Monitoring Sockeye Salmon Health in the Cedar River and Lake Washington

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This report covers the continued health monitoring of sockeye salmon in the Lake Washington basin since July 2008. The Cedar River sockeye salmon enhancement program began in 1991, with the goals of increasing the number of sockeye salmon returning to the Cedar River and providing biologists with an opportunity to study factors affecting sockeye salmon survival throughout their life history. Washington Department of Fish and Wildlife (WDFW) operates the hatchery with funding provided by Seattle Public Utilities (SPU) through the Cedar River Habitat Conservation Plan. Since the inception of the project WDFW has provided fish health monitoring for the Cedar River Hatchery located at the Landsburg diversion dam. In July of 2005 SPU also began contributing funding to support the Fish Health component.

Since the origin of this project the majority of the pathogen monitoring at the facility has been screening for the presence of the viral pathogen infectious hematopoietic necrosis virus (IHNV). This virus is present in the Lake Washington basin and caused substantial mortalities in sockeye salmon during a previous incubation project on the Cedar River. Sockeye salmon are extremely susceptible to this virus and it causes a high rate of mortality, particularly at the higher densities of artificial culture. Stringent disinfection and isolation procedures are in place at the current hatchery to avoid infection of the juveniles and minimize spread of the virus if disease should occur. Each fall adults are tested for IHNV throughout the spawning season to ascertain the prevalence of the virus. Each release of fry is also sampled for IHNV to assess the condition of the group at release. If the fish in any incubator or rearing unit show unusual behavior, or express above normal mortality prior to release, these fish are examined and tested for viral pathogens.

During most years the naturally produced sockeye salmon fry are also screened for viral pathogens during the outmigration to monitor the prevalence of IHNV in the wild. When available, fish collected at other points in their life history, such as at smoltification, are also examined for viral pathogens. Fish may be examined for other fish pathogens if there is evidence of infection or for periodic monitoring.

METHODS

Sample collection

Adults

Each year, during spawning at the hatchery, ovarian fluid (OF) from approximately 24 adults was sampled weekly and a total of 60 kidney and spleen (K/S) samples were also taken. Due to

low numbers of adult returns in 2008 and the need to open the trap to pass Chinook salmon, only 2,074 adults were collected. The majority of the broodstock were hauled from the new weir site in Renton, with a 36 fish collected by hook and line and 37 fish hauled from the SPU passage facility. All samples taken from broodstock were tested for IHNV and other regulated viral pathogens, as per requirements of the Salmonid Disease Control Policy of the Fisheries Co-Managers of Washington State.

In addition to the viral testing, on the October 20th spawning adults were also sampled for the parasite *Ceratomyxa shasta* and certain bacterial pathogens: *Aeromonas salmonicida*, the causative agent of furunculosis; *Yersinia ruckeri*, the causative agent of enteric redmouth; and *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD). These are pathogens that have been found in the Lake Washington basin and if present, could potentially impact survival of the fish.

No pathogen samples were collected from prespawning adults returning to the system in 2008. WDFW staff collected otoliths from returning adult sockeye salmon captured at the Ballard Locks by Muckleshoot Indian Tribe biologists during June and July 2008 but no abnormalities were evident in these fish. Environmental conditions were good and no mortalities were seen at the locks or elsewhere in the system. Prior year's testing did not detect any pathogens in fish collected without an indication of disease or mortality present.

Juveniles

At the hatchery 30 fry were collected from each rearing unit at release to test for IHNV. This monitoring assesses the health of the fish at release and is used to evaluate the effectiveness of the IHNV control strategies. To minimize shipping, most weeks some of the fry were held in the incubators and each week only one shipment was made to the lab. If fish in any of the incubators or rearing containers showed abnormal behavior or excessive mortality, a representative sample was sent to the lab at that time to evaluate the cause and test for virus. In addition to virology, a small number of fish from all of the rearing units were also examined microscopically to monitor for bacteria, parasites, gill condition, and other abnormalities. Due to the low number of adults collected only 2.78 million fry were released, however, with the small number of fry, the hatchery crew was able to feed all of the fry for approximately a two week period prior to release. This practice has been shown to improve the survival of the fry after release, but the high level of feeding has the potential to cause gill disease.

