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Differences in Susceptibility Among Three Stocks of Chinook Salmon, *Oncorhynchus tshawytscha,* to Two Isolates of Infectious Hematopoietic Necrosis Virus

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DIFFERENCES IN SUSCEPTIBILITY AMONG THREE STOCKS OF CHINOOK SALMON, ONCORHYNCHUS TSHAWYTSCHA, TO TWO ISOLATES OF INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS

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ABSTRACT

Fry from three stocks of chinook salmon, Oncorhynchus tshawytscha, were exposed to two water-borne isolates of infectious hematopoietic necrosis virus. One isolate was from sockeye salmon, O. nerka; the other was from chinook salmon. Fry from the two Alaskan chinook salmon stocks showed no susceptibility to the isolates. Chinook salmon fry from the Carson National Fish Hatchery at Wind River, Wash., responded significantly to both isolates ($P < 0.025$, $P < 0.05$) and had symptoms of viremia after exposure to the virus. However, we were able to isolate the virus from only a few of the Carson stock fry that died after exposure. The virus may have been destroyed; when samples were frozen before assay.

INTRODUCTION

Different salmonid. stocks[1] have different susceptibilities to pathogens. For example, chinook salmon, Oncorhynchus tshawytscha, stocks vary in their resistance to the myxosporidian Ceratomyxa shasta (see Zinn et al. 1977); and Atlantic salmon, Salmo salar, have stock-specific resistance to vibriosis (Gjedrem and Aulstad 1974) .

Both chinook salmon and sockeye salmon, Oncorhynchus nerka, are susceptible to an agent causing infectious hematopoietic necrosis (Amend et al. 1973). The agent causing this disease was originally identified in sockeye salmon as Oregon sockeye disease virus and in chinook salmon as Sacramento River chinook disease virus (Parisot et al. 1965). Because of similar morphologies, cytopathic and histopathic effects, and antigenic relationships, these viruses are now considered variants of the same agent, infectious hematopoietic necrosis virus (IHNV) (Amend and Chambers 1970; Yasutake and Amend 1972; McCain et al. 1974). Parisot et al. (1965) reported that sockeye salmon are susceptible to IHNV from either sockeye salmon or chinook salmon and that chinook salmon are not experimentally susceptible to sockeye salmon IHNV Wingfield et al. (1970) also found that chinook salmon challenged with Oregon sockeye disease virus were not overtly infected.

To test if different stocks of chinook salmon can have different susceptibilities to different IHNV isolates, we exposed fry from two Alaskan stocks and one Washington stock to IHNV isolated from chinook salmon- and to IHNV isolated from sockeye salmon.

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[1] Stock refers to "which fish to a substantial degree do not interbreed with any group spawning in a different place or in the same place at a different season" (Ricker 1972).

METHODS

The two Alaskan stocks came from the Unuk and Situk Rivers; the Washington stock came from the Carson National Fish Hatchery at Wind River. Gametes from the Alaskan stocks were taken from mature adults snagged from the spawning grounds. The gametes were transferred unmixed to the National Marine Fisheries Service research station at Little Port Walter, Alaska, where the eggs were fertilized, water hardened, and disinfected with iodophor (for method see Amend 1974). The eggs were incubated to the eyed stage at Little Port Walter and transferred to the Fish Disease Laboratory of Oregon State University, where they were again treated with iodophor and incubated to the fry stage. Eyed eggs from the Carson stock were transported from the Carson National Fish Hatchery to the Oregon Department of Fish and Wildlife Marion Forks Salmon Hatchery, where they were disinfected with iodophor and incubated to the fry stage. Carson stock fry were then transported from the Marion Forks facility to the Fish Disease Laboratory.

Each stock was challenged with two IHNV isolates: (1) chinook salmon IHNV, an isolate from an epizootic in chinook salmon at Coleman National Fish, Hatchery near Battle Creek, a tributary of the Sacramento River in northern California; and (2) sockeye salmon IHNV, an isolate from an epizootic in sockeye salmon at the Auke Creek Hatchery in Alaska. The viruses were' grown on chinook salmon embryo cell monolayers (CHSE-214) in minimal essential medium containing 5% fetal calf serum (MEM-5). When cytopathic effect was complete, the supernatant was harvested and frozen in aliquots in a Revco[2] -6O°C freezer until used. Before the experiment, a vial of each

[2] Reference to trade names does not imply endorsement by National Marine Fisheries Service, NOAA.

isolate was thawed, and the titer of infectious virus was determined using the CHSE-214 cell line. The remaining frozen aliquots of virus were used, as needed, to make up the challenge concentrations for each stock.

