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 2009. 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 and fish are examined grossly and microscopically 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 and gross abnormalities. 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. This year the last two spawns consisted of five females each week. There were extremely low

numbers of adult returns in 2009 but many of the problems with the weir had been solved and 2,916 adults were spawned. The majority of the broodstock were hauled from the new weir site in Renton, with 210 fish hauled from the SPU passage facility. All samples taken from the 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 27th and November 9th a total of 60 spawning adults were also sampled for the *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD). This is a pathogen that is endemic in Washington and can cause significant losses in sockeye salmon.

No pathogen samples were collected from prespawning adults returning to the system in 2009. WDFW staff collected otoliths from returning adult sockeye salmon captured in June and July at the Ballard Locks by biologists from the Muckleshoot Indian Tribe but no abnormalities were evident in these fish. No abnormal adult mortalities were seen at the locks or elsewhere in the system.

Juveniles

At release 60 fry were collected from each rearing unit at the hatchery to test for the presence of 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. Egg to fry survival was excellent and 4.54 million fry were released from the hatchery. This year the hatchery was again 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.

This year the WDFW Fish Health Laboratory also tested naturally produced sockeye salmon fry for the presence of IHNV. Each week from the end of January through mid March 100 fry were collected at the floating inclined-plane screen trap that is maintained on the lower river to monitor outmigration. In 2010 all hatchery produced fry were released below the fry trap so all fry collected in the trap were naturally produced. Conditions were very good in the river this winter and spring so even with the poor adult returns in 2009 it was a relatively good fry outmigration year. An estimate of natural origin fry is not yet completed, but it is expected to be greater than seven million fry.

In May 2009, sockeye salmon smolts were collected for otolith analysis using a purse seine. In the past the fish had been collected in Lake Union, but this year very few fish were caught at that site. The majority of the fish were collected at Weber Point in Lake Washington. 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 in sockeye salmon so it is emphasized during sampling of the hatchery adults. On a weekly basis OF samples were

collected individually and tested 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 or polymerase chain reaction assays. Methodology is used that will detect IHNV, infectious pancreatic necrosis virus or viral hemorrhagic septicemia virus.

The exams of the fry consisted of examining the gills and skin scrapes microscopically for bacteria, parasites, gill condition and other abnormalities. The fry were dissected and examined grossly for residual yolk material, food in the gut, whether fat was being deposited and any other abnormalities

Kidney tissues were harvested from each of 60 adults and frozen for later analysis by enzyme-linked immunosorbant assay (ELISA) to detect *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD). Results were obtained by recording the optical density (OD) of the color reaction, which indicates 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 2009 the adult return was estimated to be 21,718 sockeye salmon through the Ballard Locks. This was the lowest adult return of any year during the operation of the current hatchery. The fish examined at the locks had light infestations of copepods and occasional wounds, but no external signs of disease. It was an unusually warm and dry June but there were no reports of abnormal mortalities of sockeye salmon adults in the basin. Testing done in 2007 did not detect any pathogens in fish that had no indications of disease or mortality present.

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 IHNV was the only pathogen detected (Table 1). This virus is normally found in spawning sockeye salmon and is not an indicator of a problem. Interestingly, in the last four years very little IHNV has been detected in the adults. In two of these years virus was not detected until mid November and no virus was detected in the other two years. One of the years in which no virus was detected was 2006, which had a very large return of 458,005 fish. More virus is generally present in the later arriving fish so less virus is detected when spawning ends early, however spawning in all of these years occurred within the window of detections in prior years.

Table 1. Viral results from adult sockeye salmon

Sample date	**Viral results**
09/24/09	0/24 OF VD*
09/29/09	0/24 OF VD
10/06/09	0/24 OF VD, 0/4 pools from K/S (20 fish total in 5 fish pools) VD
10/08/09	0/8 pools from K/S (40 fish total in 5 fish pools) VD
10/12/09	0/24 OF VD
10/20/09	0/24 OF VD
10/27/09	0/24 OF VD
11/02/09	0/24 OF VD
11/16/09	0/5 OF VD
11/23/09	**4/5 OF IHNV**

* virus detected

No detectable levels of the antigen to *R. salmoninarum* were found in the spawning adults (Table 2). OD levels of all fish were similar to the negative control tissue. Sockeye salmon are very susceptible to BKD but there was no indication of disease in these fish.

Table 2. ELISA results for *R. salmoninarum* screening

OD Level	**Infection level**	**Number of fish**	**Percent of total**
> 0 .099	below low	60	100%
0.100 – 0.199	low	0	0%
0.200-0.499	moderate	0	0%
>0.450	high	0	0%

Juveniles

No major problems were encountered this year. Abnormal fish were seen in two incubators and elevated loss was seen in two rearing containers but IHNV was not detected in samples taken from any of the affected incubators or from any of the hatchery releases (Table 3). The fish in the affected incubators exhibited premature swim up, but upon examination appeared normal other than having a small amount of yolk present and mortality levels were normal. Development appeared to be accelerated in these two incubators. The two groups which had elevated mortality post ponding recovered and the fish were healthy at release. In the first instance stress was thought to be a factor and in the second instance it was speculated that the disinfectant had not been adequately flushed from the tank used to move fish.

