ORIGINAL ARTICLE

Temporally stable genetic structure of heavily exploited Atlantic herring (*Clupea harengus*) in Swedish waters

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Information on the temporal stability of genetic structures is important to permit detection of changes that can constitute threats to biological resources. Large-scale harvesting operations are known to potentially alter the composition and reduce the variability of populations, and Atlantic herring (*Clupea harengus*) has a long history of heavy exploitation. In the Baltic Sea and Skagerrak waters, the census population sizes have declined by 35–50% over the last three decades. We compared the genetic structure of Atlantic herring in these waters sampled at least two different times between 1979 and 2003 by assaying 11 allozyme and nine microsatellite loci. We cannot detect any changes in the amount of

genetic variation or spatial structure, and differentiation is weak with overall $F_{ST} = 0.003$ among localities for the older samples and $F_{ST} = 0.002$ for the newer ones. There are indications of temporal allele frequency changes, particularly in one of five sampling localities that is reflected in a relatively small local N_e estimate of c. 400. The previously identified influence of selection at the microsatellite locus *Cpa112* remains stable over the 24-year period studied here. Despite little genetic differentiation, migration among localities appears small enough to permit demographic independence between populations.

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Introduction

The ability to detect genetic differences in time and space is critical in species management and in the quest of conserving genetic variation. Biological diversity needs to be monitored over time to permit detection of changes that can constitute threats to biological resources. Conservation genetic monitoring is particularly warranted for species subject to large-scale harvesting since such operations are known to potentially alter the genetic composition and reduce the variability of populations (Conover and Munch, 2002; Laikre *et al.*, 2008). Aquatic organisms are the only major food resource of humans that is primarily harvested directly from wild populations (Ryman *et al.*, 1995), and sustainability may be compromised if population differences are overlooked by management (Ruzzante *et al.*, 2006).

Large-scale harvest operations have been demonstrated to cause genetic effects through selective removal of genotypes (Allendorf *et al.*, 2008). Over-harvest may result in extinction of local gene pools, or may reduce the genetically effective population size (N_e) through manipulation of the demographic characteristics of populations

(Ryman *et al.*, 1981; Laikre and Ryman, 1996). Reductions of N_e will result in elevated rates of genetic diversity loss.

N_e can be estimated by examining temporal changes in allele frequencies. Surprisingly small N_e estimates have been reported for some marine fish populations where the census population sizes (N) are three to five orders of magnitude larger (Hauser and Carvalho, 2008). The reasons for such low N_e compared with N have been suggested to be caused by (i) a large variance of family reproductive success ('sweepstake' recruitment; Hedgecock, 1994), (ii) high fishing pressure leading to genetic bottlenecks (Hauser *et al.*, 2002; Hutchinson *et al.*, 2003; Poulsen *et al.*, 2006), (iii) unequal sex ratios (Frankham, 1995), or (iv) genetic differences between undetected subpopulations (Palstra and Ruzzante, 2008). Empirical estimates of N_e for commercially exploited marine fish range between 560–19 535 (mean $N_e = 2085$, s.d. = 322; Palstra and Ruzzante, 2008).

The heavily exploited Atlantic herring (*Clupea harengus*) is one of the economically most important marine species. In this study, we investigate the temporal genetic stability of herring in the Skagerrak and the Baltic Sea (including the Bothnian Sea and the Bothnian Bay). Here, the estimated numbers of fish age one and older have decreased by 50% in the Bothnian Bay, increased by 290% in the Bothnian Sea, and decreased by 35% in the eastern central Baltic Sea during the past three decades (Figure 1; based on 5-year averages of the oldest compared with the most recent censuses by the Swedish National Board of Fisheries). There are no specific censuses for local

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Figure 1 Estimated population census sizes for ICES subdivision 31 (Bothnian Bay), subdivision 30 (Bothnian Sea), subdivision 25–29 (Baltic Sea), and subdivision 22–24 (Western Baltic). Source: Swedish National Board of Fisheries (*cf.* ICES subdivisions: <http://www.ices.dk/aboutus/icesareas.asp>).

Skagerrak stocks—management is merged with the western Baltic herring that feed in the Skagerrak and the census population size has decreased by 50% for this management unit since 1991 (Figure 1). Ryman *et al.* (1984) performed the first spatial genetic analysis of Atlantic herring in the North and Baltic Seas and the northeast Atlantic using allozymes. They found statistically significant allele frequency heterogeneities, but the amount of genetic differentiation was conspicuously small. Microsatellites give a consistent pattern; Larsson *et al.* (2007) showed that, with the exception for a single microsatellite locus, allozymes and microsatellites provide largely concordant information with respect to spatial genetic differentiation of Atlantic herring in this region, confirming the markedly low levels of spatial differentiation.

Jørgensen *et al.* (2005, 2008) used microsatellite loci to investigate herring structure in the Baltic Sea and observed significant associations between genetic differentiation and salinity and surface temperature. Their analyses included the outlier locus that was found to be influenced by selection by Larsson *et al.* (2007) and it is not clear to what extent their observed pattern is caused by this outlier.

The scope of this study is to examine the possible changes in genetic variation occurring over the past couple of decades among Atlantic herring populations in

the Baltic Sea and Skagerrak. We want to determine: (i) whether there is a stable spatial pattern over the time period analyzed, (ii) whether the amount of genetic variation has changed, (iii) whether the spatio-temporal pattern differs for the locus influenced by selection as compared to the others, and (iv) what the effective population sizes are. Previously, short-term temporal stability over two consecutive sampling years have been reported in herring samples from the North Sea, Skagerrak, Kattegat, and western Baltic (Bekkevold *et al.*, 2005; Mariani *et al.*, 2005), but no long-term genetic study has so far been conducted for this species and estimates of effective sizes are lacking.

Materials and methods

We analyzed and compared patterns of variation for a total of 11 allozyme and nine microsatellite loci for Atlantic herring samples collected in 1979/1980 and in 2002/2003. The genetic analysis includes 546 Atlantic herring collected during 1979 or 1980 and 1237 herring collected 2002 and/or 2003 at two locations in the Skagerrak, and three locations in the Baltic Sea (Figure 2; geographic coordinates are given in Table 2). Parts of the data represent material previously genotyped and published (*cf.* Table 1). The additional samples were genotyped using the same procedures as in our previous

studies (Ryman *et al.*, 1984; Bekkevold *et al.*, 2005; Larsson *et al.*, 2007). Tissue samples of herring scored for allozymes by Ryman *et al.* (1984) were retained, thus

Figure 2 Geographic location of sampling sites.

permitting scoring of microsatellite loci for the same individuals.

