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Population structure of herring *(Clupea pallasi)* in British Columbia determined by microsatellites, with comparisons to southeast Alaska and California

Structure des populations de hareng *(Clupea pallasi)* de la Colombie-Britannique d'après des microsatellites et comparaisons à des populations du sud-est de l'Alaska et de la Californie

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Abstract

The purpose of this study was to determine population structure of herring (*Clupea pallasi*) in British Columbia by analyzing microsatellite variation. Variation at 13 loci (Cpa6, Cpa27, Cha63, Cpa100, Cpa103, Cpa104, Cpa107, Cpa107a, Cha113, Cpa114, Cpa115, Cpa125, Cpa134) was surveyed in approximately 20,000 herring from 78 sampling locations. F_{ST} estimates by locus varied between 0.0006 and 0.0093, with the mean over all loci of 0.0032. Other than for herring spawning in Skidegate Inlet, there was no evidence of substructure for herring along the east coast of the Queen Charlotte Islands. No substructure was observed in west coast Queen Charlotte Islands herring, but herring in Louscoone Inlet at the extreme south west coast of the Queen Charlotte Islands may be distinct from herring in the east coast management unit. No convincing evidence of substructure was observed in either the North Coast or Central Coast stocks. No significant substructure was observed in herring in Johnstone Strait, although there is potential for herring spawning in the mainland inlets in the region to be distinct. In the Strait of Georgia, there was no evidence of substructure within the stock spawning along the east coast of Vancouver Island between Comox and Nanaimo. Herring spawning in Esquimault Harbour were distinct from the Strait of Georgia stock, and herring spawning in Secret Cove along the mainland coast may be distinct. Strait of Georgia herring were distinct from those spawning at Cherry Point in Puget Sound, Washington. No evidence of substructure was observed in the west coast of Vancouver Island stock. On a regional basis, herring in Johnstone Strait were distinct from those in other regions of British Columbia, but there was, on average, no significant differentiation between east and west coast Vancouver Island populations, or among Queen Charlotte Islands, North Coast, and Central Coast populations. The lack of genetic differentiation among herring stocks in British Columbia is consistent with straying rates among stocks that is sufficient to homogenize allele frequencies over broad areas. Herring spawning in southeast Alaska are distinct from those spawning further south on the Queen Charlotte Islands and in the north coast of British Columbia. Herring spawning in California are distinct from those spawning in southern British Columbia. For locations where genetically distinct populations occur, differences in timing of spawning are the main isolating mechanisms, although geographic isolation of the spawning population may also have some effect in maintaining genetic distinctiveness of the spawning population.

Résumé

L’objectif de la présente étude était de déterminer la structure des populations de hareng (*Clupea pallasi*) de la Colombie-Britannique par l’analyse de la variation des microsatellites. Pour ce faire, on a étudié la variation à 13 loci (Cpa6, Cpa27, Cha63, Cpa100, Cpa103, Cpa104, Cpa107, Cpa107a, Cha113, Cpa114, Cpa115, Cpa125 et Cpa134) chez quelque 20 000 harengs prélevés à 78 endroits. Les estimations de F_{ST} par locus variaient entre 0,0006 et 0,0093, la moyenne pour l’ensemble des loci se chiffrant à 0,0032. À part le hareng qui frayait dans l’inlet Skidegate, il n’y avait aucun signe de substructures chez le hareng de la côte est des îles de la Reine-Charlotte. De même, aucune substructure n’a été trouvée chez le hareng de la côte ouest de ces îles, quoique le hareng de l’inlet Louscoone, situé à l’extrémité sud-ouest des îles, est peut-être distinct du hareng de la côte est de cette unité de gestion. Aucun élément convaincant de la présence de substructures n’a été observé chez les stocks de la côte nord ou de la côte centrale de la province. De même, aucune substructure importante n’a été observée chez le hareng du détroit de Johnstone, bien qu’il soit possible que le hareng qui fraye dans les inlets continentaux de la région soit distinct. Dans le détroit de Georgia, aucun signe de substructure n’a été relevé chez le stock qui fraye le long de la côte est de l’île de Vancouver entre Comox et Nanaimo. Le hareng qui fraye dans le port d’Esquimalt était distinct du stock du détroit de Georgia, tandis que le hareng qui fraye dans l’anse Secret, sur la côte continentale, est peut-être distinct. Le hareng du détroit de Georgia était distinct de celui qui fraye à Cherry Point, dans le Puget Sound, dans l’État de Washington. Aucune substructure n’a été observée chez le stock de la côte ouest de l’île de Vancouver. Au niveau régional, le hareng du détroit de Johnstone était distinct de celui d’autres régions de la Colombie-Britannique, mais, en moyenne, il n’y avait pas de différence entre les populations des côtes est et ouest de l’île de Vancouver, ou entre les populations des îles de la Reine-Charlotte, de la côte nord et de la côte centrale. L’absence de différences génétiques entre les stocks de hareng de la Colombie-Britannique correspond aux taux de vagabondage entre les stocks, qui suffisent à homogénéiser les fréquences alléliques sur de vastes régions. Le hareng qui fraye dans les eaux du sud-est de l’Alaska est distinct de celui qui fraye plus au sud dans les eaux des îles de la Reine-Charlotte et celui de la côte nord de la province, tandis que le hareng qui fraye dans les eaux de la Californie est distinct de celui qui fraye dans les eaux du sud de la Colombie-Britannique. Pour ce qui est des endroits fréquentés par des populations génétiquement distinctes, les différences dans la période de fraye constituent les principaux mécanismes d’isolation des populations, quoique leur isolation géographique peut aussi avoir une certaine incidence sur la pérennité de la spécificité génétique de la population de reproducteurs.

Introduction

Delineation of population structure is fundamental to the assessment, conservation, and management of herring. Population structure of herring in British Columbia has been investigated with a variety of techniques. Early work centered on tagging (reviewed by Hay et al. 1999), with a large majority of herring returning to spawn in the region in which they were tagged (Hourston 1982). Spawning time and location were also thought to be important factors in delineating population structure (Haegele and Schweigert 1985). In British Columbia, herring are currently managed on the basis of the existence of five discrete stocks (Schweigert, 2000). The main stocks are defined as the Strait of Georgia, west coast of Vancouver Island, the Central Coast, the North Coast, and the southeast coast of the Queen Charlotte Islands. However, local population structure has been an important issue raised in the management of herring in British Columbia, with some thought that there are unique substocks or populations within these five stocks, perhaps at the level of bays or inlets.

The level of reproductive isolation, if any, among subcomponents or local populations, is uncertain. If there are discrete genetic stocks, then genetic differences should be observable among stocks, repeatable over time, and individual fish should return to spawn in the same geographic area from which they originated. Genetic differentiation at neutral genetic loci among spawning groups, indicative of restricted gene flow and independent population dynamics among the groups, is a good indicator of population structure. Moreover, if sufficient genetic differentiation is observed among populations, the genetic markers can be used to provide estimates of population or stock composition in areas of population mixing. This enables determination of catch by population with subsequent estimation of exploitation rates, allowing managers to protect less productive populations from overexploitation in regions of mixing.

Fidelity of spawning individuals to specific areas, with little exchange of spawners among areas, is a basic requirement in the designation of a “stock”. The restriction of gene flow among spawning groups that results from this fidelity enables the development over time of genetic differentiation. For a marine fish such as herring, a stock may consist of a single large, randomly-breeding aggregate, or may be subdivided into smaller groups within which mating is random, but among which there is more limited exchange of individuals. These local populations within a stock are more similar to each other than to populations in another stock complex. Analysis of genetic variation provides definitive analysis of whether or not there is restricted gene flow among putative populations and whether or not the putative populations constitute genetically distinct spawning populations. It is obviously important that the screening techniques used are able to detect genetic differentiation among putative populations should such differentiation exist. Previous analyses of genetic variation in eastern Pacific herring as determined from allozyme variation indicated that genetic differentiation was observed only over relatively large geographic areas, such as between herring from Asian and eastern Pacific regions, and perhaps within the eastern Pacific between Gulf of Alaska and more southern areas (Grant and Utter 1984). In the western Pacific Ocean, differentiation at allozymes indicated that genetic differentiation could occur on a more localized basis (Kobayashi et al. 1990), similar to the local differentiation observed in Atlantic herring (Jorstad et al. 1994; Turan et al. 1998). In a survey of mitochondrial DNA variation, local differentiation was not observed in eastern Pacific herring (Schweigert and Withler 1990), nor was any observed in ribosomal DNA sequence variation (Domanico et al. 1996). Analysis of variation at microsatellite DNA loci suggested that differentiation was detected among populations in more local areas of the eastern Pacific Ocean than with previous techniques for genetic analyses (O’Connell et al 1998b), with similar results observed in Atlantic herring (Shaw et al. 1999). Shaw et al. (1999) suggested that microsatellites uncover genetic structuring in populations that allozymes and

mtDNA studies do not detect. Beacham et al. (2001), in an analysis of microsatellite variation, found differentiation in British Columbia herring only in specific spawning locations.

The primary objective of this comprehensive study was to use microsatellite variation to investigate population structure of herring in British Columbia, in particular within the five defined stocks for management. We examined whether there are distinct "bay or inlet populations" of herring, i.e., is there genetic differentiation among herring populations in neighboring bays or inlets within currently managed stocks in British Columbia or are the currently defined stocks homogeneous genetic populations? The level of differentiation among the five defined stocks in British Columbia was also evaluated.

Materials and Methods

Collection of baseline DNA samples and laboratory analysis

Samples were obtained from adult herring immediately prior to, or at, or immediately after spawning during March to May of each year. The only exception was a juvenile sample collected in early June in Bute Inlet which was used to characterize herring from the area. Samples were collected from 75 locations in British Columbia, five in southeast Alaska, one in Washington, and two in California, with approximately 20,000 herring scored at 13 microsatellite loci (Table 1; Figs. 1-6). At the time of analysis, not all of the 2002 samples had been analyzed at all 13 loci, so some comparisons were conducted on a subset of the loci. Samples were generally obtained by a crewed vessel equipped with either a gillnet or purse seine that conducted testfishing at a particular location, and were usually collected during March in the year of spawning. The Portage Inlet/ Esquimault sample was collected from sport-caught herring in Victoria harbour. Genomic DNA was extracted using the chelex resin protocol of Small et al. (1998) from operculum punches from herring sampled between 1997 and 2002 and preserved in 90% ethanol. PCR products at 13 microsatellite loci: *Cha63*, *Cha113* (O'Connell et al. 1998a), *Cpa6, Cpa27, Cpa100, Cpa104, Cpa107, Cpa115, Cpa125, Cpa134* (Miller et al. 2001), and *Cpa103*, *Cpa107a,* and *Cpa114* (Olsen et al. pers. comm.) were size fractionated on denaturing polyacrylamide gels and allele sizes determined with the ABI 377 automated DNA sequencer and Genescan software.

