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INFECTIOUS PANCREATIC NECROSIS VIRUS — TEMPERATURE AND AGE FACTORS IN MORTALITY

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Abstract: Infectious pancreatic necrosis virus was present in brook trout in two Ontario salmonid hatcheries but did not appear to cause high mortality. Experiments and field observations showed that a number of factors were responsible for the low mortality, in particular, low water temperatures during the age of highest susceptibility of the fry and a lack of, or low level of transmission of virus during these periods of cold water temperatures.

INTRODUCTION

Infectious pancreatic necrosis (IPN), a viral fish disease can cause serious losses in populations of hatchery-reared salmonids. The disease is characterized by a rapid onset and high mortality, in some cases reaching 70% of exposed fry.[4]

An agent causing pancreatic necrosis in brook trout *(Salvelinus fontinalis)* fry has been isolated from two Ontario provincial government salmonid hatcheries and identified as infectious pancreatic necrosis virus.[3] A study initiated to establish the extent and significance of the disease in these hatcheries suggested that the disease did not present the "explosive" mortality among salmonid fry that has been reported in other areas.[4] Infectivity experiments were carried out to determine the effect of fish age and water temperature on IPN - associated mortality in brook trout in an attempt to explain the low mortality in these hatcheries.

MATERIALS AND METHODS

Infectivity trials were carried out in 75 liter enamelled washing machine tubs held in temperature controlled rooms. In all experiments approximately 80% of the water in the tubs was changed every 3 days and the immersible, air-operated, glass wool and activated charcoal filters used in each tub were changed every 6 days. This maintained the water in such a condition that the trout showed no evidence of respiratory stress from particulate or dissolved matter in the water. As a further check on water quality, whole fish were periodically fixed in Bouins fluid, and the gills were sectioned and examined for evidence of an excess of particulate material clogging lamellae, hyperplasia, or other abnormalities.

All groups of fish were fed with a dry commercial feed (Purina Trout Chow #1) and held in constant light of low intensity during the experiments. Water total hardness, expressed as ppm calcium carbonate, fluctuated between 75 and 150 ppm during the experimental period.

Infectivity was tested using two virus isolates; American Type Culture Collection (ATCC) IPN-VR #299, and IPN-Pem-Pl (one passage in rainbow trout gonad — RTG-2 cells) isolated from a provincial trout hatchery and identified as IPN virus by specific neutralization tests (Frantsi, unpublished data).

All lots of fish were checked for IPN virus before being used and the lots from which the experimental fish were taken were kept for at least 6 months at 10 C and periodically checked for IPN with negative results.

To evaluate the effect of temperature on IPN-associated mortality, three rooms were used with respective controlled temperatures of 4.5 C, 10 C and 15.5 C. Each room contained three tubs with 100, two month old brook trout fry in each tub. Infection was accomplished by feeding 10 ml of beef liver, containing

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10^3 $TCID_{50}$ virus/ml per 100 fish fed over a two day period. One group of 100 fish received IPN - Pem - Pl, a second, IPN-VR #299 and the control group, 10 ml beef liver only. The feeding was spread over the two day period since 10 ml of beef liver placed into the 75 liter test systems at one time caused cloudy conditions which could possibly lead to respiratory stress. All fish were fed the respective virus mixture, or straight liver in the case of controls, after being acclimatized for two days to the conditions in which they were to be held for the duration of the experiment.

Deaths were recorded daily for 64 days and cumulative values plotted as % of total fish. Death due to IPN was substantiated with spot checks involving virus isolation in RTG-2 cell cultures and serologic identification, using rabbit immune serum. Also, dead and moribund fry were fixed in Bouins fluid, sectioned and examined for the acute pancreatic necrosis characteristic of IPN.

At the end of the experiment, survivors in all infected groups were homogenized and individually tested for IPN virus by RTG-2 cell culture inoculation. After 64 days, to see if the mortality would increase, 50% of the remaining fish in the 4.5 C groups (control and two infected lots) were moved to the 10 C room and observed for 24 days.

To evaluate the effect of age on IPN-associated mortality, brook trout fry through to the fingerling stage, were infected by feeding IPN-Pem-Pl virus in liver. All experiments were carried out at 10 C. The trout were exposed to virus at 1, 2, 4, and 6 months post-hatching. Deaths were recorded daily for 64 days and cumulative values plotted as % of total fish.

Since the brook trout used in this study were bought from a local private hatchery, a strain of brook trout, from brood stocks supplying fish to the provincial hatcheries from which IPN has been isolated, was used to repeat some of the experiments. The fish were held at 10 C and infected with IPN-Pem-Pl virus at 2 and 4 months post-hatching and deaths recorded for 44 and 64 days respectively.

