Infectious Pancreatic Necrosis Virus in Adult Arctic Charr, *Salvelinus alpinus* (L.), in Rivers in the Mackenzie Delta Region and Yukon Territory

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by

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ABSTRACT

Souter, B.W., A.G. Dwilow, K. Knight, and T. Yamamoto. 1986. Infectious pancreatic necrosis virus in adult Arctic charr, Salvelinus alpinus (L.), in rivers in the Mackenzie Delta region and Yukon Territory. Can. Tech. Rep. Fish. Aquat. Sci. 1441: iv + 11 p.

The prevalence and distribution of IPN virus in adult Arctic charr indigenous to four river drainages in the Mackenzie Delta region and Yukon Territory was investigated over a five-year period. A total of 229 adult Arctic charr were examined of which 100 (25 males and 75 females) were positive for IPN virus for an overall prevalence of 43.7%. There was no significant difference in the prevalence of the virus between males and females. We found IPN virus neutralizing activity (80% plaque reduction titre >50) in serum from 29 of 63 virus positive and 13 of 69 virus negative Arctic charr. The acquisition of Arctic charr or their gametes from this geographical area for research or intensive culture purposes is not recommended.

Key words: Infectious pancreatic necrosis virus; Arctic charr; Mackenzie Delta region; Northwest Territories; Yukon Territory.

RÉSUMÉ

Souter, B.W., A.G. Dwilow, K. Knight, and T. Yamamoto. 1986. Infectious pancreatic necrosis virus in adult Arctic charr, Salvelinus alpinus (L.), in rivers in the Mackenzie Delta region and Yukon Territory. Can. Tech. Rep. Fish. Aquat. Sci. 1441: iv + 11 p.

Des recherches ont été effectuées sur une période de cinq (5) ans sur la prévalence et la distribution du virus de la nécrose pancréatique infectieuse (NPI) chez l'omble chevalier adulte vivant dans quatre (4) bassins hydrographiques dans la région du delta du Mackenzie et le territoire du Yukon. Au total, 229 ombles chevaliers adultes ont été examinés et cent (100) d'entre eux (25 mâles, 75 femelles) étaient porteurs du virus de la NPI, ce qui donne une prévalence globale de 43,7%. Le virus se retrouve à peu près autant chez les mâles que chez les femelles. On a constaté une activité de neutralisation du virus de la NPI (réduction des plaques de 80%, titre viral >50) dans le sérum de 29 sujets porteurs du virus sur 63 et de 13 sujets exempts du virus sur 63. Il est déconseillé de prélever des ombles chevaliers ou des gamètes d'omble chevalier dans ce secteur géographique pour des fins de recherche ou pour la culture intensive.

Mots-clés: Virus de la nécrose pancréatique infectieuse; omble chevalier; région du delta du Mackenzie; territoires du Nord-Ouest; territoire du Yukon.

INTRODUCTION

Infectious pancreatic necrosis (IPN) is principally an acute, infectious, viral disease of young, hatchery-reared rainbow trout, Salmo gairdneri Richardson, and brook trout, Salvelinus fontinalis (Mitchill), the epizootic survivors of which become life-long carriers and shedders of the virus (Wolf et al. 1963; Yamamoto 1975; Yamamoto and Kilistoff 1979). The virus exhibits a wide host range having been isolated from a variety of cultured salmonid species (Parisot et al. 1963; MacKelvie and Artsob 1969; Wolf and Pettijohn 1970; Ljungberg and Jørgensen 1973; Sano 1973), and increasingly IPN or similar viruses have been reported from a growing number of diverse freshwater and marine non-salmonid species (Sonstegard et al. 1972; Underwood et al. 1977; Stephens et al. 1980; Hudson et al. 1981; McAllister et al. 1984; Schutz et al. 1984).

Souter et al. (1984) reported the isolation of IPN virus from a feral population of Arctic charr (Salvelinus alpinus) collected from a distal tributary (Fish Creek) of the Mackenzie River in the Northwest Territories, Canada. Because of this unexpected finding, a five-year study was undertaken to determine the prevalence and distribution of IPN virus in natural Arctic charr populations indigenous to the Mackenzie Delta region and to two major rivers of the Yukon Territory. The work was carried out with the cooperation of Fish and Marine Mammal Management personnel from the Western Region, Department of Fisheries and Oceans (DFO), Winnipeg, Manitoba, and with funding support furnished by the Science Subvention Program of DFO, Ottawa.

