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UNITED STATES
DEPARTMENT OF COMMERCE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
NATIONAL MARINE FISHERIES SERVICE

ATLANTIC COASTAL FISHERIES COOPERATIVE MANAGEMENT ACT
(ACFCMA) 11-454

COMPLETION REPORT

STATE: NEW YORK

PROJECT TITLE: Interstate Management of Marine Fishery Resources in New York

GRANT NUMBER: NA76FG0345 3-ACA-043

PERIOD COVERED: October 1, 1997 to September 30, 2000

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Completion Report for Project Number NA76FG0343

Period Covered
October 1, 1997 to September 30, 2000

Introduction:

The New York State Department of Environmental Conservation is an active partner in the development and implementation of fisheries managed under Atlantic States Marine Fisheries Commission (ASMFC) Fishery Management Plans (FMP). Each FMP contains various compliance criteria, data collection needs, reporting requirements, permitting requirements, and enforcement needs. To meet these growing needs New York has chosen to supplement existing work teams, work efforts, and enforcement efforts through our Atlantic Coastal Fisheries Cooperative Management Act grant. The department has employed temporary staff to assist with ongoing projects, to assist with the issuance of permits required under the various fishery management plans, to provide monies to support enforcement activities, to provide monies to support contractual research projects and to provide resources necessary to contract for work on the Marine Permit Office computer software upgrade. This Completion report will describe activities on this Grant for the period of October 1, 1997 to September 30, 2000.

Administration of Fishery Permits for Fisheries Managed Under ASMFC Fishery Management Plans and Fishery Data Management.

During the three year period (October 1, 1997 - September 30, 2000) The Bureau of Marine Resources completed the development of a new computerized Marine Permitting system which was originally initiated during 1995. Some baseline work was completed during 1995 and 1996.

However, the initial phase of the Marine Permit Office Upgrade began in 1997 the finalization of a Business Needs Analysis of the permit office and then to employ this document in the develop of a software program that achieved those needs. Following that a contractor was hired to work with the department's Information Systems Development (ISD) Unit to create the software program and data conversion programs necessary to accept the historic data and new license information consistent with the software. During late 1999 and early 2000, the software program was rolled out for testing and reconfiguring as necessary. The completed data entry product was completed in June of 2000. At this point we began to issue permits and licenses for the year 2000. Following the issuance of the year 2000 licenses, Marine staff and ISD staff began working to develop the software for renewal of the 2000 licenses. This process was completed in late 2000. The new system successfully completed the transition from issuing permits and licenses for the year 2000 to issuing renewals for 2001. There remain several unresolved issues which will be worked on by staff from Marine Resources and ISD over the next year. These unresolved issues are not covered by this grant.

Marine Permit Office staff issue over 9,000 permits and licenses annually, including 10 special permits associated with ASMFC FMP's. Two key species involved in this permitting process were the striped bass and summer flounder. A total of five special permits were issued for these two species. Three special permits were issued for the striped bass fishery (commercial harvesters permit; party/charter boat permit; and shippers and dealers permit). Two special permits were issued for the summer flounder (commercial harvesters permit and party boat fillet permit). Staff issued 386 commercial fluke harvesters permits; 585 commercial striped bass harvesters permits; 79 party boat fluke fillet permits; 427 party/charter boat permits; and 323 shippers and dealers permits. New this year is a horseshoe crab harvesters permit which is associated with the resident and non-resident crab harvesters license. In addition, to the special permits, staff issued the following licenses: commercial lobster licenses (resident and non-resident); recreational lobster licenses; commercial crab licenses (resident and non-resident); commercial foodfish licenses (resident and non-resident) plus a variety of shellfish licenses and special vessel permits. In total, staff issued 9,010 licenses and 971 special permits for calendar year 2000. The above information is presented as an example of the numbers of licenses and permits issued and should be consider preliminary as a final verification has yet to be completed.

The major task associated with this project is the development of the computer programing necessary to consolidate all of the New York permits and licenses into one comprehensive permit that can be issued by computer. Limited progress was made during 1997 and early 1998 toward completion of the project. Significant progress was made during the last year of the three year project and the system is essentially complete. There are some minor corrections and final training to be completed. The contractor and department staff in our Albany Office of Information System Development unit have developed and refined the software packages to issue consolidated licenses. Staff assigned to the Bureau of Marine Resources have worked actively with the consultant and Albany staff to provide records and information regarding licenses and to review and test those products as produced.

At the end of the Grant Period, all hardware has been purchased and final development of the supporting software is complete. We have achieved our goal to issue one single consolidated renewal form which covers all licenses issued to an individual rather than send out multiple renewal forms. The new system also issues one single, credit card style (copy attached), license which would contain the individuals vital statistics and picture on one side and a list of valid licenses on the reverse side. Prior to development of this system, individuals carried a laminated license card for each license type they hold. Our customers, license holders, seem to be quite happy with the new license and licensing procedures afforded by this new system.

Marine Permit Office staff assisted with entering commercial striped bass harvest data and party/charter boat catch data onto computer files for subsequent reporting to National Marine Fisheries Service and ASMFC. The 1999 striped bass commercial landings data were reported to NMFS and both data sets were reported to ASMFC. In addition, temporary staff assisted with mailing summer flounder, black sea bass, and scup commercial trip limit notices to appropriate permit holders. This was done on at least ten occasions during the project period.

Support Bureau Staff in the Collection and Entry of Fisheries Independent Data for Fisheries Managed Under ASMFC Management Plans

Temporary staff were hired to assist with the fishery independent data collections related to ASMFC managed species. Specifically, one temporary staff person was hired to assist on the western Long Island juvenile striped bass survey. They assisted in all phases of the project from preparation of equipment, to field sampling, data entry, age analysis, and data QA/QC. This person also assisted when available on the ocean haul seine survey project and other unit activities. Finally, they assisted with the commercial fishery data entry and monitoring of the catch through market sampling. The results of this work are all reported separately under the grants supporting that work.

A second temporary staff person was hired to work in the permit office and assist field staff on occasion. This position remained with the permit office until a second full time employee was hired. At that point the temporary position was transferred to assist the lobster project biologist with fishery dependent data collections associated with the departments American Lobster investigations. They assisted with all phases of the project and were instrumental in successfully completing our sampling schedule for the project. Moreover, temporary staff assisted with the early stages of the 1999 western Long Island Sound American lobster die-off investigation. American lobsters were collected and shipped to various researchers investigating the potential causes of the die-off. This investigations is continuing. The results of this activity are reported separately under the grant supporting that work.

In addition, both temporary staff assisted with permit office functions as needed. For example they assisted in entering the backlog mail in renewal forms for 2000 licenses and assisted in mailings associated with quota managed species trip limit notices.

Provide Support for Law Enforcement to Refurbish Marine Law Enforcement Vessel.

Funds from this grant were assigned to the Division of Law Enforcement, (Region 1, Long Island; Region 2, New York City and Region 3 lower Hudson River and a part of Long Island Sound) to purchase several pieces of boat equipment, personnel safety items, and other items critical to Marine Law Enforcement. The items purchased will be utilized in the enforcement of New York and National Marine Fisheries Service fishing regulations. New York has a cooperative law enforcement agreement with the National Marine Fisheries Service to assist with federal enforcement activities.

During, 2000 New York's Environmental Conservation Police force was reorganized and a new Marine Enforcement unit was established. This Unit consists of six officers, four on Long Island and two in New York City. The unit will provide focused attention on Marine issues. They will be assisted by the Regional uniformed officers as needed to carry out Marine enforcement. The funds provided by this grant have been employed in Region 1 (Long Island), Region 2 (New York City) and Region 3 (Lower Hudson Valley and western Long Island Sound's north shore) to provide necessary materials for the new Marine Unit officers and other uniformed officers patrolling in the Marine District of New York.

Finally, NPS monies were assigned to the Environmental Conservation Law Enforcement unit to assist with upgrade their forty foot patrol boat. This work was completed during the second segment of the grant. That vessel is now back in service conducting essential Marine Fishery patrols around eastern Long Island and into Long Island Sound. This vessel has been used to conduct Lobster trap tag compliance patrols.

Provide For Fishery Management Planning and Implementation to Support Interstate Fishery Management Efforts.

This grant provided support to the department for Public Hearings held in conjunction with ASMFC and Federal FMP's or addendum's. Specifically, grant monies were utilized to support photocopying of documents, announcements and mailings of materials for the public hearing. Public Hearings related to Horseshoe crabs, American lobster, Winter Flounder, Summer Flounder, Tautog, Scup, Atlantic sturgeon and striped bass. Monies were used to support several mailings of information to all commercial license holders and to produce summary information about recreational laws, rules and regulations for interested parties, primarily recreational anglers.

Department staff and project staff supported by this grant entered Party/Charter boat vessel trip report data, American lobster landings data, blue crab landings data, and horseshoe crab landings data from reporting forms. The commercial landings data have been reported to NMFS and ASMFC as appropriate. The striped bass Party/Charter boat catch and harvest information for 1998 and 1999 have been reported to the ASMFC and is summarized below.

Four thousand four hundred and thirty five trips were reported on and utilized to estimate party/charter boat landings for May 8 to December 15, 1998. A total of 75,689 striped bass were reported caught. The reported harvest for 1998 was 34,242 fish which weighed an estimated 553,556 pounds. The 1998 MRFSS harvest estimate for the party/charter mode was 31,080 fish at 528,690 pounds, which is very similar to our trip reports. Other information gathered from the tripe reports are reported in New York's "Annual State Report to the Atlantic States Marine Fisheries Commission on New York's Harvest, Bycatch, and Fishery Independent Surveys for 1998.

Five thousand two hundred and sixty trip reports were received and used to estimate party/charter boat landings for May 8 - December 15, 1999. A total of 91,080 striped bass were reported caught. The reported harvest for 1999 was 41,030 fish which was estimated at 636,187 pounds. Weight was reported for approximately 24% of the harvest, which was used to estimate the total poundage caught. Mean weight of harvested fish was 15.5 pounds (Vecchio, personal communication). The 1999 MRFSS harvest estimate for the party/charter mode was 74,712 fish at 1,182,484 lbs, or a mean weight of 15.8 pounds, which is very similar to the mean weight from the NY Party/Charter trip reports. However, the catch rates do not match up as well for the 1999 fishery as they did for the 1998 fishery. No attempt was made to resolve these differences for this report. Staff working on the Annual Report to the Atlantic States Marine Fisheries Commission will examine this data and report their findings to the Commission.

Provide Support for Contractual Research covering By Catch Mortality in Marine Fish and Genetics of Long Island Sound Lobsters

A project was partially funded under this grant to examine summer flounder by catch mortality aboard party/charter boats in New York. Additional work was completed in the State of Virginia under separate funding. Results of the work are reported in the attached document entitled "Short-term Hooking Mortality of Summer Flounder in New York and Virginia" by Mark H. Malchoff and Jon Lucy.

The authors and numerous volunteers captured a total of 461 summer flounder ranging in size from 216 mm to 650 mm TL using sport fish tackle in New York and Virginia during the period of September 1997 to October 1998. Hook style, hook size, hook wound site, and presence or absence of bleeding was recorded for each capture event. Mean short-term mortality was estimated at 13.7%, with a 95% confidence interval of 10.6 - 16.7 % for the study.

