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Patterns of genetic variation in Scotia-Fundy Atlantic herring

by

Arran A. McPherson

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
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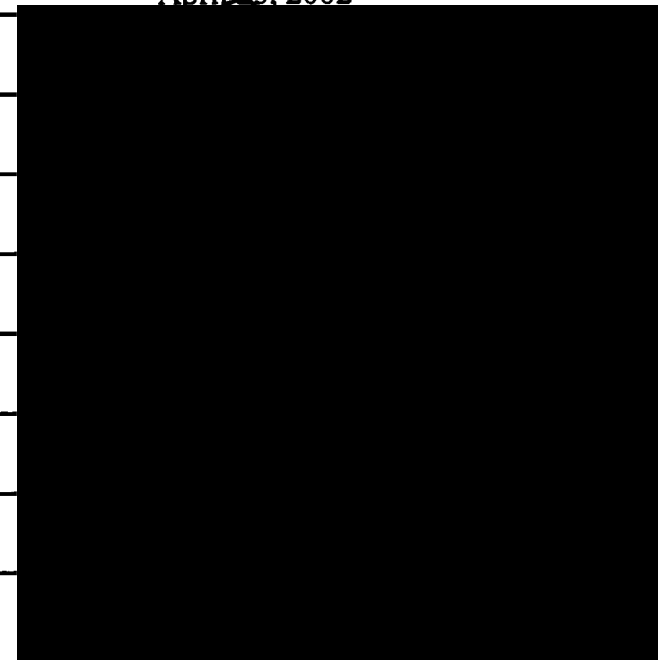
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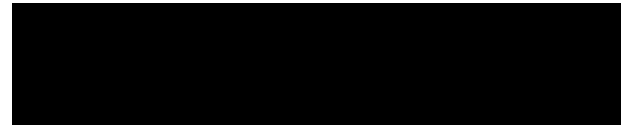
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Dedicated to the Herring Group at the St. Andrews Biological Station, St. Andrews NB
In recognition of their tireless efforts on behalf of herring

and

In memory of my mother

Dora Dawn Shepherd McPherson 1948-2001
Who would have read this work from cover to cover

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ABSTRACT

Genetic variation among Atlantic herring spawning groups and larval assemblages was assessed at a range of spatial (1000's, 100's, 10's km) and temporal (year, week, day) scales, using microsatellite DNA markers (short, tandemly repeated DNA sequences in the nuclear genome that are highly variable in repeat number). It is shown that 5 Pacific herring dinucleotide microsatellites can be amplified in Atlantic herring, and there is measurable differentiation between putative species (Pacific vs Atlantic herring; F_{ST} ~0.045), among populations at ocean basin scales (NE vs NW Atlantic; F_{ST} ~ 0.040), and at regional management scales (F_{ST} ~ <0.01), in the Scotia-Fundy herring stock complex.

Nine tetranucleotide microsatellites, the first microsatellites developed for Atlantic herring, were isolated using magnetic bead hybridization selection and were used to study 17 collections of herring (N>1400) drawn from the Scotian Shelf, the Celtic Sea, the Baltic Sea, and coastal Iceland. Differentiation was observed between NE and NW Atlantic herring (F_{ST}~0.065) and significant genetic variability was observed within NW Atlantic herring spawning groups (max F_{ST}=0.018), at a relatively small spatial scale on the Scotian Shelf. Genetic variation between Bras d'Or Lakes herring and all other collections was sufficient to hypothesize that an event associated with a small effective population size (e.g. population bottleneck) had occurred in Bras d'Or Lakes.

Temporal stability of genetic pattern, using annual collections and age data showed that among-location genetic variation was ~1.4 fold greater than within-location annual variation on the Scotian Shelf. It is shown that overlapping generations, when used for population structure analyses, may explain inconsistencies in estimates of temporal stability in marine fishes, particularly herring. There was no relationship between number of alleles found at a locus and the ability of the locus to resolve differences among the herring collections. Herring spawning waves were virtually indistinguishable from a population genetic-structure perspective, though a small difference (F_{ST}=0.0043, P=0.013) was revealed on the Scotian Shelf (Devastation Shoal) between spawners separated by 6 days within the same year class. Herring in the 2nd spawning wave were larger at age within a year-class (t-test; P<0.001). These analyses suggest that the differences can be explained by either: 1) genetically distinct Devastation Shoal populations that are temporally separated; 2) sub-annual temporal genetic patchiness; or 3) transient use of the spawning ground by different populations.

Near genetic homogeneity was observed among 14 larval herring (N>1200) collections, drawn from across the central Scotian Shelf. Genetic patterns and circulation associated with sampling locations could not easily be used to reconcile the hypothesis that larval retention or larval mixing has generated, or maintained, patterns of population structure in Scotia-Fundy herring. A suite of genetic and morphometric analyses of larval collections (coastal and offshore) indicate the larvae were likely produced by few parents. This last finding is consistent with sweepstake events, especially in light of the conflicting results (differences and lack thereof), in comparisons between larvae and the spawning adults that are presumed to have produced them.

LIST OF ABBREVIATIONS USED

Abbreviation	Definition
Bp	base pairs
BrD	Bras d'Or Lakes
BS	Barents Sea
BSA	bovine serum albumin
CTD	conductivity, temperature and depth sensor
CV	Coefficient of variation
DeSh	Devastation Shoal
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide-triphosphate
EDTA	Ethylenediaminetetraacetate
EM	Emerald Basin
EP	Eastern Passage
FAO	Food and Agriculture Organization
GeoB	Georges Bank
GrB	German Bank
He	expected heterozygosity
Ho	observed heterozygosity
HWE	Hardy Weinberg Equilibrium
IN	Inshore
Kb	Kilobase pairs
LB	10 g tryptone; 5 g yeast extract; 10 g NaCl in 1 L water; pH=7.0
MA	Maine
MDS	multidimensional scaling
mtDNA	mitochondrial DNA
N	number of samples
NAFO	Northwest Atlantic Fisheries Organization
NB	New Brunswick
NE	Northeast
NFLD	Newfoundland
NS	Nova Scotia
NW	Northwest
NWA	northwest Atlantic
OFF	Offshore
PBS	10 mM phosphate; 2.7 mM potassium chloride; 137 mM sodium chloride pH=7.0
PC	Pacific
PCR	polymerase chain reaction
RE	Restriction enzyme
RFLP	Restriction fragment length polymorphism
RV	research vessel
SB	Spectacle Buoy

ScB	Scot's Bay
Stn	Station
SSC	standard saline citrate
SW	Southwest
Ta	annealing temperature
TE	10 mM Tris; 1 mM EDTA pH=8.0
Temp	Temperature
TFH	Three Fathoms Harbour
TL	Trinity Ledge
Tris	tris(hydroxymethyl)aminomethane
V	Variance
WB	Western Bank

CHAPTER 1: GENERAL INTRODUCTION

Within the field of population ecology, the term 'population' has been defined as a self-sustaining component of a particular species whose geographical and temporal distributions can be defined (Southwood 1976). The 'population' concept has received attention in four main areas: 1) population richness (number of discrete populations) within species; 2) geographic patterns of population division; 3) absolute abundance of individuals within populations; and 4) variability among populations (Sinclair 1988). This interest in population dynamics has partly been fueled by the assumption that individuals within a population share a number of biological characters (e.g. growth rate, fecundity, morphometrics) as well as, in the case of marine fishes as reviewed by Carvalho and Hauser (1994), specific patterns of 'stock - recruitment'.

In the marine environment, ocean currents, spawning substrates, and other geographic and ecological features may provide opportunities for isolation and differentiation within species. The oceans lack obvious barriers to dispersal and migration (Waples 1998), and perhaps for this reason, many marine fishes have extended pelagic larval stages and/or migratory capabilities as adults (Harden Jones 1968). Pelagic life stages and migratory capabilities confer the opportunity to take advantage of dispersal in the marine environment. As such, delineation of populations and their geographic extent is problematic, as complex oceanographic processes, coupled with migratory capabilities of the animals themselves, may create complex spatial patterns of population structure.

Many of the basic concepts of marine fish population dynamics originate from studies on Atlantic herring (*Clupea harengus* L. e.g. Heinke 1898 reviewed by Sinclair and Solemdal 1988, Hjort 1895, 1914 reviewed by Solemdal and Sinclair 1989, Harden-Jones

1968, Cushing 1975, Iles and Sinclair 1982, Smith and Jamieson 1986) and have recognized oceanographic processes. Both Iles and Sinclair (1982) and Harden-Jones (1968) suggest that the geographic identity of each discrete spawning group is based on the fate of the larvae (retention/drift). Harden Jones (1968) hypothesized that patterns of migration (Harden Jones triangle) and passive egg and larval drift from spawning area to juvenile nursery areas, generate the observed pattern of population structure for many marine fishes. Alternatively, Iles and Sinclair (1982) proposed that larval survival depends on larvae being retained in a suitable nursery site. Larvae lost from the retention area are assumed to be lost to the spawning group and therefore fail to successfully reproduce when mature. Iles and Sinclair (1982) further argued that natal spawning ground fidelity serves as an additional mechanism contributing to population structure, but one could suggest that this mechanism is in fact sufficient to maintain population structure if fidelity is sufficiently strong.

Alternatively, Smith and Jamieson (1986) have described a conflicting explanation of herring population structure, where mixing frequently takes place among spawning groups, potentially as a result of environmental stress or fishing pressure. This exchange results in transient population structure on timescales that may be relevant to management.

The early use of Atlantic herring as a model species provides a unique opportunity to test conceptually attractive (but largely untested) hypotheses (e.g. Iles and Sinclair 1982), while investigating a contemporary management issue. With the knowledge that over-exploitation of what may be discrete populations may deplete the biodiversity of populations, thereby decreasing resiliency to environmental change, it seems timely to

address questions of population structure in herring, while investigating the potential role of oceanographic processes in generating/maintaining this structure.

Atlantic herring are a valuable (> 2.4 million metric t caught in 1999) resource in the north Atlantic ocean (Food and Agriculture Organization 2002), and recent fluctuations in catch (e.g. Stevenson 1989, Johannessen et al. 1995 and Anon 1998) have generated a need for information on the population structure of this species. In the Northwest Atlantic Fisheries Organization (NAFO) Divisions 4VWX (Scotia-Fundy region), which are managed collectively for herring, fishing takes place primarily when fish are aggregated during spawning, which varies from early spring to late autumn in the Scotia-Fundy region (Sinclair and Trembley 1984). As different spawning groups often occur in close spatial and temporal proximity, mixing of fish from what may be discrete populations has the potential to take place both prior to and after spawning. In an attempt to recognize and protect potential differences (biodiversity) among spawning groups, Canada's Department of Fisheries and Oceans has divided the 4VWX herring into three management components (Figure 1.1); however, it is recognized that there are a number of discrete spawning grounds within both the SW Nova Scotia and south shore of Nova Scotia subunits (Anon 1998). Determining whether population structure exists within and among the defined management units is necessary to avoid differential exploitation of populations. Further, the use of biological reference points developed from the aggregated data of multiple populations in fisheries models may ultimately lead to inaccurate predictions (Frank and Brickman 2000).
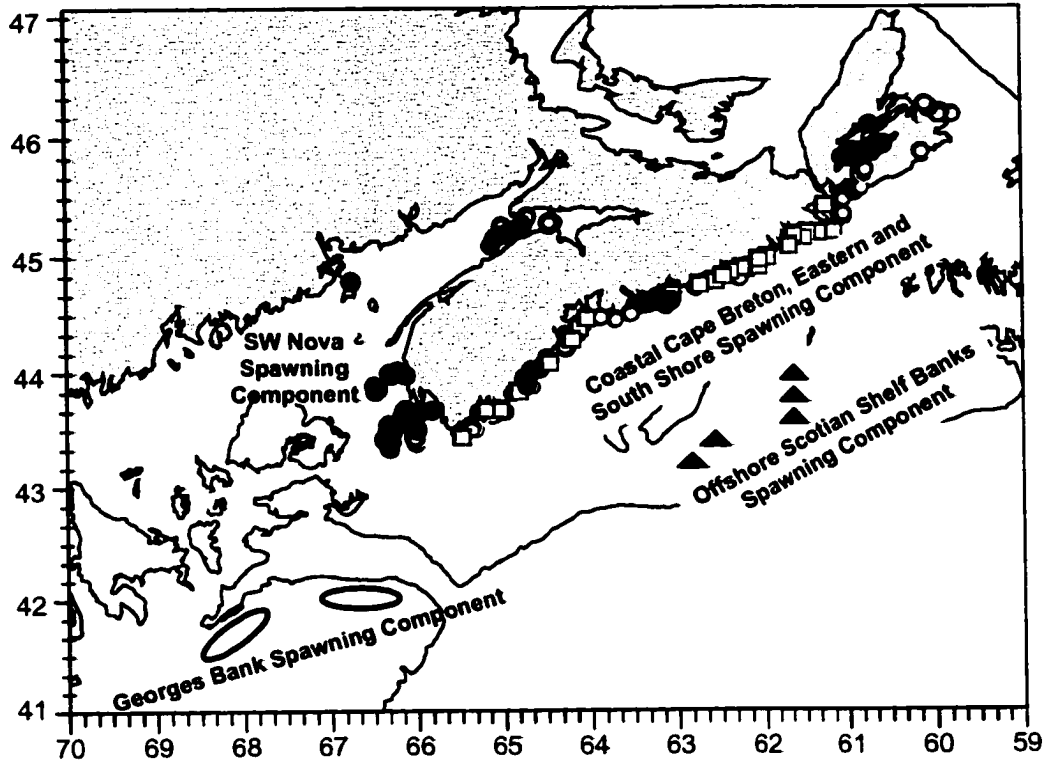
Figure 1.1
Historical (open symbols) and contemporary (filled symbols) spawning areas of Atlantic herring within the Scotia-Fundy region. Management components as defined by Department of Fisheries and Oceans are as indicated: SW Nova Scotia Spawning Component; Coastal Cape Breton, Eastern and South shore Spawning Component; and Offshore Scotian Shelf Banks Spawning Component. The Georges Bank spawning component is also indicated. Figure modified from Anon (1998).

Population structure can be delineated using a variety of techniques. Meristic, (e.g. variation in the number of vertebrae), morphometric, (e.g. variation in dimensions of body parts and/or rates of change), parasite load (e.g. variations in number and type of parasites), and demographic (e.g. age structure, fecundity, and mortality) methods have long been used in the classification of groups of marine fishes, when the means and variances of these measures have been observed to differ among populations (e.g. Messieh 1975). However, there are several limitations associated with these techniques, as the physical environment is known to influence the expression of the traits over the lifetime of the individual. This environmental influence has even greater consequence, when considering a migratory species whose range could encompass a variety of environments, hence contributing the potential for a behavioural influence on meristic, morphometric, and parasite variation among populations. Population-specific differences become difficult to interpret. By definition, selectively neutral genetic variation is not subject to confounding environmental influences, and techniques that measure this type of genetic variation may thus be the optimal tool for population discrimination of marine fish populations.

The genetic discreteness of a group of fish depends on complex evolutionary forces, including gene flow, mutation, genetic drift and natural selection. Gene flow is frequently cited as the mechanism opposing the formation of population structure in many marine fishes (e.g. Smith and Jamieson 1986), and is defined as the movement and establishment of genes into or out of a population by interbreeding, accomplished through active adult migration or larval drift, followed by maturation and spawning in "adopted" populations (MacLean and Evans 1981).

Stobo (1982) attempted to elucidate patterns of herring movement on the Scotian Shelf by initiating a multi-year tagging study of approximately 75 000 adult herring. Sexually mature (spawning stage) adult herring were tagged in the waters southwest of Nova Scotia in Aug-Sept 1974 and 1977. Tag recoveries were consistent with partial spawning ground fidelity, since 79 % of tagged fish caught in the first spawning season (post-release) were recaptured in the area where tagging took place (fish caught within 2 weeks of release were ignored), although this estimate of site fidelity decreased in consecutive spawning seasons (41% in second year, 55% in the third and 29% in the fourth to the seventh seasons). The results of tagging studies in the waters adjacent to the Newfoundland coast show a repeat spawning rate of 66-93% in herring (Wheeler and Winters 1984), demonstrating that most adults returned to the same location (presumably their spawning ground, though not necessarily their natal location).

While it has been proposed that philopatry (Iles and Sinclair 1982) is a pre-condition for genetic isolation and statistically significant population differences, there have been few studies that have considered homing precision at multiple life stages in herring over several years. Hourston (1982), however compared returns of Pacific herring at multiple life stages to spawning areas (64% return of tagged juveniles to natal areas after 3 years at sea). These results demonstrate that repeat spawning does take place, although straying from natal spawning areas is frequent. These tagging studies, however, do not elucidate the genetic consequences of dispersal – eg. the fate of the strays.

Debate over the fate of strays is not exclusive to herring. Spawning ground fidelity and straying patterns of salmon have also received considerable attention (eg. Quinn et al. 1991). In salmon, strong spawning ground fidelity has led to the formation of discrete

spawning populations (Waples 1990), despite evidence for substantial straying (eg. 9.9-27.5% for chinook salmon in the lower Columbia River; Quinn et al. 1991). The simplest explanation for the existence of genetically discrete populations, in the face of substantial straying, is that the strays rarely reproduce successfully, so gene flow is minimal.

Various biochemical markers have been used to investigate herring population structure: allozymes (eg. Jørstad et al., 1994); mitochondrial DNA (mtDNA eg. Kornfield and Bogdanowicz, 1987); and microsatellites (eg. O'Connell et al. 1998b). Ten published studies (Table 1.1) using various genetic techniques to examine population structure in Atlantic herring have essentially been unable to reject the null hypothesis of genetic homogeneity among spawning groups, on a variety of spatial scales (ocean, basin, management unit). These studies, based on allozyme and/or mtDNA markers (Table 1.1) have also shown a lack of temporal stability of genetic patterns among spawning groups of Atlantic herring (eg. Grant 1984, Ryman et al. 1984, King et al. 1987, Dahle and Eriksen 1990, Safford and Booke 1992). There are exceptions: Ridgeway et al. (1971) found few significant differences at one allozyme locus among autumn spawning populations in the northwest Atlantic; Jørstad et al. (1994) demonstrated significant differences at 3 allozyme loci between herring spawning in Balsfjord, Norway and the Atlanto-Scandian herring that dominate the coast of Norway; and Shaw et al. (1999) showed significant differences among five northeast Atlantic spawning groups with microsatellite markers. Such differences were not consistently detected with either allozyme or mtDNA methods (Turran 1997 referenced in Shaw et al. 1999).

Further, none of the studies presented in Table 1.1 fully meet the several essential criteria for population studies in migratory fishes (including herring) so their utility in

assessing the existence of population structure in Atlantic herring is somewhat compromised. Although generally more expensive and time-consuming to collect (especially on the time-scale of research grants and graduate students), it is imperative that only spawning stage herring, collected from spawning grounds, be used for genetic analysis. Many of the previous studies were not explicitly restricted to sexually mature fish in spawning condition collected from discrete spawning grounds (Table 1.1). Nor were young larvae collected at or near their source used. Herring collected from any other location or at any other life stage should not be used to assess population structure, as they may represent mixtures of different spawning groups, given the migratory nature of herring and close spatial proximity of spawning areas.

Replicate samples, collected annually, are critical in evaluating possible sources of bias and sampling artifacts in the data (Waples 1998). For example, if differences (however small) among geographically separated samples are consistent over time, one can be more confident that they represent real differences, rather than sampling artifacts. Alternatively, if relatively large differences are found among samples, but the pattern of differentiation is not consistent through time, then there are probably unrecognized biological processes involved, sampling complexities, or the data are flawed (Waples 1998). Few studies (Table1.1) with herring have had the ability to test the temporal stability of the genetic differences (eg. Kornfield et al. 1982 have suggested the differences detected by Ridgeway et al. 1971 were not temporally stable).

Despite the genetic homogeneity previously reported among local populations, Shaw et al. (1999) concluded that microsatellites were more likely to show population structure than either allozymes or mtDNA markers, presumably reflecting the greater

resolving power of microsatellites, relative to more traditional genetic markers and the recent claim that microsatellites are the most appropriate genetic tool to examine population structure (see reviews by O'Connell and Wright 1997; Neilson et al. 1997; Park and Moran 1994; Wright and Bentzen 1994).

Briefly, microsatellites are tandemly repeated DNA sequences (1-5 base pairs) of the nuclear genome that are highly polymorphic in repeat number. Each locus (series of tandem repeats) is flanked by unique DNA sequences that allow for the generation of primers to amplify the repeated sequence, using PCR (polymerase chain reaction). Variations in the length of the tandemly repeated array (the different microsatellite alleles) can then be determined by gel electrophoresis (Wright and Bentzen, 1994). Microsatellites are inherited in co-dominant Mendelian fashion and they exhibit elevated mutation rates (as compared with coding nuclear DNA) providing substantial polymorphism (many alleles). These factors, combined with an assumed absence of selection, make microsatellite markers useful in the identification and delineation of populations (Jarne and Lagoda, 1996). It has recently been suggested, however, that dinucleotide (a repeat unit of 2 base pairs) microsatellites may suffer from technical problems (as summarized in O'Reilly et al. 2000), making it difficult to determine allele sizes efficiently and precisely in all individuals.

It is noteworthy that there is a fundamental asymmetry of conclusions that can be drawn from genetic studies of the sort reported here. Stable genetic differences at neutral loci between populations are thought to constitute strong evidence that the populations are reproductively isolated but the absence of such differences does not constitute proof that the populations are not genetically, demographically, and ecologically independent

of each other (Bentzen 1998). Beyond sampling constraints, the time-scale required for differentiation of populations at neutral loci due to reproductive isolation is difficult to predict, but is thought to be on the order of 100s to 1000s of generations. Therefore, Type II error may be more important in studies of this nature due to low power associated with the statistical tests available, few loci with respect to chromosomal compliment, limited sample collections of vast population and resultant slow rate of accumulation of differences between isolated populations due to drift. In light of these limitations, models of population dynamics and conservation-oriented management strategies must address the uncertainty and obstacles (huge population sizes, sampling biases) to be faced in detecting population differences in the marine environment. Due to these obstacles and uncertainties, the articulation of specific hypotheses, the contemplation of the samples required (time and space), and the employment of methods best suited to test the hypotheses is critical to the successful application of genetic markers to fisheries questions - those related to evolution, structuring processes, conservation and management issues.

1.1 GENERAL OBJECTIVES

In this study, I assess genetic variation within and among Atlantic herring spawning groups at a number of spatial (oceans, basins, within and among management units) and temporal scales (days, weeks, years). Using these genetic differences (or lack thereof) among spawning groups as a framework, I then examine the mechanisms that may maintain the population structure observed.

Specifically, I test the null hypotheses that no significant differences exist among: 1) Pacific and Atlantic herring; 2) Northeast Atlantic herring and Northwest Atlantic herring, 3) spawning groups within the Scotia-Fundy management units (Figure 1.1); (4) years within spawning groups; (5) year-classes within collections; and (6) putative spawning waves within the same spawning season. To address these various null hypotheses, I first test five microsatellite loci developed originally for Pacific herring (O'Connell et al. 1998a) for use in Atlantic herring. I then develop tetranucleotide microsatellite loci, specific to Atlantic herring. A suite of tetranucleotide loci are then applied to spawning stage Atlantic herring collections to test the above hypotheses. The methods used to test these hypotheses are in many cases, similar. However, details of the methods in their entirety are included in the following chapters to maintain a complete record.

Using the patterns of population structure resolved in this study, I then examine the role of potential structuring mechanisms (e.g. geographic separation, retention, advection) in generating or maintaining population structure, using larval herring samples collected along the coast of Nova Scotia and offshore on the Scotian Shelf (Western Bank). These same larvae are then used to assess the potential for mixing of individuals among populations and the resulting likelihood of recolonization or resurgence of the offshore "population" in the vicinity of Western and Sable Banks, where increases in the numbers of adult herring have been documented (Harris and Stephenson 1999) after more than a decade (1970s and early 1980s) of virtual absence from groundfish research vessel (RV) survey trawl catches.

Table 1.1 – Summary of studies using a variety of genetic techniques to evaluate population structure in Atlantic herring. (S) indicates herring were explicitly reported as being in spawning stage.

Study	Site	Technique	Results	Temporal Stability
Ridgeway et al. (1971)	SW Nova Scotia, Passamaquoddy Bay Western Maine, Jeffries' Ledge MA, Massachusetts Bay, Georges Bank, Cape May	2 allozymes	limited differentiation	not tested
Kornfield et al. (1982)	Gulf of Maine (3 locales), Gulf of St. Lawrence (4 locales)	13 allozymes	negligible differentiation	
Grant (1984)	Jeffries' Ledge MA, Chaleur Bay NB Port aux Basque NFLD, Bergen, Kattegat, Baltic Sea	40 allozymes	negligible differentiation	not tested
Ryman et al. (1984)	northern Gulf of Bothnia, Baltic Sea, west Norway	13 allozymes	negligible differentiation	not tested
King et al. (1987)	Irish Sea, Celtic Sea, west of Scotland and Ireland (S)	12 allozyme	negligible differentiation	
Kornfield and Bogdanowicz (1987)	Trinity Ledge NS, Jeffries' Ledge MA, Point Escuminac NB (S)	mtDNA (6 RE)	negligible differentiation	
Dahle and Eriksen (1990)	North Sea, Skagerrak, and Kattegat (S)	mtDNA (12 RE)	no differentiation	not tested
Safford and Booke (1992)	Trinity Ledge NS, Jeffries' Ledge MA	4 allozymes	no differentiation	
Jørstad et al (1994)	Atlanto-Scandian, Balsfjord	6 allozymes	significant differentiation	not tested
Shaw et al. (1999)	Norwegian Sea, Barent's Sea, Iceland, Balsfjord, Trondheimsfjord	4 microsatellites	significant differentiation	not tested

CHAPTER 2: CHARACTERIZATION OF POPULATION STRUCTURE IN HERRING AT THREE SPATIAL SCALES

2.1 INTRODUCTION

An objective of fisheries management is to ensure the demographic variability of a stock while maintaining (even increasing) harvests. Central to this concept is the identification of "populations" that are assumed to share a number of phenotypic traits (e.g. growth rate, fecundity, morphometric measurements) as well as, in the case of marine fishes (reviewed by Carvalho and Hauser 1994), identifiable patterns of 'stock and recruitment'. Identifying population structure within and among stocks has the potential to increase our ability to anticipate the effects of harvesting on different stock components.

Population structure can be delineated using a variety of techniques. Meristic, (e.g. variation in the number of vertebrae), morphometric, (e.g. variation in dimensions of body parts and/or rates of change), parasite load (e.g. variations in number and type of parasites), and demographic (e.g. age structure, fecundity, and mortality) methods have long been used in the classification of groups of marine fishes, using differences in the means and variances of these measurements among populations (e.g. Messieh 1975). There are limitations associated with such measurements, as the physical environment is known to influence the expression of traits over the life of the individual. Convergence of non-heritable or even partially-heritable traits is possible if populations share a similar environment. This environmental influence is of greater consequence for a migratory species whose range could encompass a variety of environments; hence population-specific differences become difficult to interpret.

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Neutral genetic variation can be used to differentiate among populations, and it is not subject to confounding environmental influences. However, the utility of neutral genetic markers depends on a variety of factors, including the type and number of genetic markers used and the temporal-scale of genetic divergence among populations. The successful application of molecular genetic markers to identify population structure in freshwater and anadromous fishes that can be shown to be physically isolated (to some extent), has prompted marine fishery managers to consider genetic data in assessing population structure. Unlike the case for freshwater and anadromous fishes, however, the environment of marine species may lack obvious physical barriers to dispersal and migration (Waples 1998). Thus, delineation of marine populations with a "potentially" high capacity for gene flow (known, assumed, or otherwise) is problematic, as complex oceanographic processes, coupled with the migratory capabilities of the animals may create complex spatial patterns of population heterogeneity.

Given that relatively few successfully reproducing migrants are needed to reduce genetic divergence to low levels, and given that marine populations may be too large to diverge markedly by genetic drift (but see Hedgecock 1994), the "genetic signal" of population divergence may be small, relative to the level of "sampling noise". Therefore, measures must be taken to optimize the probability of detecting real differences (i.e., by increasing the signal to noise ratio).

Herring are an ideal model species for exploring the genetic basis of population structure, because there is more literature on herring than any other fish species, as indexed by the Food and Agriculture Organization (FAO 1999). In addition, several herring-specific population structuring mechanisms have been hypothesized (Iles and

Sinclair 1982; Smith and Jamieson 1986; McQuinn 1997) that have provided a

framework for articulating questions related to population structure. From a pragmatic

perspective, Atlantic herring (*Clupea harengus*) are a valuable resource in the north

Atlantic Ocean (> 2.4 million metric tonnes catch in 1999; FAO 2002), and recent

fluctuations in catch on historic fishing grounds (e.g. Stephenson 1997) and the

requirements for better conservation practices (e.g. Stephenson 1999) require clarification

of the population structure of this species.

Genetic analyses of Atlantic herring based on allozyme and mtDNA markers

(Dahle and Eriksen 1990 Grant 1984, Jørstad et al. 1994, King et al. 1987, Kornfield and

Bogdanowicz 1987, Ridgeway et al. 1971, Ryman et al. 1984, Safford and Booke 1992)

have been largely unable to demonstrate genetic differentiation among populations, at

management unit scales, or (in many cases) at ocean basin scales. In contrast, evidence

for homing from tagging studies (e.g. Wheeler and Winters 1984), for different

population metrics among neighbouring groups (e.g. Messieh 1975), and for predictable

variations in spawning times and locations among groups (e.g.Sinclair and Tremblay

1984) is consistent with the existence of population differentiation. Quantitative studies

that focus on measuring the degree of genetic isolation among herring populations, and

then constructing testable hypotheses about the mechanisms that can maintain genetic

isolation, are essential to resolve the inconsistency. The conflicting evidence for herring

population structure is not unique; it is shared with a variety of marine fish species.

Genetic analyses, based on variation at a suite of microsatellite loci developed for

Pacific herring (*Clupea pallasi*; O'Connell et al. 1998a), have provided evidence for

significant population structure in Alaskan herring (O'Connell et al.1998b) and NE

Atlantic herring (Shaw et al. 1999). Grant and Utter (1984) found allozyme-based differences in north Pacific herring that were not apparent in microsatellite studies of O'Connell et al. (1998b). The results of Shaw et al. (1999), demonstrating sub-basin and inter-ocean scale differences among herring, suggest that microsatellite loci may be effective in quantifying population structure in NW Atlantic herring. Microsatellite markers may detect structure at finer spatial and temporal scales than many other genetic markers due to higher levels of variability, thought to be a result of higher rates of mutation (Bentzen 1998).

Here I quantify population structure in herring at three spatial scales. Atlantic herring (NE and NW) are compared with Pacific herring to quantify the degree of differentiation between populations that are clearly isolated geographically, but whose life history similarities suggest a subspecies relationship (Svetovidov 1963, as outlined in Jørstad et al. 1994). Atlantic herring collected from the NE and NW Atlantic are then used to assess differentiation at the Atlantic basin scale, over which distances the potential for genetic exchange might be presumed to be negligible. Finally, genetic variation among herring collected from three locations (NAFO Division 4X) in the NW Atlantic are assessed at the management scale, where the potential for genetic exchange might be presumed to be greater than at the ocean basin scale.

2.2 METHODS

Spawning-stage Atlantic herring (blood and/or muscle samples) were collected (Figure 2.1) from the NW Atlantic at Spectacle Buoy (SB 43.618° N 66.124° W) and Scot's Bay (ScB 45.224° N 64.976° W). Mature fish (non-spawning) were also collected in the vicinity of Emerald Basin (EM 44.294° N 62.376° W). DNA extraction procedures

for NW Atlantic samples follow that of Ruzzante et al. (1996). Five dinucleotide

microsatellite loci, *Cha*17, *Cha*20, *Cha*63, *Cha*113 and *Cha*123 were amplified

(annealing temperatures were modified (Table 2.1) from those in O'Connell et al. 1998a)

and scored. Four microsatellites (17, 20, 63, 113- allelic data from Shaw et al. 1999;

personal communication, P. Shaw) were assayed in the NE Atlantic herring, represented

by Barents Sea (BS 70.53° N 31.583° E) and Norwegian spring spawning herring

(spawning stage), and in Pacific herring (non spawning stage) collected seaward of

Vancouver Island (PC). Single locus statistics (allele sizes, number of alleles, observed

heterozygosity) were calculated for all herring samples and conformation to Hardy-

Weinberg equilibrium (HWE) was tested for all loci in all collections (Miller 1997) to

assess random allele assortment (non-assortative mating) within populations.

