

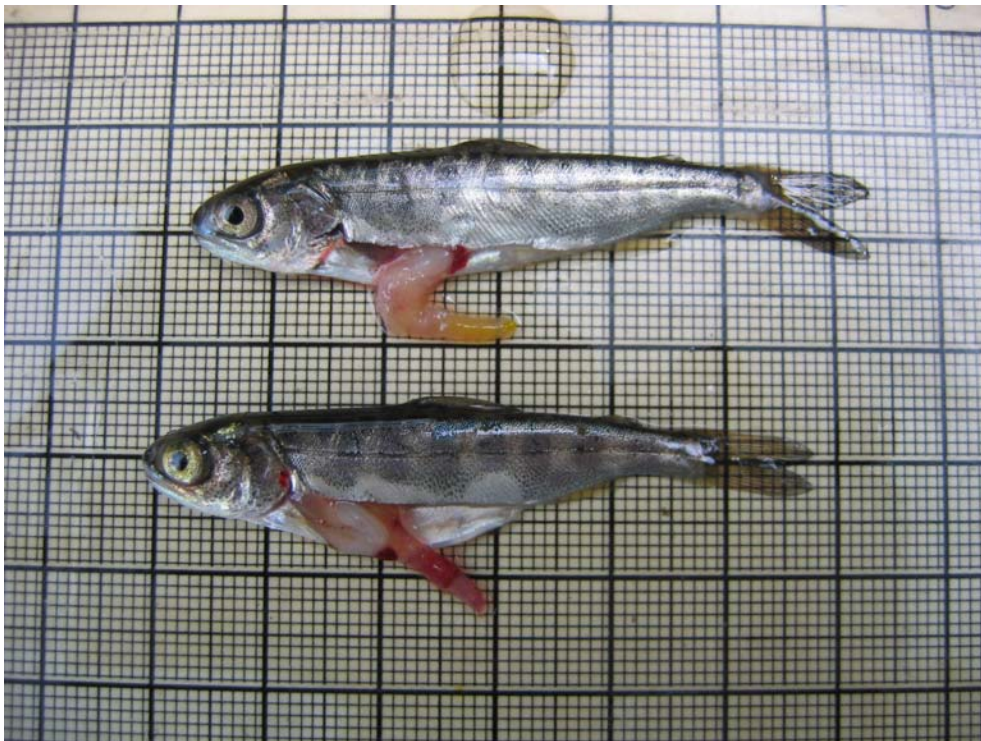
U.S. Fish & Wildlife Service

California Nevada Fish Health Center

FY2008 Technical Report:

Prognosis of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infection in Feral Klamath River juvenile Chinook salmon, May-July 2008

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May 2009



Summary: Two separate groups of feral, juvenile Klamath River Chinook Salmon (*Oncorhynchus tshawytscha*) were collected from the river on May 12, 2008 and June 25, 2008 with a sub-sample assayed initially for *Ceratomyxa shasta* (Cs) and *Parvicapsula minibicornis* (Pm) infection. The remaining fish were observed for ceratomyxosis over a 5 week period. This experiment was conducted to:

- Determine the relationship of Cs prevalence of infection (POI) for in-river collections to the disease prognosis of this sampled population.
 - Does the initial POI equal the disease incidence in the population over time?
 - Does the QPCR assay used in juvenile salmon monitoring detect all fish that will become diseased ?
- Compare infection and disease severity of fish between the two collection dates

The prevalence of *C.shasta* infection in the initial sample slightly underestimated the incidence of infection later observed from the same collection group over a 5 week period. Fish collected in June were in an advanced stage of ceratomyxosis at the time of collection. It appears that detection of *C.shasta* by QPCR is associated with greater than 80% mortality in juvenile Chinook collected from this specific reach of the Klamath R. during the spring.

The correct citation for this report is:

Fogerty Ryan. 2009. FY2008 Technical Report: Prognosis of *Ceratomyxa shasta* and *Parvicapsula minibicornis* infection in Feral Klamath River Juvenile Chinook Salmon. U.S. Fish & Wildlife Service California – Nevada Fish Health Center, Anderson, CA.

