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INFECTIOUS PANCREATIC NECROSIS VIRUS IN ATLANTIC SALMON (Salmo salar), AND BROOK TROUT (Salvelinus fontinalis), IN CENTRAL NEWFOUNDLAND

by

B.W. Souter, A.G. Dwilow and K. Knight

Central and Arctic Region

Department of Fisheries and Oceans

Winnipeg, Manitoba R3T 2N6

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ABSTRACT

Souter, B.W., A.G. Dwilow, and K. Knight. 1992. Infectious pancreatic necrosis virus in Atlantic salmon (Salmo salar), and brook trout (Salvelinus fontinalis) in central Newfoundland. Can. Tech Rep. Fish. Aquat. Sci. 1873: iv + 6 p.

A total of 513 Atlantic salmon (Salmo salar) and 92 brook trout (Salvelinus fontinalis) obtained from 11 collection sites on four rivers in central Newfoundland were tested for the presence of infectious pancreatic necrosis virus (IPNV) and other certifiable pathogens of concern. This work was undertaken as a prerequisite for an Atlantic salmon enhancement initiative proposed by the Newfoundland Environmental Resource Management Association (ERMA). Infectious pancreatic necrosis virus was isolated from Atlantic salmon and brook trout from Little Rattling Brook. The isolation would appear to represent a natural, covert infection as none of the fish from Little Rattling Brook or the other drainages exhibited clinical signs of infectious pancreatic necrosis. The virus was serologically identified as being the West Buxton IPNV serotype.

Key words: Infectious pancreatic necrosis virus; Atlantic salmon; Salmo salar; brook trout; Salvelinus fontinalis; Little Rattling Brook; Newfoundland.

RÉSUMÉ

Souter, B., A.G. Dwilow, and K. Knight. 1992. Infectious pancreatic necrosis virus in Atlantic salmon (Salmo salar), and brook trout (Salvelinus fontinalis) in central Newfoundland. Can. Tech Rep. Fish. Aquat. Sci. 1873: iv + 6 p.

On a vérifié la présence du virus de la nécrose pancréatique infectieuse (VNPI) et d'autres agents pathogènes préoccupants à certifier chez un total de 513 saumons atlantiques (Salmo salar) et de 92 ombles de fontaine (Salvelinus fontinalis) provenant de 11 sites de prélèvement répartis dans 4 rivières du centre de Terre-Neuve. Ce travail a été réalisé à titre de phase préliminaire d'une initiative de mise en valeur du saumon atlantique mise de l'avant par la Newfoundland Environmental Resource Management Association (ERMA). Le virus de la nécrose pancréatique infectieuse a été isolé chez des saumons atlantiques et des ombles de fontaine provenant du ruisseau Little Rattling. Il s'agirait d'une infection naturelle inapparente puisqu'aucun des poissons du ruisseau Little Rattling ni des autres cours d'eau ne présentait de signes cliniques de nécrose pancréatique infectieuse. Le virus a été identifié sérologiquement comme étant de sérotype West Buxton.

Mots-clés: Virus de la nécrose pancréatique infectieuse; saumon atlantique; Salmo salar; omble de fontaine; Salvelinus fontinalis; ruisseau Little Rattling; Terre-Neuve.

INTRODUCTION

Infectious pancreatic necrosis virus (IPNV) is an aquatic birnavirus having a broad host range and a wide geographic distribution (Wolf 1988). Virulent strains of the virus are capable of causing significant mortality, particularly among cultured rainbow and brook trout (Ahne et al. 1989).

The virus can persist in wild and cultured salmonid stocks through the exposure of susceptible fish to water contaminated by virus shed in the urine and feces from infected fish, and through sex products from covertly infected broodstock. Because iodophor disinfection of fertilized eggs does not eliminate the parent to progeny transmission of IPNV (Bullock et al. 1976) it is important that fish stocks be tested to determine the presence or absence of the virus in an effort to minimize the risk of transferring the virus between watersheds.