A second study partially funded by this grant examined the genetics of Long Island Sound Lobsters. Staff collected 100 American lobsters Long Island Sound, removed one leg from each animal and shipped them to the University of Maine for analysis. All work was completed and a final report received after several attempts were made to secure the final report. It is our understanding that a draft scientific paper has been prepared. A copy of the journal paper has not been made available to us for this report. A copy of the journal paper will be forward, should we receive a copy. A copy of the final report entitled "Population genetics of the American lobster, *Homarus americanus*, in the Northwest Atlantic: assessment with microsatellite and mitochondrial markers" by Dr. Yan Kit Tam and Dr. Irv Kornfield is attached.

New York State
Marine Permit Certificate

VOID

Commissioner
NYSDEC

Id: 42839	DOB: 07/16/1941	Expires: 12/31/01
	PHILIP E KARLIN	
	833 WEST MIDDLE RD	
	RIVERHEAD	NY 11901-
	RIVERHEAD	
	Gender: M Ht: 5 - 7	Wt: 165
	Eyes: BL	Hair: BR

Signature of Applicant: Phil E. Karlin

Short-Term Hooking Mortality of Summer Flounder In New York and Virginia

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Interim Report for Cornell Univ/DEC Project MOU 000024,
and Virginia Institute Marine Science Virginia Marine Resource Report 98-7

December 9, 1998

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Abstract

Summer flounder support an important recreational fishery in the Mid-Atlantic region of the United States. State fishery management agencies have imposed size and creel limits in an attempt to reduce summer flounder fishing mortality, in conjunction with overall mortality reduction guidelines as specified in amendments to the Summer Flounder, Scup and Black Sea Bass FMP jointly authored by MAFMC and ASMFC. Despite these management measures, few data are available for the estimation of angling mortality following the catch and release of sub-legal fish. The authors and numerous volunteer anglers captured a total of 461 summer flounder (216 - 650mm TL) using sport-angling tackle in New York and Virginia during the period September 1997 to October 1998. All animals were caught with barbed hooks (sizes 2/0 to 4/0) using natural baits. Hook style, hook size, hook wound site, and presence or absence of bleeding were recorded for each capture event. Mean short-term mortality was estimated at 13.7 %, with a 95% confidence interval of 10.6% - 16.7%. The presence of bleeding associated with hook removal was a significant predictor of fish death. Mortality did not differ significantly among fish caught on any of three hook types (sproat, wide-gap, and circle). These results should allow for more accurate VPA-derived spawning stock biomass estimations for the summer flounder in the Mid-Atlantic region.

Introduction

Few data are available with which to calculate the post catch-and-release angling mortality in the summer flounder fishery (Lucy and Holton,1998). Information given here may be useful to the representatives of the Summer Flounder Monitoring Committee, ASMFC Summer Flounder Board, and the Demersal Species Committee of the MAFMC. The work presented here stems from a small grant (ACFMCA funds) awarded to the senior author and funding support both from VIMS and the Virginia Sea Grant Virginia Marine Advisory Program. Additional experimentation is planned for the spring and summer of 1999. A more in-depth report will be prepared following the conclusion of the 1999 field work.

Methods

The authors and numerous volunteer anglers conducted a total of 22 hooking mortality trials in 1997 and 1998. The sampling generated a total catch of 461 summer flounder (216-650mm TL) using sport-angling tackle aboard party/charter and research boats in New York and Virginia.

In New York, 147 fish were collected in the course of 4 trials during the period July through September 1997 aboard several open boats fishing out of Captree State Park, near Fire Island Inlet in Great South Bay. During the period September though October 1998, an additional 104 fish were collected during 6 trials aboard Captree vessels specifically chartered for hooking mortality research. In Virginia, 12 trials (210 fish) were completed during the period September though October 1998. The Virginia field work was done aboard small, open research vessels on the western and eastern shore sides of lower Chesapeake Bay (south of the Rappahannock River mouth (Gwynn Island) and the Cape Charles Harbor-Kiptopeke State Park areas, respectively).

Fish were caught on medium action spinning and bait casting rods using 12-17 pound test line. Natural baits (e.g. frozen or live minnows, sometimes in combination with squid strips or cut bait) were fished on conventional bottom rigs without skirts or spinners on leaders/hooks. Following capture, individual fish were placed in onboard 378 liter aerated tanks (NY) or 113 liter containers (VA). In the Virginia field work, dissolved oxygen levels were maintained by frequent water exchange. In both states, fish densities were kept at or below 0.4 lbs/gal during fishing trials, which lasted from 1 to 4 hours. Prior to being placed in holding tanks all fish were marked with T-Bar anchor tags (Floy® Tag & Manufacturing, Inc., Seattle, WA, USA and Hallprint Ltd., Holden Hill, South AUS).

Fish were caught on one of 6 barbed hook types and sizes: 2/0 sproat (Mustad©); 3/0 circle hook (Eagle Claw© L197 and Owner© Mutu Light); 4/0 circle hook (Eagle Claw© L197); 2/0 and 3/0 wide gap (Mustad©). For each capture event, hook type, hooking location (jaw, internal but anterior to pharynx, internal and posterior to pharynx), and presence or absence of severe bleeding were recorded.

At the conclusion of each angling session, all New York fish were transferred to a 3.5 m^3 holding cage constructed of PVC drain pipe and plastic mesh (Malchoff and Heins, 1997). The Virginia holding cages were smaller (0.6m x 2.4 m x 0.3 m) totaling 0.43 m^3, and were constructed of a PVC pipe floatation collar and 2.5 cm mesh galvanized wire with fine mesh screen wire covering the cage floor. Maximum cage stocking densities in the New York and Virginia trials were 8.7 and 10.4 fish /m^2, respectively. Shade was provided by use of fine mesh black plastic screen tops (Virginia trials) or by placement of the holding cage beneath a concrete Coast Guard station pier (New York trials). All fish were held for 3 d without food, at which point mortalities were enumerated, and survivors were released.

The 72 h holding period was chosen based on previous studies in both fresh and marine systems which document that most mortality occurs during this period either from hook wounding or as a result of physiological disruptions linked to stress (Warner and Johnson 1978; Beggs et al. 1980; Wood et al. 1983; Ferguson and Tufts 1992; Matlock et al. 1993). Following conclusion of the holding period all fish were recovered, measured and enumerated by condition (i.e., dead or alive).

Water temperatures and salinities at the holding sites were recorded at the beginning and end of each trial. Water temperatures averaged 21°C in NY in 1997, and 17 °C in 1998. Virginia water temperatures averaged 25°C (range: 21 to 28°C) during the 6 week project period. Salinity in the New York trials averaged about 26 ‰ (range 25-31‰) and 21.5‰ (range 17-26‰) at the Virginia sites.

Descriptive statistics were used to estimate mean survival. Bootstrapping (1000 samples, N=461) was used to calculate a 95% confidence interval from a cumulative binomial distribution. Calculated p values were ordered (lowest to highest), enabling selection of those corresponding to $p \leq 0.25$ and $p \geq 0.975$. Fisher's exact test was used to examine whether mortality differed between the six hook styles, and whether severe bleeding was a significant predictor of mortality. Statistical analyses were conducted with SYSTAT software (SYSTAT, Inc. 1997).

Results and discussion

Length distribution of the samples are given in table 1. The majority of the fish were below the established minimum size of 381mm (15 inches).

Table 1. Length distribution (by category and state) of summer flounder samples.

State	< 380 mm (15")	381-456 mm (15"-18")	> 457 mm (18")
NY	178	44	10
VA	191	16	3

Mean short-term mortality across all of the trials was estimated to be 13.7%, with a 95% confidence interval of 10.6% - 16.7%. This estimate was similar to that observed by Lucy and Holton (1998) in flow-through tank experiments and boat live wells during fishing trials (mean mortality of 11.2% [95% CI 3.0%-23.6%] and 8.1% [95% CI 4.5%-12.6%], respectively). The presence of bleeding associated with hook removal was a significant (Fisher exact test, $p<0.001$) predictor of fish death.

Similar analysis indicated that mortality was not significantly different between trials using wide gap hooks sizes 2/0 and 3/0 and between trials with Eagle Claw©, (model L197) 3/0 and 4/0 circle hooks (Fisher's exact, $p>0.99$, and $p>0.30$ respectively).

Based on these results, data were pooled from the original six size/style hook groups into three categories as follows; sproat, which included only size 2/0; wide gap, which included sizes 2/0 and 3/0; and circle hook, including sizes 3/0 and 4/0. These pooled data similarly indicated no difference in mortality rates between the three hook types (Pearson Chi-square, $p>0.63$). (*Note: subsequent logistic regression analysis of these pooled data, along with bleeding and hook position data will be reported elsewhere*) Observed mortality estimates for each of these three hook size/style groups are summarized in table 2.

Table 2. Observed mortality of summer flounder hooked in various sites using three hook types. Hook positions: 1= external (hook point visible); 2= internal, anterior to pharynx; 3= internal, posterior to pharynx; 4= foul hooked

	Sproat			Wide Gap			Circle Hook		
Hook Position	N	# Dead	Mortality %	N	# Dead	Mortality %	N	# Dead	Mortality %
1	111	5	4.5	95	10	10.5	174	13	7.4
2	21	4	19.0	7	2	28.5	6	2	33.3
3	10	8	80.0	10	6	60.0	23	13	56.5
4	0	0	0.0	0	0	0.0	4	0	0.0
Totals	142	17	12.0	112	18	16.1	207	28	13.5

Acknowledgments

The assistance of numerous individuals was instrumental in the work reported here. For the New York trials, the authors are especially appreciative of the efforts of Chief Warrant Officer Michael Tollefson, USCG Station Fire Island; John Mantione, Pres. of the New York Fishing Tackle Trade Association, and captains and crew of the *Cap'n Andy, Lois Ann, Yankee III, Captree Queen, and Island Princess.* For the Virginia trials, appreciation goes to Mr. Wayne Pulley of Pulley's Marine, Gwynn Island, VA and Ms Laura Lohse, Town of Cape Charles Harbormaster, for dockage and live cage mooring space. Likewise, Ranger Dave Sommers, Kiptopeke State Park, provided convenient cage mooring space.

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Population genetics of the American lobster, *Homarus americanus*, in the Northwest Atlantic: assessment with microsatellite and mitochondrial markers.

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Population genetics of the American lobster, *Homarus americanus*, in the Northwest Atlantic: assessment with microsatellite and mitochondrial markers.

Abstract: Genetic variation at four hypervariable microsatellite loci suggests that the American lobster, *Homarus americanus*, may not be composed of a single panmictic population in the Northwest Atlantic Ocean. Samples included Long Island Sound, inshore Gulf of Maine, Georges Bank, Gulf of St. Lawrence, and off of Newfoundland. Microsatellite loci demonstrated extensive polymorphism in all samples of lobsters with expected heterozygosity ranging from 0.484 to 0.953. Null alleles were found at three microsatellite loci and were included in data analysis. Among populations, significant heterogeneity in allelic composition was observed for three of the four microsatellite loci. In contrast to the microsatellite loci, a polymorphic mitochondrial DNA marker showed no heterogeneity among populations. The results of these genetic characterizations suggest that the inshore and off-shore populations of lobster in the Gulf of Maine are differentiated and may merit management as separate units. By contrast, the analyses suggest that the population sample of lobster from Long Island Sound are not differentiated from those of adjacent coastal areas. However, the genetic heterogeneity detected by microsatellite markers in this study needed to be addressed with caution ; confirmation using additional markers and samples is prudent.