The following 11 allozyme loci were scored, where the nomenclature follows Shaklee *et al.* (1990), and the old locus abbreviation (to allow comparisons with results of previous studies), enzyme name, and EC number are given in parenthesis: *mAAT** (AAT-2, aspartate aminotransferase, EC 2.6.1.1), *G3PDH** (GPD-1, glycerol-3-phosphate dehydrogenase, EC 1.1.1.8), *GPI** (glucosephosphate isomerase, EC 5.3.1.9), *mIDHP** (IDH-2, isocitrate dehydrogenase, EC 1.1.1.42), *LDH-1**, *LDH-2** (lactate dehydrogenase, EC 1.1.1.27), *MDH-4** (malate dehydrogenase, EC 1.1.1.37), *sMEP** (ME-1, malic enzyme, EC 1.1.1.40), *mMEP** (ME-2, malic enzyme, EC 1.1.1.40), *PGM-1** (phosphoglucumutase, EC 5.4.2.2, formerly EC 2.7.5.1) and *SOD** (superoxide dismutase, EC 1.15.1.1). Scoring consistency with old samples was ensured by measuring the relative gel migration distances, comparing them with protocols and photographic documentation, and consulting researchers with expertise from the assays in 1979 and 1980. All these loci have earlier been shown variable in herring from this region (Andersson *et al.*, 1981; Ryman *et al.*, 1984; Larsson *et al.*, 2007).

Nine tetranucleotide microsatellite loci were scored: *Cha1017*, *Cha1020*, *Cha1027*, *Cha1202* (McPherson *et al.*, 2001), *Cpa101*, *Cpa111*, *Cpa112*, *Cpa113* and *Cpa114* (Olsen *et al.*, 2002). Scoring consistency among runs was ensured by analyzing two heterozygote control individuals

Table 1 Summary statistics by sampling locality and year for 11 allozyme and 9 microsatellite loci (*cf.* Table 2)

Region	Locality/Year	Allozymes						Microsatellites					
		n	H _T	A	AR	PA	F _{IS}	n	H _T	A	AR	PA	F _{IS}
Skagerrak	Flatbotten 58°20'N/11°15'E												
	1979	100 (60) ^a	0.129	25	2.13	1	0.006	96 (91)	0.834	149	16.10	3	0.043***
	2002	100 (99)	0.133	26	2.16		−0.019	100 (96) ^b	0.833	147	15.72	5	0.030**
	2003	120 (120)	0.133	29	2.26	2	0.051	100 (96) ^b	0.835	157	16.62	12	0.026*
Skagerrak	Måseskär 58°05'N/11°19'E												
	1979	100 (60) ^a	0.126	27	2.17		0.027	99 (86)	0.836	146	15.74	3	0.026*
	2002	100 (98)	0.132	27	2.19		−0.013	100 (93) ^b	0.841	149	15.82	5	0.045***
	2003	120 (118) ^c	0.117	28	2.15		0.048	100 (83) ^{b,c}	0.835	153	16.36	5	0.016
Baltic Sea	Himmerfjärden 59°04'N/17°45'E												
	1979/1980	82 (47) ^a	0.122	25	2.09	1	0.065	98 (63)	0.816	148	16.06	7	0.042**
	2002	100 (98)	0.129	28	2.08	1	−0.018	0	—	—	—	—	—
	2003	277 (268) ^c	0.123	34	2.19	1	−0.084***	100 (93) ^c	0.826	145	15.38	5	0.013
Baltic Sea	Kalix 65°48'N/22°43'E												
	1980	100 (100) ^a	0.119	27	2.06		−0.034	100 (85)	0.835	147	15.68	4	−0.002
	2002	100 (100)	0.121	25	1.99		−0.016	100 (96)	0.830	150	15.98	3	0.038**
	2003	120 (119) ^c	0.122	29	2.14	1	0.078*	96 (71) ^c	0.827	150	16.17	7	0.023*
Baltic Sea	Vaxholm 59°26'N/18°18'E												
	1979	100 (32) ^a	0.123	27	2.24		−0.066	100 (62)	0.814	137	14.79	4	0.009
	2002	100 (99)	0.131	31	2.37	1	0.030	0	—	—	—	—	—
	2003	100 (99)	0.130	26	2.15		0.015	96 (78)	0.813	148	15.88	9	0.020
Total		1719 (1517)	0.126	44	2.16	8	0.005	1285 (1093)	0.830	272	15.87	72	0.025***

Abbreviations: A, number of alleles; AR, mean allelic richness; H_T, gene diversity; n, number of fish (number of complete genotypes scored in parenthesis); PA, number of private alleles; F_{IS} quantifies the deviation from Hardy–Weinberg proportions (Weir and Cockerham, 1984).

Genotypes previously used by

^aRyman *et al.*, 1984.

^bBekkevold *et al.*, 2005.

^cLarsson *et al.*, 2007.

*P < 0.05; **P < 0.01; ***P < 0.001.

spanning the anticipated allelic ranges on all gels, in addition to internal and external size ladders (Mariani *et al.*, 2005). Reliability was also checked by running MICRO-CHECKER 2.2.1 (van Oosterhout *et al.*, 2004) to test for potential stuttering, large allele drop-out, or presence of null alleles.

Statistical treatment

GENEPOP version 3.4 (Raymond and Rousset, 1995) was used for calculating F -statistics (Weir and Cockerham, 1984). FSTAT version 2.9.3.2 (Goudet, 1995, 2001) was used for calculating allelic richness, testing Hardy–Weinberg proportions, and comparing pairwise genetic differences (using the program options of 5000 randomizations and not assuming Hardy–Weinberg proportions within samples). Significances of genetic differentiation were also tested using GENEPOP version 3.4 (Raymond and Rousset, 1995) and CHIFISH version 1.3 (Ryman, 2006). All these testing approaches provided a high degree of consistency, and for brevity we only report the results from FSTAT.

GDA version 1.1 (Lewis and Zaykin, 2001) was applied to examine linkage disequilibria. Differences between older and more recent samples with respect to allelic richness as well as gene diversity were tested using both the paired t -test and the non-parametric Wilcoxon rank sum test. We used BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996) applying the stepwise mutation model and the two-phase model for microsatellite loci (excluding *Cpa112* which is an outlier), and the infinite allele model for allozyme loci, with 10 000 replications to test for indications of bottlenecks. The two-phase model was run with 95% single-step and 5% multi-step mutations (*cf.* Piry *et al.*, 1999). Wilcoxon one-tailed tests were done to test for heterozygote excess.

