Monitoring Sockeye Salmon Health in the Cedar River and Lake Washington

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The Cedar River sockeye salmon enhancement program began in 1991, with the goals of increasing the number of *Oncorhynchus nerka* 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 its inception 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 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. Stringent disinfection and isolation procedures are in place at the current hatchery to avoid infection of the juveniles with this virus. Each fall adults are tested for IHNV throughout the spawning season to ascertain the prevalence of the virus. Each release of fry is also tested for IHNV to assess the condition of the group at release. If the fish in any incubator show unusual behavior, or have above normal mortality prior to release, these fish are also tested for viral pathogens. The naturally produced sockeye salmon fry are also tested for viral pathogens throughout the outmigration to monitor the prevalence of IHNV. In years where the fry outmigration is poor, such as 2005, only a limited number of fish are sampled to avoid impacting the population.

During this period, work was also initiated to screen returning adults for specific pathogens to determine whether disease plays a role in the survival of adults returning to the spawning grounds. In August 2004 moribund or dead adult sockeye salmon were noted at the Ballard Locks downstream at Shilshole Bay Marina. Two fish were recovered that were suitable for examination and only *Vibrio anguillarum*, a saltwater bacterial pathogen of fish, was detected in one fish. This bacteria is a common marine pathogen that affects salmon, generally during the warmer summer months. Subsequently, no fish were found dying in the lake, in-river losses appeared normal, and the fish held for spawning survived well and did not show clinical signs of disease, other than the typical fungus and copepods. However, the final estimate of sockeye salmon adults spawning in the basin was well below the number estimated by the lock counts. In the summer of 2005 additional testing was initiated to evaluate the health of the returning adults to determine if a pathogen might be a factor in the poor returns to the spawning grounds. A study was underway to sample otoliths from sockeye salmon adults entering the system via the fish ladder at Ballard Locks. This presented the Fish Health Lab with an opportunity to obtain pathogen samples from the adults killed for that study. Samples were subsequently taken from sockeye salmon adults

when they were spawned at the Cedar River Hatchery, after they had been holding in freshwater for several months.

METHODS

Adults

On three separate days in June, July and August of 2005 WDFW fish health staff collected samples for pathogen testing from sockeye salmon adults captured from the fish ladder at the Ballard Locks by Muckleshoot Indian Tribe biologists. On each sample day 12 fish were examined for any clinical signs of disease and tissues were collected for testing at the WDFW Fish Health Lab. Each year during spawning at the hatchery approximately 24 adults are tested weekly for IHNV and other regulated viral pathogens. In 2005 20 adults were also sampled for additional pathogen testing on each of three days spread throughout the spawning season. These samples were assayed following the same protocols that were used when immature adults were captured at Ballard Locks. The following describes the testing methodology and target pathogens.

Kidney and spleen tissues were individually harvested from each fish sampled at the locks for detection of viral pathogens. Kidney and spleen tissues were also taken from at least 20 fish spawned at the hatchery on the three spawn days with the extended sampling, but ovarian fluid (OF), which is generally a more sensitive specimen to test, was collected on the routine weekly sample days. A larger sample of kidney and spleen tissues were taken on two of the spawn days and assayed in five fish pools, as in past years, but for the third sample the 20 fish were assayed individually. These tissues are processed fresh using standard virological cell culture procedures. The diluted sample is inoculated onto CHSE 214 and EPC cell lines 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 infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV) or viral hemorrhagic septicemia virus (VHSV).

Kidney tissue was inoculated onto agar plates for detection of bacteria by culture. Tryptone yeast extract plus salts agar (TYESA) is used for detection of *Flavobacterium psychrophilum*, causative agent of coldwater disease, and *Flavobacterium columnare*, causative agent of columnaris. Brain heart infusion agar (BHIA) is used for detection of *Aeromonas salmonicida*, the causative agent of furunculosis and *Yersinia ruckeri*, the causative agent of enteric redmouth. BHIA plus salt was used for the detection of *Vibrio anguillarum*. Other bacteria can be isolated using these medias, but these are the typical bacterial pathogens that would generally be isolated in Washington. TYESA is incubated at 15°C and BHIA is incubated at 20°C and both medias are held for 7-10 days for examination for colonies of typical morphology. Identification is done using biochemical assays or, for *Flavobacterium*, typical colony and cellular morphology consistent with isolation techniques. *F. psychrophilum* can also be confirmed with an agglutination test or a polymerase chain reaction assay (PCR).