Introduction

The American lobster, *Homarus americanus*, has been one of the most important commercial fishery species along the northeast coast of United States and the east coast of Canada since the mid-1800's (Fogarty 1995). Within the Gulf of Maine, the inshore lobster population has been exploited intensively for many years; advanced fishing techniques and navigation aids have also intensified the offshore fisheries in recent years for lobsters found in regions with depths greater than 100 m (Miller 1995). Concerns have been raised about the impact of increasing fishing pressure on offshore lobster populations relative to recruitment in the inshore lobster fishery. It is important to understand the genetic relationships among lobsters from the inshore and offshore areas within the Gulf of Maine, as well as those in nearby regions, so that an effective management scheme for the American lobster fishery can be proposed.

Various methods have been applied to detect population discreteness of lobsters, particularly between inshore and offshore populations in the Gulf of Maine, southern New England areas, and the Gulf of St. Lawrence. There is conflicting evidence about population substructuring within the Gulf of Maine (Pezzack 1987), although several studies have suggested a difference between lobsters in the Gulf of Maine and the Gulf of St. Lawrence (Harding et al. 1993).

Tracey et al. (1975) applied isozyme electrophoresis to investigate genetic differences among lobster populations in the Gulf of Maine and nearby regions and observed very low level of variability (see Shaklee 1983). Kornfield and Moran (1989) used restriction enzyme analysis of mitochondrial DNA to examine variation within and among populations of American lobster collected from the Gulf of Maine, Gulf of St. Lawrence, and Atlantic slope off Delaware. They found variability within populations and suggested that a potential difference exists among samples from the Gulf of Maine and the Gulf of St. Lawrence. Recently, Harding et al. (1997) explored the nuclear genome by using random amplified polymorphic DNAs (RAPDs) to study genetic relationships among lobsters from the southern Gulf of St. Lawrence, southwestern Nova Scotia, and Georges Bank. Similar to the results of previous studies, no significant genetic substructuring of American lobsters was found. For further genetic studies, more variable, and thus presumably more sensitive markers, are needed.

Microsatellites (or simple sequence repeats) are a class of molecular markers that are abundant and widely distributed throughout eukaryotic genomes (Tautz and Renz 1984). Their ease of isolation, extensive polymorphism, and Mendelian inheritance make them attractive for population and kinship studies (Bruford and Wayne 1993; Queller et al. 1993; Jarne and Lagoda 1996). The development of microsatellite markers has proceeded for a wide variety of animals; O'Reilly and Wright (1995) reviewed the application of microsatellite markers to fisheries and aquaculture. Tam and Kornfield (1996) developed and characterized three microsatellite markers from American lobsters and demonstrated the potential usefulness of these markers for population genetic study.

The objective of the present study was to apply molecular markers to test for population subdivision (1) between inshore (Boothbay Harbor) and offshore (Georges Bank) regions within the Gulf of Maine, and (2) among populations collected throughout the Northwest Atlantic including Gulf of Maine, Long Island Sound, Gulf of St. Lawrence, and Newfoundland. Two classes of genetic markers were employed. First, four microsatellite marker loci were examined. Second, variable DNA sequences within the mitochondrial genome were identified and an informative restriction site was used to examine frequencies of two distinctive mitochondrial lineages (Kornfield and Moran 1989).

Materials and methods

Sample collection

Sampling locations (Fig. 1) include Long Island Sound (inshore, New England), Boothbay Harbor (inshore, Gulf of Maine), Georges Bank (offshore, Gulf of Maine). Prince Edward Island (southern Gulf of St. Lawrence) and Smith Sound (Newfoundland). Sample sizes, abbreviations, dates and locations of collection for the studied populations are presented in Table 1. From each lobster, a walking leg was amputated at the merus-ischium joint; samples from Boothbay Harbor were kept frozen at -80°C until analysis, while all other samples were preserved in 95% ethanol. Lobsters were released after tissues were collected.

DNA extraction

For Boothbay Harbor lobsters, a small block of leg muscle (~ 5 mm^3) was placed in 500μl digestion buffer (10mM Tris-HCl pH8.0, 10mM NaCl, 2mM EDTA, 0.1mg/ml DTT, 1% SDS, 1mg/ml proteinase K) and incubated at 65EC, with gentle agitation, for 2 hours or until the muscle was completely digested. The extract was then purified by phenol/chloroform extraction (Ausubel et al. 1989) and precipitated in 2 volumes of cold absolute ethanol.

For Long Island Sound, Georges Bank, Gulf of St. Lawrence, and Newfoundland lobsters, the Chelex extraction method (Walsh et al. 1991) was used to obtain DNA from leg muscle. Muscle was crushed in 50μl of 5% aqueous Chelex and then incubated at 100°C for 25 minutes; the supernatant was then used as sample for polymerase chain reaction (PCR).

Microsatellite amplification and data analysis

Three microsatellite loci (Hom3-2, Hom7-1, Hom8-1) developed by Tam and Kornfield (1996) and a fourth locus (Hom6-2) were used in the present study. For PCR, one of the two primer oligonucleotides was labeled with γ-^{32}P-dATP. The optimal PCR conditions for Hom3-2, Hom6-2 and Hom8-1 are 30 cycles of 30 s at 94°C, 45 s at 55°C, and 60 s at 72°C. Optimal annealing temperature for Hom7-1 is 50°C. The PCR reaction mixtures were heated at 94°C for 5 min in the initial cycle, and the extension step at 72°C lasted for 5 min during the last cycle; pH of the reaction buffer was 9.5. The amplified products were then electrophoresed through a 6% denaturing polyacrylamide gel and exposed to X-ray film for 12 hours or more at -80°C with one intensifying screen.

The size of each allele was determined by comparison to a standard M13 DNA sequence ladder on each gel. Genotypic data were tested for agreement with Hardy-Weinberg equilibrium expectations and expected heterozygosity (under the assumption of Hardy-Weinberg equilibrium), using the program GENEPOP V.3.1 (Raymond and Rousset 1995). Measures of population substructuring, F_{ST} and R_{ST}, were also calculated by the same program. Significance of the F-statistics was evaluated by using FSTAT (Goudet 1995). F_{ST} (Wright 1951) is commonly applied in population genetics to measure population differentiation. R_{ST}, which is an analog of F_{ST} (Slatkin 1995), is specifically designed for microsatellite markers such that variance of allele size is incorporated into the calculation; hence, R_{ST} should be more informative than F_{ST}. Both F_{ST} and R_{ST} estimate the proportion of genetic variation among subpopulations relative to the total variation among populations. However, Ruzzante (1998) demonstrated that R_{ST} is sensitive to unequal sample size when it is estimated as a ratio of variances, while F_{ST} estimates appear to be less affected.

Effective number of alleles was calculated after Kimura and Crow (1964). Heterozygote deficiencies (D values) were calculated as defined by Selander (1970). Estimation of null allele frequencies followed Brookfield (1996). This method estimates null-allele frequency by a maximum-likelihood approach; depending on whether null-null homozygotes can be identified with confidence, there are appropriate equations to estimate null-allele frequency based on expected heterozygosity, observed heterozygosity and the number of null-null homozygote individuals in the samples. Null alleles were included in the data analysis; allele and genotypic frequencies were adjusted using the estimated null allele frequencies. Homogeneity of allele composition between populations was examined using the randomization test in the program CHIRXC (Zaykin and Pudovkin 1993) and the Markov chain method implemented in the program GENEPOP V.3.1. Probabilities were adjusted for multiple comparisons by the Bonferroni procedure (Rice 1989). Estimates of the number of migrants between locations using the private allele method (Barton and Slatkin 1986) were also calculated by GENEPOP V.3.1.

Characterization of informative restriction site in the mitochondrial genome

From a mtDNA transfer RNA methionine gene sequence (t-RNA^{Met}) of *Homarus americanus* (GenBank Accession U29718), a primer (Lobmet 5' ATACCCCGTTTATGAGAGCA 3') was designed using the program PRIMER (Lincoln et al. 1991). Boore et al. (1995) demonstrated that ND2 is located next (3') to the methionine gene in the mitochondrial genome. However, PCR using Lobmet as the only primer amplified a 450 base pair fragment of lobster mtDNA covering partial methionine and ND2 genes. The lobster ND2 gene thus contains an inverted portion of (t-RNA^{Met}) sequence; translocation of functional t-RNA genes are not uncommon in mtDNAs (Cantatore et al. 1987). The amplified segment was cloned using the pGEM-T vector system (Promega) and sequenced using the dideoxy termination method (Sanger et al. 1977). Eight individuals from the alpha and beta mitochondrial lineages identified in Kornfield and Moran (1989) were sequenced. A single mutation (Fig. 2) was found to be informative for separating the two haplotypes. This point mutation was present within the recognition sequence of the restriction enzyme *Xcm*I. *Xcm*I recognizes the restriction site (CAAN$_5$N$_4$TGG) in one lineage but not in the other (CAAN$_5$N$_4$CGG) because of a transitional substitution. The two lobster lineages could be easily distinguished by combining PCR using Lobmet followed by *Xcm*I digestion of amplified products. 10µl of PCR products were mixed with 10µl digestion mixture (2µl 10X NEB buffer #2, 7µl dH$_2$O, 1 µl 1U/µl *Xcm*I [New England Biolabs]) and incubated in a 37°C water bath for at least 5 hours. Digested PCR products were then separated by agarose (2% Low EEO, Fisher Scientific) gel electrophoresis and visualized with ethidium bromide.

Restriction site data analysis

All Georges Bank samples (73 individuals) and about 100 individuals from each of the other four populations were examined. Individuals were assigned either type alpha (*Xcm*I site present) or type beta (*Xcm*I site absent). The number of each haplotype in each population was recorded. To compare the homogeneity of haplotype frequencies between populations, Fisher's exact test was performed using the program CHIRXC (Zaykin and Pudovkin 1993).

Results

Significant overall heterogeneity in allele composition among all populations was found for three of the four studied microsatellite loci. However, no among populations heterogeneity was observed in the frequencies of mitochondrial haplotypes.

Variation of microsatellite markers

All four microsatellite loci amplified equally well in both phenol-extracted and chelex-extracted samples. The basic statistics for each microsatellite locus in each population are presented in Table 2. While populations shared most of the alleles at all microsatellite locus, unique alleles were observed in each population. Hom7-1 is the most variable locus, with 69 alleles observed in total. Hom3-2 and Hom6-2 which are also highly variable microsatellite loci with 39 and 46 observed alleles respectively, showed differential intensity of amplified products for some samples. Generally, the small-size allele of a heterozygote was stronger in signal than the larger one, although in most cases this did not affect gel scoring. Hom8-1, which has 14 observed alleles, is the least variable locus among the four loci in this study; it showed multiple (artifact) bands which could be consistently identified and distinguished from the true allelic bands on autoradiographs. Like Hom3-2 and Hom6-2, locus Hom8-1 also showed differential intensity of the two alleles in some heterozygous individuals.

All four microsatellite loci are highly polymorphic in all populations. Expected heterozygosity for Hom3-2, Hom6-2 and Hom7-1 are above 90% in all populations, while it is above 48% for Hom8-1 (Table 2). Hom7-1, the most variable locus, agreed both with Hardy-Weinberg equilibrium (HWE) expectations and the expected abundance of heterozygotes under the assumption of HWE in all populations. For Hom3-2, both Gulf of St. Lawrence and Georges Bank populations showed significant deficiencies of heterozygotes. The Gulf of St. Lawrence population also deviated from HWE. Three populations showed both significant deviations from HWE and deficiencies of heterozygotes at Hom6-2, including Boothbay Harbor, Gulf of St.

Lawrence and Newfoundland. Hom8-1 showed HWE in all populations except Georges Bank. All samples except the Gulf of St. Lawrence population showed significant deficiencies of heterozygotes. Estimations of null allele frequencies are presented in Table 3.

