Biochemical Systematics and Ecology, Vol. 9, No. 2/3, pp. 225–232, 1981.
Printed in Great Britain.

0305–1978/81/020225–07 $02.00/0

Systematics of Irish Charr as Indicated by Electrophoretic Analysis of Tissue Proteins

A. FERGUSON

Zoology Department, The Queen's University of Belfast, Northern Ireland, UK

Key Word Index – *Salvelinus alpinus*; charr; Salmonidae; Osteichthyes; Irish fish; starch gel electrophoresis; isoelectric focusing; enzyme polymorphism; esterases; general proteins.

Abstract – The general protein patterns as produced by isoelectric focusing and the starch gel zymograms of 15 enzymes from samples of charr (*Salvelinus alpinus*) from seven Irish lakes and from autumn and spring spawning Windermere populations showed considerable homogeneity. This would suggest that all the populations examined are conspecific and descended from a common ancestor within the past 50,000 years, if not in the immediate post-glacial period. The same esterase polymorphism as described by Nyman in Scandinavian and other Arctic charr populations was noted.

Introduction

Charr (genus *Salvelinus*, family Salmonidae) are found throughout the cooler regions of the northern hemisphere. Their distribution closely parallels that of coregonine whitefish. Both groups have anadromous forms in the northern part of their range with purely freshwater populations in the deep cold lakes of the southern part. These southern populations are undoubtedly relicts of a formerly more southerly range of migratory forms during the last ice age. With the retreat of the main populations northwards at the end of the ice age, the increased salinity of the sea and the isostatic uplift of the land, these southern populations lost their migratory habits and became freshwater-resident. Thus these various populations have been reproductively isolated for at least 10,000 years.

In Ireland lacustrine populations of charr are widespread especially in the western part of the country. Although the habitat requirements of charr and coregonines seem similar, there are many more lakes inhabited by charr than by coregonines. In Ireland the latter are restricted to Lough Neagh, Lough Erne and the Shannon lakes. Maitland [1] attributes the discordance in distribution of these two groups to the later arrival of charr by which time more waters were accessible due to the increased land level.

Charr formerly inhabited more lakes in Ireland than they do at the present time. For example, charr were originally found in Lough Neagh but became extinct by about 1844 [2]. The distribution of charr in Ireland has been extensively reviewed by Went [3, 4]. In those lakes where they do occur, netting operations have shown them to be more numerous than previously suspected from angling catches.

As with the coregonines, charr show considerable plasticity in their morphological features, growth rates and way of life. As a result numerous species have been described on morphological and meristic features many of which are undoubtedly synonomous. Other workers regard these populations as representatives of a single species, the variation among populations being regarded as the result of environmental modulation of morphological features and/or differential selection and genetic drift during post-glacial isolation in various lakes. The main question, however, as put succinctly by Behnke [5] is: "How many differentiated forms of *Salvelinus* invaded the postglacial waters of Europe from glacial refugia to give rise to all of the present populations?".

The basic taxonomic work on Irish charr was carried out by Regan [6, 7] and most subsequent workers have followed his nomenclature. Although each population is unique to some extent in its morphological and meristic features, Regan recognised six groups or "species" of charr in Ireland and nine in Great Britain. However, he acknowledged [6] that all are forms of *Salvelinus alpinus* but thought it simpler "to keep the binomial nomenclature and to call the Lough Melvin char *Salvelinus grayi* in preference to *Salvelinus alpinus grayi*". This use of binomials rather than trinomial

(*Received* 29 *September* 1980; *received for publication* 7 *April* 1981)

sub-specific names has led some workers to interpret Regan's "species" too literally.

Many morphological and meristic features are plastic and subject to environmental modulation. Friend [8] has noted that charr from a lake in North Wales collected in 1865 were different in head contours from those taken in the same lake in 1954. The difficulty in separation of environmentally induced from genetically controlled variation complicates the use of morphological features in taxonomy. The use of proteins as environmentally independent characters is now of accepted systematic value [9]. Tsuyuki *et al.* [10] compared the general protein electrophoregrams of the lake trout *Salvelinus namaycush,* the brook trout *S. fontinalis,* the Dolly Varden *S. malma,* and the Arctic charr *S. alpinus,* and noted a species-specificity of the patterns, although the latter two species were very similar. Saunders and McKenzie [11] noted the considerable similarity in electrophoregrams from various populations of Arctic charr. Nyman [12] and Henricson and Nyman [13] used frequencies of a variant esterase allele to delimit three sibling species of Arctic charr in Scandinavia, two or three of which are sympatric in some lakes. Child [14] used this same esterase polymorphism and also one involving the transferrin locus, to investigate the genetic relationships of charr from four Welsh lakes.

Results

General Proteins

Separations of muscle extracts in isoelectric focusing gels of pH range 3.5–9.5 showed a maximum of 56 bands (Fig. 1). Eye extracts similarly examined revealed 47 bands, brain 41, heart 31 and liver 25. A few minor bands varied among individuals from the same lake but there were no consistent inter-lake differences in the electrophoregrams of these tissues. Since presumably the same proteins are present in several tissues it is not possible to determine the number of proteins compared. However, it would appear that in excess of 60 proteins have the same isoelectric point in all the populations examined. The consistency of position of the majority of proteins both within and between populations would suggest that polymorphism, if present, is at a very low level.

Enzymes

Of those enzymes for which staining was attempted not all gave adequate resolution. Enzymes successfully localized are listed in Table 1, together with the number of main bands resolved and the possible genetic control. In all, these enzymes represent the products of a putative 27 loci. All enzymes, with the exception of esterase, exhibited identical electrophoretic patterns both within and between lakes.