METHODS AND MATERIALS

SAMPLE COLLECTION

The Arctic charr examined in this study represented the form distributed from the lower Kuskokwin River to the Mackenzie River as characterized by McPhail (1961). Adult Arctic charr were collected in late August or early September during their migration to, or after their arrival on their spawning grounds. On one occasion a collection of presmolts was made in mid-November. The study area is shown in Fig. 1. Fish were collected from six sites involving four different river drainages and a coastal location (Fig. 2).

Samples were placed individually in numbered bags as whole fish and packed in ice, or tissue samples were asceptically removed using sterile instruments, held on dry ice and transported to the laboratory. In most instances the samples were assayed within 48-72 h from the time of capture. Tissue samples not tested within this time were held at -80°C until analysis could be performed, usually within 2-3 months from the time of capture. Sex was recorded at the time of autopsy.

VIRUS ISOLATION

The chinook salmon embryo (CHSE-214, Fryer et al. 1965) cell line was used for virus detection. Cell cultures were maintained at 20°C in 75 cm^2 plastic flasks in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum with added penicillin (100 IU/mL) and streptomycin (100 µg/mL). With the exception of the presmolt fish, all of the adult fish were assayed individually for virus. Visceral tissues from the presmolts were assayed in five fish pools. The virus isolation procedure used was that described in the Canadian Fish Health Protection Regulations: manual of compliance (Department of Fisheries and Oceans 1984). Confirmation of IPN virus isolation was by serum neutralization using polyvalent IPN virus antiserum provided by the National Fish Health Research Center, Kearneysville, West Virginia, and Connaught Laboratories Limited, Willowdale, Ontario.

SERUM

Blood samples were collected by caudal puncture and held on ice to permit clotting during transportation of the samples to the laboratory. Serum was removed by centrifugation (1000 xg, 20 min, at 4°C), dispensed into test tubes, and stored at -20°C.

Viral neutralizing activity was determined using the plaquing method described by Wolf and Quimby (1973) and the plaque reduction technique of Jørgensen (1973). The results are expressed as 80% plaque reduction titres (PRT) (Habel 1969) against the IPN virus isolated initially from Fish Creek Arctic charr (Macdonald et al. 1983; Souter et al. 1984).

SERUM FRACTIONATION

To determine the nature of the antiviral activity present in the Arctic charr serum, a high titred (80% PRT > 10 000) serum sample was fractionated using the sucrose density gradient ultracentrifugation method of Burke and Nisalak (1982) as modified by Kelly and Nielsen (1985). Fourteen fractions were collected and each was assayed against 10^6 $TCID_{50}$ of homologous IPN virus to determine the extent of neutralizing activity. Two previously fractionated protein markers, porcine thyroglobulin (19S), and bovine gamma globulin (7S) were used for comparative purposes.

RESULTS

SAMPLE COLLECTION AND VIRUS ISOLATION

A summary of the collection dates, locations, and the prevalence of IPN virus in all of the fish sampled is shown in Table 1. The virus was prevalent in adult Arctic charr procured from all six collection sites used during the course of this five year investigation. A total of 229 adult fish (60 males and 169 females) were assayed for IPN virus of which 100 (25

males and 75 females) or 43.7% were found to be infected (Table 1). However, the freeze-thaw lability of this virus (unpublished data) may have resulted in the inactivation of virus in some of the frozen samples. Therefore, the prevalence of IPN virus is probably higher than reported.

Samples were collected randomly and an overall sex ratio favored females approximately 3:1. A X^2 test of the null hypotheses of equal probabilities in all groups showed there was no significant difference ($P>0.05$) between males and females in the prevalence of IPN virus (Table 2). Virus was not isolated from any of the pooled tissue samples prepared from the presmolt fish obtained from Big Fish River, November, 1981.

Repeated sampling from two drainages (Fish Creek/Rat River and Big Fish River) over a four year period indicated that there was no significant difference ($P>0.05$, X^2 test) in the prevalence of IPN virus in adult Arctic charr indigenous to the respective drainages (Table 3).

