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Genetic diversity of coastal Northwest Atlantic herring populations: implications for management

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Analysis of nine tetranucleotide microsatellite loci for Atlantic herring at five locations in the Northwest Atlantic including the Bras d'Or Lakes shows considerable genetic variation and significant population structure within the Coastal Nova Scotia management component, and among coastal populations and herring collected from Georges Bank. However, results are also consistent with gene flow across the Gulf of Maine. The magnitude of differentiation between the Bras d'Or Lakes sample and all others considered was sufficient to warrant further investigation. These data support the precautionary spawning-ground based management approach implemented in this area.

Key words: Atlantic herring; microsatellite; genetic diversity; Bras d'Or Lakes.

INTRODUCTION

Within species genetic diversity can be partitioned into variation within and among populations. It is necessary to maintain both types of variation to minimize the frequency of extirpation of local populations and to sustain species stability since genetic diversity is a requisite for evolutionary adaptation to a changing environment (Hedrick & Miller, 1992).

Atlantic herring *Clupea harengus* L. exhibit considerable variation among spawning groups: spawning intervals range from early spring to late autumn (Sinclair & Tremblay, 1984) and takes place over a range of environments (inshore, offshore and estuarine). Herring are thought to exhibit natal spawning site fidelity (Blaxter, 1985) that results in predictable patterns of migration to and from spawning grounds, although Hay *et al.* (2001) have recently re-estimated Pacific herring *Clupea pallasii* (Valenciennes) fidelity rates as 'high' for large areas (e.g. 10 000 km^2) but 'lower' for small geographic areas (100 km^2) of the scale considered here. Hypothesized spawning site fidelity and the predictable nature of spawning time contributed to the assertion that herring spawning groups are in fact discrete (Iles & Sinclair, 1982; Sinclair, 1988). However, several authors have cited evidence that is difficult to reconcile with the 'discrete' population theory: syntheses of the opposing arguments (Smith &

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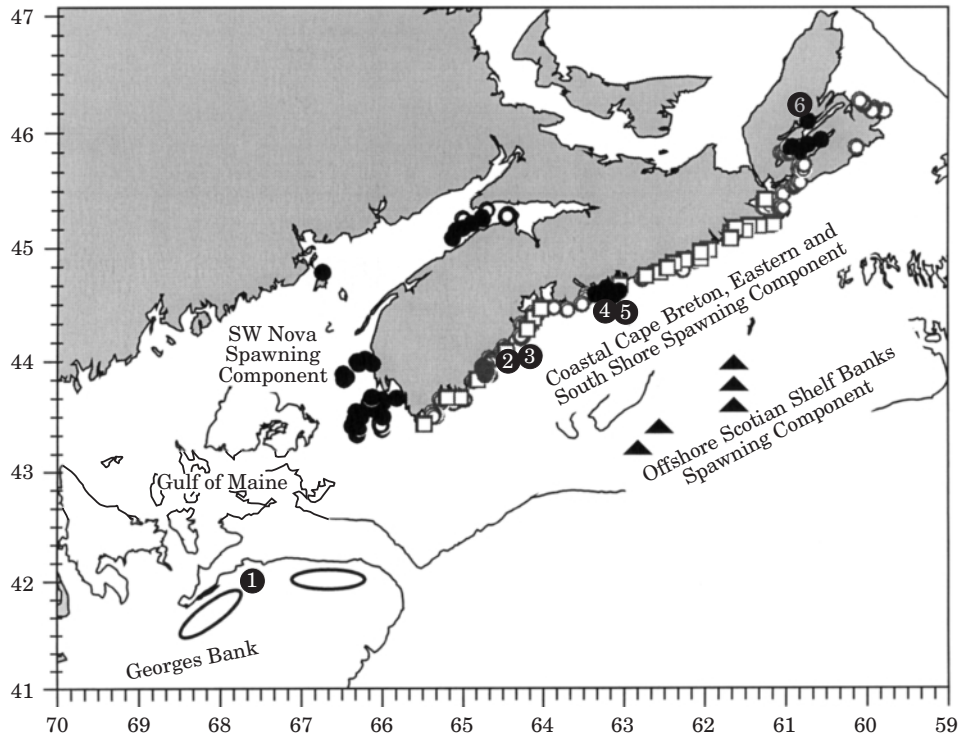

FIG. 1. Atlantic herring management components, locations of spawning groups (historical and present), and sampling sites. 1, Georges Bank; 2, Devastation Shoal 1; 3, Devastation Shoal 2; 4, Three Fathoms Harbour; 5, Eastern Passage; 6, Bras d'Or Lakes. Amended from Stephenson *et al.* (2001) ●, 1996/1997 Fishery; ○, □, historical; ▲, other sources; ⬭, Georges Bank.

Jamieson, 1986) and attempts to reconcile the considerable information into conceptual models can be found in Stephenson (1991), McQuinn (1997), and Stephenson *et al.* (2001).

Management of Atlantic herring in the Northwest Atlantic off Nova Scotia [Northwest Atlantic Fisheries Organization (NAFO) Division 4WX fishing area] assumes stock complexity as the area has been subdivided into three management components (Fig. 1) and the assessment strategy (Stephenson *et al.*, 1999*a*) explicitly attempts to protect individual spawning groups within these components from over-exploitation while attempting to maintain the spatial and temporal diversity observed in spawning. The Coastal Nova Scotia management component includes many small spawning grounds, larger spawning grounds (e.g. Eastern Passage) that support a commercial fishery, and a number of relatively isolated spawning grounds in the Bras d'Or Lakes region of Cape Breton. The management strategy in this area is a relatively new initiative, however, and critical questions remain unanswered including whether or not this management strategy reflects the scale of herring population structure and can be successful in protecting within species diversity.

