Table 5). This estimate is derived from the following low and high contributor data applied to the return numbers: 1. average spore / scraping, prevalence of myxospores detection (POI), summation of spore estimates per site. We assume that carcass proximity to mainstem polychaete populations in the “infectious zone (Shasta to Seiad)” will influence myxospores transmission to polychaetes to a greater degree than overall watershed myxospores counts. An additional uncertainty in applying this data to transmission models is the unknown spore viability of each sample.

Table 5. Projected myxospores input into the sampled sites in 2011.

		<u>Bogus</u>	<u>Shasta</u>	<u>Klamath</u>
A	mean spore - LOW	122,464	76,323	39,803
	mean spore - HIGH	4,559,225	1,950,676	3,180,525
B	POI - LOW	0.2	0.24	0.32
	POI-HIGH	0.02	0.13	0.07
C	Return	522	11,400	4963
D	BxC- LOW	104	2736	1588
	BxC - HIGH	10	1482	347
	DxA - LOW	12,785,242	208,819,728	63,213,532
	DxA- HIGH	47,598,309	2,890,901,832	1,104,946,190
	sub-totals	60,383,551	3,099,721,560	1,168,159,723
	all site total	4,328,264,833		

In summary, an estimated $\geq 92\%$ of the carcasses were infected (DNA positive) with some stage of *C.shasta* and myxospores detected in 22 – 38% of the surveyed carcasses. Only 2- 12% of the survey carcasses were considered to be high spore contributors (>500,000). This data is similar to the 2008 and 2009 surveys.

Acknowledgements:

Morgan Knechtle and the CDFG carcass crew for access to Bogus and Shasta weir samples and Arcata FWO carcass crew for collection of Klamath carcasses.

Reference:

Baumsteiger J and JL Kerby. 2009. Effectiveness of salmon carcass tissue for use in DNA extraction and amplification in conservation genetic studies. North American Journal of Fisheries Management 29: 40 – 49.

Hallett, S. L. and J. L. Bartholomew. 2006. Application of a real-time PCR assay to detect and quantify the myxozoan parasite *Ceratomyxa shasta* in water samples. Diseases of Aquatic Organisms 71:109-118.

Appendix 1.

Site, collection date, carcass sex, and myxospores/scraping data for Shasta R., Klamath R., and Bogus creek collections in 2011.

site1	site2	sam-date	sex	spore/scrape
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	0
WEIR	SHASTA	10/25/2011	M	1827
WEIR	SHASTA	10/25/2011	M	3116
WEIR	SHASTA	10/25/2011	M	24,375
WEIR	SHASTA	10/25/2011	M	24435
WEIR	SHASTA	10/25/2011	M	54375
WEIR	SHASTA	10/25/2011	M	171250
WEIR	SHASTA	10/25/2011	M	232500
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0

WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	0
WEIR	SHASTA	11/1/2011	M	13125
WEIR	SHASTA	11/1/2011	M	37841
WEIR	SHASTA	11/1/2011	M	42308
WEIR	SHASTA	11/1/2011	M	48750
WEIR	SHASTA	11/1/2011	M	121349
WEIR	SHASTA	11/1/2011	M	146953
WEIR	SHASTA	11/1/2011	M	372698
WEIR	SHASTA	11/1/2011	M	624957
WEIR	SHASTA	11/1/2011	M	1518750
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	0
WEIR	SHASTA	11/9/2011	M	1397
WEIR	SHASTA	11/9/2011	M	2411
WEIR	SHASTA	11/9/2011	M	3914
WEIR	SHASTA	11/9/2011	M	6685
WEIR	SHASTA	11/9/2011	M	140833
WEIR	SHASTA	11/9/2011	M	2687031
WEIR	SHASTA	11/9/2011	M	2971964
	KLAMATH	10/25/2011	F	0
	KLAMATH	10/25/2011	M	0
	KLAMATH	10/25/2011	F	0
	KLAMATH	10/25/2011	F	0
	KLAMATH	10/25/2011	F	0
	KLAMATH	10/25/2011	F	0