SERUM ANTI-IPN VIRUS ACTIVITY

Serum was collected from 132 Arctic charr and assayed by the plaque reduction method to determine levels of IPN virus neutralizing antibodies (Table 4). Antibody titres obtained from both virus positive and negative Arctic charr were similar to those reported by Wolf and Quimby (1969), and Jørgensen (1973) for immunized adult rainbow trout. Twenty-nine of 63 virus positive, and 13 of 69 virus negative Arctic charr produced neutralizing titres greater than 50 indicating that significant antibody had been produced.

SERUM FRACTIONATION

The sucrose density gradient profile for the fractions of a selected, high titred (80% PRT>10 000) serum sample assayed against the homologous virus isolate is shown in Fig. 3. A peak of virus neutralizing activity was present in the lower portion of the gradient where trout immunoglobulin (16S) would be expected to band (Dorson 1972; Jørgensen 1973). No attempt was made to determine if the lower neutralizing titres (50-500), in particular those obtained from the IPN virus negative fish, were the result of a 16S immunoglobulin or of a 6S natural antiviral inhibitor similar to that found in the serum of normal rainbow trout (Jørgensen 1973; Hill and Dixon 1977; and Kelly and Nielsen 1985).

DISCUSSION

The results of this study suggest that IPN virus is enzootic in Arctic charr populations indigenous to the river drainages of the Mackenzie Delta region and the Yukon north slope. Because salmonids have never been cultured in the Northwest Territories (R. Moshenko, Department of Fisheries and Oceans, Western Region, Winnipeg, MB, personal communication) or the Yukon Territory (T. Young, Department of Fisheries and Oceans, Pacific Region, Whitehorse, YK, personal communication), and stocking practices have been restricted to the southern reaches of both the Northwest Territories (Falk and Low 1981), and the Yukon Territory (T. Young, personal communication) it is highly unlikely that the virus was contracted from infected hatchery-reared fish. Therefore, the Arctic charr populations surveyed are believed to represent naturally infected carrier populations. How and when these fish became infected with the virus is a matter of speculation. However, these results suggest that the virus is capable of persisting at consistently high levels within the respective populations. This was particularly evident with the repeated Fish Creek/Rat River and Big Fish River collections. The close proximity of fish to each other on the spawning grounds and the overwintering areas together with the parent to progeny transmission of the virus via the gametes undoubtedly contribute to the persistence and maintenance of the high prevalence levels observed in these fish.

Unfortunately we were unable to ascertain what, if any, impact the virus has had on the respective populations, and in particular the underyearling component of the various populations. The only sampling of presmolt fish was negative for virus. These fish may not have been infected, or, if they were the virus could have been latent or diluted out due to the pooling of tissue samples for assay purposes.

The virus neutralizing titres present in Arctic charr serum were similar to those reported by Wolf and Quimby (1969) and Jørgensen (1973) for immunized adult rainbow trout. Detectable antibody was found in serum from both virus positive and virus negative fish. However, 34 of 63 virus positive fish demonstrated little (80% PRT<50), if any, antibody production. This result raises the questions of how, when, and where did these fish contract the virus, and what degree of protection is conferred by antibody under these environmental conditions. Conversely, 13 of 50 virus negative Arctic charr produced antibody titres greater than 50. This finding suggests that these particular fish had effectively neutralized and eliminated the virus; or possibly the virus was neutralized during the assay procedure, thereby, creating a false negative result. Unfortunately, the antibody titres obtained during this study represent virus neutralizing activity at only one point in time. Therefore, it is impossible to know what influence factors such as age, temperature, and environment had on antibody development and production in these fish. Further research, under controlled laboratory conditions would be required to provide answers to these questions.

The results of this study are significant for a number of reasons. Firstly, regional and territorial fisheries managers, and transplant committees should be cognizant of these findings. Appropriate measures should be taken to ensure that the virus is not inadvertently transferred to non-enzootic areas where it could pose a problem to nonexposed feral salmonid populations. Secondly, IPN virus has been

stated not to occur in Alaska (Burke 1981). However, limited tagging studies have demonstrated that some interdrainage exchange of nonspawning Arctic charr has taken place between the Firth River (Yukon Territory) and both the Kongakut and Canning Rivers in Alaska (McCart 1980). Because of this migration to nonnatal streams, it is conceivable that IPN virus could be transferred to Arctic charr indigenous to the river drainages of the Alaskan north slope, if the virus is not already present. Lastly, because of the high prevalence and wide distribution of IPN virus in the Arctic charr populations involved in this study, the authors recommend that Arctic charr or their gametes not be considered for import from this geographical area for research and/or intensive culture purposes.