Kidney tissues were individually harvested for detection of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD). BKD is a very serious disease in salmonids and can cause significant losses in sockeye salmon. Smears were made on slides to screen for the bacteria by a fluorescent antibody technique. Kidney tissue was also harvested and frozen for later

assay by enzyme-linked immunosorbant assay (ELISA) for detection of the antigen to *R. salmoninarum*. The samples were thawed and diluted 1:4 with PBS and assayed by the ELISA plate method. Results are obtained by recording optical density of a color reaction, which indicates relative levels of antigen to *R. salmoninarum* in the sample. The fluorescent antibody technique detects the actual bacteria. The ELISA assay is a more sensitive test, but it is possible to have contaminants that can produce false positives.

A section of hindgut was taken from each fish and frozen for later screening by wet mount for spores of the parasite *Ceratomyxa shasta*, the causative agent of ceratomyxosis. This parasite is known to cause significant mortalities in salmonids in some watersheds. *C. shasta* was first isolated from wild steelhead and cutthroat smolts in the Lake Washington basin in 2002. Subsequent testing has indicated that this pathogen is not likely to be present in high levels, but *C. shasta* has the potential to cause serious fish losses. A sample of the hindgut was also placed in alcohol for later testing for this parasite by the PCR assay. This test is used for confirmation and can also detect the parasite before spores are formed or when infection levels are very low.

Several other samples were taken for possible eventual screening or confirmation of pathogens. Smears were taken from kidney and spleen tissues for microscopic exam. Kidney, spleen and liver tissues were preserved in Davidson's fixative for possible histological exam. A sample of kidney was also preserved in alcohol for later testing by PCR. This tissue sample will most likely be used to test for *Parvicapsula minibicornis*, but can alternately be used for other pathogens if needed. The internal parasite *P. minibicornis* has caused significant mortalities some years in sockeye salmon returning to spawn in British Columbia.

Juveniles

Each year approximately 30 fry are collected from each incubator or 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 control strategies. Most weeks some of the fry were held in the incubators and only one shipment was made to the lab in order to minimize shipments. The fry were shipped to the Fish Health Lab alive, euthanized upon receipt and processed whole in five fish pools. 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. In addition to virology, a small number of fish from the majority of the releases were also examined microscopically to monitor for bacteria, parasites, gill condition, and other abnormalities. Rearing space is limited at this hatchery, but because the low returns in 2005 resulting in a smaller egg take of 6.9 million eggs, a large portion of the fry were fed for a one to two week period prior to release. This practice has been shown to improve the survival of the fry after release. However, the intensive feeding has the potential cause gill disease so the gill condition was evaluated.

Most years the WDFW Fish Health Lab has also taken weekly samples of 100 sockeye salmon fry from the floating inclined-plane screen trap that is maintained on the lower river to monitor outmigration. These fish are also processed whole in five fish pools and tested for virus. Although there is no way to separate the naturally produced fry from the hatchery fry, sampling has been done on nights that were at least two nights after the last hatchery release. Due to the small outmigration during this sampling period only one sample was taken in early February of 2006 and it was collected the night before the first hatchery release.

RESULTS AND DISCUSSION

Adults

During the summer of 2005 the adult return was estimated to be 87,023 sockeye salmon through the Ballard Locks. The fish examined at the locks had light infestations of copepods, occasional wounds, but no clinical signs of disease such as hemorrhaging or abnormalities of the internal organs. This was a much lower return of adults than had been forecast, indicating poor ocean survival. Returns to the river were somewhat lower than anticipated but there were no reports of moribund or dying adults this year.