Allele frequency distribution

The allele frequency distribution for each locus is shown in Figs. 3 - 6. Hom3-2, Hom6-2 and Hom7-1 showed multimodal distributions with several alleles occurring in relatively high frequencies. Hom8-1 had a unimodal distribution with a common allele of frequency greater than 50% in all samples. All four loci showed discontinuous distributions in allele size, particularly Hom8-1 which has three gaps each with more than 60 base pairs (bp). Alleles with only one bp difference were observed in Hom7-1. The allele-frequency distributions in each of the four loci appear very similar across populations. No differences in allele distributions were observed between male and female lobsters or between small-size and large-size lobsters within a sampling area (data not shown).

Population comparisons

When all populations were compared together, Hom3-2, Hom6-2 and Hom8-1 all showed highly significant heterogeneity among populations. Data analysis was conducted with and without the inclusion of null alleles, but both analyses gave similar results. The inclusion of null alleles strengthened the heterogeneity between populations in several cases, particularly at locus Hom8-1. The results of randomization tests for homogeneity using the program ZAYKIN were the same as the homogeneity tests using Markov chain resampling method in GENEPOP V.3.1. (Table 4). For both Hom3-2 and Hom7-1, there is no significant difference in any pairwise comparison of allele composition among populations. For Hom6-2, after Bonferroni correction for multiple comparisons, significant difference in allele compositions were found between Georges Bank and other populations except the Gulf of St. Lawrence samples, and also between Boothbay Harbor and Newfoundland samples. For Hom8-1, Boothbay Harbor population showed significance differences with all other populations; Georges Bank also showed differences with all other samples except the Newfoundland population. Thus in a general sense, the Georges Bank sample appeared somewhat distinctive.

Table 5 presents the F_{ST} and R_{ST} estimates for each pairwise population comparison over all loci. R_{ST} estimates are more variable and, in general, greater than the corresponding F_{ST} estimates. In most cases, the estimates are less than 0.01 which indicates negligible genetic differentiation between populations. Table 5 also shows the probability that F_{ST}, F_{IS} and F_{IT} differed from 0. None of the F_{IS} and F_{IT} values was significant, but the F_{ST} values were found to be significant in several comparisons, mostly those including Boothbay Harbor, Georges Bank and Newfoundland populations. The regression of $F_{ST}/(1-F_{ST})$ and $R_{ST}/(1/R_{ST})$ for pairs of studied populations on the logarithm of distance between populations (Rousset 1997) indicated no significant relationship between the estimates of divergence and distance (Fig. 7). Table 6 shows the estimated number of migrants between populations per generation.

Restriction enzyme analysis

Partial sequences of the ND2 region of the mitochondrial genome revealed variability within American lobster populations (Fig. 2). *Xcm*I could clearly distinguish the two lineages. The relative abundance of these lineages in populations are presented in Table 7; Table 8 presents the results of the Fisher's Exact Tests for homogeneity between populations. There was no significant heterogeneity among all populations when considered together ($p = 0.083$). Boothbay Harbor samples showed differences in haplotype proportions from both the Gulf of St. Lawrence and Newfoundland samples, but these differences are not significant after Bonferroni correction.

Discussion

The two types of molecular markers applied in the present study gave conflicting results. While mitochondrial marker demonstrated no genetic heterogeneity among locations, microsatellite markers demonstrated subtle but significant genetic heterogeneity among American lobsters collected over a broad region in the Northwest Atlantic, including Long Island Sound, inshore Gulf of Maine (Boothbay Harbor), offshore Gulf of Maine (Georges Bank), southern Gulf of St. Lawrence (Prince Edward Island), and Newfoundland (Smith Sound). This latter finding is intriguing but needed to be taken with caution. This finding contradicts the results of previous studies (Harding et al. 1997; Kornfield and Moran 1989) which suggested no significant genetic differentiation among lobster populations in the Gulf of Maine and nearby regions. Earlier allozyme studies by Tracey et al. (1975), while suggesting differentiation among some localities, have been disputed on methodological grounds (Shaklee 1983) and could be critiqued from the perspective invoking natural selection (Avise 1994). Compared to other molecular markers, the four microsatellite loci used in the present study are extremely variable and thus should be highly sensitive to population dynamic process like founder effects and genetic drift. The results from the present study can contribute to the understanding of genetic relationships among lobster populations and may therefore have implications for the management of the American lobster fishery in the Northwest Atlantic. For example, the genetic relationships between inshore and offshore lobster populations in the Gulf of Maine have been raised and have remained an unresolved issue since the late 1960s (Saila and Flowers 1969; Tracey et al. 1975; Pezzack 1987).

Microsatellite polymorphism

The extremely polymorphic nature of the microsatellite markers in the present study (heterozygosity ranges from 0.354 to 0.976 and number of alleles from 14 to 69) makes the markers highly informative in genetic studies. One potential disadvantage in employing such highly variable markers is the requirement of extremely large sample sizes necessary to adequately sample the genotypes present in the populations. The sample size of about 100 individuals is too small to capture all of the possible genotypes in Hom3-2, Hom6-1 and Hom7-1. However, it is not necessary to examine all possible genotypes since populations can be compared based on the frequencies of the relatively common alleles in the gene pools. On the other hand, small sample size may limit the ability to detect private alleles which exist in a single population only. For such variable loci, the smaller the sample size, the higher the chance of observing differences between populations simply because of sampling heterogeneity. However, based on 100 individuals, if homogeneity is found between populations, it is likely that increasing the sample size would continue to demonstrate homogeneity.

Tam and Kornfield (1996) demonstrated by inheritance analysis that null alleles exist at both Hom3-2 and Hom8-1. From the data of the present study (Tables 2 and 3), Hom6-2 also appears to possess null alleles. Null alleles are alleles that could not be amplified because of mutation(s) in the primer sites necessary for PCR (Pemberton et al. 1995). Depending on the frequencies of null alleles in the samples, they may cause deviations from Hardy-Weinberg equilibrium expectations and/or deficiencies of heterozygotes; underestimating or ignoring null-allele frequency may mislead data analysis and subsequent inferences. The frequencies of null alleles were generally less than 5% in most of the populations, but null allele frequencies over 10% were observed for Hom6-2 in Boothbay Harbor and for Hom8-1 in Georges Bank (Table 3). No significant deficit of heterozygotes was found in Hom7-1 in any population and the estimation of null allele frequency in this locus is about 1% or less (Table 3). One approach to investigate the actual proportion of null alleles in the gene pool is to examine the genotypes of ovigerous females and their offsprings. Since eggs are attached to the female's abdomen for a prolonged period during their development, mother-offspring comparisons can reveal the presence of parental null alleles. Thus, of a large number of females are examined, accurate estimates of null alleles frequencies may be obtained. Alternatively, new primers for the PCR reactions could be designed such that mutated priming sites are avoided.

In order to examine the possibility that the heterozygote deficiencies observed in the samples are the results of population substructuring within a location, genetic data has been divided according to either sex or size classes and tested for heterogeneity within a single population. These tests have been done for samples collected from Boothbay Harbor, Newfoundland, and Long Island Sound (data not shown). No genetic heterogeneity was observed between male and female lobsters, and between small and large size lobsters in these regions. Therefore, a temporal stability in allele frequencies was demonstrated.

Tests of genetic homogeneity among population gave similar results whether null alleles were included or not. When all populations were considered together and tested for genetic heterogeneity, three (Hom3-2, Hom6-2, Hom8-1) of the markers demonstrated significant differences ($p = 0.002, <0.001, <0.001$, respectively). However, the most variable marker (HOM7-1) showed genetic homogeneity among regions. Since sample size varied among populations and some microsatellite markers demonstrated a lack of Hardy-Weinberg equilibrium and a deficiency of homozygotes in some populations, the comparison of all populations needed to be approached with caution. Exhaustive, a posteriori test of homogeneity indicated that no differences in the allele compositions of Hom3-2 exist between any pair of populations despite overall significant heterogeneity at this locus (Table 4). In contrast, for both Hom6-2 and Hom8-1, significant differences in allele compositions were found for both Boothbay Harbor and Georges Bank samples when compared with most of the other populations. The inclusion of null alleles in these latter comparisons only strengthened the differences in these two areas (and others). The result for Hom3-2 indicated that there are subtle differences in the genetic makeup of individual populations although these small differences are not individually distinguishable.

The relatively variable R_{ST} values in Table 5a are most likely affected by the differences in sample size between populations. For the present study, F_{ST} may be a more appropriate measure than R_{ST} because of the relatively short period of potential population separation (perhaps 2,300 generations since glaciation); this period may not be sufficient for many new mutations to accumulate with high frequencies in the gene pool, particularly with the large population sizes of lobsters throughout their observed range. Table 5b shows that when considering all loci in pairwise population comparisons, none of the F_{IS} or F_{IT} values is significantly different from zero. This implies that the F_{ST} values which are significantly greater than zero reflect real differentiation between populations, even though individual F_{ST} values in Table 5a are less than 0.01. These F-statistics reflect the same conclusion as genetic heterogeneity tests: subtle, but detectable genetic differences are present among the studied lobster populations. The lack of a significant linear relationship between the extent of genetic differentiation and geographic distance (Fig. 7) demonstrates the absence of isolation by distance and may indicate that all studied populations may have very limited gene flow with each other. The small number of migrants relative to the large population size in each region (Table 6) also suggests that gene flow through migration between locations is very limited. However, these numbers needed to be treated with caution as the sample sizes in the present study may not allow us to visualize all private alleles in each location. Genetic distance was not estimated in the present study because it has been demonstrated that genetic distance is more susceptible to small sample size and it is less sensitive than F_{ST} and R_{ST} when microsatellite markers are used in population studies (Ruzzante 1998).

Mitochondrial polymorphism

In molecular studies, it is particularly prudent to examine a hypothesis with more than one data set to see if results are concordant. The mitochondrial genome provides information independent from that of nuclear loci such as microsatellites. Two distinct lineages of mitochondrial DNA found by Kornfield and Moran (1989) in American lobsters were not significantly different in relative frequencies between Gulf of Maine and Gulf of St. Lawrence populations. However, frequency homogeneity could be more accurately assessed if the sample size of the original study were larger. In the present study, a restriction site with the mitochondrial ND2 gene provided an efficient way to study more samples from different locations.

Kornfield and Moran (1989) suggested that the two lineages (about 1% sequence divergence) originated well before invasion of the Gulf of Maine and the Gulf of St. Lawrence following glacial withdrawal approximately 14,000 years ago. Since the mitochondrial genome is haploid and maternally inherited in lobsters, the effective population size of markers on the mitochondrial genome is one fourth of the nuclear genome; it should thus be more sensitive than the nuclear genome to population dynamic processes such as gene flow and genetic drift (Avise 1994; Ward and Grewe 1995). Further, it is unlikely that two or more founding mtDNA lineages will survive by chance beyond 4N generations, where N is the effective population size (Avise 1994). If the founding populations in the Gulf of Maine and in the Gulf of St. Lawrence were small and no gene flow occurred between the two areas since invasion of the two regions, the populations would respond to genetic drift independently, and thus might now differ in the proportions of mtDNA haplotypes. In contrast to the results with microsatellite markers, homogeneous frequency distributions of the two mitochondrial lineages were found among all sampling locations. This suggests that (1) there has been gene flow among these areas and/or (2) that isolation has not been long enough to complete the lineage-sorting process, implying that the initial population sizes in these regions may have been large and contained representatives of many mtDNA lineages. In view of the results from the microsatellite markers in this study, the latter hypothesis is appealing.