Data Analysis

Each population at each locus was tested for departure from Hardy-Weinberg equilibrium using the Genetic Data Analysis (GDA) software (Lewis and Zaykin 2001). Tests of genetic differentiation utilizing pairwise comparisons among samples were conducted using GENEPOP (Raymond and Rousset 1995) with the Markov-chain approach using χ^2 probability values. The dememorization number was set at 1,000, and 50 batches were run for each test with 1,000 iterations/batch. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice, 1989). F_{ST} estimates for each locus were calculated with GDA, and the standard deviation of the estimate for an individual locus was determined by jackknifing over samples and for all loci combined by bootstrapping over loci. F_{ST} (or the coancestry coefficient) is the correlation of genes of different individuals in the same population and can range from 0 to 1. The formula is: $F_{ST} = \delta_p^2/[p(1-p)]$ where δ_p^2 is the variance over samples in the frequency of allele A, and p is the average sample frequency of allele A. Cavalli-Sforza and Edwards (1967) chord distance (CSE) was used to estimate distance among samples, with a neighbor-joining dendrogram generated with PHYLIP (Felsenstein 1993).

Hierarchical analyses of allele frequency variation with stocks were conducted with GDA using a nested random effects model. These analyses allowed the testing for each locus and for all loci combined of the significance of regional effects relative to variance among populations within region, as well the population effects relative to variance among sampling

years within populations. Variance components of population differences and annual variation within populations were determined for each of the five managed stocks of herring in British Columbia, as well as herring from the west coast of the Queen Charlotte Islands and from Johnstone Strait. For some areas, such as the North Coast and Strait of Georgia, the geographic place name of site of sample collection varies, but the geographic proximity of many of the samples was quite close, and thus should have sampled the same population, if it exists. These pooled samples were considered to represent a single putative population in the hierarchical analyses. Negative variance components were set to zero in the relative diversity analysis. As samples were derived from a number of sites in British Columbia, putative populations were defined within each stock. For the east coast of the Queen Charlotte Islands, the Selwyn Inlet samples were defined as a single putative population, as well as the Section Cove samples, the Skincuttle Inlet samples, the Skaat Harbour and Juan Perez Sound samples. Skidegate Inlet samples were also considered as a separate population. West Coast Queen Charlotte Islands populations were defined as Port Louis, Inskip Channel, and Rennell Sound. Two putative populations were defined for the North Coast, one north of Prince Rupert (Big Bay, Venn Passage, Tree Bluff, Ryan Point) and one southwest (Gurd Island, Kitkatla Inlet). Four putative populations were defined for the Central Coast, one near Swindle Island (East Higgins, Kitasu Bay), one near Bella Bella (Spiller Channel, Powell Anchorage, Dundivan Inlet, Stryker Bay, and Gale Passage), one near Rivers Inlet (Illahie Inlet, Pruth Anchorage, Gikumi Point, Fairmile Passage, and Kwakshua Channel), and one near Smith Inlet (Takush Harbour, Smith Inlet). Population structure of herring sampled near Johnstone Strait was also evaluated, with putative populations defined near Port Hardy (Hardy Bay, Beaver Harbour), Thompson Sound (1997, 1999), and Loughborough Inlet and Bute Inlet. For the Strait of Georgia stock, three putative populations were defined along the east coast of Vancouver Island. These consisted of samples taken near Denman and Hornby Islands (Longbeak Point, Henry Bay , Norman Point , Whalebone Point, Bowser, Chrome Island, Komas Bluff , Fanny Bay, Metcalf Bay, Baynes Sound, and Lambert Channel), near Parksville (Qualicum, French Creek and Parksville Bay), and near Nanaimo (Jessie Island, Hammond Bay, Sharpe Point, and Link Island). For the west coast of Vancouver Island, seven putative populations were defined. These were near Barkley Sound (Spilling Islet, Castle Rock , Bryant Islands, and Forbes Island), in Sydney Inlet (Sydney Inlet, Young Bay), in Nootka Sound (Nootka Sound, McKay Pass), in Hesquiat Harbour, in Esperanza Inlet, in Klaskish Inlet, and in Winter Harbour. In southeast Alaska, four populations were defined: Kirk Point, Seymour Canal, south Sitka, and north Sitka. Two populations were defined for California: San Francisco Bay (Sausalito Bay) and Tomales Bay.

Hierarchical analyses of allele frequency variation was conducted among herring from seven regions in British Columbia. The basic design was a nested analysis of variance, with regions, populations within regions, sampling years within populations, and remainder as the sources of variation. Skidegate Inlet samples were not included in the east coast of the Queen Charlotte Islands stock as the population was distinct. The Portage Inlet/Esquimault Harbour samples were not included in the Strait of Georgia stock as it was distinct. Hierarchical analyses of variance were also conducted incorporating populations from either Alaska or California with appropriate populations from British Columbia (northern populations with the comparison with Alaskan populations, southern populations with the comparison with California populations).

Results

Variation within stocks

All microsatellite loci surveyed were highly polymorphic, with the number of alleles per locus ranging from 29 at Cpa6 and Cpa27 to 63 at Cpa104, with an average of 40 alleles per locus (Table 2). Expected heterozygosity estimates were very similar among sampling sites, ranging from 0.84 to 0.89 (Table 1). Genotypic frequencies at each locus within sampling sites generally conformed to Hardy-Weinberg equilibrium (HWE) expectations, with the exception of Cpa115. Conformance to HWE expectations indicates that null or nonamplifying alleles were not present at the loci surveyed, and that the samples were comprised of single genetic populations. At Cpa115, about 47% of the sampling sites were not in HWE. One or more nonamplifying allele(s) was likely present at this loci, or unequal amplification of alleles may have resulted in the failure to detect large alleles in some heterozygous individuals. The mean F_{ST} value over 83 samples and 13 loci surveyed was 0.0032 (0.0030 with Cpa115 excluded), with significant single-locus values observed at 11 loci (Table 2).

Population structure within stocks

If substructuring of the five currently managed stocks of herring in British Columbia is present, then genetic differences among putative populations within stocks must be larger than annual variation within populations. This implies that there is a high degree of homing to the spawning location, and that genetic differences or genetic signal are greater than annual variation or sampling noise. Gene diversity analysis was used to determine the magnitudes of variation among samples within putative populations and among putative populations within stocks for herring in British Columbia. For herring along the east coast of the Queen Charlotte Islands, significant population differentiation was observed when herring spawning in Skidegate Inlet, Selwyn Inlet, Section Cove, and Skincuttle Inlet were compared, with the differentiation largely observed at Cpa27 (Table 3). Allele frequencies of Skidegate Inlet herring at Cpa27 were significantly different when compared with herring spawning at more southern locations along the east coast of the Queen Charlotte Islands and at somewhat earlier times ($P<0.05$). As Skidegate Inlet is outside of the current management area for this stock, comparisons were conducted among herring spawning in Selwyn Inlet, Section Cove, Skaat Harbour/Juan Perez Sound, and Skincuttle Inlet. No differentiation was observed at any of the 13 loci surveyed. Variation was, on average, greater among sample years within populations than among the three putative populations (Table 3). There is no evidence that herring spawning in Selwyn Inlet, Section Cove, or Skincuttle Inlet constitute a genetically distinct population in any of these three locations.

Along the west coast of the Queen Charlotte Islands, three populations based upon multiple years of sampling were defined for gene diversity analysis: (Rennell Sound, Port Louis, and Inskip Channel). As single samples were only available from Kano Inlet and Peel Inlet, these locations were not included in the analysis. No differentiation was observed at any of the 13 loci surveyed. Variation was, on average, greater among sample years within populations than among the three putative populations (Table 4). Thus, there was no evidence of stock structure for herring in these three locations. Similarly, there was no consistent differentiation between populations on the east (except Skidegate Inlet) and west coasts of the Queen Charlotte Islands (Table 4). Variation attributable to coastal differentiation was less than 10% of variation attributable to annual variation within sampling sites. Louscoone Inlet is at the extreme south west portion of the Queen Charlotte Islands, and has been considered as part of the management zone for the east coast. However, differentiation was observed at up to two loci for the single sample collected from Louscoone Inlet in 2002 compared with all east coast sampling sites, and indeed some differentiation was observed with some of the more northern

sites on the west coast (Table 5). The earlier timing of spawning (late February) of herring at this site may perhaps account for the differentiation, but this should be confirmed with additional samples.

Two putative populations were considered for the North Coast herring stock, with the two populations about 60 km apart. The gene diversity analysis indicated that about 99.9% of the variation occurred within populations, with no differentiation observed at any locus between the two putative populations (Table 6). None of the observed variation was attributable to population differentiation. There is no convincing evidence to indicate the existence of genetically discrete spawning populations within the North Coast herring stock. However, allele frequencies of the 2002 Wilson Inlet sample, located in the southern portion of the management zone, were differentiated from the Kitkatla population at Cha104 and Cha107 loci ($P<0.05$). Repeat sampling will be necessary to confirm the stability of this differentiation.

Four putative populations were considered for the Central Coast herring stock, with the two most distal populations about 120 km apart. As with the North Coast stock, about 99.9% of the variation occurred within populations, with no significant differentiation observed among the four putative populations (Table 7). Annual variation within populations was greater than any differentiation among putative populations. There is little evidence to indicate the existence of genetically discrete spawning populations within the Central Coast herring stock. However, the herring spawning in Rivers Inlet (southern area of the management zone) may be distinct from portions of the Central Coast herring stock, particularly herring spawning in the more northern portion of the management zone (Table 8). Additional sampling will be required to confirm this potential differentiation.

In Johnstone Strait, three putative populations were considered, one near Port Hardy, one in Thompson Sound, and one in the southern portion of the region (Loughborough Inlet, Bute Inlet). No significant differentiation was observed among these three putative populations, although there were some differences observed at the Cha27 locus (Table 9). More extensive sampling will be required to evaluate differentiation among herring in this region.