RESULTS

Temperature

The Pem-Pl strain produced the greatest mortality, 74% at 10 C and 46% at 15.5 C (Fig. 1). In contrast, VR #299 produced a loss of only 31% at 15.5 C and mortality at 10 C and 4.5 C did not exceed that of uninoculated controls; similar to Pem-Pl at 4.5 C.

At the end of the experiment, virus was isolated from roughly 25% of all survivors (controls were negative) with no differences observed between the individual temperature groups. The control and infected fish which were moved after 64 days, from 4.5 C to 10 C did not show an increase in mortality over the 24 day observation period.

Fish Age

Pem-Pl strain virus caused 83% mortality in 1 month old fish but progressive lower losses among 2 and 4 month old fish. The response of 6 month old fish was negligible (Fig. 2).

The mortality in provincial hatchery-strain fish compares very closely to that seen in the trout used in other experiments (Fig. 3).

DISCUSSION

From the experiments presented it appears that IPN mortality decreases with the age of the fish and that there is an optimum temperature at which greatest mortality occurs. This decrease in mortality with increasing temperature appears to be a situation paralleling that reported by Amend[1] in sockeye salmon infected with infectious hematopoietic necrosis (IHN) virus.

Whether IPN-VR #299 shows its greatest mortality at 15 C because it was isolated from and adapted to different conditions than Pem-Pl or because of its different history in tissue culture is unknown.

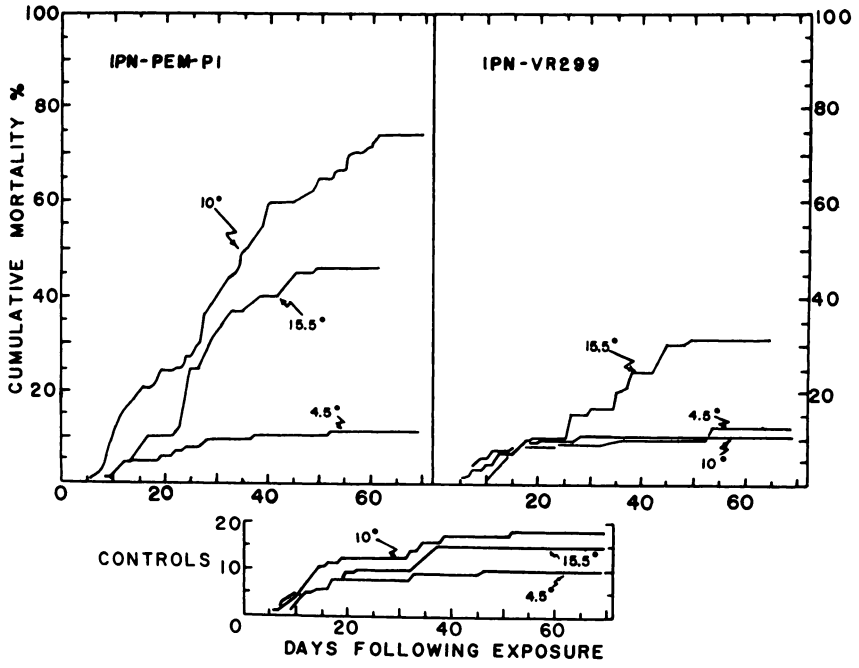
Mortality data from one of the hatcheries from which IPN has been isolated is shown in Fig. 4. In 1967 the mortality attributed to IPN did not exceed 3%, in 1968 not more than 2% and in 1969 not

more than 1% of the total fish in the hatchery. In 1969 IPN virus was isolated and characteristic corkscrew whirling and extensive pancreatic necrosis were observed in the fry just before the peak of mortality occurred. In 1967 and 1968, similar peaks occurred and although virus was isolated from these lots of fish after the time of the mortality peaks, the mortality was presumed due to IPN.

We believe that the low mortality in the hatcheries under study is due to two main factors (a) the water temperature during the period of greatest IPN susceptibility of the fry (up to 4 months) is cold enough to prevent mortality and (b) by the time the water temperature rises to a point where IPN virus could kill the fish, many of the trout are less susceptible because of increased age and prior exposure to the virus.