The presence of loci potentially affected by selection ('outliers') was investigated with the test of Beaumont and Nichols (1996), where the null distribution was created using FDIST2 (Beaumont, 2002). Concordance in F_{ST} estimates among samples taken at different times was investigated using paired t -tests and pairwise correlation plots.

If effects of geographic separation and time are acting simultaneously, these two factors are not properly estimated with conventional gene diversity analyses. To evaluate possible interaction between them, a two-factor variance analysis is a more proper design (rather than multiple hierarchical analyses). We investigated the significance of geographical region (where the two regions consist of the Baltic Sea and Skagerrak) and time separation as well as the interaction between them using an orthogonal modification of Nei's genetic diversity analysis with a modified analysis of variance (*cf.* Johannesson and Tatarenkov, 1997). The overall G_{ST} is divided into the following components: $G_{\text{regiontotal}}$ variation among regions; $G_{\text{timetotal}}$ variation among time periods; $G_{\text{timexregion}}$ variation due to an interaction between regions and time; $G_{\text{stimerregion}}$ average variation among samples within combinations of regions and time period (residual). The samples from the locality Himmerfjärden were excluded to provide a balanced number of samples from each region. Multiple samples from the same locality 2002/2003 were pooled.

The variance effective population size (N_e) was assessed with TEMPOFS and the unbiased estimator F_S using sample plan II, as the individuals were sampled destructively before reproduction and not returned to the populations (Jorde and Ryman, 2007). The assessment was done in two ways; first by comparing allele frequencies in the samples separated by 22–24 years ('long-term N_e '), which corresponds to approximately five herring generations. (Bias due to overlapping generations is expected to be minimized when temporal samples are spaced more than four generations apart (Waples and Yokota, 2007).) Second, we used allele frequency differences between consecutive cohorts and corrected this 'short-term N_e ' by multiplying with the ratio C (a correction term obtained from life table data) divided by G (generation time) according to Jorde and Ryman (1995). C and G were calculated from stock and gamete production estimates in Arrhenius and Hansson (1993). $G = 5.25$ for all samples, $C = 11.74$ for Flatbrotten and Måseskär, 15.21 for Himmerfjärden and Vaxholm, and 16.09 for Kalix. Only cohorts in which 20 aged individuals or more had been genotyped were included when estimating N_e with the Jorde–Ryman method (1995). The cohort sample sizes and statistics are summarized in Appendix 1.

Short-term N_e was further estimated with the linkage disequilibrium method of Waples (2006) using LDNE (Waples and Do, 2008). Alleles occurring in frequencies of $<2\%$ were pooled as rare alleles may otherwise increase bias (Waples, 2006). Confidence intervals were estimated with the jackknife method.

We also estimated long-term N_e with a likelihood method using MLNE version 2.0 (Wang and Whitlock, 2003), both assuming a migration rate m of 0 (Wang, 2001) and jointly estimating N_e and m (Wang and Whitlock, 2003). When jointly estimating N_e and m with this method, the source population of the immigrants is assumed to be an infinitely large population with constant allele frequencies. The allele frequencies of the source population were obtained by pooling all samples from 1979/1980 and 2002/2003 except from the population for which N_e was to be estimated. The likelihood methods require a user specified upper limit for N_e , which we set at 35 000 after checking that similar results were obtained for limits in the range 5000–35 000 (the upper bound was limited by computer capacity). Finally, we estimated N_e using Nei and Tajima's (1981) estimator F_C also using MLNE.

Results

A total of 44 alleles were observed for the allozymes and 272 for the microsatellites (Table 1), corresponding to averages of 4.0 and 30.2 alleles per locus for the two groups of loci, respectively. Missing genotypes accounted for 1.5% of the allozyme data and 3.3% of the microsatellite data distributed over all loci, likely due to the original quality of samples; retrospectively omitting individuals with one or more missing genotypes did not change the results. Expected heterozygosities were in the range 0.117–0.133 (allozymes) and 0.813–0.841 (microsatellites; Table 1). Gene diversity and allelic richness have not changed significantly over the time period examined.

Table 2 Summary statistics for allozyme and microsatellite loci covering the five localities sampled 1979/1980 and 2002/2003 (multiple samples pooled by locality)

Locus	Old samples—1979/1980							New samples—2002/2003						
	n	H _T	A	AR	F _{IS}	F _{ST}	F _{IT}	n	H _T	A	AR	F _{IS}	F _{ST}	F _{IT}
<i>Allozymes</i>														
<i>mAAT*</i>	482	0.029	2	1.80	−0.012	−0.003	−0.014	1232	0.035	4	1.87	−0.018	0.001	−0.018
<i>G3PDH*</i>	419	0.008	4	1.38	−0.003	0.001	−0.002	1237	0.011	4	1.41	−0.005	0.001*	−0.004
<i>GPI*</i>	482	0.535	7	5.12	−0.027	−0.001	−0.027	1235	0.539	6	4.90	0.002	0.000	0.002
<i>mIDHP*</i>	447	0.035	3	1.77	−0.023	0.007	−0.016	1230	0.037	4	2.16	−0.014	0.002	−0.013
<i>LDH-1*</i>	362	0.062	2	1.98	−0.038	0.004	−0.034	1237	0.073	3	2.20	−0.016	0.000	−0.015
<i>LDH-2*</i>	482	0.058	2	1.87	−0.038	0.010**	−0.028	1237	0.042	2	1.83	−0.025	0.005*	−0.020
<i>MDH-4*</i>	482	0.498	3	2.11	0.031	−0.002	0.029	1233	0.496	2	2.00	−0.004	0.002	−0.003
<i>sMEP*</i>	482	0.011	2	1.33	−0.009	0.006	−0.003	1237	0.006	4	1.26	−0.003	0.001*	−0.002
<i>mMEP*</i>	482	0.021	4	1.85	−0.006	−0.001	−0.007	1236	0.029	4	2.08	−0.009	0.000	−0.010
<i>PGM-1*</i>	481	0.074	3	2.60	0.026	−0.002	0.024	1235	0.101	6	2.59	0.018	0.001	0.018
<i>SOD*</i>	447	0.032	2	1.70	−0.019	0.003	−0.017	1234	0.024	3	1.65	−0.021	0.014***	−0.006
Total/Mean	459	0.124	34	2.14	−0.002	0.000	−0.002	1235	0.127	42	2.18	−0.003	0.001***	−0.001
<i>Microsatellites</i>														
<i>Cha1017</i>	471	0.820	13	9.39	0.076***	0.000	0.076***	782	0.814	15	9.22	0.071***	0.004*	0.074***
<i>Cha1020</i>	471	0.914	28	16.44	−0.013	0.006	−0.007	776	0.919	36	17.60	0.014	0.003***	0.017
<i>Cha1027</i>	471	0.937	28	19.63	0.051***	0.008***	0.059***	778	0.934	32	19.07	0.045***	0.001**	0.046***
<i>Cha1202</i>	482	0.778	14	10.10	−0.022	0.001	−0.022	779	0.761	17	9.74	−0.014	0.000	−0.014
<i>Cpa101</i>	473	0.914	27	17.25	0.017	0.003	0.020	773	0.917	35	17.50	0.016	0.001*	0.017*
<i>Cpa111</i>	464	0.413	8	5.60	−0.046	0.010***	−0.036	778	0.440	16	6.45	0.035	0.001***	0.036
<i>Cpa112</i>	462	0.858	35	15.82	0.061***	0.035***	0.094***	764	0.853	42	16.36	0.031*	0.036***	0.066***
<i>Cpa113</i>	471	0.933	22	17.19	0.015	0.003***	0.018	781	0.934	28	17.49	0.027**	0.004***	0.031***
<i>Cpa114</i>	472	0.917	27	16.63	0.034*	0.000	0.034**	777	0.917	28	16.23	0.020*	0.000	0.020*
Total/Mean	471	0.832	202	14.23	0.023***	0.007***	0.030***	776	0.832	249	14.40	0.027***	0.006***	0.033***
All microsatellites except <i>Cpa112</i>	472	0.828	167	14.03	0.018**	0.003***	0.022***	778	0.829	207	14.16	0.027***	0.002***	0.028***
All allozymes and microsatellites except <i>Cpa112</i>	464	0.420	201	7.14	0.015**	0.003***	0.018***	1042	0.423	249	7.22	0.020***	0.002***	0.022***