Two zones of esterase activity were present in separations of muscle extracts. The more anodal zone consisted of a single band of the same electrophoretic mobility in individuals from all lakes. The slower zone exhibited three phenotypes: a single fast-moving band, a single slow-moving band, or both bands present simultaneously. This is typical of the pattern of protein polymorphism given by a locus with two co-dominant alleles. Comparison of muscle and plasma samples from Lough Melvin charr showed that the same polymorphism is present in plasma. Thus this esterase polymorphism is undoubtedly the same one as described by Nyman [12, 15] in Scandinavian and other Arctic charr populations. Using the terminology of Allendorf and Utter [16] based on relative mobilities, the allele responsible

TABLE 1. ENZYMES EXAMINED, ENZYME COMMISSION NUMBERS, ELECTROPHORETIC PATTERNS AND GENETIC INTERPRETATIONS

Enzyme	EC No.	Tissue examined †	No. of major bands	Possible no. of loci
Adenylate kinase	2.7.4.3	L	2	2
Alcohol dehydrogenase	1.1.1.1	L	1	1
Enolase	4.2.1.11	M	5	3
Esterase	3.1.1.[*]	M	2/3	2
Gluycose-6-phosphate dehydrogenase	1.1.1.49	H	2	2
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	L	1	1
Glycerol-3-phosphate dehydrogenase	1.1.1.8	M	2	1
Lactate dehydrogenase	1.1.1.27	H,L,M	9	4
Malate dehydrogenase	1.1.1.37	M	3	2
Phosphoglucose isomerase	5.3.1.9	M,L	6	3
Phosphoglucomutase	2.7.5.1	M	2	2
Phosphomannose isomerase	5.3.1.8	L	1	1
Sorbitol dehydrogenase	1.1.1.14	L	1	1
Valyl-leucine peptidase	3.4.11.[*]	M	1	1
Xanthine dehydrogenase	1.2.1.37	L	1	1

† H = heart; L = liver; M = skeletal muscle.

(3)

Transactions of the American Fisheries Society 129:782–796, 2000

The Brief Period of Spring Migration, Short Marine Residence, and High Return Rate of a Northern Svalbard Population of Arctic Char

ODD A. GULSETH*

Brattøra Research Center, Department of Zoology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway, and Norwegian Polar Institute, N-9296 Tromsø, Norway

KJELL J. NILSSEN

Brattøra Research Center, Department of Zoology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

Abstract.—Arctic char *Salvelinus alpinus*, in the high-Arctic archipelago of Svalbard, entered the Dieset River (79°10′N) immediately after ice breakup in late June (1991–1993), and within 48 h almost half the migrating population had left the lakes where they spent the winter. The majority of the anadromous char descended into the sea within 3 weeks of the melt. The temporal pattern of emigration was independent of body size. The average residence time at sea of the char was 33.6 d, and the maximum was 56 d. The duration of the seawater sojourn was independent of body size. However, the combined time of downstream migration and marine residence was inversely related to body length in early migrating char. Fish that migrated to the sea early tended to stay there longer. The overall return rate ranged from 33.3% for the smallest (15.1–20 cm) to 75.0% for the largest (45.1–50 cm) char, averaging 51.5%. The average return rate for fish shorter than 25.1 cm (first-time migrants) was 42.5%. The upstream run started in mid-July, peaked in August, and was completed by the beginning of September. In contrast to the seaward migration, there was a structured size-precedence in the upstream run, with the large char entering the river first, followed by char of intermediate size and then the smallest anadromous char. The results indicate insignificant immigration to this high-Arctic watercourse. The marked local adaptation to the extreme physical conditions restricted these char to a narrow migrational window. Because this is the first comprehensive study on migratory behavior in anadromous Svalbard char, the results are a valuable contribution to fishery science and management of individual populations in the most northern Eurasian area.

The Arctic char *Salvelinus alpinus* has a northern circumpolar distribution of landlocked, resident and anadromous populations (Johnson 1980). Ellesmere Island (82°N, 70°W) in Canada (Johnson 1980; Reist et al. 1995) and the northern part (80°N) of the Svalbard archipelago (74–81°N) north of Norway (Hammar 1985) represent the northern limit of their distribution. Anadromy is one aspect in the complex life history of this species, which reaches its greatest abundance north of the polar circle, where anadromous animals and resident forms live sympatrically in some drainages (Johnson 1980, 1995). In contrast with populations of Pacific salmon *Oncorhynchus* spp. and Atlantic salmon *Salmo salar*, anadromous Arctic char may make short but repeated journeys out to sea during summer (Dempson and Kristofferson 1987; Randall et al. 1987; Berg and Berg 1993). After spending 3–8 years in rivers or lakes, first-time migrants enter the sea to feed (Nordeng 1961; Johnson 1980; Berg and Jonsson 1989; Finstad and Heggberget 1993) on reaching a threshold of 15–18 cm in body length (Nordeng 1961; Delabbio et al. 1988; Jensen 1994; Finstad and Heggberget 1995). The seaward-migrating population consists of first-time and repeat migrants. Normally, both immature and maturing char exhibit this seasonal migration but at some locations char may stay in freshwater throughout the year before they spawn (Johnson 1980). Arctic char are iteroparous and adults mature on an intermittent basis (Johnson 1980; Dutil 1986). Age and body length at sexual maturity range from 4 to 10 years and from 30 to 65 cm, respectively (Nordeng 1961; Johnson 1980). During their annual feeding excursion at sea, char are reported to exhibit high growth rates, and immature individuals may double their body weight (Mathisen and Berg 1968; Johnson 1980; Berg and Berg 1989; Finstad and Heggberget 1993, 1995).

* Corresponding author: odd.gulseth@chembio.ntnu.no

Received May 28, 1999; accepted December 9, 1999

Molecular Ecology (1998) 7, 209–223

Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine lakes

P. C. BRUNNER,*† M. R. DOUGLAS* and L. BERNATCHEZ

Zoologisches Museum, Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland, †Département de biologie, GIROQ, Université Laval, Saint-Foy (Québec) G1K 7P4, Canada

Abstract

Despite geographical isolation and widespread phenotypic polymorphism, previous population genetic studies of Arctic charr, *Salvelinus alpinus*, have detected low levels of intra- and interpopulation variation. In this study, two approaches were used to test the generality of low genetic diversity among 15 Arctic charr populations from three major drainages of the central Alpine region of Europe. First, a representative subsample of each drainage was screened by PCR–RFLP analysis of mtDNA using 31 restriction enzymes. All individuals but one shared an identical haplotype. In contrast, microsatellite DNA variation revealed high levels of genetic diversity within and among populations. The number of alleles per locus ranged from six to 49, resulting in an overall expected heterozygosity from 0.72 ± 0.09 to 0.87 ± 0.04 depending on the locus. Despite evidence for fish transfers among Alpine charr populations over centuries, genetic diversity was substantially structured, as revealed by hierarchical Φ statistics. Eighteen per cent of total genetic variance was apportioned to substructuring among Rhône, Rhine, and Danube river systems, whereas 19% was due to partitioning among populations within each drainage. Cluster analyses corroborated these results by drainage-specific grouping of nonstocked populations, but also revealed damaging effects of stocking practices in others. However, these results suggest that long-term stocking practices did not generally alter natural genetic partitioning, and stress the importance of considering genetic diversity of Arctic charr in the Alpine region for sound management. The results also refute the general view of Arctic charr being a genetically depauperate species and show the potential usefulness of microsatellite DNAs in addressing evolutionary and conservation issues in this species.