In the summer of 1991 the Newfoundland Environmental Resources Management Association (ERMA) proposed collecting Atlantic salmon eggs from three rivers in central Newfoundland for enhancement purposes. The eggs were to be hatched and reared at an incubation facility situated on Noel Paul's Brook, a tributary of the Exploits River, prior to release back into the rivers of origin. Prior to the proposed egg collections, the Department of Fisheries and Oceans required that a sample of fish from each of the selected rivers undergo disease analysis to avert the possible introduction or transfer of fish pathogens. This work was undertaken with funding provided by the Atlantic Fisheries Adjustment Program and the assistance of LeDrew, Fudge and Associates Ltd., St. John's, Newfoundland.

This report describes the isolation and identification of IPNV from Atlantic salmon and brook trout from one of the three rivers in central Newfoundland selected as potential sources of seedstock for Atlantic salmon enhancement purposes.

MATERIALS AND METHODS

SAMPLE COLLECTION

The four rivers selected for investigation were: Little Rattling Brook, Gander River (Northwest and Southwest Gander rivers), and Terra Nova River, and Exploits River (Noel Paul's Brook) the proposed site for incubation and early rearing of stocks from the other three rivers. Fish were collected by electrofishing from three separate locations on each river. The exception was Little Rattling Brook where bottom substrate and the apparent lack of fish of sufficient size resulted in collections from two locations only (Fig. 1). Samples of 60 fish, preferably Atlantic salmon 5-15 cm in length, were to be collected. The samples were separated by species, placed in labelled plastic bags, packed on ice and shipped to arrive in Winnipeg within 24 h of capture.

VIRUS ISOLATION

All samples were received in the laboratory within 24 h of the time of collection and were processed immediately. The chinook salmon embryo (CHSE-214) and rainbow trout gonadal (RTG-2) cell lines were used for virus isolation. Cell cultures were maintained at 18°C in 75 cm^2 plastic flasks in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum with added penicillin (100 IU/mL) and streptomycin (100 µg/mL). In most instances kidney, spleen, pyloric caeca-pancreas and gill tissues were assayed in five fish pools. The virus isolation methodology described in the Fish Health Protection Regulations: manual of compliance (Department of Fisheries and Oceans 1984) was used for virus detection. When suspect cytopathic effect was observed monovalent IPNV antisera (Connaught Laboratories, Willowdale, Ontario) was used to confirm the identity of the virus isolates.

SEROTYPING OF VIRUS ISOLATES

The serotype of the IPNV isolates was determined by plaque-neutralization testing with serotype-specific rabbit serum. Serial two-fold dilutions of the heat-inactivated (56°C, 30 min) rabbit antiserum were made in MEM. Antibody titres were determined by a modified plaque-inhibition method, as previously described (Kelly and Nielsen 1990). For each assay at 15°C, the reciprocal of the greatest dilution of antibody that resulted in an 80% reduction in the number of virus plaques per 0.1 ml of inoculum was recorded as the antibody end-point titre (Habel 1969).

RESULTS

SAMPLE COLLECTION AND VIRUS ISOLATION

A summary of the sampling sites, dates, numbers of each species collected and IPNV detections is shown in Table 1. A total of 605 fish (513 Atlantic salmon and 92 brook trout) were collected from the four rivers investigated. No clinical evidence of infectious pancreatic necrosis was observed in any of the fish tested, and only the fish from Little Rattling Brook were found to be infected with the virus. The brook trout (3 of 3 pools positive) from Rattling Lake, and both the Atlantic salmon (single pool positive) and brook trout (9 of 11 pools positive) from Frozen Ocean Lake were covertly infected

with the virus.

SEROTYPING OF VIRUS ISOLATES

The brook trout and Atlantic salmon IPNV isolates were serologically identical to IPNV West Buxton as indicated by the similar neutralization titres (Table 2). The neutralization titre for West Buxton antisera was 16,384 for the homologous virus, and 16,384 and 8,192 respectively for the brook trout and Atlantic salmon virus isolates.

DISCUSSION

Cultured salmonids have never been introduced into Little Rattling Brook (Vern Pepper, Department of Fisheries and Oceans, Newfoundland Region, St. John's, Nfld , personal communication). Hence, these findings would appear to represent a natural, persistent infection of IPNV in wild Atlantic salmon and brook trout inhabiting the Little Rattling Brook drainage in central Newfoundland. The results also suggest a high prevalence of the virus in the brook trout population in this river as 12 of 14 visceral tissue pools were positive for the virus.