Differentiation among groups of spawning herring along the coast of Nova Scotia has been attempted using a variety of techniques. Meristic (variation in the number of vertebrae), morphometric (variation in body part dimensions and

TABLE I. Sampling details of Atlantic herring collections. Location numbers as in Fig. 1

Location number	Location name	Sample size	Date sampled	Latitude	Longitude	Predominant year-classes
1	Georges Bank	75	29 Oct. 1999	41°59·77′	67°41·5′	80% 1994 20% 1995
2	Devastation Shoal 1	65	7 Oct. 1998	43°53·48′	64°44·30′	100% 1992
3	Devastation Shoal 2	65	13 Oct. 1998	43°52·13′	64°43·13′	100% 1992
4	Three Fathoms Harbour	60	3 Oct. 1998	44°37·38′	63°10·70′	8% 1991 81% 1992
5	Eastern Passage	75	4 Oct. 1999	44°37′	63°11′	10% 1991 39% 1992 44% 1993
6	Bras d'Or Lakes	71	24 Apr. 2000	46°05·20′	60°44·70′	

or rates of change), and demographic (age structure and mortality) methods have been used to discriminate among groups of marine fishes when the means and variance of these measurements have been observed to differ among populations (Messieh, 1975). The limitation of such techniques is that the characters used may be influenced by environmental variation and therefore exposure to a variety of environments (especially in the early life stages) may contribute to variation in these traits among populations. Thus population-specific differences, that are influenced by environmental conditions, become difficult to quantify and interpret (Swain & Foote, 1999).

Analyses of neutral genetic variation have been shown to be useful in differentiating among fish populations (Ruzzante *et al.*, 1998) and neutral genetic variation is generally thought to be less affected by environmental fluctuations (e.g. temperature differences of several degrees) at least at fisheries management time-scales (decades to centuries).

In this paper, the genetic variation of Atlantic herring, within the Coastal Nova Scotia management component is examined and (age data provided by Fisheries & Oceans Canada) the ability of the contemporary management scheme in the NAFO 4WX fishing area to preserve existing genetic diversity is questioned.

MATERIALS AND METHODS

Tissue samples (blood, fin and muscle) were collected from Atlantic herring at several spawning sites along the southern coast of Nova Scotia (Fig. 1) in 1998, 1999 and 2000 (Table I). Samples were also collected on Georges Bank (across the Gulf of Maine) and were included in these analyses for comparison. As herring found in the Bras d'Or Lakes are in a relatively unique environment (inland sea with restricted access to the Atlantic Ocean, limited flushing, and low salinity) the opportunity was taken to investigate the potential effect of this restrictive environment on the genetic variation within this population.

Care was taken to ensure that tissues were collected only from herring that were in spawning condition, and therefore were members of an *in situ* spawning population. A second collection was obtained in the Devastation Shoal region, 6 days after the first. The samples from Baddeck Bay in the Bras d'Or Lakes were collected from spring-spawning herring. All other samples (Georges Bank, Eastern Passage, Three Fathoms

Harbour and Devastation Shoal) were collected from autumn-spawners. The age of each fish was determined by counting annuli on the otoliths. (Age data provided by Fisheries & Oceans Canada.)

DNA was isolated using Qiagen DNeasy genomic DNA extraction kits. Nine microsatellite loci (*Cha*1027, *Cha*1020, *Cha*1059, *Cha*1202, *Cha*1017, *Cha*1045, *Cpa*108, *Cpa*113, and *Cpa*102) were amplified using polymerase chain reaction (PCR). PCR amplification and electrophoresis conditions for *Cha* and *Cpa* loci are given in McPherson *et al*. (2001) and Olsen *et al*. (2002) respectively. DNA fragments were visualized and sized using an FMBIO II fluorescent imaging system (Hitachi).

Departures from Hardy Weinberg expectations were tested at each sample location using GENEPOP (Raymond & Rousset, 1995). *P*-values for each comparison were estimated using the Markov chain method with 2000 dememorizations, 200 batches and 2000 iterations per batch for each test. Multilocus combinations of single-locus tests within samples were performed using Fisher's (1954) method.

Pairwise F_{ST} estimates (Wright, 1951, as amended by Weir & Cockerham, 1984) were calculated using Genetix (Belkhir, 2000) and 1000 permutations were used to estimate the probability of departure from the null hypothesis of no difference. An Exact test (GENEPOP: Raymond & Rousset, 1995) was also employed to assess the statistical significance of allele frequency differences at individual loci, between pairs of populations. *P*-values were calculated using a Markov chain method described as above.

Patterns of genetic differences between samples were illustrated using multidimensional scaling techniques (NCSS). F_{ST} estimates between samples were used to create a two dimensional map displaying the relative positions of sample collections, based on their genetic dissimilarities.

A Mantel test employing the great circle distance between populations and F_{ST} estimates were used to test for an isolation by distance relationship between populations (Genetix; Belkhir, 2000).

RESULTS

All the loci were polymorphic in all sample populations and one or more alleles were amplified at >95% of samples at each locus. The average number of alleles per locus per sample ranged from 5 to 30 and observed heterozygosities (*Ho*) ranged from 0·183 to 0·965 (Table II). Observed heterozygosity (*Ho*) averaged over loci did not differ among populations (ANOVA; $P=0{\cdot}96$), although the Bras d'Or Lakes collection had the lowest mean *Ho* (0·77) and the lowest mean variance relative to the other populations. Furthermore, Bras d'Or had the fewest number of alleles, on average, although the allele deficit was not significant (ANOVA; $P=0{\cdot}98$) when means of all populations were compared statistically.