Keywords: Arctic charr, conservation, microsatellite DNA, mtDNA, population genetics, *Salvelinus alpinus*

Received 2 May 1997; revision received 1 September 1997; accepted 16 October 1997

Introduction

The distribution, diversity, and genetic structure of northern biota have been considerably influenced by the repeated coverage of northern Europe and central mountain ranges (e.g. Alps, Pyrenees) by Pleistocene ice sheets (Hantke 1978). These dramatic climate oscillations undoubtedly resulted in habitat and range alterations by forcing species into suitable refugia during colder conditions and allowing recolonization and dispersal during warmer interglacials. Hewitt (1996) considered these processes instrumental to Quarternary population divergence in northern temperate regions.

The Arctic charr, *Salvelinus alpinus* L. (Teleostei; Salmonidae), has long intrigued biologists as a prime example for population differentiation in northern fishes. This freshwater and anadromous fish has a Holarctic

Correspondence: P. C. Brunner. Department of Biology, Arizona State University, Tempe, Arizona, 85287-1501, USA. Fax: +1-602-965-0362; E-mail: patbrun@zoolmus.unizh.ch

Mitochondrial DNA and Conservation of an Aboriginal Arctic Char (*Salvelinus alpinus oquassa*) from Floods Pond, Maine

Irv Kornfield

Department of Zoology and Center for Marine Studies, University of Maine, 5751 Murray Hall, Orono, ME 04469-5751, USA

and Frederick W. Kircheis

Marine Department of Inland Fisheries and Wildlife, 650 State Street, Bangor, ME 04401-5654, USA

Kornfield, I., and F.W. Kircheis. 1994. Mitochondrial DNA and conservation of an aboriginal Arctic char (*Salvelinus alpinus oquassa*) from Floods Pond, Maine. Can. J. Fish. Aquat. Sci. 51: 62–67.

Periods of low water in Floods Pond, Maine, USA, during spawning seasons for an endemic population of landlocked Arctic char, *Salvelinus alpinus oquassa*, have contributed to several year-class failures. To determine the genetic uniqueness of these fish, samples of Arctic char from five isolated lakes in New England and eastern Canada were examined by restriction endonuclease analysis of mitochondrial DNA (mtDNA) and compared with samples of lake trout (*Salvelinus namaycush*) and Arctic char from Scandinavia. Results suggest that (1) Arctic char of eastern North America should all be considered members of *Salvelinus alpinus oquassa*, (2) char from Floods Pond possess a unique mtDNA banding pattern for one restriction enzyme not observed in fish from any other sampled locality (this difference delineates a distinct lineage of Arctic char whose preservation is warranted on genetic grounds), and (3) unique banding patterns did not characterize any other studied char population, including geographic variants (these populations may be viewed as genetically homogeneous, and none warrant individual protection based upon our genetic characterizations). We contend that genetically identified lineages in the early stages of divergence warrant preservation.

Les périodes de basses eaux dans le lac Floods Pond (Maine, États-Unis) pendant les saisons de ponte d'une population endémique d'ombles chevaliers confinés en eau douce (*Salvelinus alpinus oquassa*) ont joué un rôle dans plusieurs échecs des classes annuelles. Pour déterminer le caractère génétiquement distinct de ces poissons, nous avons examiné des échantillons d'ombles chevaliers provenant de cinq lacs isolés de Nouvelle-Angleterre et de l'est du Canada en effectuant une analyse de l'ADN mitochondrial par l'endonucléase de restriction, et nous avons comparé les résultats à ceux obtenus sur des échantillons de touladi (*Salvelinus namaycush*) et d'omble chevalier de Scandinavie. Les résultats permettent d'émettre les opinions suivantes : 1) les ombles chevaliers de l'est de l'Amérique du Nord doivent être tous considérés comme des membres de la sous-espèce *Salvelinus alpinus oquassa*, 2) les ombles de Floods Pond possèdent un spectre de bandes de l'ADN mitochondrial tout à fait particulier pour une enzyme de restriction qui n'a pas été observée chez des poissons provenant d'un autre endroit échantillonné (cette différence fait apparaître une lignée distincte d'omble chevalier dont la préservation se justifie pour des raisons d'ordre génétique et 3) les spectres de bandes n'ont pas permis de différencier les autres populations d'ombles étudiées, même dans le cas des variantes géographiques (on peut considérer que ces populations sont génétiquement homogènes, et qu'aucune ne justifie une protection individuelle basée sur des caractéristiques génétiques). Nous soutenons que des lignages génétiquement identifiés qui se trouvent dans les premiers stades de divergence méritent d'être préservés.

Received March 26, 1993
Accepted August 18, 1993
(JB862)

Reçu le 26 mars 1993
Accepté le 18 août 1993

The Arctic char[1], *Salvelinus alpinus*, is a taxonomically complex group of salmonid fishes widely distributed around the world at high northern latitudes. Populations in several places over the range of the species possess distinctive morphologies that distinguish them from other local char. In the eastern and southern areas of North American char distribution, there are 11 waters in Maine, USA (Kircheis 1980, 1986), three in New Brunswick, Canada (W. Hooper, New Brunswick Department of Natural Resources, Fredericton, N.B., personal communication), and approximately 100 in southeast Quebec, Canada (Dumont 1982), that have natural populations of landlocked Arctic char.

Of particular interest are the silver char[2] of Floods Pond, Maine, a natural population of landlocked fish character-

[1]We cannot agree upon the appropriate spelling of "char(r)". The American Fisheries Society (Robins et al. 1991) prefers "char" whereas several major texts on this taxon use "charr" (Balon 1980; Johnson and Burns 1984; Kawanabe et al. 1989). Many Scandanavian authors, who for many years preferred "char", are now using "charr" (Anonymous 1992). The International Society of Arctic Char Fanatics (ISACF) allows both "char" and "charr" in its proceedings but objects to using both spellings in the same paper (Hammar 1985). The subject has been reviewed by Morton (1955).