F_{ST} estimates (Wright 1951, as amended by Weir and Cockerham 1984) of

population structure were calculated using F-STAT (Goudet 1996), assuming an infinite

allele model of microsatellite mutation. R_{ST} (Slatkin 1995) estimates were calculated

using R_{ST} -CALC (Goodman 1997) assuming a stepwise mutation model.
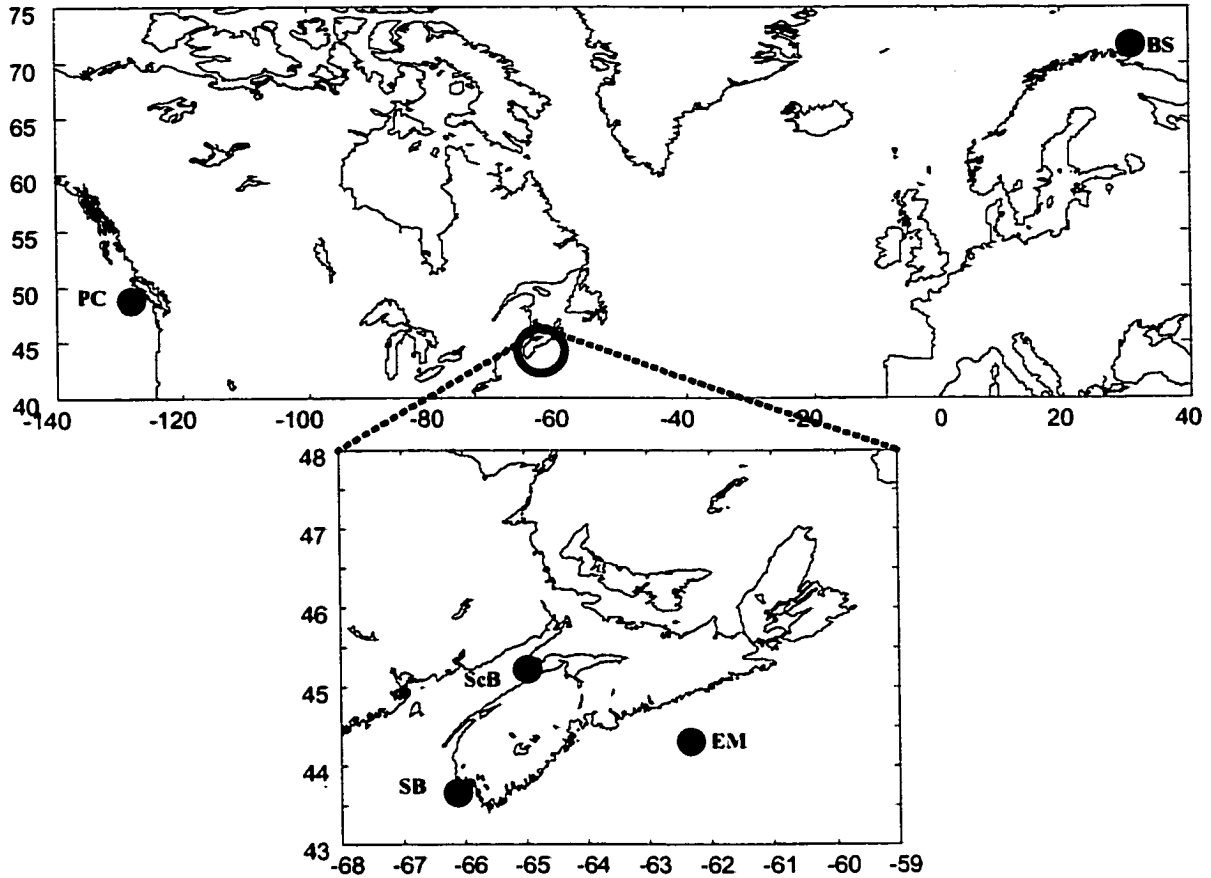
Figure 2.1
Sample locations of Atlantic and Pacific herring. Abbreviations are as follows: Pacific
(PC) herring collected seaward of Vancouver Island; Atlantic herring from the Barents
Sea (BS); Atlantic herring collected from Emerald Basin (EM); Atlantic herring collected
from Spectacle Buoy (SB); and Atlantic herring collected from Scot's Bay (ScB).

Allele size data were expressed in terms of standard deviation units from the mean for R_{ST} analyses, as recommended by Goodman (1997), to minimize effects of unequal variance among loci and unequal sample sizes. Permutation tests (1000 re-sampling trials per comparison) were used to determine significance values for all tests. I also employed an Exact test (Raymond and Rousset 1995) to assess the statistical significance of locus-specific allele frequency differences between pairs of population samples (Miller 1997), providing an assessment of discriminatory utility for each locus.

Alleles (base pairs; bp) were binned at each locus, prior to parameter estimation, to facilitate inter-lab comparisons of microsatellite data. The number of bins and bin-widths were determined at each locus by progressively increasing the number of bins and then comparing the binned frequency distribution to the original non-binned distribution of all samples pooled for each locus individually. The fewest number of bins (and thus maximum bin width) that provided a frequency distribution not significantly different from the original distribution (Kolmogorov-Smirnov $P>0.05$) was chosen to establish an objectively based classification criterion. In all cases, bin-widths were found to meet this criterion at 4 bp. Consequently, "allele classes" differ by 4 bp for each locus in all comparisons.

2.3 RESULTS

Single locus statistics (allele sizes, number of alleles, observed heterozygosity) were similar across all samples (Table 2.1) and results for the NW Atlantic (SB, ScB, and EM) are comparable to those reported for Pacific and NE Atlantic herring (O'Connell et al. 1998b, Shaw et al. 1999), with the exception of one locus (*Cha*20) in the Barents Sea,

Table 2.1
Descriptive statistics for microsatellite DNA analysis of herring samples showing sample size (N), the number of alleles per locus, annealing temperature of locus (Ta °C), range of allele sizes in base pairs (bp), observed heterozygosity and the χ^2 value/probability associated with Hardy-Weinberg equilibrium (HWE) estimates (Miller 1997).

	Sample		
	Pacific Vancouver N=30	NE Atlantic Barents Sea N=50	NW Atlantic Emerald Basin N=40 Scot's Bay N=50 Spectacle Buoy N=49
Cha17 Ta=52			
No. of alleles	21	22	23
Allele size (bp)	96-154	90-152	96-170
Heterozygosity	0.93	0.94	0.89
HWE	0.01/0.99	1.44/0.23	0.94/0.33 EM 0.02/0.64 ScB 1.65/0.19 SB
Cha20 Ta=52			
No. of alleles	15	27	25
Allele size (bp)	108-180	96-158	96-186
Heterozygosity	0.77	0.72	0.93
HWE	0.020/0.66	8.62/**0.003**	0.16/0.69 EM 0.11/0.65 ScB 0.49/0.48 SB
Cha63 Ta=52			
No. of alleles	17	13	13
Allele size (bp)	128-166	126-156	130-174
Heterozygosity	0.77	0.84	0.88
HWE	0.01/0.99	0.50/0.50	0.70/0.4 EM 0.64/ 0.46 ScB 1.23/0.27 SB
Cha113 Ta=50			
No. of alleles	18	16	18
Allele size (bp)	100-150	104-134	94-132
Heterozygosity	0.87	0.77	0.93
HWE	0.54/0.46	3.26/0.06	0.01/0.94 EM 0.5/0.5 ScB 0.02/0.90 SB
Cha123 Ta=50	N/A	N/A	
No. of alleles			23
Allele size (bp)			152-222
Heterozygosity			0.93
HWE			0.5/0.5 EM 0.45/0.49 ScB 0.30/0.58 SB

no population deviated significantly from Hardy-Weinberg Equilibrium - i.e. consistent with samples being drawn from within randomly mating populations.

All pairwise R_{ST} and F_{ST} estimates between the Pacific (PC) and each of the four Atlantic (BS, SB, ScB, and EM) collections were significant (Table 2.2). Significant population differentiation, as inferred by both F_{ST} and R_{ST} analyses, was also observed between the NE and NW Atlantic (see Table 2.2; pairwise comparisons between the NE and each of the NW samples). At the smallest spatial scale investigated (within the NAFO Division 4X region) significant structuring, using both F_{ST} and R_{ST}, was observed within herring from Spectacle Buoy and Emerald Basin, though the magnitude of this difference was half that observed at the basin scale. No significant differences were observed between Scot's Bay herring and either Emerald Basin or Spectacle Buoy herring.

An Exact test showed locus-specific significant differences among populations (Table 2.3). All loci were useful (i.e. reported significant differences) in discriminating among Atlantic and Pacific herring. However, as the geographic scale of the comparisons decreased from ocean scale to management unit scale, fewer loci were of discriminatory value. In fact, two to three loci showed significant comparisons and included Cha123 - a locus not used by Shaw et al. (1999). When single-locus pairwise comparison results were pooled over all loci, the same population pairs showed significant differences as did F_{ST} and R_{ST}.

Table 2.2
Pairwise F_{ST} above the diagonal (θ/p) and R_{ST} below diagonal (ρ/p) estimates of population structure in herring collected from the northeast (Barents Sea), northwest (Spectacle Buoy, Emerald Basin and Scot's Bay) Atlantic and the Pacific. All significant results remain significant at p<0.05 after Bonferroni correction (Manly 1985).

Location	Spectacle Buoy	Emerald Basin	Scot's Bay	Pacific	Barents Sea
	5 loci	5 loci	5 loci	4 loci	4 loci
Spectacle Buoy		0.008/p<0.01	0.006/p=0.11	0.047/p<0.01	0.039/p<0.01
Emerald Basin	0.039/p=0.002		0.005/p=0.16	0.046/p<0.01	0.060/p<0.01
Scot's Bay	0.025/p=0.15	0.013/p=0.21		0.042/p<0.001	0.028/p<0.01
Pacific	0.098/p<0.0001	0.068/p=0.001	0.073/p<0.001		0.060/p<0.01
Barents Sea	0.193/p<0.0001	0.290/p<0.0001	0.180/p<0.001	0.185/p<0.0001	

Table 2.3

Pairwise Exact test results for allele frequency differentiation (Raymond and Rousset 1995) at each locus. PC=Pacific; BS=Barents Sea; EM=Emerald Basin; SB=Spectacle Buoy ScB=Scot's Bay. Shaded areas indicate significant differences (p<0.05) between samples for that locus. * indicates combined Exact test probability over all loci is significant at p<0.001. N/A: no data available for one of the populations at this locus.

Locations	Locus Cha 17	Cha 20	Cha 63	Cha 113	Cha123
*PC-BS	P=0.0020			P<0.001	N/A
*PC-EM	P=0.055			P=0.002	N/A
*PC-ScB	P=0.008			P<0.001	N/A
*PC-SB	P=0.007			P<0.001	N/A
*BS-EM	P=0.56	P=0.037	P<0.001	P<0.001	N/A
*BS-ScB	P=0.31			P<0.001	N/A
*BS-SB	P=0.053			P<0.001	N/A
EM-ScB	P=0.65	P=0.43	P=0.100	P=0.04	P=0.049
*EM-SB	P=0.88	P=0.24		P=0.071	P=0.050
ScB-SB	P=0.65	P=0.12	P=0.14	P=0.002	P=0.050

2.4 DISCUSSION

These results show significant differences in herring at three spatial scales,

ranging from inter-ocean (Atlantic vs Pacific) to those within a NW Atlantic management

unit. The differences among Atlantic and Pacific herring agree with Svetovidov (1963),

as outlined in Jørstad et al. (1994), who argued a subspecies relationship, based on an

analysis of biological traits, with Grant (1984) who argued a distinct species relationship

from genetic (allozyme) information, and with Domanico et al. (1996), who estimated a

3.1 million year divergence time (corresponding to the mid-Pliocene) between Atlantic

and Pacific herring, assessed with ribosomal DNA sequence variation.

The differences detected between NE and NW Atlantic herring are inconsistent

with the results of Grant (1984), who was unable to establish the existence of structure at

basin scales, using 40 allozyme loci. These results show that microsatellite loci can be

used to resolve population structure at ocean basin scales. At the finest geographic scale

considered (NAFO Division 4X in the NW Atlantic), differences were observed between

herring from Emerald Basin and herring caught at Spectacle Buoy. While it is necessary

for these differences to be tested in subsequent years to be of direct use for management

purposes, these results reveal microsatellite-based genetic evidence for management scale

population structure of herring in the NW Atlantic.

The implications of management scale population differences are in some ways

unique for 4X herring, as Scotia-Fundy herring are currently managed using an "in

season management approach", as described by Stephenson et al. (1999). Each spawning

ground is assessed (in season) and spawning-ground-specific quotas are established

accordingly. The results presented here lend support to the precautionary management

currently employed. However, for most other marine fish management scenarios, there has not been this degree of precautionary management. The failure to recognize discrete populations within a stock complex may explain both the collapse and recovery failure in many marine fish populations (Frank and Brickman 2000). In fact, those authors suggest that when biological reference points are developed from aggregate (stock) data representing distinct (unit) populations, and are then employed in conventional assessment models, the results are likely to be inaccurate and non-conservative.

However, the results of the within management unit comparisons should be interpreted with caution. As herring are thought to exhibit spawning ground fidelity (potentially to their natal site), and are known to mix at other seasonal stages (e.g. feeding), it is essential that spawning stage herring collected from the spawning ground be used to characterize population structure. While I can be reasonably confident that the Pacific herring do not to mix with either the NE or the NW Atlantic populations, the assumption of no mixing is problematic within the 4X management unit comparisons, due to the close geographic proximity of sampling locations. This is particularly important when sampling different spawning groups that may overlap in time and space, outside the spawning period or location, as is typically the case for comparisons at management unit scales. Thus, the state of the Emerald Basin herring sample (not in spawning condition) limits the utility of these results, beyond providing evidence for small-scale population structure in the Nova Scotia and Bay of Fundy management unit. In addition, the issue of temporal stability must be addressed as the next step in avoiding the sampling artifacts possible with such a highly migratory fish (Waples 1998). However, migration and mixing among adults does not necessarily imply reproductive

mixing (i.e. gene flow) among putative populations, especially when considering those that exhibit spawning-ground fidelity.

Although differences were detected at all spatial scales observed, the magnitude of differences observed at the species/subspecies comparison (F_{ST} <0.05) of Atlantic and Pacific herring reflect less differentiation than expected. This may be due to the high rates of mutation assumed for microsatellite loci and the likelihood of convergence of allele sizes. F_{ST} was designed for use with characters that are considerably less polymorphic than microsatellites. Nevertheless, it is routinely applied to allele frequency data generated by any of several molecular markers. The statistic can be interpreted as a ratio of the expected heterozygosity of an individual in an equivalent random mating total population minus the expected heterozygosity of an individual in an equivalent random mating sub-population, relative to the expected heterozygosity of an individual in an equivalent random mating total population. Thus, F_{ST} effectively estimates heterogeneity within sub-populations relative to the total population. In doing so, the magnitude of the statistic is influenced by high levels of heterozygosity. Consider an extreme example involving 2 populations, each with an expected heterozygosity of 95% but with no alleles in common. The maximum pairwise F_{ST} value possible would be 0.05 (i.e. less than or equal to the homozygosity). Therefore, as Hendrick (1999) illustrated, the actual upper limit of the F_{ST} statistic is limited by the homozygosity (1-heterozygosity). Therefore, should I consider standardizing the F_{ST} value reported against the maximum F_{ST} possible given the observed homozygosities? If shown to be robust, this may prevent misleading interpretations of small F_{ST} values, that may correspond to very significant differences among groups of marine fish as responsible managers faced with difficult decisions may

not fully appreciate the subtleties of the statistic that is easily and mistakenly interpreted (in its most frequently used form) as the proportion of the maximum possible differentiation.

When applying F_{ST} to address marine fish population structure, the magnitude of the error associated with each estimate is of importance. For marine species with potentially high gene flow, the expected F_{ST} error estimates (due to non-random sampling) can be of a similar magnitude to the estimate itself when small samples sizes are used (<50). Therefore, following Waples (1998), expected random sampling error (1/2n) should be estimated for a given sample size (n) and used when interpreting results. For example, the significant ($P<0.05$) Emerald Basin to Spectacle Buoy comparison had an estimated F_{ST} value of 0.008 and the potential sampling error associated with that estimate was 0.005, which is close the estimated F_{ST} value. Thus, temporally replication would be useful to substantiate the results reported here.

Because of the complexities involved with interpreting results averaged over loci (i.e. the error is dependent on sample size and independent of the significance of F_{ST}), I advocate considering the pair-wise population comparisons at each locus (e.g. Exact test; Table 2.3). Significant differences at two to four loci were found in each pairwise comparison suggesting that all populations (including those within management unit 4X) are at least partially reproductively isolated and should therefore be considered distinct if differences can be shown to be reproducible and temporally stable. Bentzen (1998) advocates that if even one of several loci yield a significant result, it may be biologically meaningful, given the obstacles (huge population sizes, sampling biases) faced in detecting legitimate population differences in the marine environment.

In summary, dinucleotide microsatellites developed for use in Pacific herring were successfully applied to NW Atlantic herring. Highly significant species-level differences were observed between Atlantic and Pacific herring. However, differences between NE and NW Atlantic herring were of equal or greater magnitude. Given the estimates of divergence time between Pacific and Atlantic herring, I suggest that homoplasy may be responsible for the level of differentiation detected between Atlantic and Pacific herring relative to the pan Atlantic comparisons. A small (but significant) difference was also detected at the spatial scale of the management unit. These analyses were dependent on one sample of non-spawning fish and should therefore be interpreted with caution.

CHAPTER 3: ISOLATION AND CHARACTERIZATION OF TETRANUCLEOTIDE MICROSATELLITES IN ATLANTIC HERRING

3.1 INTRODUCTION

Genetic analyses of Atlantic herring, based on allozyme and mtDNA markers have detected limited genetic differentiation among populations at different spatial scales, whereas genetic structuring has been reported (Northeastern Atlantic herring: Shaw et al. 1999; Northwest Atlantic herring: Chapter 2) using dinucleotide microsatellite loci developed for Pacific herring (*Clupea pallasi*; O'Connell et al. 1998a).

Dinucleotide microsatellites have a two base-pair repeat motif and are the most frequently used class of microsatellites (Jarne and Logada 1996). This may be due to their great abundance in most genomes and their resulting ease of isolation. Beckmann and Weber (1992) estimate one microsatellite locus every 30 Kb of DNA in mammals. However, shortcomings of these types of markers include difficulty in the reliability of scoring alleles, due to overlapping PCR induced artifacts (Figure 3.1). Therefore, additional loci, less prone to PCR induced stutter and specific to Atlantic herring will be useful for future applications.

The objective of this research was to isolate and characterize tetranucleotide microsatellites from Atlantic herring, which if successful, would be the first microsatellite markers developed for this species, to better address questions related to population structure and the maintenance of population structure in herring.

3.2 METHODS

Microsatellites were isolated as outlined in Hamilton et al. (1999), with minor modifications. DNA was obtained from ethanol-preserved Atlantic herring muscle, using

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phenol-chloroform extraction methods. DNA (assuming three different concentrations) was digested with HaeIII, dephosphorylated, and ligated to the double stranded SNX linker complex (Hamilton et al. 1999), in the presence of XmnI. Ligation products were hybridized to 5' biotinylated oligonucleotides ((GACA)$_4$ and (GATA)$_4$) at 48°C for 30 minutes, then complexed with streptavidin-coated magnetic beads (Dynabeads; Dynal), previously washed with PBS-0.1%BSA. The bead-hybridization mixture was incubated at 43°C for one hour to allow beads to bind to DNA-oligo hybrids and then washed 3 times (using a new tube for each wash) with decreasing concentrations of standard saline citrate (SSC; 2X = 0.3 M sodium chloride, 30 mM sodium citrate, pH 7.0; 1X, 0.5X with 0.5ng·µl^{-1} of forward linker primer) at 48°C to remove free (non-bound) DNA. Following washes, enriched DNA was released after incubation with 30 µl TE (10 mM Tris; 1 mM EDTA) at 95°C for 15 minutes. However, this denaturation step was not necessary to amplify the enriched DNA because both the DNA recovered from the beads and the bead-hybrid mixture were successfully used as templates in PCR reactions. PCR (0.01% Tween 20, 75 mM Tris-HCl pH 8.8, 20 mM (NH$_4$)$_2$SO$_4$, 2.5 mM MgCl$_2$.0.8 mM dNTPs, 10 pM forward SNX primer; 0.2 U Taq DNA polymerase MBI in a final reaction volume of 20 µl) was carried out in a Techne Genius thermocycler as follows: 1x92°C for 5 minutes, 40x (94°C for 45 seconds, 62°C for 1 minute, and 72°C for 2 minute), 1x 72°C for 30 minutes. PCR products were purified using Qiaquick PCR columns (Qiagen), and ligated into the TOPO 2.1 TA vector (Invitrogen). Invitrogen ONESHOT Escherichia coli were transformed with recombinant plasmids and plated at low density on warm LB plates containing 50 µg·ml^{-1} ampicillin.

Table 3.1

Core repeat, primer sequences (5'-3'), Genbank accession number, annealing temperature (Ta °C), range of allele sizes (bp), number of alleles, and observed (H_o) and expected heterozygosity (H_e) in 50 Atlantic herring from each of Scot's Bay (Northwest Atlantic) and Celtic Sea (Northeast Atlantic). Deviations from Hardy-Weinberg expectations are indicated as [+] $P<0.05$ and * significant after Bonferroni correction. Genotypic disequilibrium detected [1] Scot's Bay $P =0.014$.

Locus	Core Repeat	Primers	Accession Number	Ta °C	Range	#Alleles	He	Ho
1005[1]	GACA	L:TgCAAgATAgAgTCACAg R:ggggACAgAACCAACTTCAC	AF304359	52	120-232	31	0.93/0.94	0.80[+]/0.72*
1014	GATA	L:TCCTAAACCAACCCCTgTgA R:ATTATTgTgTTAAATTTgACAgACC	AF304360	58	136-460	56	0.98/0.98	0.78*/0.84*
1017	GATA	L:ggTCTCATTATCTTCTCACTCTTTTg R:TCTCCCCTATgTgTATTgTTTTACTgTg	AF289096	55	162-206	14	0.83/0.83	0.82/0.66[+]
1020	GACA	L:CCTggAgAgACAgATAgAAAA R:gAgTTTAgCAgACgCTTTA	AF289095	55	160-224	19	0.93/0.93	0.98/0.88
1027	GACA	L:ATTCAACCCCTACAAgC R:TgAggCAgCAgACgATACAC	AF290885	60	110-202	24	0.93/0.93	0.98*/0.92
1045	GATA	L:CATTAgggATggCTCTgC R:CCAgAAAAgAAgTCCCAgATg	AF304361	57	76-208	32	0.92/0.94	0.90/0.96
1059[1]	GACA	L:CATCTACCACCTCCgACTCC R:AATCTAAAggAAgCCCACTC	AF289094	54	66-108	12	0.66/0.72	0.66/0.70
1202	GACA	L:TTTCCgTTACACTTTCACATCg R:gTgCCTCAgTTTTCACATACA	AF304363	52	92-148	14	0.80/0.85	0.90/0.88
1235	GACA/GATA	L:gCCCCTCCCTCTgTCTTTTA R:AcgATggAggTAgTgTgC	AF304362	58	128-404	53	0.97/0.98	0.85*/0.88*

Plasmid DNA was prepared using a Qiaprep spin miniprep kit (Qiagen) and cycle-

sequencing was performed using a universal reverse primer, Thermo-SequenaseII

(Amersham Pharmacia Biotech) chemistry and the following conditions: 25 cycles of

96°C for 30 s, 50°C for 15 s and 60°C for 1 minute on a Techne Genius thermocycler.

Sequence was determined using an ABI 373A DNA sequencer. Of the 72 cloned DNA

segments sequenced, 56 contained tetranucleotide microsatellites, of which 42 were

unique (Table 3.1).

Prior to the selection of loci to be screened for variability, flanking regions were

scrutinized for the presence of repetitive elements. To be of optimal value, loci should be

free of repetitive DNA regions in the flanking sequence of the microsatellite and should

include few if any intervening bases within the repeat motif (imperfect repeats). Loci

possessing the characters described above could exhibit heterogeneity in repeat units,

thereby creating non-uniform sizes of PCR-amplified alleles that would complicate size

determination of alleles and subsequent interpretation of data. To avoid these

complications, loci displaying post-PCR product length variation that did not conform to

expected patterns based on repeat length, were not considered further.

Primers flanking the microsatellite regions were designed with the assistance of

Primer3 (Rozen and Skaletsky 1998) and Gene Runner (Ver.3 Hastings Software).

Microsatellites were PCR-amplified (see Table 3.1 for specific annealing temp) using the

following conditions: 5x (92°C for 1 minute, Ta-(1-5 °C) for 30 seconds, 72°C for 15

seconds); 25x (92°C for 1 minute, Ta for 30 seconds, 72°C for 15 seconds); 72°C for 30

minutes on a PTC-100 MJ Research thermocycler. Reaction mixes contained ~ 20-100 ng

template DNA, 0.01% Tween 20, 75 mM Tris-HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 2.5 mM

$MgCl_2$ 0.8 mM dNTPs, 5 pM of each primer (R primer 5' end-labeled with a fluorophore e.g. fluorescein or HEX), and 0.1 U *Taq* DNA polymerase (MBI) in a final reaction volume of 10 µl. Amplified products and a 4bp allelic ladder were electrophoresed at 850 V on 0.8 mm 8% denaturing-polyacrylamide gels for ~ 1 hour. DNA fragments were visualized on an FMBIO II fluorescent imaging system (Hitachi Software Engineering).

Twenty-four loci were screened for variability (Figure 3.2) and extensive optimization methods were attempted; eight loci were monomorphic. Of the remaining loci, nine (Table 3.1) could be consistently amplified (>98%) and yielded repeatable genotypes for test individuals (n=100 herring; e.g. Figure 3.3). Therefore, these nine loci were tested for genotypic disequilibrium and departure from Hardy Weinberg expectations (Genepop; Raymond and Rousset 1995) in 50 Atlantic herring from each of Scot's Bay (Northwest Atlantic) and Celtic Sea (Northeast Atlantic).

3.3 RESULTS AND DISCUSSION

The magnetic bead hybridization selection protocol used here yielded an 80% enrichment for tetranucleotide microsatellites (Figure 3.2). Array lengths (alleles) varied from 10 to greater than 50 repeats. The loci selected for consideration were polymorphic (12-56 alleles per locus) and exhibited high levels of observed heterozygosity (0.66 to 0.98). Loci with > 50 repeats were not considered for use, as larger alleles require prohibitively lengthy eletrophoresis to be fully resolvable.

Association between loci, measured as genotypic disequilibrium, was observed between *Cha*1005 and *Cha*1059 in the Scot's Bay population (Table 3.1). While this

association was not seen in the Celtic Sea population, the possibility of physical linkage

(and thus lack of independence) of these loci cannot be dismissed.

With the exception of *Cha*1005, *Cha*1014 and *Cha*1235, the loci showed no

significant deviation from Hardy-Weinberg expectations (Table 3.1). The large range in

allele sizes at *Cha*1005, *Cha*1014, and *Cha*1235 (e.g. in excess of 275 bases for *Cha*1014

and *Cha*1235) may lead to PCR-based bias because efficient amplification of the smaller

allele effectively out-competes amplification of the larger allele and results in upper allele

drop out. Therefore, these loci may be of greater value for applications where

conformation to Hardy-Weinberg expectations are not assumed or necessary. In the

following chapters, six (and five-Chapter 7) tetranucleotide microsatellites developed for

Atlantic herring and 3 tetranucleotide microsatellites developed (Olsen et al. 2001) for

Pacific herring are used.

Typical Dinucleotide Microsatellite locus
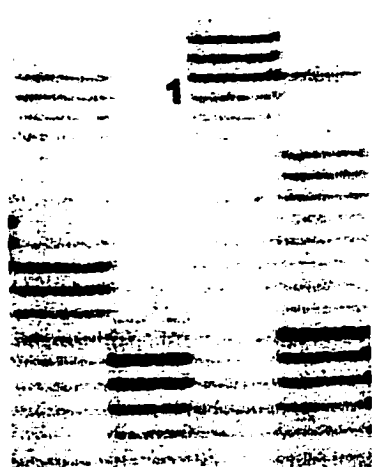
Typical Tetranucleotide Microsatellite locus
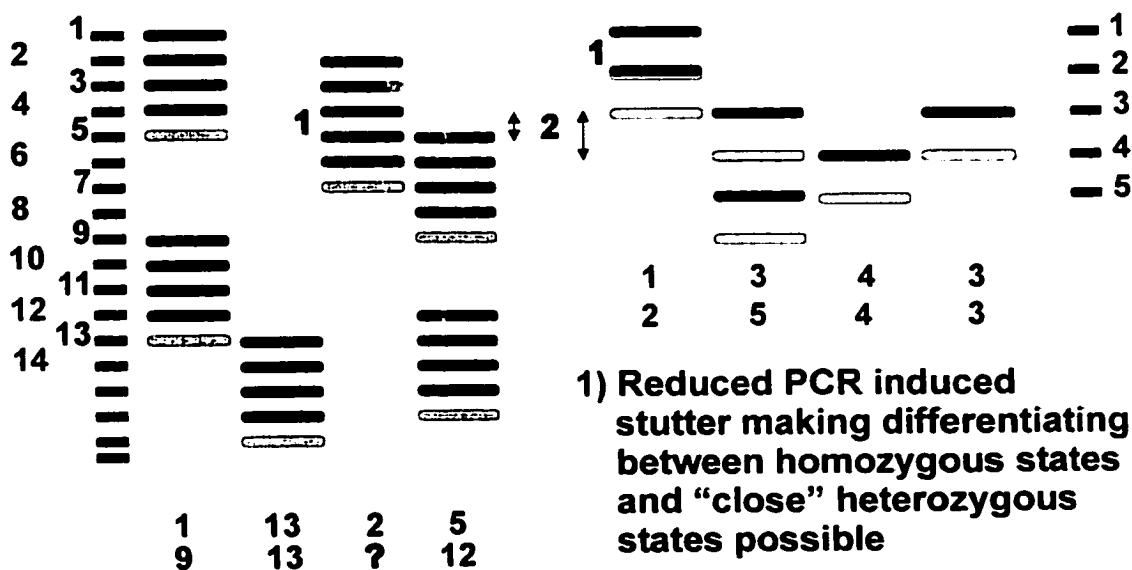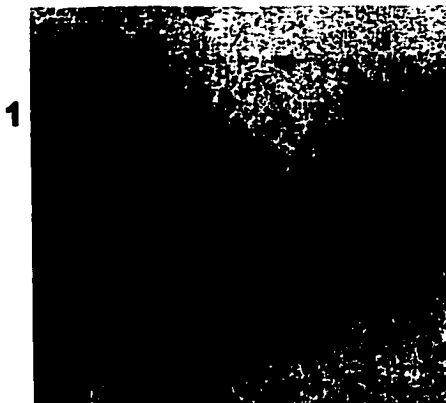
1) **Reduced PCR induced stutter making differentiating between homozygous states and "close" heterozygous states possible**

2) **Greater separation (distance) between alleles**

Figure 3.1 Schematic illustrating practical advantages of tetranucleotide microsatellites over dinucleotide microsatellites. Top panels show typical dinucleotide and tetranucleotide microsatellites from gel images and bottom panels show a diagram of the banding patterns observed for each individual. Allelic ladders appear beside both diagrams to illustrate the derivation of genotypes listed below each individual. In both cases. four individuals are being represented. There is reduced PCR induced stutter (represented by faint horizontal lines beneath the main band) in the tetranucleotide example and less ambiguity in assigning genotype.

No tetranucleotide
microsatellites

0.2

Duplicated
tetranucleotide
microsatellites

0.2

Contained unique
tetranucleotide
microsatellites

0.6

PCR primers
were designed

0.45

0.02 Too many repeats

0.08 Imperfect or
compound repeats

0.3

No flanking
sequence

0.15

Contained repetitive
elements
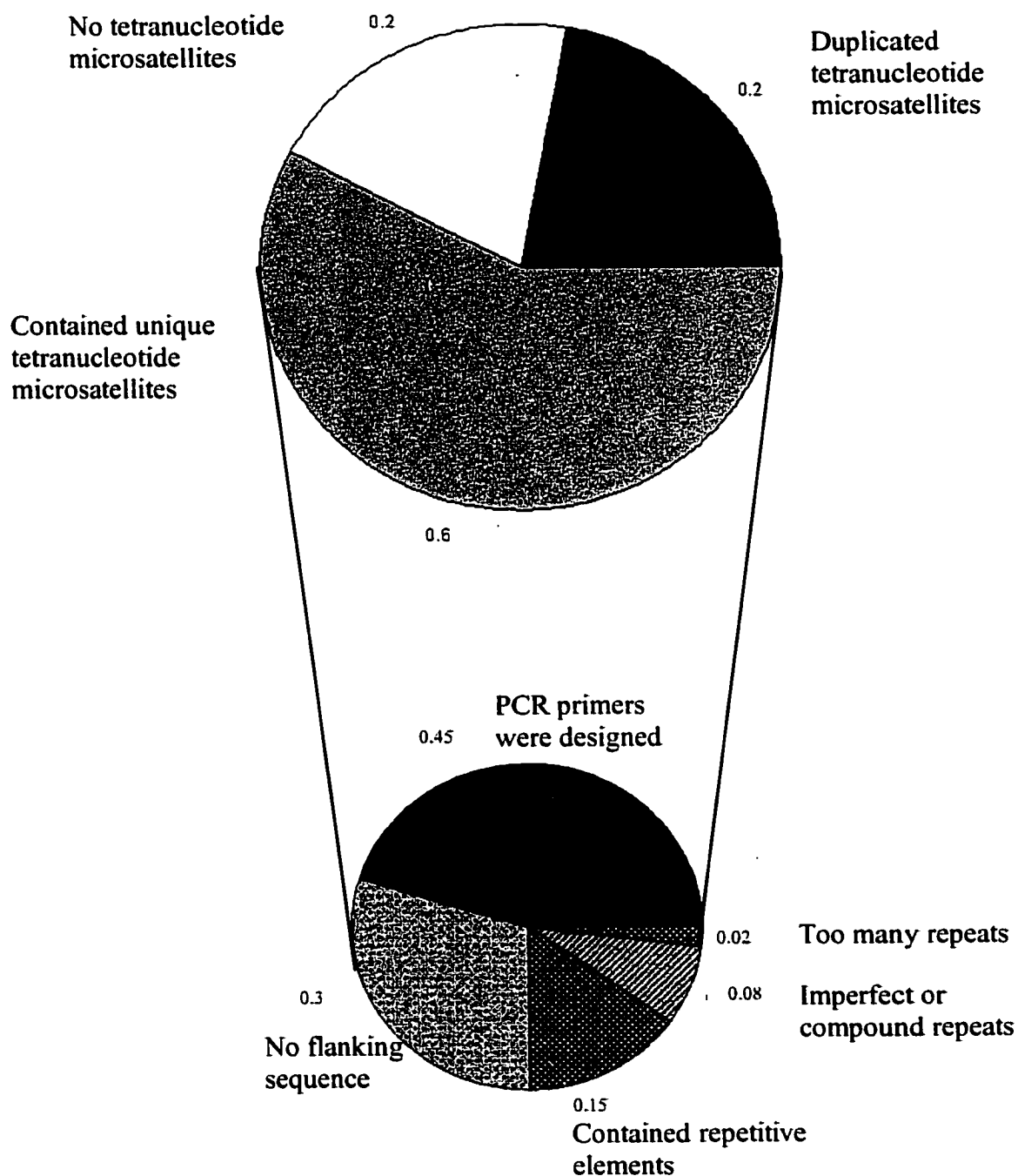
Figure 3.2
Schematic illustrating microsatellite development. The upper pie chart shows proportions
of sequences examined that contained duplicated or unique tetranucleotide microsatellites
or neither. The lower pie chart shows the proportion of the unique microsatellites that
were excluded from PCR trials and the explanations for exclusion.
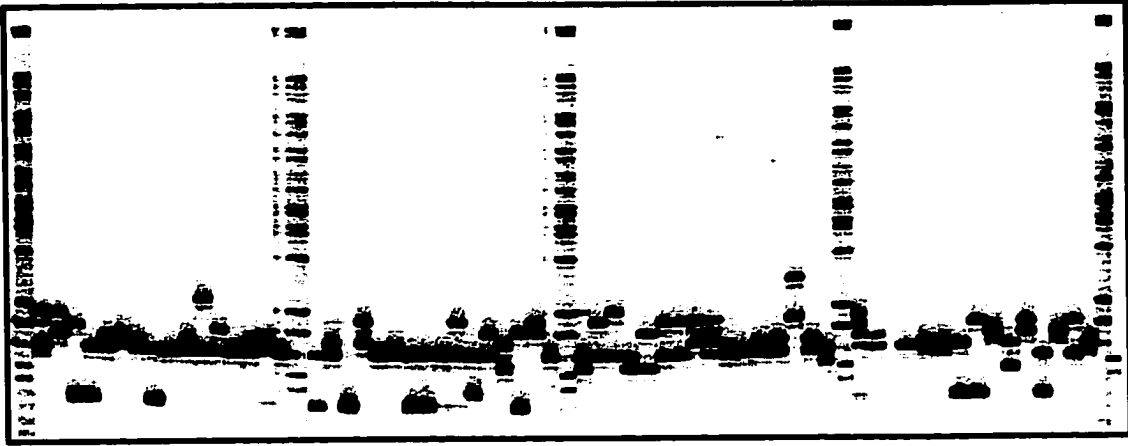
Figure 3.3
Gel image showing *Cha*1202 for 64 Northwest Atlantic herring samples interspersed among five allelic standards (lanes with multiple bands used to score sample allele sizes). Each vertical lane with one or two bands represents an individual genotype (homozygote or heterozygote respectively).