When results from all loci were combined for each population, no population departed from Hardy–Weinberg (HW) expectations. However, there were several single locus results that deviated from HW expectations: Georges Bank—*Cha*1045 ($P=0{\cdot}011$) and *Cpa*102 ($P=0{\cdot}011$); Devastation Shoal 2—*Cpa*102 ($P=0{\cdot}011$) and *Cpa*108 ($P=0{\cdot}03$); Eastern Passage—*Cha*1045 ($P=0{\cdot}005$); Bras d'Or Lakes—*Cpa*102 ($P=0{\cdot}03$).

In the Eastern Passage collection, there were sufficient individuals from 2-year-classes (1992 and 1993) to test for temporal stability of year-classes ($n=30$ and 33, respectively). When 1992 and 1993 collections were compared using multi-locus F_{ST}, no differences were detected ($F_{ST}=-0{\cdot}002$; $P=0{\cdot}76$). Therefore, in the following analyses, all herring from Eastern Passage were pooled.

Multi-locus F_{ST} analyses revealed significant substructuring between Bras d'Or Lakes samples and all others (Table III). The magnitude of these differences

TABLE II. Levels of genetic variation observed at nine microsatellite loci within six Atlantic herring samples: sample size (*n*); number of alleles detected at each locus; observed (*Ho*) and expected (*He*) heterozygosity within samples are indicated

Locus	GeorgesBk *n*=75	DevSh1 *n*=65	DevSh2 *n*=65	ThrFathHr *n*=60	EasternPass *n*=75	Brasd'Or *n*=71
*Cha*1027						
No. of alleles	23	20	20	22	23	19
Ho	0·933	0·935	0·918	0·900	0·933	0·902
He	0·928	0·925	0·935	0·922	0·939	0·888
*Cha*1202						
No. of alleles	11	10	12	8	11	10
Ho	0·693	0·729	0·662	0·638	0·783	0·788
He	0·733	0·727	0·639	0·684	0·739	0·742
*Cha*1059						
No. of alleles	13	9	10	9	13	11
Ho	0·681	0·774	0·639	0·672	0·739	0·648
He	0·707	0·766	0·718	0·680	0·722	0·714
*Cha*1017						
No. of alleles	10	10	11	10	10	8
Ho	0·730	0·903	0·813	0·737	0·767	0·783
He	0·814	0·831	0·824	0·815	0·834	0·764
*Cha*1020						
No. of alleles	20	19	17	18	20	17
Ho	0·959	0·918	0·898	0·860	0·905	0·864
He	0·921	0·910	0·920	0·914	0·915	0·856
*Cha*1045						
No. of alleles	21	21	22	19	21	20
Ho	0·919*	0·807	0·869	0·811	0·761**	0·883
He	0·893	0·901	0·895	0·895	0·897	0·911
*Cpa*113						
No. of alleles	19	17	19	22	20	19
Ho	0·960	0·907	0·892	0·982	0·931	0·879
He	0·930	0·925	0·922	0·934	0·927	0·921
*Cpa*102						
No. of alleles	30	27	28	29	30	26
Ho	0·958*	0·887	0·923*	0·965	0·960	0·939*
He	0·936	0·919	0·918	0·929	0·935	0·883
*Cpa*108						
No. of alleles	7	9	5	7	7	6
Ho	0·500	0·617	0·295*	0·576	0·573	0·183
He	0·470	0·569	0·301	0·542	0·522	0·197
Mean No. of alleles	17·11	15·77	16	16	17·22	15·11
Mean *Ho*	0·815	0·831	0·768	0·793	0·817	0·763

*$P<0·05$ but nonsignificant after Bonferroni procedure. **$P<0·01$.

(reflected by high F_{ST} values and highly significant *P*-values) suggest that the Bras d'Or herring are distinct from all other sample collections included in this study.

Additional significant ($P<0·05$) pair-wise population differences (based on F_{ST}) were observed between the two Devastation Shoal collections, between Eastern Passage and Georges Bank, between Eastern Passage and Devastation

TABLE III. Pair-wise F_{ST} estimates between samples above the diagonal

	Georges Bank	Devastation Shoal 1	Devastation Shoal 2	Three Fathoms Harbour	Eastern Passage	Bras d'Or Lakes
Georges Bank	—	0·0015	0·0014	0·0002	0·0025*	**0·0110****
Devastation Shoal 1		—	0·0043*	0	0·0007	**0·0119****
Devastation Shoal 2			—	0·0028*	0·0031*	**0·0110****
Three Fathoms Harbour				—	0·0009	**0·0095****
Eastern Passage					—	**0·0140****
Bras d'Or Lakes						—

*P<0·05; **P<0·001. Results significant after Bonferroni corrections for table-wide significance are in bold.

Shoal 2, and between Devastation Shoal 2 and Three Fathoms Harbour. However, these results are nonsignificant after Bonferroni corrections (Table III).

Exact tests revealed locus-specific differences among populations (Table IV) and the majority of the differences were found at three loci; (*Cha*1020, *Cpa*102 and *Cpa*108). All highly significant pair-wise comparisons ($P<0{\cdot}001$) included the Bras d'Or Lakes sample, with the Eastern Passage–Bras d'Or and Georges Bank–Bras d'Or comparisons having three of nine loci at $P<0{\cdot}001$.