Abbreviations: A, number of alleles; AR, mean allelic richness; H_T, total gene diversity; n, number of individuals genotyped.

F_{ST} represents the fixation index quantifying genetic differentiation over the sampling localities at the two time periods. F_{IT} and F_{IS} measure the degree of deviation from Hardy–Weinberg expectations in the total material and within localities, respectively (Weir and Cockerham, 1984).

*P < 0.05; **P < 0.01; ***P < 0.001.

Conformance to Hardy–Weinberg expectations

When considering each locus–location combination separately only one of 128 allozyme F_{IS} values indicated significant deviation from Hardy–Weinberg expectations, and it did not remain significant after applying the Bonferroni correction (critical P = 0.05/128 = 0.0004). For the microsatellites, 20 of 117 locus/locality F_{IS} values showed significance, but they did not persist after Bonferroni correction (critical P = 0.05/117 = 0.0004). When pooling the information from multiple loci and/or samples, there is a significant heterozygote deficiency at the microsatellites (F_{IS} = 0.025, P < 0.001), but not at the allozymes (F_{IS} = 0.005; Table 1). There was no evidence for large allele drop-out or stuttering for any microsatellite locus according to the MICRO-CHECKER results. The heterozygote deficiencies were most prevalent for *Cha1017* and *Cha1027*; the locus specificity indicates possible presence of null alleles rather than a Wahlund effect. These two loci do not appear to be outliers in other respects and omitting them did not change estimates of differentiation (F_{ST}).

Testing for linkage disequilibrium, bottleneck, and selection

There are no apparent indications of linkage disequilibrium. There are a total of 2580 pairwise locus combinations (20 loci) within localities. Of these, 78 showed

significant deviation from linkage equilibrium (c. 3% of the tests) when accounting for potential within locus Hardy–Weinberg deviations. None of the significances remained after Bonferroni correction. There are no indications of bottlenecks in any of the samples for neither allozymes (0.90 < P < 0.98) nor microsatellites (two-phase model: 0.37 < P < 0.90; stepwise mutation model: 0.63 < P < 0.99).

The outlier analysis using the method of Beaumont and Nichols (1996) identifies the microsatellite locus *Cpa112* as an outlier whereas the other loci show no indication of selection influence. This outlier locus has a conspicuously higher F_{ST} than other loci, indicating divergent selection, concurring with the observation by Larsson *et al.* (2007). Two alleles, *Cpa112*(306) and *Cpa112*(344), exhibit higher allele frequencies in regions with lower salinity. This is seen in the older samples as well as in the newer ones. As *Cpa112* appears to be influenced by selection, this locus is excluded in some of the subsequent analyses.

Spatial and temporal genetic structure

Overall, there are no large genetic heterogeneities. Although weak, there is a significant spatial genetic difference both in the older and the newer samples (Table 2). F_{ST} over all the 19 loci (excluding *Cpa112*) and five localities is 0.003 (95% CI = 0.001–0.005) for the old

Table 3 Significance levels of temporal changes in Atlantic herring samples based on 19 loci (the outlier *Cpa112* excluded)

Locality	Temporal changes			
	Long term 1979/1980–2002/2003	Short term 2002–2003	Between cohorts ^a	
			Old cohorts	New cohorts
Flatbrotten	NS	*	**	***
Måseskär	***	NS	NS	NS
Skagerrak samples	***	*	**	***
Himmerfjärden ^b	NS	NS	—	NS
Vaxholm ^b	***	NS	NS	NS
Kalix	NS	NS	—	NS
Baltic samples	NS	NS	NS	***

Abbreviation: NS, non-significant.

^aTesting among present cohorts, number of cohorts vary between localities (cf. Appendix 1).^bSample from 2002 was only genotyped for allozymes.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.**Table 4** Two-factor variance analysis (orthogonal design) of gene diversity at 19 loci (excluding *Cpa112*) for eight samples of Atlantic herring from two time periods distributed over four localities and two regions (Baltic Sea and Skagerrak)