CHAPTER 4: GENETIC DIVERSITY OF COASTAL NORTH-WEST ATLANTIC HERRING POPULATIONS: IMPLICATIONS FOR MANAGEMENT

4.1 INTRODUCTION

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

Atlantic herring (*Clupea harengus*) exhibit considerable variation among spawning groups. Spawning intervals range from early spring to late autumn (Sinclair and Tremblay 1984) and spawning takes place over a range of environments (inshore, offshore, and estuarine). Herring are thought to exhibit natal spawning site fidelity (Blaxter 1985), resulting in predictable patterns of migration to and from spawning grounds (e.g. Harden Jones 1968), but Hay et al. (2001) have recently re-estimated Pacific herring (*Clupea pallasi*) fidelity rates as "high" for large areas (e.g. 10 000 km^2) and "lower" for small geographic areas (100 km^2), of the scale considered here. Hypothesized spawning site fidelity and the predictable nature of spawning time have contributed to the assertion that herring spawning groups are discrete (e.g. Iles and Sinclair 1982; Sinclair 1988). However, some authors have cited evidence that is difficult to reconcile with the "discrete" population theory. A synthesis of the opposing arguments (e.g. Smith and Jamieson 1986) and an attempt to reconcile the considerable information can be found in Stephenson (1991) and McQuinn (1997).

Management of Atlantic herring in the northwest Atlantic, off Nova Scotia (NAFO Division 4WX fishing area), explicitly assumes stock complexity, as the area has

been subdivided into three management components (Figure 4.1). The assessment

strategy (Stephenson et al. 1999a) consequently attempts to protect individual spawning

groups within these components from over-exploitation, in an effort to maintain the

spatial and temporal diversity observed in spawning. The Coastal Nova Scotia

management component includes dozens of small spawning grounds and larger spawning

grounds (e.g. Eastern Passage) that support a commercial fishery, as well as a number of

relatively isolated spawning grounds (e.g. Bras d'Or Lakes region of Cape Breton). The

management strategy in this area is a relatively new initiative and two critical questions

remain unanswered: (1) does this management strategy reflect the correct scale of herring

population structure? (2) Can it be successful in protecting genetic diversity within the

management area?

In this chapter, I examine the genetic variation of Atlantic herring within the

Coastal Nova Scotia management component using microsatellite markers and ask

whether the contemporary management scheme in the NAFO 4WX fishing area is

commensurate with the goal of preserving existing genetic diversity.

4.2 METHODS

Tissue samples (blood, fin and/or muscle) were collected from Atlantic herring at

several spawning sites along the southern coast of Nova Scotia (Figure 4.1) in 1998, 1999

and 2000 (Table 4.1). Samples were also collected on Georges Bank (across the Gulf of

Maine) and were included in these analyses. Herring found in the Bras d'Or Lakes were

also investigated as these are in a relatively unique environment (inland sea with

restricted access to the Atlantic Ocean, limited flushing, and low salinity) and I wanted to

Table 4.1

Sampling details of Atlantic herring collections. Location numbers are shown in Figure 4.1.

Location Number	Location Name	Sample Size	Date Sampled (d-m-y)	Latitude N Longitude W	Predominant Year-classes
1	Georges Bank	75	29-10-99	41° 59.77 67° 41.50	80% 1994 20% 1995
2	Devastation Shoal 1	65	07-10-98	43° 53.48 64° 44.30	100% 1992
3	Devastation Shoal 2	65	13-10-98	43° 52.13 64° 43.13	100% 1992
4	Three Fathoms	60	03-10-98	44° 37.38 63° 10.70	8% 1991 81% 1992
5	Eastern Passage	75	04-10-99	44° 37 63° 11	10% 1991 39% 1992 44% 1993
6	Bras d'Or Lakes	71	24-04-00	46° 05.20 60° 44.70	no data

examine 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 (data provided by St. Andrews Biological Station, Department of Fisheries and Oceans).

DNA was isolated using Qiagen DNeasy genomic DNA extraction kits. Nine tetranucleotdie microsatellite loci, (6 developed for Atlantic herring, as outlined in Chapter 3: *Cha*1027, *Cha*1020, *Cha*1059, *Cha*1202, *Cha*1017 and *Cha*1045; 3 developed for Pacific herring, as described in Olsen et al. 2002: *Cpa*108, *Cpa*113, and *Cpa*102) were amplified using polymerase chain reaction (PCR). PCR amplification and electrophoresis conditions are described in Chapter 3 and in Olsen et al. (2002). DNA fragments were visualized and sized with an FMBIO II fluorescent imaging system (Hitachi).

Departures from Hardy Weinberg expectations (HWE) were tested at each sample location, using GENEPOP (Raymond and Rousset 1995). *P*-values for each comparison were estimated, using the Markov chain method with 2000 dememorization steps, 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.
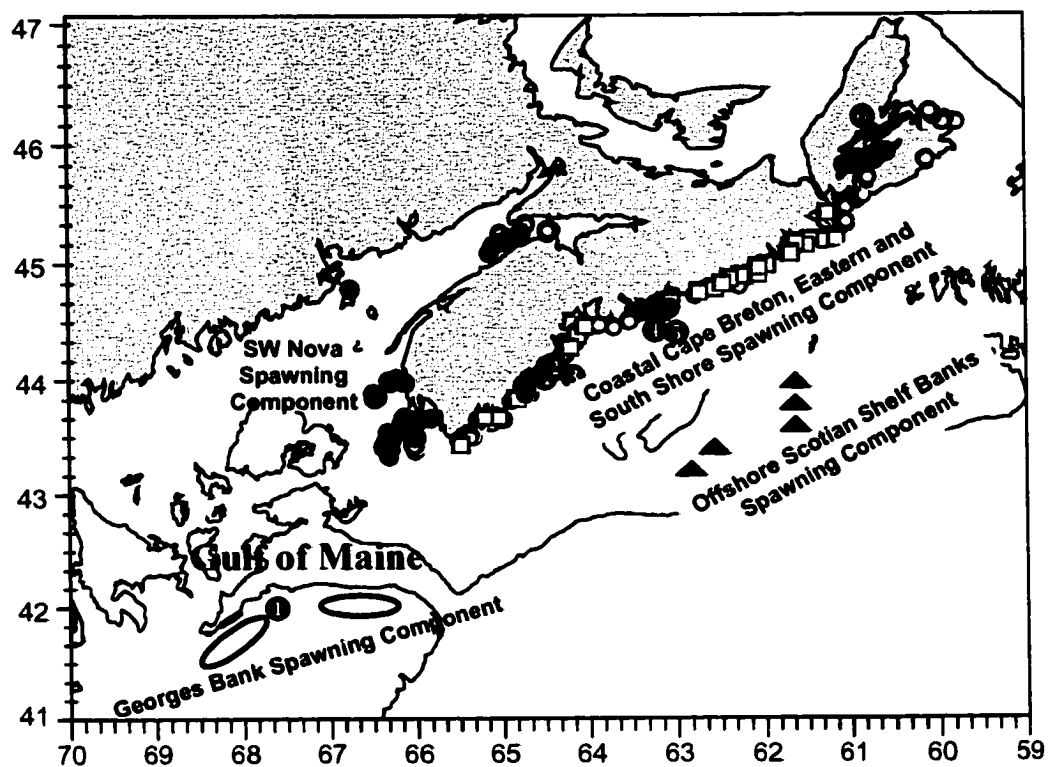
Figure 4.1
Chart showing Atlantic herring management components, spawning locations (as per Figure 1.1) and sampling sites. 1) Georges Bank; 2 and 3) Devastation Shoal1 and 2; 4) Three Fathoms Harbour; 5) Eastern Passage; 6) Bras d'Or Lakes. Amended from Anon. (1998).

Pairwise F_{ST} estimates (Wright, 1951, as amended by Weir and 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 and Rousset, 1995) was also employed to assess the statistical significance of single-locus allele frequency differences, 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 (NCSS97; 1999). 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 shortest marine distance between populations and F_{ST} estimates were used to test for an isolation by distance relationship between populations (Genetix; Belkhir, 2000).

4.3 RESULTS

All of the loci were polymorphic in all sample populations, and one or more alleles were amplified in greater than 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 4.2). Observed heterozygosity (Ho), averaged over loci did not differ among populations ($ANOVA\ P = 0.96$), although the Bras d'Or Lakes collection had the lowest mean Ho (0.77) and the lowest average variance in allele size, relative to the other populations. Furthermore, Bras d'Or had the fewest alleles, on

average, although the allele deficit was not significant *(ANOVA P* = 0.98) when means of all populations were compared statistically.

When results from all loci were combined for each population, no population departed from HWE. However, there were several single locus results that deviated from HWE: Georges Bank-*Cha*1045 (*P* = 0.011) and *Cpa*102 (*P* = 0.011); Devastation Shoal 2-*Cpa*102 (*P* = 0.011) and *Cpa*108 (*P* = 0.03); Eastern Passage-*Cha*1045 (*P* = 0.005); and Bras d'Or Lakes-*Cpa*102 (*P* = 0.03).

In the Eastern Passage collection, there were sufficient individuals from two year-classes (1992 and 1993) to test for temporal stability of year-classes (n=30 and 33 respectively). When 1992 and 1993 collections were tested for genetic differentiation using multi-locus F_{ST}, no differences were detected (F_{ST}= -0.002; *P*=0.76), so in the following analyses, all herring from Eastern Passage are pooled.

Multi-locus F_{ST} analyses revealed significant differentiation between Bras d'Or Lakes samples and all others (Table 4.3). The magnitude of these differences (reflected by higher pairwise 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 (*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 Shoal2, and between Devastation Shoal2 and Three Fathoms Harbour. However, these results are not significant, after Bonferroni corrections (Table 4.3). Exact tests revealed locus-specific differences among populations (Table 4.5) and the majority of the differences were found at three loci; (*Cha*1020, *Cpa*102 and *Cpa*108). All of the highly significant pair-wise

Table 4.2

Levels of genetic variation observed at nine microsatellite loci within six Atlantic herring collections: sample size (N); number of alleles detected at each locus; observed (Ho) and expected (He) heterozygosity within collections are indicated. *P<0.05 but nonsignificant after Bonferroni procedure. **P<0.01

LOCUS	GeorgesBk N=75	DevSh1 N=65	DevSh2 N=65	ThrFathHr N=60	EasternPass N=75	Brasd'Or N=71
Cha1027						
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
Cha1202						
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
Cha1059						
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
Cha1017						
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
Cha1020						
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
Cha1045						
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
Cpa113						
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
Cpa102						
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
Cpa108						
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

Table 4.3
Multi-locus pair-wise F_{ST} estimates between collections of Atlantic herring.

	Georges Bank	Devastation Sh.1	Devastation Sh.2	Three Fathom Hr.	Eastern Passage	Bras d'Or
Georges Bk	-	0.0015	0.0014	0.0002	0.0025*	**0.0110****
Devast. Sh.1		-	0.0043*	0	0.0007	**0.0119****
Devast. Sh.2			-	0.0028*	0.0031*	**0.0110****
Three Fath. Hr.				-	0.0009	**0.0095****
Eastern Pass.					-	**0.0140****
Bras d'Or						-

*P<0.05; ** P<0.001 Results significant after Bonferroni corrections for table-wide significance are in bold.

Table 4.4

P-values of single locus pairwise genic comparisons among collections of Atlantic herring. Results significant after Bonferroni corrections for table-wide significance are in bold.

					Locus				
Comparison	1027	1202	1059	1017	1020	1045	270	102	162
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**

MDS Map
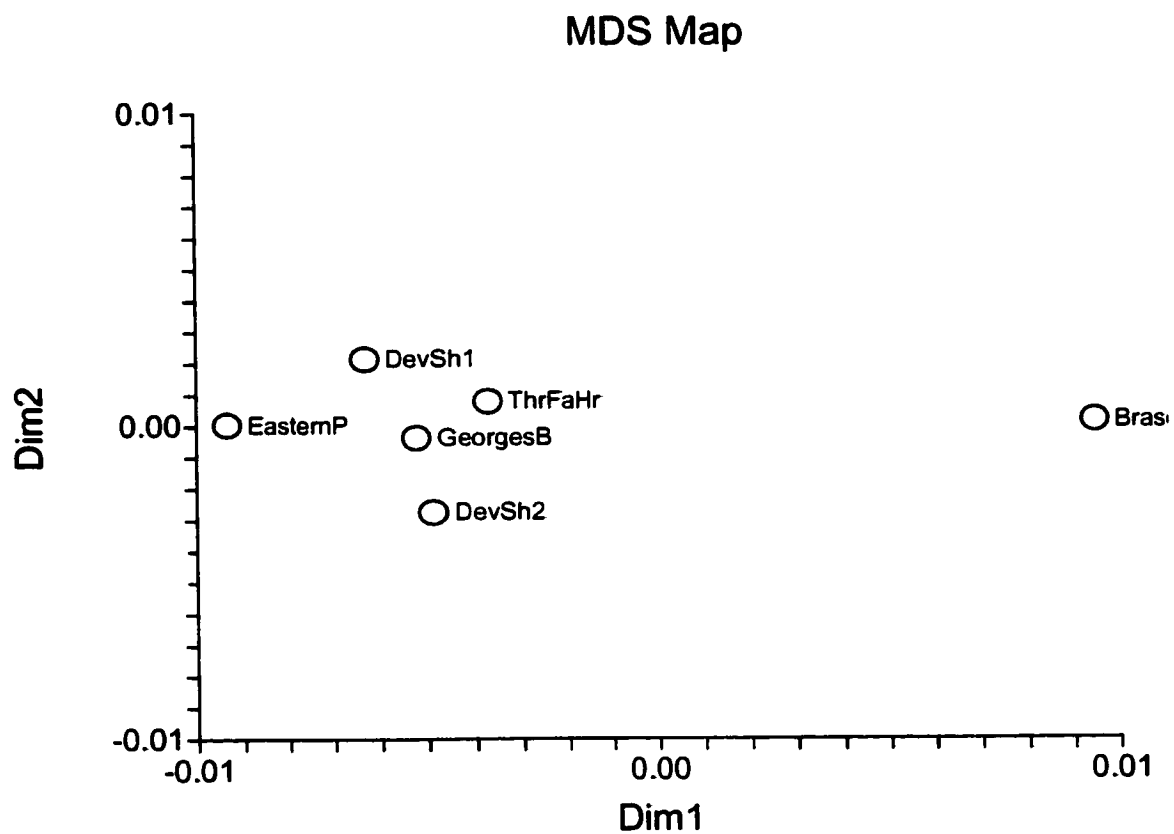
Figure 4.2
Multidimensional scaling of pair-wise F_{ST} estimates between collections of Atlantic herring from Georges Bank (GeorgesB), Eastern Passage (EasternP), Three Fathoms Harbour (ThrFaHr), Devastation Shoal1 &2 (DevSh1, DevSh2), and Bras d'Or Lakes (Bras). Dimension one (Dim 1) explains 68% of the variation whereas Dimension two (Dim 2) explains 2%.

comparisons ($P<0.001$) involved the Bras d'Or Lakes sample - with Eastern Passage vs Bras d'Or and Georges Bank vs Bras d'Or comparisons having three of nine loci significant at $P<0.001$.

The patterns of genetic differences among samples are illustrated using multidimensional scaling of pair-wise F_{ST} estimates (Figure 4.2). The Bras d'Or Lakes sample is clearly separate from the remaining herring samples considered here. Within the other collections, Eastern Passage is modestly divergent from the Devastation Shoal-Three Fathoms Harbour- Georges Bank complex, within which there is little differentiation. A Mantel test using pair-wise F_{ST} estimates and shortest marine distance between collections showed no ($P>0.4$) linear relationship, and thus no evidence of isolation by distance.

4.4 DISCUSSION

4.41 WITHIN POPULATION VARIATION

The range in heterozygosity detected in this study is somewhat broader, but is on average similar to that reported for dinucleotide microsatellites in Pacific herring (O'Connell et al. 1998a) and Atlantic herring from both sides of the Atlantic (Shaw et al. 1999, Chapter 2).

Samples did not depart from HWE when all loci were combined. P-values of five of the six significant single locus tests for conformance to HWE ranged from 0.05 to 0.01: Cha1045 (Georges Bank), Cpa102 (Georges Bank, Devastation Shoal2, and Bras d'Or Lakes) and Cpa108 (Devastation Shoal2 $P=0.035$) 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 :(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 7 of 9 loci, I concluded that, overall, these data conform to HWE.

The large number of alleles generally observed at microsatellite loci in marine fish species (see O'Connell and Wright 1997 and references therein), 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 (e.g. Kornfield and Bogdanowicz 1987) highlight the need for replicate sampling over time, when assessing population structuring. Although there were no formal annual temporal replicates reported in this chapter, I was able to include a "replicate" sample of the same year-class from 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, though this difference was not significant after Bonferroni correction.

Observations from fishers and herring assessment reports (Stephenson et al. 1999b, 2000) indicate that the residence time on the grounds for spawning herring is less than ten days. There are insufficient specific data with which to test this residence time

(however this issue is addressed in Chapter 6), but it is generally accepted that herring vacate the spawning ground soon after releasing their eggs or milt (Blaxter and 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 (*T-test*; P<0.001) greater average length (306.6 mm), relative to the first collection (301.2 mm), and the differences detected are likely related to a slightly different population of fish (based on size at age) 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-classes 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, I felt justified in pooling across year-classes and assuming temporal stability, at least at this location. There were insufficient numbers to test for year-class differences in all other locations (but see Chapter 5 for an extensive treatment of temporal stability).

4.42 AMONG POPULATION VARIATION

Multi-locus F_{ST} analyses and exact tests of allele frequencies revealed significant differences (after Bonferroni correction) between the Bras d'Or Lakes collection and all others. 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 and 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 relatively unique environment for Northwest Atlantic herring in the Scotia-Fundy region. Restricted access alone would lead one to hypothesize limited gene flow between the Bras d'Or Lakes herring and Coastal Nova Scotia herring.

Multidimensional scaling of F_{ST} estimates shows three groupings of the herring collections: the Bras d'Or Lakes, Eastern Passage (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 herring, all others were collected in October of 1998 and 1999 and these fish therefore share the same spawning season. Bras d'Or Lakes herring are spring-spawning, and this different reproductive timing may contribute to the genetic isolation of these fish and the magnitude of the differentiation detected between 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 and observed greater differences between fish populations with different spawning seasons within the Gulfs of Maine and St. Lawrence than between herring collected from each of 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 of complex stock structure of Atlantic herring in this area (Scott 1975, Crawford et al. 1982, Stephenson and Gordon 1991) based on phenotype. For example, Stephenson and 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. Scott (1975) used a host of meristic characters (e.g. number of vertebrae, gill rakers, pectoral-, dorsal-, and anal fin-rays) to discriminate among Bras d'Or fish and fish collected elsewhere (Southwest Nova Scotia, Escuminac, Northumberland Strait, Gulf of St. Lawrence). In addition, Crawford et al. (1982) observed 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, one could conclude that the Bras d'Or Lakes herring are morphologically, behaviourally, and genetically 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 do not conform to an isolation by distance model that predicts an inverse relationship between geographic distance and gene flow. An isolation by distance relationship is expected if gene flow decreases as geographic distance among samples increases (assuming no physical barriers exist). The Bras d'Or Lakes herring may experience physical barriers as the Bras d'Or Lakes samples are thought to be partially isolated from immigration/emigration, but 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 Lakes so migration out of Bras d'Or (and therefore gene flow) is possible. An isolation by distance

relationship was tested amongst all samples, excluding the Bras d'Or Lakes. Again, no

isolation by distance relationship was detected ($P<0.3$). Therefore, in this study, a simple

isolation by distance model based F_{ST} estimates must be rejected.

Because lower allele numbers and average heterozygosities were observed in the

Bras d'Or Lakes collection, I hypothesised that the population may have undergone a

reduction in effective population size. A low effective population size may enhance the

effects of random drift, so genetic divergence may accrue faster in small populations than

in large populations. Hansen et al. (2000) used gametic-phase disequilibria between loci

to estimate the number of breeders in a population. I therefore attempted a similar

analysis to assess reductions in effective population size. Random genetic drift may

create gametic-phase disequilibrium between unlinked loci in populations that have

become greatly reduced. I examined incidence of linkage disequilibrium (as estimated by

GENPOP; Raymond and Rousset, 1995) between each pair of loci in each population.

Three locus-pair comparisons were found to be significant at $P<0.01$. Of these, two

comparisons were from the Bras d'Or Lakes sample (*Cha*1027 and *Cha*1045 $P=0.005$;

*Cha*1027 and *Cha*1017 $P=0.001$) and the loci involved were not shown to be associated

in any other comparison provided here or previously (Chapter 3). 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 Bras d'Or Lakes herring (or

at least this sample of herring) having a relatively low effective population size.

Further, 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 is

heterozygosity in small populations (Nei et al. 1975), populations that have undergone
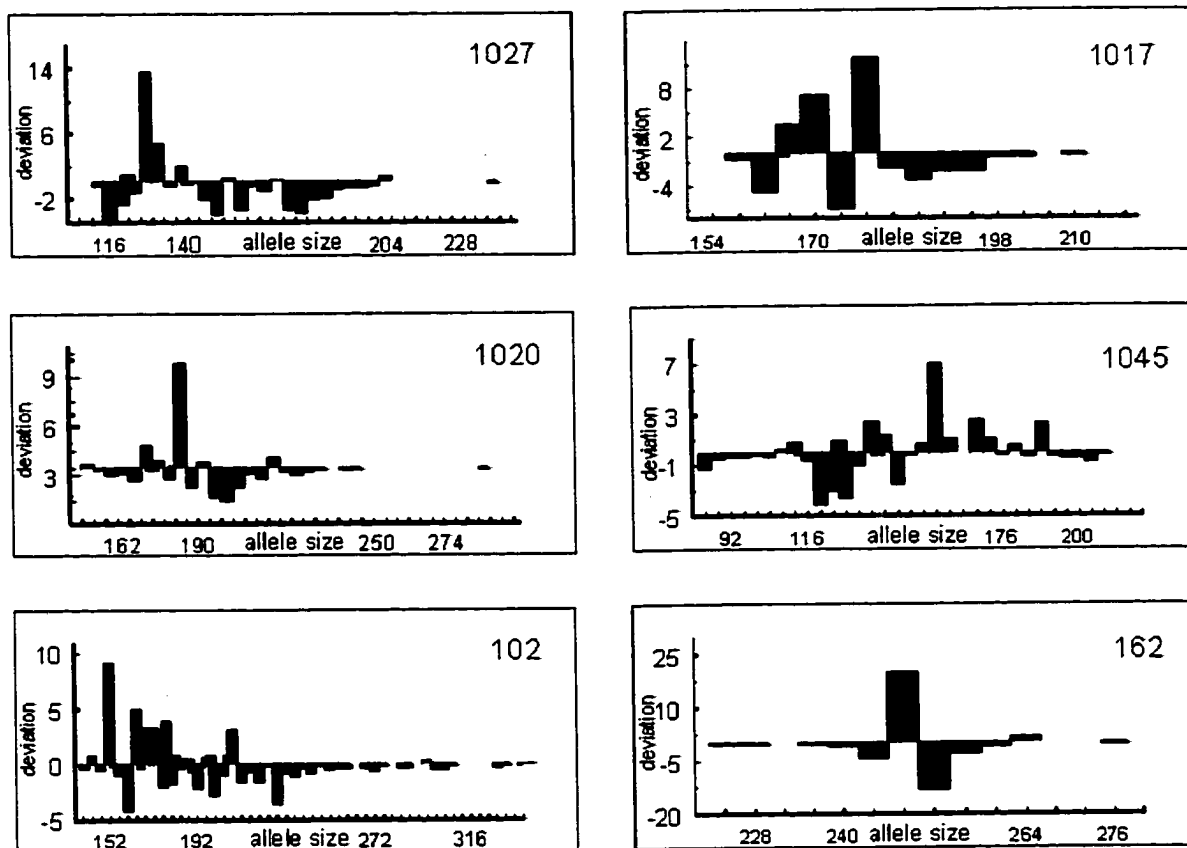
Figure 4.3
Allele deviations between Bras d'Or Lakes herring and all other herring collections in the Scotia-Fundy region at locus Cha1027, Cha1017, Cha1020, Cha1045, Cpa102, and Cpa108. Number of alleles were averaged over all populations (excluding Bras d'Or Lakes) and subtracted from those of Bras 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 samples.

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*). 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 anomalies are revealed (Figure 4.3). 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 and 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 one might expect to detect genetic changes), concomitant with an increase in fishing effort over the previous decade.

4.43 MANAGEMENT IMPLICATIONS

Considerable genetic diversity was detected within the Coastal Nova Scotia management component (comprised of Bras d'Or Lakes, Eastern Passage, Devastation Shoal and Three Fathoms Harbour) and the magnitude of differentiation within this management component is in some cases greater than the differences among these collections and Georges Bank (included for comparison). The marked differences between the Bras d'Or Lakes and all others, again suggests reproductive isolation of this

spawning group – an interpretation that is consistent with analyses based on meristics and on differences in spawning time. 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 in agreement with that of Kornfield and 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 other populations (in concert with differences detected using a suite of other measures as described above) clearly implies that special management consideration for the Bras d'Or herring is warranted. The evidence is sufficient to provoke a serious re-consideration of their inclusion in the Coastal component.

The occurrence of geographically distinct spawning groups, predictable spawning times (Sinclair and Tremblay 1984), detectable meristic differences (Messieh 1975), and evidence for homing from tagging studies (Wheeler and 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 summarised as an "in-season, survey, assess, then fish" protocol (Stephenson et al. 1999). The primary herring spawning locations are surveyed and assessed each season, prior to fishing, in an attempt to allocate spawning location-specific quotas and avoid overexploitation of any

individual spawning ground. Why? Because Stephenson et al. (2001) hypothesized that population structure of these different spawning grounds should relate to discontinuities in herring spawning and larval distributions (i.e. exchange would be highest among spawning groups that share a larval nursery area and lowest among spawning groups that are more physically separated).

In light of the genetic differences observed in herring in the present study, (albeit based on samples with limited temporal replication), it seems reasonable to conclude that the contemporary management of herring in the Scotia-Fundy region is rational and precautionary. However, the greater variation detected within the coastal management component considered here than between this management component and Georges Bank suggests that the management divisions may warrant re-examination.

CHAPTER 5: TEMPORAL STABILITY AND SPATIAL PATTERNS OF GENETIC VARIATION IN ATLANTIC HERRING

5.1 INTRODUCTION

Atlantic herring (*Clupea harengus*) is one of the most studied marine fishes (FAO 2000) and is found on both sides of the northern Atlantic Ocean. To the west, it ranges from Greenland to Cape Cod, and to the east from the Bay of Biscay to the Arctic coasts of Norway, Russia and the Baltic Sea. Herring fisheries in the Atlantic have undergone large (orders of magnitude) fluctuations in annual yield, and this has led to considerable interest in documenting population structure and dynamics, in an effort to minimize unequal exploitation of what may be largely independent stock components.

Attempts to delineate the population structure of herring have frequently focused on the use of a few polymorphic loci or a few populations or both (e.g. Kornfield and Bogdanowicz 1987; Safford and Booke 1992; Turan et al. 1998; Chapter 4). The majority of these cited studies conclude that despite the existence of what appear to be discrete spawning components (Sinclair and Tremblay 1984) and the widely assumed high degree of spawning site fidelity thought to be characteristic of herring (Iles and Sinclair 1982), gene-flow would seem to be sufficient among spawning components to prevent Atlantic herring from becoming genetically differentiated (e.g. Kornfield *et al.* 1982). Alternatively, it has been postulated that herring may only have begun the process of differentiation in recent evolutionary times, so that resolvable genetic differences have yet to evolve (e.g. Grant 1984), though this idea appears difficult to test.

By contrast, rejections of the null hypothesis of no genetic differentiation among small numbers of populations of northeast (NE; Shaw et al. 1999) and northwest (NW; Chapter 4) Atlantic herring have been reported. However, the geographic distances

encompassed by the samples used in most contemporary studies was generally low, and the potential for temporal variation of allele frequencies within location was not explicitly assessed. Establishing the temporal stability of patterns of genetic differentiation (using spawning stage specimens) among populations is essential for quantifying population structure; i.e. the inferences deduced from spatial patterns of variation may be misleading if such patterns vary over short (interannual) time scales.

Kornfield et al. (1982) reported significant temporal heterogeneity among annual collections of NW Atlantic herring, but a later study by King et al. (1987) of NE Atlantic herring demonstrated temporal stability across eight year-classes and three developmental stages. Both studies used a suite of allozyme markers, but it is possible that the contrasting results stem from the different methods used to assess temporal stability: multiple-year collection comparisons (Kornfield et al. 1982) vs. cross-cohort comparisons within a single year (King et al. 1987).

To address this contention, I used nine tetranucleotide microsatellites to examine the pattern of genetic relationships among Atlantic herring across a varying spatial scale, ultimately focusing on the coastal Nova Scotia and Bay of Fundy region. Microsatellite variation in samples from spawning aggregations of NW Atlantic herring are compared, and the resulting patterns are interpreted within the context of processes (e.g retention) hypothesized to generate population structure.

Beyond spatial population structure analyses (likely important to conservation issues), temporal variation of genetic patterns of differentiation is explored, as spawning aggregations are sampled at several locations in successive years. Further, samples within

a location are also categorized by cohort (year-class) and compared to provide an additional measure of temporal stability within location.

5.2 METHODS

5.21 LABORATORY PROCEDURES

Tissue samples (blood, fin and/or muscle) were collected from spawning stage Atlantic herring at ten locations along the Scotian Shelf, in the Bay of Fundy, and in the Gulf of Maine (Figure 5.1) in 1998, 1999 and 2000 (Table 5.1). Analyses of a subset of these samples (Bras d'Or Lakes, Georges Bank, Devastation Shoal, Eastern Passage and Three Fathoms Harbour) were previously reported in Chapter 4, in relation to the management of intra-species genetic diversity along the southern coast of Nova Scotia. Herring from the vicinity of Iceland, the Celtic and Baltic Seas were also included in the analysis to provide comparisons of differentiation across the species range. Individuals from spawning aggregates were preferentially collected to maximize the possibility that a spawning population was represented. In all but one case, herring were of spawning stage (Icelandic herring were collected from a pre-spawning assemblage).

Herring from a subset of the above locations were collected in multiple years (Table 5.1) to assess the temporal stability of allele frequencies. Cohort information (determined using the number of annuli on the otoliths collected from the same individuals used for genetic analyses) was also collected (age data provided by St. Andrews Biological Station, Fisheries and Oceans Canada).

DNA was isolated from tissue samples using Qiagen DNeasy genomic DNA extraction methods and nine microsatellite loci (*Cha*1027, *Cha*1020, *Cha*1059, *Cha*1202,

Table 5.1
Sampling details of Atlantic herring collections. Location numbers as in Figure 5.1.