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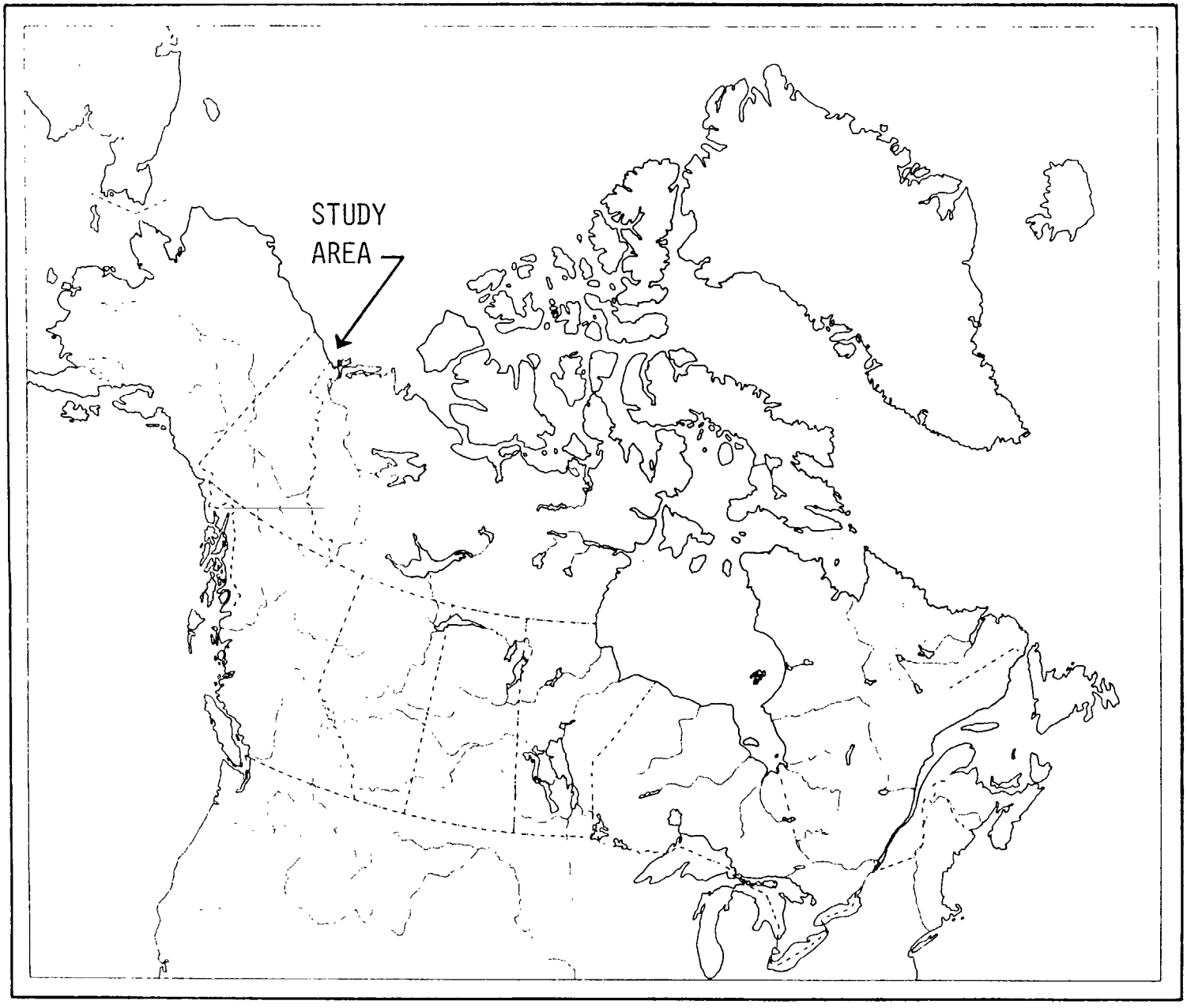

Fig. 1. Map of the Northwest and Yukon territories showing the location of the study area.