[2]Silver char is used as the common name for these fish instead of the former "Sunapee char". We have shown the Floods Pond char to be distinct from the Sunapee Lake char (F.W. Kircheis, I. Kornfield, and S. Seyoum, unpublished data). Local anglers have long referred to the Floods Pond char as "silver trout".

TABLE 1. Collection localities for samples of fish used in mtDNA comparisons.

Taxon and locality	Sample size	Latitude	Longitude
S. alpinus oquassa			
Big Reed Pond, Maine, USA	10	46°21′N	69°03′W
Floods Pond, Maine, USA	30	44°43′N	68°28′W
Gardiner Lake, Maine, USA	17	46°58′N	68°53′W
Lac Godin, Quebec, Canada	18	47°57′N	71°20′W
Walton Lake, New Brunswick, Canada	20	45°30′N	65°20′W
S. alpinus alpinus			
Jonsvann, Norway	30	63°22′N	10°37′W
S. namaycush			
Clearwater Lake, Manitoba, Canada	3	54°30′N	101°00′W

ized by unique breeding color (Kircheis 1989). Like much of the Arctic char species complex (Behnke 1984, 1989), the taxonomic status of the Floods Pond fish has been the subject of considerable unresolved debate.

Several genetic studies have been conducted to clarify the systematics of Arctic char populations by conventional electrophoretic analysis (Kornfield et al. 1981; Andersson et al. 1983; Magnusson and Ferguson 1987; Partington and Mills 1988). Allozyme studies have been able to differentiate subspecies of char but have provided no information on differentiation among populations. Studies of *Salvelinus* species using restriction analysis of mitochondrial DNA (mtDNA) were conducted by Grewe et al. (1990). In this work, landlocked populations of Arctic char could be clearly distinguished from anadromous populations; comparisons of Arctic char from Great Britain with samples from Floods Pond revealed extremely high similarities. Restriction analysis of ribsomal DNA (Phillips et al. 1992) produced similar results. Thus, while some genetic investigations have convincingly demonstrated genetic isolation of sympatric morphs, the status of allopatric populations, such as the char of Floods Pond, has not been resolved.

Floods Pond is a 260-ha, 50-m-deep oligotrophic lake located in central Maine. Floods Pond contains a native, relict population of landlocked Arctic char and serves as the sole water source for the City of Bangor, Maine, and seven neighboring communities. A total of 54 000 people depend upon the pond for their water, and in 1992, approximately 22.2 million L per day was used. Past water use demands have contributed to periodic lowering of water levels. Three times in the past 10 yr, there was little or no char reproduction in Floods Pond because of exposure of the spawning site, a single 0.3-ha bed of glacial cobble located in shallow water. In addition, in 5 yr during the same 10-yr period, low water levels reduced the size of the spawning area, potentially limiting char reproduction.

Future demands for water could impact the breeding habitat of the silver char in Floods Pond. In 1989, the legislature of the State of Maine directed the Bangor Water District and the Maine Department of Inland Fisheries and Wildlife to cooperate in the development of a management plan that would ensure long-term survival of the silver char while continuing to allow use of Floods Pond as a public water supply. In response to this charge, it was important to clarify the taxonomic status of this fish. The principal goal of our study was to investigate the genetic distinctiveness of the char from Floods Pond to resolve their formal taxonomic status and provide information critical for long-term conservation and management. This was conducted at two levels: (1) comparison with other landlocked populations in eastern North America and (2) comparison with a European char population in Norway. Furthermore, to place these results in the proper context, comparison of Arctic char with lake trout, *Salvelinus namaycush*, was also conducted. We here report the results of these comparisons and consider them within a conservation context.

Materials and Methods

Tissue samples were obtained from fish collected from five Arctic char populations in eastern North America: blueback char (Big Reed Pond, Gardner Lake, Maine, and Walton Lake, New Brunswick), silver char (Floods Pond), and Quebec red char (Lac Godin, Quebec). On the basis of traditional taxonomy, fish from this New England/eastern Canada region are all considered to represent the Arctic char subspecies *Salvelinus alpinus oquassa* (Behnke 1989). Samples of *S. alpinus alpinus*, the western European subspecies, were obtained from a landlocked population in Norway. Samples of lake trout, a species that is closely related to but distinct from Arctic char, were obtained from a State of Maine fish hatchery (Table 1).

Tissues from all specimens were dissected from freshly killed fish and transported frozen on dry ice or liquid nitrogen to the University of Maine. Specimens were kept frozen at −80°C for up to 4 mo prior to processing.

mtDNA was extracted from liver and egg tissues by standard protocols and purified by cesium chloride ultracentrifugation (Lansman et al. 1981; Dowling et al. 1990). Purified samples were dialyzed and stored in an ultracold freezer prior to use. Aliquots of purified mtDNA were digested with a battery of 18 informative restriction endonucleases (Kornfield and Bogdanowicz 1987). Many of these enzymes had recognition sequences of four base pairs (bp) which resulted in the production of a large number of cleavage fragments: *Alu*I (recognition sequence, bp, 4), *Ava*I (4.6), *Ava*II (4.6), *Bst*EII (6), *Cfo* (4), *Dde*I (4), *Eco*RII (4.6), *Hae*III (4), *Hin*cII (5.3), *Hin*fI (4), *Mbo*I (4), *Msp*I (4), *Nci*I (4.6), *Nco*I (6), *Rsa*I (4), *Sau*96I (4), *Sty*I (5.3), and *Taq*I (4). Resulting restriction fragments were end-labeled with

TABLE 2. mtDNA restriction fragment phenotypes observed for all sampled localities. Enzymes composing composite phenotypes are listed (left to right) as in Materials and Methods. *Sty*I (bold) discriminates the Floods Pond population.