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Introduction:

In 2008, two groups of feral, juvenile Klamath River Chinook salmon were captured at the Kinsman trap site and transported to the California-Nevada Fish Health Center's Wet Laboratory for a five week observation period. Ceratomyxosis was observed in mortalities and moribund fish from both the 12-May and the 25-June group.

The primary objective of this study was to determine the relationship between the prevalence of *Ceratomyxa shasta* infection at capture to the subsequent incidence and severity of Ceratomyxosis in the same population (prognosis of infection). These fish were also assayed for *Parvicapsula minibicornis* infection.

Methods:

Two separate groups were collected on 12-May and 25-June and consisted of 162 fish and 178 fish respectively. The May group was of natural origin and the June group was composed of unknown mixture (Iron Gate Hatchery release began on 5-27). Fish were returned to the California-Nevada Fish Health Center wet laboratory in a 50-gallon transport tank of Coleman National Fish Hatchery water. Each group was held in an 800L circular tank supplied with 7.6 L/min flow of ozonated 17° C water for five weeks of observation. Both groups were held for 24 hours to remove post capture mortalities. These 24h post-capture mortalities were not included in the study. Fish received a 10-min prophylactic bath of 1 mg L⁻¹ furanase treatment during transport and additional treatments as needed to reduce columnaris (*Flavobacterium columnare*).

After the initial 24 hours post-capture, 30 fish from each group were randomly netted from the tank and sampled for Quantitative Polymerase Chain Reaction (QPCR) analysis of both Cs and Pm. Ten additional fish were collected for histological analysis. These 40 fish were referred to as the "time-zero" (T⁰) sub-sample and represented a standard in-river monitoring collection. Fish were fed frozen bloodworms to satiation twice daily. Tanks were checked twice per day for moribund or dead fish. Presumptive cause of death (if known) was documented and each fish was given a unique identifying code. Any fish that did not die by end of five week observation was considered a survivor. Any fish that was moribund (example weak swimming behavior with clinical signs of infection) or died after the T⁰ sample was considered a "mortality". Mortalities were documented for signs of clinical ceratomyxosis (CI), columnaris (Fc), or with no symptoms (NS). If fish were diagnosed with both CI and Fc, they were documented as having died with signs of clinical ceratomyxosis. All moribund or recent mortalities (gills still pink), had kidney and intestines preserved in Davidson's fixative for 24 hours and transferred to 70% EtOH for histological analysis as well as sub-samples of intestines and kidneys frozen and processed for QPCR analysis. Fish which survived the 5-week observation period were euthanized and sampled for Cs and Pm QPCR analysis (Nichols et al. 2008).

QPCR analysis was conducted on all samples collected from the following types of fish: 1) time zero, 2) survivor, 3) mortalities with clinical signs, and 4) a subset of mortalities without clinical signs (every 5th fish chosen in a chronologically manner).

For histology, a composite infection and disease rating was developed based on the degree of tissue inflammation associated with the presence of the parasites. A similar histology rating system was used in Klamath River monitoring studies since 2004 (Nichols and Foott 2006; Nichols et al. 2007; Nichols and True 2007; Nichols et al. 2008). Cs infections were rated as **clinical** (parasite present and inflammatory tissue in >33% of the intestine section), **subclinical** (parasite present, but inflammatory tissue in <33% of intestine section) or uninfected (no *C. shasta* detected). Similarly Pm infections were rated as clinical (parasite present and glomerulonephritis in >33% of the kidney section), subclinical (parasite present, and glomerulonephritis in <33% of the kidney section) or uninfected (no *P. minibicornis* detected).

In the QPCR assay, cycle threshold (C_T) values were calculated by the SDS software (v 1.3.1, Applied Biosystems). Validation studies examining the dynamic range and endpoint of the assays indicated a C_T of 38 and minimum change in normalized fluorescent signal of at least 10,000 units defines a positive test for the *P. minibicornis* assay (True et al. 2009). Previous assay validation studies, using DNA plasmid controls and naturally infected fish tissue, determined a similar assay threshold for the *C. shasta* assay. It should be noted that these thresholds are relatively conservative statistically, and therefore slightly underestimate the true infection incidence of both parasites. There is an inverse relationship between Ct value and DNA concentration (lower Ct = higher DNA, more parasites in the sample).