Comparison with previous studies

Harding et al. (1993) used morphological characteristics of the first larval stage of American lobsters to separate populations from the Gulf of St. Lawrence as distinct from lobsters in the Gulf of Maine and the Atlantic coast of Nova Scotia. They also reviewed earlier studies which all suggested a difference between lobsters found in the Gulf of St. Lawrence and in the Gulf of Maine. The distinction between the two areas noted by Harding et al. (1993) may or may not be genetic, as it could have originated from ecological, physiological, or environmental (fishing) factors. Microsatellite analyses revealed significant heterogeneity between Gulf of St. Lawrence and Gulf of Maine (both Boothbay Harbor and Georges Bank) for one out of four tested loci:

Hom8-1 (Table 4). These results are consistent with the idea that there is some genetic isolation between these two regions.

Harding et al. (1993) also recognized that one-way introductions of lobsters from the Gulf of St. Lawrence into the Gulf of Maine have occurred. In fact, an introduction of lobsters from the Gulf of St. Lawrence to Boothbay Harbor, Maine, took place in May, 1995 (J. Krouse, personal communication), six months before samples were collected from the Boothbay Harbor region for the present study. There is thus the possibility that samples collected around Boothbay Harbor may include transplanted lobsters. While we cannot exclude the possibility that our Boothbay Harbor sample includes transplanted lobsters, we find this idea unappealing given the very large natural population size in the region. Allele frequency heterogeneity between Gulf of St. Lawrence and the Gulf of Maine populations is probably not sufficiently large to generate a Wahlund Effect (Hartl and Clark 1989) even with extensive mixing. Regardless, we cannot exclude the possibility that long-term transplantation activities from the Gulf of St. Lawrence have not had an impact on the gene pool of lobsters in the Gulf of Maine and southern New England area in general. Harding et al. (1997) provided an enlightening description of transplantation activities since the turn of the twentieth century.

Common alleles shared among populations are indistinguishable when introduced by dispersal or by transplantation activities. Private alleles, those unique to a single population, could be used to track introductions if they occurred in high frequencies in the donor population. If patterns of gene flow were unidirectional, it would be expected that source populations should display lower levels of genetic diversity than the recipient populations. More explicitly, since lobster larval dispersal is unidirectional from the Gulf of St. Lawrence to the Gulf of Maine, we may expect to observe fewer unique alleles in that upstream population. No such pattern was found in this study. The lobster fishery in the Gulf of Maine has been strong and stable in recent decades (Fogarty 1995); the effective population size of lobsters in the Gulf of Maine is in the millions. The genetic composition of such a large population, in principle, should not be altered by limited transplantation of lobsters from other regions. Regardless, more extensive investigations should be conducted on genetic impact of lobster transplantation activities.

The early life history of *H. americanus* includes three larval stages and one postlarval stage, all of which are planktonic (Factor 1995b). These planktonic stages, which usually last four to six weeks, may serve as a dispersal phase in the life history of American lobsters. Katz et al. (1994) used a simulation model to suggest that later active and directional swimming behavior of the postlarvae may also play an important role in dispersal. While larval dispersal could be one of the mechanisms that link lobster populations, movements of adult lobsters may also facilitate genetic exchange over board areas. Many studies have demonstrated long-distance (>100 km) migration of adult lobsters (Krouse 1980; Campbell et al. 1984; Pezzack and Duggan 1986; Campbell 1989). Considering the dispersal potential of larvae and the migration ability of adult lobsters, it would be expected that American lobsters within the Gulf of Maine and in the nearby areas would be genetically homogeneous. However, the present study demonstrated that the number of migrants among locations is limited when compared to the wild population size (Table 6). This small number of migrants probably could not result in homogenization of private alleles which exist only in a particular region. Furthermore, this limited number of migrants also challenges the idea that larval dispersal may serve as an effective way to homogenize the gene pool of American lobsters. Unseen barriers such as physiological stress, unsuitable habitats, or the availability of food may prevent massive long-distance dispersal and, in particular, successful subsequent gene flow.

Most recently, Harding et al. (1997) studied RAPDs to evaluate genetic relationships among American lobster populations from the southern Gulf of St. Lawrence, southwestern Nova Scotia, and Georges Bank. No significant differences in the frequencies of RAPD bands were found among all samples after Bonferroni corrections (Rice 1989) were applied. Thus, unlike our findings, the results from RAPD analysis suggest gene flow between lobster populations in the Gulf of St. Lawrence and the Gulf of Maine. From their data, Harding et al. (1997) estimated that the magnitude of differentiation between these two regions could reflect movement of less than 5 migrants per generation. This number is even smaller than those estimated with microsatellites (Table 6). While the present study demonstrated subtle but significant genetic differences among lobster populations, these findings may have resulted from the use of microsatellite markers which have a different form of mutation and faster mutation rate than other nuclear genes. It is possible that the application of a very large number of informative RAPD markers may detect significant genetic differences among lobster populations.

Hypotheses for population differentiation

When considering all microsatellite loci in the present study, Georges Bank has significant F_{ST} values with all other populations except Long Island Sound (Table 5b); the Boothbay Harbor population has significant F_{ST} values with both Georges Bank and Newfoundland populations; Newfoundland has significant F_{ST} values with all other populations except the Gulf of St. Lawrence. The lobster populations in the Northwest Atlantic thus seem to be subdivided into several local populations with somewhat restricted gene flow. The results of the present study support the idea that lobsters in the inshore and offshore regions within the Gulf of Maine belong to different populations and that limited migration (i.e. gene flow) occurs each generation. While lobsters in Newfoundland should also be regarded as a distinctive population from others, no detectable differences were found between Newfoundland and the Gulf of St. Lawrence populations. In view of this latter observation, we may predict that there would be no genetic substructuring of lobster population within the Gulf of St. Lawrence. Multiple locations within Gulf of St. Lawrence should be sampled and studied to challenge this prediction. Lobsters in Long Island Sound and southern Gulf of St. Lawrence share genetic compositions with most of the other studied populations; Long Island Sound lobsters do not appear to represent a distinct population.

Several hypotheses are available to explain significant genetic structuring of American lobsters observed in this study, including: (1) differential selection pressure on the genetic markers, (2) the lack of sufficient time to accumulate and stabilize new mutants, and (3) founder effects.

The first hypothesis is unlikely because microsatellite markers are generally believed to be selectively neutral (Queller et al. 1993). Changes in the frequency of alleles among populations would be expected if populations experienced different environments, and if microsatellite alleles responded differentially to these environments. It is certainly the case that the populations sampled here have distinct local fishing histories; differences in gear, catch sizes, fishing seasons, etc. have generated different demographic effects. For examples, offshore lobsters on Georges Bank, which are generally much larger than those inshore in the Gulf of Maine, are probably composed of multiple cohorts, many of them much older than those inshore. These older, mixed populations have experienced many more environments than the relatively uniform inshore populations. However, since these environmental differences do not directly influence the relative fitness of microsatellite alleles, they cannot alter the frequencies of alleles among population. If a neutral marker were linked to a gene which were under strong selective pressure in a population, the allele frequency distribution of the marker in the population would become distinct from other populations. However, in the current study, the genetic differences detected among populations involved multiple loci, rather than a single locus. Thus selection pressure is an extremely unlikely explanation for the observed heterogeneity.

The second hypothesis concerns the time since separation of populations and rate of mutation at microsatellite loci. Lobsters invaded the Gulf of St. Lawrence - Gulf of Maine region with glacial withdrawal about 14,000 years before present, or approximately 2,300 generations ago. Even though microsatellites are known to have a very high mutation rates, i.e. 10^{-2} to 10^{-5} in humans (Jarne and Lagoda 1996), mutation is still a stochastic event and new mutations can only increase in frequency very slowly. It is therefore not surprising to see that all populations share most of the common alleles at each locus while only a limited number of private alleles were found in each population. The slight genetic heterogeneity detected among populations could be due to the differences in distribution of private alleles. Private alleles may originate from new mutations accumulated after isolation of populations, or may be present initially in a restricted gene pool immediately after isolation. The former scenario seems unlikely given the relatively short time since glacial withdrawal. Regardless, significant overall heterogeneity was observed even when private alleles were excluded from the analysis.

The final hypothesis concerns founder effects. When lobster populations became fragmented after the invasion of the Gulf of Maine, Gulf of St. Lawrence, and other regions following glacier withdrawal, the extent of changes in genetic makeup among locations depended on the founding population sizes and the associate spectrum of genetic variations they retained. The very large population sizes of lobsters currently observed may be due to the expansion of individual founding populations in each location. If there is rapid population growth immediately following the founding event, private alleles in the initial gene pool of each population may be retained despite low initial frequencies. Thus, the subtle genetic heterogeneity now observed among lobster populations may reflect the allelic compositions of the founding populations prior to population expansion; this may imply that the ancestral population encompassed an unusually rich diversity of allelic variants. Given the relatively short time of separation among, and the small number of private alleles detected within each population, this last hypothesis seems to be more compelling than the others.

Stock concept and lobster fishery management

Effective management of the fishery relies on an accurate understanding of the biology of the species, the response of the species to commercial exploitation, and the relationships and interactions among regional subpopulations. Numerous aspects of the biology of *Homarus americanus* have been studied comprehensively (Cobb and Phillips 1980a, 1980b; Factor 1995a). Fogarty (1995) and Miller (1995) provide detailed reviews of lobster fishery management and regulations in both United States and Canada. Reviewing evidence from tagging, morphometrics studies, electrophoretic studies, fishery landing trends, and differences in demographic parameters of lobsters in different regions, Ennis (1986) suggested the existence of different stocks, although the precise boundaries of these stocks could not be defined. While morphometric (Harding et al.

1993) and demographic differences (Campbell and Robinson 1983) may reveal regional heterogeneity, fishery landing trends (Campbell and Mohn 1983) may reflect the ultimate regional response to all biotic and abiotic factors. The microsatellite markers in the present study demonstrated that genetic heterogeneity exists among lobster populations between (1) inshore and offshore regions within the Gulf of Maine; and (2) the Gulf of Maine and nearby regions. However, mitochondrial marker in the study demonstrated no heterogeneity among populations. Given that sample size varied among regions and not all microsatellite markers demonstrated heterogeneity among populations, the results from microsatellite needed to be taken with caution. The subtle heterogeneity being detected may be apparent or transient rather than real. Additional markers and samples are needed to confirm the findings of this study. Although no genetic markers were identified which could distinguish individual populations, the overall genetic heterogeneity among locations suggests limited gene flow among the studied regions. In the absence of genetic markers, other attributes can be developed as means of identifying intraspecific units using unique morphological, physiological, ecological, and demographic characteristics. For management purposes, if the genetic heterogeneity observed here is confirmed by further studies, lobsters in the Gulf of Maine should not be treated as a single, panmictic population: inshore and offshore lobster fisheries should be regulated separately. While it is reasonable to presume that offshore regions may be a refuge for the older and larger lobsters, these lobsters are not likely to serve as a brood stock for the inshore or the whole Gulf of Maine population. Indeed, our data suggest that recruitment of inshore lobsters may not rely on the offshore lobster population.

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Table 1. Collection details for the studied populations.