Taxon	n[a]	Locality[b]	Composite phenotype
S. alpinus oquassa	22	Floods Pond	AAAAAAAAAAAAAAAAA
	8	Big Reed Pond	AAAAAAAAAAAAAAAACA
	7	Gardiner Lake	AAAAAAAAAAAAAAAACA
	5	Lac Godin	AAAAAAAAAAAAAAAACA
	7	Walton Lake	AAAAAAAAAAAAAAAACA
	1	Gardiner Lake (1)	AAAAAAAAAADAAAAACA
	1	Gardiner Lake (2)	AAAAAAAAABAAAAAACA
	1	Gardiner Lake (3)	AAAAAAAAAABAAAAACA
S. alpinus alpinus	1	Jonsvann (1)	BBAAAAAAAAAAABBBBB
	1	Jonsvann (2)	BDAAAAAABAAAABABBB
	1	Jonsvann (3)	BDAAAAAAAAACBBABBB
	2	Jonsvann (4)	BDAAAAAAAAAAABABBB
	2	Jonsvann (5)	BDAAAAAAAAAECBABBB
S. namaycush	2	Maine	CCBBBCBBCCCDEACCEC

[a]For restriction enzymes that were polymorphic for eastern North American samples, the number of individuals examined was greater than that indicated for composite phenotypes.

[b]Numbers in parentheses indicate locality-specific variant phenotypes.

TABLE 3. mtDNA restriction phenotypes for *Sty*I (estimated fragment sizes are given in base pairs).

A	B	C	D	E
1736	1565	1736	1736	2192
1565	1474	1565	1565	1314
1474	1314	1474	1474	1048
1314	1212	1314	1314	1003
1003	1003	1003	1003	841
933	742	742	742	796
742	688	688	688	742
688	609	521	562	688
521	521	468	521	562
468	468	410	468	521
358	410	358	358	468
292	358	292	292	358
232	292	232	232	232
	232			

[^{32}P]NTPs and separated by agarose electrophoresis on gels containing molecular weight standards (Maniatias et al. 1982). Restriction banding patterns were visualized by autoradiography. For a number of restriction enzymes, sample sizes were reduced when banding patterns were identical among all North American samples.

Banding patterns were recorded and restriction fragment phenotypes were assigned to all individuals. Intrapopulation variation and interpopulation differentiation in restriction profiles were quantified by standard mathematical procedures (Upholt 1977; Nei and Li 1979; Nei 1987). Fragment data and estimates of sequence divergence were summarized using phenetic (Sneath and Sokal 1973; Rohlf 1988) and maximum-likelihood techniques (Felsenstein 1988, 1989).

Results

A total of 230 cleavage fragments were visualized by digestion with the 18 restriction enzymes. Given an approximate size of 16 800 bp for the mtDNA of *Salvelinus* (Grewe et al. 1990), about 5.9% of the mtDNA genome of these samples was examined. The choice of restriction enzymes used in this study was designed to maximize the detection of differences among char samples; the use of more conservative (six-base) enzymes was limited. The number of bases assayed here was more than four times that in the study of Grewe et al. (1990), thus enhancing sensitivity of the assays.

In all, 13 mtDNA banding patterns or phenotypes were observed among the samples of Arctic char (Table 2). For the North American localities, three of the populations (Big Reed Pond, Lac Godin, and Walton Lake) exhibited a single phenotype that was identical for all individuals; Quebec red char (Lac Godin) had the same phenotype as the blueback char. Fish with this phenotype were common in Gardner Lake. However, this locality also possessed additional banding phenotypes which did not occur in the other samples; three additional phenotypes from Gardner Lake occurred in single individuals and each differed from the common pattern by a single band.

All fish examined from Floods Pond had identical phenotypes that differed from those of all other Arctic char for banding produced by the enzyme *Sty*I (Table 3). This unique pattern, "A", possessed one additional fragment (933 bp) which was not present in the "C" phenotype that was common to all other eastern populations (Fig. 1). The banding phenotypes for Floods Pond char produced by all other restriction enzymes were identical to the common patterns observed in other eastern population samples. Details on restriction fragment patterns for other enzymes may be obtained from the senior author upon request.

Sequence divergence estimates based upon shared banding patterns were calculated for all composite mtDNA phenotypes. Percent nucleotide sequence divergence (p) among phenotypes for char from New England and eastern Canada was very small (p = 0.02–0.12%, mean = 0.07%). Despite having a unique cleavage pattern for *Sty*I, char from Floods Pond exhibited very low sequence divergence with the cleavage phenotype for blueback char and Quebec red char sam-

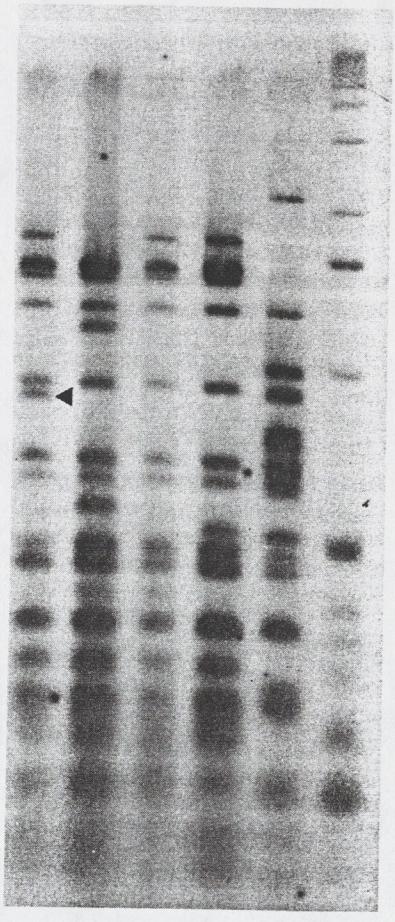

FIG. 1. Autoradiograph of mtDNA char populations digested with the restriction enzyme *Sty*I. Cleavage phenotypes (left to right) are A, B, C, D, and E. Note the band (arrowhead) that is present in "A" but absent from all other patterns. Geographic distribution of banding patterns (phenotypes) is given in Table 3. The banding pattern in the far right lane is a molecular weight size standard (BRL, Bethesda, Md.).

ples (p = 0.03%). By contrast, sequence divergence between the North American subspecies of Arctic char, *S. a. oquassa*, and the Scandinavian subspecies, *S. alpinus alpinus*, was larger (p = 0.42–0.78%, mean = 0.50%). Sequence divergence between Arctic char and lake trout averaged 3.55%.