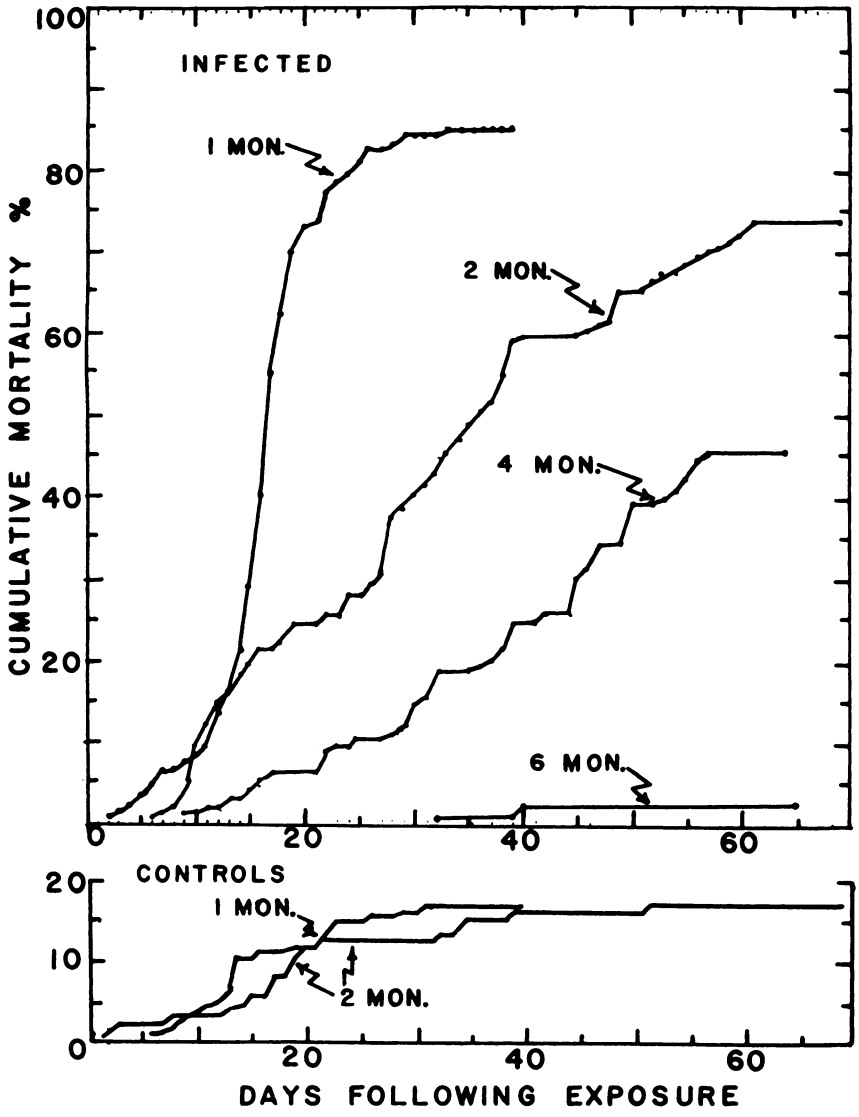
Average monthly temperatures for 1967-1969 in 4 Ontario salmonid hatcheries are shown in Fig. 5. A and B hatcheries are located in southern Ontario and C and D in northern Ontario. C and D are the hatcheries from which IPN has been isolated and C is the hatchery from which the mortality curve in Fig. 4 was derived. The temprature in hatchery C does not rise above 7 C until late April, at which time smaller numbers of fry are susceptible because of their increased age (3 months). The water temperatures in hatchery D rarely rise above 7 C. Examination of data in hatchery D revealed no peaks of mortality during the summer period 1967-1969, nor has virus been isolated from fry, although it has been isolated from some year classes of adult fish. A and B hatcheries in Southern Ontario have warmer temperatures earlier in the trout fry's life, a situation in which IPN virus could possibly cause explosive mortality, were it to be introduced.