Most years the WDFW Fish Health Laboratory has also collected periodic samples of approximately 100 sockeye salmon fry from the floating inclined-plane screen trap that is maintained on the lower river to monitor outmigration. In 2009 all hatchery produced fry were released below the fry trap so all fry collected in the trap were naturally produced. With the poor adult returns in 2008 and high flows this winter it was a poor fry outmigration year. An estimate of natural origin fry is not yet completed, but it is expected to be less than 1.5 million fry. Initial outmigration was quite low and only one sample of naturally produced fry was collected on March 2, 2009.

In May 2009, a purse seine was used in Lake Union on a weekly basis to collect sockeye salmon smolts for otolith analysis. A portion of these fish were also examined for a general health and tested for viral pathogens.

Laboratory analysis

OF is generally a more sensitive specimen to test for IHNV so it is emphasized during sampling of the hatchery adults. On a weekly basis OF samples were collected and tested individually to determine the prevalence of IHNV in the population. K/S tissues collected from adults at the hatchery were combined in 5 fish pools to screen for a viral pathogen (infectious pancreatic necrosis virus) that is not detected in the OF. All fry samples were sent to the Fish Health Laboratory live, euthanized with MS-222 and processed whole, in pools of up to five fish, to assay for viral pathogens. K/S tissues were collected from the smolts and assayed individually for viral pathogens. All viral samples were processed fresh and assayed using standard cell culture procedures. The diluted sample is inoculated onto CHSE 214 and EPC cell lines and observed for a minimum of 14 days at 15°C to monitor for the cytopathic effect from viruses. Confirmation of any viral isolates is done using specific antibody tests. Methodology is used that will detect IHNV, infectious pancreatic necrosis virus or viral hemorrhagic septicemia virus.

Sections of hindgut was collected from 20 adults and frozen for later examination by microscopy to screen for spores typical of *C. shasta*. To detect *Y. ruckeri* and *A. salmonicida*, brain heart infusion agar plates were inoculated with kidney tissue from 20 adults and incubated at 20°C for 7-10 days with examination for colonies of typical morphology. Kidney tissues were harvested from each of 48 adults and frozen for later analysis by enzyme-linked immunosorbant assay (ELISA). Results were obtained by recording the optical density (OD) of the color reaction, which indicated relative levels of an antigen produced by the bacteria *R. salmoninarum*. The results are reported here by a summary of levels of OD. Although there is not an absolute correlation to disease, OD levels indicate the level of infection of a fish.

RESULTS AND DISCUSSION

Adults

During the summer of 2008 the adult return was estimated to be 34,000 sockeye salmon through the Ballard Locks. The fish examined at the locks had light infestations of copepods and occasional wounds, but no external signs of disease. There were no reports of abnormal mortalities of sockeye salmon adults in the basin. Mortality levels were normal in the adults that were held at the hatchery for spawning. No signs of disease were evident in the fish spawned at the hatchery and no fish pathogens were detected other than *R. salmoninarum*, the causative agent for bacterial kidney disease. No IHNV was detected in the returning adults during this period indicating a lower prevalence of virus in the system Table 1.). This is only the second year since the beginning of this project that virus has not been detected in the adults (no virus was detected in 2006), however, IHNV is generally present in the later arriving fish and the hatchery only spawned fish through mid November.

Table 1. Viral results from adult sockeye salmon

Sample date	Viral results
10/06/08	0/24 OF VD*
10/13/08	0/23 OF VD
10/20/08	0/24 OF VD 0/12 pools from K/S (60 fish total in 5 fish pools) VD
10/22/07	0/24 OF VD
10/27/08	0/26 OF VD
11/03/08	0/24 OF VD
11/12/08	0/24 OF VD
11/17/08	0/24 OF VD

* virus detected

No *C. shasta*, *A. salmonicida*, or *Y. ruckeri* were detected in any of the adults tested. It is possible that the bacterial pathogens could have been present earlier in the year and caused mortality, but it is unlikely that the pathogen would not be present at spawning if this was the case. Due the senescence of adult salmon an infection will continue to progress rather than be cleared.