Locus	n	H _S	H _T	G _{ST}	G _{regiontotal}	G _{timetotal}	G _{timexregion}	G _{stimerregion}
<i>mAAT</i> *	100–220	0.030	0.030	0.0023	0.0016*	0.0001	0.0001	0.0006
<i>G3PDH</i> *	72–220	0.010	0.010	0.0043	0.0014	0.0001	0.0005	0.0024
<i>GPI</i> *	100–220	0.537	0.538	0.0032*	0.0005	0.0001	0.0009	0.0018
<i>mIDHP</i> *	100–220	0.042	0.042	0.0052 [†]	0.0001	0.0003	0.0004	0.0044
<i>LDH-1</i> *	60–220	0.058	0.058	0.0034	0.0007	0.0008	0.0000	0.0019
<i>LDH-2</i> *	100–220	0.053	0.054	0.0104**	0.0063*	0.0008	0.0001	0.0031
<i>MDH-4</i> *	100–220	0.495	0.497	0.0037	0.0021 [†]	0.0000	0.0001	0.0015
<i>sMEP</i> *	100–220	0.007	0.007	0.0081 [†]	0.0019	0.0002	0.0005	0.0055
<i>mMEP</i> *	100–220	0.023	0.023	0.0026	0.0004	0.0007	0.0001	0.0015
<i>PGM-1</i> *	100–220	0.089	0.090	0.0040*	0.0000	0.0019 [†]	0.0006	0.0014
<i>SOD</i> *	100–220	0.033	0.034	0.0087**	0.0051*	0.0009	0.0002	0.0025
<i>Cha1017</i>	93–199	0.811	0.816	0.0064 [†]	0.0028	0.0002	0.0007	0.0028
<i>Cha1020</i>	89–200	0.908	0.915	0.0077***	0.0034	0.0004	0.0006	0.0033
<i>Cha1027</i>	94–200	0.929	0.937	0.0084***	0.0021	0.0008	0.0016	0.0039
<i>Cha1202</i>	92–199	0.764	0.768	0.0045**	0.0021 [†]	0.0006	0.0002	0.0018
<i>Cpa101</i>	89–199	0.912	0.917	0.0051 [†]	0.0011	0.0004	0.0010	0.0025
<i>Cpa111</i>	93–200	0.430	0.433	0.0080***	0.0004	0.0006	0.0012	0.0058
<i>Cpa113</i>	95–200	0.926	0.932	0.0062***	0.0026	0.0003	0.0008	0.0024
<i>Cpa114</i>	90–197	0.913	0.917	0.0045	0.0003	0.0006	0.0006	0.0030
Average		0.420	0.422	0.0059***	0.0019*	0.0004	0.0008	0.0028

Abbreviations: G_{ST}, overall variation among samples; H_S, average heterozygosity within samples; H_T, total heterozygosity; n, sample sizes. G_{ST} was further divided into the following parameters: G_{regiontotal} variation among regions; G_{timetotal} variation among time periods; G_{timexregion} variation due to an interaction between regions and time; G_{stimerregion} average variation among samples within combinations of regions and time period (residual). For further details see text and Johannesson and Tatarenkov (1997).

[†]0.05 < P < 0.1; * P < 0.05; ** P < 0.01; *** P < 0.001.

samples and 0.002 (95% CI = 0.001–0.003) for the new ones, and both are highly significant. At both occasions is the major part of the divergence due to differences between the Baltic and the Skagerrak where multilocus F_{ST} is 0.003 (old samples) and 0.002 (new samples) as well. Within the Skagerrak, we find an F_{ST} = 0.001, which is significant in the new samples (uncorrected P = 0.003) but not in the old ones. There is spatial genetic heterogeneity among the three localities within the Baltic in 1979/1980 (multilocus F_{ST} = 0.002***) and 2002/2003 (multilocus F_{ST} = 0.001***). Overall pairwise multilocus F_{ST} s ranged between 0–0.006 for the 1979/1980 samples and between 0–0.008 for the 2002/2003 samples.

With respect to temporal heterogeneity, there are both short-term and long-term differences at some localities (Table 3, Appendix 1). Multilocus F_{ST} ranged between 0–0.004 over time within the localities. The samples from Flatbrotten 2002 vs 2003 show a significant short-term

temporal shift (F_{ST} = 0.001, P = 0.036). However, this low significance level warrants dismissal of the difference if a Bonferroni correction is applied. Long-term temporal changes were found when comparing the old samples with newer ones both at Måseskär (F_{ST} = 0.002, P < 0.001) and Vaxholm (F_{ST} = 0.004, P < 0.001). There is an overall long-term difference when χ^2 test results for temporal heterogeneity at each locality are combined (P = 0.002; the outlier *Cpa112* excluded; Ryman *et al.*, 2006). Significant differences between consecutive cohorts were only observed at Flatbrotten (old cohorts F_{ST} = 0.005, P = 0.002; new cohorts F_{ST} = 0.003, P < 0.001).

In the orthogonal analysis of variance, there is no interaction between the two factors time and region, nor is there a significant effect of time (Table 4). The only significant orthogonal component is the variation among regions (G_{regiontotal} = 0.0019; multilocus P = 0.014) that explains 32% of the overall genetic variation among

Figure 3 Pairwise multilocus F_{ST} estimates for the old samples compared with the pairwise multilocus F_{ST} estimates in the new samples. When *Cpa112* is included (filled circles) $r^2 = 0.91$ ($P < 0.001$), with *Cpa112* excluded (open circles) $r^2 = 0.65$ ($P = 0.005$).

samples ($G_{ST} = 0.0059$). The variation among the time periods is concordant for microsatellites and allozymes and only explains 6.8% (non-significant).

The spatial structure does not appear to have changed over the time interval examined, and the similarity is particularly obvious at *Cpa112* (Figure 3). There is a strong and highly significant positive correlation between the 10 pairwise F_{ST} s among localities of old and new samples ($r^2 = 0.91$, $P < 0.001$). *Cpa112* contributes primarily to this correlation computed over all loci, but the effect remains also when excluding this outlier locus ($r^2 = 0.65$, $P = 0.005$).

Estimates of N_e

Under the assumption of $m = 0$, the observation of a significant temporal allele frequency change is necessary to obtain an estimate of effective size with a finite upper confidence limit. The short-term temporal shift at Flatbrotten is significant, as are the long-term changes at Måseskär and Vaxholm (Table 3), whereas no temporal changes can be observed at Himmerfjärden or Kalix. Pooling samples within the Baltic and Skagerrak separately yielded significant temporal changes in Skagerrak but only short-term temporal change between the newer cohorts in the Baltic.

The short-term N_e estimate for Flatbrotten yields $N_e = 138$ (95% CI = 63–infinity) based on consecutive cohorts sampled in 1979 and $N_e = 356$ (95% CI = 178–infinity) based on cohorts sampled in 2002 and 2003 (Table 5). The linkage disequilibrium method estimates somewhat higher although similar effective population sizes. The samples retrieved from Skagerrak in 2002 and 2003 yield an estimated short-term $N_e = 1806$ (95% CI = 981–7977) with the linkage disequilibrium method (Table 5).