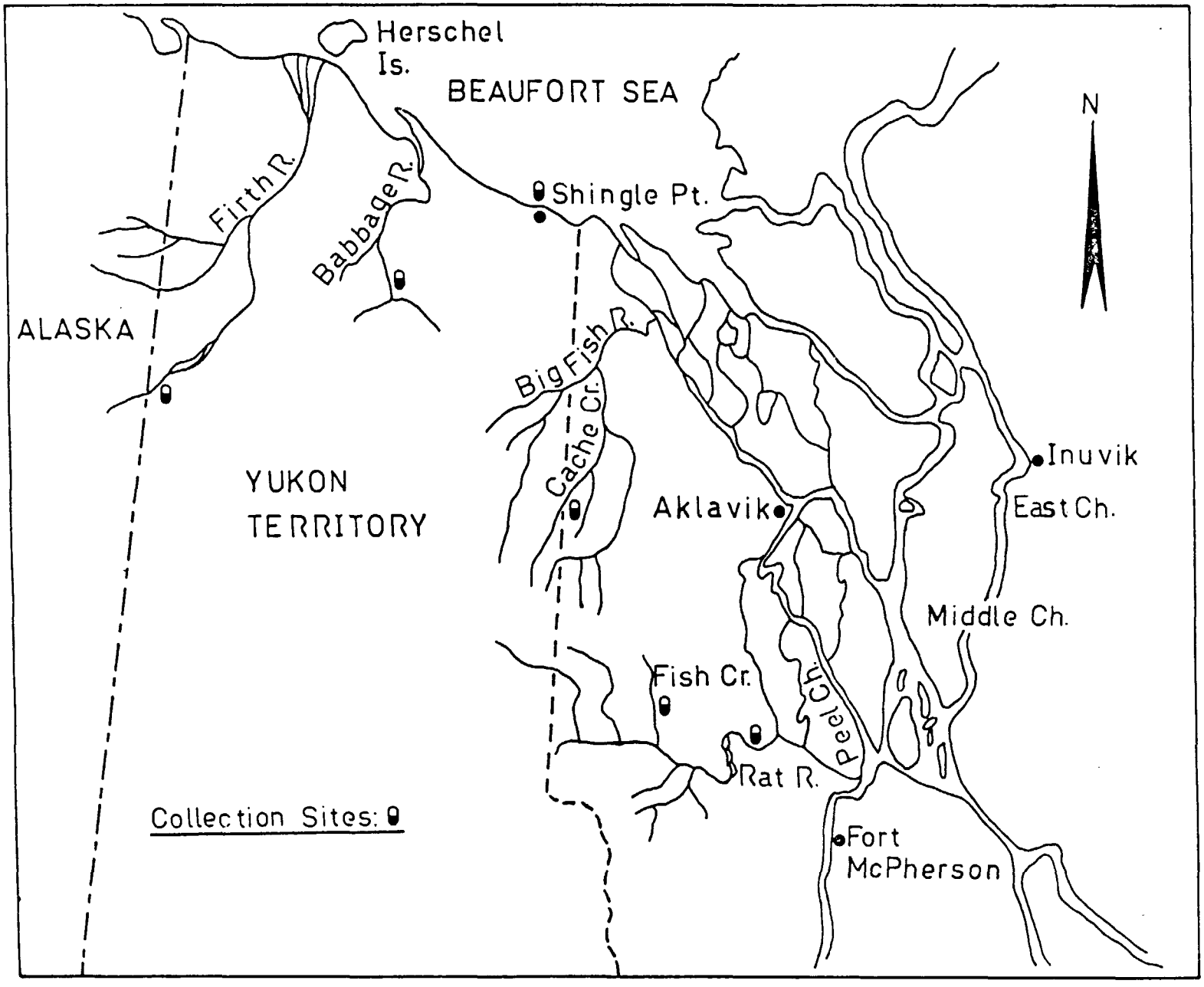

Fig. 2. Map of the Mackenzie Delta region and the Yukon north slope showing the Arctic charr collection sites.