A summary of IPNV detections from both wild and cultured salmonid stocks prepared by Shaw and Hart (1990) shows the wide distribution of this virus in the province of Newfoundland. Since 1978, IPNV has been isolated from a variety of salmonid species inhabiting 16 different river systems, two lakes/ponds and two private cage culture/hatchery operations. The isolation of IPNV from Atlantic salmon and brook trout inhabiting Little Rattling Brook expands the known geographical distribution of the virus in central Newfoundland.

In the Maritime region of Canada the West Buxton serotype of IPNV appears to be the predominant serotype of virus. At least one additional serotype (Canada 2/3) has previously been identified from Newfoundland (Kelly et al. 1991). To our knowledge the serological identity(ies) of other Newfoundland IPNV isolates mentioned by Shaw and Hart (1990) has not been established.

It should be noted that the apparent absence of IPNV in fish from the three other rivers we investigated should not be interpreted as meaning that fish from these rivers are free of the virus. In fact the virus has been previously isolated on three occasions from Exploits River Atlantic salmon and brook trout (Cone and Moore 1981; Shaw and Hart 1990), and on one occasion from brook trout from Terra Nova River (Shaw and Hart 1990). Our inability to detect IPNV in fish from both of these rivers may have been due to the fact that the fish we received were not infected with the virus, or the virus titre in these fish was at a level not detectable by the methods used. However, our results and the findings of previous investigators indicate that the indigenous salmonid stocks from three of the four rivers we investigated are infected with IPNV.

The concern of the Department of Fisheries and Oceans was to prevent the egg-borne introduction of pathogens into Noel Paul's Brook from the selected rivers providing seed stock. Although IPNV has been detected in Exploits River fish stocks, the virus has never been detected in fish from Noel Paul's Brook. Therefore, Atlantic salmon eggs from Terra Nova River and Little Rattling Brook should not be transferred to the Noel Paul's Brook incubation facility. The only acceptable procedure would be to transfer eggs from Gander River broodstock to Noel Paul's Brook and return of the fingerlings for stocking in the Gander River.

Little is known of the impact of IPNV on wild salmonid stocks. Therefore, it is important to ensure that salmonid stocks, not previously exposed to the virus, are not placed at risk through the introduction of the virus as a result of stocking or enhancement activities.