On the basis of the long-term allele frequency change with the unbiased estimator F_S , the N_e estimate for Vaxholm is 419 (95% CI = 275–879) and the corresponding estimate for Måseskär is 630 with an upper limit of infinity (Table 6). The lower confidence limits for the other localities are approximately in the low thousands. N_e point estimates obtained with the F_C estimator are alike the ones with F_S . The likelihood method results in very similar N_e estimates, when assuming $m = 0$, as the other approaches based on long-term shifts (Table 6). The long-term N_e estimate for Vaxholm is 711 (95% CI = 380–2418), and for Måseskär 1552 (95% CI = 755–11 691). For the other localities, the 95% upper bound is greater than the limit imposed on this method.

When jointly estimating N_e and m , the observed allele frequency change is assumed to reflect the effects of both genetic drift and migration, resulting in lower estimates of N_e . When using this approach, an upper confidence limit is obtained for all samples, with the lowest estimate at Vaxholm ($N_e = 276$, 95% CI = 155–556). Vaxholm and Måseskär also have the highest migration rate estimates of 3–5%.

Discussion

Although Baltic Sea and Skagerrak herring is heavily fished, we cannot see indications of major changes in the spatial genetic structure or amount of genetic diversity in these regions between 1979/1980 and 2002/2003. Spatial genetic structure remains weak with overall $F_{ST} = 0.003$ (95% CI = 0.001–0.005) among locations for the older samples and $F_{ST} = 0.002$ (95% CI = 0.001–0.003) for the newer ones. The major component of this divergence refers to differences between the two major regions Baltic vs Skagerrak in line with earlier

Table 5 Estimates of short-term effective population size (N_e) for Atlantic herring using (i) the temporal method accounting for overlapping generations and (ii) the linkage disequilibrium method

Locality	Sample year(s)	Consecutive cohorts (Jorde and Ryman, 1995, 2007) N_e	Linkage disequilibrium (Waples, 2006) N_e
Flatbrotten	1979	138 (63– ∞)	716 (309– ∞)
	2002/2003	356 (178– ∞)	3816 (861– ∞)
Måseskär	1979	∞ (152– ∞)	∞ (1 419– ∞)
	2002/2003	∞ (1030– ∞)	1981 (754– ∞)
Skagerrak samples	1979	497 (169– ∞)	∞ (1153– ∞)
	2002/2003	∞ (1951– ∞)	1806 (981–7977)
Himmerfjärden	1979/1980	NA	∞ (0– ∞)
	2002/2003	448 (121– ∞)	∞ (1171– ∞)
Kalix	1980	NA	∞ (663– ∞)
	2002/2003	417 (163– ∞)	4063 (841– ∞)
Vaxholm	1979	∞ (231– ∞)	1553 (390– ∞)
	2002/2003	840 (116– ∞)	∞ (859– ∞)
Baltic samples	1979/1980	∞ (238– ∞)	∞ (1600– ∞)
	2002/2003	1468 (106– ∞)	∞ (2165– ∞)

Abbreviation: NA, not available (ages were missing).
95% CI are given in parentheses. See text for details.

Table 6 Estimates of long-term effective population size (N_e) and migration rate (m) based on temporally separated samples of Atlantic herring from five locations

Locality	Method (assuming $m = 0$)			Method (assuming $m > 0$)	
	F_{ST} (Jorde and Ryman, 2007) N_e	Likelihood (Wang, 2001) N_e	F_{ST} (Nei and Tajima, 1981) N_e	Likelihood (Wang and Whitlock, 2003)	
				N_e	m
Flatbrotten	∞ (949– ∞)	> 35 000 (2169–> 35 000)	∞	1515 (671–6577)	0.009 (< 0.001–0.021)
Måseskär	630 (288– ∞)	1552 (755–11 691)	795	543 (311–1158)	0.027 (0.011–0.046)
Skagerrak samples	2914 (704– ∞)	9576 (2602–> 35 000)	2834	1582 (866–3600)	0.008 (0.005–0.015)
Himmerfjärden ^a	∞ (2267– ∞)	> 35 000 (1333–> 35 000)	∞	1624 (577–6725)	0.009 (< 0.001–0.027)
Kalix	∞ (2570– ∞)	> 35 000 (2753–> 35 000)	∞	3062 (953–5785)	0.004 (< 0.001–0.015)
Vaxholm ^a	419 (275–879)	711 (380–2418)	492	276 (155–556)	0.049 (0.023–0.099)
Baltic samples	∞ (3775– ∞)	12 280 (3345–> 35 000)	6119	2146 (1194–4409)	0.007 (0.004–0.012)

Note that an upper limit of 35 000 is imposed on the likelihood methods. 95% CI are given in parentheses. See text for details.

^aSample from 2002 was only genotyped for allozymes and was therefore excluded.

studies (Ryman *et al.*, 1984; Bekkevold *et al.*, 2005; Ruzzante *et al.*, 2006; Larsson *et al.*, 2007). The stability of the spatial structure is supported by the strong correlation between old and new samples with respect to multilocus F_{ST} s among pairwise localities when considering the 19 loci presumed to be selectively neutral ($r^2 = 0.65$).

Temporal stability of genetic structure

There are indications of long-term temporal allele frequency change within the total Baltic Sea-Skagerrak area. These long-term genetic changes are mainly observed at Måseskär in the Skagerrak and Vaxholm in the Baltic. We also observe a short-term genetic change at Flatbrotten in the Skagerrak. Short-term allele frequency shifts were not observed in an earlier study on herring in this area (Bekkevold *et al.*, 2005). A difference between cohorts (such as noted at Flatbrotten in this study) has also been observed in the North Sea (Mariani *et al.*, 2005).

We have previously shown that the microsatellite locus *Cpa112* is subject to selection or closely linked to a locus under selection (Larsson *et al.*, 2007). Our present results

do not contradict these findings. We observe a conspicuously stronger correlation of pairwise F_{ST} s among sampling localities for *Cpa112* ($r^2 = 0.93$, $P < 0.001$) than for any of the presumed neutral loci (r^2 ranging from 0.0003 to 0.476 with P -values between 0.03 and 0.96). Thus, our present results suggest that the selective regime has not changed over the 24-year period studied here, and that there has not been enough migration to counteract natural selection.