Overall, this was considered a very successful year for the hatchery with the fry released in good condition. Gill condition was very good in all of the fish examined and the majority of the fry were showing some deposition of fat. 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. All fry were fed again this year and yolk was present in only one of the fish examined at release. Since the fish do not emerge volitionally there will be some variation in development.

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

Sample date	Vessel	Rearing	Sample type	Virus results	Visual exam
02/05/10	A-4	incubator	diagnostic	0/2 pools VD (10 fish)	Loss after ponding, but no abnormalities detected
02/05/10	A-11	incubator	diagnostic	0/2 pools VD (10 fish)	Early swimup in both incubators, no abnormalities detected, both groups with small yolk present
02/05/10	A-12	incubator	diagnostic	0/2 pools VD (10 fish)	
02/08/10	S-20	fed	routine	0/12 pools VD (60 fish)	normal
02/09/10	A-3	fed	routine	0/12 pools VD (60 fish)	normal
02/09/10	A-8	fed	diagnostic	0/4 pools VD (19 fish)	Loss after ponding, no fish examined
02/16/10	A-4	fed	routine	0/12 pools VD (60 fish)	normal
02/16/10	A-5	fed	routine	0/12 pools VD (60 fish)	normal
02/17/10	A-6	fed	routine	0/12 pools VD (60 fish)	normal
02/17/10	A-7	fed	routine	0/12 pools VD (60 fish)	normal
02/18/10	A-9	fed	routine	0/12 pools VD (60 fish)	normal
02/18/10	A-10	fed	routine	0/12 pools VD (60 fish)	normal
02/22/10	A-8	fed	routine	0/12 pools VD (60 fish)	normal
02/22/10	A-12	fed, C-4	routine	0/12 pools VD (60 fish)	Normal
02/25/10	A-11	fed	routine	0/12 pools VD (60 fish)	Normal
02/25/10	A-12	fed, C-3	routine	0/12 pools VD (60 fish)	Normal
03/04/10	A-15	fed, C-1	routine	0/12 pools VD (60 fish)	Normal
03/04/10	A-15	fed, C-2	routine	0/12 pools VD (60 fish)	Normal
03/08/10	A-13	fed	routine	0/12 pools VD (60 fish)	Normal
03/08/10	A-14	fed	routine	0/12 pools VD (60 fish)	Normal
03/09/10	A-16	fed	routine	0/12 pools VD (60 fish)	Normal
03/09/10	A-17	fed, C-5	routine	0/12 pools VD (60 fish)	Normal
03/09/10	A-17	fed, C-6	routine	0/12 pools VD (60 fish)	Normal
03/09/10	A-18	fed, C-4	routine	0/12 pools VD (60 fish)	Normal
03/09/10	A-18	fed, C-7	routine	0/12 pools VD (60 fish)	Normal
03/11/10	A-19/20	fed, R-1	routine	0/12 pools VD (60 fish)	Normal
03/11/10	A-19/20	fed, R-3	routine	0/12 pools VD (60 fish)	Normal
03/15/10	A-21	fed	routine	0/12 pools VD (60 fish)	Normal
03/22/10	A-22	fed, C-1	routine	0/12 pools VD (60 fish)	Normal
03/22/10	A-22	fed, C-2	routine	0/12 pools VD (60 fish)	Normal
03/29/10	A-23	fed	routine	0/12 pools VD (60 fish)	Normal
04/02/10	S-20	fed	routine	0/12 pools VD (60 fish)	Normal
04/9/10	S-19	fed	routine	0/12 pools VD (60 fish)	Normal

No virus was detected in the naturally produced sockeye salmon fry (Table 4). Due to good river conditions survival of the wild fry was very good this season and more intensive sampling occurred. Historically, if virus is present, it is detected beginning early in the outmigration so sampling was discontinued after seven weeks of not detecting the virus. The fish were only examined grossly, but all appeared normal. The first two samples included a number of fish that had significant amounts of yolk present but the later samples had fewer fish with yolk present, and only in small amounts. IHNV was last detected in the naturally produced fry in the 2008, however, only one sample was assayed in 2009.

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

Sample date	**Virus results**	**Visual exam**
01/25/10	0/20 pools VD detected (100 fish)	no microscopic exams
02/02/10	0/20 pools VD detected (100 fish)	Same
02/09/10	0/20 pools VD detected (100 fish)	Same
02/16/10	0/20 pools VD detected (100 fish)	Same
02/22/10	0/20 pools VD detected (100 fish)	Same
03/01/10	0/20 pools VD detected (100 fish)	Same
03/09/10	0/20 pools VD detected (100 fish)	Same
03/16/10	0/20 pools VD detected (100 fish)	Same

All smolts collected 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**
05/11/10	0/20 K/S VD detected	no microscopic exams, fish appeared normal with no clinical signs of disease
05/18/10	0/20 K/S VD detected	Same
05/25/10	0/20 K/S VD detected	Same