In the Strait of Georgia, spawning is concentrated along the east coast of Vancouver Island, with minor spawning in the eastern Strait of Georgia along the mainland coast of British Columbia. Unlike the Queen Charlotte Islands, where samples were collected from the same inlet over time, most samples from the Strait of Geogia have only been collected from one specific location in one year (Table 1). However, the geographic proximity of many of the samples was quite close, and thus should have sampled the same population, if it exists. In order to minimize the geographic spread of samples from a putative population, gene diversity analysis was restricted to samples along the east coast of Vancouver Island. Four putative populations were evaluated, with geographic locations adjacent to Denman and Hornby Islands, Parksville, Nanaimo, and Victoria. The gene diversity analysis indicated significant differentiation at seven loci was observed, indicative of strong population structure, but that all of the differentiation was due to the Portage Inlet/Esquimault Harbour population (Table 10). When this population was not considered to be part of the Strait of Georgia, no differentiation was observed at any locus among the remaining three putative populations (Table 10). Indeed, on average, there was no variation at all attributable to population differentiation. There is no evidence of genetic substructure in herring along the east coast of Vancouver Island between Denman and Hornby Islands and Nanaimo. These samples were pooled and considered as a single population and compared with single samples from Bargain Harbour and Secret Cove along the mainland coast. No differentiation was observed between the east coast of Vancouver Island population and the Bargain Harbour sample, but significant differentiation was observed at five of the 13 loci surveyed between the Vancouver Island population and the Secret Cove sample (Table 11). However, as only a single sample was available from the Secret Cove location, it was not possible to evaluate whether this differentiation is stable over

time. It is not possible to evaluate whether the differentiation is due to sampling error or population genetic differentiation. Significant differentiation was also observed between the Strait of Georgia population and herring from Cherry Point in Washington (Table 11). Significant differentiation was also observed between the Cherry Point sample and the Portage Inlet/Esquimault Harbour population, the geographically closest Canadian population. Herring in Esquimault harbour and Cherry Point are distinct from the Vancouver Island population.

Seven putative populations were considered for the west coast of Vancouver Island herring stock, with the populations defined by inlets or sounds along the coast. The gene diversity analysis indicated that about 99.9% of the variation occurred within populations, with no differentiation observed at any locus (Table 12). On average, there was no variation at all attributable to population differentiation. There is no evidence that there are genetically unique populations of herring spawning along the west coast of Vancouver Island.

In southeast Alaska, there was some evidence to suggest that herring spawing in Seymour Canal were genetically different than those spawing in the other three locations samples (Kirk Point and Mary Island considered as the same location) (Table 1). Six of the 13 loci surveyed approached statistical significance ($0.05<P<0.10$). No differentiation was observed among herring spawning in the other three locations, but additional sampling will be required to confirm the distinctiveness of the Seymour Canal population.

In California, no differentiation was observed between herring spawning in Sausalito Bay and Tomales Bay at any locus, indicative of probable gene flow between herring spawning in these two locations.

Structure among stocks

Seven putative regional stocks of herring were defined for the gene diversity analysis: east coast Queen Charlotte Islands, west coast Queen Charlotte Islands, North Coast, Central Coast, Johnstone Strait, east coast Vancouver Island, and west coast Vancouver Island. As there were distinct populations in Skidegate Inlet and Portage Inlet/Esquimault Harbour, these were considered to be separate from the seven regional putative stocks, and not included in the analysis. Significant differentiation at three loci was observed in the gene diversity analysis, indicative of some structure among herring in British Columbia (Table 13). The source of the differentiation was evaluated for subsets of the stocks. It has been previously noted that there was no consistent differentiation between herring populations on the east and west coast of the Queen Charlotte Islands, as was the case for populations on the east and west coasts of Vancouver Island. No consistent differentiation was observed between Queen Charlotte Islands, North Coast, or Central Coast stocks. However, differentiation was observed between the Johnstone Strait stock and herring in more northern and southern regions in British Columbia. In general, weak regional structure of herring in British Columbia is further illustrated in Fig. 7, as samples from specific stocks did not generally cluster together as a group, but were instead distributed among stocks. The Portage Inlet/Esquimault Harbour sample contained the most distinctive herring sampled in our survey.

Comparisons were possible between herring spawning in northern British Columbia and southeast Alaska. With four populations defined from southeast Alaska and six populations from the east coast of the Queen Charlotte Islands (four populatlions, Skidegate Inlet not included, Table 3) and North coast (two populations, Table 6), gene diversity analysis indicated that there was significant differentiation between herring in southeast Alaska compared with those in northern British Columbia at eight of 13 loci: Cpa27 ($F_{1,8}=10.14$, $P<0.05$), Cpa63 ($F_{1,8}=5.87$, $P<0.05$), Cpa103 ($F_{1,8}=8.25$, $P<0.05$), Cpa107 ($F_{1,8}=7.78$, $P<0.05$), Cpa107a ($F_{1,8}=9.43$, $P<0.05$), Cpa114 ($F_{1,8}=9.59$, $P<0.05$), Cpa115 ($F_{1,8}=19.86$, $P<0.01$), and Cpa125 ($F_{1,8}=7.14$, $P<0.05$), with the overall comparison significant ($F_{1,8}=8.00$, $P<0.05$). Herring spawning in northern British Columbia were generally distinct from those in southeast Alaska.

Although there was no differentiation between herring spawning in San Francisco Bay and Tomales Bay in California, herring from California were very distinct from those in southern British Columbia. For example, significant differentiation was observed at eight of 10 loci surveyed in a comparison between California herring and those from the west coast of Vancouver Island, with the overall comparison highly significant ($F_{1,7}$=27.30, P<0.01). Observed genetic differentiation was likely a result of geographic isolation coupled with differences in timing of spawning in the two areas.

Discussion

High levels of genetic diversity were observed in the Pacific herring samples surveyed in our study, with an observed average heterozygosity over 13 loci of 0.86, very similar to other microsatellite loci surveyed in Pacific herring by O'Connell et al. (1998b), and comparable to microsatellite variation in Atlantic herring (*C. harengus*) (Shaw et al. 1999). There was no reduction in heterozygosity at any sampling site that would indicate a recent population bottleneck. Although population sizes of herring in British Columbia declined to low levels during the 1960s (Hourston 1980), the declines in population size were not extensive enough to have reduced heterozygosity or the number of alleles at a locus substantially.

With the exception of the Portage Inlet/Esquimault Harbour population, virtually no population structuring was observed within the five currently managed stocks of herring in British Columbia, with 99.6-99.9% of the variation contained within samples, with virtually none of the variation distributed among putative populations within stocks. There is little evidence for genetically discrete populations of herring spawning in different bays or even inlets within local stocks or management groupings. In particular, along the east coast of the Queen Charlotte Islands, there is no evidence of genetic differentiation among herring spawning in Selwyn Inlet, Section Cove, Skincuttle Inlet, Skaat Harbour, or Juan Perez Sound. However, herring spawning in Louscoone Inlet (considered part of the east coast management zone) on the extreme southwest portion of the Queen Charlotte Islands may be distinct from herring spawning on the east coast of the Queen Charlotte Islands. In the Central Coast, the potential difference of River Inlet herring needs to be fruther evaluated. In the Strait of Georgia, herring spawning in Esquimault Harbour were clearly distinct from herring spawning in more northern areas along the east coast of Vancouver island in the Strait of Georgia. If management areas are intended to include only one discrete population, the south western boundary of the Strait of Georgia management area may need to be evaluated. Herring spawning at Secret Cove may also be distinct, but further samples need to be analyzed from this site to conclude that it is a distinct population. The other locations where there either are or may be genetically distinct populations, Skidegate Inlet on the east coast of the Queen Charlotte Islands, and Cherry Point in Washington are not included in current boundaries for stock assessment and management.

In order for local population differentiation to occur, herring spawning in a particular area must home and be isolated from other spawning herring through differences in timing of spawning, or the location of spawning must be isolated from other spawning areas, or both may occur. Differences in timing of spawning may have led to some genetically discrete local populations of herring. Herring spawning in Skidegate Inlet spawn later than in other locations sampled in British Columbia and southeast Alaska (samples collected April 15-18, 1998; May 7, 1999), and this later timing of spawning has provided enough reproductive isolation for genetic differentiation to occur. Herring spawning at Secret Cove in the Strait of Georgia tend to spawn somewhat earlier than in other locations in the Strait (sample collected March 2, 1999), perhaps enough of an isolating mechanism to allow for genetic differentiation. Herring spawning at Cherry Point, Washington were sampled May 2, 2000, approximately two months after

spawning typically begins in the Strait of Georgia. This difference in timing of spawning has likely led to the observed genetic differentiation between Strait of Georgia herring and those from Cherry Point. Geographic isolation may have also led to differentiation of local populations. Herring spawning in Esquimault Harbour at the extreme southern end of Vancouver Island move in to Portage Inlet (where the samples were collected) prior to and just after spawning. As there is a restricted distribution of spawning herring in this area, geographic isolation of the spawning population may have led to differentiation of a local population. Esquimault Harbour/Portage Inlet herring also spawn in late March, later than herring in most other locations in the Strait of Georgia, and thus both geographic isolation and differences in timing of spawning may be isolating mechanisms to maintain the distinctiveness of these populations.

There was a lack of genetic differentiation among the five regional stocks of herring assessed and managed in British Columbia, but all appear to be different from herring spawning in Johnstone Strait mainland inlet locations. Lack of differentiation between putative stocks can occur even if gene flow among stocks is restricted if the stocks have only recently diverged, and not enough time has passed since divergence to allow sufficient genetic drift to occur to lead to stock differentiation. Alternatively, significant gene flow among putative stocks can lead to homogenization of allele frequencies, even if the putative stocks have been separated for thousands of years. In British Columbia, tagging data has indicated that homing to management areas has been about 85% (Hourston 1982). Additional analyses of all tagged herring indicated that homing ranged from 53% of released tagged fish in Johnstone Strait to 97% of tagged fish released off the west coast of Vancouver Island (Hay et al. 1999). However, tagged fish released from one of six regions and that were at large for one year or longer were usually recovered in all other regions. These levels of gene flow, if the migrants are successful at reproducing, will lead to homogenization of allele frequencies, and is the most likely explanation for the lack of genetic differentiation among putative stocks of herring in British Columbia. Although there was a lack of genetic differentiation among the five putative stocks of herring in British Columbia, all herring should not be considered as a single stock for assessment and management. There is straying among putative herring stocks, but the levels of straying may not be sufficient to offset overexploitation of herring in specific regions. Therefore, exploitation of herring should continue to be distributed geographically in British Columbia, in order to ensure maintenance of diversity that may not have been detected in our survey of microsatellite loci.

Genetic structuring of Alaskan herring populations has been reported by O'Connell et al. (1998b), and for Atlantic herring in Norwegian waters and the Barents Sea by Shaw et al. (1999). Both studies were based on microsatellite surveys where only one year of samples was analyzed, and conclusions drawn on population structure without a measure of within population variation available. Subsequent sampling of the Alaskan putative populations indicated that temporal or annual variation was as large or larger than any differentiation between putative local populations, and thus characterization of separate populations in the Gulf of Alaska was not warranted (J. Olsen, Alaska Department of Fish & Game, Anchorage, pers. comm.). However, the difference between Bering Sea and Gulf of Alaska stocks was maintained over time. Demonstration that population differentiation is persistent over time increases the likelihood that the appropriate population structure has been elucidated (Waples 1998).