KLAMATH	10/25/2011	F	0
KLAMATH	10/25/2011	F	8,437.5
KLAMATH	10/25/2011	M	28,125.0
KLAMATH	10/25/2011	M	154,387.5
KLAMATH	10/25/2011	F	1,348,825.0
KLAMATH	10/25/2011	M	1,752,000.0
KLAMATH	11/1/2011	M	0
KLAMATH	11/1/2011	F	0
KLAMATH	11/1/2011	M	0
KLAMATH	11/1/2011	F	0
KLAMATH	11/1/2011	M	0
KLAMATH	11/1/2011	F	0
KLAMATH	11/1/2011	M	0
KLAMATH	11/1/2011	M	52,150.0
KLAMATH	11/1/2011	F	64,125.0
KLAMATH	11/1/2011	F	105,800.0
KLAMATH	11/1/2011	M	1,147,525.0
KLAMATH	11/1/2011	F	13,182,956.3
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	M	0
KLAMATH	11/8/2011	F	0
KLAMATH	11/8/2011	F	2,150.0
KLAMATH	11/8/2011	F	22,612.5
KLAMATH	11/8/2011	F	1,225,087.5
KLAMATH	11/8/2011	F	1,876,225.0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	M	0
KLAMATH	11/15/2011	M	0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	F	0
KLAMATH	11/15/2011	F	1131.25
KLAMATH	11/15/2011	F	2968.75
KLAMATH	11/15/2011	F	5418.75
KLAMATH	11/15/2011	F	1731056.25
KLAMATH	11/22/2011	M	0

	KLAMATH	11/22/2011	M	0
	KLAMATH	11/22/2011	M	0
	KLAMATH	11/22/2011	F	0
	KLAMATH	11/22/2011	M	0
	KLAMATH	11/22/2011	M	6703.125
	KLAMATH	11/22/2011	M	63426.13636
WEIR-	BOGUS	10/25/2011	M	0
R2	BOGUS	10/25/2011	F	0
WEIR-	BOGUS	10/25/2011	M	0
R2	BOGUS	10/25/2011	F	0
WEIR-	BOGUS	10/25/2011	M	0
R2	BOGUS	10/25/2011	F	0
WEIR-	BOGUS	10/25/2011	F	0
R2	BOGUS	10/25/2011	F	0
WEIR-	BOGUS	10/25/2011	M	0
R2	BOGUS	10/25/2011	M	0
WEIR-	BOGUS	10/25/2011	M	0
R2	BOGUS	10/25/2011	F	0
WEIR-	BOGUS	10/25/2011	F	0
R2	BOGUS	10/25/2011	M	1594
WEIR-	BOGUS	10/25/2011	M	19863
R2	BOGUS	10/25/2011	M	64331
WEIR-	BOGUS	10/25/2011	F	82694
R2	BOGUS	10/25/2011	F	271875
WEIR-	BOGUS	10/25/2011	F	488663
R2	BOGUS	10/25/2011	F	4559225
WEIR-	BOGUS	11/1/2011	M	0
R2	BOGUS	11/1/2011	M	0
WEIR-	BOGUS	11/1/2011	F	0
R2	BOGUS	11/1/2011	F	0
WEIR-	BOGUS	11/1/2011	M	0
R2	BOGUS	11/1/2011	M	0
WEIR-	BOGUS	11/1/2011	F	0
R2	BOGUS	11/1/2011	F	0

R2				
WEIR-R2	BOGUS	11/1/2011	F	0
WEIR-R2	BOGUS	11/1/2011	M	0
WEIR-R2	BOGUS	11/1/2011	F	0
WEIR-R2	BOGUS	11/1/2011	F	0
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WEIR-R2	BOGUS	11/1/2011	M	0
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WEIR-R2	BOGUS	11/1/2011	F	53625
WEIR-R2	BOGUS	11/1/2011	M	93713
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WEIR-R2	BOGUS	11/9/2011	M	0
WEIR-R2	BOGUS	11/9/2011	M	0
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WEIR-R2	BOGUS	11/9/2011	M	0

WEIR- R2	BOGUS	11/9/2011	M	5063
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