Tests of the three stocks were not conducted concurrently because of different spawning times and incubation-temperature regimes; however, all fry were exposed approximately 14 d after yolk absorption when they averaged 0.5 g wet weight. Fry from each stock were divided into seven groups (one control group and six challenge groups) that consisted of two duplicate lots of 50 fry. Each lot was placed in a separate 8-l glass jar containing 2 1 of water. Three groups of fry were exposed to sockeye salmon IHNV at 10^3, 10^4, and 10^5 plaque-forming units per ml; three other groups were exposed to the same amounts of chinook salmon IHNV. The appropriate amount of virus in 14 ml of MEM-5 was added to each jar, and 14 ml of sterile MEM-5 was added to each control lot. Fry were held in the jars for 24 h,. and air was bubbled into the water to provide adequate dissolved oxygen. Each lot of fish was then transferred to a 50-l tub that received 0.2 l/min waterflow. The fish were held 21 d after viral challenge. Dead fish were removed daily and frozen at -18ºC for later examination. Water temperature was maintained at 12ºC throughout. the' challenge and subsequent holding periods. The fry were fed Oregon Moist Pellets several times daily (except during the challenge) from the time of yolk absorption until the end of the experiment.

Dead fish removed from the challenge groups were assayed for presence of IHNV. Each fish was ground with a mortar and pestle in 10 ml of Hank's Basal Salt Solution . The sample was then centrifuged and 0.5 ml of supernatent added to 2 ml of a mixture of MEM and 2000 units penicillin, 2000 units streptomycin, 500 µg gentamicin, and 1000 µg Mycostatin. The

supernatant and antibiotic mixture were stored overnight at 5°C, then innoculated onto the CHSE-214 cell monolayers. After the cell cultures were incubated for 14 d at 15°C, we examined them for viral cytopathic effect.

Results from the tests were fitted to a simple linear-regression model to test for response within each stock to challenge concentrations of each of the viral isolates. Because the low incidence of deaths indicated a Poisson distribution, a square-root transformation was used on each observation of numbers of deaths (Steel and Torrie 1960). Log_{10} (dosage plus 1) was used as the independent variable. Analysis-of-covariance F-test for common slope (Snedecor and Cochran 1956) was used to test for differences among the three stocks in their response to concentrations of each viral isolate.

RESULTS

More Carson stock fry than Alaskan stock fry died after exposure to chinook salmon IHNV (Table 1). As many as 15 fish died in one lot of Carson stock fry. No more than two fish in any Unuk lot or three fish ‘in any Situk lot died after exposure. Numbers of deaths in the Carson stock exposure groups increased significantly ($P < 0.025$) with increased challenge concentrations of chinook salmon IHNV. There was not, however, a significant relationship between number of deaths and challenge concentrations of chinook salmon IHNV for either Alaskan stock (Table 2). Analysis of covariance showed a significant difference ($P = 0.005$, Table 2) among the three stocks in their responses to different challenge concentrations of chinook salmon IHNV

For the two Alaskan stocks and the Carson stock, no more than four fry died in any lot exposed to sockeye salmon IHNV (Table 1). Numbers of deaths in the Carson groups exposed to this isolate increased significantly with challenge concentrations ($P < 0.05$), whereas numbers of deaths ‘in the

Table 1. --Number of dead fish in, replicate lots of 50 fry from three stocks of chinook salmon challenged with two different infectious hematopoietic necrosis virus (IHNV) isolates.

Challenge level (PFU[1]/ml)	Number of deaths					
	Unuk stock		Situk stock		Carson stock	
	CHINOOK SALMON IHNV					
Control	0	1	0	1	0	0
10^3	1	2	0	1	0	2
10^4	1	2	1	3	4	7
10^5	0	1	1	2	12	15
	SOCKEYE SALMON IHNV					
Control	0	1	0	1	0	0
10^3	0	1	2	3	2	4
10^4	1	2	1	1	1	1
10^5	1	2	0	2	2	3

[1] Plaque-forming units.

Table 2. Regression analysis of the response of the three chinook salmon stocks to viral challenge concentrations. The data were fitted to the model y = A + Bx, where y was the square-root transformation of the numbers of deaths and X was the $\log_{10}$ (dosage + 1). Probabilities for B ≤0 were! determined by I-tests. The three regression equations for each viral isolate: were then tested by analysis-of-covariance F-test for probability of common slope. If no difference in slopes was indicated, analysis of covariance was; used to test for common intercepts assuming common slope.