Collection Number	Collection Name	Sample Size	Date Sampled (m-y)	Spawning Season
1	Georges Bank (GeoB)	75	10-99	autumn
2	Scot's Bay98 (ScB) 99 00	120 75 56	08-98 08-99 08-00	autumn
3	Spectacle Buoy98 (SpB) 99	50 100	06-98 06-99	summer
4	Trinity Ledge (TL)	150	09-98	autumn
5	German Bank98 (GrB) 00	148 164	08-98 08-00	autumn
6	Devastation Shoal (DeSh)	127	10-98	autumn
7	Eastern Passage (EP)	75	10-99	autumn
8	Three Fathoms Hr (TFH)	60	10-98	autumn
9	Bras d'Or Lakes (BrD)	71	04-00	spring
10	Western Bank (WB)	75	10-00	autumn
11	Iceland	55	06-00	summer
12	Celtic Sea	50	02-99	winter
13	Baltic Sea	50	04-01	spring
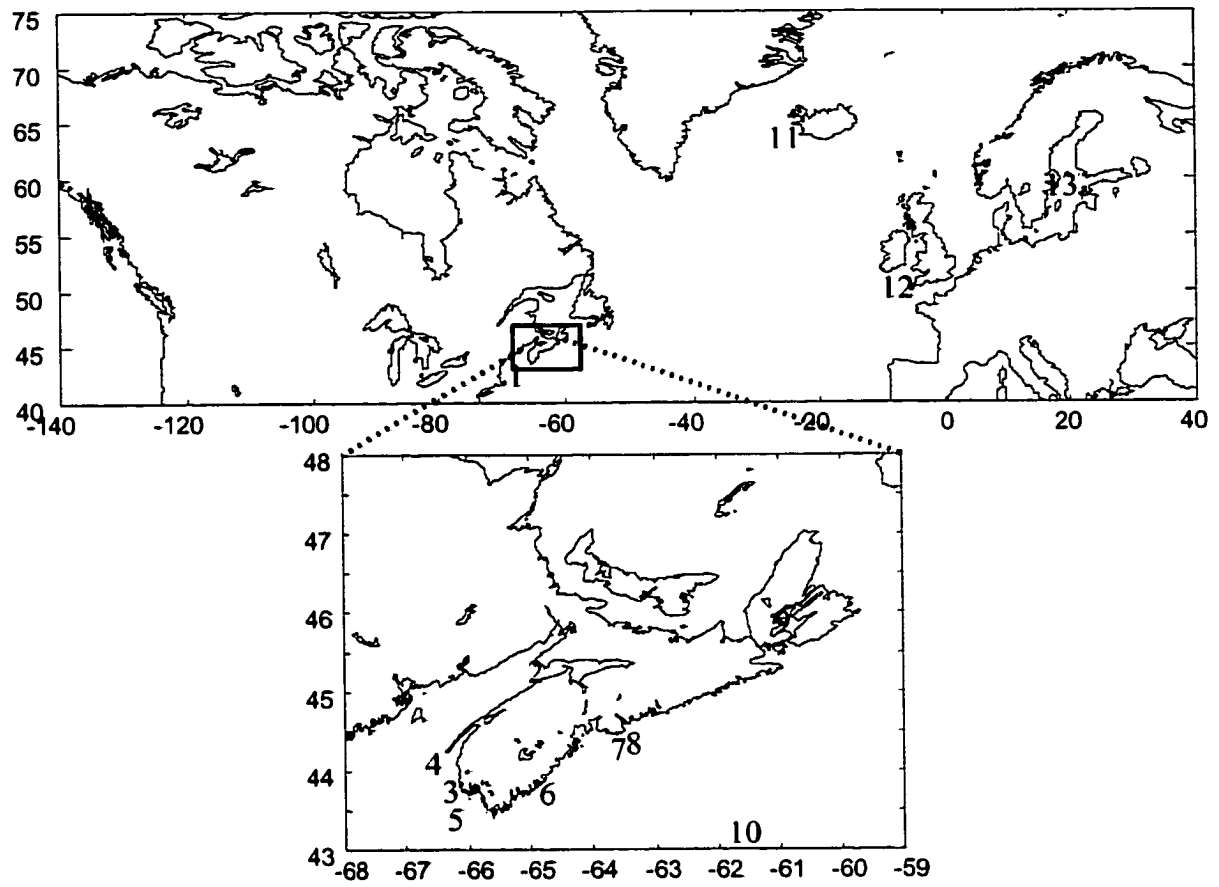
Figure 5.1
Chart of sample locations. Numbers correspond to Table 5.1

*Cha*1017, *Cha*1045, *Cpa*108, *Cpa*113, and *Cpa*102) were amplified using PCR. PCR

amplification and electrophoresis conditions for the *Cha* and *Cpa* loci are provided in

Chapter 3 and in Olsen *et al.* (2002) respectively. DNA fragments were visualized and

sized using an FMBIO II fluorescent imaging system (Hitachi).

5.22 DATA ANALYSES

Departures from Hardy Weinberg equilibrium (HWE) were tested for each locus

and collection using GENEPOP v3.1d (Raymond and Rousset 1995). Significance (*P*-

value) for each comparison was estimated using the Markov chain method with 2000

dememorization steps, 200 batches and 2000 iterations per batch.

F_{ST} estimates (Wright, 1951 as amended by Weir and Cockerham 1984) among

and between collections and year-classes were calculated, using Genetix (Belkhir 2000),

and 1000 permutations were used to estimate the probability of departure from the null

hypothesis of F_{ST} = 0. Multidimensional scaling (MDS) of pair-wise F_{ST} estimates was

used to create a two dimensional illustration of the relative dissimilarities among

collections (NCSS97 Hintze 1998). R_{ST} estimates (Slatkin 1995) between NE and a

random subset of NW Atlantic collections were also calculated using R_{ST}-Calc

(Goodman 1997) and 1000 permutations.

I employed an Exact test (GENEPOP: Raymond and Rousset 1995) to assess the

statistical significance of allele frequency differences at individual loci between pairs of

collections. Significance was calculated using the Markov chain method, as above.

Single-locus results were also used to test the null hypothesis that the resolving power of

individual microsatellite loci (as measured by the number of significant differences

detected) is not dependent on the number of alleles observed at each locus. Therefore, for

each locus that did not significantly depart from HWE, the number of alleles was tabulated and regressed on the number of significant pair-wise comparisons for each locus using Exact test results, as above.

Temporal stability of allele frequencies was assessed using samples collected from the same location in consecutive years and from samples collected in the same year that comprised multiple cohorts of herring. In collections that had sufficient individuals (nominally 15) from at least two year-classes, the individuals were pooled across collections (from the same location) according to year-class. Cohorts were then compared within each location to assess the temporal stability of the allele frequencies.

Spatial patterns of genetic variation were investigated using a Mantel test (compares slope of the linear regression created from the realised data to the distribution of slopes-generated by randomization procedures-under the null model of no spatial pattern). The shortest marine distance between populations and F_{ST} estimates were used to test for a linear isolation by distance relationship between populations (Genetix; Belkhir 2000).

5.3 RESULTS

All loci were polymorphic in all sample collections, and levels of average heterozygosity per collection ranged from 0.763 to 0.827 (Table 5.2). The total number of alleles detected across all Atlantic herring samples ranged from 14 at *Cpa*108 to 75 at *Cpa*102. Of the 153 single-locus tests for conformation to HWE, 19 had significance values of <0.05 but only one (Devastation Shoal; *Cha1020*) test showed significant (*P*=0.0007) departure from expectation, following Bonferroni corrections for multiple

Table 5.2

Single locus statistics for all sample collections showing number of samples (N), average observed heterozygosity (*Ho*) and average number of alleles per collection are reported. Probability of conformance to HWE is listed for each collection at each locus; P<0.05 in bold; * significant departure from HWE after Bonferroni correction.

	Locus								
	1027	1202	1059	1017	1020	1045	113	102	108
GeorgesBank N=75; *Ho*=0.815 # alleles=17.11	0.365	0.082	0.328	0.664	0.941	**0.004**	0.615	**0.021**	0.846
Scot'sBay98 N=120; Ho=0.817 # alleles=19.00	0.058	0.227	0.360	0.248	0.530	0.335	0.193	0.529	0.999
Scot'sBay99 N=75; Ho=0.820 # alleles=16.89	0.761	0.736	**0.032**	**0.002**	0.237	0.071	0.271	0.572	0.195
Scot'sBay00 N=56; Ho=0.786 # alleles=15.33	0.620	0.291	0.773	0.895	0.376	0.081	0.233	0.382	0.168
SpectacleBuoy98 N=50; Ho=0.813 # alleles=15.89	0.056	0.758	0.119	0.372	0.244	0.182	0.285	0.354	0.158
SpectacleBuoy99 N=100; Ho=0.827 # alleles=17.00	0.839	0.734	0.719	0.710	0.994	0.811	0.242	**0.047**	0.518
TrinityLedge N=150; Ho=0.812 # alleles=20.22	0.051	0.332	**0.018**	**0.005**	0.092	0.728	03931	0.755	0.492
GermanBank98 N=148; Ho=0.799 # alleles=17.56	0.103	0.061	**0.045**	0.167	**0.036**	0.187	**0.039**	0.864	0.248
GermanBank00 N=164; Ho=0.810 # alleles=18.89	0.080	0.380	0.412	0.174	0.228	0.423	0.359	0.933	**0.019**
DevastationShoal N=127; Ho=0.798 # alleles=19.00	0.164	0.705	0.405	0.695	**<0.001***	0.400	0.498	0.080	**0.037**
EasternPassage N=75; Ho=0.817 # alleles=17.00	0.202	0.451	0.193	0.174	0.547	**0.004**	0.631	0.841	0.114
ThreeFathomsHr N=60; Ho=0.796 # alleles=16.00	0.368	0.401	0.188	0.069	0.512	0.212	0.969	0.930	0.839
Brasd'Or Lakes N=71; Ho=0.763 # alleles 15.11	0.303	0.482	0.150	0.122	0.482	0.422	0.129	0.091	0.387
WesternBank N=75; Ho=0.799 # alleles=17.56	0.612	0.509	0.172	0.292	0.215	0.405	0.591	**0.018**	0.751
Iceland N=55; Ho=0.806 # alleles=15.22	0.459	0.901	0.378	**0.044**	0.317	0.128	0.535	0.761	0.797
CelticSea N=50; Ho=0.805 # alleles=16.44	0.141	0.055	0.796	**0.014**	**0.048**	0.911	0.722	**0.018**	0.999
BalticSea N=50; Ho=0.783 # alleles=14.78	0.380	0.383	0.908	0.872	0.529	0.212	0.386	0.070	0.491

tests (Table 5.2). Multi-locus combinations of single-locus tests within samples (following Fisher 1954) indicated that only the 1998 German Bank collection departed from HWE (P=0.005), following Bonferroni correction.

5.31 SPATIAL PATTERN

At the largest spatial scale, all other collections differed from the Baltic Sea collection according to F_{ST} estimates (F_{ST} ranged from 0.0048 to 0.0123, with P values of 0.025 and <0.00001 respectively). Irish Sea and Icelandic collections also differed from the majority of others (68% and 62% respectively). Multidimensional scaling (Figure 5.2) of pairwise F_{ST} estimates between NE and NW Atlantic herring (pooled by location, with Spectacle Buoy and Bras d'Or Lakes removed – see below) show that the NW Atlantic herring separate from the NE Atlantic herring along dimensions 1 and 2. NW Atlantic collections are most similar to the Icelandic collections, then to the Celtic Sea sample, and most distant from the Baltic sample. This range in relative similarity is what would be predicted, based on geographic distance from the NW Atlantic. Further, given that the magnitude of differentiation within the NW Atlantic is comparable to the magnitude of differentiation between Icelandic and Celtic Sea samples (based on dimension 1, where the majority of the variance is represented), there is more than twice the variation within NE Atlantic sample collections (including Baltic) than within all NW Atlantic collections. The magnitude of this variation is also reflected when the degrees of substructure in the NE and NW are compared (Table 5.3). In fact, based on F_{ST}, there is two fold more substructure within the NE collections than in the NW collections. However, NE and NW Atlantic samples were not collected at a comparable geographic

MDS Map
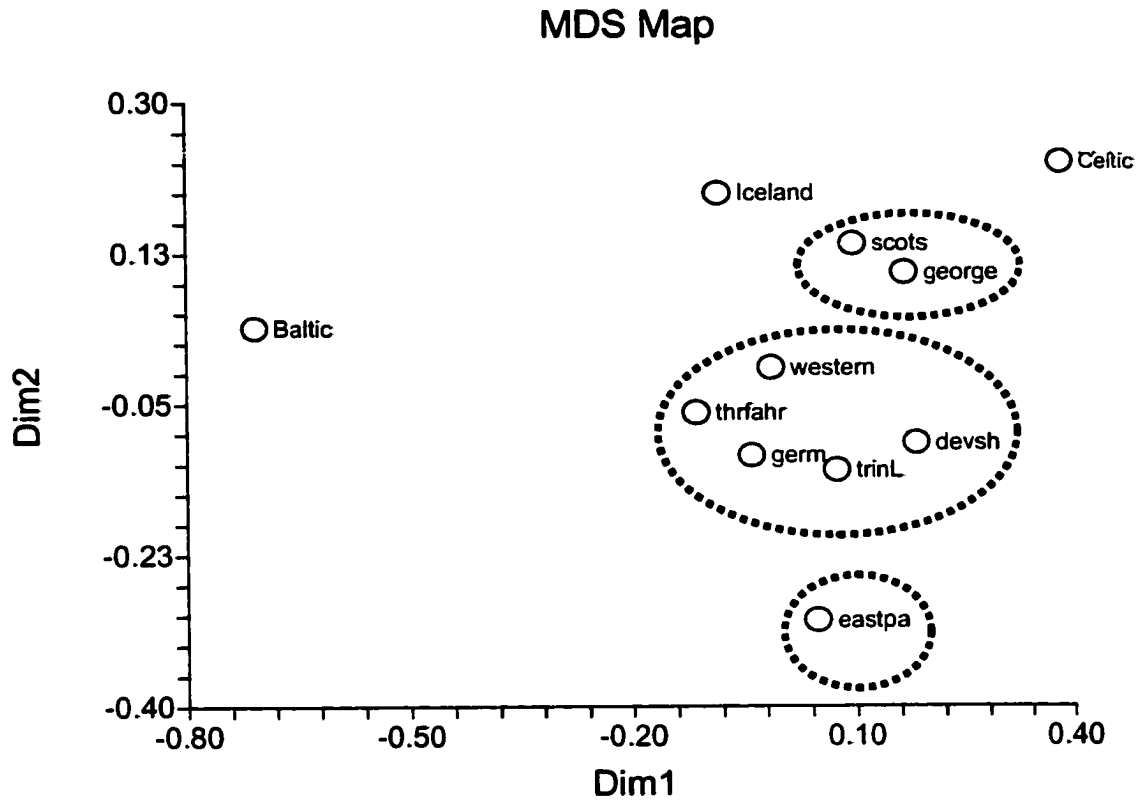
Figure 5.2
Multi-dimensional scaling illustration of F_{ST} dissimilarities among herring samples from the Celtic Sea (Celtic), Iceland, Baltic Sea (Baltic) and northwest Atlantic herring collections collapsed by location (scots = Scot's Bay; george = Georges Bank; western = Western Bank; thrfahr = Three Fathoms Harbour; germ = German Bank; trinL = Trinity Ledge; devsh = Devastation Shoal; eastpa = Eastern Passage). Dashed lines define the hypothesized quasi-isolated grouping. Dimension 1 (Dim 1) r=0.69; Dimension 2 (Dim 2) r=0.05.

Table 5.3

F_{ST} estimates of each locus among all herring collections, among northeast Atlantic collections, among northwest Atlantic (that represents spatial substructure), among years within northwest Atlantic (that represents temporal substructure) and the ratio of spatial to temporal substructure.

Locus	Among all Pops	Among Northeast Collections	Among Northwest Collections	Among Years	Spatial/ Temporal
*Cha*1027	0.0027	0.0027	0.0026	0.0004	6.50
*Cha*1202	0.0028	0.0180	0.0013	0	-
*Cha*1059	0.0021	0.0104	0.0009	0	-
*Cha*1017	0.0003	0	0.0020	0	-
*Cha*1020	0.0039	0.0084	0.0040	0.0009	4.44
*Cha*1045	0.0008	0.0017	0.0027	0.0041	0.66
*Cpa*102	0.0012	0.0044	0.0002	0.0009	0.22
*Cpa*113	0.0018	0.0073	0.0014	0.0002	7.00
*Cpa*108	0.0009	0.0059	0.0125	0.0179	0.70
All	**0.0024**	**0.0053**	**0.0026**	**0.0018**	**>1.44**

scale and therefore conclusions related to these comparisons of substructure within NE

and NW Atlantic herring cannot easily be drawn. When the NW Atlantic samples were

pooled and compared to Baltic, Icelandic, and Irish Sea herring, all comparisons revealed

significant structure (Table 5.4) based on F_{ST}. The magnitude of these differences

between NE and NW Atlantic collections were greater than any within region (NE or

NW) substructure estimate. R_{ST} estimates (Table 5.4) were in most cases lower than F_{ST}.

The largest R_{ST} estimates were between Icelandic and Baltic (inconsistent with F_{ST}) and

Icelandic and NW Atlantic herring (consistent with F_{ST}). In fact, the Icelandic and Baltic

comparison yielded the second largest difference between samples estimated using R_{ST}

(0.021) and the second lowest difference using F_{ST} (0.0045).

Multi-locus F_{ST} analyses revealed significant population structure within

the Scotia-Fundy region of the NW Atlantic Ocean (Table 5.5). Most notably, herring

collected from the Bras d'Or Lakes (most eastern collection) were different from all other

collections considered, with pairwise F_{ST} estimates ranging from 0.0037 to 0.0142. In all

but one comparison (Scot's Bay 2000), differences remained significant after Bonferroni

corrections for multiple tests. Eastern Passage is the next most eastern collection site

within the Scotia-Fundy region and F_{ST} estimates were significant between herring from

this location and herring collected from the interior of the Bay of Fundy (Scot's Bay and

Spectacle Buoy) and the Gulf of Maine (Georges Bank). In addition, differences were

observed between Spectacle Buoy98 and many of the herring collections from the

southwest Nova Scotia region (Trinity Ledge, German Bank, Devastation Shoal, Three

Fathoms Harbour), Georges and Western Banks, and from Spectacle Buoy99. The

Table 5.4

Above diagonal are pairwise estimates of F_{ST} detected between all Northwest Atlantic herring collections pooled (n=1322) and Baltic (n=50), Celtic (n=50) and Icelandic (n=55) herring. Below diagonal are pairwise estimates of R_{ST} detected between a random sample (n=50) of all Northwest Atlantic herring collections pooled and Baltic, Celtic and Icelandic samples as above. *indicates P<0.05; ** indicates P<0.0001.

	Northwest Atlantic	Baltic	Celtic	Icelandic
NWA	0	0.0656**	0.0681**	0.0652**
Baltic	0.009*	0	0.0085**	0.0045*
Celtic	0.008*	0.005	0	0.0035*
ICE	0.029*	0.021*	0.006	0

MDS Map
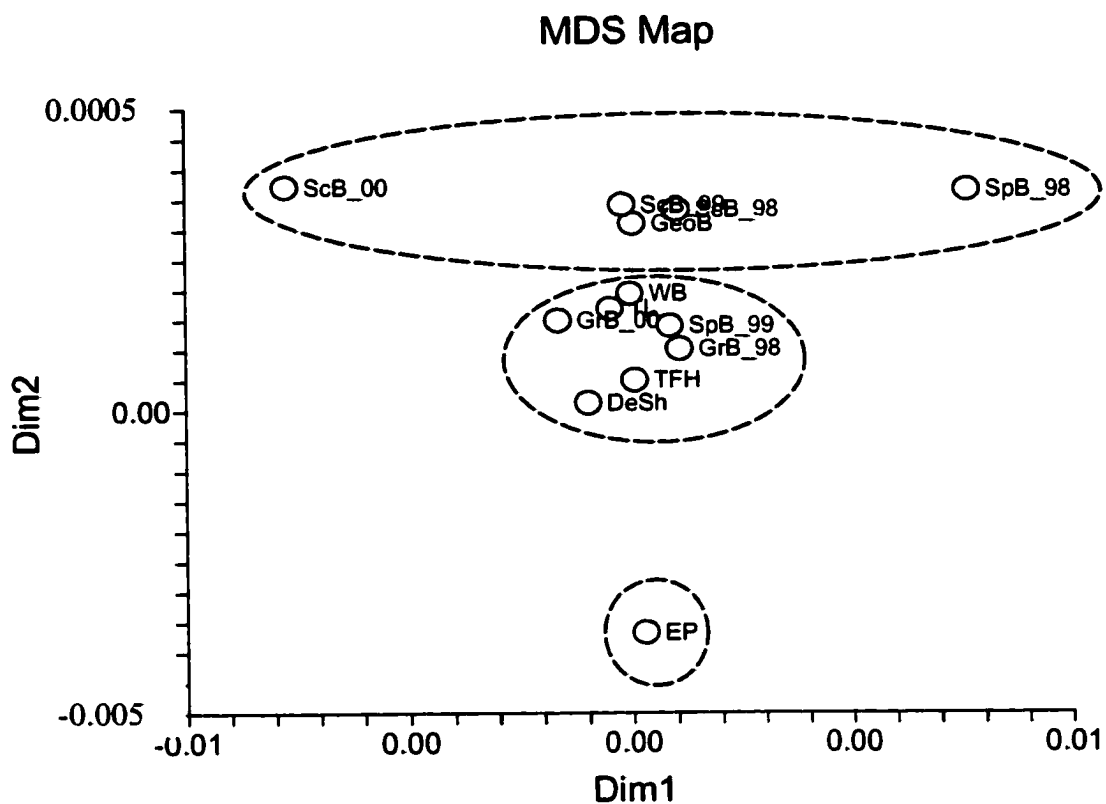
Figure 5.3

Multi-dimensional scaling illustration of F_{ST} dissimilarities among all (excluding Bras d'Or Lakes) NW Atlantic herring samples (Abbreviations as in Table 1). Dashed lines define hypothesized grouping discussed in text. Dimension 1 (Dim 1) r=0.66; Dimension 2 (Dim 2) r=0.04.

majority (56%) of these tests remain significant after the sequential Bonferroni correction was applied.

When Bras d'Or Lakes herring (discernable from all others; see Chapter 4) were removed from the comparative analyses and pair-wise F_{ST} estimates among spawning groups were visualized using MDS, three quasi-isolated groupings were apparent along dimension-2 (Figure 5.3): the sole Eastern Passage collection; a group that included the majority of the Southwest Nova Scotia spawning groups (German Bank, Devastation Shoal, Three Fathoms Harbour, Trinity Ledge, Spectacle Buoy99) as well as Western Bank; and a group that consisted of Georges Bank, Scot's Bay and Spectacle Buoy98. The relative associations between these groupings and the NE Atlantic collections are shown in Figure 5.2. Because collections from Spectacle Buoy were assigned to two different groups and because inclusion of Bras d'Or lakes samples collapses the relative positions of all NW Atlantic collections, these samples were omitted from Figure 5.2.

A Mantel test using pair-wise F_{ST} estimates from NW Atlantic herring comparisons and shortest marine distance among sample locations resulted in no linear relationship (P=0.72) and thus no clear evidence of isolation by distance. Further, no linear relationship was detected between the number of alleles observed at each locus and the number of significant single-locus pair-wise results generated for each locus respectively (P=0.96).

5.32 TEMPORAL PATTERN

In three locations, samples were collected in sequential years to test the temporal stability of allele frequencies: Scot's Bay 1998, 1999 and 2000; Spectacle Buoy 1998 and 1999; and German Bank 1998 and 2000. Within Scot's Bay no differences (based on

multi-locus F_{ST}) were found between '1998 and 1999' and '1999 and 2000' (Table 5.5). However, significant differences were observed between Scot's Bay 1998 and Scot's Bay 2000 (F_{ST}= 0.0051; P=0.001). At Spectacle Buoy, the 1998 and 1999 collections were marginally different (F_{ST}=0.0036; P=0.02) and at German Bank, no differences were revealed between the 1998 and 2000 collections, based on F_{ST} (Table 5.5).

When the relative levels of differentiation within and among sample locations (annual samples) were compared at each locus, in six of nine cases, there was greater substructure among NW Atlantic locations than within location over successive years. When averaged, this corresponded to a greater than 1.4 fold increase in spatial variation relative to temporal variation.

Single-locus comparisons (n=1296 tests) using exact tests also revealed a degree of temporal stability within location. However, differences between Scot's Bay 1998 and 2000 were found at both Cha1059 (P=0.04) and Cpa108 (P=0.00009), while between 1999 and 2000, only one locus showed a significant result (Cpa108; P=0.003). There were no differences between the 1998 and 1999 collections at any locus. Differences were detected at three loci in the Spectacle Buoy: Cha1045 (P=0.0009), Cpa113 (P=0.035) and Cpa102 (P=0.04); and German Bank: Cha1020 (P=0.03), Cha1045 (P=0.0009) and Cpa108 (P=0.006); comparisons. In three of the cases where single-locus differences were observed among collections, one of the two populations involved departed from HWE at that same locus, prior to Bonferonni corrections: Scots Bay 2000 at Cha1059; German Bank 1998 at Cha1020; and German Bank 2000 at Cpa108.

Four cohorts were represented in the German Bank collections, three in the Trinity Ledge and Scot's Bay collections, and two in the Eastern Passage collections.

Table 5.5

Pairwise F_{ST} estimates between Northwest Atlantic Scotia-Fundy herring. Abbreviations as in Table5.1. Bold font indicates a P value <0.05 and * denotes comparisons that remain significant after sequential Bonferroni corrections. Outlined boxes indicate comparisons where the magnitude and significance of differences detected are generally temporally stable.

Sample	ScB98	ScB99	ScB00	SpB98	SpB99	TL	GrB98	GrB00	DeSh	EP	TFH	BrD	WB
GeoB	0.0006	0	0.0034	0.0031	0.0011	0.0003	0.0013	0.0003	0.0005	0.0026	0.0002	0.0107*	0.0008
ScB98		0.0004	0.0051*	0.0021	0.0007	0.0013	0.001	0.00001	0.0025*	0.0032*	0.0004	0.0115*	0
ScB99			0.0020	0.0027	0.00005	0	0.0004	0.0006	0	0.0023	0.00005	0.0098*	0
ScB00				0.0123*	0.0056*	0.0029	0.0056*	0.0014	0.0041*	0.0075*	0.0051*	0.0037	0.0034
SpB98					0.0036	0.0042*	0.0029	0.006*	0.006*	0.007*	0.0049	0.0183*	0.0034
SpB99						0	0.0001	0.0007	0.0015	0.0021	0.0006	0.0122*	0.0007
TL							0.0003	0	0	0	0.0004	0.0115*	0
GrB98								0.0003	0.001	0.001	0.00008	0.0126*	0
GrB00									0.0012	0.0015	0	0.009*	0.0003
DeSh										0.0010	0.0003	0.0095*	0.0008
EP											0.0009	0.0142*	0.0002
TFH												0.0095*	0
BrD													0.0089*
WB													

F_{ST} analyses revealed no differences among year-classes. In addition, single-locus exact tests revealed very few differences between cohorts within each sample location. Exceptions included: Scot's Bay (*Cha*1027:1992 vs1993 year- classes, $P=0.007$); German Bank (*Cha*1027: 1991 vs 1994; 1992 vs 1994 and *Cha*1202: 1992 vs 1994, $0.05<P>0.02$). Only the Scot's Bay comparison remained significant subsequent to Bonferroni correction. The reader should note that fewer single locus differences were detected when samples were classified by cohort and differences detected among cohorts were not found at the same loci as among samples classified by collection year.

Temporal stability of single-locus pair-wise exact test results were then tabulated (Appendix A) and classified by: (i) collection year; and (ii) cohort for a subset of NW Atlantic herring populations. In the Scot's Bay comparisons (by collection year), *Cha*1020 showed consistent differences from both Bras d'Or Lakes and Eastern Passage in 1998, 1999 and 2000. However, there were many single-locus comparisons that were significant in one (62%) and two (27%) years only. With respect to the test with Spectacle Buoy, Georges Bank differed in both collection years (1998 and 1999) at *Cha*1045; *Cha*1020 and *Cpa*113 were different from Eastern Passage in both 1998 and 1999 as well; and *Cha*1027, *Cha*1020, *Cpa*102 and *Cpa*108 consistently revealed differences between Bras d'Or Lakes and Spectacle Buoy collections.

German Bank 1998 and 2000 collections differed from Georges Bank at *Cha*1202 and *Cha*1045; from Eastern Passage at *Cha*1020 and *Cha*1045; from Western Bank at *Cpa*113; and Bras from d'Or Lakes at *Cha*1202, *Cha*1020, *Cpa*102, and *Cpa*113.

When herring samples were grouped by cohort (age), similar patterns to those above were noted (Appendix A). For the Scot's Bay collections and all cohorts, *Cha*1020

and *Cpa*108 differed from Bras d'Or Lakes. Thus, *Cha*1020 identifies differences in all Scot's Bay and Bras d'Or comparisons, regardless of the temporal classification scheme, as all Scot's Bay collections (ScB 1998, 1999, and 2000) and all year-classes (ScB4, 5, and 6) differ from Bras d'Or Lakes at this locus.

Comparisons involving Eastern passage consistently differed across cohorts from Georges Bank (*Cha*1020), Devastation Shoal (*Cha*1027), Three Fathoms Hr (*Cha*1020 and *Cha*1027) and Bras d'Or Lakes (*Cha*1027, *Cha*1020, *Cpa*102, and *Cpa*108). In the Trinity Ledge and German Bank year-class comparisons, Bras d'Or Lakes differed from each cohort consistently and at the same locus(i). For Trinity Ledge, these differences accounted for 50% of the total single-locus differences observed.

5.4 DISCUSSION

The results above demonstrate not only population structure in herring at three very different spatial scales: basin-, shelf-, and bank-scales (using nine markers on spawning stage fish and samples that conform to HWE), but also aspects of temporal stability, as differentiation among collections exceeded annual variation within populations. Further, despite the general agreement between different methods for assessing temporal stability, comparisons by cohort yielded fewer significant differences than simply pooling all cohorts and considering each multiple year comparisons. Thus, inferences should be made with caution when pooling samples with overlapping year-classes.

Atlantic herring displayed a high degree of allelic diversity with an average heterozygosity reaching 0.80 - slightly less than that reported by O'Connell et al. (1998a) for Pacific herring and Shaw et al. (1999) for NE Atlantic herring using dinucleotide

microsatellites developed for Pacific herring (O'Connell *et al.* 1998b). All collections (n=17), with the exception of German Bank98, conformed to HWE when all loci were combined (following Fisher 1954). Single-locus tests showed departure from HWE only in the Devastation Shoal collection at *Cha*1020, after Bonferroni corrections. Given the large number of tests conducted, the degrees of significance and the ensuing corrections, I conclude that, overall, these data conform to HWE.

The majority of the significant single-locus differences among collections were found at a subset of the loci - 64% of the single-locus exact test comparisons with $P<0.01$ were found at four loci: *Cha*1027, 1020, 1045 and *Cpa*108. Perhaps surprisingly, these loci are not the most variable and *Cpa*108 was the least variable locus used. Further, there was no linear relationship between the number of alleles at each locus and the frequency of detecting differences in pair-wise tests at that locus. The inference is clear – the number of alleles observed at a given locus provides no predictive power for the ability of the locus to reveal differences among sample collections, at least in Atlantic herring when using these markers. Is this finding generally applicable? Ferguson and Danzmann (1998) used the results of a simulation to conclude that increasing the number of alleles per locus does not increase the probability of detecting significant differences among closely related yet significantly differentiated populations. The same conclusion was drawn empirically by Allendorf and Seeb (2000) who compared the amount of genetic differentiation observed for different classes of genetic markers, and found that their data (and 75% of comparable studies they reviewed) provided no evidence for differences in the amount of divergence revealed (via F_{ST}) among sockeye salmon populations using low (protein) vs higher (nuclear DNA) variability markers.

When the degree of substructure estimated by each locus is compared (Table 5.3), opposing trends are observed between the NE and NW Atlantic assemblages. *Cha*1059 and 1202 (the second and third least variable loci) reveal the most substructure within NE Atlantic herring collections. These loci exhibited the least amount of variation (7th and 8th of 9 loci) within the NW Atlantic collections. In contrast to the generality proposed above, it may be that the ability of each locus to detect differences is location-specific and using a locus that is discriminative within a narrow geographic range does not imply that the same locus will be so in another location.

5.41 SPATIAL PATTERN

Unlike Grant (1984), who showed that the magnitude of pan-Atlantic differentiation (based on 40 protein coding loci) in herring was not greater than that estimated among regions on either side of the Atlantic, I observed highly significant pan-Atlantic differences based on F_{ST}. I then inferred that transoceanic interbreeding among herring is rare. The inability to demonstrate difference between NE Atlantic collections and *all* NW Atlantic collections reported by Grant (1984) may be attributable to balancing selection on at least some of the allozyme loci (e.g. Pogson et al. 1995) or the lack of divergence through drift, whereas divergence in microsatellite allele frequencies presumably arises from both drift and mutation, so divergence of microsatellite DNA accrues more quickly. However, when the NW Atlantic collections in this study were pooled, the differentiation between NE and NW Atlantic herring was consistent with the magnitude of pan-Atlantic differences reported for other marine fishes using mtDNA analyses (e.g. Atlantic cod; Carr and Crutcher 1998); although, it has been suggested (e.g.

Carr and Crutcher 1998) that F_{ST} results derived from microsatellite markers underestimate the degree of transatlantic differentiation.

It has been suggested (e.g. deWoody and Avise 2000; Forbes et al. 1995) that R_{ST} (calculated from the variance in allele sizes whereas F_{ST} is typically related to variance in allele frequencies) may be a better predictor of differentiation among populations when the historical separation between populations is long (and therefore substantial population differentiation is expected) as the effect of mutation is thought to become more important than the effect of migration. Conversely, when little differentiation is expected, F_{ST} is thought to out perform R_{ST} as a metric of differentiation as migration is likely more important is these situations and mutation can generally be ignored is separation of populations is thought to be recent. However, in this study, the opposite pattern has been demonstrated. Estimates of differentiation between NE and NW Atlantic herring populations were almost exclusively greater using F_{ST} despite the assumed historical separation between NE and NW. Therefore, I conclude that the application of R_{ST} does not necessarily provide additional insights as suggested (e.g. deWoody and Avise 2000; Forbes et al. 1995).