Detectable levels of the antigen to *R. salmoninarum* were detected in the spawning adults (Table 2.). Only one fish had OD levels that would be indicative of disease (OD = 2.964). Sockeye salmon are very susceptible to BKD but finding this amount of infection typically does not indicate a significant disease problem in an adult population. As with other pathogens *R. salmoninarum* levels will increase as the fish become senile.

Table 2. ELISA results for 10/29/09 *R. salmoninarum* screening

OD Level	Infection level	Number of fish	Percent of total
> 0 .099	below low	45	93.7%
0.100 – 0.199	low	2	4.2%
0.200-0.499	moderate	0	0.0%
>0.450	high	1	2.1%

Juveniles

No major problems were encountered this year. There were only minor impacts on gill condition in one of the fed groups and the majority of the fry were showing some deposition of fat. Abnormal fish were seen in one incubator but IHNV was not detected from fish in that incubator or any of the hatchery releases (Table 3.). The fish in the affected incubator exhibited premature swim up, but upon examination appeared normal other than having a small amount of yolk present and mortality levels were normal.

Overall, this was considered a very successful year for the hatchery with the fry released in good condition. The fish accepted feed well and showed good growth. A starter feed of Rangen soft moist feed was used again this year and it continued to perform well. Gill condition at release was good even with intensive feeding. Yolk was present in very few of the fish at release since all fry were fed this year.

Table 3. Health screening results from hatchery produced sockeye salmon fry

Sample date	Vessel	Rearing	Sample type	Virus results	Visual exam
02/09/09	A-2	incubator	diagnostic	0/6 pools VD (30 fish)	fish up and swimming early, but no abnormalities detected other than small amount of yolk present
02/11/09	S-20	Fed	routine	0/6 pools VD (30 fish)	normal, one fish with a little yolk
02/23/09	A-1	Fed	routine	0/6 pools VD (30 fish)	normal, one fish with a little yolk
02/23/09	A-2	Fed	routine	0/6 pools VD (30 fish)	normal
03/04/09	A-3	Fed	routine	0/6 pools VD (26 fish)	normal
03/04/09	A-4/5	Fed	routine	0/6 pools VD (30 fish)	normal, one fish with a little yolk
03/04/09	A-17	Fed	routine	0/6 pools VD (30 fish)	normal
03/09/09	A-6	Fed	routine	0/6 pools VD (30 fish)	normal
03/11/09	A-7/8	Fed	routine	0/6 pools VD (30 fish)	normal, gills slightly swollen
03/11/09	A-18	Fed	routine	0/6 pools VD (30 fish)	normal
03/17/09	A-9	Fed	routine	0/6 pools VD (30 fish)	normal
03/25/09	A-10	Fed	routine	0/6 pools VD (30 fish)	normal
03/25/09	A-11	Fed	routine	0/6 pools VD (30 fish)	normal
03/25/09	A-12	Fed	routine	0/7 pools VD (31 fish)	normal
04/03/09	A-14	Fed	routine	0/6 pools VD (30 fish)	normal
04/03/09	A-15	Fed	routine	0/6 pools VD (30 fish)	normal
04/03/09	S-19	Fed	routine	0/6 pools VD (30 fish)	normal

No virus was detected in the naturally produced sockeye salmon fry (Table 4.). Fry were only sampled on one day this season, primarily due to the poor outmigration numbers. This sample was not taken until early March. Often virus is only detected early in the outmigration, but it has been detected this late in past years.

Table 4. Health screening results from naturally produced sockeye salmon fry

Sample date	Virus results	Visual exam
03/02/09	0/20 pools VD detected (100 fish)	no microscopic exams, fish appeared normal with small amounts of residual yolk in some fish

All smolts collected in Lake Union appeared healthy with no observable clinical signs of disease and no virus was detected (Table 5.). Virus testing has been performed on smolts or presmolts periodically since the beginning of this project and no virus has been detected to date. This is an indicator of good health since the stress of smoltification is known to cause expression of virus, often combined with other bacterial diseases.

Table 5. Health screening results from outmigrating sockeye salmon smolts

Sample date	**Virus results**	**Visual exam**
05/07/09	0/10 K/S VD detected	no microscopic exams, fish appeared normal with no clinical signs of disease
05/12/09	0/30 K/S VD detected	same
05/19/09	0/30 K/S VD detected	same
05/26/09	0/30 K/S VD detected	same