Relationships among char samples were visualized by clustering the estimates of sequence divergence among mtDNA banding phenotypes (UPGMA; Sneath and Sokal 1973). The resultant phenogram (Fig. 2) depicts the low divergence among eastern North American samples of char. The population from Floods Pond is embedded within a cluster of eastern phenotypes. However, because of the high similarity among these fish, the ordering of groups within this cluster is largely arbitrary. By contrast, these phenotypes were distinct from the samples representing the subspecies from Scandinavia. Finally, all Arctic char are genetically well separated from lake trout. Associations among char mtDNAs were statistically examined by bootstrapping (100 replicates) of banding patterns (Felsenstein 1988). The resulting maximum-likelihood network was similar to the phenogram; the North American phenotypes were significantly separated from the Scandinavian phenotypes (p = 100%, results not shown).

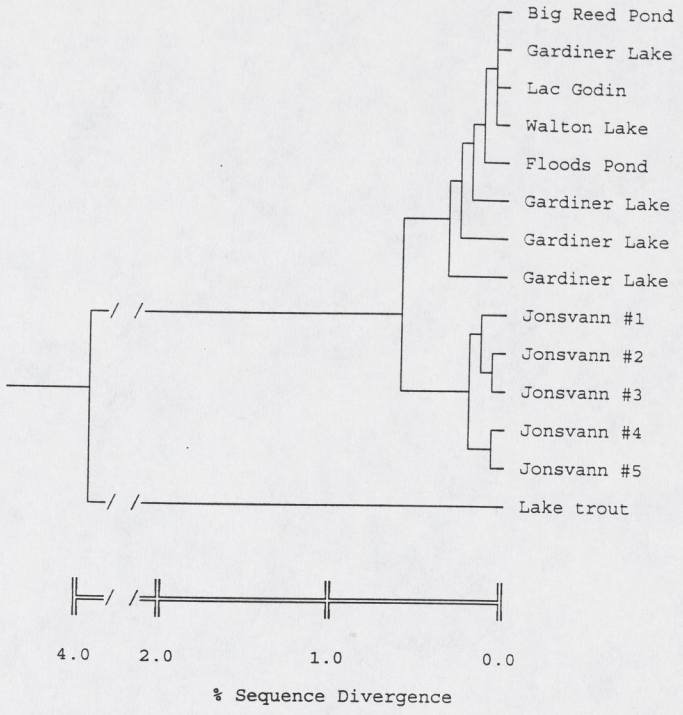

FIG. 2. Relationships among char populatons based on observed mtDNA phenotypes. Clustering was performed on estimates of genetic divergence obtained from mtDNA banding patterns. Cophenetic correlation = 0.997.

Discussion

Systematic Implications

It is clear from the mtDNA analyses conducted here and from the molecular studies of Grewe et al. (1990) and Phillips et al. (1992) that populations of Arctic char from eastern North America are of very recent origin. Given the geological history of the region (Borns 1988), the isolated populations we studied probably originated after glacial withdrawal less than 15 000 yr ago. The results from earlier electrophoretic studies are consistent with this idea (Kornfield et al. 1981; Andersson et al. 1983; Magnusson and Ferguson 1987; Partington and Mills 1988). Because of the many caveats associated with application of a molecular clock to mtDNA (Moritz et al. 1987; Hillis and Moritz 1990; T.E. Dowling, Arizona State University, Tempe, Ariz., personal communications), we will not use it to estimate absolute separation time.

By all characterizations, the samples of char from eastern North America are very closely related. The low levels of estimated sequence divergence and the absence of stable or significant clustering (Fig. 2) are similar to observations made among conspecific populations in a diversity of fishes including other salmonids (see Avise et al. (1987) and Gonzalez-Villasenor and Powers (1990) for reviews). None of the char populations that we studied should be distinguished by subspecific or specific rank. Formal taxonomic status is not warranted for the char of Floods Pond, Maine blueback char, or Quebec red char.

As a group, the fishes from eastern North America are readily distinguishable from the other subspecies in this study represented by specimens of landlocked char from

Norway. The average sequence divergence between char from eastern North America (silver, blueback, and Quebec red) and Norway was $p = 0.50\%$. This level of divergence is consistent with that observed between geographically isolated populations of freshwater fishes, including subspecies (Gonzalez-Villasenor and Powers 1990; Meyer et al. 1990). Grewe et al. (1990) compared mtDNA samples of *S. alpinus oquassa* and *S. alpinus alpinus* (Floods Pond versus Lake Windermere, England) and recorded lower divergence between them ($p = 0.22\%$) than we did. However, their estimate may have been conservative in view of the number of sites assayed. In a maximum-likelihood analysis, samples from the two subspecies formed groups that were statistically distinguishable. The extent and pattern of differentiation between these groups strongly support their recognition as representatives of discrete subspecies.

Despite their overall genetic similarity to neighboring populations, the char of Floods Pond possess a unique mtDNA banding pattern for one restriction enzyme. Our earlier allozyme studies (Kornfield et al. 1981) did not indicate any differences in heterozygosity among populations. By contrast, fixation of a mtDNA variant in the Floods Pond char suggests that the population went through a bottleneck in the past (see Leberg 1992). The difference in the *Sty*I phenotype is equivalent to a change in one base pair among the estimated 1000 bases examined. Simple extrapolation to the entire mtDNA sequence of 16 800 bp suggests the existence of 15 additional base changes. While mtDNA is generally used as a proxy to infer the extent of genetic differences that occur in nuclear DNA, it is not realistic to numerically extrapolate to this class of DNA. Differentiation of mtDNA generally implies parallel differentiation at the nuclear level (Karl and Avise 1992), but mtDNA is a much more sensitive indicator of population size effects.