At a smaller spatial scale, Bras d'Or Lakes herring differed from all others included in this study, with the exception of one collection in one year – Scots Bay 2000. The degree of differentiation between the Bras d'Or Lakes "population" and all others comes as no surprise, as these herring inhabit a relatively closed and unique lake-estuary environment. Additional attributes of the Bras d'Or herring are discussed in Chapter 4. The magnitude of the differences presented here are inconsistent with frequent mixing of herring in and out of the Bras d'Or Lakes and subsequent successful reproduction.

However, it is possible that the Bras d'Or collection was sampled from a resident population and thus does not preclude migratory spawners elsewhere in the lake system.

There are also significant differences among many of the Scotian Shelf herring collections, and this may be most easily explained by considering the putative populations as belonging to one of three groups, within which there are limited differences. The first group is made up of only Eastern Passage herring, the most eastern population examined on the Scotian Shelf other than the Bras d'Or Lakes. A second group is comprised of the herring collected south of Eastern Passage, along the coast of Nova Scotia to Trinity Ledge and offshore to Western Bank. The third group is comprised of Georges Bank herring and all three Scot's Bay collections, representing the interior Bay of Fundy and Gulf of Maine. The one exception to this explanation is the Spectacle Buoy98 collection, assigned to the Bay of Fundy and Gulf of Maine complex, based on F_{ST} (and MDS). The Spectacle Buoy99 collection falls within the second group, and the location of Spectacle Buoy suggests inclusion with the second.

With the exception of Scot's Bay 2000 and Spectacle Buoy 1998, the groupings I describe above separate along Dimension 2 (Figure 5.3). While there are many differences (based on F_{ST} estimates) among collections between groups (Table 5.5), there are few instances of within-group significant comparisons after corrections for multiple tests (e.g. Scot's Bay 1998 and 2000), but the magnitudes of these differences are, in some cases, greater than among-group comparisons.

5.42 RECONCILIATION WITH HERRING POPULATION CONCEPTS

Given the spatial pattern of differentiation, in concert with a lack of evidence for isolation by distance (even when Bras d'Or Lakes herring are excluded because the

potential for mixing is arguably limited), it is reasonable to consider both evolutionary (Iles and Sinclair 1984) and management-based (Stephenson et al. 1999) alternative stock concepts that attempt to address patterns of population structuring in herring. Iles and Sinclair (1984) predicted that the number of geographically stable larval retention areas determines the number of genetically distinct herring stocks. Accordingly, each distinct gene pool is postulated to envelop all those spawning groups whose larval and post-larval stages come to share (and remain in) the same area of distribution. In this case, that argument would necessitate the existence of at least four larval retention sites on the Scotian Shelf and Gulf of Maine region. As the Bras d'Or Lakes have limited connections with the Atlantic Ocean (Petrie 1999), it is reasonable to consider the Bras d'Or Lakes as a "stable" larval retention area, with limited opportunities for larval dispersal. The inner Bay of Fundy and coastal Gulf of Maine herring complex may also comprise a single larval retention zone, as the residual circulation in the Gulf of Maine area indicates a re-circulation with potential connections to the interior Bay of Fundy (as modeled by Greenberg 1983). The herring complex that extends south of Eastern Passage to the waters beyond southwest Nova Scotia could also conceivably share a larval retention area if the Nova Scotia coastal current (flowing southwest along the coast of Nova Scotia) is considered a larval retention area. It is possible that herring spawned along the coast get entrained into the coastal current and are therefore mixed with larvae from other spawning groups along the coast (Reiss et al. 2000). The association of the Western Bank herring collections with this southwest Nova Scotia complex may also be explained by the dispersal pattern of herring larvae and the attendant circulation on the Scotian Shelf, as reported by Reiss et al. (2000), who demonstrate a connection between

the offshore banks and the Coastal current (this is addressed in Chapter 7). The relative

distinctness of herring collected from Eastern Passage is not easily explained. It is

possible that larvae from this location are not entrained and advected in the coastal

current, or alternatively, that natal spawning site fidelity in this location is sufficiently

strong to preclude straying in the face of mixing at the larval stage. I can test neither

hypothesis but Eastern Passage is the main (and commercially exploited) spawning

location of the many tens of spawning locations along the coast (Stephenson et al. 2000).

The most recent conceptual model of population structure for herring in the

Scotia-Fundy region, proposed by Stephenson et al. (2001) and generalized in Smedbol

and Stephenson (2001), organizes herring spawning groups into complexes that share

larval retention areas. Limited genetic exchange among complexes is postulated, along

with greater exchange among spawning groups within complexes. Spawning groups that

exchange individuals (and presumably genes) with some frequency are considered to

form a cell - an intermediate classification (in terms of relative exchange) - somewhere

between spawning group and complex.

Of the population pair-wise comparisons (based on F_{ST}), only three significant

differences (after corrections for multiple tests) occur within what would be considered

complexes, as defined above and in Figure 5.3. However, none of these comparisons can

be corroborated with the temporal analyses; in fact, one of these temporal differences is

within a spawning group: Scot's Bay98 and 2000. It appears that although I cannot reject

the presence of "cells" within complexes, the degree of exchange may be sufficiently

high to preclude quantifiable genetic structure at this intermediate level. Alternatively, it

is possible, though not easily tested, that measurable differences have yet to accrue, despite reproductive isolation.

It has been suggested that under migration-drift equilibrium conditions, the values and variability in pair-wise F_{ST} estimates will increase with geographic distance assuming migration rate is a function of distance (e.g. Hutchison and Templeton 1999). It is therefore possible that equilibrium conditions in NWA herring have not yet been attained, and the lack of isolation by distance reported here may be attributable to the absence of equilibrium. However, patterns of population structure do not appear to be random with respect to location (Figure 5.3 and discussion above) as differences detected among populations are largely consistent with impediments to dispersal (gene-flow) other than geographic distance (e.g. water circulation). As such, in a dynamic marine environment, the lack of isolation by distance may not realistically be used to identify nonequilibrium conditions.

5.43 TEMPORAL PATTERNS OF VARIATION

As genetic divergence among locations was 1.4 fold greater than among years at a single location (Table 5.3), I concluded that there is temporal stability of the genetic patterns seen. This measure of temporal stability may be influenced by the inclusion of anomalously differing populations or the paucity of temporal replicates at each location and therefore, additional methods for assessing temporal stability of genetic variation were also explored in some detail (i.e. differences within and among locations as estimated by F_{ST} and exact test analyses).

Kornfield et al. (1982) concluded that it was doubtful that intergenerational or year-class differences contribute significantly to population differentiation, due to the presence of overlapping generations in the collections and because herring are iteroparous. However, Kornfield et al. (1982) demonstrated a lack of temporal stability among annual collections from the Gulf of St. Lawrence and Gulf of Maine, whereas King et al. (1987) demonstrated temporally stable genetic patterns of variation in North Sea herring, when fish were compared by cohort. The contrasting results and the respective conclusions that follow may reflect the different grouping protocols used to assess temporal variation.

Although a study to test this suggestion optimally would include samples for each representative cohort sampled across multiple years allowing a comparison of cohorts through time, obtaining the requisite material would be difficult, in practise. Realizing the inherent limitations in such data, the findings reported here are consistent with a protocol difference explanation (as above). F_{ST} analyses revealed no differences among any of the cohorts (where I had sufficient numbers for comparison) collected from the same location in different years, whereas, marginal differences ($P=0.02$) were detected between Spectacle Buoy 98 and 99, and significant ($P=0.001$) differences were detected between Scot's Bay98 and 2000 when samples were compared by year of collection. Further, when the youngest year-class (1996), which only appears in the Scot's Bay2000 collection and represents 38% of that sample, is removed from the comparison of collection year analysis, the Scot's Bay2000 collection no longer differed from the Scot's Bay98 collection, and the remaining samples collapsed the MDS illustration along

dimension 1 (Figure 5.3), strengthening the relationship between the interior Bay of Fundy and Gulf of Maine group I defined above.

I suggest this result reflects unequal cohort representation within different samples. But do these results also reflect a temporal shift of allele frequencies that may have periodicity (as in pink salmon e.g. Aspinwall 1974) or do they describe a "de-correlation" scale of genetic cohesiveness at a temporal scale of approximately 3 years (as there were 3 year-classes represented in the other Scot's Bay collections)? As there were insufficient samples representing the 1996 cohort, direct comparisons with the other Scot's Bay cohorts are precluded.

Alternatively, there are a number of additional explanations for the discrepancy among Scot's Bay comparisons: (1) the sample sizes were greater in the collection year comparison than in the cohort comparisons, and as such, the discrepancy may be related to sample size; (2) type I error due to multiple tests that is not accounted for via the Bonferroni method; and (3) selective (non-random) mortality with respect to allele frequencies over time. I am unable to address (1) or (2) with the data at hand. For (3), however, if one or more loci were susceptible to selection (the genotypic composition of the year-class changed over time), it would be conceivable that no differences would be detected when cohorts were pooled, because one would be pooling the cohorts from multiple collection years. Pooling across collections to assemble cohorts may mask evidence of selective mortality. It would be optimal to compare the cohorts within a single sample to the same cohort from a subsequent collection (a full cohort analysis as prescribed by Ward and Grewe; 1994). Unfortunately, I had insufficient samples in the majority of locations to adequately assess this possibility. However, I did manage to

isolate two different cohorts (n>30) in Scot's Bay for three candidate loci (*Cha*1020,

1027, *Cpa*108) using the method described above and I compared their allele frequency

distributions in one subsequent year. There were no significant differences between the

distributions (Kolmogorov-Smirnov P>0.2 for all tests).

I concluded that unequal representation of cohorts within samples may lead to the

erroneous dismissal of veritable genetic patterns and suggest inclusion of cohort

comparisons when testing hypotheses related to temporal stability. When comparing

samples based on collection year, one is in fact implicitly assuming that there are no

differences among cohorts, which is the underlying justification for pooling the cohorts

and considering the sample as a whole. It seems problematic (tautological?) to assume the

very thing one is attempting to test. Cohort information is likely critical, if not

imperative, when assessing temporal stability in marine fishes.

When the temporal stability of single-locus Exact test comparisons across

locations are considered, based on collection year and cohort respectively, it is clear that

the significant single-locus results are not temporally consistent in the majority of cases.

There were, however, some cases where patterns were consistent: German Bank98 and

2000, where 72% of all single-locus pair-wise differences were shared by both

collections; all Bras d'Or Lakes comparisons (e.g. Bras d'Or Lakes and all other

collections), where at least one locus showed significant differences that were consistent

through time, regardless of classification scheme (collection year or cohort). These

results (and others listed in Table 5.5) suggest that significant differences often occur at

the same locus(i) through time regardless of method used for temporal comparisons.

In light of these patterns, I conclude that herring in the NW Atlantic are in some way spatially structured and support the precautionary management approach employed in this area. The lack of significant differentiation between cohorts from the same location (and in most cases between different collections in subsequent years), and the greater among-location than temporal within-location genetic variability are more consistent with temporal stability of genuine genetic differences than with chaotic genetic patchiness often associated with marine species (as reviewed by Larson and Julian 1999).

CHAPTER 6:GENETIC VARIATION AMONG ATLANTIC HERRING SPAWNING WAVES

6.1 INTRODUCTION

The quantification of sub-annual genetic variation may have important implications, especially within spawning groups of a species assumed to have a philopatric life history (e.g. Wheeler and Winters 1984). Chaotic genetic patchiness has been observed in marine populations when the genetic composition of samples (assumed to be representative of populations) has been observed to vary as much over time at one location as over large distances (Purcell et al. 1996, David et al. 1997), even when the sampling strategy seemed optimal e.g. spawning fish collected from the spawning grounds were used (i.e. Kornfield et al. 1982 who considered Atlantic herring). An interest in small-scale, seemingly unpatterned, genetic heterogeneity –"chaotic genetic patchiness" - among and within local populations (Johnson and Black 1982, Hedgecock 1994, Larson and Julian 1999, Chapman et al. 1999) has resulted.

There are a number of adaptive and chance-related processes that may generate this heterogeneity. In particular, it has been proposed (e.g. Hedgecock 1994; sweepstakes hypothesis) that temporal genetic variation in marine organisms may result from large variance in reproductive success among adults such that resulting year-classes comprises the progeny of only a small proportion of the spawning population. This "instantaneous [genetic] drift effect" (David et al. 1997) results in reduced genetic variation among recruits within a year-class, differences between the genetic composition of recruits and the spawning population presumed (in its entirety) to have produced them, and subsequent differences in the genetic composition of the recruits over time to the extent that different portions of the adult population contribute the successful progeny at

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different times (normally different years). Although the so-called "sweepstakes" hypothesis described above is conceptually appealing (see Ruzzante et al. 1996) the role sweepstakes events may play in generating apparent temporal instability has rarely been tested.

An alternative (and perhaps a more testable) scenario may be responsible for the heterogeneity frequently observed among annual collections of marine organisms from the same location: is it possible that the observations of temporal genetic patchiness reflect the existence (and sampling) of distinct temporally spaced populations that share a spawning ground? If a spawning location supports distinct populations that are separated temporally (within a spawning season) and is sampled in consecutive years, collections may be drawn from different populations (that may be genetically distinct). Therefore, what is perceived as temporal instability of genetic patterns may in fact represent comparisons between sympatric populations. Therefore, in light of the relatively unstable inter-annual temporal genetic variation detected elsewhere in herring (Pacific herring, McConnell et al. 1998b; Atlantic herring, Kornfield et al. 1982, Kornfield and Bogdanowicz 1987), the potential for sympatric, distinct populations at a sub-annual temporal scale was tested.

Herring (*Clupea harengus*) are iteroparous. Their spawning times are relatively predictable and herring are assumed to exhibit natal spawning site fidelity (Sinclair and Tremblay 1984). It has also been suggested that herring spawn in temporally discrete cohorts (defined here as an assemblage of spawning fish) separated by several days to several weeks (Ware and Tanasichuk 1989) and the observations of discontinuous spawning (as inferred by maturity of fish) over time on spawning grounds (Stephenson et

al. 2000) support this suggestion. There are, however, few estimates of residence time (based on field observations) for a given spawning ground.

A coordinated cohort of spawners using the spawning ground at the same time has been called a "spawning wave" (Hay 1985). Lambert (1984, and references therein) suggested that peak landings of spawning herring in northwest Atlantic waters coincide with the origin of measurably discrete larval cohorts, and concluded that spawning groups (spawners associated with a particular location) segregate into spawning waves. Do these spawning waves of herring represent genetically distinct sympatric populations? Here, I use the aforementioned spawning wave phenomenon in Atlantic herring at different locations within a given year to investigate the potential for sub-annual genetic variation using nine microsatellite loci.

6.2 METHODS

Tissue samples (blood or muscle) from spawning-stage herring were collected on two occasions from four annually occupied spawning locations on the Scotian Shelf in 1998: German Bank, Devastation Shoal, Trinity Ledge, and Scot's Bay (Figure 6.1). These spawning locations vary greatly in absolute biomass ranging from ~15 (Devastation Shoal) to 450 (German Bank) thousand tonnes in 1999 (Stephenson et al. 2000). Intervals between the collections, presumed to represent different spawning waves in the same location, ranged between six and fifteen days (Table 6.1) and collections from each location are assumed to be a random representation of the fish on the spawning ground that were susceptible to the seine. Data from the Devastation Shoal collection are reported in Chapter 4, though for a different purpose. Length measurements, sex
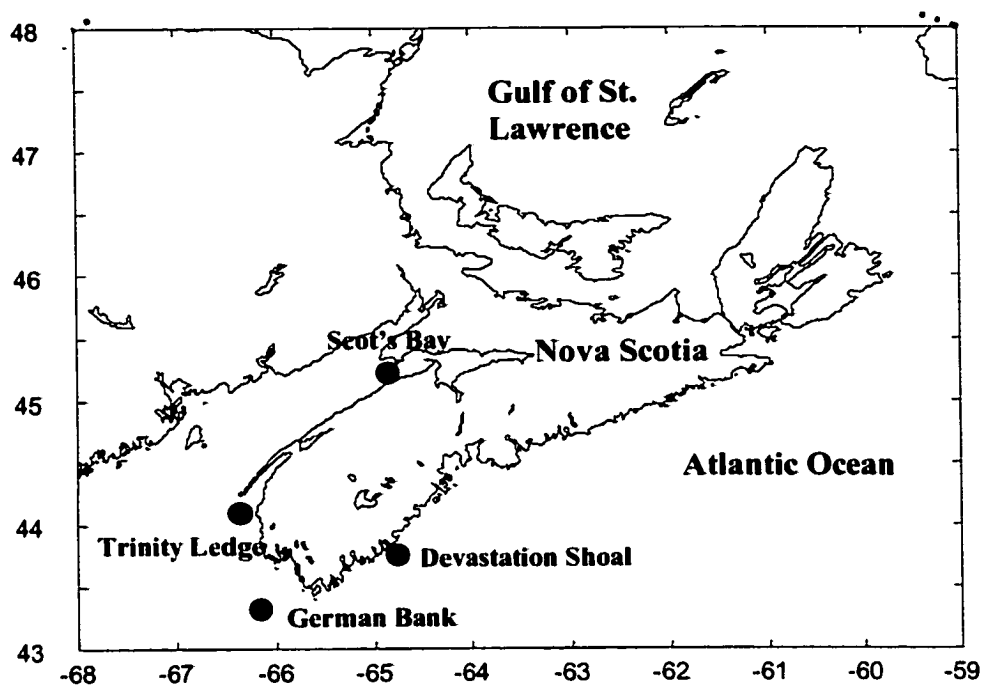
Figure 6.1
Chart of spawning grounds on the Scotian Shelf where Atlantic herring samples were collected from spawning waves in 1998.

Table 6.1 Number of herring sampled (N), date of collection, mean age and range, mean length and range, and proportion of females in each spawning wave collection.

	N	Date of collection (y-m-d)	Mean Age yr (range)	Mean Length mm (range)	% Female
Collection					
Devastation Shoal 1	62	98-10-07	6.0 (6 only)	301.2 (274-331)	44.0
Devastation Shoal 2	65	98-10-13	6.0 (6 only)	306.6 (285-345)	47.7
Scot's Bay 1	50	98-07-30	5.1 (4-6)	271.9 (228-309)	74.0
Scot's Bay 2	70	98-08-08	4.7 (3-7)	264.5 (230-306)	50.0
German Bank 1	73	98-08-19	5.7 (4-9)	296.0 (246-340)	69.3
German Bank 2	75	98-09-02	5 (4-9)	281.2 (233-341)	55.3
Trinity Ledge 1	75	98-08-27	4.9 (3-9)	274.4 (227-335)	52.0
Trinity Ledge 2	75	98-09-23	4.3 (3-6)	262.3 (234-301)	26.7

determination, and year-class information (determined using the number of annuli on the otoliths) for all individuals were provided by St. Andrews Biological Station, Department of Fisheries and Oceans. DNA was isolated using Qiagen DNeasy genomic DNA extraction methods. Nine microsatellite loci (as in Chapters 4 and 5; *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) for all samples. PCR amplification and electrophoresis conditions are given in Chapter 3 and Olsen *et al.* (2002). DNA fragments were visualized and sized using an FMBIO II fluorescent imaging system (Hitachi).

Departures from HWE were tested for each locus and collection using GENEPOP (Raymond and 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.

Pair-wise F_{ST} estimates between collections (Wright, 1951 as amended by Weir and Cockerham, 1984) were calculated using Genetix (Belkhir, 2000), and 1000 permutations were used to estimate the probability of departure from the null hypothesis of genetic homogeneity. F_{ST} estimates were also used to compare temporal and spatial genetic variation among the spawning wave collections. Nei's distance (1978), as calculated by Genetix (Belkhir 2000), was used to generate genetic dissimilarities used for multidimensional scaling (MDS; NCSS97 Hintze 1998). Linear regression techniques were used to test the null hypothesis of no linear relationship between degree of differentiation between spawning waves from the same location (as measured by F_{ST}) and time interval between collections. I also employed Exact tests (GENEPOP: Raymond and

Table 6.2

Single locus statistics for sequential spawning waves of herring showing number of sampled (N), average observed heterozygosity (*Ho*) and average number of alleles per collection. Probability of conformance to HWE is listed for each collection at each locus; Pooled refers to results of conformation to HWE when data from spawning waves in each location are pooled. P<0.05 in bold.

Sample	Locus								
	1027	1202	1059	1017	1020	1045	113	102	108
Devastation Shoal 1 N=62 *Ho*=0.83 # alleles =15.78	0.669	0.611	0.568	0.876	0.136	**0.041**	0.121	0.556	0.201
Devastation Shoal 2 N=65 *Ho*=0.77 # alleles =16	0.141	0.454	0.289	0.758	**0.041**	0.801	0.462	**0.014**	**0.029**
Pooled DS	0.164	0.705	0.403	0.695	**<0.01**	0.400	0.498	0.079	**0.037**
Scot's Bay 1 N=50 *Ho*=0.84 # alleles =15.78	**0.047**	0.902	0.195	0.644	0.495	0.179	0.226	0.382	0.106
Scot's Bay 2 N=70 *Ho*=0.80 # alleles =16.22	0.408	**<0.01**	0.442	0.361	0.779	**0.044**	0.492	0.954	0.638
Pooled SB	0.058	0.227	0.360	0.248	0.529	0.335	0.233	0.529	0.168
German Bank 1 N=73 *Ho*=0.80 # alleles =17.44	**0.031**	0.093	0.084	0.285	**<0.01**	0.723	0.156	0.765	0.478
German Bank 2 N=75 *Ho*=0.80 # alleles =17.22	0.169	0.291	0.557	0.196	0.606	0.375	0.062	0.268	0.651
Pooled GB	0.102	0.061	**0.045**	0.166	**0.036**	0.187	**0.039**	0.864	0.742
Trinity Ledge 1 N=75 *Ho*=0.82 # alleles =16.89	0.684	0.427	0.231	0.069	0.325	0.472	0.953	0.789	0.299
Trinity Ledge 2 N=75 *Ho*=0.80 # alleles =18	**0.033**	0.890	0.065	**<0.01**	0.155	0.802	0.854	0.971	0.854
Pooled TL	0.051	0.332	**0.018**	**<0.01**	0.091	0.727	0.931	0.755	0.518

Rousset, 1995) to assess the statistical significance of allele frequency differences at

individual loci, between pairs of sample locations. P-values were calculated using a

Markov chain method, described as above.

6.3 RESULTS

Significant deviations ($P<0.05$) from HWE were observed in 11 of the 72 (15.3%)

single locus tests within spawning waves (Table 6.2) from each location. When spawning

waves were pooled by location and tested for deviations from HWE, 7 of 36 (19.4%)

significant deviations were detected.

A significant difference ($F_{ST}=0.005$, $P=0.003$) was observed between the two

Devastation Shoal spawning waves that were separated by 6 days. The difference

between the two Scot's Bay spawning waves separated by 8 days was smaller and

marginal ($F_{ST}=0.003$ $P=0.040$). Only the Devastation Shoal comparisons remained

significant after Bonferroni adjustments for multiple tests. In fact, the substructure

observed at Devastation Shoal (as estimated by F_{ST}) exceeded the magnitude of

substructure (as estimated by F_{ST}) when all spawning wave collections were pooled

($F_{ST}=0.0009$). No differences (based on F_{ST} estimates) were detected between either the

German Bank spawning waves, separated by 14 days, or between the Trinity Ledge

collections, separated by 15 days. Multidimensional scaling of Nei's genetic distance

(1978) illustrates that the spawning waves from the same location tend to group closely

(at least in one dimension), although the Devastation Shoal spawning waves are a clear

exception, as they are separated in both dimensions (Figure 6.2). I also note that three of

the four second spawning waves appear to be loosely associated (Figure 6.2). I therefore
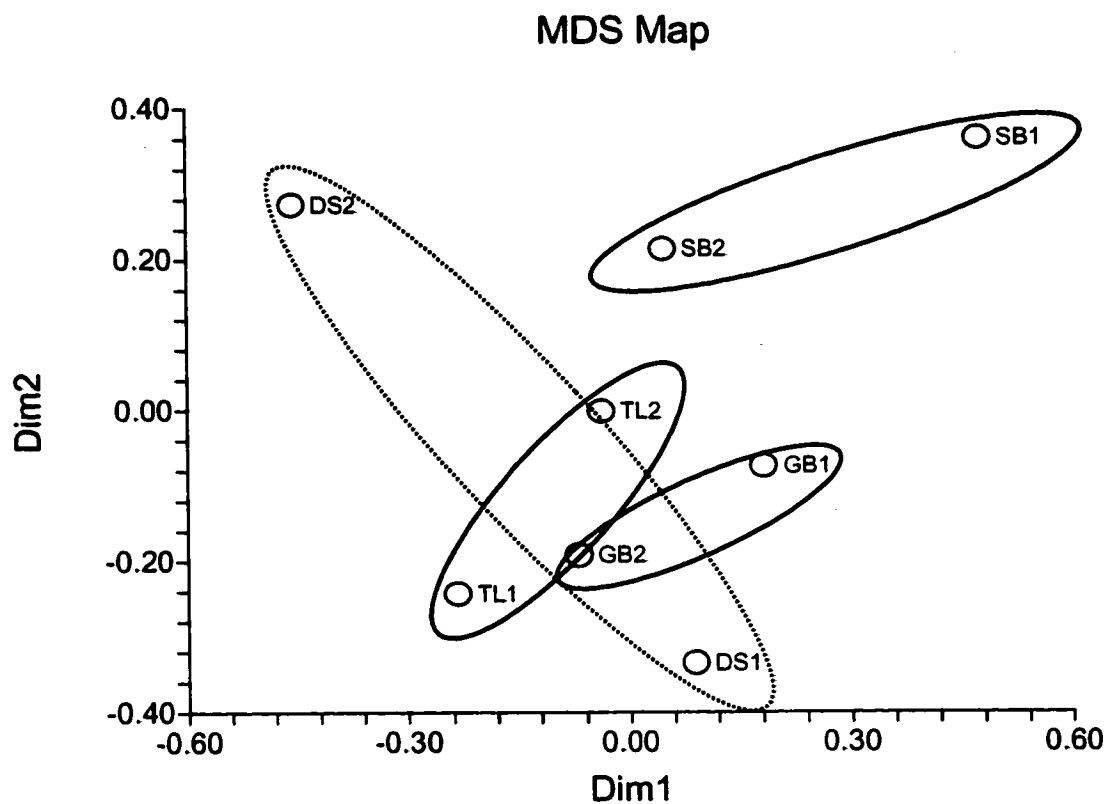
Figure 6.2
 Multi-dimensional scaling of genetic distances among spawning waves from Devastation Shoal (DS); Trinity Ledge (TL), Scot's Bay (SB), and German Bank (GB). The numbers 1 and 2 refer to the first and second spawning wave collections respectively. Collections from the same spawning location are as indicated by ovals. Broken oval reflects significant differences between spawning wave collections. Dimension 1 r=0.63; Dimension 2 r=0.07.
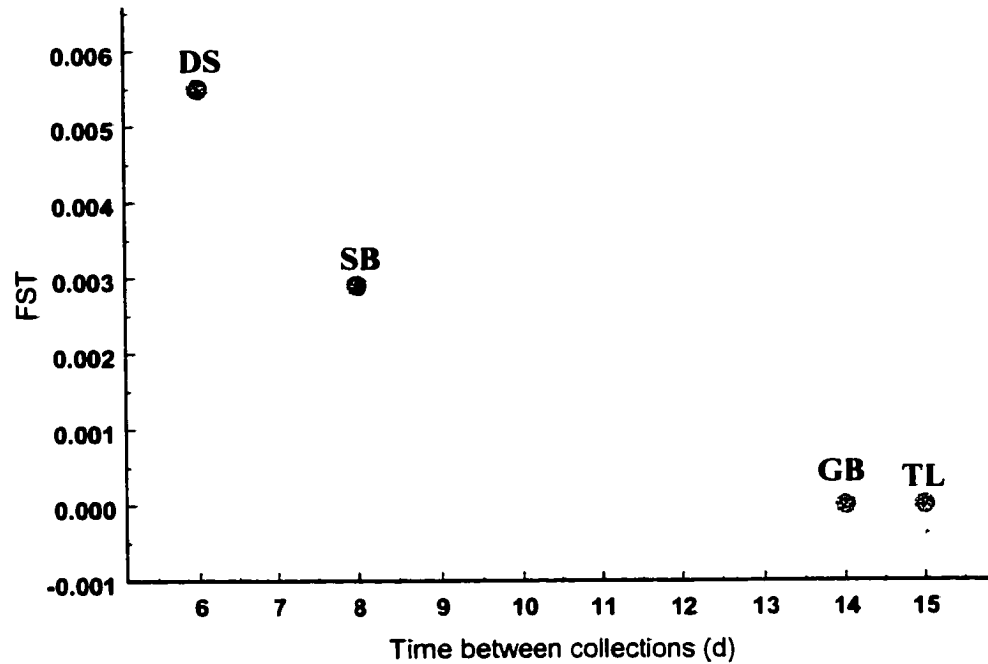
Figure 6.3
Scatter-plot of pairwise F_{ST} estimates between spawning waves in each location and time interval between spawning wave collections. DS = Devastation Shoal, SB = Scot's Bay, GB = German Bank, and TL = Trinity Ledge.

partitioned collections into first and second spawning waves (independent of location of collection). Slightly less substructure (within all samples pooled) was detected (F_{ST}=0.0007) when samples were grouped in this manner. F_{ST} estimates between waves at each location decreased as the time interval between spawning wave collections increased (Figure 6.3; r^2=0.928; P=0.03; N=4), though I consider the data far too limiting to draw strong inferences.

Single locus comparisons between spawning waves revealed differences at several loci: *Cha*1027 (P=0.0005) and *Cpa*108 (P=0.015) between the Devastation Shoal waves; *Cha*1017 (P=0.004) between German Bank waves; and *Cha*1059 (P=0.030), *Cha*1017 (P=0.032) and *Cpa*102 (P=0.040) between the Scot's Bay spawning waves. There were no single locus differences between the spawning wave samples collected on Trinity Ledge.

6.4 DISCUSSION

The results presented above demonstrate little, if any, genetic variation between what are presumed to be spawning waves of Atlantic herring on three different spawning grounds on the Scotian Shelf that have traditionally supported a commercial fishery and are known to be occupied annually by Autumn-spawning herring. However, genetic differences were revealed at a sub-annual (days) scale at a fourth location, Devastation Shoal; a spawning ground which has not shared the same level of exploitation. Although the spawning-wave phenomenon has been recognized in herring for decades, few documented observations exist (although see Lambert 1984 for northwest Atlantic

herring) and the potential for genetic variability among waves within the spawning season has not, to my knowledge, been explored.

In general, pooling of spawning waves did not reduce the number of significant deviations from Hardy Weinberg expectations. In fact, the number of significant deviations from HWE increased by 4.1% when spawning waves were pooled. This may be due to the resulting increase in stastical power due to larger sample sizes, or alternatively may reflect some degree of population admixture (e.g. Wahlund effect) when spawning waves are pooled. The second Devastation Shoal collection and the pooled German Bank spawning waves each exhibited three ($P<0.05$) departures from HWE, which suggests that spawning waves from each spawning grounds may not be homogeneous elements of the same randomly mating "population".

Differences (based on F_{ST}) between spawning waves were observed in two of the four multi-locus comparisons: marginal differences between the two Scot's Bay collections; and differences, significant after corrections for multiple tests, between the two Devastation Shoal collections. Single locus Exact tests revealed differences in three of four spawning wave comparisons, with only Trinity Ledge demonstrating no differences between spawning waves at any locus(i). However, only one single locus comparison remained significant following corrections for multiple tests - *Cha*1027 in the comparison between Devastation Shoal spawning waves.

Multidimensional scaling (Figure 6.2) of genetic distance among spawning waves illustrates the genetic similarities among collections and one might argue that all but the Devastation Shoal spawning waves are in relatively close proximity, which is consistent with the F_{ST} results. It seems reasonable to suggest, at a minimum, that these data are

consistent with temporal stability (with respect to genetic variation) among spawning waves at German Bank and Trinity Ledge. As the genetic difference between the Scot's Bay spawning waves are marginal, after corrections for multiple tests, it too may be interpreted as illustrating temporally stable spawning waves. However, the existence of temporally separated distinct populations cannot be refuted in these locations due to the limited sampling (e.g. sampling was not continued throughout the entire spawning season). Alternatively, the Devastation Shoal spawning waves differed (and remained different subsequent to corrections for multiple tests) and the difference exceeds the spatial differences among all collections. In fact, further analyses that compare all samples reveal differences among all other collections (save Trinity Ledge; F_{ST}=0.0015 and F_{ST}=0.0019; P>0.1 for first and second spawning wave respectively) and the second Devastation Shoal spawning wave.

Although limited by a small sample size, and contrary to what might have been predicted, an inverse relationship was found between the time interval separating spawning wave collections and the F_{ST} estimates between waves from each location. In particular, the Devastation Shoal collections were separated by only 6 d (the shortest interval between all waves in the collections) and were found to be the most different. This observation is consistent with a rapid (< 6 d) turn-over of fish on this spawning ground.

It has been reported that the largest and oldest herring tend to dominate spawning in the first wave, with smaller fish spawning in subsequent waves (Hay 1985). This is reflected in all other locations described here (Table 6.1), but greater than 80% of the individuals in both spawning waves on Devastation Shoal were from the same 1992 year-

class. When fish from the 1992 year-class only were compared between waves at Devastation Shoal (a direct assessment of temporal stability in a year-class between spawning waves), a difference was detected (F_{ST}=0.0043 P=0.013) as reported in Chapter 4. Because this year-class was compared directly at Devastation Shoal, the significant result is unlikely to be attributable to unequal year-class representation (in concert with a high degree of reproductive variance).