Divergent views have been expressed on population structure of Atlantic herring. In one case, discrete herring populations were thought to exist, with larval retention in the spawning area and general homing to the natal spawning area the mechanisms maintaining population distinctiveness (Iles and Sinclair 1982). Conversely, Smith and Jamieson (1986) suggested that herring populations fluctuate in size and the range occupied, and that straying among spawning

populations do not result in distinct local populations. Alternatively, McQuinn (1997) suggested that there is a metapopulation structure in herring, with local population discreteness maintained by homing to initial spawning areas, not necessarily natal spawning areas, while new recruits to a population learn migration patterns and spawning areas from existing spawners within the population. Although genetically distinct local populations of herring exist in British Columbia, they are the exception, with differences in timing of spawning the main isolating mechanism. In these populations, discreteness is maintained by homing to natal spawning grounds, with a low rate of straying into the population, as there are few herring spawning in adjacent locations at the same time. For most herring in British Columbia, spawning is concentrated in March, and straying among spawning populations is substantial enough to prevent the development of genetically distinct populations in most areas.

Recommendations

1. In the Strait of Georgia management unit, herring spawning at Esquimault Harbour/Portage are genetically distinct from those further north in the management unit. If management units are intended to include only one genetically discrete group of herring, the south western boundary of the Strait of Georgia management area may need to be evaluated.

2. Although there was no consistent genetic differentiation among the five defined stocks of herring in British Columbia, prudent management action suggests that herring continue to be assessed and managed on a five-stock basis. In particular, exploitation should be distributed over herring in all five regions as appropriate, and not concentrated on herring in a specific region.

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Table 1. Population sample, collection years, total number of fish analyzed, expected heterozygosity (H_e), and observed heterozygosity (H_o) for 83 samples of herring in ten regions in southeast Alaska, British Columbia, Washington, and California.

Population	Years	N	H_e	H_o
	Southeast Alaska			
North Sitka	2000	92	0.88	0.87
South Sitka	2000, 2001	266	0.88	0.87
Kirk Point	2000, 2001	193	0.87	0.86
Mary Island	2000	93	0.88	0.87
Seymour Canal	2000, 2001	245	0.88	0.87
	Queen Charlotte Islands - east coast			
Skidegate Inlet	1998, 1999	227	0.87	0.84
Selwyn Inlet	1998, 1999, 2000	398	0.88	0.86
Section Cove	1998, 1999	418	0.88	0.85
Skincuttle Inlet	1998, 1999, 2000,2001	636	0.87	0.86
Haswell Bay	2000	158	0.88	0.89
Skaat Harbour	2000	166	0.88	0.86
Juan Perez Sound	2001	113	0.84	0.85
	Queen Charlotte Islands - west coast			
Rennell Sound	1999, 2002	165	0.87	0.87
Port Louis	1999, 2001, 2002	304	0.87	0.87
Inskip Channel	1999, 2001, 2002	355	0.87	0.86
Peel Inlet	1999	92	0.87	0.83
Kano Inlet	2002	108	0.88	0.85
Louscoone Inlet	2002	183	0.88	0.88
	North Coast			
Big Bay	1999	143	0.87	0.84
Venn Passage	1999	147	0.88	0.84
Tree Bluff	2000	159	0.88	0.84
Gurd/Kitkatla	1999, 2001	318	0.87	0.86
Kitkatla	2000	171	0.87	0.87
Ryan Point	2001	186	0.88	0.88
Wilson Inlet	2002	118	0.88	0.88
Hunt’s Inlet	2002	116	0.87	0.85

	Central Coast			
Spiller Channel	1999, 2000, 2001	514	0.86	0.86
Powell Anchorage	1999	69	0.88	0.85
Dundivan Inlet	1999	260	0.88	0.85
Gale Passage	2000	175	0.88	0.89
Illahie Inlet	1999	122	0.88	0.83
Pruth Anchorage	1999	146	0.86	0.84
East Higgins	2000	192	0.88	0.83
Kitasu Bay	2000, 2001	363	0.87	0.87
Smith Inlet	2000	159	0.88	0.88
Stryker Bay	1999	117	0.88	0.85
Takush Harbour	1999	129	0.88	0.86
Fairmile Pass.	2001	102	0.88	0.89
Gikumi Point	2001	178	0.88	0.88
Kwakshua Chn.	2001	90	0.88	0.88
Rivers Inlet	2001	89	0.89	0.89
	Johnstone Strait			
Beaver Harbour	1999	145	0.88	0.84
Hardy Bay	2000	172	0.89	0.83
Wakeman Sound	1999, 2001	98	0.88	0.88
Thompson Sound	1997, 1999	179	0.89	0.84
Loughborough Inlet	1997, 1999	147	0.89	0.82
Bute Inlet	1999	358	0.89	0.87
	Strait of Georgia			
Longbeak Point	1997	95	0.88	0.86
Henry Bay	1999	150	0.88	0.84
Norman Point	1999	118	0.88	0.85
Whalebone Point	1999	261	0.88	0.84
Bowser	1999	260	0.88	0.86
Chrome Island	2000	137	0.86	0.84
Komas Bluff	2000	171	0.87	0.86
Fanny Bay	2000	66	0.84	0.87
French Creek	1999	236	0.88	0.84

Parksville Bay	2000	127	0.87	0.86
Hammond Bay	2000	149	0.87	0.87
Jessie Island	1999	164	0.88	0.86
Link Island	1999	166	0.88	0.84
Portage Inlet	1999, 2001	246	0.85	0.84
Bargain Harbour	1999	125	0.88	0.85
Secret Cove	1999	175	0.87	0.85
Lambert Channel	2001	174	0.87	0.89
Baynes Sound	2001	175	0.86	0.86
Qualicum	2001	180	0.87	0.86
Metcalf Bay	2001	176	0.88	0.88
Sharpe Point	2002	200	0.88	0.87
	Vancouver Island – west coast			
Winter Harbour	1999, 2001, 2002	452	0.88	0.86
Klashish Inlet	1999, 2002	248	0.88	0.85
Nootka Sound	1999, 2000, 2001	500	0.87	0.86
McKay	1999	94	0.88	0.85
Hesquiat Harbour	1999, 2001	173	0.86	0.86
Syndey Inlet	1999, 2000	366	0.88	0.86
Esperanza Inlet	1999, 2000, 2001	522	0.88	0.87
Spilling Island	1997	154	0.88	0.82
Castle Rock	1999	136	0.87	0.83
Bryant Island	1999	151	0.88	0.84
Forbes	2000	161	0.87	0.85
Young Bay	2001	182	0.87	0.88
	Washington			
Cherry Point	2000	164	0.90	0.86
	California			
Sausalito Bay	2001, 2002	363	0.87	0.85
Tomales Bay	2001, 2002	360	0.88	0.86

Table 2. Number of alleles, expected heterozygosity (H_e), observed heterozygosity (H_o), percent significant Hardy-Weinberg equilibrium tests (HWE, N=83 tests), and F_{ST} (standard deviation in parentheses) among 83 herring samples for 13 microsatellite loci.

Locus	Alleles	H_e	H_o	HWE	F_{ST}
Cpa6	29	0.71	0.68	7.2	0.0030 (0.0005)
Cpa27	29	0.73	0.71	2.4	0.0093 (0.0030)
Cha63	36	0.88	0.89	1.2	0.0013 (0.0005)
Cpa100	48	0.91	0.89	1.2	0.0008 (0.0003)
Cpa103	30	0.83	0.81	1.2	0.0006 (0.0003)
Cpa104	63	0.96	0.97	1.2	0.0016 (0.0004)
Cpa107	34	0.88	0.87	2.4	0.0118 (0.0004)
Cpa107a	42	0.78	0.74	6.0	0.0071 (0.0011)
Cha113	32	0.87	0.86	3.6	0.0004 (0.0005)
Cpa114	31	0.84	0.83	3.6	0.0011 (0.0006)
Cpa115	47	0.90	0.86	47.0	0.0036 (0.0008)
Cpa125	52	0.94	0.94	3.6	0.0015 (0.0004)
Cpa134	55	0.91	0.89	2.4	0.0006 (0.0002)
All loci	40	0.88	0.86		0.0032 (0.0005)

Table 3. Hierarchical gene-diversity analysis of five putative populations of herring along the east coast of the Queen Charlotte Islands: Skidegate Inlet (sampled in 1998, 1999), Selwyn Inlet (1998,1999, 2000), Section Cove (1998, 1999), Skaat Harbour 2000/Juan Perez Sound 2001, and Skincuttle Inlet (1998, 1999, 2000, 2001) for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

	With Skidegate Inlet			Without Skidegate Inlet		
Locus	Within pops	Among years within pops	Among pops	Within pops	Among years within pops	Among pops
Cpa6	0.9952	0.0022	0.0026	0.9953	0.0026	0.0021
Cpa27	0.9879	0.0000	0.0121**	0.9998	0.0001	0.0001
Cha63	0.9982	0.0016	0.0002	0.9980	0.0018	0.0002
Cpa100	0.9996	0.0000	0.0004	0.9997	0.0000	0.0003
Cpa103	0.9990	0.0010	0.0000	0.9988	0.0012	0.0000
Cpa104	0.9992	0.0002	0.0006	0.9996	0.0003	0.0001
Cpa107	0.9956	0.0019	0.0025	0.9990	0.0010	0.0000
Cpa107a	0.9980	0.0020	0.0000	0.9982	0.0018	0.0000
Cha113	0.9992	0.0000	0.0008	0.9991	0.0000	0.0009
Cpa114	0.9990	0.0010	0.0000	1.0000	0.0000	0.0000
Cpa115	0.9967	0.0018	0.0015	0.9978	0.0022	0.0000
Cpa125	0.9992	0.0007	0.0001	0.9992	0.0007	0.0001
Cpa134	0.9997	0.0003	0.0000	0.9993	0.0007	0.0000
All	0.9979	0.0008	0.0013	0.9992	0.0008	0.0000

** indicates $P < 0.01$.

Table 4. Hierarchical gene-diversity analysis of: a) three putative populations of herring along the west coast of the Queen Charlotte Islands: Port Louis (sampled in 1999, 2001, 2002), Inskip Channel (1998, 2001, 2002), and Rennell Sound (1999, 2002), and b) west versus east coast Queen Charlotte Islands populations for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