Population	(Latitude, Longitude)	Abbreviation	Sample Size	Male to Female Ratio	Size Range (mm)[*]	Date of collection
Long Island Sound, Southern New England	(41.05N, 72.58W)	LIS	109	2:1	**64 – 102**	November, 1996
Boothbay Harbor, Inshore Gulf of Maine	(43.09N, 69.30W)	BH	114	1:1	**46 –119**	July, 1995
Georges Bank, Offshore Gulf of Maine	(41.50N, 67.50W)	GB	69	NI[**]	**NI[**]**	October, 1995 April, 1996
Prince Edward Island, Southern Gulf of St. Lawrence	(46.20N, 63.20W)	GSL	127	1:1	**NI[**]**	October, 1995
Smith Sound, Newfoundland	(48.20N, 53.50W)	NFL	125	1.3:1	**83-107**	July, 1997

[*] carapace length in mm

[**] NI: not enough information

Table 2. Variation of four microsatellite loci among five populations of *Homarus americanus*. Null alleles are not included in this table. Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

	LIS	BH	GB	GSL	NFL
Hom3-2					
N	109	114	62	106	124
n	31	33	26	27	30
n_e	14.38	13.23	14.05	12.23	9.96
H_o	0.908	0.921	0.871	0.792	0.879
H_e	0.935	0.929	0.938	0.922	0.903
D	-0.029	-0.009	-0.071	-0.141	-0.027
P(HW)	0.245	0.355	0.170	0.007**	0.174
P(DH)	0.099	0.138	0.038*	0.000**	0.157
Hom6-2					
N	108	76	65	127	124
n	33	36	31	31	30
n_e	10.08	12.75	18.10	13.20	9.75
H_o	0.831	0.790	0.925	0.817	0.806
H_e	0.906	0.928	0.952	0.929	0.902
D	-0.083	-0.149	-0.028	-0.121	-0.106
P(HW)	0.203	0.002**	0.714	0.002**	0.038*
P(DH)	0.091	0.000**	0.229	0.010**	0.000**

Hom7-1					
N	106	105	69	100	125
n	41	45	35	37	43
n_e	**19.18**	**17.48**	**18.71**	**15.71**	**18.19**
H_o	**0.943**	**0.962**	**0.957**	**0.920**	**0.976**
H_e	**0.952**	**0.948**	**0.953**	**0.942**	**0.949**
D	-0.009	0.015	0.004	-0.023	0.028
P(HW)	0.236	0.775	0.118	0.065	0.831
P(DH)	0.228	0.481	0.092	0.181	0.914

Hom8-1					
N	108	96	65	127	124
n	7	7	9	9	9
n_e	2.41	2.61	2.37	2.20	1.93
H_o	0.565	0.542	0.354	0.520	0.411
H_e	0.595	0.620	0.582	0.548	0.484
D	-0.050	-0.126	-0.392	-0.051	-0.151
P(HW)	0.068	0.330	0.000**	0.572	0.180
P(DH)	0.045*	0.009**	0.000**	0.110	0.009**

Abbreviation used in the table: N, number of studied individuals; n, number of observed alleles; n_e, effective number of alleles (Kimura and Crow 1964); H_o, observed heterozygosity; H_e, expected heterozygosity under Hardy-Weinberg equilibrium; D, deviation from expected heterozygosity, $((H_o-H_e)/H_e)$; P(HW), probability of fit to Hardy-Weinberg equilibrium; P(DH), probability of deficit in heterozygotes. * and **, statistical significant at $P > 0.05$ and $P > 0.01$, respectively.

Table 3. Estimation of null allele frequencies for each microsatellite locus in each population. Calculation after Brookfield (1996). Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland. **Inheritance studies previously demonstrated the presence of null alleles at Hom3-2 and Hom8-1 (Tam and Kornfield 1996).**

	LIS	BH	GB	GSL	NFL
Hom3-2					
N	109	114	62	106	124
nn	0	1	0	1	0
Freq	0.014	0.027	0.035	0.084	0.013
Hom6-2					
N	108	76	65	127	124
nn	0	2	0	0	0
Freq	0.039	0.120	0.014	0.058	0.049
Hom7-1					
N	106	105	69	100	125
nn	0	0	0	0	0
Freq	0.005	0.000	0.000	0.011	0.000
Hom8-1					
N	108	96	65	127	124
nn	0	0	0	0	0
Freq	0.019	0.048	0.144	0.018	0.049

Abbreviation used in the table: N, number of examined individuals; nn, observed number of null-allele homozygotes; Freq, estimated frequency of null allele in the sample.

Table 4. Probability (p) of homogeneity in allele composition from Markov chain resampling. Null alleles frequencies are included in the data analysis. Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

Populations		Hom3-2	Hom6-2	Hom7-1	Hom8-1
All populations		**0.002***	**<0.001****	**0.412**	**<0.001****
LIS	BH	0.096	0.301	0.143	<0.001[b]
LIS	GB	0.157	<0.001[b]	0.951	<0.001[b]
LIS	GSL	0.056	0.867	0.329	0.184
LIS	NFL	0.097	0.280	0.388	0.017
BH	GB	0.608	<0.001[b]	0.524	<0.001[b]
BH	GSL	0.067	0.168	0.319	0.005[a]
BH	NFL	0.020	0.004[a]	0.142	<0.001[b]
GB	GSL	0.284	0.011	0.545	<0.001[b]
GB	NFL	0.086	<0.001[b]	0.487	0.007
GSL	NFL	0.009	0.629	0.850	0.348

* significant at $\alpha = 0.05$ ** significant at $\alpha = 0.01$

[a] significant at $\alpha = 0.05$ after Bonferroni correction ($\alpha = 0.005$ for 10 comparisons).

[b] significant at $\alpha = 0.01$ after Bonferroni correction ($\alpha = 0.001$ for 10 comparisons).

Table 5. (a) F_{ST} and R_{ST} estimates over all microsatellite loci. (b) Probability that F_{ST}, F_{IS} and F_{IT} is greater than 0. Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

(a)

Populations		F_{ST}	R_{ST}
All populations		0.0040	0.0140
LIS	BH	0.0026	0.0118
LIS	GB	0.0041	0.0189
LIS	GSL	0.0016	-0.0018
LIS	NFL	0.0034	-0.0030
BH	GB	0.0067	0.0679
BH	GSL	0.0026	0.0097
BH	NFL	0.0079	0.0108
GB	GSL	0.0045	0.0315
GB	NFL	0.0078	0.0127
GSL	NFL	0.0019	0.0010

(b)

Populations		F_{ST}	F_{IS}	F_{IT}
LIS	BH	0.034	0.049	0.030
LIS	GB	0.010	0.083	0.037
LIS	GSL	0.066	0.038	0.023
LIS	NFL	0.005*	0.175	0.115
BH	GB	0.002*	0.072	0.018
BH	GSL	0.029	0.043	0.024
BH	NFL	<0.001**	0.145	0.052
GB	GSL	0.002*	0.057	0.031
GB	NFL	<0.001**	0.245	0.109
GSL	NFL	0.036	0.138	0.109

* significant at $\alpha = 0.05$ after Bonferroni correction ($\alpha = 0.005$ for 10 comparisons).

** significant at $\alpha = 0.01$ after Bonferroni correction ($\alpha = 0.001$ for 10 comparisons).

Table 6. Estimate of number of migrants per generation using private alleles. Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

Populations	BH	GB	GSL	NFL
LIS	15	11	10	15
BH	-	8	15	12
GB		-	12	10
GSL			-	23

Table 7. Distribution of alpha and beta mitochondrial haplotypes in each studied population. Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

Populations	Total	alpha	beta	Proportion of alpha
LIS	107	80	27	0.748
BH	111	76	35	0.685
GB	71	57	14	0.803
GSL	109	88	21	0.807
NFL	86	72	14	0.837

Table 8. Probability of homogeneity in haplotype frequencies between populations estimated from Fisher's exact tests. Abbreviation of locations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

Populations	**BH**	**GB**	**GSL**	**NFL**
LIS	0.369	0.470	0.330	0.158
BH	-	0.089	0.045[a]	0.020[a]
GB		-	1.000	0.677
GSL			-	0.710
NFL				-

[a] $p < 0.05$, but not significant after Bonferroni correction.

Fig. 1. Locations of the studied populations. Sampling locations are indicated by "*" on the map. Abbreviations: LIS, Long Island Sound; BH, Boothbay Harbor; GB, Georges Bank; GSL, Gulf of St. Lawrence; NFL, Newfoundland.

Fig. 2. Partial sequences of the mitochondrial ND2 gene in *Homarus americanus*. Nucelotides in bold represent the informative mutation which can distinguish the two haplotypes described in Kornfield and Moran (1989.) Recognition sequence *Xcm*I is indicated with an overline. DNA samples A1-A4 and B1-B4 defined the alpha and beta haplotypes, respectively, based on previous restriction analysis.

Fig. 3. Allele frequency distribution of microsatellite locus Hom3-2 in the five studied *Homarus americanus* populations.

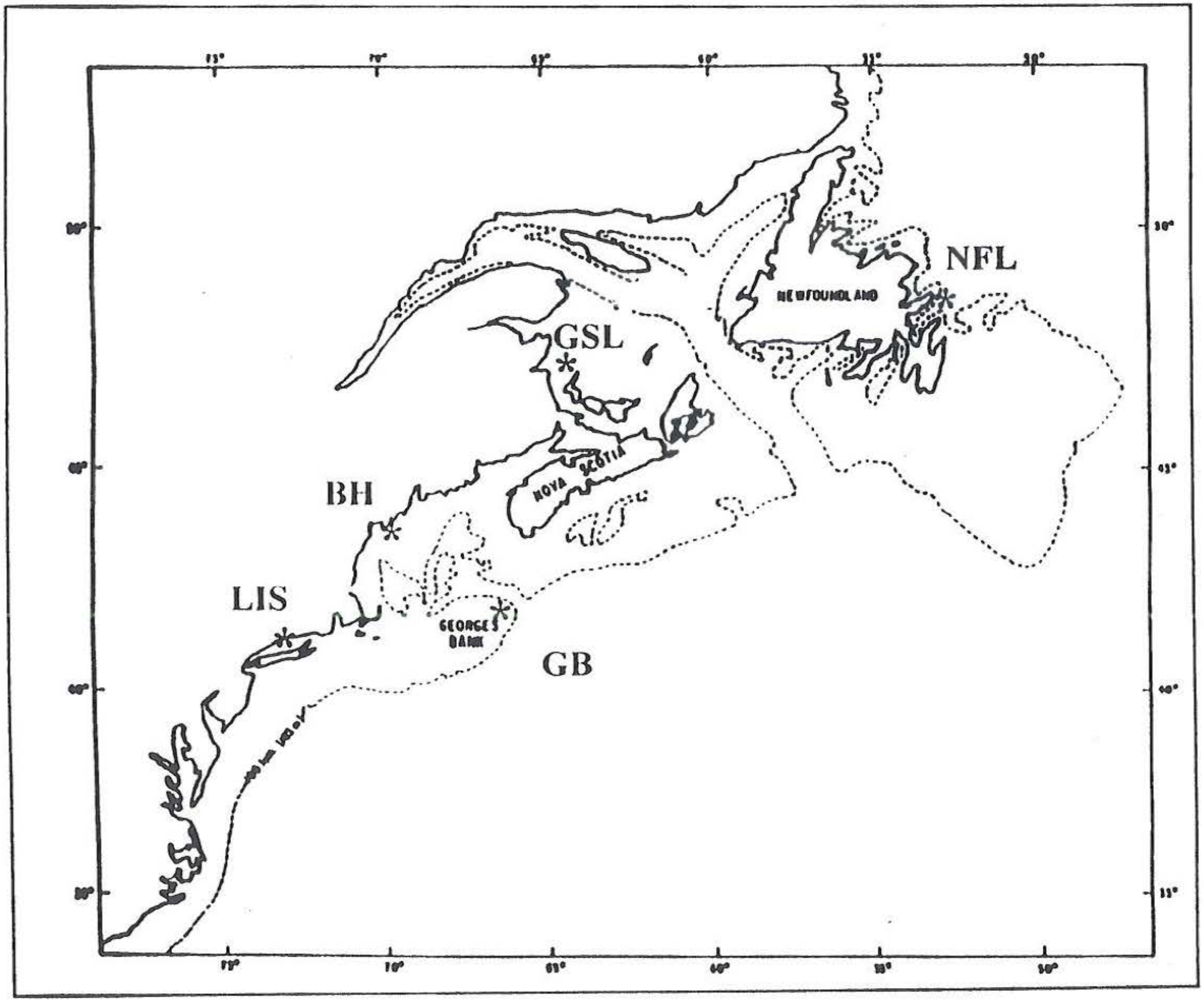
Fig. 4. Allele frequency distribution of microsatellite locus Hom6-2 in the five studied *Homarus americanus* populations.