Results and Discussion:

12-May Group

The 12-May group had 22 fish removed as transport mortalities leaving 140 fish at the start of the experiment. The 25-June group had 47 fish removed as post-capture mortalities leaving 131 fish at the start of the experiment (Table 1).

Table 1- Accounting of fish in the 12-May and the 25-June groups.

12-May Group		25-June Group	
# Fish Collected	162	# Fish Collected	178
24-H Post Capture Morts	22	24-H Post Capture Morts	47
Time Zero Samples	40	Time Zero Samples	40
<hr/>			
# Fish at start of Exp.	100	# Fish at start of Exp.	91
Experimental Morts	82%(82/100)	Experimental Morts	95%(86/91)
Survivors	18%(18/100)	Survivors	5%(5/91)

The 12-May group exhibited a peak in daily mortality on day 13. Mortalities occurred through all five weeks of observation. (Figure 1). Similarly, day 13 post-

collection was the peak daily mortality for the 25-June group with the last mortality occurring on day 19 (Figure 2). The rate of mortality during the 1st 14d post-capture was approximately 1.9x greater in the 25 -June than the 12-May group.

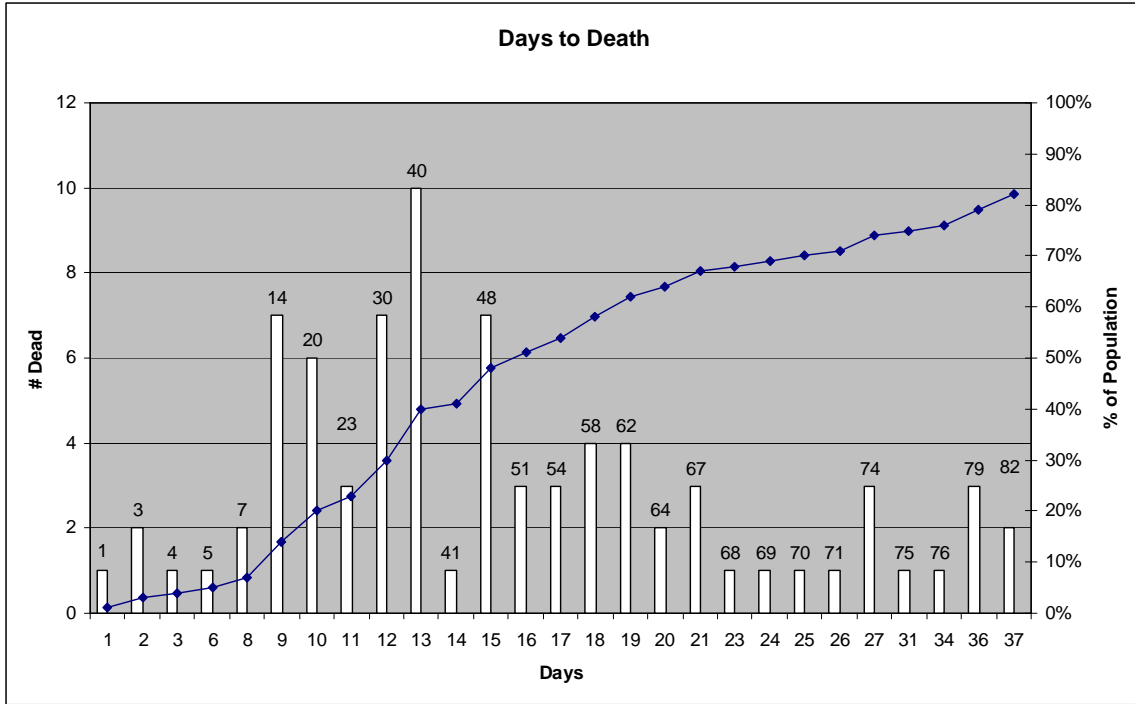


Fig. 1- Daily (bar) and cumulative (number above each bar and line) mortality for the 12-May group.