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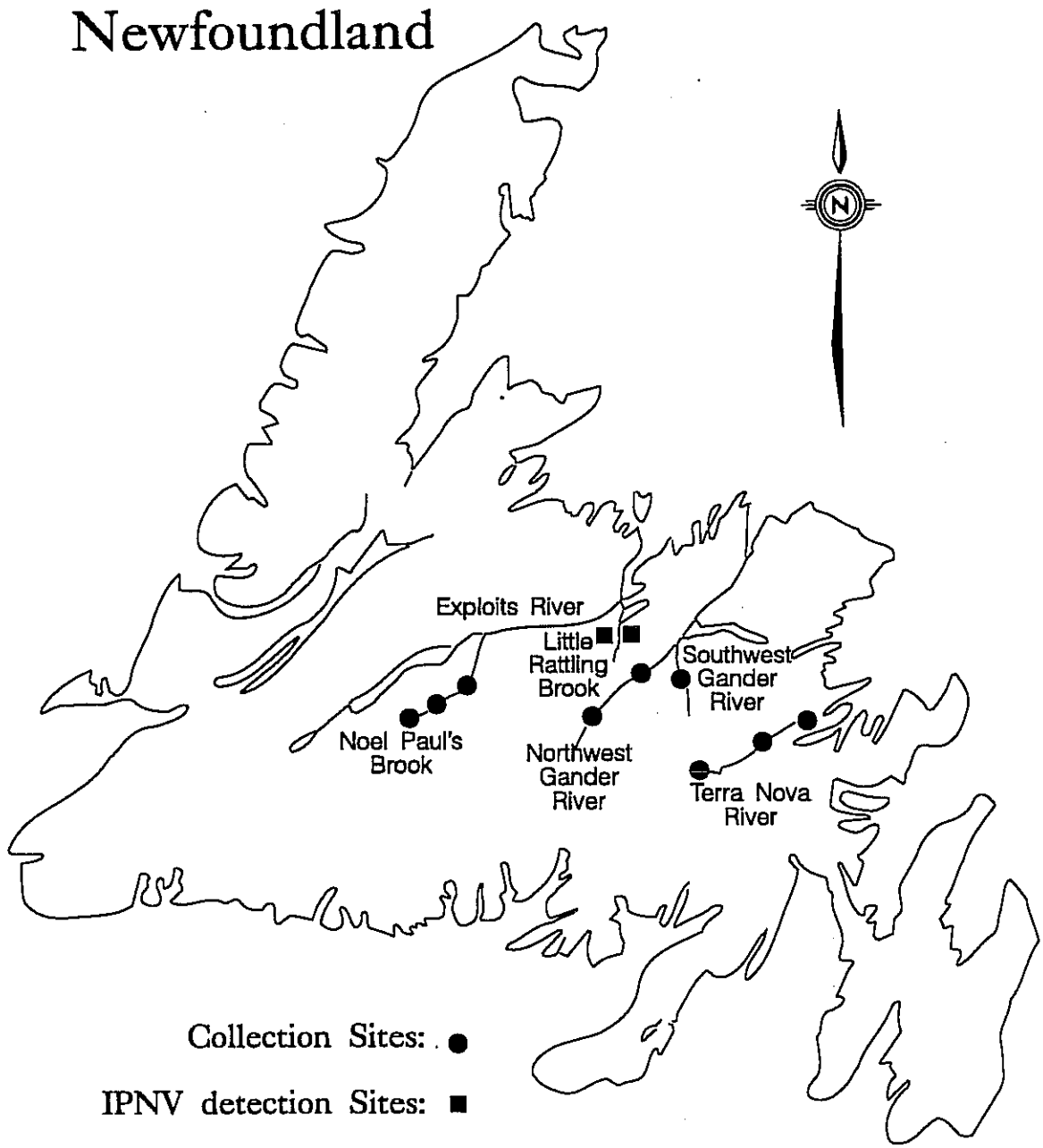

Fig. 1. Map of Newfoundland showing the collection locations and the IPNV detection sites.

Table 1. Summary of collection sites, species, numbers sampled and IPNV isolations.

Location	Date	Atlantic salmon	Brook trout	IPNV Isolation Atl. sal.	Bkt
Exploits River:					
Noel Paul's Incubation Facility	July 2/91	60	0	-	NA[1]
Meelpaeg Crossing	"	60	0	-	NA
Lake Douglas	"	60	0	-	NA
Little Rattling Brook:					
Rattling Lake	July 2/91	0	17	N/A	3/3[2]
Frozen Ocean Lake	"	5	55	1/1	9/11
Gander River:					
Northwest Gander River (at Bay d'Espoir Highway)	July 8/91	60	0	-	NA
Northwest Gander River (at Gander Lake)	"	60	0	-	NA
Southwest Gander River (at Dead Wolf Brook)	"	60	0	-	NA
Terra Nova River					
Maccles Brook (near Trans Canada Highway)	July 9/91	60	0	-	NA
Terra Nova River (upstream of Terra Nova Lake)	July 8/91	42	6	-	-
Terra Nova River at Lake St. John	"	46	14	-	-
Total		513	92	1/1	12/14

[1] Samples not obtained
[2] 3 of 3 pools positive for IPN virus

Table 2. Serological identification of Newfoundland IPNV isolates[a,b].

Antisera[c]	Virus			
	Rattling Lake BKT-1	Frozen Ocean Atl. sal-1	Frozen Ocean BKT-2	West Buxton
Rabbit Anti IPNV West Buxton[d]	16384	8192	16384	16384

[a] Table 2 was furnished, courtesy of Dr. R. Kelly, who performed the immunological testing for birnavirus serotypes previously described by Caswell-Reno et al. (1989).

[b] 80% end-point, plaque-reduction method

[c] Previously heat-inactivated at 56°C for 30 min.

[d] Specific rabbit antisera to Birnavirus Serotype A-1 or IPNV West Buxton