Quebec red char from Lac Godin and char from Big Reed Pond, Gardner Lake, and Walton Lake had no distinctive mtDNA banding patterns. From the perspective of evolutionary genetics, the apparent absence of DNA phenotypes which could distinguish any of these populations means that there is no compelling reason for preserving the genetic material of any particular lake. That is, it does not matter exactly which populations are conserved as long as the genetic lineage of Arctic char that is common to all of these populations is preserved. By this reasoning, if the mtDNA from Floods Pond char were indistinguishable from that of the other populations, there would be no genetic justification for preservation of the population as distinct from other char in the region. We appreciate the perspective that theoretically all populations should be protected, since they may harbor undiscovered unique differences. Pragmatically, demonstrated uniqueness provides a legitimate basis for advocating conservation of a population. The presence of a mtDNA banding phenotype in all individuals from Floods Pond ($n = 22$) and its absence from all other North American fishes studied ($n = 52$) justifies the conclusion that these fish represent a distinct genetic lineage of North American Arctic char. This lineage is not present in the descendants of char from Sunapee Lake that occur in Idaho (F.W. Kircheis, I. Kornfield, and S. Seyoum, unpublished data). Thus, protecting the Floods Pond *lineage* of Arctic char is warranted on genetic grounds.

We note that conventional methodologies have also consistently demonstrated the uniqueness of the fish from Floods Pond. Waters (1960) determined that the Floods Pond population was significantly different from five blueback char populations based on serological studies and an examination of meristic characters. Qadri (1974), although he considered the Quebec red char *Salvelinus marstoni*, the blueback char, and the Floods Pond char all the same suspecies, found significant differences in the Floods Pond char for 42.8% of the meristic characters he examined. Vladykov (1954) concluded that the Quebec red char and the blueback char were the same subspecies but that the so-called Sunapee char, *Salvelinus aureolus*, was unique. Kendall (1905) felt that the blueback char from Rangeley Lake and Rainbow Lake (Maine) were "quite different" from the silver char of Floods Pond.

Conservation Implications

The challenge of protecting the char from Floods Pond is driven by two competing, but not mutually exclusive, perspectives: the statuatory requirement for preserving the Floods Pond char in its present habitat and the need for a potable water supply for a growing human population. Three approaches are being taken to achieve accommodation. First, consumers are being asked to conserve water and some have been alerted to the possibility that they may have to develop alternative sources of supply. Second, artificial spawning substrates have been recently constructed in Floods Pond; these areas may permit natural reproduction during periods of low water. Finally, silver char have been introduced into a number of waters in Maine (Kircheis 1989). At least one of these introductions, at Long Pond, has resulted in the establishment of a stable, reproducing population. To the degree that Long Pond remains free of introduced competitors and predators, the genetic lineage of silver char is now protected.

A question of more global implication is raised by the results of this study. We found that the silver char in Floods Pond have a unique mtDNA composition which mandates their protection. However, there are other, geographically close char populations that are in little danger and are extremely similar genetically to the silver char. Some feel that protecting this degree of difference does not warrant large amounts of money and possible inconveniences to humans. Consistent with this perspective, Morowitz (1991) suggested that extinction is an inevitable result of human population growth and that if economics is to be used as an argument for protection, then the degree of uniqueness must be considered.

While it may be true that the silver char are only slightly different from other populations, we are the custodians of this difference. If water usage were to have continued without regard for the char, a unique line of genetic diversity might be lost. If this were only a single incident in the region, then consequences would be minimal. But when considered in the context of the extinction of the Monadnock trout in New Hampshire (Kendall 1912), the blueback char of Rangeley, Maine (Kendall 1914), the char of Sunapee Lake (Newall 1958), and unknown and uncounted other "types" of fish, the cumulative impact is substantial. We suggest that the silver char of Floods Pond is an appropriate symbol for preservation.

Acknowledgements

Tissue samples were obtained through the generous contribu-

tions of David Basley (Maine), Stan Georges (Quebec), Gilles Godin (New Brunswick), William Hooper (New Brunswick), James Lucas (Maine), Odd Terje Sandlund (Norway), and Joan Trial (Maine). Much of the laboratory work was performed by Seifu Seyoum. Paul Moran assisted with autoradiography. Peter Grewe, Cornell University, provided unpublished data and helpful comments on some of our findings. Malcolm Hunter, John Moring, Joan Trial, and Kendall Warner critiqued early drafts of the manuscript. James Reist and another, unnamed reviewer provided critical comments on the final draft. This study was supported by the Bangor Water District, the Nature Conservancy (Maine Chapter), the Maine Department of Inland Fisheries and Wildlife, and the New Hampshire Department of Fish and Game. Data analysis and manuscript preparation were supported by award 07G-GTSS-930323 from the Maine Science and Technology Commission as part of NSF grant EHR 91-08766.

References

ANDERSSON, L., N. RYMAN, AND G. STAHL. 1983. Protein loci in the Arctic charr, *Salvelinus alpinus* L.: electrophoretic expression and genetic variability patterns. J. Fish Biol. 23: 75–94.

ANONYMOUS. 1992. Nordic Journal of Freshwater Research, Institute of Freshwater Research, S-178 93 Drottningholm, Sweden. 67.

AVISE, J.C., J. ARNOLD, R.M. BALL, E. BIRMINGHAM, T. LAMB, J.E. NEIGEL, C.A. REEB, AND N.C. SAUNDERS. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18: 489–522.

BALON, E.K. [ED.] 1980. Charrs, salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands. 928 pp.

BEHNKE, R.J. 1984. Organizing the diversity of the Arctic charr complex, p.3–21. *In* L. Johnson and B.L. Burns [ed.] Biology of the Arctic Charr: Proceedings of the International Symposium on Arctic Charr, Winnipeg, Manitoba, May 1981. University of Manitoba Press, Winnipeg, Man. 584 p.

BEHNKE, R.J. 1989. Interpreting the phylogeny of *Salvelinus*, p. 35–48. *In* H. Kawanabe, F. Yamazake, and D.L.G. Noakes [ed.] Biology of charrs and masu salmon. Physiol. Ecol. Jpn. Spec. Vol. 1.

BURNS, H.W. 1988. The making of Maine: the advance and retreat of the last great glacier. Habitat: J. Maine Audubon Soc. 5: 22–25.

DOWLING, T.E., C. MORITZ, AND J.D. PALMER. 1990. Nucleic acids II: restriction site analysis, p. 250–317. *In* D.M. Hillis and C. Moritz [ed.] Molecular systematics. Sinauer Associates, Sunderland, Mass.

DUMONT, P. 1982. Dispersion post-glaciare de l'omble chevalier d'eau douce (*Salvelinus alpinus*) dan le Québec meridional. Naturaliste Can. (Rev. Ecol. Syst.) 109: 229–234.