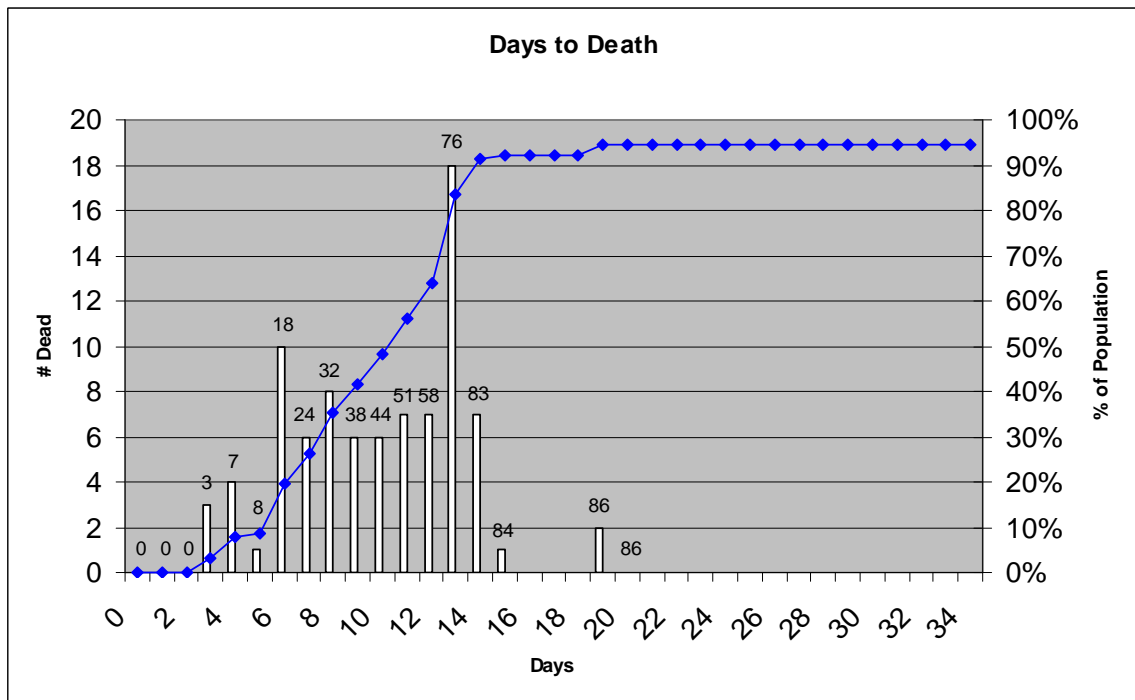


Fig. 2- Daily (bar) and cumulative (number above each bar and line) mortality for the 25-June group.

The 12-May group had 35% (n=29) of fish die with signs of clinical ceratomyxosis, 18% died with signs of columnaris (n=15), and 46% (n=38) died with no obvious symptoms. The 25-June group experienced 58% (n=47) mortality with signs of clinical ceratomyxosis, 7% (n=6) mortality with signs of columnaris, and 41% (n=33) mortality with no obvious symptoms (Table 2). The higher percentage of the 25-June population dying with signs of clinical ceratomyxosis suggests a higher parasite load of *C. shasta* at the time of collection.

Table 2- Summary of clinical signs observed from mortality in the 12-May and 25-June Groups.

12-May Group	25-June Group
29% died with Clinical signs of Ceratomyxosis (29/100)	52% died with Clinical signs of Ceratomyxosis (47/91)
15% died with Clinical signs of Columnaris (15/100)	7% died with signs of Columnaris (6/91)
38% died with no clinical signs (38/100)	36% died with no clinical signs (33/91)
18% of fish survived 5-week observation period (18/100)	5% of fish survived 5-week observation period (5/91)

In the 12-May group, *C. shasta* was detected by QPCR in 66.7% (20/30) of the T⁰ fish, 87.1% (27/31) of mortalities, and 55.6% (10/18) of the survivors. In the 25-June group, *C. shasta* was detected by QPCR in 73.3% (22/30) of the T⁰ fish, 90.6% (29/32) of mortalities, and 0% (0/5) of survivors (Figure 3). In the 12-May group, *P. minibicornis* was detected by QPCR in 90% (27/30) of T⁰ fish, 96.8% (30/31) of mortalities, and 100% (18/18) of survivors. In the 25-June group, *P. minibicornis* was detected by QPCR in 100% (30/30) of T⁰ fish, 100% (26/26) of mortalities, and 100% (5/5) of the survivors. (Figure 4)