Chinook salmon stock	A	B	r^2	$P_B \leq 0$	Probability common slope	Probability common intercept
			CHINOOK SALMON IHNV			
Unuk	0.702	0.051	0.033	P >0.35		
Situk	0.405	0.163	0.284	P >0.15		
Carson	-0.395	0.690	0.759	P <0.025		
Analysis of covariance					P = 0.005	--
			SOCKEYE SALMON IHNV			
Unuk	0.399	0.152	0.296	P >0.15		
Situk	0.749	0.065	0.042	P >0.35		
Carson	0.182	0.296	0.640	P <0.05		
Analysis of covariance					P = 0.320	P = 0.748

Unuk and Situk groups did not (Table 2). Analysis of covariance did not indicate a significant difference between either the slopes or the intercepts of the regression equations describing the responses of the three stocks to viral challenge concentration.

External symptoms of IHNV viremia include petechial hemorrhages at the base of the fins, dark coloring, exophthalmia, and fecal casts (Amend et al. 1973). All of these symptoms were observed in some of the Carson fish after exposure to either sockeye salmon or chinook salmon IHNV. None of these symptoms was observed in the fish from the two Alaskan stocks.

We were able to isolate IHNV from only a few of the dead fish removed from the experimental groups. Viruses were isolated from 3 of 40 Carson fish that had been exposed to chinook salmon IHNV. No virus was isolated from fish removed from any of the Unuk or Situk groups or from Carson fish that had been exposed to sockeye salmon IHNV.

DISCUSSION

Fry from the Carson stock exhibited greater susceptibility to IHNV than fry from the Alaskan stocks. There was no evidence that the Alaskan stocks were susceptible to either IHNV isolate: few fish died after exposure to the virus; no significant relationship between mortality and challenge level of virus was detected; and no fish had symptoms of viremia. Susceptibility of the Carson fish to chinook salmon IHNV was clearly indicated by (1) increased mortality in the exposed groups, (2) a significant relationship between mortality and challenge level, and (3) observed symptoms of viral infection. After exposure to sockeye salmon IHNV, only a few Carson fish died; however, susceptibility to this isolate was suggested by the significant relationships between challenge levels and mortality, and challenge levels and observed symptoms of viremia.

The low rate of viral reisolation from dead Carson fish is not consistent with the interpretation that the fish were killed by IHNV. The virus may have been destroyed when the fish were stored in the freezer. To test this hypothesis, we tried to reisolate IHNV from sockeye salmon fry that had died in a confirmed IHNV epizootic. The fry from the epizootic had been stored in the same freezer for a similar period as the Carson chinook salmon fry--we were unable to isolate the virus from these fish. Other workers have found that IHNV rapidly loses its activity in frozen tissue samples (Burke[3]).

Because we were unable to isolate the virus from any Carson fish dying after exposure to sockeye salmon IHNV, the evidence that this stock of fish is susceptible to sockeye salmon IHNV is not conclusive. The suggestion that the Carson chinook salmon might be overtly susceptible to IHNV from sockeye salmon is contrary to results reported by Parisot et al. (1965) and Wingfield et al. (1970). To determine whether some chinook salmon are overtly susceptible to sockeye salmon IHNV, this experiment should be repeated with an emphasis on the reisolation of the virus from dead fish. Fish with symptoms of viremia should be processed immediately to determine presence of virus and thereby eliminate the possibility of loss of virus during freezing.

Because IHNV is ubiquitous in sockeye salmon in Alaska (Grischkowsky and Amend 1976; Grischkowsky[4]), any chinook salmon stock that is susceptible to IHNV in general, and to sockeye salmon IHNV in particular, would be a poor choice for a hatchery stock in Alaska. In 1977, the Alaska Department of Fish and Game stopped rearing Carson chinook salmon at the Crystal Lake Hatchery at Petersburg because IHNV was

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[3] John Burke, virology specialist , Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99502, pers. commun. 1980.

detected in returning Carson adults.. This study supports their decision in two ways: (1) by demonstrating that the Carson chinook salmon stock is overtly more susceptible to chinook salmon IHNV than the two Alaskan chinook salmon stocks tested and (2) by suggesting that the Carson chinook salmon stock may be susceptible to sockeye salmon IHNV.

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[4] Dr. Roger Grischkowsky, Chief Fish Pathologist, Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99502, pers. commun. 1980.

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