The patterns of genetic differences among samples are illustrated using multidimensional scaling of pair-wise F_{ST} estimates (Fig. 2). The Bras d'Or Lakes sample is clearly separate from the remaining herring considered here. Within the non-Bras d'Or collection of samples, Eastern Passage is moderately removed from the Devastation Shoal–Three Fathoms Harbour–Georges Bank group, within which there is little differentiation.

A Mantel test using pair-wise F_{ST} and great circle distance between samples detected no significant ($P=0{\cdot}55$) linear relationship between geographic distances among sample collections and estimates of population differentiation.

DISCUSSION

WITHIN POPULATION VARIATION

The range in heterozygosity detected in this study is somewhat broader, but on average similar to that reported for dinucleotide microsatellites in Pacific herring (O'Connell *et al*., 1998*a*) and Atlantic herring from both sides of the Atlantic (Shaw *et al*., 1999; McPherson *et al*., 2001).

Samples did not depart from Hardy–Weinberg expectations (HWE) when all loci were combined. *P*-values of five of the six significant single locus tests for conformance to HWE ranged from between 0·05 to 0·01: *Cha*1045 (Georges Bank), *Cpa*102 (Georges Bank, Devastation Shoal 2, and Bras d'Or Lakes) and *Cpa*108 (Devastation Shoal 2 $P=0{\cdot}035$) and were not significant after sequential Bonferroni corrections for table-wide significance. Only Eastern Passage at *Cha*1045 remained significant after Bonferroni corrections. Four of the six departures from HWE were common to two locations and thus may be associated with population specific processes such as a (1) a Wahlund (1928) effect where heterozygosity is decreased as a result mixing of differentiated sub-samples or (2) a high variance in reproductive success (Hedgecock, 1994). Conversely, five of the departures occurred at two loci, alternatively suggesting locus specific effects, such as the occurrence of one or more segregating null alleles. Given the large number of tests conducted, the statistical degree of significance and ensuing corrections, and the general absence of significance at seven of nine loci, it was concluded that, overall, these data conform to HWE.

The large number of alleles generally observed at microsatellite loci in marine fish species like herring (O'Connell & Wright, 1997), the decreased magnitude of differentiation typically observed among marine fish populations relative to freshwater and anadromous species (Ward *et al*., 1994), and the lack of temporal stability often observed in studies of marine systems (O'Connell *et al*., 1998*b*) highlights the need for replicate sampling over time when assessing population structuring. Although there were no temporal replicates collected in the present study, it was possible to include a ' replicate ' sample of the same year-class from

TABLE IV. *P*-values of single locus pairwise genic comparisons. Results significant after Bonferroni corrections for table-wide significance are in bold

Comparison	Locus								
	1027	1202	1059	1017	1020	1045	270	102	108
Georg-DevSh1	0·061	0·467	0·150	0·125	0·228	0·113	0·579	0·335	0·532
Georg-DevSh2	0·138	0·082	0·562	0·485	0·584	0·391	0·495	0·490	0·298
Georg-ThrFaHr	0·721	0·705	0·425	0·125	0·441	0·318	0·573	0·008	0·455
Georg-EastPass	0·123	0·149	0·295	0·041	0·002	0·087	0·110	0·432	0·909
Georg-Brasd'Or	0·005	0·103	0·126	0·038	**<0·0001**	0·035	0·309	**<0·0001**	**<0·0001**
DevSh1-DevSh2	0·664	0·492	0·084	0·206	0·231	0·564	0·510	0·388	0·016
DevSh1-ThrFaHr	0·703	0·417	0·012	0·972	0·896	0·479	0·742	0·008	0·456
DevSh1-EastPass	0·004	0·552	0·043	0·891	0·008	0·263	0·815	0·016	0·774
DevSh1-Brasd'Or	0·085	0·385	0·291	0·014	0·018	0·086	0·677	0·006	**<0·0001**
DevSh2-ThrFaHr	0·172	0·250	0·229	0·127	0·252	0·794	0·352	0·140	0·014
DevSh2-EastPass	0·054	0·043	0·923	0·036	0·567	0·557	0·278	0·575	0·049
DevSh2-Brasd'Or	0·007	0·189	0·307	0·147	**<0·0001**	0·075	0·081	0·098	0·055
ThrFaHr-EastPass	0·050	0·728	0·591	0·227	0·002	0·461	0·059	0·389	0·375
ThrFaHr-Brasd'Or	0·229	0·034	0·248	0·004	0·032	0·505	0·334	**0·0004**	**<0·0001**
EastPass-Brasd'Or	0·003	0·192	0·612	0·018	**<0·0001**	0·104	0·112	**0·0007**	**<0·0001**

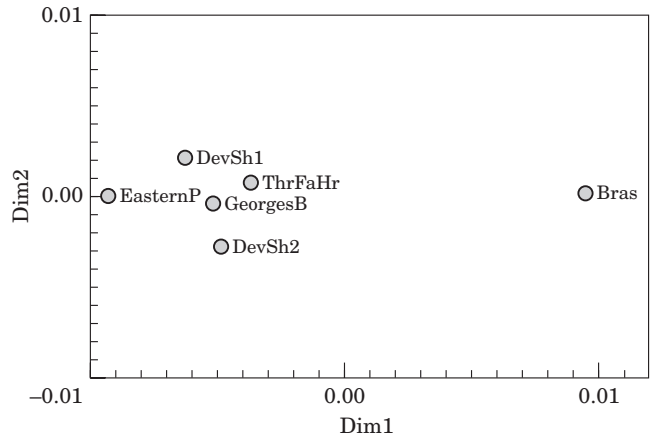

FIG. 2. Multidimensional scaling of pair-wise F_{ST} between samples. Sample locations are Georges Bank (GeorgesB), Eastern Passage (EasternP), Three Fathoms Harbour (ThrFaHr), Devastation Shoal 1 and 2 (DevSh1, DevSh2), and Bras d'Or Lakes (Bras). Dimension one (Dim1) explains 68% of the variation whereas dimension two (Dim2) explains 2%.