Although of obvious interest from a conservation management perspective, investigations of the stability of spatial structure of marine fish are relatively scarce. What was previously considered to represent spatial structuring in the European eel (*Anguilla anguilla*), has since been suggested to reflect temporal heterogeneities (Dannewitz *et al.*, 2005). In line with the results of this study, temporally stable spatial structuring has been reported in Atlantic cod (*Gadus morhua*) populations, despite large reductions in census sizes (Ruzzante *et al.*, 2001; Poulsen *et al.*, 2006). A close relative of the herring, the European sardine or pilchard (*Sardina pilchardus*), was also found to exhibit stable, although very weak, spatial structure (Laurent and Planes, 2007).

Genetic diversity

We could not detect any significant change in genetic diversity measured as expected heterozygosity or allelic richness over the approximate two decades studied here. Herring has a long history of exploitation and the fisheries have been characterized by fluctuating abundances (MacKenzie *et al.*, 2002), making it difficult to know what to expect in regards to changes of genetic diversity. We cannot make comparisons with non-exploited populations like Hauser *et al.* (2002) did with the New Zealand snapper (*Pagrus auratus*) for which they found a significant decrease in heterozygosity and mean number of alleles.

Similarly, a decrease in genetic diversity was detected in a comparison before and after heavy exploitation of orange roughy (*Hoplostethus atlanticus*; Smith *et al.*, 1991). In contrast, there was no significant loss of genetic diversity over 40 years and with declining population sizes in the thornback ray (*Raja clavata*; Chevolut *et al.*, 2008). However, as with our herring data, these samples do not predate major exploitation so genetic diversity may have been lost before sampling. Genetic diversity can also be lost but subsequently increase due to immigration (e.g. Hutchinson *et al.*, 2003). We may be unaware of declines and subsequent increases in diversity (or vice versa) occurring between our sampling occasions. Long-term sequential sampling schemes (although typically financially and logistically difficult to sustain) can therefore be essential when examining temporal trends to detect possible changes of genetic diversity related to exploitation.

Large populations have a higher capacity to retain rare alleles than small ones. Therefore, when a large population decreases drastically, there is a disproportionate risk of losing alleles compared with small populations experiencing a similar relative decline. During this study period, herring populations in for example the Bothnian Bay have declined from two billions to one billion (Figure 1). At mutation-drift equilibrium, assuming a mutation rate of 10^{-4} , and an N_e/N ratio between 0.001–1%, such a decline is expected to result in at least a 50% reduction of the number of alleles (cf. Ryman *et al.*, 1995). However, we did not find any reduction of allelic richness (Table 2). Possible explanations for this include (i) that equilibrium has not yet been reached, (ii) previous fluctuations of population size before our study have reduced the number of alleles (e.g. Grant and Bowen, 1998), or (iii) lack of power to detect loss of low frequency alleles. Obviously, the populations cannot be in mutation-drift equilibrium as they are still declining, and the relative importance of the two other explanations cannot be determined.

Short-term effective population size

Using allele frequency shifts between consecutive cohorts and correcting for overlapping generations provides smaller point estimates of N_e and smaller values for the lower confidence limits than the linkage disequilibrium method (Table 5). It is uncertain what effect overlapping generations (as is the case for Atlantic herring) can have on the linkage disequilibrium method. Regardless, it is not possible to separate the estimates from infinity, and this is also true for Flatbotten where we observe a significant short-term temporal allele

frequency shift. There is no compelling evidence for either an increase or decrease of N_e over time.

Long-term effective population size

Overall, the effective population sizes over the 24-year period studied here appear to be rather large, and the upper confidence intervals indicate that many of these estimates cannot be separated from infinity (Table 6). It is difficult to estimate N_e for large populations (Waples, 1989; Skalski, 2007); the sample sizes in such situations need to be large as well (Luikart *et al.*, 1999; Ovenden *et al.*, 2007). Nevertheless, our N_e estimates are still relatively small compared with the estimated census sizes, which are in the billions for these management areas (cf. Figure 1). Conspicuously small N_e/N ratios have been observed in several marine fishes (Hauser and Carvalho, 2008), and our present observation is consistent with those findings.

Allele frequency changes at the localities Måseskär and Vaxholm are reflected in relatively small N_e estimates of for example 630 and 419 (using the F_S estimator of Jorde and Ryman, 2007). For Måseskär, the upper limit is infinity with this estimator, but for Vaxholm the 95% CI = 275–879. The other methods consistently estimate a low effective size for this locality. Why would there be a small N_e in Vaxholm? One explanation would be that a local herring population exists in this area that is relatively small. Physically isolated and genetically differentiated populations have been observed both in Atlantic and Pacific herring (*Clupea pallasii*; McPherson *et al.*, 2004; Small *et al.*, 2005).

An alternative explanation for the low N_e estimate at Vaxholm is that the 'large' allele frequency shifts is not caused by genetic drift but by immigration from, or replacement by, a genetically distinct population (genetic swamping). We do not consider this explanation unlikely; F_{ST} between Vaxholm 1979 and 2002/2003 is 0.004, which is of the same magnitude as observed among localities within the Baltic.

Demographic independence

Despite the minuscule amount of spatial differentiation between localities, the herring populations in the Baltic-Skagerrak regions may still be demographically independent. Demographic independence between populations is suggested to occur at migration rates of 10% or less (Hastings, 1993; Palsbøll *et al.*, 2007). Assuming migration-drift equilibrium, we expect $F_{ST} \approx 1/(4N_e m + 1)$ (Crow and Kimura, 1970). This means that our F_{ST} estimates of c. 0.002 both within and between regions would correspond to about 100 genetically effective migrants per generation or more, implying demographic independence at effective population sizes of 1000 or more. Most of our N_e estimates seem to meet this criterion. Similarly, using the MLNE approach we estimate migration rates to be only a few percentages or less (Table 6).

The subtle genetic population structure found in this study does not challenge the view of a metapopulation structure in herring proposed by McQuinn (1997) where local population integrity is maintained by repeat but not necessarily natal homing to spawning areas; recruits learn migration patterns from adults. The genetic population structure observed in these herring samples

can also conform to a 'mixed structure metapopulation model' (Harrison and Taylor, 1997). In this model, most subpopulations are connected by high rates of exchange, whereas more isolated subpopulations occur within the metapopulation. Both geographic isolation and differences in timing of spawning can facilitate distinctiveness. The evidence is not overwhelming, but the mixed structure metapopulation model can explain the local allele frequency shifts at Vaxholm and Måseskär and the resulting relatively small local effective population sizes.