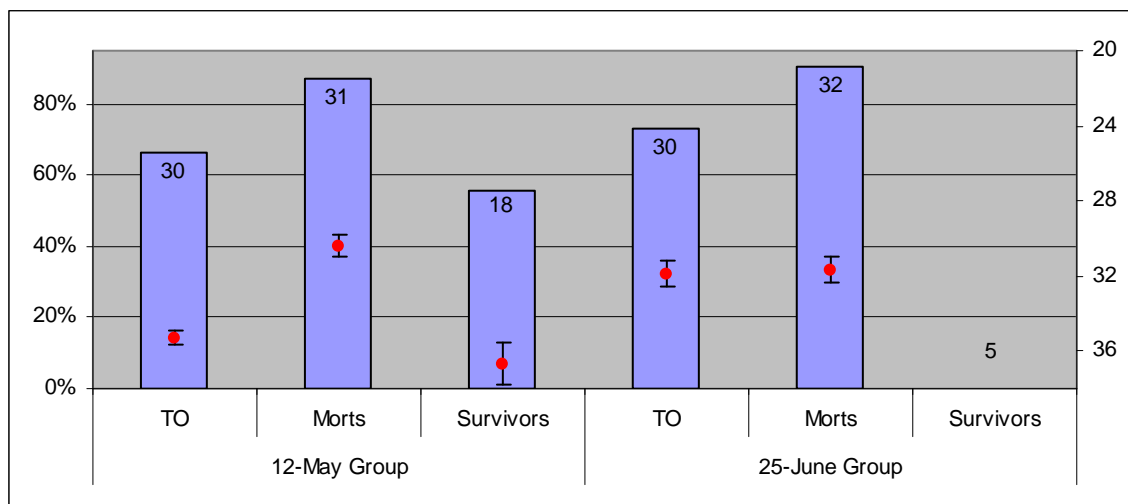


Figure 3- Incidence of *Ceratomyxa shasta* infection assayed by QPCR. Sample number is at top of each bar. Red dots indicate average Cycle Threshold (Ct) per group. Left Y-axis is percentage of sample group infected and right Y-axis is Ct score. Error bars represent +/- SE of the Ct mean.

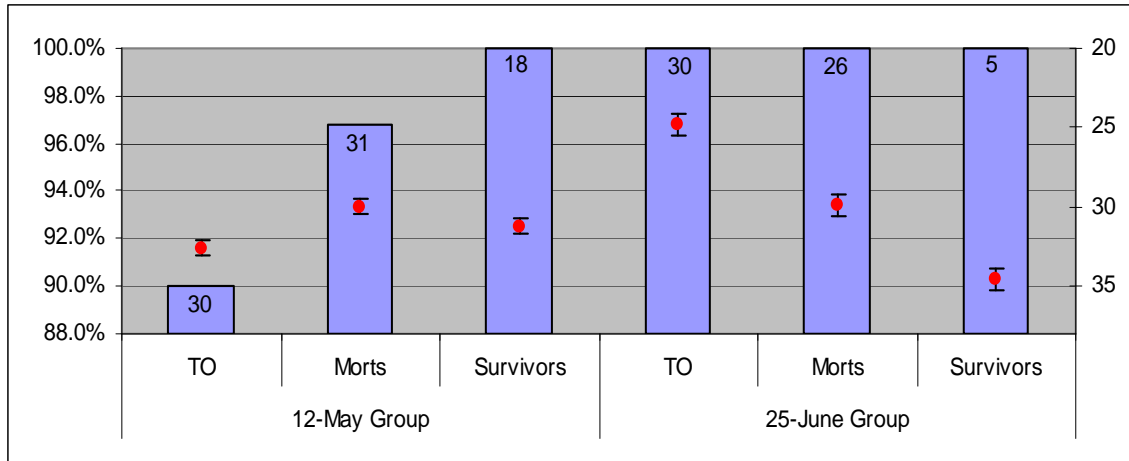


Figure 4- Incidence of *P. minibicornis* infection assayed by QPCR. Sample number is at top of each bar. Left Y-axis is percentage of sample group infected and right Y-axis is Ct score. Red dots indicate average Cycle Threshold (Ct) per group. Error bars represent +/- SE of the Ct mean.