Devastation Shoal that was collected 6 days subsequent to the first sample. Pair-wise F_{ST} estimates revealed a small (F_{ST}=0·0043; P=0·013) difference between the two collections. However, this comparison was not significant after Bonferroni correction.

Given that the differences between samples were small, the collections were made 6 days apart, and the fish analysed were from the same year-class and stage of maturity, it seems reasonable to suggest that the differences may be attributable to (a) an F_{ST} estimate within the range of sampling noise associated with estimating a parameter from a sample of individuals (Waples, 1998); (b) patchiness, due to, for example among family variation in reproductive success; or (c) use of the Devastation Shoal spawning ground by different spawning fish within the time-scale of 6 days. Observations from fishers and herring assessment reports (Stephenson *et al.*, 1999*b*, 2000) indicate that the residence time on the spawning ground for herring is less than 10 days. There are insufficient data with which to test this residence time but it is generally accepted that herring vacate the spawning ground soon after releasing their eggs or milt (Blaxter & Hunter, 1982). Length measurements of the two sample populations showed that although drawn from the same year class (1992), the second Devastation Shoal collection had a significantly (ANOVA; P<0·001) greater average length (307 mm) relative to the first collection (301 mm) and therefore the differences detected are likely a result of slightly different fish moving onto the spawning ground. Therefore the two Devastation Shoal samples remained separated for the remaining analyses.

In addition to the Devastation Shoal collections, there were sufficient numbers of both the 1992 and 1993 year-class in the Eastern Passage collection to test for temporal stability among year-classes. As there were no differences detected between 1992 and 1993 Eastern Passage herring, it seemed reasonable to pool across year-classes and assume temporal stability, at least in this location. There were insufficient numbers to test for year-class differences in all other locations.

AMONG POPULATION VARIATION

Multi-locus F_{ST} analyses revealed highly significant differences ($P<0{\cdot}001$ and significant after Bonferroni corrections were applied) between the Bras d'Or Lakes sample and all others. This result suggests that there may be restricted gene flow between the Bras d'Or Lakes herring and all others. Further, single locus Exact tests of allele frequencies showed the same pattern as all comparisons having $P<0{\cdot}001$ (significant after Bonferroni corrections were applied) were between Bras d'Or Lakes and other sample populations.

The Bras d'Or Lakes are relatively isolated from the sea. The main connection to the Atlantic Ocean is by the long (30 km) and narrow (minimum 1 km) Great Bras d'Or Channel (Gurbutt & Petrie, 1995). The combination of restricted oceanic access and freshwater inflow keeps the salinity of the Lakes in the range of 20–26‰ (well below the oceanic average for the Northwest Atlantic) and creates a unique environment for Northwest Atlantic herring. Due to the restricted access, one would hypothesize limited geneflow between the Bras d'Or Lakes herring and Coastal Nova Scotia herring.

Based on the multidimensional scaling of F_{ST} estimates, there appears to be three groupings of the herring samples considered here: the Bras d'Or Lakes sample, the Eastern Passage sample (most different from the Bras d'Or Lakes and marginally different from Devastation Shoal and Georges Bank), and a group that encompasses Georges Bank and the southern shore of Nova Scotia (Devastation Shoal, Three Fathoms Harbour) within which there are limited differences.

With the exception of Bras d'Or Lakes, all other herring considered were collected in October of 1998 and 1999; therefore these fish share the same spawning season. Bras d'Or Lakes herring are spring-spawning and this different reproductive timing may contribute to the isolation of these fish and the magnitude of the differentiation detected among this putative population and all others. Genetic differentiation based on spawning season has also been detected by Kornfield *et al.* (1982) who used allozyme loci to illustrate greater differences between fish populations with different spawning seasons in the Gulf of St Lawrence than between the Gulf of Maine and the Gulf of St Lawrence.

The differences observed between Bras d'Or Lakes and other samples are consistent with earlier suggestions that there is a complex stock structure of Atlantic herring in this area (Scott, 1975; Crawford *et al.*, 1982; Stephenson & Gordon, 1991). For example, Stephenson & Gordon (1991) were able to discriminate between herring from Bras d'Or Lakes and Georges Bank by assessing differences in the number of pectoral fin rays, gill rakers, and pyloric caecae in different sample populations in this same region. Scott (1975) used a host of meristic characters (number of vertebrae, gill rakers, pectoral, dorsal, and anal fin-rays) to discriminate among Bras d'Or herring and all of the other groups he considered (Southwest Nova Scotia, Escuminac, Northumberland Strait, Gulf of St Lawrence). In addition, Crawford *et al.* (1982) detected significantly greater numbers of fin rays and gill rakers from herring collected from a coastal Nova Scotia spawning group (in the vicinity of Eastern Passage) relative to Bras d'Or Lakes herring. It appears that the null hypothesis of no significant difference between Bras d'Or herring and all others in the 4WX and surrounding regions can be rejected on the basis of spawning time, on any of a

suite of meristic measures, as well as on a number of genetic measures. Given the magnitude of the differences detailed here, it is concluded that the Bras d'Or Lakes herring are probably differentiated from the other Coastal Nova Scotia spawning groups, even in the absence of support from temporal replicates.

