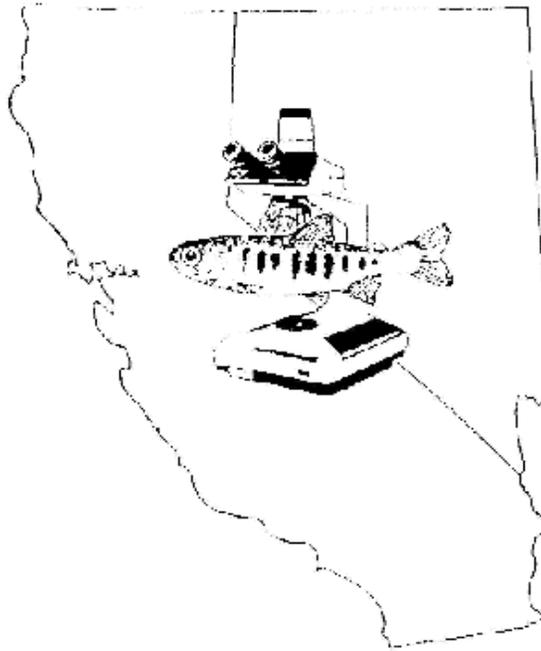


FY2000 Investigational Report :
Lack of experimental evidence for IHN virus
transmission from infected hatchery to natural
Chinook salmon juveniles in the Sacramento River



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Abstract - Coleman National Fish Hatchery (CNFH) has a long history of infectious hematopoietic necrosis (IHN) disease in its juvenile chinook salmon (*Oncorhynchus tshawytscha*) that can result in high fish mortality and the subsequent release of large numbers of IHNV exposed juveniles. The transmission of IHNV to wild or natural chinook populations in the Sacramento River system from infected hatchery fish is a concern for resource managers. In this study, natural chinook juveniles were cohabitated with experimentally IHNV-infected hatchery chinook at ratios of 1:1, 1:10, and 1:20 for either 5 minutes or 24 hours. Additional natural chinook salmon were held in cages within the exposure tanks. During the 7 d post-exposure rearing period, a portion of each natural group was stressed daily. These exposures were designed to simulate brief and “worst case” natural fish contacts with a massive hatchery release of infected fish. Virus was not detected by tissue culture assays from any natural chinook in the 3 experiments. The inability to detect virus in the tissues of exposed natural fish, regardless of their duration of exposure, ability to directly interact with infected fish, or post-exposure stress indicates a low ecological risk to natural populations if infected hatchery fish are released into the Sacramento River. Unique characteristics of the host – pathogen relationship should be evaluated for each situation when developing risk assessments.

Introduction

Pathogen transmission from cultured fish to wild fish is one element within the controversy over the impact of aquaculture on natural systems (Hedrick 1998, Schramm and Piper 1995). Infectious hematopoietic necrosis virus (IHNV) has been a significant chinook salmon (*Oncorhynchus tshawytscha*) disease problem at Coleman National Fish Hatchery (CNFH) since it began operations in the 1940's (Ross et al. 1960). Epizootics have been common in the fall-run chinook salmon production with high mortality and subsequent release of large numbers of IHNV exposed juveniles. Recently, the National Marine Fisheries Service has placed specific conditions on CNFH chinook releases due to the potential risk of virus transmission to the endangered winter -run chinook juveniles (1999 interim Biological Opinion). There appears to be a distinct difference between a hatchery epizootic and experimental viral challenges with the local IHNV strain. Constant viral exposure and high densities of hosts may be required for efficient transmission as the Sacramento R. strain of IHNV shows relatively low virulence. Previous studies have shown that 30 min static bath challenges with 5.7×10^3 Plaque Forming Units / mL (1997 CNFH isolate) produce only 10 % cumulative mortality over 19 days (unpublished data by author).

The objective of this study was to determine, if during simulated river encounters, IHNV could be transmitted from infected hatchery to natural chinook juveniles. It was partially funded by an interagency agreement with U.S. Bureau of Reclamation (11230-1937-0191, \$13,000).

Methods:

Three separate experiments were conducted with natural juvenile chinook salmon from the Upper Sacramento River collected by the U.S. Fish & Wildlife Service (USFWS) Northern Central Valley Fish & Wildlife Office and juvenile hatchery chinook salmon from CNFH. The term natural is used in this report to denote a feral fish from a watershed with hatchery influence that may or may not have genetic background derived from cultured fish. The natural juveniles were captured in Rotary Screw traps below Red Bluff Diversion Dam (river km 391) and transported to the USFWS California-Nevada Fish Health Center's wet laboratory in Anderson, California. Laboratory effluent is treated with 4 mg / L chlorine for 12 min, de-chlorinated with activated charcoal filters, and discharged into a gravel leach field. Within 3 h of arrival at the laboratory, 4 - 5 natural fish were placed directly into 177 L tanks and a similar number held in a 40 m³ cage inside the tanks. After 1 h, IHNV -infected hatchery fish were introduced into the tank. The caged group was included to distinguish waterborne from physical contact transmission. Nipping behavior or fish-to-fish contact could be a route of viral transmission as high concentrations of IHNV has been detected in cutaneous mucus (unpubl. FHC data, LaPatra et al. 1989). Unexposed natural chinook salmon were also tested for virus in each experiment. The ratio of cohabitated natural fish to infected hatchery fish was 1:1 for experiment 1 (9-10March2000), 1:10 for experiment 2 (15 - 16March 2000), and 1:20 for experiment 3 (03-04April2000). The 1:20 ratio was based on the natural to hatchery fall run chinook smolt ratio estimated from rotary screw trap collections at Red Bluff Diversion Dam following the 1999 CNFH release (P. Gaines, Northern Central Valley Fish & Wildlife Office, personnel communication).

Exposures occurred in 1 m diameter circular tanks supplied with 17 L / min flow of 10 - 13°C untreated, Battle creek water. This flow was determined to be the minimum that produced an upstream swimming orientation. The center of the tanks contained a 25 cm circular divider to encourage circular swimming and the water level was held at a depth of 25 cm. Total volume that the fish occupied was 177 L. A quarter of the surface in each tank was covered to encourage schooling. Hatchery fish were infected with a 0.1 mL intraperitoneal injection of supernatant harvested from cultures of epithelioma papulosum cyprini (EPC) cells infected with an isolate from a 1997 IHNV epizootic in juvenile CNFH chinook salmon. The supernatants used for injections in the 3 experiments contained 1.2 – 6.3 x 10⁶ Plaque Forming Units (PFU)/mL of virus. Five to ten days post-injection, hatchery fish were marked in a 40 min bath of 0.02 g / L Bismark brown dye. Infected fish were used for the exposures within 4 days of the marking operation.

Exposure times of 5 min (4 replicates / experiment) and 24 h (2 replicates per experiment) were chosen to simulate both a brief and a "worst-case" river interaction, respectively. After each exposure, all fish were captured and the unmarked natural chinook salmon were moved to 15 L flow-through aquaria supplied with aerated, 12 - 14° C water. A randomly selected half of each natural chinook exposure group was subjected to a 30 s netting stress for the first 3 days of captivity (Barton et al. 1987). The natural salmon were sampled for virus isolation at 7 days post-exposure and the

hatchery fish immediately after the cohabitation. A triangular section comprised of gill, anterior kidney, liver, muscle and skin was processed from each fish for virus isolation (Thoesen 1994). The triangular section was produced by cutting a cross-section of the fish at the level of the operculum and diagonally from the ventral aspect of this incision point to the dorsal surface. This method precluded most of the stomach and intestinal tract from the tissue pool. One hundred microliter of a 40x dilution was inoculated onto replicate wells of a drained, EPC culture pretreated with 7% polyethylene glycol solution (Batts and Winton 1989). The 48 well EPC plates were incubated at 15°C for 14 days. A blind pass culture was started at 6 days post-inoculation for each natural salmon sample. In experiments 2 and 3, two fish pools of infected hatchery chinook tissues were assayed for virus.

Results :

Natural chinook salmon used in experiment 1 and 2 were approximately 25 -30 % smaller in fork length (mean, 38 mm and 41 mm, respectively) than the infected hatchery fish (mean, 55 mm). We observed that the smaller natural fish stayed near the surface and center of the tanks. The injected hatchery fish used for experiment 1 were asymptomatic, however, each was determined to be infected with IHNV (Table 1). The mean virus concentration in their tissue pools was 1.2×10^4 PFU / g. Many of the injected hatchery chinook used in experiment 2 demonstrated IHNV clinical signs (exophthalmus, darken color, weaken condition) and all tested viral positive (Table 1). Separate gill and kidney-liver samples were viral tested from a sub-sample of 8 hatchery fish from experiment 2. Mean virus concentration was 2.8×10^4 PFU / g in gill and 1.4×10^4 PFU / g in the kidney / liver tissue. Natural chinook, resembling out-migrant smolts (increased silvering, > 60mm fork length), were selected for experiment 3. They were similar in size to their infected hatchery cohorts (means, 69 and 63 mm, respectively). Most of the infected hatchery fish in experiment 3 showed clinical signs of infection and approximately 30% of the 24 h exposure group died prior to the end of the cohabitation period. All injected hatchery fish from experiment 3 tested positive for virus (Table 1). No virus was isolated from any natural chinook salmon in all 3 experiments (Table 1). There were 2 natural salmon mortalities from both experiments 2 and 3. Columnaris lesions were seen in a number of experiment 3 natural fish after 7 days of captivity.