In the 12-May group, *C. shasta* was detected by histology in 10% (1/10) of T⁰ fish and 88.2% (15/17) of mortalities. In the 25-June group, *C. shasta* was detected by histology in 50% (5/10) of T⁰ fish and 56.5% (13/23) of mortalities (Figure 5). In the 12-May group, *P. minibicornis* was detected by histology in 80% (8/10) of T⁰ fish and 100% (17/17) of mortalities. In the 25-June group *P. minibicornis* was detected by histology in 100% (10/10) of T⁰ fish and 100% (23/23) of mortalities. (Figure 6).

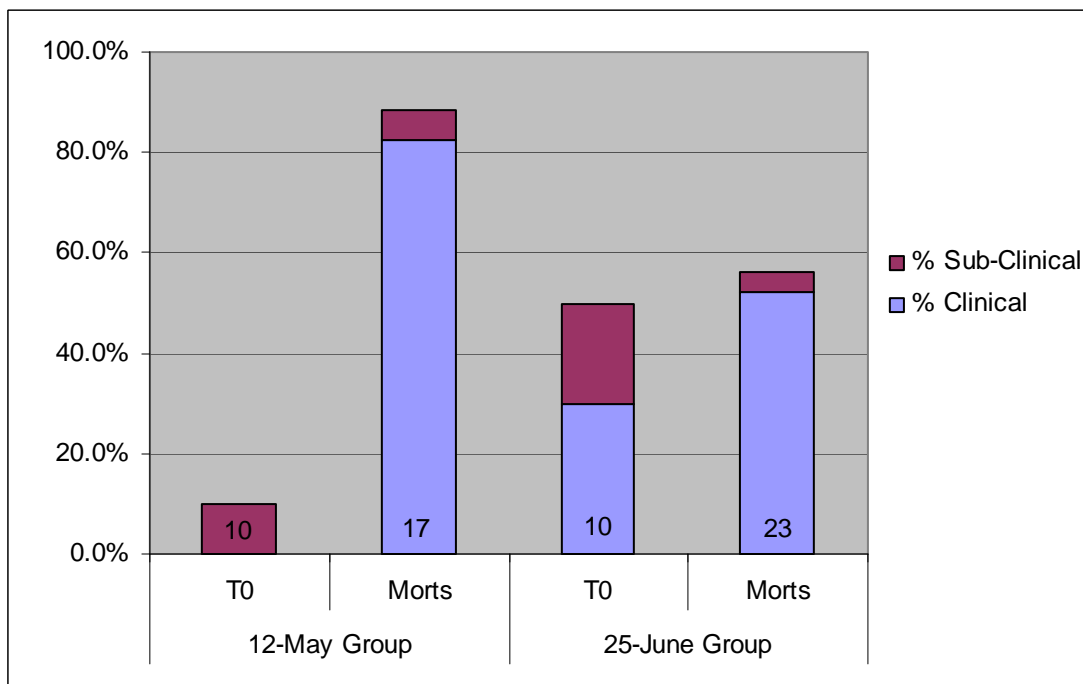


Figure 5- Incidence of *Ceratomyxa shasta* infection and significant intestinal lesion (Clinical) assayed by histology. Sample numbers are at base of each bar.

