Bull. Eur. Ass. Fish Pathol. 7 (4), 97, 1987

SUSCEPTIBILITY OF COHO *(Oncorhynchus kisutch)* AND CHINOOK *(Oncorhynchus tshawytscha)* SALMON HYBRIDS TO EXPERIMENTAL INFECTIONS WITH INFECTIOUS HEMATOPOIETIC NECROSIS VIRUS (IHNV)

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Sockeye or kokanee *(Oncorhynchus nerka)* and chinook *(O. tshawytscha)* salmon and rainbow or the anadromous steelhead trout *(Salmo gairdneri)* are the principal species susceptible to natural infections with infectious hematopoietic necrosis virus (IHNV) in North America (Pilcher and Fryer, 1980). Coho salmon *(O. kisutch)* have traditionally been resistant to infections even in hatcheries where they are raised with susceptible species. However, in the last two years adult coho salmon infected with IHNV have been found returning to the Trinity River Hatchery in northern California (LaPatra et al. 1987). This hatchery has had well established populations of returning coho and chinook salmon for many years and they begin entering the facility in October and continue through January. Although chinook salmon at this facility have been known to be IHNV positive each year since 1969 (Wingfield and Chan, 1970) only recently has virus been detected in coho salmon.

After the Trinity Hatchery has taken the needed number of eggs for production quotas from returning adult coho and chinook salmon, the gates are closed and the remaining adults in the river spawn naturally at the base of the impassable dam. The high concentrations of spawning adults of both species near the hatchery may explain why hybrids between the two species have been observed at Trinity Hatchery in enzyme electrophoresis studies (personal communication, B. Bentley, Department of Animal Science, University of California, Davis, California, USA).

The susceptibility to virus infection of hybrids between resistant and susceptible species has been studied both with viral hemorrhagic septicemia virus (VHSV) and IHNV. Dorson and Chevassus (1985) found that hybrids of brook *(Salvelinus fontinalis)* and rainbow trout were totally resistant to VHSV. Chen (1984) and Parsons et al., (1986) in similar studies with coho salmon and rainbow trout hybrids found increased resistance to IHNV. We could find no reports of studies examining the susceptibility of coho and chinook salmon hybrids to IHNV and this prompted us to conduct the study reported here.

Four groups of fish were utilized in this study. Coho (mean wt. 9.0 g) and chinook salmon (mean wt. 12.9 g) were obtained from Darrah Springs Hatchery, a facility with no history of IHNV infections. Two groups of hybrids between the species

were also obtained from the same location. One group (mean wt. 8.7 g) was a result of crossing a male chinook with a female coho and the second (mean wt. 25.0 g) were progeny of a male coho crossed with a female chinook salmon. Fish from each of the four groups were divided equally and placed into three replicate 120 l rectangular tanks (25-32 fish/tank) receiving 12°C well water. Because of a shortage of chinook salmon, only 15 fish were placed into each of the replicate tanks. All fish in one replicate from each of the four groups were then injected intraperitoneally (i.p.) with 3.0 x 10^6 PFU of IHNV grown in chinook salmon embryo cells (CHSE-214). This isolate of IHNV was recovered from Trinity coho adults in 1985. Fish in the second set of replicates received an i.p. injection of 3.7 x 10^6 PFU of virus propagated in the same manner as above but recovered from Trinity chinook adults in 1984.

Mortalities were collected daily and the concentrations of the virus in the pooled kidney and spleen samples determined by plaque titration on the EPC cell line derived from common carp *(Cyprinus carpio)*. In selected samples, the concentrations of virus in the liver and brain were also determined. At 7 days post injection between 2 and 5 fish were removed from each tank and placed in Bouin's fixative for later histological processing.

Starch gel electrophoresis of muscle and liver enzymes were used to confirm the hybrid nature of the fish used in this study.

Experimental challenges with IHNV showed that chinook and both hybrid groups were susceptible to virus infections (Table 1). Concentrations of virus in the tissues of dead fish were 10^5-10^6 PFU/g and clinical signs (both gross and microscopic) indicated the susceptibility of the chinook and both hybrid groups to IHNV.

One coho salmon injected with the chinook salmon strain of IHNV died during the course of the study (Table 1). This fish died as a result of IHNV as shown by clinical signs of infection, both grossly and microscopically, and recovery of virus from the tissues. Virus concentrations of 3.0 x 10^6, 1.8 x 10^5, 6.0 x 10^3 PFU/g were found in the kidney-spleen, liver and brain respectively. However, a sample of five coho salmon from the same tank on day seven failed to show any signs of infection or virus presence.