In further contrast to Hay's (1985) observations, it has been shown (Chapter 4) that herring from the 1992 year-class in the second wave at Devastation Shoal were significantly larger than herring from the 1992 year-class in the first spawning wave (t-test; P<0.001), consistent with the suggestion of rapid population turnover on the spawning ground. Taken together, the genetic and morphometric differences between Devastation Shoal spawning waves and the interval between waves can be used to estimate the replacement period of spawners at approximately 6 d or less (at least at this location). This estimate of replacement period is similar in magnitude to the shortest (8 d) estimates of residence time, calculated by Lambert (1984) using length differences among larval cohort distributions.

It is clear from the literature that reliable (accurate and precise) estimates of residence time of herring on their respective spawning grounds are limited. Yet such estimates would seem to be essential for effective resource management. Estimates of spawning biomass (generated using hydroacoustic techniques) are often (e.g. as in NAFO Division 4WX) used to infer population size for which exploitation limits are set for each spawning ground (Stephenson et al. 1999). Thus, if the residence time varies across the

spawning locations or deviates from the estimate used, or both, then faulty estimates may result.

With the exception of the Devastation Shoal collections, the first spawning wave at all other locations were composed of older and larger fish on average (Table 6.1) as predicted by Hay (1985). In addition (again with the exception of Devastation Shoal), the first spawning wave had a greater proportion of females than in the second. Ware and Tanasichuk (1989) suggest that wave-spawning is determined by differences in size-at-age and that herring of all sizes begin to mature at the same time, but that larger fish mature faster and spawn earlier, (a finding reported by both Hay 1985 and Lambert 1987). This prediction should be testable. The size composition of the collection of spawning fish should determine how spawning is distributed over time (inter-wave interval). In general, duration of spawning on each spawning ground should be protracted in years when the size range of the stock is large, if the samples truly reflect spawning waves of the same population. However, if sympatric populations were sampled, no relationship between size composition of the sample and time between spawning interval would be expected. A positive relationship was detected between length differences of spawning waves from each location and the time interval (the minimum estimate of spawning interval) between the collections ($R^2=0.89$; $P=0.02$ However, as sampling was not conducted throughout the spawning season at each location, I cannot discount the possibility that spawning extended beyond these time estimates, so the inferred spawning interval (time between spawning wave collections) represents a minimum estimate.

Management of herring in these areas has explicitly targeted the maintenance of spatial and temporal distributions of spawning to preserve the intra-specific biodiversity

within spawning groups. In light of these results, further consideration of the potentially phenotypically (different spawning times and length at age, albeit at one location) and genetically different spawning waves may be necessary within the management scheme to circumvent the erosion of biodiversity.

As few differences were observed between three of four pairs of spawning waves, can I infer that distinct sympatric populations do not exist and therefore cannot be responsible for generating the observed temporal instability reported for herring? I am uncertain and remain cautious because the herring collected 6 days apart and within a few km of each other on Devastation Shoal showed clear genetic and morphometric difference, including within year-class. At least with the data in hand, I suggest that the Devastation Shoal difference between waves may either: 1) reflect the presence of distinct Devastation Shoal populations that are temporally separated by 6 days or less; or 2) reflect sub-annual temporal genetic patchiness for which I cannot attribute a mechanism but which may involve non-random sampling or, 3) reflect a transient use of a spawning ground by a different population of herring – in which case the assumption of natal spawning site fidelity often assumed for herring would be invalid (e.g. the Trinity Ledge population is using Devastation Shoal as a spawning ground). Instantaneous sample collections (virtually simultaneous collections from the same location) and continuous sampling over the complete spawning season at these spawning grounds are necessary (but often difficult to co-ordinate) to quantify the degree of genetic variability attributable to sampling noise or random chaotic patchiness at this narrow temporal scale.

CHAPTER 7: GENETIC ANALYSES OF EARLY LIFE STAGES OF ATLANTIC HERRING: RECOLONIZATION, RESURGENCE, AND RETENTION REVISITED

7.1 INTRODUCTION

The historical record shows that herring in the North Atlantic have undergone large fluctuations in catch (c.f. Sinclair and Solemdal 1988, Stephenson and Kornfield 1990, Johannessen et al. 1995 and Anon 1998). Commercial extinctions of herring "stocks" have occurred; some of these stocks have rebounded (e.g. Georges Bank; Stephenson and Kornfield 1990) and some have not (e.g. Downs; Cushing 1992). The reappearance of several stocks and the fluctuations documented in others have fueled interest in the relative 'connectivity' of different spawning groups (Stephenson et al. 2001) and the associated likelihood of resurgence of the extirpated 'population(s)' or colonization by other 'population(s)' of the habitat once used by an extirpated population.

Increased numbers of adult herring (Harris and Stephenson 1999) have been documented in the vicinity of Western and Sable Banks (denoted by the 50m isobath; Figure 7.1), where herring were caught in high numbers by a multi-national fleet prior to the extension of national jurisdiction in 1977 (Stephenson et al. 2000) but were virtually absent from groundfish survey catches in the late 1970s and early 1980s. These and the observations of Reiss et al. (2000), who collected small larvae offshore in 1997 and 1998, led me to question whether these observations reflected resurgence of an existing population (centered on Western and Sable Banks), or re-colonization of the Scotian Shelf from herring normally found elsewhere in the North Atlantic. It may be possible to examine this question using the larvae collected by Reiss et al. (2000) and adult
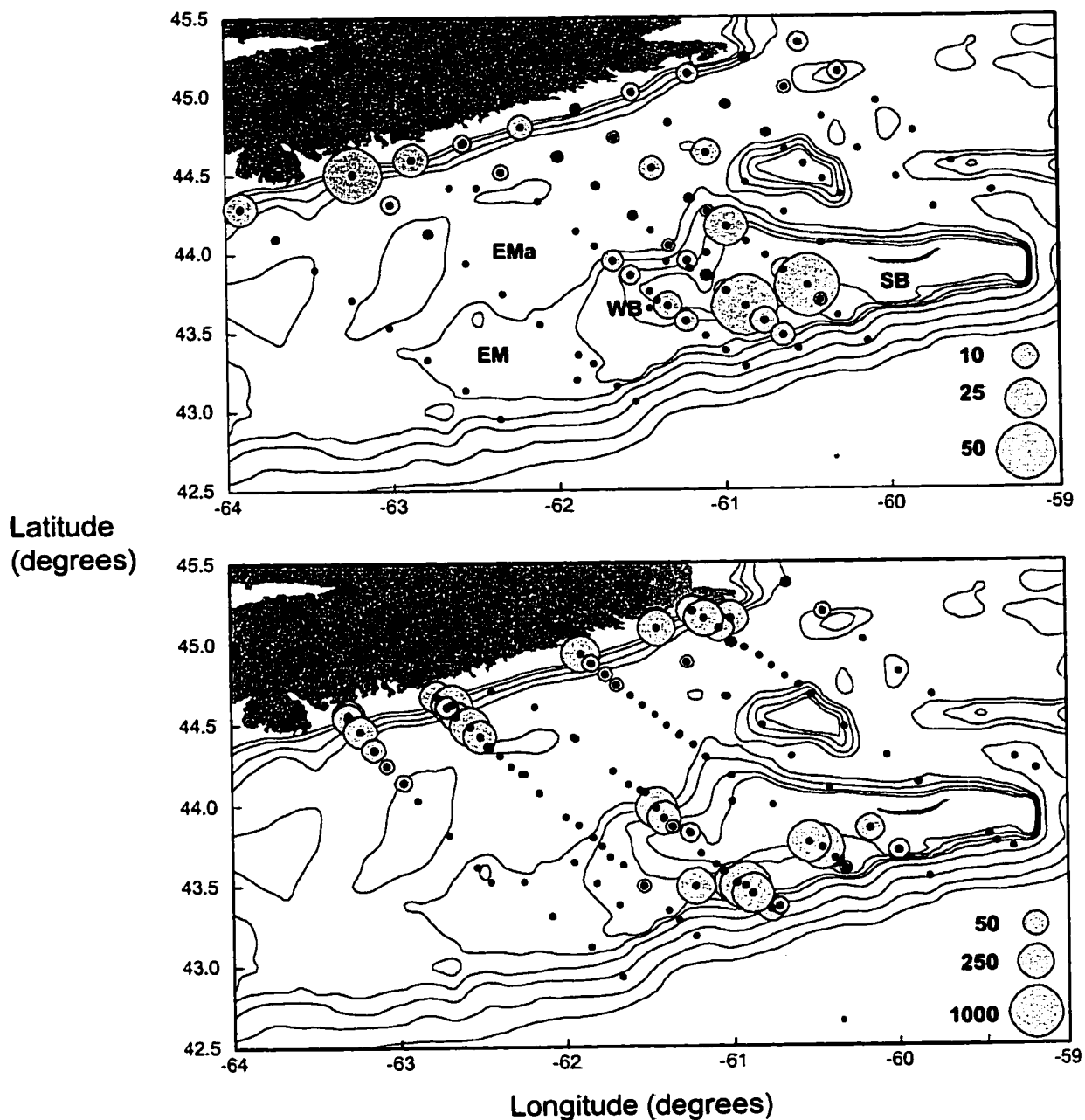
Figure 7.1
Bathymetric (50, 80, 100, 150, 200, 500, 1000 and 1500 m isobaths) chart of the central Scotian Shelf showing the spatial distribution of larval Atlantic herring abundance (shaded circles; number·$(100 \text{ m}^3)^{-1}$) at sampling stations (solid circles) in November of: a) 1997 and b) 1998. Features include Emerald Bank (EM), Emerald Basin (EMa), Western (WB) and Sable (SB) Banks. Amended from Reiss et al. 2000.

herring from spawning grounds inshore and offshore. The reappearance of herring on the offshore banks (by whatever process) and the occurrence of larvae in neighbouring locations also provides an opportunity to test the generalities proposed by Iles and Sinclair (1982), in what is generally referred to as the "retention hypothesis". Iles and Sinclair (1982) hypothesize that genetically distinct herring occur in geographically discrete regions, defined by physical features (e.g. fronts) that serve to retain larvae in a suitable habitat. Although herring are thought to exhibit natal spawning-site fidelity (Wheeler and Winters 1984), Sinclair (1988) further postulated that larvae "lost" from their retention zone would be "lost" to their natal population and be at a reproductive disadvantage (i.e., will not reproduce successfully) in their "adopted" population. Such a process will limit gene flow among spawning groups (populations).

The distribution of herring larvae on the Scotian Shelf shown in Figure 7.1 coincides with spawning locations inshore and offshore, each characterized by different spawning habitats that experience different circulation patterns (including recirculation and possibly retention around Western Bank and advection into the Nova Scotia coastal current that flows from northeast to southwest along the coast of Nova Scotia). Here I define retention as physical processes leading to reduced dispersion of larvae following Reiss et al. (2000) that eventually results in a size (age) frequency distribution that is skewed toward larger sizes. The larvae associated with these different hydrographic environments (inshore – advection and offshore - retention) may allow me to examine the relative roles of advection and retention in maintaining population structure (e.g. if larvae are retained offshore, are they genetically distinct as predicted by Iles and Sinclair; if

larvae are not retained and are distinct, how is this reconciled with the predictions of Iles and Sinclair?).

When examining questions of population differentiation using samples collected over time and space, it is essential to recognize the disparity between effective and observed population size and the temporal genetic patchiness of very abundant marine organisms that can result from a large variance in reproductive success among individuals (Hedgecock 1994). In extreme cases, successful recruits may be the progeny of a small proportion of the population whose reproductive output is in synchrony with (unknown) oceanographic conditions conducive to spawning, larval development and recruitment. This is generally known as the sweepstakes hypothesis. Although Ruzzante et al. (1996) attempted to test this proposition in analyses of Atlantic cod, (*Gadus morhua*), the same authors reported that larval cohorts (defined by length and age) within a location were genetically indistinguishable from each other and from adults collected from the same location and they concluded that larvae were generated from the same "population", which seems inconsistent with a sweepstakes model. Here, cohorts of larvae (defined by length) collected at the same location are compared and subsequently compared to the spawning adults that represent the population presumed to have produced the larvae to test for sweepstakes events in Atlantic herring (e.g. no differentiation between cohorts and among adults and cohorts would be inconsistent with sweepstakes events).

Given the wide and relatively stable distributions of adult spawning herring in coastal regions of the Scotian Shelf (Anon 1998; Figure 1.1), the relatively recent increase in adult and spawning herring in the offshore region, the distribution of recently hatched larvae offshore and inshore on the Scotian Shelf in 1997 and 1998, and the

availability of larval samples from multiple inshore and offshore locations in 1997 and

1998, an opportunity arises to address a suite of questions that relate the processes

occurring during the early life history stage of herring to patterns of population structure.

Thus, in this Chapter, two years of larval herring samples from different regions of the

Scotian Shelf, (along the south eastern coast of Nova Scotia and in the vicinity of

Western Bank) are compared. I use larval size and genetic analyses based on

microsatellite data to assess the potential for the mixing of individuals among populations

and the resulting likelihood of population colonization versus resurgence. Genetic

analyses are used to infer connectivity among populations indirectly, avoiding problems

associated with direct measurements, that are often time-consuming (e.g., mark and

recapture techniques) and expensive (e.g., telemetry). Larvae assumed to have been

collected from different oceanographic environments are compared and an attempt is

made to reconcile the results with the retention hypothesis of Iles and Sinclair (1982).

Larval cohorts from the same location are compared and comparisons among those

cohorts and spawning adults collected along the coast of Nova Scotia and offshore (i.e.

presumed to represent the "putative" populations that produced larvae in question) may

provide elucidation of the relevance to resurgence/colonization process, the incidence of

sweepstakes events, and temporal stability.

7.2 METHODS

7.21 SAMPLE COLLECTIONS

Larval herring were collected from stations (Figure 7.1) across the central Scotian

Shelf, aboard the *CCGS Alfred Needler* (18 to 28 November 1997; 18 to 21; 23-28

November 1998) and *CCGS Hudson* (25 October to 10 November 1997).
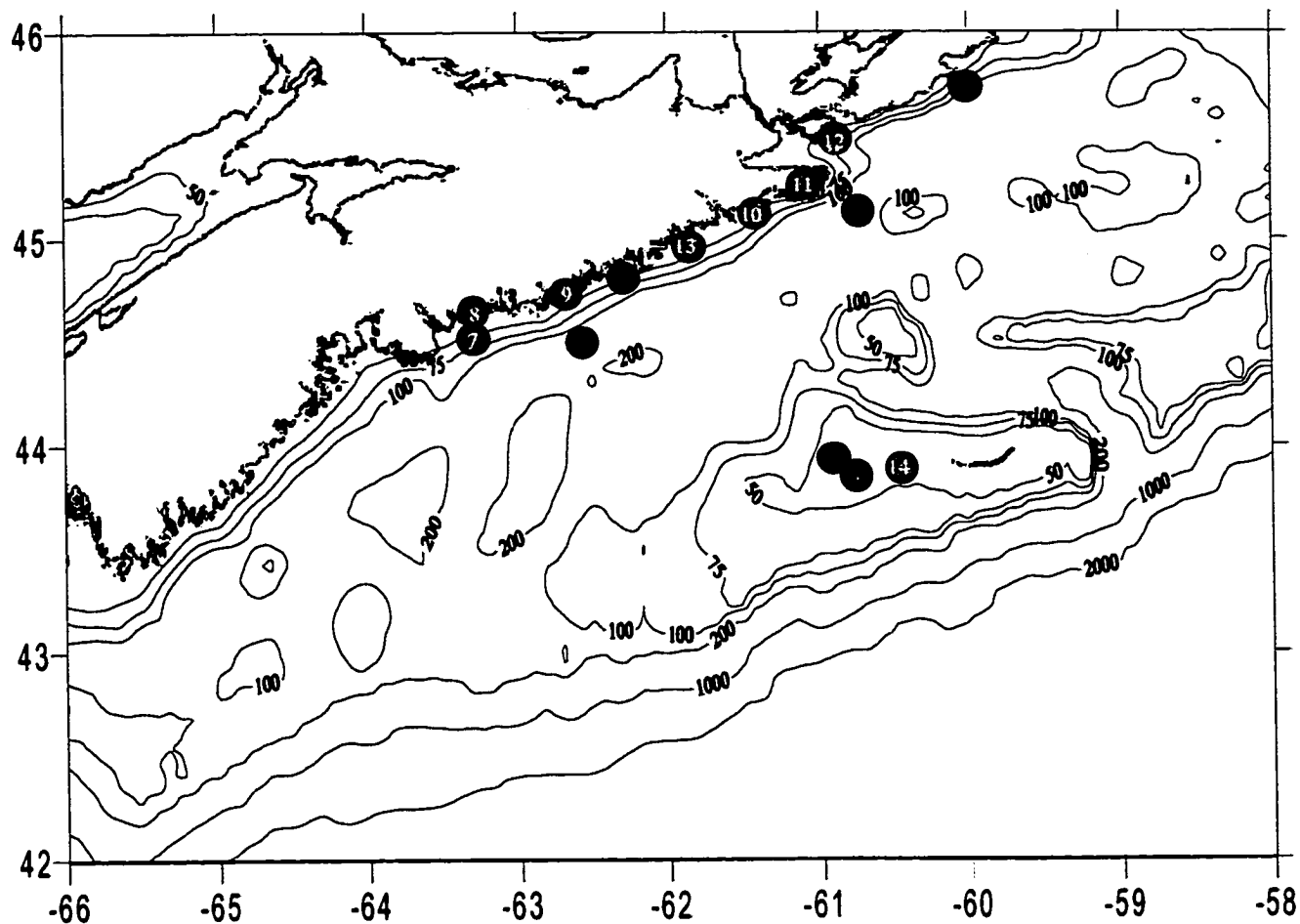
Figure 7.2
Bathymetric (m) chart showing sample locations of larval herring collections on the
central Scotian Shelf where numeric identifiers correspond to stations in Table 7.1.
Samples collected in 1997 (1-6) are indicated by black filled circles and samples
collected in 1998 (1-14) by grey filled circles.

Hydrographic data were collected at all but one station, using a Seabird SBE-25 conductivity, temperature, and depth sensor (CTD), deployed to within ~ 5 m above bottom. The regional flow field for the surface layer was estimated by Reiss et al. (2000), using the CTD (density) data, the dynamic height method of Sheng and Thompson (1996), and the variational algorithm of Grotov et al. (1998; see Panteleev et al. 2001).

Larvae were sampled using the netminder-II BONGO system (Bedford Institute of Oceanography, Dartmouth, NS), consisting of a 61 cm diameter bongo frame, fitted with 333 μm mesh-nets and an electronics package that transmitted pressure, temperature, pitch, roll, and filtered volume data through the conducting tow cable. On retrieval, starboard-side net samples were preserved in 70-85% ethanol (final concentration). All fish larvae were removed from the plankton samples and larval herring were identified and enumerated. Herring larvae from stations representing offshore and inshore samples in each year (Figure 7.2) were removed from plankton samples and total length was measured to the nearest 0.1 mm using SIGMASCAN imaging software and a dissecting microscope. The first ten herring larvae from each station (Stn) were measured three times to assess the precision associated with measurements. No correction for larval shrinkage was applied. Sample collections from offshore and inshore were preferentially selected to address questions (outlined above). Tissue samples (blood, fin and/or muscle) were collected from spawning stage Atlantic herring on German Bank (southwest Nova Scotia) in 1998, near Eastern Passage (inshore-along the eastern coast of Nova Scotia) in 1999, and on Western Bank in 2000. Analyses of these samples were previously reported in Chapter 5 and were discussed within the context of broad-scale patterns of population structure in North Atlantic

herring and are used here to characterize the genetic composition of successful recruits at each location.

7.22 LABORATORY ANALYSES

DNA was isolated from whole larvae and adult tissues using Qiagen DNeasy genomic DNA extraction methods and eluted in 50 μl TE buffer (Qiagen). Eight microsatellite loci (*Cha*1027, *Cha*1020, *Cha*1059, *Cha*1202, *Cha*1017, *Cpa*108, *Cpa*113, and *Cpa*102) were amplified using PCR. PCR amplification and electrophoresis conditions for the *Cha* and *Cpa* loci are provided in Chapter 3 and in Olsen *et al.* (2002) respectively. DNA fragments were visualized and sized using an FMBIO II fluorescent imaging system (Hitachi). Due to the amount of DNA extracted from each larva, reruns of individuals were often not possible. Therefore, samples that amplified at fewer than four loci were excluded from analyses.

7.23 RESURGENCE VS COLONIZATION

Departures from HWE were tested for each locus and larval collection (where N> 25) using GENEPOP v3.1d (Raymond and Rousset 1995). Significance (*P*-value) for each comparison was estimated using the Markov chain method with 2000 dememorizations, 200 batches and 2000 iterations per batch. Association between pairs of loci within each sample collection, measured as genotypic disequilibrium, was tested using GENEPOP v3.1d (Raymond and Rousset 1995; significance estimated as above) to identify collections of larvae that may have been produced by a relatively small number of individuals.

Table7. 1
Summary statistics for all sample collections (with N>25) showing number of herring (N), year of collection, median length of larvae in sample, average observed heterozygosity (Ho) and average number of alleles per collection. Probability of conformance to HWE is listed for each collection at each locus; P<0.05 in bold; * significant departure from HWE after Bonferroni correction. Genotypic disequilibrium between pairs of loci within sample collections are indicated: † and ‡ P<0.01 and € P<0.001. IN denotes samples comprising Inshore and OFF denotes samples comprising Offshore classification for subsequent analyses.

			Locus							
		Median length of larvae	1027	1202	1059	1017	1020	113	102	108
1997	1. IN N=96; Ho=0.780 # alleles=17.13	8.7 mm	0.158	0.306†	0.977	0.909†	0.648	0.286	0.132	0.772
	2. OFF N=60; Ho=0.785 # alleles=16.13	7.0 mm	**0.022**	0.481	0.263	0.070	0.481	0.385	**0.002**	0.464
	3. IN N=28; Ho=0.749 # alleles=12.63	10.2 mm	0.951	0.241†	0.109†	**0.015**	0.222	0.712	0.554	0.191
	4. OFF N=69; Ho=0.778 # alleles=15.75	7.6 mm	0.302	0.677	**0.014**	0.388	0.529	0.982	0.349	0.093
	5. IN N=6	16.2 mm								
	6. IN N=8	18.8 mm								
1998	7. IN N=33; Ho=0.825 # alleles=13.63	6.8 mm	**0.001**	0.302	0.560	0.124	0.526	0.205	0.543	0.973
	8. IN N=78; Ho=0.806 # alleles=15.63	8.2 mm	0.114	0.714	0.198	0.869	0.786	0.093	**0.031**	0.651
	9. IN N=98; Ho=0.783 # alleles=17.38	7.9 mm	**0.005**	0.677†	0.387‡	0.089†	0.277	0.356	0.137	0.178‡
	10. IN N=190; Ho=0.800 # alleles=21.13	7.4 mm	0.598	0.099	0.594	0.821	0.340	0.201	0.464	0.504
	11. IN N=61; Ho=0.783 # alleles=15.75	8.2 mm	0.225	0.163†	0.984	0.667	0.133	0.325†	0.219	0.733
	12. IN N=193; Ho=0.780 # alleles=20.25	8.2 mm	0.334	0.516	0.057	0.229	0.080†	0.220	0.127	0.328†
	13. IN N=80; Ho=0.805 # alleles=18.38	8.4 mm	**0.003€†**	0.366€	0.052†	0.463‡	0.264	0.081	0.586	0.443‡
	14. OFF N=233; Ho=0.795 # alleles=21.63	7.1 mm	0.790	0.415	**<0.001***	0.508	0.817	**0.030**	**0.018†**	0.290†

Larvae were then recategorized into four groups, based on location and year of sample collection: offshore (in the vicinity of Western Bank) vs inshore (along the eastern coast of Nova Scotia) in 1997 and 1998 (Table 7.1). Larvae from stations with <25 individuals were only used (due to sample size limitations) when pooled in inshore vs offshore comparisons. As differentiation between inshore and offshore collections may be inconsistent with colonization of the offshore from proximate (inshore) spawning locations, multi-locus F_{ST} estimates (Wright, 1951 as amended by Weir and Cockerham, 1984) between inshore and offshore sample collections (as indicated in Table 7.1) in each of 1997 and 1998 were calculated using Genetix (Belkhir, 2000) and 1000 permutations were used to estimate the probability of departure from the null hypothesis of F_{ST}=0. Larval collections were also compared (using F_{ST} estimates) with three spawning stage adult herring collections (Chapter 5) from the vicinity of Western Bank and along the southwest coast of Nova Scotia. Multidimensional scaling (NCSS97 Hintze 1998) of F_{ST} was used to illustrate relative differences among collections in two dimensions. An Exact test was also employed (GENEPOP: Raymond and Rousset, 1995) to assess the statistical significance of allele frequency differences at individual loci between pairs of collections. Significance was calculated using the Markov chain method as above.

Potential cohorts (larvae that are presumed to result from a single spawning event or multiple spawning events in close temporal proximity) were identified using length frequency distributions and all larvae > 10 mm were removed from each collection to minimize the probability of more than one larval cohort within the collection. Analyses were then repeated (as above) to identify any potential effects of cohort admixture.

7.24 ROLE OF RETENTION AND ADVECTION

The potential role of the Nova Scotia coastal current in mixing larvae from different sources (assuming passive advection of larvae into and in the coastal current) was investigated by regressing the coefficient of variation of larval lengths and the average number of alleles for each coastal collection in 1998 (sample size was too limited in 1997) on the relative position along the coast (and therefore potential position within the coastal current). As above, larvae > 10 mm were removed from each 1998 collection to minimize the probability of multiple cohorts being considered. Subsequent to exclusion of larvae > 10 mm, the average number of alleles at each coastal station was re-calculated and compared to the previous calculations. The degree of substructure (as estimated by F_{ST}) pre - and post - exclusion of larvae > 10 mm, within collections in 1998 was also compared to assess the potential effect of combined cohorts.

An assignment test was used to assess the potential for multiple sources of larvae (other than collection site) within collections in 1998. To define the reference (baseline) genetic information for assignment analyses, only larvae < 7.5 mm in length (larvae are reported to hatch at ~7 mm; Lambert 1984) were used to ensure that all larvae originated at (or in very close proximity to) the collection site. The probability of belonging to each site was calculated for all individual larvae within each reference group, using a Bayesian approach for estimating allele frequencies (as performed by Geneclass; Cornuet et al. 1999). Degree of successful assignment was then compared to assignments expected by chance to assess the ability of the test to correctly assign an individual to its baseline collection. Larval herring >7.5 mm (potentially originating elsewhere) were then assigned

to the baseline collection for which they had the highest probability of belonging, given their genotypes.

A second test of alternate larval origin, based on larval length, was then conducted and compared to the genetic assignment test results. A larval herring growth rate of 0.2mm per day (average reported by Lambert 1984 for larval herring in St. Mary's Bay, Nova Scotia) and a mean circulation pattern, as generalized in Reiss et al. (2000), were used to investigate potential sources (other than collection site) for herring larvae >7.5 mm in 1998, to determine whether or not exchange between offshore and inshore was possible (i.e., can larvae collected offshore have originated inshore and vice versa?). The utility of these mean surface circulation estimates is dependent on the assumption that the larvae collected are derived from the surface (mixed) layer, an assumption also made by Reiss et al. (2000). The potential for alternate origins of larvae from coastal collections in 1998 was also evaluated. The average growth rate of 0.2 mm per day was applied to back-calculate where larvae might have originated, had they been entrained within the coastal current. Larvae were then compared (genetically using F_{ST}) to their alternate origin location, where sufficient numbers (N>25) allowed.

7.25 SWEEPSTAKES EVENTS

Differences between cohorts of larvae collected from the same location or differences between larval cohorts and the adults assumed to have produced them (or both) might occur as a result of sweepstakes events (see Chapter 6). Larval collections from offshore stations in the vicinity of Western Bank (Stn-2, -4 and -14 in Figure 7.2) in 1997 and 1998 were compared (using F_{ST} estimates) with spawning stage adult herring collected in 2000 from Western Bank (N=75) to investigate the potential for sweepstake

events and temporal stability. Larvae >10 mm (and therefore assumed to represent a different cohort) in each of these larval collections were isolated and compared to the remaining cohort (<10 mm larvae). In one inshore location in 1998 (Stn-7 and -8; Figure 7.2), samples were collected five days apart, providing an additional opportunity to test for differences among collections (which I treated as different spawning events). Further, spawning stage adult herring were collected inshore from virtually the same location in 1999 (Eastern Passage N=75) as Stn-7 and -8 in 1998 and were compared to larval collections to test for shifts in allele frequencies over time.

7.3 Results

7.31 SAMPLE COLLECTIONS

Larval herring assemblages of high concentration were associated with the flanks of Western and Sable banks and along the coast of Nova Scotia (Figure 7.1) in each of 1997 and 1998. In 1998, a discontinuity in larval distributions inshore and offshore can be identified, as there is a general absence of herring larvae at all stations sampled between the coast of Nova Scotia and the offshore banks. Herring were most concentrated (> 50 per 100 cubic meters of water sampled) along the southern flank of Western Bank (Figure 7.1) in both years. The various size-classes of herring were not limited to specific water masses, although Reiss et al. (2000) concluded that the highest concentrations of small larvae were associated with the slightly cooler and fresher water masses and larger herring were equally associated with all water masses. Stations representing offshore and inshore collections in each year were selected for further analyses (Figure 7.2). The spatial distribution of the size-classes of herring larvae

indicates multiple spawning locations/populations (Figure 7.2) as small larvae

(consistent with recent emergence) were observed in multiple locations.

The median length of larvae (Table 7.1) within each station ranged from 6.8 (Stn-

7, inshore in 1998) to 18.8 mm (Stn-6, inshore 1997), suggesting that there is

considerable age (as inferred from size) variation among larvae within and among

stations. Length frequency distributions of larval herring (Figure 7.3) show multiple

cohorts at stations 3, 5, and 6, along the coast in 1997. Size distributions at Stations 1, 2,

and 4, collected along the coast and offshore in 1997 appear to reflect a single cohort.

Within the 1998 collections, Stn-7, -11, -13, and –14, collected along the coast and

offshore also appear to be dominated by one cohort. In contrast, Stn-8, -9, -10, and -12

(collected along the coast in 1998) encompass larval length ranges consistent with

multiple cohorts. When only one cohort is observed, the mode of the length distribution

falls between 6 and 9 mm - consistent with samples being comprised of recently hatched

larvae.

7.32 RESURGENCE VS COLONIZATION

All loci were polymorphic in all sample collections, and levels of average

heterozygosity per collection ranged from 0.780 to 0.825 (Table 7.1). Of the 96 single-

locus tests for conformation to HWE, 11 had significance values of <0.05 and only one

test (Stn-14, collected in the vicinity of Western Bank; *Cha1059*) showed significant

($P<0.001$) departure from HWE, following Bonferroni correction for multiple tests

(Table 7.1). Multi-locus combinations of single-locus tests within samples (following

Fisher 1954) also indicated that collections from Stn-2 and -14 (both collected in the
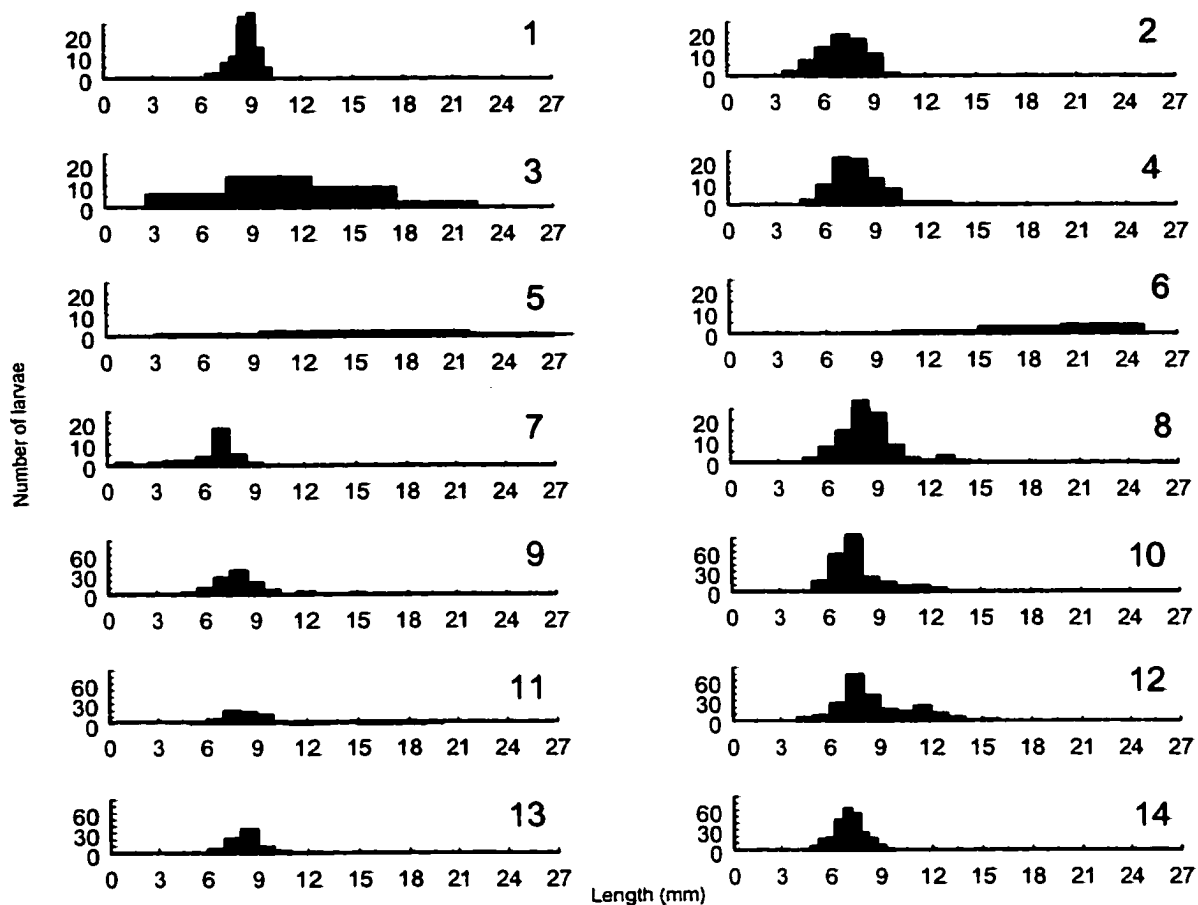
Figure 7.3
Length frequency distributions of larval herring collected in 1997 and 1998 at different coastal and offshore stations on the Scotian Shelf. Station numbers in each panel correspond to those in Figure 7.2 and Table 7.1. Larvae less than 6 mm were fractured and not considered in length-dependent analyses. Note that different abundance (number of larvae) scales are used.

vicinity of Western Bank) departed from HWE ($P=0.0001$), following Bonferroni correction, suggesting that individuals were not sampled from a single, large, randomly mating population. Significant associations among 10 pairs of loci within sample collections were observed (Table 7.1). Three pairs of associated loci occurred in larvae collected at Stn-13 and two associated pairs occurred in those collected at Stn-9; both located along the coast of Nova Scotia in 1998.