Another factor contributing to low mortality, based on field observations,



1. Mortality caused by IPN-Pem-PI and ATCC VR #299 in 2 month old brook trout fry, held at 4.5 C, 10 C and 15.5 C.

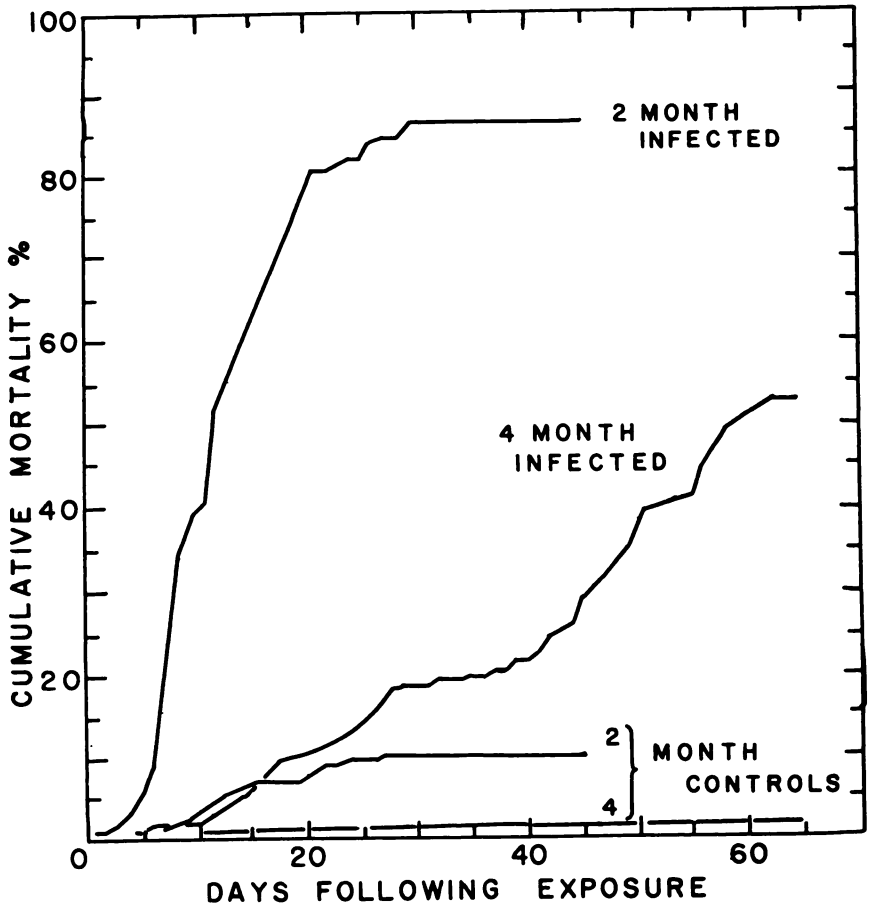
may be that low water temperatures have a depressing effect on the infectivity and/or transmission of the virus as well as on host response. This may partially explain the failure to isolate IPN virus from fry in hatchery D. In hatchery C, in February (average temperature — 2.5 C), the yearling brook trout, some of which are IPN carriers, are brought to the hatchery building from a holding pond located downstream from the main operation. These yearlings are placed in troughs or raceways adjacent to newly-hatched, IPN-susceptible, brook trout fry.



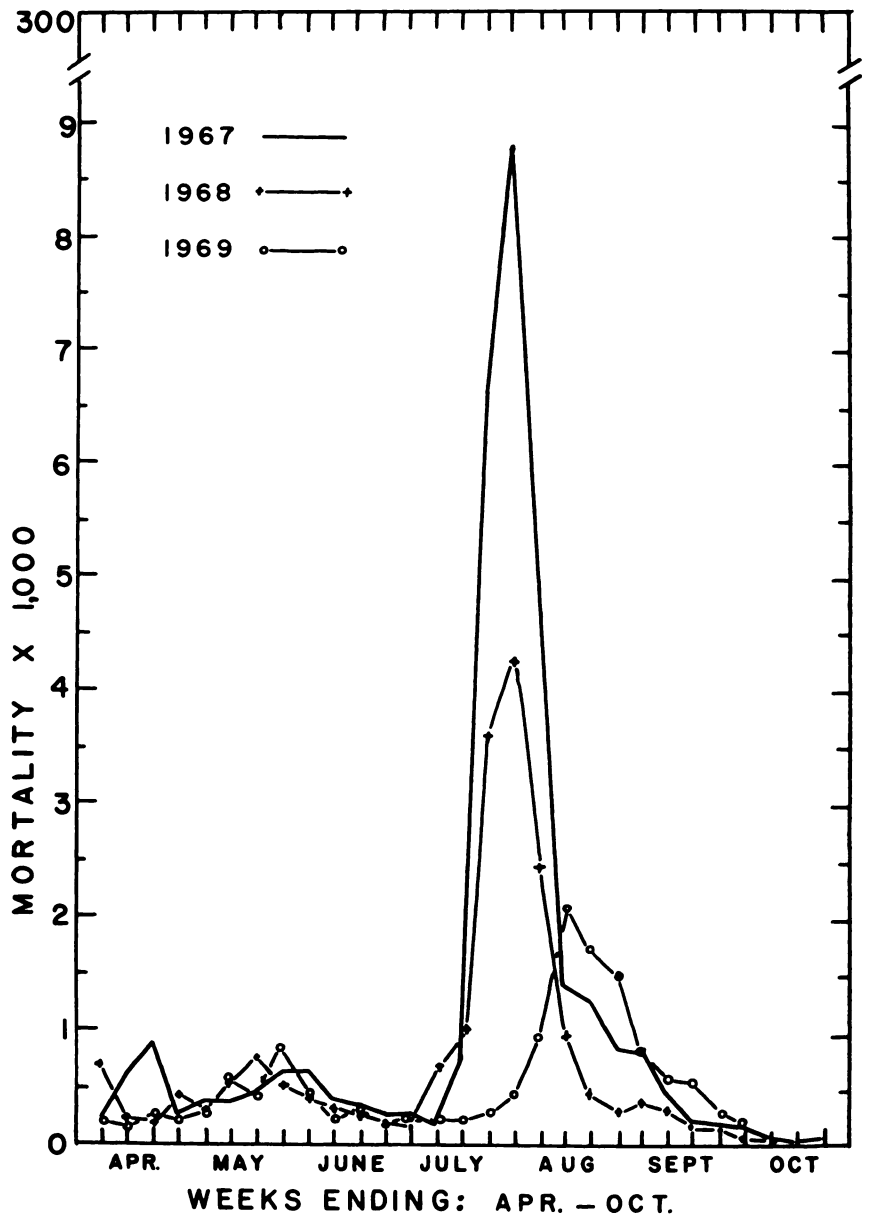
2. Mortality caused by IPN-Pem-Pl at 10 C in brook trout of different ages.