	Along west coast			West versus east coast			
Locus	Within pops	Among years within pops	Among pops	Within pops	Among years within pops	Among pops within coasts	Between coasts
Cpa6	0.9951	0.0049	0.0000	0.9953	0.0034	0.0007	0.0006
Cpa27	0.9964	0.0036	0.0000	0.9986	0.0014	0.0000	0.0000
Cha63	0.9937	0.0063	0.0000	0.9960	0.0034	0.0000	0.0006
Cpa100	0.9996	0.0000	0.0004	0.9996	0.0000	0.0004	0.0000
Cpa103	0.9968	0.0032	0.0000	0.9979	0.0016	0.0000	0.0005
Cpa104	0.9980	0.0020	0.0000	0.9992	0.0008	0.0000	0.0000
Cpa107	0.9986	0.0014	0.0000	0.9988	0.0011	0.0000	0.0001
Cpa107a	0.9970	0.0030	0.0000	0.9976	0.0020	0.0000	0.0004
Cha113	0.9964	0.0019	0.0017	0.9989	0.0000	0.0011	0.0000
Cpa114	0.9976	0.0024	0.0000	0.9996	0.0004	0.0000	0.0000
Cpa115	0.9987	0.0013	0.0000	0.9979	0.0019	0.0000	0.0002
Cpa125	0.9971	0.0029	0.0000	0.9985	0.0015	0.0000	0.0000
Cpa134	0.9969	0.0031	0.0000	0.9984	0.0014	0.0000	0.0002
All	0.9973	0.0027	0.0000	0.9985	0.0014	0.0000	0.0001

Table 5. Probability of homogeneity of allele frequencies estimated from pairwise probability tests between Louscoone Inlet and other Queen Charlotte Islands samples, with probabilities derived from GENEPOP version 3.1 with the Markov-Cpain approach using χ^2 probability values (Raymond and Rousset 1995). Values considered statistically significant are in bold type.

Population	Cpa6	Cpa27	Cpa63	Cpa100	Cpa104	Cpa107	Cpa113	Cpa115	Cpa125	Cpa134
East Coast										
Skidegate	0.0366	**0.0000**	0.8820	0.3058	**0.0021**	**0.0000**	0.6125	**0.0000**	**0.0006**	0.1364
Selwyn Inlet	0.0138	**0.0000**	0.4712	0.7704	0.7979	0.7669	0.4114	0.1744	0.0553	0.3610
Haswell	**0.0033**	**0.0044**	0.4454	0.9505	0.2096	0.3134	0.5505	0.7032	0.3677	0.0381
Skaat/Juan	**0.0022**	**0.0000**	0.6940	0.8770	0.1794	0.8751	0.1695	0.2697	0.0646	0.0125
Section Cove	**0.0006**	**0.0002**	0.4081	0.1864	0.0619	0.2464	0.7480	0.0738	0.0083	0.1274
Skincuttle	0.0152	**0.0000**	0.1379	0.6043	0.8018	0.9288	0.3302	0.0467	0.0057	0.1623
West Coast										
Port Louis	**0.0038**	0.0435	0.5196	0.7849	0.3644	0.7318	0.5555	0.0330	0.1500	**0.0043**
Rennell Snd	0.3405	**0.0044**	0.6342	0.9658	0.4777	0.1237	0.3545	0.1240	0.1832	0.1100
Kano Inlet	0.2130	0.7195	0.3013	0.3930	0.6504	0.3459	0.3373	0.5924	0.2192	0.8786
Inskip Chan.	0.0537	0.0072	0.3095	0.3953	0.8721	0.4222	0.4608	0.0554	0.1091	0.0087
Peel Inlet	0.0560	0.1124	0.2078	0.3337	0.2760	0.7162	0.9191	0.0173	0.1524	0.5845

Table 6. Hierarchical gene-diversity analysis of two putative populations of herring along the north coast of British Columbia: Population 1 (Big Bay 1999, Venn Passage 1999, Tree Bluff 2000, Ryan Point 2001) and Population 2 (Gurd Island 1999, 2001, Kitkatla Channel 2000) for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

Locus	Within Populations	Among years within pops	Among pops
Cpa6	0.9989	0.0011	0.0000
Cpa27	0.9992	0.0004	0.0004
Cha63	0.9954	0.0046	0.0000
Cpa100	0.9990	0.0004	0.0006
Cpa103	0.9977	0.0022	0.0001
Cpa104	0.9997	0.0001	0.0002
Cpa107	0.9999	0.0001	0.0000
Cpa107a	0.9987	0.0013	0.0000
Cha113	0.9986	0.0014	0.0000
Cpa114	0.9991	0.0009	0.0000
Cpa115	0.9991	0.0005	0.0004
Cpa125	0.9995	0.0005	0.0000
Cpa134	0.9997	0.0003	0.0000
All	0.9989	0.0011	0.0000

Table 7. Hierarchical gene-diversity analysis of four putative populations of herring along the central coast of British Columbia: Population 1 (East Higgins 2000, Kitasu Bay 2000, 2001), Population 2 (Spiller Channel 1997, 1999, 2000, Powell Anchorage 1999, Dundivan Inlet 1999, Stryker Bay 1999, Gale Passage 2000), Population 3 (Illahie Inlet 1999, Pruth Anchorage 1999, Gikumi Point 2001, Fairmile Passage 2001, Kwakshua Channel 2001), and Population 4 (Takush Harbour 1999, Smith Inlet 2000) for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

Locus	Within Populations	Among years within pops	Among pops
Cpa6	0.9973	0.0027	0.0000
Cpa27	0.9987	0.0002	0.0011
Cha63	0.9989	0.0011	0.0000
Cpa100	0.9996	0.0004	0.0000
Cpa103	0.9991	0.0008	0.0001
Cpa104	0.9996	0.0001	0.0003
Cpa107	0.9997	0.0002	0.0001
Cpa107a	0.9997	0.0002	0.0001
Cha113	0.9998	0.0001	0.0001
Cpa114	0.9971	0.0029	0.0000
Cpa115	0.9974	0.0019	0.0007
Cpa125	0.9997	0.0001	0.0002
Cpa134	0.9992	0.0008	0.0000
All	0.9990	0.0009	0.0001

Table 8. Probability of homogeneity of allele frequencies estimated from pairwise probability tests between Rivers Inlet and other Central Coast samples, with probabilities derived from GENEPOP version 3.1 with the Markov-Cpain approach using χ^2 probability values (Raymond and Rousset 1995). Values considered statistically significant are in bold type.

Population	Cpa6	Cpa27	Cpa63	Cpa100	Cpa104	Cpa107	Cpa113	Cpa115	Cpa125	Cpa134
East Higgins	0.0629	**0.0001**	0.6713	0.6944	0.0542	**0.0004**	0.5264	0.2732	0.1909	0.2075
Kitasu Bay	0.5150	**0.0001**	0.5060	0.1864	0.0337	0.0105	0.7692	0.7965	0.2498	0.0151
Spiller Channel	0.2669	**0.0000**	0.1463	0.5117	**0.0000**	**0.0000**	0.3878	0.0438	0.1967	0.0329
Stryker Bay	0.0223	**0.0021**	0.7770	0.4815	**0.0012**	0.2399	0.5425	0.1421	0.3470	0.4361
Powell	0.8186	0.0111	0.5649	0.3869	0.0778	**0.0036**	0.3325	0.3114	0.8654	0.3472
Anchorage										
Dundivan Inlet	0.9904	**0.0001**	0.1217	0.1384	**0.0000**	**0.0001**	0.6467	**0.0003**	0.5817	0.0116
Gale Passage	0.6426	**0.0019**	0.5101	0.8314	0.1328	**0.0000**	0.4744	0.3667	0.7729	0.0144
Illahie Inlet	0.4245	0.0080	0.2631	0.5487	0.0228	0.0383	0.6740	0.4049	0.1548	0.3690
Pruth Anchorage	0.3124	**0.0007**	0.0492	0.0188	0.0167	**0.0000**	0.8938	0.0405	0.2608	0.0695
Fairmile Pass.	0.8141	0.0096	0.4845	0.8670	0.0454	0.2360	0.5555	0.5541	0.5686	0.0731
Gikumi Point	0.4322	0.0635	0.9082	0.3813	0.0101	0.0092	0.3849	0.0371	0.6817	0.0092
Kwakshua Chn.	0.7496	0.0110	0.8078	0.4731	0.0055	0.0224	0.4670	0.3445	0.5314	0.2958
Smith Inlet	0.4506	0.2585	0.6971	0.8179	0.0623	**0.0013**	0.4941	0.2784	0.6550	0.2240
Takush Harbour	0.3729	0.0111	0.9281	0.3964	0.0389	0.0461	0.7010	0.6301	0.5450	0.0064

Table 9. Hierarchical gene-diversity analysis of three putative populations of herring in Johnstone Strait: Population 1 (Beaver Harbour 1999, Hardy Bay 2000), Population 2 (Thompson Sound 1997, 1999), and Population 3 (Loughborough Inlet1997, Bute Inlet1999) for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

Locus	Within Populations	Among years within pops	Among pops
Cpa6	0.9968	0.0027	0.0005
Cpa27	0.9926	0.0027	0.0047
Cha63	0.9970	0.0030	0.0000
Cpa100	0.9995	0.0005	0.0000
Cpa103	0.9993	0.0004	0.0003
Cpa104	0.9999	0.0001	0.0000
Cpa107	0.9955	0.0013	0.0032
Cpa107a	0.9886	0.0101	0.0015
Cha113	0.9991	0.0009	0.0000
Cpa114	0.9998	0.0002	0.0000
Cpa115	0.9963	0.0020	0.0017
Cpa125	0.9996	0.0004	0.0000
Cpa134	0.9997	0.0000	0.0003
All	0.9990	0.0019	0.0008

Table 10. Hierarchical gene-diversity analysis of four putative populations of herring along the east coast of Vancouver Island: Population 1 (Longbeak Point 1997, Henry Bay 1999, Norman Point 1999, Whalebone Point 1999, Bowser 1999, Chrome Island 2000, Komas Bluff 2000, Fanny Bay 2000, Metcalf Bay 2001, Baynes Sound 2001, Lambert Channel 2001), Population 2 (French Creek 1999, Parksville Bay 2000, Qualicum 2001), Population 3 (Jessie Island 1999, Link Island 1999, Hammond Bay 2000, Sharpe Point 2002), Population 4 (Portage/Equimault 1999, 2001) for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

	With Portage/Esquimault			Without Portage/Esquimault		
Locus	Within pops	Among years within pops	Among pops	Within pops	Among years within pops	Among pops
Cpa6	0.9964	0.0013	0.0023	0.9985	0.0015	0.0000
Cpa27	0.9728	0.0004	0.0268**	0.9995	0.0005	0.0000
Cha63	0.9986	0.0004	0.0010	0.9995	0.0005	0.0000
Cpa100	0.9983	0.0000	0.0017*	0.9999	0.0000	0.0001
Cpa103	0.9980	0.0020	0.0000	0.9978	0.0022	0.0000
Cpa104	0.9992	0.0002	0.0031*	0.9999	0.0001	0.0000
Cpa107	0.9956	0.0010	0.0397**	0.9990	0.0010	0.0000
Cpa107a	0.9930	0.0030	0.0040*	0.9971	0.0029	0.0000
Cha113	0.9989	0.0004	0.0007	0.9993	0.0005	0.0002
Cpa114	0.9963	0.0035	0.0002	0.9961	0.0039	0.0000
Cpa115	0.9924	0.0007	0.0069*	0.9993	0.0007	0.0000
Cpa125	0.9992	0.0001	0.0028*	0.9996	0.0002	0.0002
Cpa134	0.9995	0.0005	0.0000	0.9994	0.0006	0.0000
All	0.9979	0.0011	0.0067**	0.9991	0.0009	0.0000

* indicates $P<0.05$, ** indicates $P < 0.01$.