Table 1.

Prevalence of IHNV infection in juvenile natural and injected hatchery chinook salmon. Natural salmon were cohabitated or held in cages with injected hatchery fish for either 5 min. or 24 h. Half of each natural cohabitation group was subjected to netting stress for the first 3 d of the 7 d post-exposure period. Data report as number positive / total number of samples per exposure group.

	Natural chinook salmon		
<u>5 minutes</u>	<u>1 : 1</u>	<u>1 : 10</u>	<u>1 : 20</u>
cohabitated + stress	0 / 8	0 / 8	0 / 8
cohabitated	0 / 8	0 / 8	0 / 8
caged	0 / 6	0 / 8	0 / 8
<u>24 hours</u>			
cohabitated + stress	0 / 4	0 / 5	0 / 4
cohabitated	0 / 4	0 / 5	0 / 3
caged	0 / 4	0 / 4	0 / 8
no exposure	0 / 1	0 / 34	0 / 17
	Injected hatchery chinook salmon		
	16 / 16	80 / 80*	98 / 98*

* Two fish pools

Discussion:

The inability to detect virus in the tissues of exposed natural chinook salmon juveniles, regardless of their duration of exposure, ability to directly interact with infected fish, or post-exposure stress indicates a low ecological risk to natural populations if infected hatchery fish are released into the Sacramento River. We base this assertion on 3 factors specific to the Upper Sacramento River including:

- 1) The low probability of virus transmission from infected hatchery to natural chinook juveniles in the river environment.
- 2) IHN virus is present in the river throughout the entire year and is endemic to all 4 runs of Upper Sacramento River chinook salmon. This strain has electrophoretic, virulence, and species susceptibility characteristics that are unique from Columbia River basin and Alaskan isolates (Hsu et al. 1986, LaPatra et al. 1993a).
- 3) The low virulence of the Sacramento R. strain of IHNV would reduce the likelihood of an initial infection progressing to a disease state in unstressed juveniles.

While we cannot absolutely rule out viral infection in the natural salmon used in the study, our exposure system should have detect IHNV transmission if the concentration of waterborne virus was 10^3 PFU / mL or greater. In a pilot experiment, IHNV could be isolated from gill - liver- kidney tissue pools of hatchery juveniles 4 days after a 5 minute exposure to 2.5×10^3 PFU / mL. Yamamoto et al. (1992) demonstrated by immunohistochemistry that IHNV rapidly multiplies in the epidermal cells of the skin and gill when the tissues were exposed to high levels of the virus. Virus could be detected immediately after exposure through the conclusion of the 4 day experiment. The infection subsequently spread into the dermis and subcutaneous tissues. Helmick et al. (1995) reported that 24 h post-immersion challenge, rainbow trout mucus secreting cells in the esophageal / cardiac stomach region became necrotic and showed signs of viral replication. These data increase our confidence in detecting IHNV if the fish were exposed to relatively high levels of the virus.

Reno (1998) modeled the effects of changing a given pathogen's transmission coefficient, β , on the expected number of animals infected over time in a susceptible population. Low β values were unlikely to result in an epizootic. Factors such as host resistance and population density, pathogen virulence and exposure dose, and water characteristics were stated to influence β . Innate resistance mechanisms such as epidermal / mucus barriers and non-specific defenses, have been linked to IHNV resistance (Helmick et al. 1995). Stimulation of the non-specific immune system (e.g. lysozyme, phagocytosis, and acute phase serum proteins) from glucan injections were associated with enhanced protection from IHNV challenge (LaPatra et al. 1998). There is the remote possibility that some portion of the natural chinook juveniles had been previously exposed to the virus and developed humoral immunity (La Patra et al. 1993b). Conversely, high density rearing stress and handling events in a hatchery could reduce immune defense mechanisms (Wedemeyer 1997). Chou et al. (1999) report that heavy metal exposure and salinity shock significantly increased mortality in

groupers challenged with a infectious pancreatic necrosis virus of low virulence.

An ozone water disinfection system is now in operation at CNFH and should significantly reduce the threat of waterborne fish diseases such as IHNV. As mentioned above, the Sacramento R. strain of IHNV demonstrates only moderate virulence and appears to require a combination of chronic virus exposure and a high density of susceptible hosts for the occurrence of an epizootic (Parisot and Pelnar 1962). Unlike hatchery production fish reared in raceways, juvenile chinook in the Sacramento River system would not be living in continuous, high density conditions. Also unlike a raceway environment, the dilution effects of significantly larger volumes and flows within the river would act to reduce β . As demonstrated in this study, the risk of pathogen transmission and subsequent disease must be evaluated for each specific situation.

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