In 1969, IPN virus was not isolated from the fry until 6 months later, in mid July, (temperature — 11.4 C) and then only from fry in a raceway directly adjacent to a lot of IPN carrier fish, which had not yet been stocked, in spite of the fact that many fry in other raceways had been located just as close to the carriers during the preceding 6 months period as the fry which became infected. Fry and yearling carriers had been moved throughout the hatchery during counting, preparation for stocking, etc. The mortality was approximately 5% of the total fish in this raceway. The IPN virus was isolated from, and confined to, this one raceway lot of fry probably because of stringent control measures carried out by hatchery personnel.

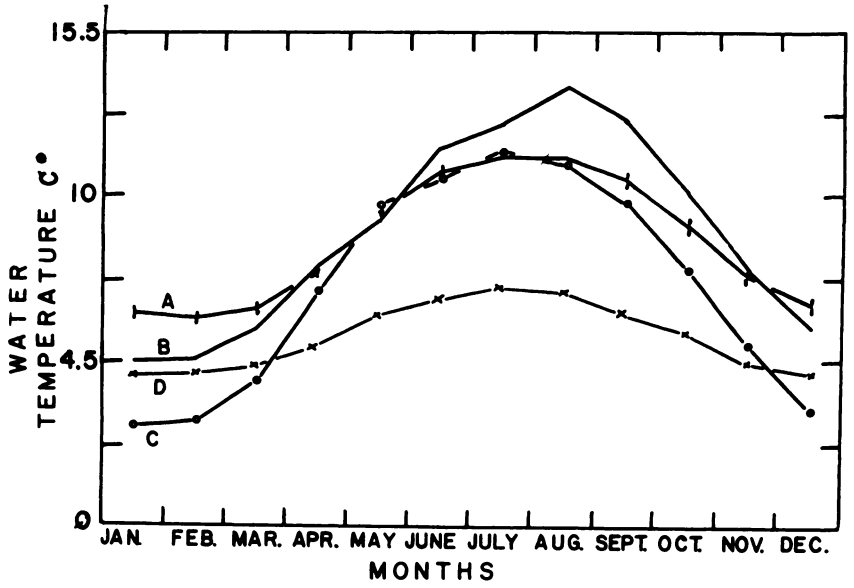
It is possible that more fish than we were able to detect, during periods of colder water temperatures may have been infected or exposed to virus but because of this pre-exposure or pre-infection, they may have been protected when the water warmed. This probability was demon-



3. Mortality caused by IPN-Pem-PI in brook trout from brood stock which supply the Ontario Government hatcheries from which IPN was isolated. Infected at 2 and 4 months of age and held at 10 C.



4. Mortality among 300,000 brook trout in hatchery C from 1967-69 inclusive.



5. Average monthly temperatures for 1967-1969 in 4 Ontario salmonid hatcheries. A and B are located in southern Ontario, C and D are in northern Ontario. IPN has been isolated from brook trout in hatcheries C and D.

strated earlier, with the movement of fish from 4.5 C to 10 C with no increase in mortality.

The cold water temperatures of Ontario, and possibly other areas of Canada, in spite of effecting a slow rate of growth in poikilotherms, may be beneficial in tnrms of preventing disease mortality. This present work substantiates this theory.

Acknowledgements

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