Table 11. Probability of homogeneity of allele frequencies estimated from pairwise probability tests derived from GENEPOP version 3.1 with the Markov-Cpain approach using χ^2 probability values (Raymond and Rousset 1995). Values considered statistically significant are in bold type. Sample comparisons are:

1) Secret Cove versus combined ECVI samples (18 locations)
2) Bargain Harbour versus combined ECVI samples (18 locations)
3) Cherry Point versus combined ECVI samples (18 locations)
4) Cherry Point versus Portage Inlet

	Sample Comparison			
Locus	1	2	3	4
Cpa6	0.0449	0.7418	**0.0021**	**0.0002**
Cpa27	**0.0000**	0.9622	**0.0001**	**0.0000**
Cha63	0.5196	0.9314	0.6228	0.0613
Cpa100	0.0588	0.2526	0.0194	**0.0005**
Cpa103	0.5317	0.1541	0.0736	0.0991
Cpa104	0.0542	0.7396	**0.0000**	**0.0000**
Cpa107	**0.0000**	0.5510	**0.0000**	**0.0000**
Cpa107a	**0.0024**	0.4267	**0.0000**	**0.0025**
Cha113	0.3815	0.2181	0.8486	**0.0032**
Cpa114	0.3396	0.1684	**0.0034**	**0.0040**
Cpa115	**0.0000**	0.1220	**0.0000**	**0.0000**
Cpa125	**0.0000**	0.9163	**0.0000**	**0.0027**
Cpa134	0.7862	0.3764	0.1428	0.1426

Table 12. Hierarchical gene-diversity analysis of seven putative populations of herring along the west coast of Vancouver Island: Population 1 (Spilling Islet 1997, Castle Rock 1999, Bryant Islands 1999, Forbes Island 2000), Population 2 (Sydney Inlet 1999, 2000, Young Bay 2001), Population 3 (Nootka Sound 1999, 2000, 2001, McKay Pass 1999), Population 4 (Hesquiat Harbour 1999, 2001), Population 5 (Esperanza Inlet 1999, 2000, 2001), Population 6 (Klaskish Inlet 1999, 2002), and Population 7 (Winter Harbour 1999, 2001, 2002) for 13 microsatellite loci. The relative diversity owing to sampling years within populations and among populations is indicated.

Locus	Within Populations	Among years within pops	Among pops
Cpa6	0.9986	0.0013	0.0001
Cpa27	0.9988	0.0012	0.0000
Cha63	0.9998	0.0001	0.0001
Cpa100	0.9998	0.0000	0.0002
Cpa103	1.0000	0.0000	0.0000
Cpa104	0.9992	0.0007	0.0001
Cpa107	0.9996	0.0004	0.0000
Cpa107a	0.9986	0.0012	0.0002
Cha113	0.9993	0.0007	0.0000
Cpa114	0.9995	0.0005	0.0000
Cpa115	0.9989	0.0008	0.0003
Cpa125	0.9996	0.0003	0.0001
Cpa134	1.0000	0.0000	0.0000
All	0.9995	0.0005	0.0000

Table 13. Hierarchical gene-diversity analysis of seven putative stocks of herring in British Columbia: east coast Queen Charlotte Islands, west coast Queen Charlotte Islands, North Coast, Central Coast, Johnstone Strait, east coast Vancouver Island, and west coast Vancouver Island for 13 microsatellite loci. The relative diversity owing to sampling years within stocks, among years within populations, among populations within stocks, and among stocks is indicated.

Locus	Within Stocks	Among years within populations	Among populations within stocks	Among stocks
Cpa6	0.9978	0.0020	0.0001	0.0001
Cpa27	0.9969	0.0013	0.0005	0.0013*
Cha63	0.9983	0.0015	0.0000	0.0002
Cpa100	0.9960	0.0000	0.0002	0.0038*
Cpa103	0.9991	0.0008	0.0000	0.0001
Cpa104	0.9996	0.0004	0.0000	0.0001
Cpa107	0.9994	0.0008	0.0001	0.0002
Cpa107a	0.9973	0.0022	0.0000	0.0005
Cha113	0.9989	0.0006	0.0005	0.0000
Cpa114	0.9985	0.0015	0.0000	0.0000
Cpa115	0.9983	0.0012	0.0003	0.0002
Cpa125	0.9977	0.0004	0.0001	0.0018*
Cpa134	0.9999	0.0006	0.0000	0.0001
All	0.9982	0.0011	0.0000	0.0007*

* $P<0.05$

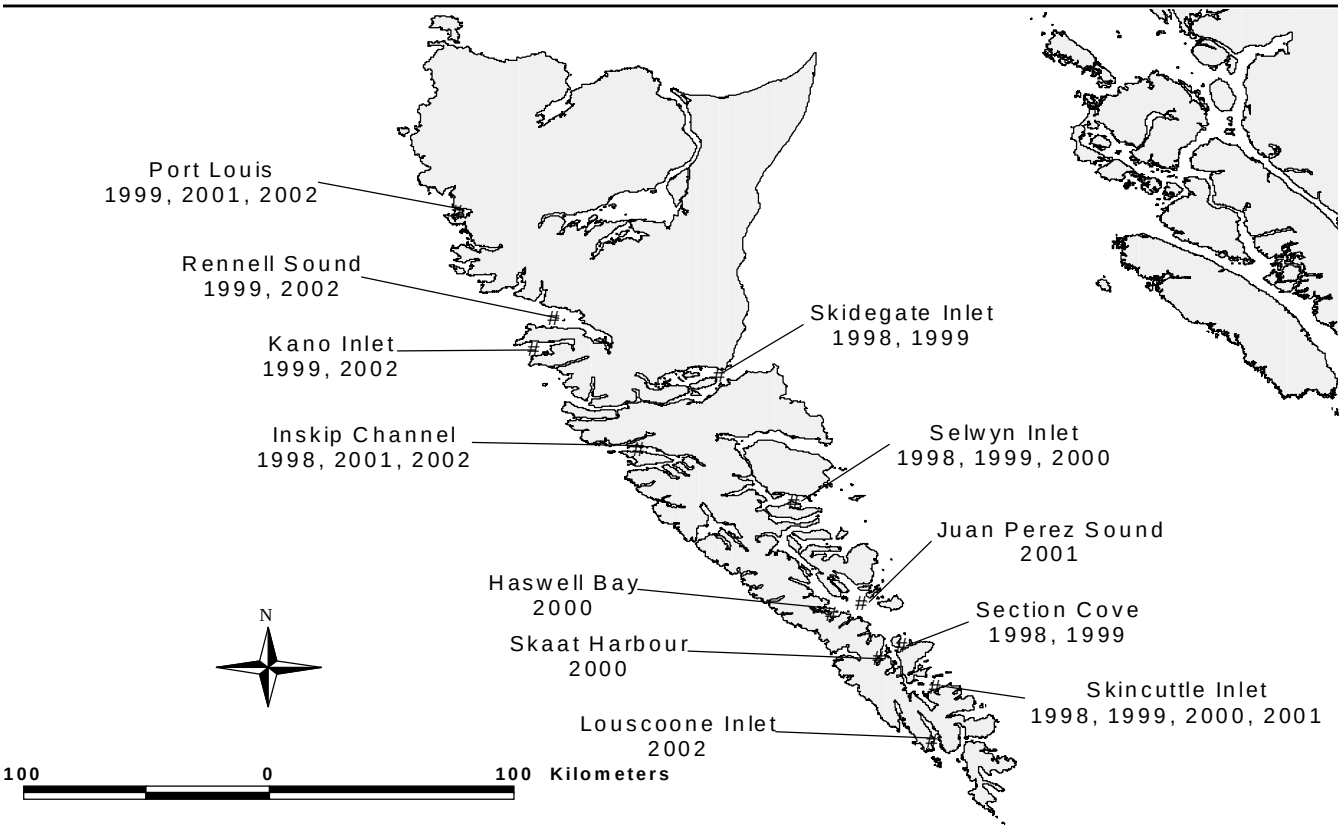

Figure 1: Sampling locations for genetic analysis from 1998 - 2002 in the Queen Charlotte Islands herring assessment region**.**