No significant differences (based on F_{ST} estimates) were observed in the pairwise comparisons among combined inshore and offshore larval collections in 1997 and 1998 (Table 7.2); in fact, greater differentiation (~2 to 4 fold increase in F_{ST}) was found between inshore collections in 1997 and 1998 than among inshore and offshore collections from either year. When individual collections were compared (using all samples comprised of ≥25 individuals), 18 pairwise comparisons differed at $P<0.05$ and seven of these comparisons remained significant after Bonferroni adjustments for multiple comparisons (Table 7.3). All seven cases included the larvae collected at Stn-8 located midway along the coast of Nova Scotia. Stn-8 is not unique in either abundance or length distribution of larvae (Table 7.1 and Figure 7.3); i.e. the overlapping collection location, the abundance of larvae, and the size of larvae are each directly comparable to Stn-9 and few collections differ genetically from Stn-9. Exact test results are consistent with F_{ST} analyses; all comparisons (and several others) with significant F_{ST} results also yield single-locus Exact test differences at locus Cha1017. In fact, differences at Cha1017 may be the result of the 4-5 fold greater frequency of one of 20 alleles occurring at this locus in Station 8 larvae (Figure 7.4).

Table 7.2
Pairwise F_{ST} estimates between inshore and offshore larval herring collections in 1997 and 1998 on the Scotian Shelf (N; sample size). F_{ST} estimates are above diagonal and P values associated with these comparisons are below the diagonal.

	Samples Collection			
	1997 Inshore N=138	1997 Offshore N=129	1998 Inshore N=733	1998 Offshore N=233
1997 Inshore		0.00012	0.00056	0.00018
1997 Offshore	0.405		0	0
1998 Inshore	0.111	0.631		0.00031
1998 Offshore	0.361	0.783	0.161	

Table 7.3
Pairwise F_{ST} estimates between all collections of larval herring (N>25) are above the diagonal. Pairwise F_{ST} estimates between 1998 larval collections with larvae >10 mm removed are below diagonal. P<0.05 in bold and *P<0.00001 and significant after Bonferroni adjustment for multiple tests.

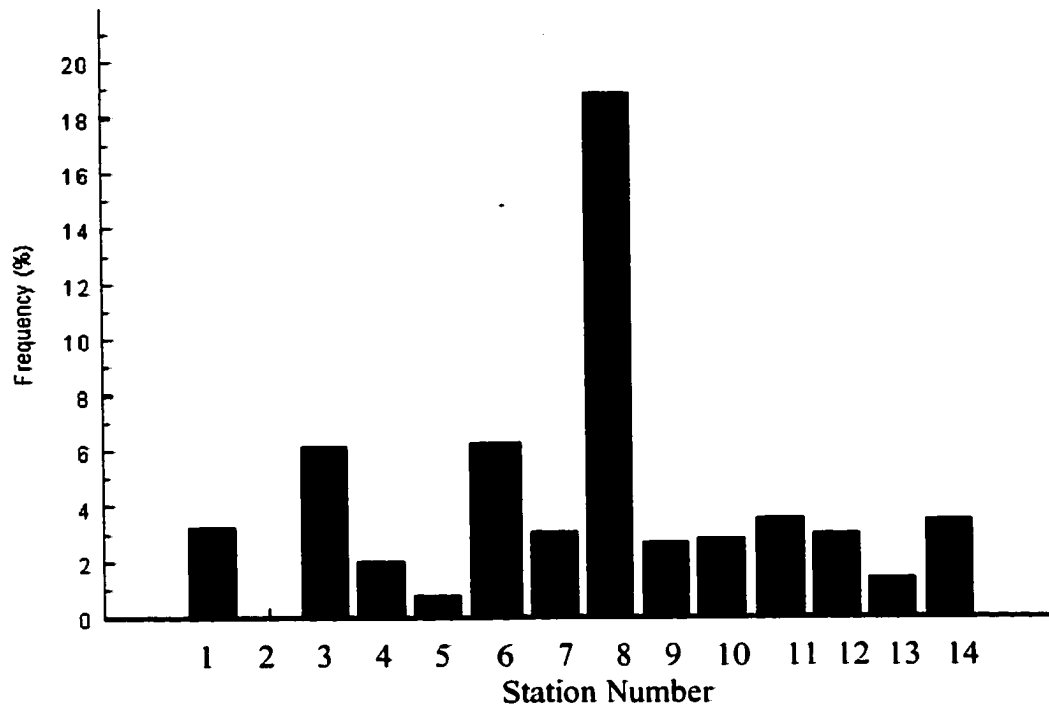|						Sample							
	1	2	3	4	7	8	9	10	11	12	13	14
1		0.0004	0	0.0007	0.00078	**0.0061***	0.0012	0.0014	0.0019	**0.0023**	**0.0025**	0.0002
2			0	0.0003	0	**0.0026**	0.0015	0	0	0.0003	0.0017	0.00003
3				0	0	0.0011	0	0.0012	0	0.0031	0.0002	0.0007
4					0	**0.0060***	0.0012	0.0003	0	0	**0.0039**	0
7						0.0053	0.0005	0	0.0001	0.0024	0.0010	0.0012
8					0.0044		**0.0076***	**0.0068***	0.0053	**0.0072***	**0.0066***	**0.0043***
9					0.0005	**0.0074***		**0.0015**	0.0009	0.0012	0.0020	0.0014
10					0	**0.0066***	0.0015		0.0006	0	0.0020	0.0005
11					0.0007	0.0055	0.0017	0.0012		0.0012	0.0048	0.0003
12					0.0029	**0.0060***	0.0012	0	0.0022		0.0028	0.0007
13					0.0009	**0.0068***	0.0025	0.0021	**0.0061***	0.0026		**0.00311**
14					0.0012	0.0039	0.0013	0.0006	0.0013	0.0003	**0.0033***	

Figure 7.4
Frequency of the 162 allele in locus *Cha*1017 within all larval herring collections inshore and offshore on the Scotian Shelf in 1997 and 1998.

Adult samples collected from Western Bank in 2000, did not differ from offshore larval collections in either 1997 nor 1998, and Western Bank adults differed only from Stn-8 larvae (F_{ST}=0.0034; P=0.001). However subsequent to Bonferroni adjustments, this difference was not significant. Adult samples collected in the vicinity of Eastern Passage (which corresponds to the approximate location of larval collections at Stn-7 and -8) differed from Stn-1 (F_{ST}=0.0025; P=0.041), -4 (F_{ST}=0.0027; P=0.042), -8 (F_{ST}=0.0081; P=<0.0001), -10 (F_{ST}=0.0024; P=0.012), -12 (F_{ST}=0.0023; P=0.015), and -14 (F_{ST}=0.0028; P=0.008). However, only the collection taken in approximately the same location as Eastern Passage, (Stn-8 –which also has the largest pairwise F_{ST} value) differs after Bonferroni adjustment, and this is in contrast to what might be expected as these larvae were hypothesized to be the progeny of the adult population spawning in this Eastern Passage area. Adult herring collected on one of the main southwest Nova Scotia spawning locations, German Bank, differed from larvae collected at both Stn-8 and -13 at P<0.05, but only the comparison with Stn-8 remained significant after Bonferroni correction. Multidimensional scaling (Figure 7.5) of these relative differences illustrated that the Eastern Passage adult collections and the Stn-8 larval collection (samples collected from virtually the same location) are found at opposite ends of dimension 1. Conversely, offshore larval collections, adults from Western Bank and adults from Southwest Nova Scotia spawning grounds overlap in both dimensions.

In 1998, substructure within pooled collections did not vary when larvae >10 mm (<10% of data; Figure 7.3) in length were excluded (F_{ST}=0.00209 before exclusion of larvae as compared to F_{ST}=0.00203 after exclusion). However, in three pairwise comparisons between 1998 collections, exclusion of >10 mm larvae generated different
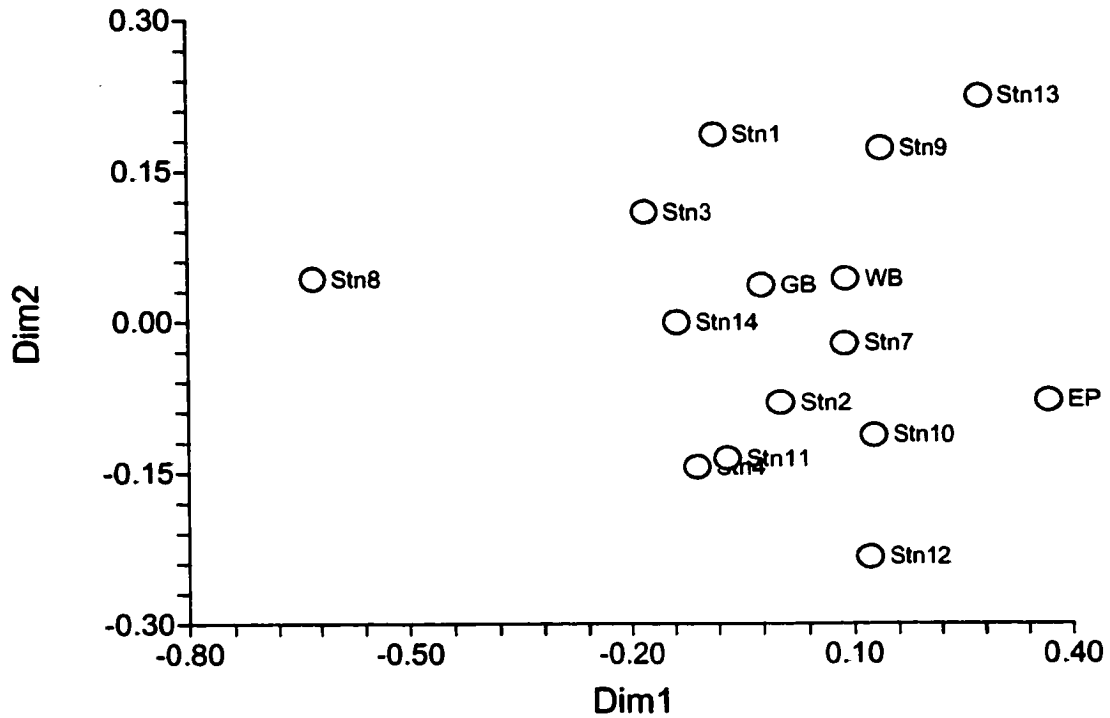
Figure 7.5

Multidimensional scaling of pairwise F_{ST} for all sample collections of larval herring on the Scotian Shelf where N ≥ 25. Also included are Eastern Passage (EP), Western Bank (WB) and German Bank (GB) adult collections (Figure 5.1).

results: comparisons between larvae from Stn-13, collected inshore, and those from Stn-14, collected offshore, and between Stn-8 and -13 (both collected inshore) were significant (after Bonferroni adjustment) while Stn-8, collected inshore, and Stn-14, collected offshore, were no longer significant (after Bonferroni adjustment), when the larger cohort was excluded.

7.33 ROLE OF RETENTION AND ADVECTION

The coefficient of variation of larval lengths for each coastal collection in 1998 increased (r^2=0.79; P=0.007) with distance northeast upstream of the coastal current along the coast (Figure 6). When the number of alleles at each sample location was regressed on relative distance (Figure 7.6), a positive and marginally significant relationship (r^2=0.49; P=0.082) resulted; i.e. there was an increase in both the coefficients of variation of larval lengths and the average number of alleles eastward along the coast, which is the opposite to what I would have predicted if larvae from multiple sources (with the potential for different alleles) were mixing as one moves downstream in the coastal current. When larvae > 10 mm were excluded and number of alleles compared (pre - and post-exclusion), significantly fewer alleles were detected after excluding >10 mm larvae (T-test; P=0.019) and the slope of the average number of alleles vs relative distance decreased (0.397 as compared to 0.489). Less variation was explained by relative distance (r^2=0.427; P=0.111) with the larger (>10 mm) larvae excluded.

Between 0% (Stn-11) and 31% (Stn-10) of the baseline samples (comprised of larvae <7.5 mm) were correctly assigned, using an assignment test. Assignment success of 13% at each station can be explained by chance alone. In light of these results, few
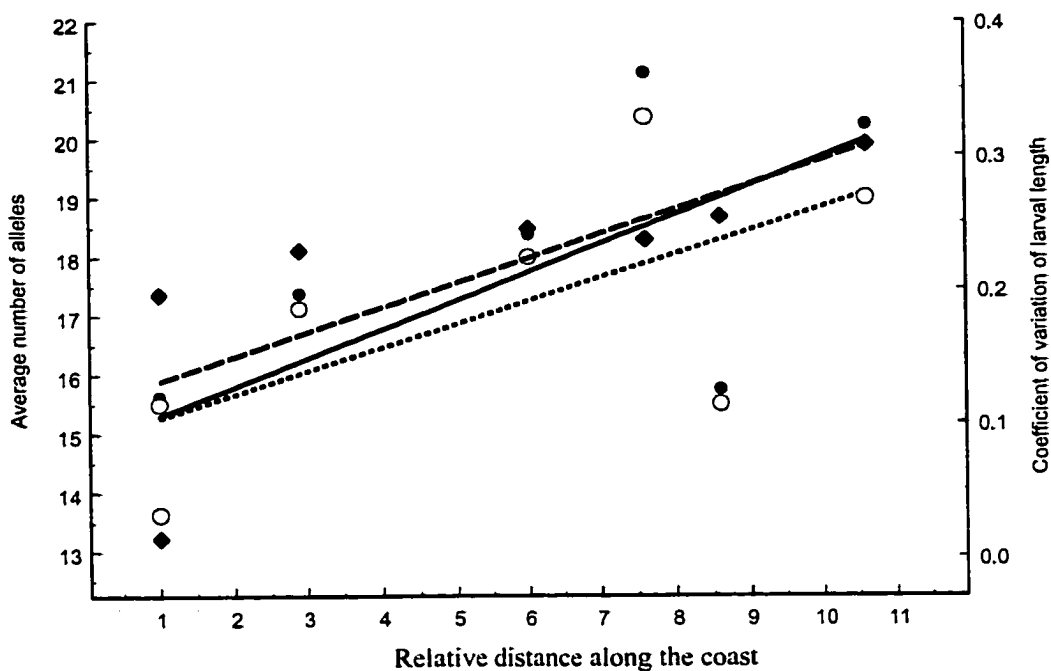
Figure 7.6
Coefficient of variation of larval lengths (filled diamonds) and average number of alleles
at each 1998 coastal collection (filled circles; larvae > 10 mm removed - open circles) as
a function of the sample location relative to Stn-7. Relative distance increases eastward
along the coast of Nova Scotia. The long dashed line represents the linear regression of
coefficient of variation with distance, the solid line represents the linear regression of
average number of alleles (all samples) with distance, and the short dashed line represents
the linear regression of average number of alleles with larvae > 10 mm removed and
distance.
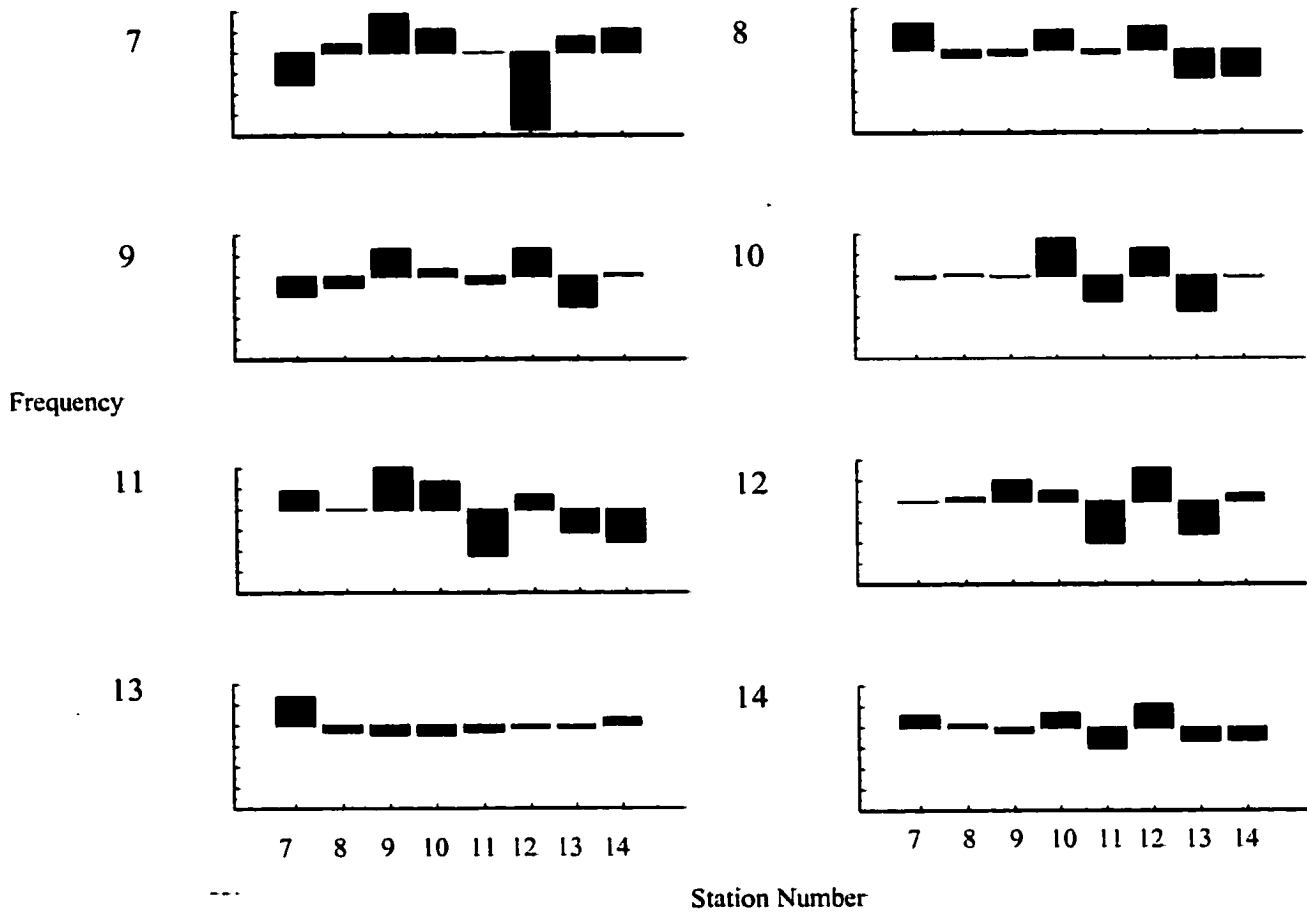
Figure 7.7 Relative proportions of herring larvae ≥7.5 mm from each station in 1998 (station ID to the left of each frequency distribution) that were assigned to each station along the x axis subtracted from the relative proportion of larvae <7.5 mm (reference larvae) from each station (station IS to the left of frequency distribution) that were assigned to stations along x-axis.

inferences (if any) may be drawn from this analysis. The assignment test performed

slightly better when assigning large larvae to reference populations; e.g. between 6.7%

(Stn-11) and 32% (Stn-14) of larvae >7.5 mm were assigned to the collection within

which they were found (Figure 7.7). As there are no physical (circulation) mechanisms

(that I know of) to facilitate the movement of larvae from downstream in the coastal

current to upstream in the coastal current or from inshore to offshore, I attempted to

identify larvae that may have originated upstream of the coastal current and had been

advected and sampled downstream, or larvae that originated offshore that had been

transported inshore. Based on assignment results, the majority (66%) of larvae >7.5 mm

from Stn-7 had the greatest probability of belonging to the reference collection (larvae

<7.5 mm) at Stn-12, located well northward along the coast. Due to the paucity of data

(N=4), larvae >7.5 mm from Stn-7 cannot be genetically compared to Stn-12. Based on

the lengths of these larvae and the distance between stations, the larvae would have to

have been transported at least 20·km d^{-1} (assuming a larval growth rate of 0.2 mm d^{-1})

southwest along the coast if they had originated at or near Stn-12. This is approximately

equivalent to the maximum seasonal (winter) mean current velocity modelled by Hannah

et al. (2001) and is greater than what coastal current velocities estimated elsewhere (e.g.

Reiss et al. 2000) would allow. Eight (13%) larvae >7.5 mm collected at Stn-8 were

assigned to each of Stn-11 and -13, also upstream in the coastal current. Larvae from Stn-

9 were assigned to Stn-13 (N=9; 14.5%), Stn-11 (N=7; 11%) and Stn-12 (N=14.5%).

Again, numbers of individuals were insufficient to compare these larvae to their potential

alternate source, using genetic methods, and coastal current velocities of record

magnitude would be necessary to explain the transport of these larvae along the coast in the period estimated based on larval length (inferred growth rate).

Eighteen percent (N=15) of larvae from Stn-10 were assigned to Stn-11 and of larvae >7.5 mm from Stn-13, 20% (N=13) were assigned to Stn-10 and 17% (N=11) were assigned to Stn-12. In contrast to the above, larvae collected from Stn-10 and -13 and assigned to upstream locations, would have been required to travel a minimum 1.8 km·d^{-1} to have originated in the assigned locations upstream in the coastal current.

As many as 35% of larvae >7.5 mm from each inshore station were assigned to the offshore Stn-14. Therefore, the number of days post-hatch were calculated and compared to estimates of offshore-to-inshore circulation, presented by Reiss et al. (2000), based on a particle tracking model using geostrophic flow. Reiss et al. (2000) modeled particles released on the southern half of Western Bank (the presumed offshore spawning location) and estimated that after 20 days of passive transport, no particles reached the coast. According to the flow-fields estimated by the same authors, larvae would follow an indirect path (determined largely by bathymetric steering) and therefore a single estimate of transport velocity is unrealistic. Thus, larvae assigned as having an offshore source must be at least 20 days of age for the offshore origin to be reasonable. A total of 11 larvae assigned to offshore and collected from six inshore stations were sufficiently large (i.e. >11.5 mm as calculated by 7.5 mm + 0.2 mm d^{-1}) to have come from an offshore source. Stn-12 had the most offshore-assigned larvae (N=5) that were large enough to have originated offshore.

The mixed layer circulation modeled by Reiss et al. (2000) cannot be reliably used inshore along the coast, as the geostrophic model employed provides artifactually

strong eastward flows along the coast. Only weak eastward currents most inshore along the coast have ever been observed (Thompson and Sheng 1997).

7.34 SWEEPSTAKES EVENTS

No significant differences were detected (using F_{ST} or exact tests) among larval collections from Western Bank in 1997 (Stn-2 and -4), 1998 (Stn-14), and spawning-stage adult collections in 2000. There was no indication of multiple cohorts within any of these larval collections (Figure 7.3) and they were all of similar size (age), with median lengths ranging from 7 to 7.6 mm (Table 7.1). Only 6 individual larvae (from Stn-4) were >10 mm and therefore no comparisons (number of alleles) could be made across the putative cohorts.

In contrast to the homogeneity detected among offshore samples, Stn-7 and –8, taken 5 d apart, differed at P=0.02 (F_{ST}=0.0053). Further, larvae from Stn-8 differed (F_{ST}=0.008; P<0.0001) from adult samples collected at Eastern Passage (the same general location) in 1999 - one year following the larval collections. Adult samples collected in 1999, however, did not differ from the first collection of larvae in that location at Stn-7.

The differences (based on F_{ST} estimates) among adult and larval collections may reflect temporal shifts in allele frequencies, perhaps at sub-annual scales. Single locus Exact tests were used to identify which locus (or loci) was responsible for generating the differences between these adult and larval collections. As described above, all significant differences between Stn-8 and other collections primarily occur at *Cha*1017 and the frequency distribution of alleles at this locus showed that larvae from Stn-8 have a single allele (162 bp) that occurs in greater frequency (Figure 7.4). I suggest that these larvae

may be the progeny of few individuals (relative to the other collections) and at least one parent possessed the 162-allele at *Cha*1017. Alternatively, selection (and association of the *Cha*1017 locus with a locus subject to this selection) occurring at the early life stages of herring at Stn-8 may be responsible for increased frequency of the 162 bp allele.

There was no indication of multiple cohorts (based on length) within either of inshore Stn-7 or -8 (Figure 7.3) and only seven larvae (from Stn-8) were >10 mm. Therefore, no comparisons (number of alleles) could be made across putative cohorts. Fewer alleles were detected in the two larval collections (Stn-7 and -8) than in the adult Eastern Passage collection, and may reflect fewer parental fish producing the larval collections than for the adult Eastern Passage collection.

7.4 DISCUSSION

7.41 RESURGENCE OR COLONIZATION

Atlantic herring larvae were primarily associated with the eastern coast of Nova Scotia and the Western-Sable regions of the Scotian Shelf during the autumn spawning season of 1997 and 1998. The abundance of small larvae (<7.5 mm) at multiple collection locations likely reflects multiple spawning events along the coast and at least one spawning location in the vicinity of Western and Sable Banks, especially as the hatch size of herring in Nova Scotia waters is ~ 7mm (Lambert 1984).

Conformance of collections to HWE is generally consistent with samples being drawn from large, randomly mating populations. Deviations from HWE may reflect population subdivision, selection, inbreeding, phenotypic assortative mating, the presence of null alleles or any combination of these (Chakraborty and Jin 1992). If null alleles

were responsible for the significant deficiency of heterozygotes observed in larvae from Stn-2 and -14 (both from offshore but in different years), it is reasonable to assume that the null alleles would have been present within the other larval samples, as well. The absence of heterozygote deficiency in the other larval collections suggests that null alleles are unlikely to have been the cause of departures from HWE observed in the two collections. It appears that the results more likely reflect population sub-structure, selection, or unequal parental contributions to larvae within the collections.

Ten sets of associated loci (inferred by linkage disequilibrium using GENEPOP; Raymond and Rousset, 1995) within collections were detected at P<0.01. Three pairs of associated loci were observed at Stn-13 (inshore), two pairs were detected at Stn-2 (offshore), and one pair in each of Stn-1, -3, -11, -12 and -14 (inshore and offshore stations). The loci involved had not been shown to be associated previously (Chapter 3). After Bonferroni adjustments for multiple tests, one significant association remained (Stn 13). The associated loci can be used to infer that the larvae in at least one collection may have been generated by relatively few parents (i.e. non-random association of alleles). Finally, the association between loci in conjunction with the deviations from HWE observed are indications that large randomly mating populations may not have generated larval collections.

The significant deviations from HWE and associations between loci are consistent with larvae produced from relatively few breeders and thus, larvae may not be representative of the total population. Therefore, in the comparisons made here, chances of statistically significant results may be inflated (termed the Allendorf-Phelps effect) because the sampling does not conform to assumptions implicit in the null hypothesis

(specifically, that individuals sampled have been drawn randomly from and represent the total population of interest). It is possible to adjust the null hypothesis (as described in Waples 1998), to account for additional complexities of sampling associated with the Allendorf-Phelps effect, but implementation of this adjustment requires prior knowledge of the number of breeders responsible for producing the larvae. Because erroneously estimating the number of breeders producing each larval collection may greatly affect the analyses conducted and the implications that follow, I assume that the Allendorf-Phelps effect has not considerably biased these results and can therefore be ignored (following Ruzzante et al. 1996).

The virtual absence of herring on the offshore banks of the Scotian Shelf during the late 1970s and the subsequent increase in adult herring over the last decade (Harris and Stephenson 1999) is consistent with an increasing population in that habitat. Does this recent increase reflect resurgence of an existing (local) population or colonization from a neighbouring population(s)? Rejecting either hypothesis (resurgence or colonization) is a difficult task, as direct estimates of dispersion and migration rates are difficult to make, especially within a dynamic oceanographic context. In this study, I have attempted to combine an indirect method of estimating dispersion (by assuming passive drift of larvae) and migration, through the use of genetic analyses at eight tetranucleotide microsatellite loci, along with morphometric data on larvae and the oceanographic circulation pattern within the geographic regions where the larvae were collected.

Although migration rates can be estimated using these genetic data, the analyses presented here serve only to test the degree of isolation (or structure) among sample

collections, whereas genetic estimates of gene flow necessarily rely on a mathematical relationship between genetic structure (e.g. F_{ST} or some similar statistic) and the inferred rate of gene flow. These latter estimates implicitly assume that the ecological properties of the populations from which the genetic data are taken match the assumptions (often unrealistic) of the theoretical model upon which the mathematical relationship is based. Estimates of gene flow, then, depend on assumptions of population structure and the subsequent use of the estimates to infer population structure is tautological (Smedbol et al. 2002). Even in the optimal circumstances, when all assumptions are met, population structures estimates, and by extension estimates of gene flow, are subject to sampling error, which can be large. For many applications, measures of genetic structure are valuable, but their transformations to quantitative estimates of gene flow or dispersal can often be misleading (Whitlock and McCauley 1998).

The results presented here (paucity of differentiation between larvae collected inshore and offshore in either of 1997 or 1998) cannot distinguish between either colonization or resurgence. Neither the movement of fish from inshore to offshore (colonization) and resulting homogenization of what variation among groups may have existed, nor a historical mixing between inshore and offshore populations, and the resulting potential for resurgence of the offshore "spawning group" can be rejected. Herring collected prior to the reduction in numbers offshore are necessary to help resolve the question. If I assume that there has been sufficient time for isolation to have taken place (in the absence of mixing) and differences to have accrued given the differentiation detected in Chapter 5, mixing (as illustrated in Figure 7.5) across the shelf (from multiple sources - at possibly at the adult stage) is the simplest explanation for no differentiation

among the offshore larval collections and all inshore larval collections (with the exception of Stn-8 that differed from all others), which mirrors the pattern among adults from Western Bank (i.e. they differed only from Stn-8). Colonization of the offshore spawning habitat from one source population seems less likely.

Greater subdivision (as reflected by F_{ST} estimates) was detected within the inshore collections than between inshore and offshore larval collections - consistent with the existence of more than one distinct spawning group of herring along the coast of Nova Scotia – and reflected by differences between Stn-8 and others. A similar conclusion was drawn in Chapter 5, where differences in adult collections along the coast of Nova Scotia were reported. Results based on genetic analyses of larvae show all significant comparisons among collections of larvae involved Stn-8 - located midway along the coast of Nova Scotia. This location appears anomalous as the adults collected at this location (in a different year) differed from other coastal adult collection and also differed from Stn-8 larvae. In fact, as illustrated in Figure 7.5, these samples (Stn-8 and Eastern Passage) are found at opposite ends of dimension 1 and represent the largest pair-wise difference reported in this study. In summary, the only collection that is unlikely to have been a source for repopulation of the offshore banks (at least not in great numbers) are those herring that produced the larvae with an origin at or near Stn-8.

Substructure within larval collections in 1998 did not vary (ΔF_{ST}=0.00006) with the exclusion of larvae >10 mm in length (N<75 removed, less than 10% of larvae in 1998), suggesting little if any effect of different cohorts within collections. However, exclusion of these larger larvae yielded three different pair-wise comparison results that alternatively suggest some degree of inter-cohort variation. In particular, with the larger

larvae removed, the offshore collection at Stn-14 is less different from Stn-8. This may be due to the larger larvae contributing variance to each of the two collections (once they are removed, the difference dissipates) or alternatively may be related to selection.

7.42 ROLE OF RETENTION AND ADVECTION

The inshore and offshore spawning regions where larvae and spawning adults were collected are characterized by different spawning habitats and different circulation features. Offshore, there is the potential for recirculation (and possibly retention) around the Bank within the anticyclonic flow (Reiss et al. 2000) observed on Western Bank (Taggart et al. 1996). Inshore, there is the potential for advection into and subsequent containment within the Nova Scotia coastal current (Reiss et al. 2000). In either case, the larval abundance distributions are consistent with those of Iles and Sinclair (1984), who postulated that herring occur in geographically distinct regions, defined by physical features (Figure 7.1), which according to the discontinuities in larval abundance may be the Nova Scotia coastal current and Western/Sable bank.

Similar relations (one significant and one marginally significant) were observed between relative distance along the coast of Nova Scotia (from which I inferred increased advection into the current in a downstream progression) and each of average number of alleles and coefficient of variation in larval length at 1998 inshore collections. Both the coefficient of variation of larval lengths ($P=0.007$) and number of alleles ($P=0.08$) within collections increased with relative distance up the coast, inconsistent with the proposed larval mixing within the coastal current by advection of larvae from spawning grounds inshore and upstream of collection sites. It must be conceded, however, that the collections used here, may have been sampled inshore of the coastal current. When larvae

>10 mm were excluded, the slope of the relation between number of alleles and distance decreased slightly, consistent with the larvae >10 mm comprising a different cohort, which when removed - decreases the number of alleles. However, regardless of significance, the positive slopes may be used to infer that changes (post-exclusion of larger larvae) result from the presence of a different cohort from the same location and not the mixing of larvae from multiple upstream stations in the coastal current. The role of mixing within the coastal current in generating or homogenizing population structure therefore seems dubious based on these analyses.