Fig. 5. Allele frequency distribution of microsatellite locus Hom7-1 in the five studied *Homarus americanus* populations.

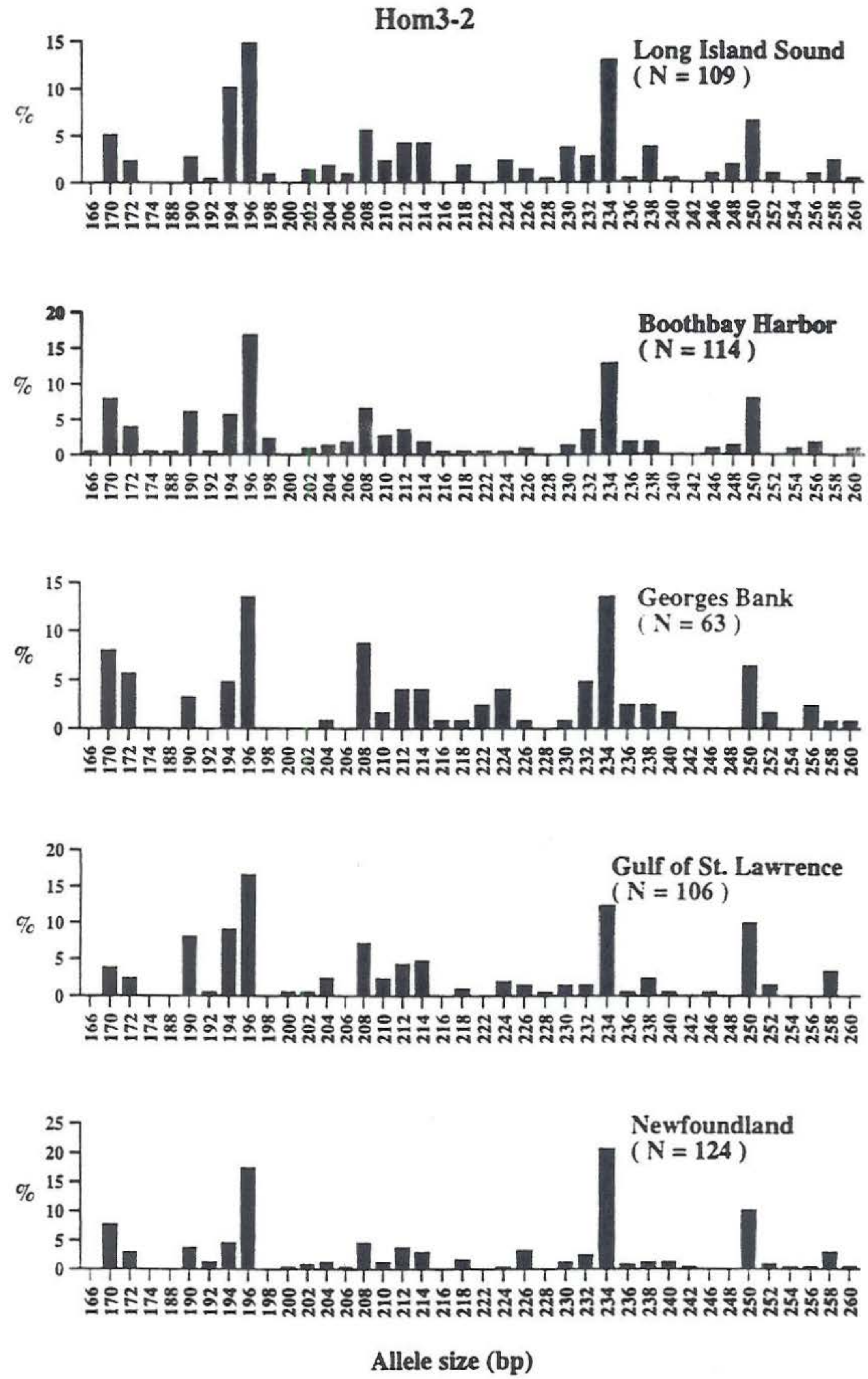
Fig. 6. Allele frequency distribution of microsatellite locus Hom8-1 in the five studied *Homarus americanus* populations.

Fig. 7. Relationships between estimates of genetic differentiation (a) $F_{ST}/(1-F_{ST})$ and

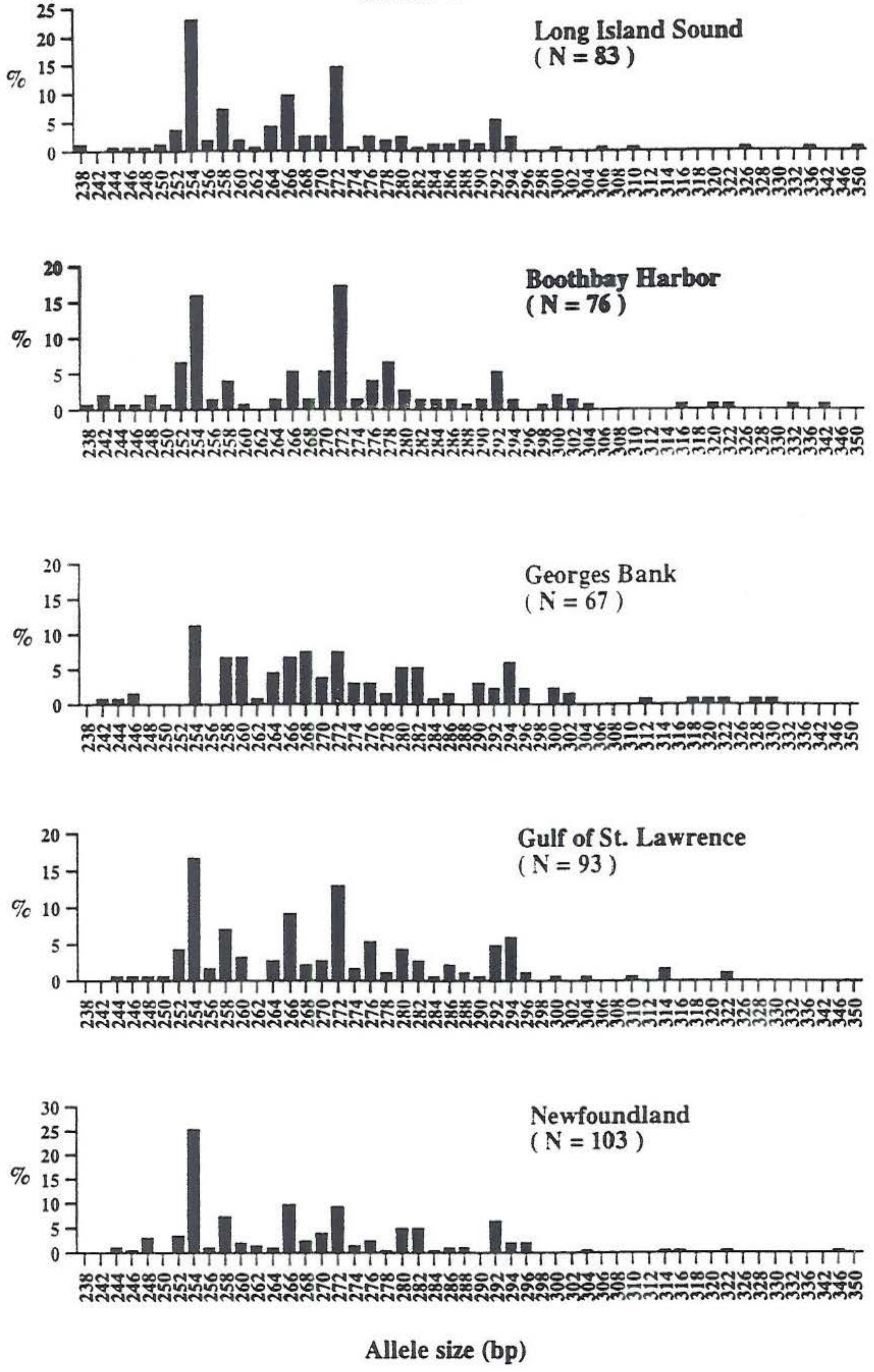
(b) $R_{ST}/(1-R_{ST})$ versus logarithm of geographic distance.