Testing has been completed for viral pathogens, bacterial pathogens by culture, *R. salmoninarum* by ELISA and FAT, and *C. shasta* by wet mount. No pathogens were detected in these tests from any of the immature adults collected at Ballard Locks (Table 1.). The only pathogen detected in the spawning adults was IHNV. This finding of IHNV is typical of most populations of sockeye salmon, which express IHNV as they become senile. No clinical signs were present that indicated disease, either for *P. minibicornis* or *C. shasta*, but the PCR samples are being held for later testing. No findings from any of the testing to date indicate a pathogen is responsible for the lower than expected adult return, either at the locks or for the returns to the river. It is possible that there was an earlier disease episode in the lake, but it would be unusual not to have some evidence of moribund fish at the time or infected fish surviving to spawn. WDFW will continue to monitor the returning sockeye salmon adults, particularly those in distress, to ascertain what role disease may play in the loss of any adults prior to spawning.

Table 1. Adult sockeye pathogen results

Sample date	**Viral results**	**Bacteriology results**	***R. salmoninarum* by ELISA and FAT**	***C. shasta* spores**
06/23/05	0/12 from K/S virus detected	0/12 bacterial pathogens	0/12 in detected range	0/12 detected
07/14/05	0/12 from K/S virus detected	0/12 bacterial pathogens	0/12 in detected range	0/12 detected
08/02/05	0/12 from K/S virus detected	0/12 bacterial pathogens	0/12 in detected range	0/12 detected
09/28/05	0/24 from OF virus detected			
10/03/05	0/24 from OF virus detected			
10/12/05	0/24 from OF virus detected 0/5 pools from K/S (5 fish/pool) virus detected	0/20 bacterial pathogens	0/20 in detected range	0/20 detected
10/17/05	0/24 from OF virus detected			
10/24/05	0/24 from OF virus detected			
10/26/05	0/7 from K/S (5 fish/pool) virus detected	0/20 bacterial pathogens	0/20 in detected range	0/20 detected
10/31/05	**3/24 OF IHNV positive**			
11/07/05	**8/24 OF IHNV positive**			
11/14/05	**22/24 OF IHNV positive**			
11/22/05	**16/18 OF IHNV positive**			
11/28/05	**18/24 OF IHNV positive** **17/20 K/S IHNV positive**	0/20 bacterial pathogens	0/20 in detected range	0/20 detected

Juveniles

In general the health of the fry was very good at release (Table 2.). There were only minor impacts on gill condition in some of the fed groups and the majority of the fry were showing some deposition of fat. Several incubators suffered fish losses but in all but one cases the loss was attributed to a water interruption or poor flow patterns in the incubators. The fry in one incubator, vertical stack #17 containing 63,000 fry, suffered a loss due to IHN and the remaining fish were destroyed. This group of fish suffered a substantial loss just prior to ponding that was initially believed to be due to a water interruption and the remaining fry were ponded. The severe loss continued for several days after ponding and then diminished. At this point the viral testing from the fry in this incubator resulted in 6 of 6 pools positive for IHNV. Although loss had subsided, the remaining fry were destroyed to avoid spreading IHNV to other groups and the fish in the river.

The source of the virus in the infected fry is unknown, but there are two probable sources. Although rare, there is the potential for virus to be transferred from the adult, possibly from inadequate disinfection. The level of virus was very high in the adults that were spawned on the day that produced these fry. Cedar River Hatchery uses very thorough disinfection procedures involving rinsing and waterhardening in iodophor, but it is possible that with very high titers of virus, not all virus was destroyed. The other source is horizontal transmission through the water supply. Cedar River Hatchery uses water collected from a number of springs that anadromous fish cannot enter. However, these spring pools are adjacent to the river and are not covered. Other animals can move from the river into the pools, potentially carrying infected fish or virus from the river. The epidemic in this incubator occurred very late, making it less likely that horizontal transmission from the adults occurred, but naturally produced fry would be present in the river at this time. Although no virus was detected in the naturally produced sockeye salmon fry this year (Table 3.), the sample size was very small. Prior years' testing has frequently detected the presence of IHNV in the naturally produced fry.

Overall, this was considered a very successful year with the fry released in good condition. The fish accepted feed well and had good growth, even with the short rearing period. There was some yolk retention on a small portion of the fish, but this is normal for fry that can't emerge volitionally.