The marked differences detected between the Bras d'Or herring and all others are not consistent with an isolation by distance model which predicts an inverse relationship between geographic distance among samples and gene flow. An isolation by distance relationship is expected if gene flow decreases as geographic distance among samples increases with homogeneous barriers (or lack thereof) among samples. This may not be the case here, as the Bras d'Or Lakes samples are thought to be partially but not completely isolated from immigration and emigration; Bras d'Or spring-spawners are thought to make up a portion of the 4Vn winter purse seine fishery (Denny *et al.*, 1998) outside of the Bras d'Or Lakes. An isolation by distance relationship was tested amongst all samples, excluding the Bras d'Or Lakes. Again, no significant isolation by distance relationship was detected ($P<0{\cdot}2$). Therefore, in this study, as the geographic distance among samples increases, there is no corresponding increase in differentiation as estimated by F_{ST}.

Because low allele numbers and low average heterozygosities were observed in the Bras d'Or Lakes collection it was hypothesized that the population had undergone a reduction in effective population size. A low effective population size may have enhanced the effects of random drift. Allele frequencies fluctuate much more quickly (due to drift) at neutral loci when the population numbers are small, therefore changes in allele frequencies (and resulting differentiation) may accrue faster in small populations than in larger populations. Hansen *et al.* (2000) used gametic-phase disequilibria between loci to estimate the number of breeders in a population. Random genetic drift may create gametic-phase disequilibrium between unlinked loci in populations that have become drastically reduced. Therefore, this method was used in an attempt to detect reductions in effective population size. Incidence of linkage disequilibrium (as estimated by GENPOP; Raymond & Rousset, 1995) was examined between each pair of loci in each population. Three locus-pair comparisons were found to be significant at $P<0{\cdot}01$. Of these, two comparisons were from the Bras d'Or Lakes sample (*Cha*1027 and *Cha*1045 $P=0{\cdot}005$; *Cha*1027 and *Cha*1017 $P=0{\cdot}001$) and the loci involved were not shown to be associated in any other comparison here or previously (McPherson *et al.*, 2001). Although this analysis is by no means conclusive, the increased incidence of gametic disequilibrium detected in the Bras d'Or herring may be interpreted as consistent with having experienced a reduction in effective population size.

An additional consequence of a reduction in effective population size may be a reduction in the frequency of rare alleles. As rare alleles are lost more rapidly than heterozygosity in small populations (Nei *et al.*, 1975), populations that have undergone major reductions in effective population size may show a paucity of rare alleles (even when heterozygosities are not reduced). Withler *et al.* (2000) used this technique to detect historical reductions in population size of sockeye salmon *Oncorhynchus nerka* (Walbaum). When allele numbers at each locus are averaged across all populations (excluding the Bras d'Or Lakes) and subtracted from the Bras d'Or Lakes allele numbers, Bras d'Or Lakes allele specific

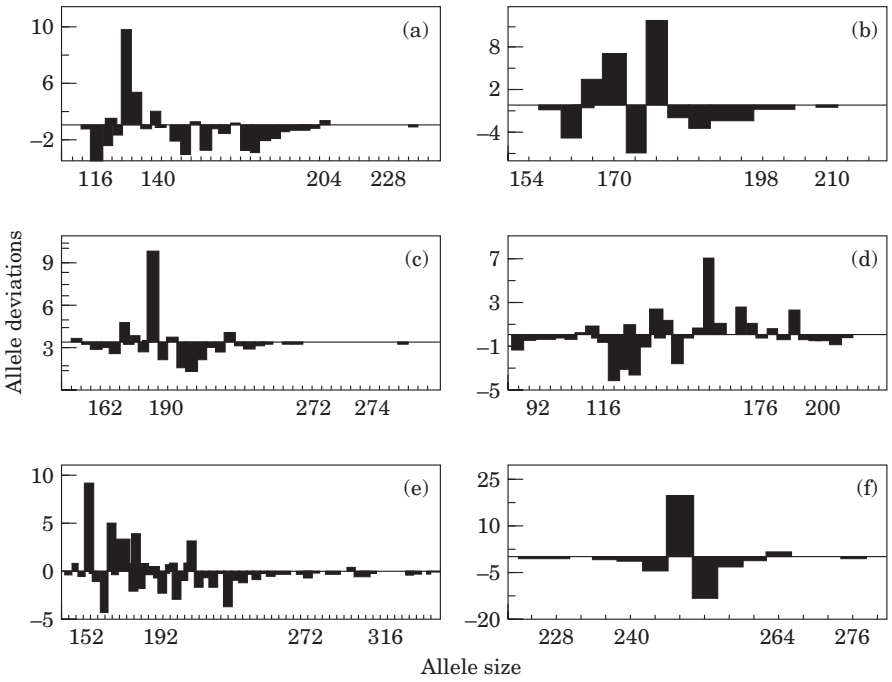

FIG. 3. Allele deviations between Bras d'Or Lakes and all other populations at locus *Cha*1027 (a), *Cha*1017 (b), *Cha*1020 (c), *Cha*1045 (d), *Cpa*102 (e), and *Cpa*108 (f). Number of alleles were averaged over all populations (excluding Bras d'Or Lakes) and subtracted from those of Bras d'Or Lakes sample. Negative deviations represent instances where one or more populations (excluding Bras d'Or) have alleles that are absent or less frequent in the Bras d'Or sample.

anomalies are revealed (Fig. 3). Thus, negative deviations represent instances where one or more populations (excluding Bras d'Or) have alleles that are less frequent or absent in the Bras d'Or Lakes. In general, the Bras d'Or Lakes herring have more restricted allele frequency distributions, with many small negative deviations at the tails of the distributions that may indicate a reduction of rare alleles consistent with a historical reduction in effective population size.