Conclusions

In summary, we conclude:

- (1) The amount of gene diversity and the number of alleles in Atlantic herring in Swedish waters have not decreased even though the census population sizes have declined during the time period investigated here.
- (2) The spatial structure of the investigated Atlantic herring populations did not change between 1979/1980 and 2002/2003. The differentiation is weak with $F_{ST} = 0.003$ (95% CI = 0.001–0.005) for the older samples and $F_{ST} = 0.002$ (95% CI = 0.001–0.003) for the newer ones.
- (3) Temporal genetic changes are fairly small and spatial genetic structure, particularly between the two main regions (Baltic Sea vs Skagerrak), accounts for most of the allele frequency variation. Of the overall genetic variation among samples ($G_{ST} = 0.0059$), 32% is explained by the difference between the Baltic and the Skagerrak ($P = 0.014$), whereas only 6.8% is explained by temporal genetic differences (non-significant).
- (4) Although there is little long-term temporal allele frequency change within the total area, such changes are observed at Måseskär in the Skagerrak and Vaxholm in the Baltic. Short-term temporal changes are only observed at Flatbotten in the Skagerrak.
- (5) Generally, the effective population sizes appear to be rather large and can in many cases not be separated from infinity. The exceptions are Måseskär and Vaxholm where the long-term temporal genetic changes are reflected in relatively small local N_e estimates. There is no compelling evidence for either an increase or decrease of N_e over time.
- (6) The generally large effective population sizes, in combination with our estimates of low degrees of genetic divergence, and low migration rates support the potential for demographic independence among populations in this region.
- (7) The previously identified influence of selection at the microsatellite locus *Cpa112* remains stable over the 24-year period studied here.

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Appendix 1

Descriptive statistics and pairwise comparisons for 11 allozyme and eight microsatellite presumed neutral loci of the cohorts in temporally separated samples of Atlantic herring from five locations

Locality	Cohort	n	H_E	H_O	AR	Pairwise F_{ST} between cohorts below diagonal, nominal P-values above											
						1974	1975	1976	1977	1993	1994	1995	1996	1997	1998	1999	2000
Flatbrotten	1974	20 (18)	0.445	0.440	4.83	—	0.004	0.045	—	—	—	—	—	—	0.058	0.059	0.125
	1975	24 (23)	0.447	0.408***	4.77	0.004	—	0.009	—	—	—	—	—	—	0.280	0.013	0.034
	1976	43 (42)	0.444	0.436	4.91	0.006	0.004	—	—	—	—	—	—	—	0.027	0.088	0.094
	1998	106 (96)	0.445	0.436	4.86	0.002	−0.002	0.002	—	—	—	—	—	—	—	0.043	0.009
	1999	56 (52)	0.448	0.437	4.91	0.004	0.003	0.002	—	—	—	—	—	—	0.001	—	0.006
	2000	48 (42)	0.457	0.450	4.89	0.009	0.003	0.004	—	—	—	—	—	—	0.005	0.003	—
Måseskär	1974	25 (24)	0.443	0.440	4.98	—	0.061	0.594	—	—	—	—	—	—	0.313	0.869	0.454
	1975	54 (54)	0.452	0.436*	4.88	0.000	—	0.310	—	—	—	—	—	—	0.001	0.011	0.002
	1976	21 (21)	0.430	0.421	4.68	−0.001	−0.002	—	—	—	—	—	—	—	0.006	0.083	0.147
	1998	115 (110)	0.451	0.440	4.92	−0.001	0.002	0.005	—	—	—	—	—	—	—	0.411	0.392
	1999	52 (47)	0.443	0.426*	4.86	−0.002	0.002	0.003	—	—	—	—	—	—	−0.001	—	0.664
	2000	36 (27)	0.434	0.426	4.92	−0.002	0.003	0.000	—	—	—	—	—	—	0.000	−0.003	—
Himmerfjärden	1993	24 (12)	0.447	0.445	4.91	—	—	—	—	—	0.868	0.295	0.849	0.382	0.879	0.529	0.409
	1994	39 (20)	0.443	0.445	4.94	—	—	—	—	−0.006	—	0.533	0.908	0.335	0.575	0.427	0.133
	1995	45 (18)	0.422	0.434	4.60	—	—	—	—	0.001	−0.003	—	0.589	0.000	0.151	0.088	0.179
	1996	56 (20)	0.445	0.441	4.75	—	—	—	—	−0.003	−0.001	0.005	—	0.252	0.448	0.041	0.462
	1997	40 (8)	0.418	0.411	NA ^a	—	—	—	—	0.008	0.005	0.017	0.008	—	0.031	0.061	0.195
	1998	57 (10)	0.444	0.455	NA ^a	—	—	—	—	−0.004	−0.005	0.004	0.003	0.010	—	0.444	0.303
	1999	34 (0)	0.144	0.134	NA ^a	—	—	—	—	0.004	0.012	0.015	0.018	0.012	0.006	—	0.049
	2000	55 (2)	0.458	0.471	NA ^a	—	—	—	—	0.002	0.002	0.004	−0.002	0.005	0.003	0.010	—
Kalix	1997	30 (25)	0.442	0.434	4.63	—	—	—	—	—	—	—	—	—	0.065	0.234	—
	1998	76 (62)	0.442	0.426**	4.90	—	—	—	—	—	—	—	—	0.003	—	0.429	—
	1999	78 (73)	0.434	0.421*	4.84	—	—	—	—	—	—	—	—	0.000	−0.001	—	—
Vaxholm	1975	22 (22)	0.442	0.442	4.69	—	—	0.032	0.171	—	—	—	—	0.065	0.004	0.001	—
	1976	33 (33)	0.436	0.450	4.91	—	− 0.002	—	0.151	—	—	—	—	0.092	0.066	0.204	—
	1977	23 (22)	0.431	0.427	4.85	—	0.008	0.000	—	—	—	—	—	0.001	0.002	0.086	—
	1997	36 (26)	0.430	0.418	4.74	—	0.009	0.008	0.015	—	—	—	—	—	0.305	0.461	—
	1998	62 (29)	0.431	0.432	4.87	—	0.003	0.003	0.007	—	—	—	—	0.004	—	0.573	—
	1999	71 (19)	0.434	0.417*	4.70	—	0.004	−0.001	0.000	—	—	—	—	0.002	−0.002	—	—

Abbreviations: AR, allelic richness based on minimum sample size of 12; *n*, sample size (no. of individuals scored for microsatellites in parenthesis); H_E , expected heterozygosity; H_O , observed heterozygosity; departure from Hardy–Weinberg equilibrium noted with * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Significant pairwise differentiation between cohorts from the same locality are marked in bold. Bonferroni corrections have not been applied.

^aNot available, not included in allelic richness calculations since no or very few microsatellites were genotyped.