The retention of larvae spawned offshore and subsequent natal spawning site fidelity when mature (e.g. Iles and Sinclair 1982) would likely lead to reproductive isolation between inshore and offshore spawning populations. Alternatively, transport of the same offshore larvae to the inshore (e.g. Reiss et al. 2000), without subsequent natal spawning site fidelity, might serve to homogenize genetic variation between inshore and offshore herring. In investigating the plausibility of retention of offshore larvae, I considered the potential for offshore-to-inshore transport. Such a process may lead to genetic homogeneity between inshore and offshore if the larvae successfully reproduced in their adopted population - although Sinclair (1988) suggested these "vagrants" would be less successful in their "adopted" environment. Of all larvae collected inshore in 1998, 11 larvae, whose probability of origin based on assignment tests, was greatest for offshore, were sufficiently large (>11.5 mm) to have traveled from offshore to inshore. Five of these 11 larvae (which was the maximum from any one station) were collected at Stn-12, which is one of the most eastern locations sampled. Larvae from offshore would have, therefore, needed to travel across the shelf, into coastal current and then northeast

along the coast, inshore of the coastal current. This seems unlikely within the period of 40 days, the maximum period possible for transport, based on the maximum length of the largest larvae assigned to offshore and collected at Stn-12. It seems much more likely that the connection between offshore and inshore has been (is) realized by the movement and subsequent reproductive success of adult herring or mixing of larvae across the shelf in concert with "leaky" spawning site fidelity. These mechanisms are therefore inconsistent with the strict natal spawning site fidelity commonly assumed for herring but are consistent with genetic analyses of adult herring in these regions (Chapters 5, 6), indicating a considerable degree of mixing among spawning groups on the central Scotian Shelf and in the area southwest of Nova Scotia.

Thus, the genetic patterns and associations presented here, along with distributions of recently hatched and older larvae, and their associations with circulation features, suggest that the model put forth by Iles and Sinclair (1984) may not be strictly valid. Iles and Sinclair (1984) predicted that the number of genetically distinct herring stocks is determined by the number of geographically stable larval retention areas. Accordingly, each distinct gene pool is postulated to envelop all those spawning groups whose larval and post-larval stages come to share (and remain in) the same area of distribution. The data presented here are consistent with few genetic differences across spawning grounds with different physical environments that have different larval distributions. It is therefore difficult to reconcile these results with the "retention hypothesis", as it was originally proposed. It is possible that homogeneity is maintained by adult migration and not larval mixing but that is also at odds with the predictions of

Iles and Sinclair (1982). The simplest reconciliation can only be formed by redefining the retention area to encompass the entire Scotian Shelf.

7.43 SWEEPSTAKES EVENTS

Although minimal in absolute magnitude, F_{ST} estimates among inshore collections in 1997 and 1998 were 1.8 and 4.6 fold greater than those between inshore and offshore comparisons in 1997 and 1998, respectively, indicative of considerable annual heterogeneity along the inshore. This heterogeneity may be due to high variance in progeny production (i.e. sweepstakes; Hedgecock et al. 1982) among individuals from year to year. Such reproductive variance, when coupled with the potential for protracted spawning waves in herring (Lambert 1984; Hay 1985, Chapter 6) may be responsible for generating complex patterns of population variation as proposed by Stephenson et al. 2001, especially when considering larval samples, as there is no way of knowing, a priori, which larvae will survive to adulthood.

When larval samples collected from Western-Sable were compared to spawning stage adults collected from that same location (albeit from different years), no differences were detected. In fact, F_{ST}'s of ~ 0 were estimated in adult vs both 1997 (Stn-2, offshore) and 1998 (Stn-14, offshore) larvae and $F_{ST}=0.00038$ (comparable to the estimates among inshore collections in 1997 and 1998) between adults spawners and Stn-4 (offshore in 1997). If reproductive variance was responsible for small fluctuations in allele frequencies on an annual or sub-annual basis, one would expect less genetic diversity within the recruits relative to the adult spawning population presumably responsible for producing them. Ruzzante et al. (1996) also compared larvae (cod) collected in the

vicinity of Western Bank with adults from the same vicinity and showed no differentiation between larvae and adults, subsequently concluding that this finding reflected temporal stability of cod and no evidence of "sweepstakes" events within larvae collected from a single water mass.

I had a limited opportunity to assess this phenomenon in another location in 1998, where two inshore collections of larvae were taken in the same location: Stn-7 and -8 within 5 d. In this case, the genetic analyses revealed differences (Table 7.3) between these two samples and between Stn-8 and many samples east of this location (Table 7.3). Further, larvae from Stn-8 also differed (F_{ST}=0.008; P<0.0001) from adults collected from this same location (or in very close proximity) in 1999 - one year after. Adult samples collected in 1999, however, did not differ from the first collection of larvae in that location - Stn-7. Single locus Exact tests were used to identify which locus (or loci) was responsible for generating the differences between these adult and larval collections. All significant differences between Stn-8 and other collections primarily occur at *Cha*1017. Upon examination of the frequency distributions of this locus, it became apparent that larvae from Stn-8 have a single allele that occurs in greater frequency – 162 bp (Figure 7.4). In parallel to the situation offshore where there was evidence of few parents, I suggest that the larvae collected at Stn-8 may be the progeny of few individuals (with at least one parent possessing the 162 allele at *Cha*1017). However, the potential role of selection (where favorable alleles that are associated with some "advantage" are over represented at a certain lifestage) in generating the pattern of distribution of the 162 bp allele cannot easily be ascertained or tested. To test the contention that the larvae carrying the 162 bp allele at *Cha*1017 may be progeny of few parental individuals, I

examined the length distributions these larvae collected at Stn-8. The variance (V) and

coefficient of variation (CV-used to standardize for any size differences between 162

and non–162 bp allele carriers) in larval length was considerably smaller within the

"cohort" that carried the 162 allele at *Cha*1017: 1.9 and 0.16 (V and CV respectively)

relative to 2.7 (V) and 0.2 (CV) for the station as a whole and 2.8 (V) and 0.2 (CV) for

non-162 bp allele carriers at this Stn. These results indicate that larvae with the 162-allele

are more similar in size (consistent with an increased probability of relatedness among

these individuals).

Further, these results may reflect the incidence of spawning waves in herring (as

described by Lambert 1984), as larvae from the same location collected within 5 days

differ as much as samples collection across the shelf. Unlike Ruzzante et al. (1996), the

results presented here may be consistent with sweepstakes events, as only one of the two

larval collections were indiscernible from the spawning adults collected from the same

location. There are important implications of these analyses. Sweepstakes events (or

reproductive variance generated by some other mechanism) may be responsible for the

temporal genetic heterogeneity and seemingly chaotic genetic patchiness (small-scale,

seemingly un-patterned, genetic heterogeneity among and within local populations)

previously documented for herring (e.g. Kornfield et al. 1982) and other species (Johnson

and Black 1982, Hedgecock 1994, Larson and Julian 1999, Chapman et al. 1999). From a

management perspective, these data suggest that herring may depend on the reproductive

output of a small number of individuals and imply that changes in management of adult

stages may have unpredictable consequences (related to recruitment), especially when the

series of environmental events that culminate in sweepstakes are unknown but likely vary from year to year.

To summarize, the genetic homogeneity in herring larvae collected across most of the central Scotian Shelf may reflect either a colonization or resurgence-based mechanism for the increase in adult herring abundance and recently emerged larvae observed in the vicinity of Western and Sable Banks. However, one inshore collection of larvae (Stn-8) was sufficiently different from all others, including the offshore, to preclude it as a source for the repopulation of the offshore banks. The genetic patterns and the associated circulation processes presented here are difficult to reconcile with the retention hypothesis, as defined by Iles and Sinclair (1984), or with larval mixing into and along the coastal current as proposed by Reiss et al. (2000). Larval collections (both inshore and offshore) may have been produced by few parents as indicated by numerous analyses, both genetic and morphometric. This finding is therefore consistent with the incidence of sweepstakes events, especially in light of the conflicting results (differences and lack thereof) of comparisons between larvae and the spawning adults that are presumed to have produced them.

8. THESIS SUMMARY

In this thesis, I have shown that dinucleotide microsatellite loci, originally developed for Pacific herring can be amplified for use in Atlantic herring. I have subsequently shown that these markers can be used to draw inferences about species/subspecies of herring, as well as populations of herring at ocean basin and shelf (regional management) spatial scales. Differences were revealed at each of these spatial scales and pan-Atlantic differentiation was larger ($F_{ST} \sim 0.040$) than previously reported. I suggest that these observations are related to the use of microsatellite DNA markers that are thought to mutate (generating new alleles) more rapidly than other genetic methods (e.g. allozyme, mtDNA sequence) employed over the last several decades. Despite the demonstrated utility of the dinucleotide microsatellites, few were available (n=5-6), and recent studies have concluded that dinucleotide makers are often associated with increased error in allele designation, most frequently a function of artifacts generated during the polymerase chain reaction process used to amplify the marker, and scoring of the allele sizes with only 2 bp separating putative alleles. To circumvent the above problems associated with dinucleotide microsatellites, I developed nine new tetranucleotide microsatellites to further the analyses of population structure in Atlantic herring. The rationale for the development is based on recent studies that show that tetranucleotide microsatellites allow for increased precision when scoring alleles. These markers were found to be highly polymorphic (Ho ~ 0.6 to 0.9). When a suite of tetranucleotide loci were then applied to predominantly spawning stage herring (17 collections; N>1400) drawn from the Scotian Shelf, the Celtic sea, the Baltic Sea, and a region adjacent to the eastern coast of Iceland, to assess population structure across the

species range, differentiation was observed between NE and NW Atlantic herring (F_{ST}~0.065), which confirmed the results generated using the dinucleotide loci. Significant patterns of genetic variability were detected among NW Atlantic herring spawning groups (max F_{ST}=0.018) on a relatively small spatial scale (Scotian Shelf). I therefore concluded that NW Atlantic herring are in some way spatially structured and support the precautionary management approach employed in this area. Specifically, four semi-isolated groupings (within which little divergence was detected) were identified: (1) Bras d'Or Lakes, (2) Coastal Nova Scotia (i.e. Eastern Passage), (3) Southwest Nova Scotia, (4) interior of the Bay of Fundy and Georges Bank, and these groups generally agree with the management components delineated by Department of Fisheries and Oceans in this area. Further, the genetic differences between the Bras d'Or Lakes herring and all other collections were greater than that of any other comparisons among NW Atlantic herring, sufficient to hypothesize that an event associated with a small effective population size (e.g. population bottlenecks) had occurred in the Bras d'Or Lakes. However, the potential role of selection in generating this pattern of differentiation cannot be discounted. I then assessed the temporal stability of the genetic patterns observed on the Scotian Shelf, using both annual collections and age-data (provided by St. Andrews Biological Station, DFO). Genetic variation among locations was greater than 1.4 fold that of annual genetic variation within location. The four groupings were generally found to be temporally stable, using two methods of assessment: comparisons of collections made in the same location in consecutive years and comparisons among cohorts (annual) within location. Temporal stability of genetic patterns is essential when using genetic data to identify populations, as inferences based on spatial patterns may be

inaccurate if such patterns vary considerably over time. In the contemporary literature, assessing temporal stability using one method is desirable; use of two methods is extremely rare. Based on the analyses of cohort data, I hypothesize that overlapping generations in sample collections (when used for population structure analyses) may explain the inconsistencies in temporal stability in marine fishes, particularly herring. A more conclusive test of this hypothesis would require large (N>50) sample sizes of each cohort within each collection on the spawning ground. When I decreased the temporal scale of resolution to include the examination of putative spawning waves within a single season, little genetic variation was detected between spawning waves, but a small difference (F_{ST}=0.005, P=0.003) was revealed at one location (Devastation Shoal) between spawning waves separated by 6 days and comprised of almost exclusively the same year class. Herring from the second spawning wave at Devastation Shoal were also larger when comparing within a year-class (T-test; P<0.001). I hypothesize that this length difference, in conjunction with genetic analyses, is consistent with a ~ 6 day turn-over rate on the spawning ground and either 1) the presence of distinct Devastation Shoal populations that are temporally separated by 6 days or less 2) sub-annual temporal genetic patchiness, generated by an unknown mechanism or 3) transient use of a spawning ground by different populations of herring. Discriminating among these explanatory mechanisms would require more thorough sampling of each spawning ground within the spawning season (e.g. continuous sampling throughout the spawning window). Herring larvae collected across most of the central Scotian Shelf were examined both genetically and morphometrically. Virtual genetic homogeneity was observed in herring larvae (14 collections; N>1200) and genetic patterns and the

associated circulation mechanisms inherent to sampling locations were difficult to reconcile with the retention hypothesis (which suggests that retention of larvae in suitable nursery sites contributes to the maintenance of patterns of population structure in herring). I also found no evidence to support the proposition that larval mixing within the Nova Scotia coastal current was responsible for population patterns in herring. Perhaps the model of population structure for herring, suggested by Stephenson et al. (2001) that predicts a gradient in separation among spawning areas based on the degree of connection of larval distributions is appropriate to describe population structure of Scotia-Fundy Atlantic herring. Unfortunately, I was not able to test the population structure observed in adult samples comprehensively with these larvae, due to the restricted range of larval sampling. Numerous analyses (both genetic and morphometric) indicated that several larval collections (occurring both inshore and offshore) were likely produced by a few parental individuals each. In light of the conflicting results (differences and lack thereof) of comparisons between these larvae and the spawning adults that are presumed to have produced them, I considered these findings consistent with the incidence of sweepstakes events (or temporal genetic patchiness). Repeated sampling of spawning adults throughout the spawning interval, in concert with repeated sampling of the larvae as they emerge, all replicated in consecutive years may be required to test this contention. Based on the data presented here, I suggest that NW Atlantic herring are spatially structured. Further, I hypothesize that complexes of spawning groups in close geographic proximity may exchange individuals, which raise questions about strict natal spawning site fidelity (often assumed) in herring. However, the isolating mechanisms that generate and maintain population differences remain elusive.

9. APPENDIX A Temporal stability of single-locus pair-wise exact test results sorted by collection year and by year-class. All significant (P<0.05) comparisons are listed for a subset of NW Atlantic populations: locus/P-value. Comparisons significant at either each collection at the same location or at all year-classes at the same location are in bold font.

		N	GeorgesB 75	EasternP 75	DevSho 127	ThreeFath 75	WesternB 75	BrasDor 67
Collection Year	ScB98	120	1202/0.037 1045/0.028	1027/0.003 1202/0.010 1017/0.007 **1020/0.015** 1045/0.030	1202/0.041 1059/0.023 1017/0.040 108/0.025	1017/0.011		1027/0.008 1202/0.019 1017/0.007 **1020/0.006** 1045/0.002 108/<0.001
	ScB99	75		**1020/0.005** 102/0.016		102/0.039		1017/0.008 **1020/0.004** 102/0.006 108<0.001
	ScB00	56	1059/0.04 108/0.003	1027/0.035 **1020/0.005** 108/<0.001	1027/0.008 1020/0.039 108/0.008	1059/0.005 108/<0.001	1059/0.016 108/<0.001	**1020/0.013** 102/0.004
	SpB98	50	**1045/0.001** 108/0.011	1017/0.028 **1020/0.037** 1045/<0.001 **113/0.032** 108/0.018	1045/<0.001 108/0.001	1017/0.008	1017/0.020 1045/<0.001	**1027/0.002** 1059/0.041 **1020/0.006** 1045/<0.001 **102/0.003** **108/<0.001**
	SpB99	100	**1045/0.016**	1027/0.002 **1020/0.020** **113/0.023**	1202/0.040	1045/<0.001	270/<0.001	**1027/0.002** 1202/0.011 **1020/<0.001** 113/0.001 102/0.003 **108/<0.001**
	GrB98	148	1202/0.014 1045/0.007	**1020/0.002** 1045/0.049	1027/0.031 108/0.002		**113/0.022**	1027/<0.001 **1202/0.005** **1020/<0.001** **102/0.005** **108/<0.001**
	GrB00	164	**1202/0.008** **1045/0.003**	**1020/0.001** **1045/0.029**	102/0.024	1045/0.032	**113/0.027**	**1202/0.015** 1017/0.024 **1020/0.006** 1045/0.008 **102/0.002** **108/<0.001**
Year-class	ScB4	45	1045/0.01	1027/0.021 1202/0.016				1027/0.020 1202/0.033 1017/0.004 **1020/0.009** 1045/0.014 102/0.025 **108/0.002**
	ScB5	38	1202/0.011	1202/0.043 1017/0.010 1020/0.011	1202/0.017	1202/0.023 1017/0.011	1017/0.045	1202/0.043 1059/0.019 1017/0.002 **1020/0.039** **108/<0.001**
	ScB6	35	102/0.022		1059/0.028 102/0.007 108/0.036		102/0.029	1027/0.019 **1020/0.043** 102/0.015 **108/<0.001**
	EP6	33	**1020/0.044** 1045/0.016		**1027/<0.001**	1027/0.013 **1020/0.026**		**1027/0.002** **1020/<0.001** **102/0.021** **108/0.001**
	EP7	30	1027/0.013 **1020/0.007**		**1027/0.012** 1020/0.041	**1027/0.015** **1020/0.021**	1020/0.048	**1027/0.001** 1017/0.008 **1020/0.002**

						102/<0.001 **108/<0.001**
TL4	77		1017/0.015 1020/0.005			1027/0.003 **1017/0.011** **1020/<0.001** 1045/0.011 **102/0.005** **108/<0.001**
TL5	31					1027/0.003 **1017/0.029** **1020/<0.001** **102/0.007** **108/<0.001**
TL6	30	1017/0.049	1017/0.026	1017/0.030 113/0.022	1017/0.014	1017/0.010	1017/0.021 1020/0.036 **102/0.008** **108/<0.001**
GrB4	36	1202/0.039	1020/0.010	1027/<0.001 108/0.004	1027/0.006		**1027/<0.001** 1202/0.035 1020/0.009 **102/<0.001** **108/<0.001**
GrB5	50		108/0.010	108/0.002	113/0.039		**1027/0.001** 1017/0.032 1020/0.001 **102/<0.001** **108/<0.001**
GrB6	42	1045/0.017	102/0.049	102/0.027	1027/0.012 102/0.011		**1027/<0.001** 1202/0.013 1017/0.006 **102/0.036** **108/<0.001**
GrB7	16					102/0.013	1027/0.036 1020/0.028 **102/0.020** **108/<0.001**

10. APPENDIX B

GLOSSARY

Allee effects - Allee effects are ecological phenomena associated with collapsed populations. The mechanisms underlying Allee effects are physiological and behavioural. These result in decreased reproduction at low population sizes. E.g. difficulty finding a mate, breakdown in social patterns and migration routes, difficulty in fending off predators in schooling fish. Both compensatory and depensatory stock and recruitment relationships assume that the curve should pass through the origin, despite lack of data at low population levels. A S-R relationship incorporating Allee effects would have a non-zero intercept defining a species-specific SSB threshold associated with absolute recruitment failure. Under the assumptions of Allee effects, when a substock falls below its critical value, any self-regeneration capabilities have been lost and stock rebuilding can only occur through immigration. Dispersal of offspring or adults from adjacent substocks or populations may fulfill this recolonization role.
In Frank and Brinkman (2000), the loss of spawning components from NW Atlantic herring and cod demonstrate unplanned, negative consequences of an aggregated management scale. The loss of subunits from stock complex aggregated management has been predicted in a recent modeling study. Frank and Brinkman simulated the dynamics of a stock complex comprising a number of sub-stocks with differing stock-recruitment relationships. The model demonstrated that for populations comprising multiple units (and each unit exhibits depensatory population dynamics—where per capita reproductive success decreases with decreasing stock size), it was possible to extirpate subpopulations in a situation where the aggregate stock-recruitment relationship appears to be compensatory (increasing per capita reproductive success with decreasing stock size). The end result of such dynamics is that subpopulation extinction would be well under way before analyses of aggregate data would question the health of the complex.

Allele- the basic unit of inheritance. Each character is composed of a series of loci, and at each locus there may be one to many alleles, each allele contributing to the genotype and in some cases, the phenotype. More generally and in non-coding regions of DNA, each location where there is a DNA polymorphism can be considered a locus, and the specific polymorphisms are the alleles.

Allozyme – heritable, functionally similar but separable forms of enzymes encoded by one or more loci

Assignment tests – tests in which individuals are assigned to the population where the likelihood of their genotype is highest

Bayesian Statistics (Analysis) - Bayesian analysis is an approach to statistical analysis that is based on the Bayes law, which states that the posterior probability of a parameter p is proportional to the prior probability of parameter p multiplied by the likelihood of p derived from the data collected. This increasingly popular methodology represents an alternative to the traditional (or frequentist probability) approach: whereas the latter

attempts to establish confidence intervals around parameters, and/or falsify a-priori null-hypotheses, the Bayesian approach attempts to keep track of how a-priori expectations about some phenomenon of interest can be refined, and how observed data can be integrated with such a-priori beliefs, to arrive at updated posterior expectations about the phenomenon.

A good metaphor (and actual application) for the Bayesian approach is that of a physician who applies consecutive examinations to a patient so as to refine the certainty of a particular diagnosis: The results of each individual examination or test should be combined with the a-priori knowledge about the patient, and expectation that the respective diagnosis is correct. The goal is to arrive at a final diagnosis which the physician believes to be correct with a known degree of certainty.

Bias- a systematic departure of the estimated mean from the true or expected value

Bonferroni correction – correction made when a series of statistical tests are performed. The correction adjusts the individual probability values such that the overall probability (of Type 1 error) remains at 5%

Bootstap – principle is that if we do not know the true distribution of the difference between an unknown parameter and some estimator of the parameter, we can approximate it by the distribution of the difference between an estimate obtained from a particular data set and estimates computed from different imaginary data sets generated using information provided by the sample. In nonparametric bootstraps, data sets are generated by sampling with replacement within the real data set. The parametric is similar except that one generates imaginary datasets according to a probability distribution whose parameters are estimated from the real dataset.

Codominant inheritance – 2 alleles are both expressed in a heterozygote. E.g in microsatellites, both alleles (one from each parent) are resolvable. Both alleles are separately manifested in the phenotype. Classification depends on the level at which we examine the character: organismal, biochemical or molecular.

Cycle-sequencing – a combination of PCR and sequencing where the annealing and extending steps are performed over and over again using the same template (similar to how PCR amplifies a large amount of product from the same template). This enables sequencing from a much smaller amount of template than before. Automated sequencers use different coloured, fluorescently labeled, dideoxy (that stop the growing sequence) terminators that enable all four sequencing reactions to be run in a single lane.

DNA (deoxyribonucleic acid) - The genetic material of most living organisms, which is a major constituent of the chromosomes within the cell nucleus and plays a central role in the determination of hereditary characteristics by controlling protein synthesis in cells. Nucleic acids are polymers of nucleotides that are composed of an nitrogenous base, a 5 carbon sugar (deoxyribose in DNA) and a phosphate group. There are 2 families of

nitrogenous bases: the pyrimidines (six membered ring of carbon and nitrogen atoms – C, T, U); and the purines (six membered rings fused to a five membered ring – A, G). Nucleotides are joined by covalent bonds called phosodiester linkages between the phosphate group of one nucleotide and the sugar of the next. The double helix is held together by H bonds between the bases. The molecule is negatively charged.

DNA sequencing Sanger Dideoxy-termination method – a primer is used to initiate the synthesis of new strands of DNA. Unlike PCR, only one primer is used and thus only one of the two strands is synthesized. In addition to the regular nucleotides, modified (dideoxy) nucleotides are added to the sequencing reaction. The modification of these nucleotides prevents the formation of the phosphate bond that would link the next nucleotide of the chain, thus the synthesis is terminated upon the addition of the modified nucleotide. A sequencing reaction comprises four identical syntheses reaction carried out in separate tubes with a different modified nucleotide added to each of the four tubes. All four regular nucleotides are present, but the modified nucleotides are present in a ratio that favours their incorporation at low frequency. The result is a population of fragments of different sizes, each with a dideoxy nucleotide at the terminal point. These fragments are either radioactively or fluorescently labeled and are separated on a high resolution electrophoretic gel.

Dynamic height – height of an isobar relative to an assumed constant pressure surface

Effective population size – a measure of the number of individuals making a genetic contribution to the next generation. **OR** The size of the ideal population (discrete generations, random mating, constant population size) that would undergo the same amount of genetic drift as the actual population. Population genetic theory provides several methods to estimate Ne from allele frequency data: including temporal change in allele frequencies, gametic disequilibrium and heterozygote excess methods.

Exact test – tests not affected by an imperfect knowledge of some parameter value. Fisher's exact test is the first (historically) permutation test. It is used with two samples of binary data, and tests the null hypothesis that the two samples are drawn from populations with equal but unknown proportions of "successes". A permutation test involves the shuffling of observed data to determine how unusual an observed outcome is. A typical problem involves testing the hypothesis that two or more samples might belong to the same population. The permutation test proceeds as follows: 1. Combine the observations from all the samples 2. Shuffle them and redistribute them in samples of the same sizes as the original samples. 3. Record the statistic of interest. 4. Repeat 2-3 many times. 5. Determine how often the resampled statistic of interest is as extreme as the observed value of the same statistic.

Tests are exact, in that they are based on an distribution and exact probabilities can be calculated without information of the unknown parameter. Exactness of a test does not imply that they tell the truth about some hypothesis, since the outcome of the analysis can only be a statement about the probability of events.. E.g. HWE, linkage disequilibrium

Extirpation – extinction of a local distribution

Fisher's method of combining probabilities – combines probabilities from different experiments (that are assumed to be independent). This approach is advocated by Sokal and Rohlf. Natural log probability values from each experiment are summed and multiplied by –2 based on the fact that –2 lnP is distributed as chi square . With 2 degrees of freedom associated with each probability value, the total value can be analyzed using a table of critical values of the chi squared distribution.

Fronts: transition regions between water masses (e.g. mixed and stratified water masses)

Full-sibs – individuals that share 2 parents

Geostrophic flow -A type of movement where the Coriolis force balances exactly the horizontal pressure force and all other forces are minimal and can be ignored (e.g. advection and friction)

Gene flow – movement and establishment of genes into non-indigenous genepools. In subdivided populations, gene flow introduces new alleles and is a source of variation, it increases Ne, and allows local populations to adjust to environmental change by utilizing new variability.

Genetic distance (Nei) – genetic distance between pairs of samples that depends on the sum of the products of frequencies of those alleles shared between 2 populations

Genetic drift – variation in allelic frequencies caused by random sampling (reproduction) from a finite population

Genetic hitch-hiking – occurs when a more-or-less selectively neutral allele at one locus travels through generations because of linkage with an allele at another locus that has a selective advantage. Hitch-hiking is genetic correlation through linkage; genetically correlated characters evolve together when either or both are genetically correlated with fitness. Hitch-hiking is, however, a general phenomenon that occurs irrespective of the effects of genes involved on fitness. For example, an inferior, deleterious allele may spread through a population because it is associated with another allele at a different locus, "whose superiority overrides its own inferiority."

Genetic patchiness - small-scale, seemingly un-patterned, genetic heterogeneity among and within local populations

Genotype – the genetic constitution of an organism at one or many loci

Half-sibs – individuals that share one parent

Hardy Weinberg equilibrium – the equilibrium attained at a single locus after one generation of random mating (meeting various assumptions)

Heritability – ratio of the total genetic variance to the phenotypic variance

Heterozygosity – the presence of alternate alleles at a given locus in a diploid individual

Homozygosity – the presence of the same allele at a given locus in a diploid individual

Homoplasy – co-occurrence of alleles that are identical in state and not identical by descent

Inbreeding – the mating of related individuals

Iteroparous - Producing offspring in successive, e.g., annual or seasonal batches, as is the case in most fishes. Opposite of semelparous

Life history trait – a trait that is directly associated with fitness, such as the development time, fecundity, etc.

Linkage – the presence of 2 loci on the same chromosome. Because of recombination, the 2 loci still may be in linkage equilibrium – if they are greater than 50 map units apart

Linkage disequilibrium – the nonrandom association between 2 loci. This can be caused by physical linkage due to the 2 loci being on the same chromosome, or to disassortative mating

Locus – the unit of inheritance. Each character or polymorphism location is make up of one to many loci, at which there are one to two alleles.

Mantel test- a randomization test for the comparison of the correlation between 2 variables. Compares the slope of the linear regression created from the data to the distribution of slopes generated by randomization procedures under the null model of no spatial pattern.

Maximum likelihood – a statistical method based on the assumption that the best estimates are those which maximize the probability of obtaining the observed data-set These methods find the parameters that maximize the probability of obtaining the observed data under a certain model. For example, a statistical model is devised that gives the probability of obtaining the observed allele frequencies given some set of demographic and mutation parameters. It can then be determined which parameter values maximize the likelihood of obtaining the observed dataset. Maximum likelihoods use the raw data and not summary statistics or functions of the data which can waste information and introduce bias.

Microsatellite – short segments of nuclear DNA in which a specific motif (2-6 bases long) is repeated. Origin of name: satellite DNAs received their name from centrifugation experiments. Several fragments floated as satellite bands above a main band due to their

lighter density compared to the denser randomized DNA. When sequenced, they were found to be repetitive. Microsatellites are small, repetitive sequences.

Mitochondrial DNA – haploid, circular DNA (~16000 bp) molecule found in the mitochondria organelles. Mitochondria are cytoplasmically inherited and the cytoplasm of an ovum is derived from the female, thus mtDNA is generally maternally inherited.

Multidimensional scaling – analysis that attempts to detect meaningful underlying dimensions that allow the researcher to explain observed similarities or dissimilarities between the investigated objects.

Mutation – the change from one allelic type to another

Null Alleles – existing alleles that are not observed using standard assays (e.g. as a result of mutations at primer sites).

Phenotype – the phenotypic appearance of the organism, consisting of the interaction between the genotype and the environment

Polymerase Chain Reaction - The PCR involves replicating DNA which are flanked by regions of known sequences. Synthetic oligonucleotide primers (20-30 bases) that are complimentary to each of the flanking regions are needed. These are combined with a small sample of genomic DNA and free deoxynucleotides, a reaction buffer, and Taq polymerase. During a series of heating and cooling cycles, the DNA is denatured into single stranded molecules, the two primers anneal to their complementary sequences on either side of the target region, and the DNA polymerase replicates the region downstream from each primer. The amount of target DNA doubles with each cycle until micrograms of product are generated.

Population - a self-sustaining component of a particular species whose geographical and temporal distributions can be defined. Individuals within a population are assumed to share a number of biological characters (e.g. growth rate, fecundity, morphometrics) as well as, in the case of marine fishes, specific patterns of 'stock - recruitment'.

Power – the probability of rejecting the null hypothesis when it is false

Precautionary Approach – states that there should be more caution when information is uncertain, unreliable or inadequate, and that the absence of adequate scientific information should not be used as a reason for postponing or failing to take conservation action. The precautionary approach implements conservation measures even in the absence of scientific certainty that fish stocks are being overexploited. In a fisheries context, the precautionary approach is receiving considerable attention throughout the world primarily because the collapse of many fisheries resources is perceived to be due to the inability to implement timely conservation measures without scientific proof of overfishing. Thus, the precautionary approach is essentially a reversal of the "burden of proof.".

Random mating – mating takes place at random with respect to the gene/locus in question. The chance of an individual mating with another having a prescribed genotype is equal to the frequency of that genotype in the population.

Randomization test – a statistical test based on the randomization of the data set and recalculation of the test statistic. The randomization is done many times and from this, the probability of the observed value of the test statistic is obtained.

Restriction enzyme – an enzyme that cleaves DNA when it recognizes a particular sequence (usually 4-8 bp long)

Sampling error – amount by which the statistic being estimated deviates from the parameter

Selection - a bias in survival or fertility between genotypes within generations. The frequency of a particular allele will change and the rate of change of that allele will depend mathematically on the fitness advantage conferred by that allele. How can we detect selection? 1. selection causes changes in genotype frequencies, the generally good fit to HWE provides indirect evidence for a lack of significant selective forces 2. Selection may also be detectable through examination of the distribution of differentiation across loci: genetic divergence arising from restricted gene flow tends to affect all loci simultaneously, whereas selectively determined divergence is typically observed at one or only a few loci.

Statistic - A measure calculated from a sample of data. Contrast "statistic" (drawn from a sample) with "parameter," which is a characteristic of a population. For example, the sample mean is a statistic; the population mean is a parameter of a population.

Sweepstakes Hypothesis – variation in reproductive success that is made possible by great fecundity and high early mortality of many marine organisms. A small minority of individuals can replace the entire population in each generation by a sweepstakes-chance matching of reproductive activity with oceanographic conditions conducive to spawning, fertilization, larval survival and successful recruitment.

Sympatric- Occurring in the same area; capable of occupying the same geographic ranges without loss of identity by interbreeding.

Variational Algorithm. As opposed to recommendations based on statistical evidence or theoretical reasoning, *algorithms* are completely defined, finite sets of steps, operations, or procedures that will produce a particular outcome. For example, with a few exceptions, all computer programs, mathematical formulas, and (ideally) medical and food recipes are *algorithms*.

Variational algorithms attempt to maximize or minimize some value, property or equations by varying inputs. In this case, gradients in dynamic height showed flow

direction---however to estimate the circulation on the Scotian Shelf more fully, geostrophic flow was estimated using a variational interpolation algorithm (Panteleev). This method is superior in resolving small-scale circulation features because weighting functions related to the correlation scales are neither fixed nor set a priori. The current is calculated by applying the thermal wind equations to the irregularly distributed observations of T and S. The resultant velocity estimates are put into the variational algorithm that treats the interpolation of the velocity field as a weak constraint problem. The technique assimilates the velocity information by minimizing a series of cost functions that include divergence, nonlinear advection of the resulting velocity fields and a smoothing function. As the surface currents are virtually geostrophic on the Scotian Shelf in autumn, and as the along-current spatial correlation scale is greater than the across-current scale, minimizing the nonlinear velocity-advection terms has both a physical and a mathematical justification.

Wahlund effect – heterozygosity is decreased (compared to expected under HWE) as a result of mixing of differentiated sub-samples

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