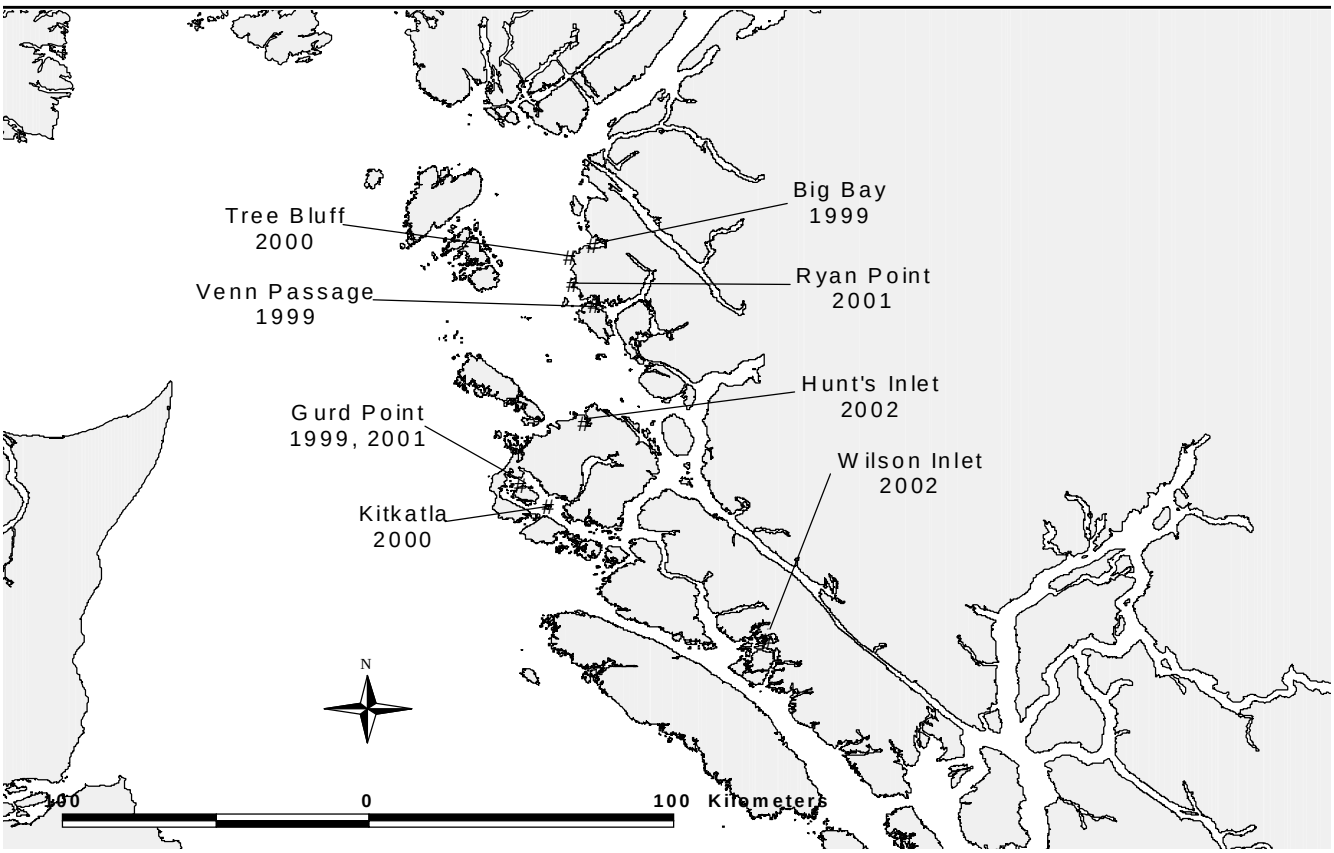

Figure 2: Sampling locations for genetic analysis from 1999 - 2002 in the Prince Rupert herring assessment region.

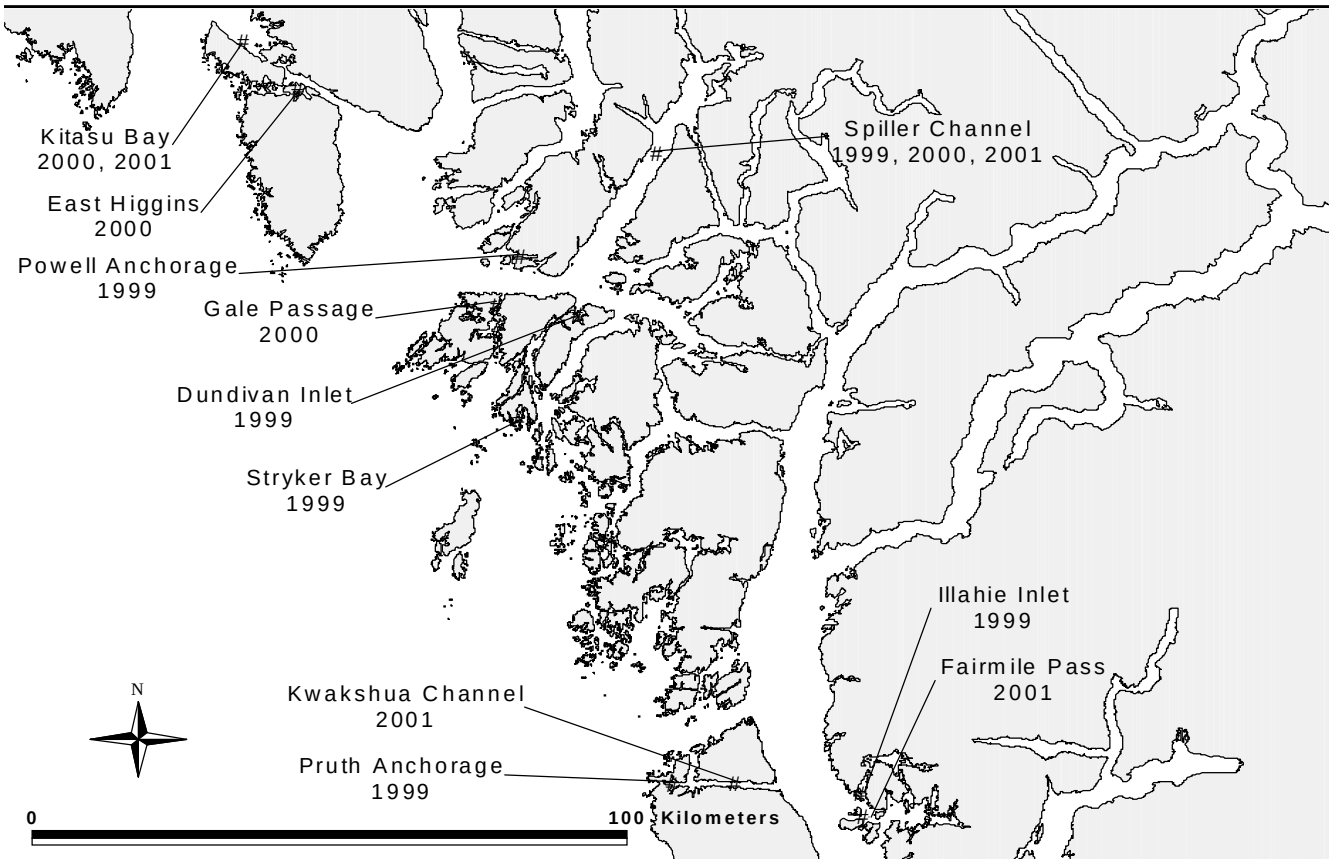

Figure 3: Sampling locations for genetic analysis from 1999 - 2002 in the Central Coast herring assessment region.

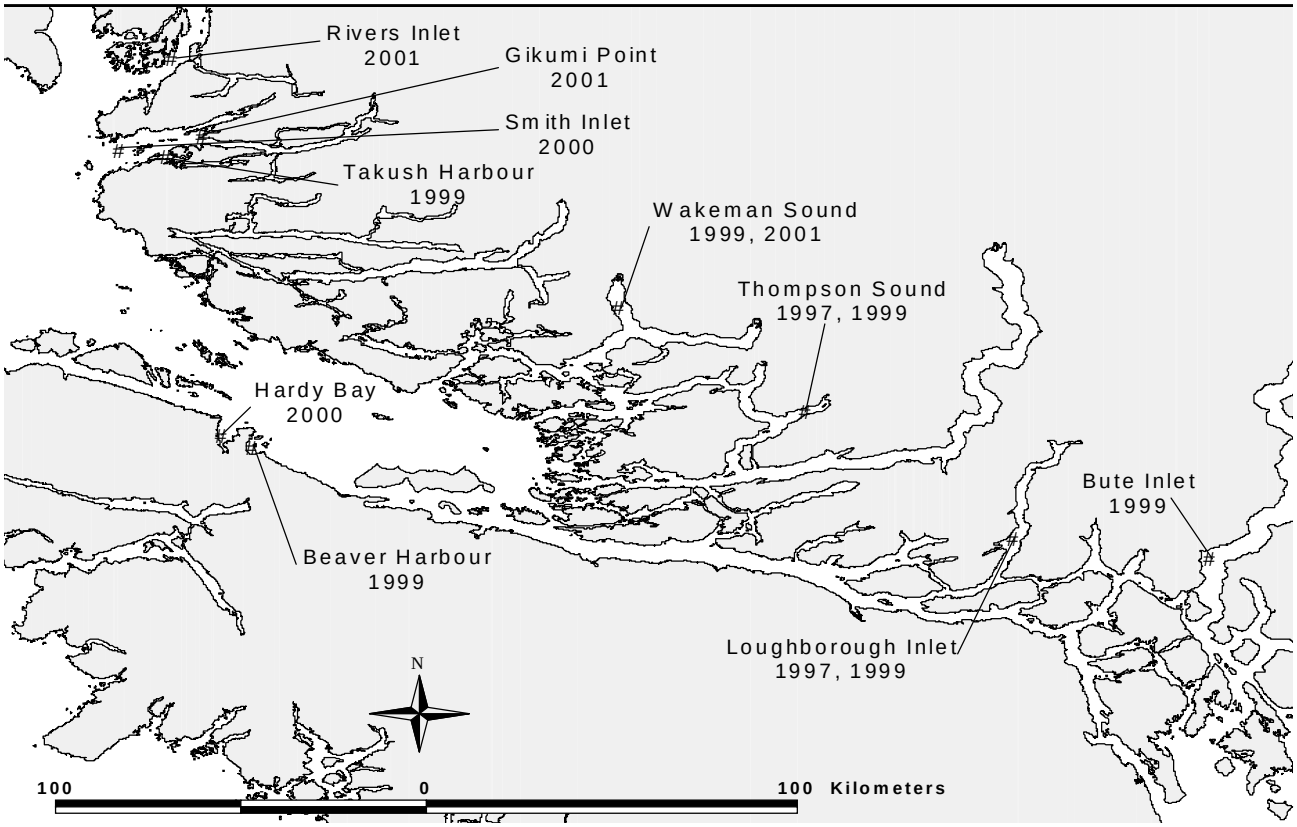

Figure 4: Sampling locations for genetic analysis from 1997 - 2002 in the Johnstone Strait region.