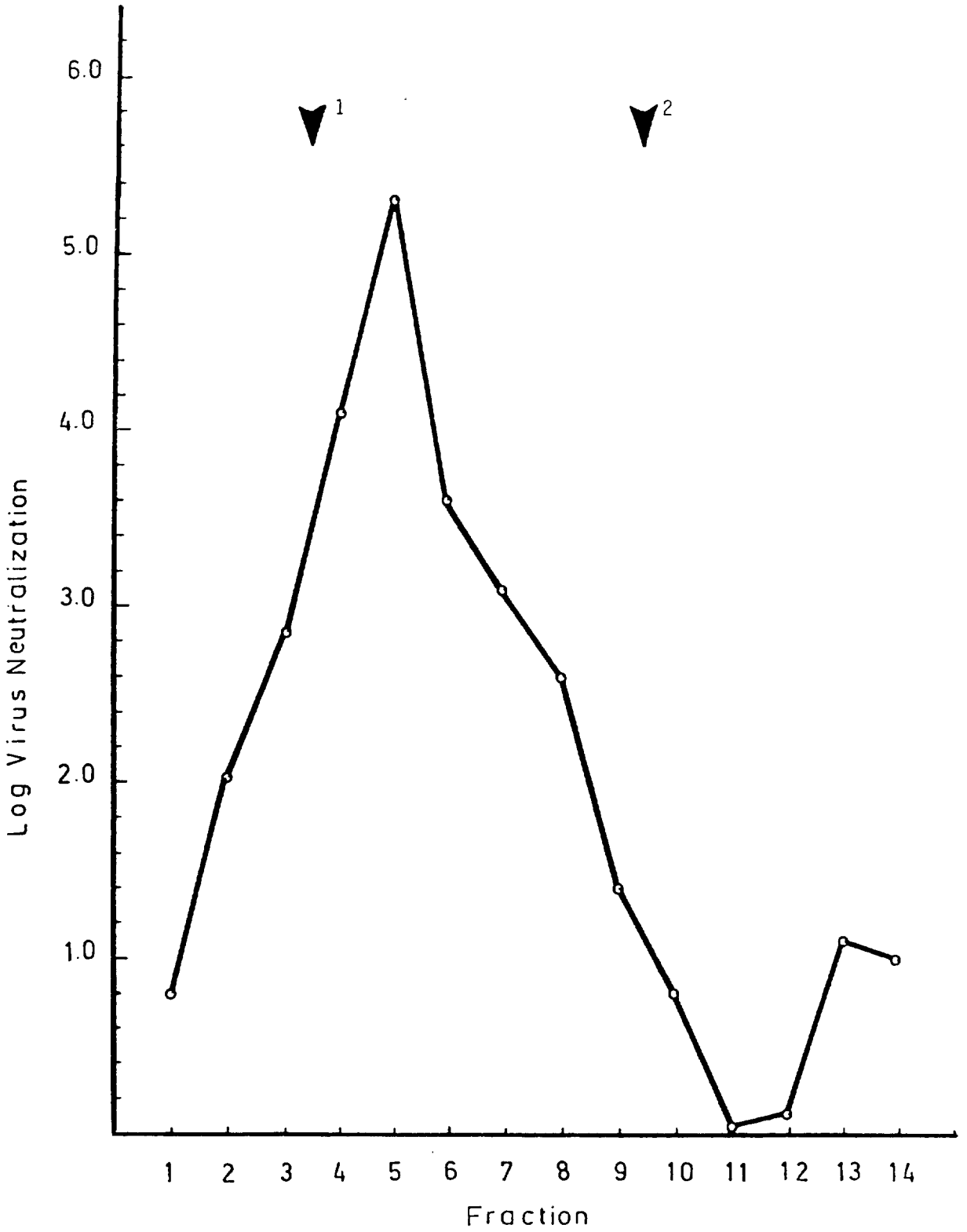

Fig. 3. Arctic charr serum fractionation by sucrose density gradient ultracentrifugation.

[1]position of porcine thyroglobulin (19S) marker
[2]position of bovine gamma globulin (7S) marker
(fractionation proceeded from left to right)

Table 1. Summary of collection dates, locations, sex, numbers sampled and percent positive for IPN virus.

Date	Location	Sex	Number Sampled	No. IPNV Positive	Percent Positive
Nov '81	Big Fish R.	N/A[1]	60	0	0
Sept '82	Fish Cr.	M	3	0	0
"	"	F	17	7	41.2
Sept '82	Big Fish R.	M	3	0	0
"	"	F	7	2	28.6
Aug '83	Rat R.	M	8	3	37.5
"	"	F	35	18	51.4
Aug '83	Fish Cr.	M	1	1	100.0
"	"	F	6	4	66.6
Sept '83	Big Fish R.	M	4	2	50.0
"	"	F	14	6	42.9
Aug '84	Shingle Pt.	M	2	1	50.0
"	"	F	5	2	40.0
Aug '84	Big Fish R.	M	20	8	40.0
"	"	F	37	13	35.1
Sept '84	Big Fish R.	M	3	3	100.0
"	"	F	7	6	85.7
Sept '84	Rat R.	M	0	0	0
"	"	F	5	3	60.0
Sept '84	Babbage R.	M	9	5	55.5
"	"	F	2	0	0
Sept '84	Firth R.	M	5	2	40.0
"	"	F	5	4	80.0
Aug '85	Rat R.	M	0	0	0
"	"	F	23	7	30.4
Aug '85	Big Fish R.	M	2	0	0
"	"	F	6	3	50.0
Total[2]			229	100	43.7