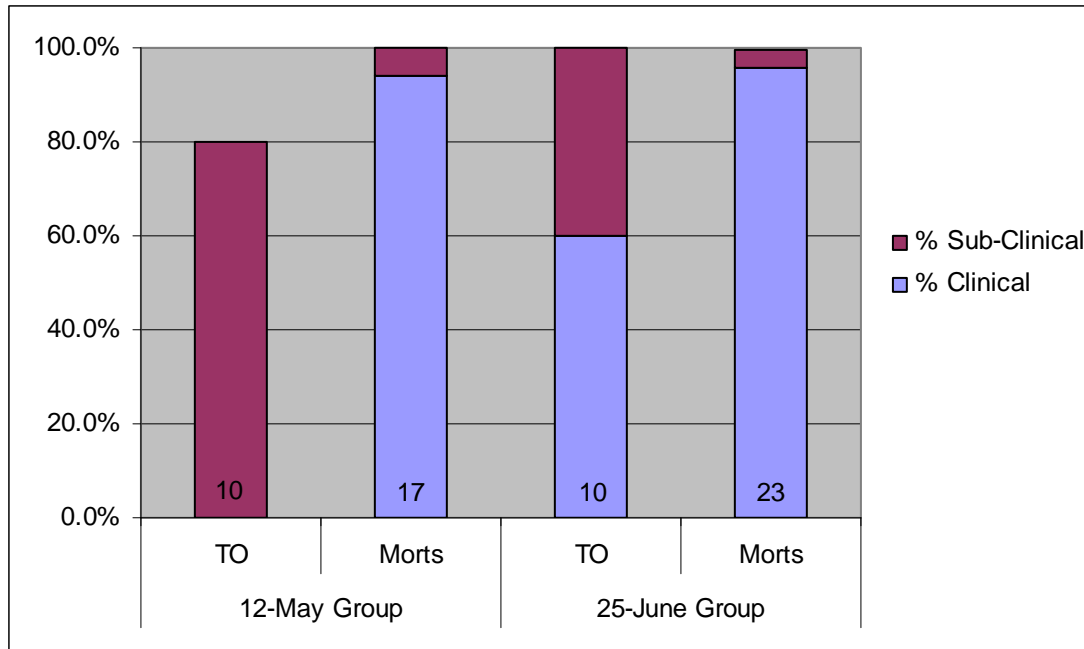


Figure 6- Incidence of *Parvicapsula minibicornis* infection and significant kidney lesion (Clinical) assayed by histology. Sample numbers are at base of each bar.

In both collections, the initial estimate of Cs infection (POI) underestimated the population's later incidence of infection by 5-9% (Table 3).

Table 3- Cs POI in Time zero (T⁰) fish compared to IOI in Morts and Survivors

	TO	Morts	Survivors
QPCR (12-May)	66.7% (20/30)	87.1% (27/31)	55.6% (10/18)
Histology (12-May)	10%(1/10)	88.2% (15/17)	
QPCR (25-June)	73.3% (22/30)	90.6% (29/32)	0% (0/5)
Histology (25-June)	50% (5/10)	56.5% (13/23)	

It appears that the 25-June fish were impacted by disease to a greater degree than the 12-May collection group and were in an advance state of disease at the time of collection. The 25-June group exhibited both a higher incidence of infection for both Cs and Pm, greater parasite DNA concentration (lower Ct values), and greater rate of mortality than the 12-May group. Mean day to death for the 12-May group was 19.9 days (SE +/- 1.1) compared to 10.6 (SE +/- 0.5) for the 25-June fish. At 14 days post-capture, 41% (41/100) of the 12-May population had died compared to 84% (76/91) of the 25-June population at the same time period post-capture. It is noteworthy that the 5 survivors in the 25-June group were QPCR negative for Cs.

Furanase treatment for columnaris appeared to be more successful with the June group than the May fish. Columnaris was observed in only 7% (6/91) of the June group mortalities compared to 15% (15/100) in the May group. This further illustrates that the 25-June group were dying at a faster rate due to ceratomyxosis than the 12-May group. The 12-May group experienced 14% (22/162) transport mortality compared to 26% (47/178) in the 25-June group. It is likely that many of these transport mortalities could be associated with ceratomyxosis as well as handling.

ACKNOWLEDGMENTS

I wish to acknowledge significant contributions by biologists with the USFWS Arcata FWO for fish collection; Scott Foott, Ken Nichols, and Ron Stone, CA-NV Fish Health Center for processing and reading histology slides, additional lab assistance, and editorial comments.

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Journal of Fish Disease (early 2009 publication date).