Table 2. Hatchery sockeye salmon fry results

Sample date	Vessel	Treatment	Sample type	Virus results	Visual exam
02/06/06	S-1	fed	routine	0/6 pools (30 fish) VD*	normal
02/06/06	S-2	fed	routine	0/6 pools (30 fish) VD	normal
02/06/06	S-3	started feeding	diagnostic	0/6 pools (30 fish) VD	fish in top tray had delayed development but otherwise appeared normal
02/07/06	S-4	before feeding	diagnostic	0/7 pools (28 fish) VD	gills swollen, no pathogens detected
02/14/06	A-1	fed	routine	0/6 pools (30 fish) VD	normal
02/14/06	A-2	fed	routine	0/6 pools (30 fish) VD	normal
02/14/06	S-3 2nd test	fed	routine	0/6 pools (30 fish) VD	gills slightly swollen and pale
02/21/06	S-4 2nd test	fed	routine	0/6 pools (30 fish) VD	normal

* VD = virus detected

Table 2. Hatchery sockeye salmon fry results (continued)

Sample date	Vessel	Treatment	Sample type	Virus results	Visual exam
02/21/06	A-3	fed	routine	0/6 pools (30 fish) VD	normal
02/22/06	A-9	unfed	routine	0/6 pools (30 fish) VD	gills somewhat swollen
02/22/06	A-10	unfed	routine	0/6 pools (30 fish) VD	normal
02/22/06	A-13	before ponding	diagnostic	0/12 pools (60 fish) VD	normal
02/22/06	A-14	before ponding	diagnostic	0/3 pools (15 fish) VD	normal, but some fry still had yolk present
02/22/06	A-15	before ponding	routine	0/5 pools (23 fish) VD	normal
02/22/06	A-4	fed	routine	0/6 pools (30 fish) VD	normal
02/22/06	A-5	fed	routine	0/6 pools (30 fish) VD	normal
02/22/06	A-6	fed	routine	0/6 pools (30 fish) VD	normal
02/22/06	A-7	fed	routine	0/6 pools (30 fish) VD	normal
02/22/06	A-8	unfed	routine	0/6 pools (30 fish) VD	normal
02/27/06	A-16	unfed	routine	0/6 pools (30 fish) VD	No exam
02/27/06	A-17	unfed	routine	0/6 pools (30 fish) VD	No exam
02/27/06	A-18	unfed	routine	0/6 pools (30 fish) VD	No exam
02/27/06	A-19	unfed	routine	0/6 pools (30 fish) VD	No exam
03/01/06	A-11	fed	routine	0/6 pools (30 fish) VD	No exam
03/01/06	A-12	fed	routine	0/6 pools (30 fish) VD	No exam
03/06/06	A-13 2nd test	fed	routine	0/6 pools (30 fish) VD	normal, but some fish didn't start feeding
03/06/06	A-14 2nd test	fed	routine	0/6 pools (30 fish) VD	normal
03/07/06	A-25	fed	routine	0/6 pools (30 fish) VD	normal
03/07/06	A-26	fed	routine	0/6 pools (30 fish) VD	normal
03/14/06	A-20	fed	routine	0/6 pools (30 fish) VD	
03/14/06	A-21	fed	routine	0/6 pools (30 fish) VD	
03/14/06	A-22	fed	routine	0/6 pools (30 fish) VD	
03/20/06	A-23	fed	routine	0/6 pools (30 fish) VD	normal
03/20/06	A-24	fed	routine	0/6 pools (30 fish) VD	normal
03/27/06	A-27	fed	routine	0/6 pools (30 fish) VD	normal
03/27/06	S-17	after ponding	diagnostic	**6/6 pools IHNV positive** (30 fish)	not live when received, suspected suffocation initially
04/10/06	S-18	fed	routine	0/6 pools (30 fish) VD	
04/10/06	S-19	unfed	routine	0/6 pools (29 fish) VD	
04/10/06	S-20	unfed	routine	0/6 pools (30 fish) VD	

Table 3. Naturally produced sockeye salmon fry viral results

Sample date	Virus
02/06/06	0/20 pools (100 fish) virus detected