Approximately equivalent concentrations of both the coho and chinook strains of IHNV were used in the challenges. Among the four groups challenged with IHNV, the hybrids made from the coho male and chinook female were the most susceptible to virus-induced mortality with either strain of IHNV (Table 1). It is not known why this particular parental-cross might have resulted in a susceptibility greater to IHNV infection than pure chinook salmon tested. It however, demonstrates that hybridizations may result in species as susceptible as the parental stocks used for the crosses.

Resistance within and among certain species of salmonids to virus infections has been observed. The potential for using selective breeding for virus resistance has been demonstrated for IHNV in sockeye salmon (Amend and Nelson, 1977; McIntyre and Amend, 1978) but these approaches have not been fully exploited. Hybrids made between

virus resistant and susceptible species have been tested and shown to have either complete or intermediate resistances to virus infections (Chen, 1984; Dorson and Chevassus, 1985; Parsons et al., 1986). In our study however, hybrids made between coho and chinook salmon were susceptible to IHNV infections. The presence of naturally occurring hybrids between the two species at the Trinity River Hatchery and the presence of coho adults returning with IHNV is under further investigation. In the next year we will examine the enzyme electrophoresis profiles and virus content of adult salmon returning to the hatchery to ascertain whether naturally occurring hybrids are returning as IHNV infected adults.

Acknowledgements

This work was supported by Wallop-Breaux funds administered by the California Department of Fish and Game. The assistance of the hatchery personnel at the Trinity River and Darrah Springs Hatcheries was especially appreciated.

References

Amend, D.F. and Nelson, J.R. 1977. Variation in the susceptibility of sockeye salmon *Oncorhynchus nerka* to infectious hematopoietic necrosis virus. J. Fish Biol., 11: 567-573.

Chen, M.F. 1984. Comparative susceptibility of salmonid fish and salmonid fish cell lines to five isolates of infectious hematopoietic necrosis virus and biological properties of two plaque variants. Ph.D. Thesis. Oregon State University, Corvallis, Oregon, USA. 124 pp.

Dorson, M. and Chevassus, B. 1985. Resistance of triploid rainbow trout x brook trout hybrids to viral hemorrhagic septicemia. Proceedings of the 2nd International Conference of the European Association of Fish Pathologists, 2-5 September, Montpellier, France pp. 115.

LaPatra, S.E., Fryer, J.L., Wingfield, W. and Hedrick, R.P. 1987. Transmission of infectious hematopoietic necrosis virus (IHNV) between adult species of salmon: management strategies for anadromous broodstock. Fish Health Section Newsletter, 15(2): 7.

McIntyre, J.D. and Amend, D.F. 1978. Heritability of tolerance for infectious hematopoietic necrosis in sockeye salmon *(Oncorhynchus nerka)*. Trans. Am. Fish. Soc., 107: 305—308.

Parsons, J.E., Busch, R.A., Thorgaard, G.H. and Scheerer, P.D. 1986. Increased resistance of triploid rainbow trout x coho salmon hybrids to infectious hematopoietic necrosis virus. Aquaculture, 57: 337—343.

Pilcher, K.S. and Fryer, J.L. 1980. The viral diseases of fish: a review through 1978. CRC Critical Reviews in Microbiology, 7: 287—364.

Wingfield, W.H. and Chan, L.D. 1970. Studies on the Sacramento river chinook disease and its causative agent. In: S.F. Snieszko (Editor), A Symposium on Diseases of Fish and Shellfishes. Special Publication No. 5, American Fisheries Society, Washington D.C. pp. 307—318.

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Table I. Mortality among chinook, coho and hybrid salmon following intraperitoneal injections of infectious hematopoietic necrosis virus isolates from adult chinook and coho salmon.

	No. dead*/No. total (%)			
	Host Species			
Virus**	Coho	Chinook	Coho Male x Chinook Female	Chinook Male x Coho Female
Coho IHNV	0/24 (0)	7/15 (47)	7/32 (22)	2/21 (10)
Chinook IHNV	1/31 (3)	12/14 (86)	31/32 (97)	19/23 (83)
No virus	0/30 (0)	1/15 (7)	0/20 (0)	0/20 (0)

* The kidney and spleen from nearly all mortalities was examined for the concentrations of IHNV by plaque titration on EPC cells. With certain fish, titrations of the virus content of the liver and brain were also determined.

** The viruses used in this study were isolated from adult chinook and coho salmon returning to the Trinity Hatchery in 1984 and 1985 respectively. Fish received intraperitoneal injections with 3.0×10^6 PFU of the coho strain or 3.7×10^6 PFU of the chinook strain of IHNV. Both viruses were grown in CHSE-214 cells. Control fish received an equal volume of balance salt solution with no virus. Water temperature during the 19 days following injection was a constant 12°C.