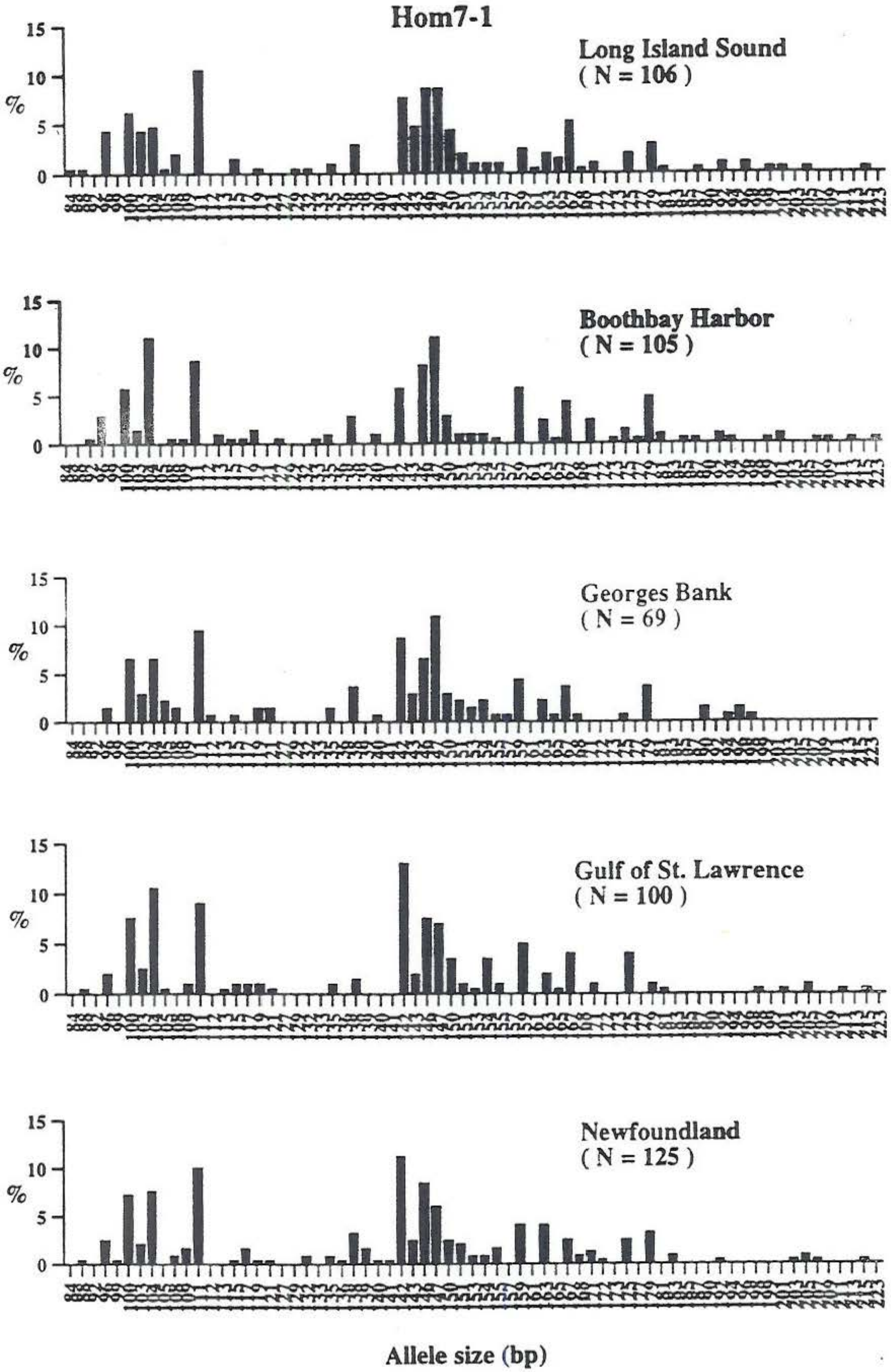

75°
70°
65°
60°
55°
50°
50°
45°
40°
35°
NFL
NEWFOUNDLAND
GSL
NOVA SCOTIA
BH
LIS
GEORGES BANK
GB

```
B1      GGAGCAATTTTTTGAATGGTTATGAGCACAATAGATTGGATTCAATTTATTCCTTCTATA   60
B2      ............................................................
B3      ............................................................
B4      ............................................................
A1      ............................................................
A2      ............................................................
A3      ............................................................
A4      ............................................................


                   ---------------
B1      ACTTGAGGGAACCAAAAATGAAACGGAGCTGCTCCTAGTTTGAGAAGAAGAGCAAGGGCT  120
B2      ............................................................
B3      ............................................................
B4      ............................................................
A1      .......................T....................................
A2      .......................T....................................
A3      .......................T....................................
A4      .......................T....................................


B1      AGACAAAGCGGTGCAAAACTGGGGGAAATTAATATAATTGAGGCTGAAAAAATAATAATA  180
B2      ............................................................
B3      ............................................................
B4      ............................................................
A1      ............................................................
A2      ............................................................
A3      ............................................................
A4      ............................................................


B1      GCAGATCCCAAAGCTTGGACAAGAAAGTATTTAAGTGCAGACTCGGAAGAATATTGATTA  240
B2      ..................................................N.........
B3      ........T...................................A...............
B4      ........T...................................A...............
A1      ........T...................................A...............
A2      ........T...................................A...............
A3      ........T...................................A...............
A4      ........T...................................A...............


B1      TTTTTTGTNATAATTNAAGGAATAAAAGATAAAAGATTTAATTCTAAGCCTGCTCATGCC  300
B2      ........A......A............................................
B3      ........A......A............................................
B4      ........A......A............................................
A1      ....................G.......................................
A2      ........A......A............................................
A3      ........A......A............................................
A4      ........A......A............................................
```


Hom3-2
Long Island Sound
(N = 109)
Boothbay Harbor
(N = 114)
Georges Bank
(N = 63)
Gulf of St. Lawrence
(N = 106)
Newfoundland
(N = 124)
%
0
5
10
15
20
25
166
170
172
174
188
190
192
194
196
198
200
202
204
206
208
210
212
214
216
218
222
224
226
228
230
232
234
236
238
240
242
246
248
250
252
254
256
258
260
Allele size (bp)

Hom6-2


Long Island Sound
(N = 83)
Boothbay Harbor
(N = 76)
Georges Bank
(N = 67)
Gulf of St. Lawrence
(N = 93)
Newfoundland
(N = 103)
%
0
5
10
15
20
25
30
238
242
244
246
248
250
252
254
256
258
260
262
264
266
268
270
272
274
276
278
280
282
284
286
288
290
292
294
296
298
300
302
304
306
308
310
312
314
316
318
320
322
326
328
330
332
336
342
346
350
Allele size (bp)

Hom7-1
Long Island Sound
(N = 106)
Boothbay Harbor
(N = 105)
Georges Bank
(N = 69)
Gulf of St. Lawrence
(N = 100)
Newfoundland
(N = 125)
15
10
5
0
%
84
223
Allele size (bp)

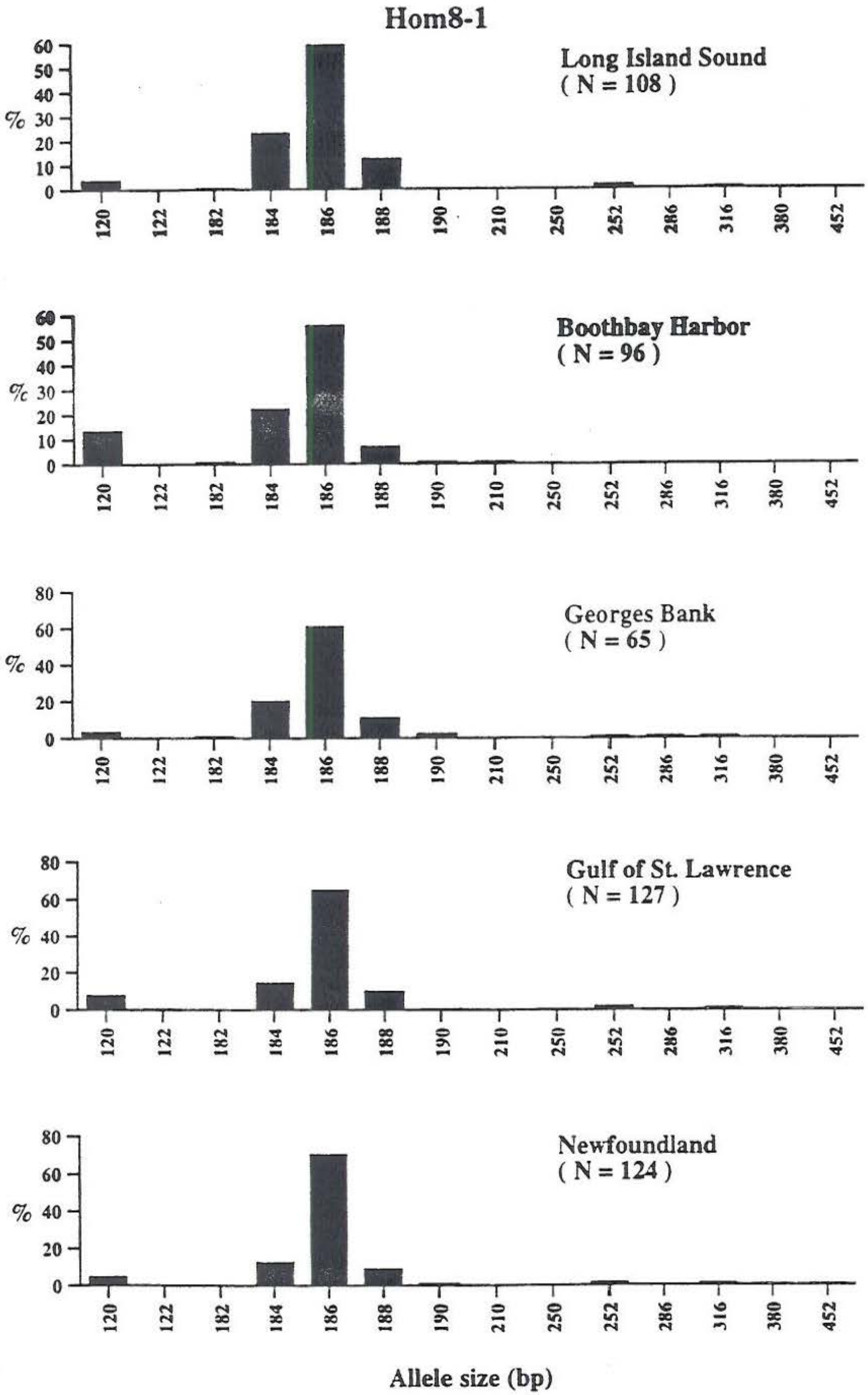
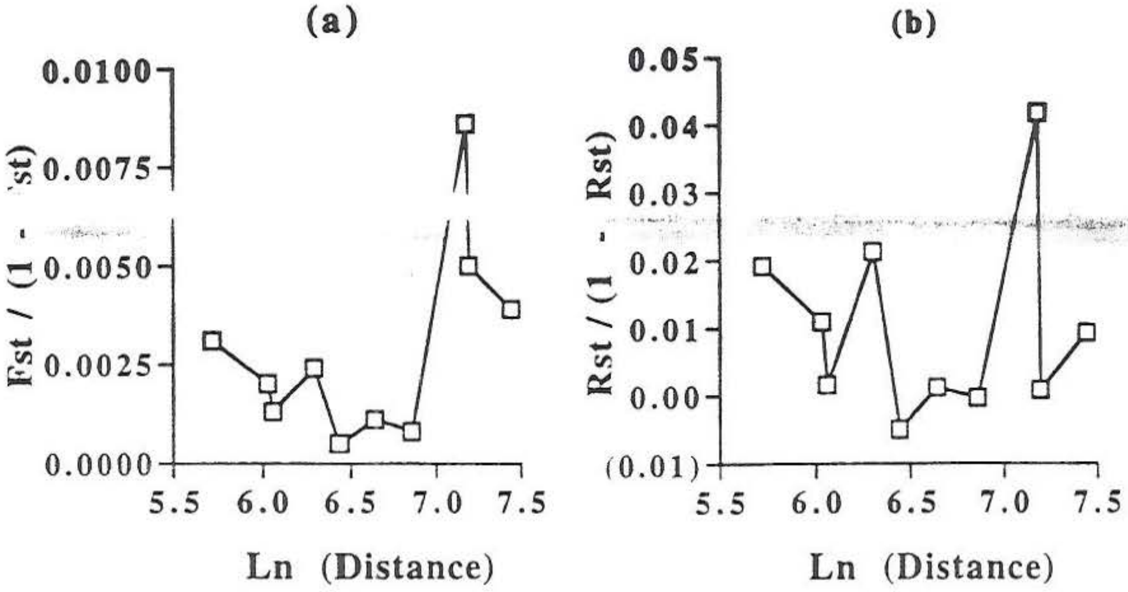

Hom8-1
Long Island Sound
(N = 108)
Boothbay Harbor
(N = 96)
Georges Bank
(N = 65)
Gulf of St. Lawrence
(N = 127)
Newfoundland
(N = 124)
%
0
10
20
30
40
50
60
80
120
122
182
184
186
188
190
210
250
252
286
316
380
452
Allele size (bp)

Fig. 7



(a)
0.0100
0.0075
0.0050
0.0025
0.0000
Fst / (1 - Fst)
5.5
6.0
6.5
7.0
7.5
Ln (Distance)
(b)
0.05
0.04
0.03
0.02
0.01
0.00
(0.01)
Rst / (1 - Rst)
5.5
6.0
6.5
7.0
7.5
Ln (Distance)