Although the history of the Bras d'Or fishery is largely undocumented, a reduction in herring abundance in the Bras d'Or Lakes has been suggested (Denny *et al.*, 1998). An enhanced survey of the Bras d'Or Lakes herring fishery in 1996 confirmed a decrease in abundance (although not to the level at which genetic changes would be expected to be detected) concomitant with an increase in fishing effort in the region over the previous decade.

MANAGEMENT IMPLICATIONS

Considerable genetic diversity was detected within the Coastal Nova Scotia management component and the magnitude of differentiation within this management component is in some cases greater than the differences among these samples and Georges Bank (included for comparison). The marked differences between the Bras d'Or Lakes and all others, suggest reproductive isolation of this spawning group, an interpretation that is consistent with analyses based on meristics. The relatively limited differentiation (albeit in most cases, based on one year-class and or sample collection) detected between Eastern Passage,

Georges Bank, Devastation Shoal and Three Fathoms Harbour suggest gene flow across the Bay of Fundy and Gulf of Maine. This finding is consistent with that of Kornfield & Bogdanowicz (1987) who concluded, based on mtDNA RFLP analyses, that there was no evidence for the existence of genetically distinct herring populations in the Gulf of Maine.

The magnitude of the significant genetic differentiation between the Bras d'Or herring and all others, suggests that at a minimum, special management consideration for the Bras d'Or herring, within the current management strategy, is warranted. There also may be sufficient genetic differences between the Bras d'Or Lakes and all others to provoke re-consideration of their inclusion in the Coastal component.

The occurrence of geographically distinct spawning groups, predictable spawning times (Sinclair & Tremblay, 1984), detectable meristic differences (Messieh, 1975) and evidence for homing from tagging studies (Wheeler & Winters, 1984), have been repeatedly invoked to suggest that spawning populations of herring may be genetically distinct. It was this body of evidence, and suspicions of an erosion of intra-species biodiversity and concerns for the consequences, that led to the conception and imposition of the contemporary management strategy in NAFO 4WX that can be summarized as an ' in-season, survey, assess, then fish ' protocol (Stephenson *et al.*, 1999*b*). Within this management regime, main herring spawning locations are surveyed and assessed each season, prior to fishing in an attempt to avoid overexploitation of any individual spawning ground.

In light of the differences detected in the present study, albeit based on samples with limited temporal replication, it seems reasonable to conclude that there is considerable genetic variation within the Coastal Nova Scotia management component and these results support the precautionary, spawning-ground based, management approach adopted in this region. However, the greater variation detected within a management component than among the coastal management component and Georges Bank suggests that these management divisions may warrant re-examination using the inferred levels of gene flow among them.

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References

Belkhir, K. (2000). GENETIX, logiciel sous Windows pour la génétique des populations. Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France.

Blaxter, J. H. (1985). The herring: a successful species? *Canadian Journal of Fisheries and Aquatic Sciences* **42,** 1228–1234.

Blaxter, J. H. & Hunter, J. R. (1982). The biology of clupeoid fishes. *Advances in Marine Biology* **20,** 1–223.

Crawford, R. H., Webber, D. M. & Boutlier, G. (1982). *The Biology of Herring from Bras d'Or Lake Cape Breton, Nova Scotia.* Manuscript and technical report series. Nova Scotia Department of Fisheries. 82-04.

Denny, S., Clark, K. J., Power, M. J. & Stephenson, R. L. (1998). The status of the herring in the Bras d'Or Lakes in 1996–1997. *Canadian Stock Assessment Secretariat Research Document* 98/80.

Fisher, R. A. (1954). *Statistical Methods for Research Workers*, 12th edn. Edinburgh: Oliver & Boyd.

Gurbutt, P. A. & Petrie, B. (1995). Circulation in the Bras d'Or Lakes. *Estuarine, Coastal, and Shelf Science* **41,** 611–630.

Hansen, M. M., Nielson, E. E., Ruzzante, D. E., Bouza, C. & Mensberg, K. D. (2000). Genetic monitoring of supportive breeding in brown trout (*Salmo trutta* L.) using microsatellite DNA markers. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 2130–2139.

Hay, D. E., McCarter, P. B. & Daniel, K. S. (2001). Tagging of Pacific herring *Clupea pallasi* from 1936–1992: a review with comments on homing, geographic fidelity, and straying. *Canadian Journal of Fisheries and Aquatic Sciences* **58,** 1356–1370.

Hedgecock, D. (1994). Does variance in reproductive success limit effective population sizes of marine organisms? In *Genetics and Evolution of Aquatic Organisms* (Beaumont, A., ed.), pp. 122–134. London: Chapman & Hall.

Hedrick, P. W. & Miller, P. S. (1992). Conservation genetics: techniques and fundamentals. *Ecological Applications* **2,** 30–46.

Iles, T. D. & Sinclair, M. (1982). Atlantic herring: stock discreteness and abundance. *Science* **215,** 627–633.

Kornfield, I., Sidell, B. D. & Gagnon, P. S. (1982). Stock definition in Atlantic herring (*Clupea harengus harengus*): genetic evidence for discrete fall and spring spawning populations. *Canadian Journal of Fisheries and Aquatic Sciences* **39,** 1610–1621.

Kornfield, I. & Bogdanowicz, S. M. (1987). Differentiation of mitochondrial DNA in Atlantic herring, *Clupea harengus. Fisheries Bulletin* **85,** 561–568.