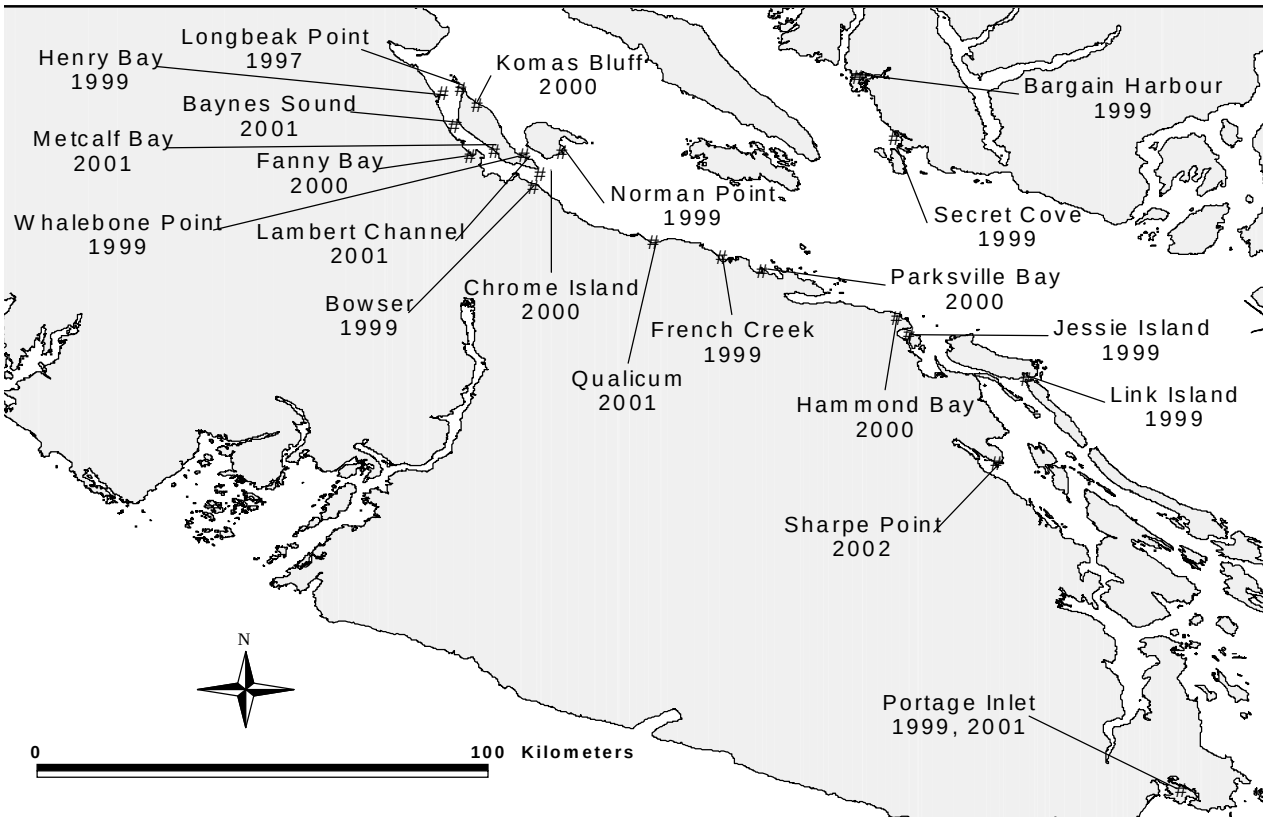

Figure 5: Sampling locations for genetic analysis from 1997 - 2002 in the Strait of Georgia herring assessment region.

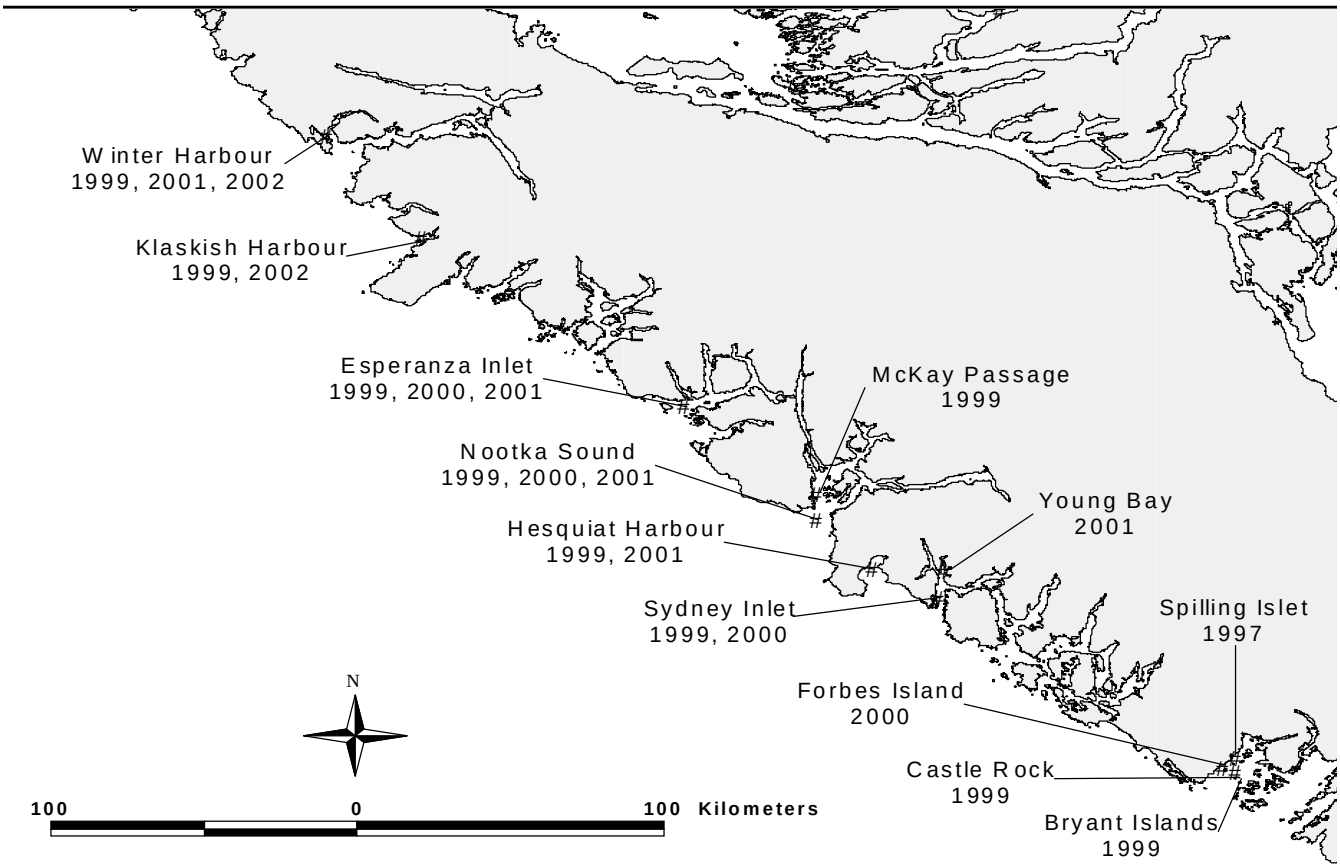

Figure 6: Sampling locations for genetic analysis from 1997 - 2002 in the west coast of Vancouver Island herring assessment region.

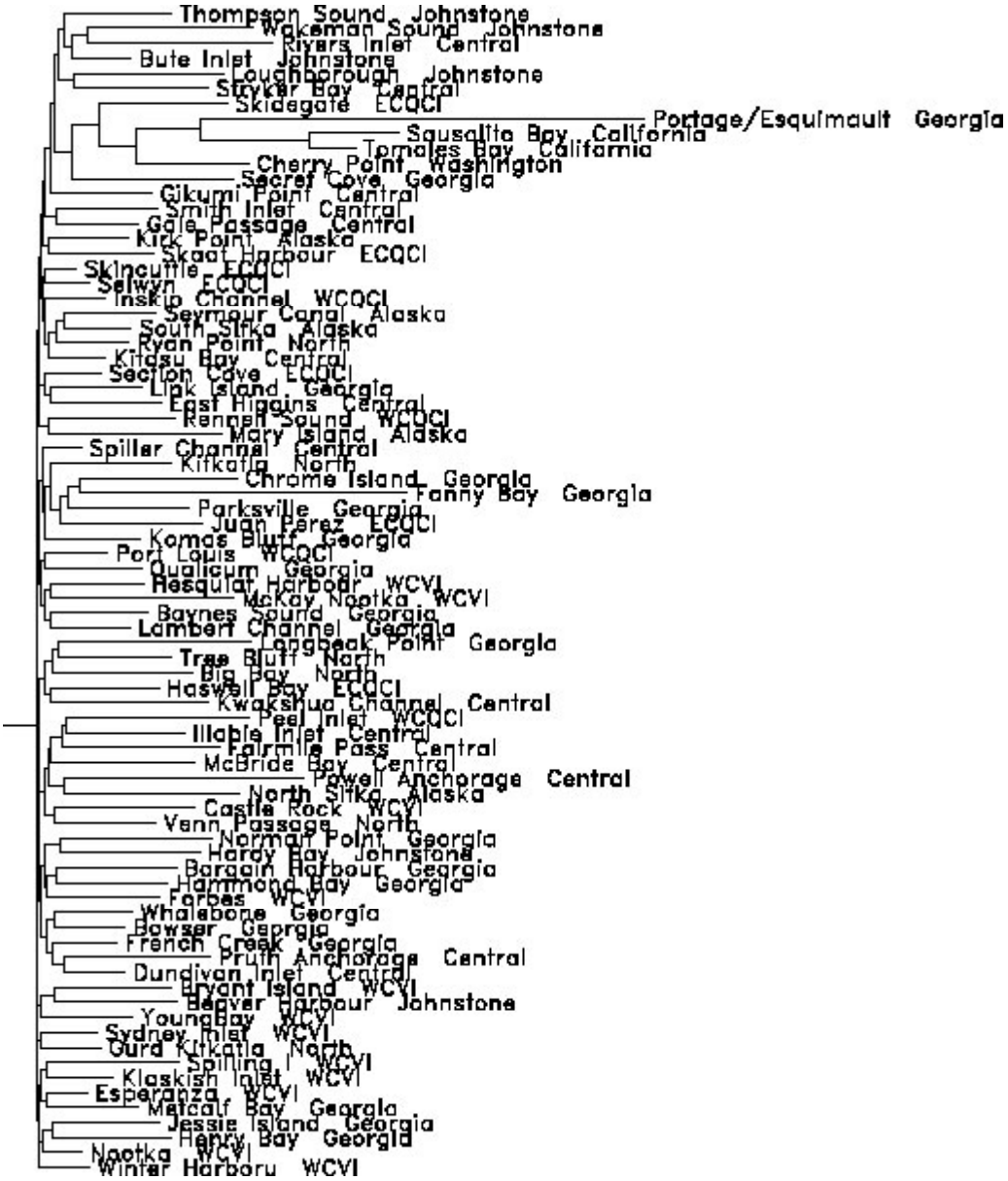

Figure 7. Neighbor-joining dendrogram based on Cavalli-Sforza and Edwards (1967) chord distance for herring from 78 locations in southeast Alaska, British Columbia, Washington, and California. Only locations in which data from all 13 loci were available at the time of the analysis were included in the analysis.