[1]Presmolts, no sex determination
[2]Excluding presmolt collection Nov.'81 Big Fish River

Table 2. Comparison of the prevalence of IPN virus between male and female Arctic charr.

Date	Location	Number of fish IPNV positive/no. examined		
		Males	Females	Total
Sept '82	Fish Cr.	0/3 (0.0)	7/17 (41.2±11.9)[1]	7/20 (35.0±10.7)
Sept '82	Big Fish R.	0/3 (0.0)	2/7 (28.6±17.0)	2/10 (20.0±12.6)
Aug '83	Rat R.	3/8 (37.5±17.1)	18/35 (51.4± 8.4)	21/43 (48.8± 7.6)
Aug '83	Fish Cr.	1/1 (100.0)	4/6 (66.6±19.2)	5/7 (71.4±17.0)
Sept '83	Big Fish R.	2/4 (50.0±25.0)	6/14 (42.9±13.2)	8/18 (44.4±11.7)
Aug '84	Shingle Pt.	1/2 (50.0±35.4)	2/5 (40.0±21.9)	3/7 (42.4±18.7)
Aug-Sept '84	Big Fish R.	11/23 (47.8±10.4)	19/44 (43.2± 7.5)	30/67 (44.8± 6.1)
Sept '84	Rat R.	0/0 (0.0)	3/5 (60.0±21.9)	3/5 (60.0±21.9)
Sept '84	Babbage R.	5/9 (55.5±16.6)	0/2 (0.0)	5/11 (45.5±15.0)
Sept '84	Firth R.	2/5 (40.0±21.9)	4/5 (80.0±17.9)	6/10 (60.0±15.5)
Aug '85	Rat R.	0/0 (0.0)	7/23 (30.4± 9.6)	7/23 (30.4± 9.6)
Aug '85	Big Fish R.	0/2 (0.0)	3/6 (50.0±20.4)	3/8 (37.5±17.1)
Total		25/60 (41.7±6.4)	75/169 (44.4± 3.8)	100/229 (43.7±3.3)

[1]Percent positive ± standard deviation

Table 3. Comparison of the prevalence of IPN virus between the Fish Creek/Rat River and Big Fish River drainages.

Year	River Drainage	
	Fish Cr./Rat R.	Big Fish R.
1982	7/20[1](35.0±10.7)[2]	2/10 (20.0±12.6)
1983	26/50 (52.0± 7.1)	8/18 (44.4±11.7)
1984	3/5 (60.0±21.9)	30/67 (44.8± 6.1)
1985	7/25 (30.4±30.3)	3/8 (37.5±17.1)
Total	43/100(43.0± 5.0)	43/103(41.7± 4.9)

[1]No. IPNV positive/no. examined
[2]Percent positive ± standard deviation

Table 4. Eighty percent plaque reduction titres for Arctic charr sera.

Titre	IPN Virus Positive A. charr								
	FCr '82	BFR '82	RR '83	BFR '83	RR '84	BFR '84	BR '84	FR '84	RR '85
<10[1]	1		6	3		11	5	5	1
10-50			2						
50-100			1	1		1			
100-500			3	2	3			1	1
500-1000	2	1	1	1					
1000-1500	2		1						
1500-2000			1			1			
2000-2500		1							
2500-3000	1								
3500-4000		1		1					
>10,000	1		1						

Titre	IPN Virus Negative A. charr								
	FCr '82	BFR '82	RR '83	BFR '83	R R '84	BFR '84	BR '84	FR '84	RR '85
<10	13	7	13	5		3	6	4	3
10-50						2			
100-500					1				4
500-1000			1						
1000-2000									3
2000-2500				2					
5000-10,000					1				
>10,000						1			

[1]reciprocal values
FC=Fish Creek; BFR=Big Fish River; RR=Rat River; BR=Baggage River; FR=Firth River