McPherson, A. A., O'Reilly, P. T., McParland, T., Jones, M. W. & Bentzen, P. (2001). Rapid isolation of tetranucleotide microsatellites in Atlantic herring (*Clupea harengus*). *Molecular Ecology Notes* **1,** 31–32.

McQuinn, I. H. (1997). Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries* **7,** 297–329.

Messieh, S. (1975). Delineating spring and autumn herring populations in the southern Gulf of St. Lawrence by discriminant function analysis. *Journal of the Fisheries Research Board of Canada* **32,** 471–477.

Nei, M., Maruyama, T. & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution* **29,** 1–10.

O'Connell, M. & Wright, J. M. (1997). Microsatellite DNA in Fishes. *Reviews in Fish Biology and Fisheries* **7,** 331–363.

O'Connell, M., Dillon, M. & Wright, J. (1998*a*). Development of primers for polymorphic microsatellite loci in the Pacific herring (*Clupea harengus pallasi*). *Molecular Ecology* **7,** 358–259.

O'Connell, M., Dillon, M., Wright, J. M., Bentzen, P., Merkouris, S. & Seeb, J. (1998*b*). Genetic structuring among Alaskan Pacific herring (*Clupea pallasi*) populations identified using microsatellite variability. *Journal of Fish Biology* **53,** 150–163.

Olsen, J. B., Lewis, C. J., Kretschmer, E. J., Wilson, S. L. & Seebr, J. E. (2002). Characterization of fourteen tetranucleotide satellite loci derived from Pacific herring. *Molecular Ecology Notes*, in press.

Raymond, M. & Rousset, F. (1995). GENEPOP: a population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86,** 248–249.

Ruzzante, D. E., Taggart, C. T. & Cook, D. (1998). A nuclear DNA basis for shelf and bank-scale population structure in Northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. *Molecular Ecology* **7,** 1663–1680.

Scott, J. (1975). Meristics of herring (*Clupea harengus*) from the Canadian maritimes area. *Fisheries Marine Services Technical Report* **599,** 24.

Shaw, P., Turan, C., Wright, J., O'Connell, M. & Carvalho, G. (1999). Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*) with direct comparison to allozyme and mtDNA data. *Heredity* **83,** 490–499.

Sinclair, M. (1988). *Marine Populations. An Essay on Population Regulation and Speciation*. Seattle: University of Washington Press.

Sinclair, M. & Tremblay, M. (1984). Timing of spawning on Atlantic herring (*Clupea harengus harengus*) populations and the match-mismatch theory. *Canadian Journal of Fisheries and Aquatic Sciences* **14,** 1055–1065.

Smith, P. J. & Jamieson, A. (1986). Stock discreteness in herring: a conceptual revolution. *Fisheries Research* **4,** 223–234.

Stephenson, R. L. (1991). Stock discreteness in Atlantic herring: a review of evidence for and against. In *Proceedings of the International Herring Symposium* (Wespestad, V., Collie, J. & Collie, E., eds), pp. 659–666. Fairbanks: University of Alaska.

Stephenson, R. L. & Gordon, D. J. (1991). Comparison of meristic and morphometric characters among spawning aggregations of Northwest Atlantic herring (*Clupea harengus* L.). In *Proceedings of the International Herring Symposium* (Wespestad, V., Collie, J. & Collie, E., eds), pp. 279–296. Fairbanks: University of Alaska.

Stephenson, R., Rodman, K., Aldous, D. & Lane, D. (1999*a*). An in-season approach to management under uncertainty: the case of the SW Nova Scotia herring fishery. *ICES Journal of Marine Science* **56,** 1005–1013.

Stephenson, R. L., Power, M. J., Clark, K. J., Melvin, G. D., Fife, F. J., Paul, S. D., Harris, L. E. & Boates, S. (1999*b*). 1999 evaluation of 4VWX herring. *Canadian Stock Assessment Secretariat Research Document* 99/64.

Stephenson, R. L., Power, M. J., Clark, K. J., Melvin, G. D., Fife, F. J., Scheidl, T., Waters, C. L. & Arsenault, S. (2000). 2000 evaluation of 4VWX herring. *Canadian Stock Assessment Secretariat Research Document* 2000/065.

Stephenson, R. L., Clark, K. J., Power, M. J., Fife, F. J. & Melvin, G. D. (2001). Herring stock structure, stock discreteness and biodiversity. In *Herring2000: Expectations for a New Millennium* (Funk, F., Blackburn, J., Stephenson, R., Toresen, R. & Witherill, eds), pp. 559–571. University of Alaska Sea Grant, AK-SG-01-04, Fairbanks.

Swain, D. P. & Foote, C. J. (1999). Stocks and chameleons: The use of phenotypic variation in stock identification. *Fisheries Research* **43,** 113–128.

Wahlund, S. (1928). The combination of population and the appearance of correlation examined from the stand-point of the study of heredity. *Hereditas* **11,** 65–106 (in German).

Waples, R. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89,** 438–450.

Ward, R. D., Woodwark, M. & Skibinski, D. O. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *Journal of Fish Biology* **44,** 213–232.

Weir, B. & Cockerham, C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.

Wheeler, J. & Winters, G. (1984). Homing of Atlantic herring (*Clupea harengus*) in Newfoundland waters as indicated by tagging data. *Canadian Journal of Fisheries and Aquatic Sciences* **41,** 108–177.

Withler, R. E., Le, K. D., Nelson, R. J., Miller, K. M. & Beacham, T. D. (2000). Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon (*Oncorhynchus nerka*) populations of the Fraser River, British Columbia, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 1985–1998.

Wright, S. (1951). The genetical structure of populations. *Annals Eugenics* **15,** 323–335.