FELSENSTEIN, J. 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. 22: 521–565.

FELSENSTEIN, J. 1989. PHYLIP (phylogenetic inference package, computer software), version 3.2. University of Washington, Seattle, Wash.

GONZALEZ-VILLASENOR, L.I., AND D.A. POWERS. 1990. Mitochondrial DNA restriction-site polymorphisms in the teleost *Fundulus heteroclitus* support secondary integradation. Evolution 44: 27–37.

GREWE, P.M., N. BILLINGTON, AND P.D.N. HEBERT. 1990. Phylogenetic relationships among members of *Salvelinus* inferred from mitochondrial DNA divergence. Can. J. Fish. Aquat. Sci. 47: 984–991.

HAMMAR, J. 1985. Proceedings of the Third ISACF Workshop on Arctic Char, 1984. Institute of Freshwater Research, 8-170 11 Drottningholm, Sweden. p. 4.

HILLIS, D.M., AND C. MORITZ [ED.] 1990. Molecular systematics. Sinauer Associates, Sunderland, Mass.

JOHNSON, L., AND B. BURNS [ED.] 1984. Biology of the Arctic Charr: Proceedings of the International Symposium on Arctic Charr, Winnipeg, Manitoba, May 1991. University Manitoba Press, Winnipeg, Man. 584 p.

KARL, S.A., AND J.C. AVISE. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science (Wash., D.C.) 256: 100–102.

KAWANABE, H., F. YAMAZAKI, AND D.L.G. NOAKES [ED.] 1989. Biology of Charrs and Masu Salmon: Proceedings of the International Symposium on Charrs and Masu Salmon. Physiol. Ecol. Jpn. Spec. Vol. 1: 711 p.

KENDALL, W.C. 1905. Letter to the editor. Maine Sportsman, Feb. p. 117.

KENDALL, W.C. 1912. Fishes and fishing in Sunapee Lake. U.S. Bur. Fish. Doc. No. 783: 96 p.

KENDALL, W.C. 1914. The fishes of New England. The salmon family. Part 1. The trout of charrs. Mem. Boston Soc. Nat. Hist. 8(1): 103 p.

KIRCHEIS, F.W. 1980. The landlocked charrs of Maine: the Sunapee and the blueback, p. 749–755. *In* E.K. Balon [ed.] Charrs, salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

KIRCHEIS, F.W. 1986. Landlocked Arctic charr management plan. Maine Department of Inland Fisheries and Wildlife, Augusta, Maine.

KIRCHEIS, F.W. 1989. History of landlocked Arctic charr management in Maine, U.S.A., p. 615–623. *In* H. Kawanabe, F. Yamazake, and D.L.G. Noakes [ed.] Biology of Charrs and masu salmon. Physiol. Ecol. Jpn. Spec. Vol. 1.

KORNFIELD, I., K.F. BELAND, J.R. MORING, AND F.W. KIRCHEIS. 1981. Genetic similarity among endemic Arctic char (*Salvelinus alpinus*) and implications for their management. Can. J. Fish. Aquat. Sci. 38: 32–39.

KORNFIELD, I., AND S.M. BOGDANOWICZ. 1987. Differentiation of mitochondrial DNA in Atlantic herring, *Clupea harengus*. Fish. Bull. 85: 561–568.

LANSMAN, R.A., R.O. SHADE, J.F. SHAPIRA, AND J.C. AUISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. II. Techniques and potential applications. J. Mol. Evol. 17: 214–226.

LEBERG, P.L. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution 46: 477–494.

MAGNUSSON, K.P., AND M.M. FERGUSON 1987. Genetic analysis of four sympatric morphs of Arctic char, *Salvelinus alpinus*, from Thingvalavatn, Iceland. Environ. Biol. Fishes 20: 67–73.

MANIATIAS, T., E.F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning — a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MEYER, A., T.D. KOCHER, P. BASASIBWAKI, AND A.C. WILSON. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature (Lond.) 346: 550–553.

MORITZ, C., T.E. DOWLING, AND W.M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18: 269–292.

MOROWITZ, H.J. 1991. Balancing species preservation and economic considerations. Science (Wash., D.C.) 253: 752–754.

MORTON, W.M. 1955. Charr or char — history of a common name for *Salvelinus*. Science (Wash., D.C.) 121(3155): 874–875.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, N.Y.

NEI, M., AND W.H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. U.S.A. 76: 5269–5273.

NEWELL, A.E. 1958. The life history and ecology of the Sunapee trout, *Salvelinus aureolus* (Bean). New Hampshire Fish and Game Department, Concord, N.H. 17 p.

PARTINGTON, J.D., AND C.A. MILLS. 1988. An electrophoretic and biometric study of Arctic charr, *Salvelinus alpinus* (L.), from ten British lakes. J. Fish Biol. 33: 791–814.

PHILLIPS, R.B., K.A. PLEYTE, AND M.R. BROWN. 1992. Salmonid phylogeny inferred from ribosomal DNA restriction maps. Can. J. Fish. Aquat. Sci. 49: 2345–2353.

QADRI, S.U. 1974. Taxonomic status of the *Salvelinus alpinus* complex. J. Fish. Res. Board Can. 31: 1355–1361.

ROBINS, C.R., R.M. BAILEY, C.E. BOND, J.R. BROOKER, E.A. LACHNER, R.N. LEA, AND W.B. SCOTT. 1991. Common and scientific names of fishes from the United States and Canada. Spec. Publ. 20. 5th ed. American Fisheries Society, Bethesda, Md.

ROHLF, F.J. 1988. NTSYS-pc, numerical taxonomy and multivariate analysis system, version. 1.4. Exeter Publishing, Setauket, N.Y.

SNEATH, P.H.A., AND R.R. SOKAL. 1973. Numerical taxonomy. W.H. Freeman, San Francisco, Calif.

UPHOLT, W.B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic Acids Res. 4: 1257–1285.

VLADYKOV, V.D. 1954. Taxonomic characters of the eastern North American chars (*Salvelinus* and *Cristivomer*). J. Fish. Res. Board Can. 11: 904–932.

WATERS, C.S. 1960. A study of the life history and taxonomic position of the blueback trout (*Salvelinus oquassa* Girard). M.S. thesis, University of Maine, Orono, Maine. 90 p.