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RESEARCH ARTICLE

Effective size and genetic composition of two exploited, migratory whitefish (*Coregonus lavaretus lavaretus*) populations

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Abstract Large scale harvesting and other anthropogenic activities have caused severe population declines in many commercially important fish populations, but accurate information about census and effective population size is often hard to come by. Available evidence suggests that in marine fishes, effective population size (N_e) is often several orders of magnitude smaller than census size, such that intensively harvested populations may be particularly vulnerable to loss of genetic diversity. The European whitefish (*Coregonus lavaretus*) has a long history of heavy exploitation in the Baltic Sea, and the Finnish commercial catch of the species has been substantially reduced, despite high fishing effort. We investigated the temporal genetic stability of migratory whitefish populations from two Finnish rivers (Tornionjoki and Kiiminkijoki), sampled at least twice between 1981 and 2006, by assaying variability in 21 microsatellite loci. Our results suggest a small, albeit significant ($F_{ST} = 0.004$; $p = 0.008$) and temporally stable, degree of differentiation between rivers. However, in contrast to earlier reports, heterochronous runs (ascending groups) from Tornionjoki did not exhibit significant genetic divergence. Bayesian estimates of N_e suggest substantial declines from historic levels dating to ca 250 years. Yet despite a probable decrease in census population size over the study period, we detected no significant change in contemporary N_e. Within group genetic diversity appeared largely unchanged over this time frame; however, we detected a trend towards decreased differentiation between spawning groups (rivers) since the 1980s. These results are discussed in light of stocking programs and conservation of genetic diversity of natural populations.

Keywords Fisheries stock assessment · Genetic monitoring · Effective population size (N_e) · Population bottleneck · Supplemental breeding · Stocking

Introduction

Over the past few decades, several commercially important fish stocks have declined as a result of anthropogenic activities such as overexploitation, population translocation and habitat degradation. The collapse of Northern cod (*Gadus morhua*) provides one of the most dramatic examples of the consequence of overfishing (Hutchings 2000; Hutchings and Baum 2005), whilst Atlantic salmon (*Salmo salar*) has suffered worldwide from habitat destruction and fragmentation, such as effects from river damming (Thorstad et al. 2008). In 1997 it was estimated that 60 % of major marine fisheries were either overexploited, depleted, or in a state of collapse (FAO 1997), and more recent estimates suggest that this figure has increased to approximately 80 % (FAO 2009), leading to a prediction of total collapse of all current fisheries by the year 2048 if

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exploitation continues at its current rate (Worm et al. 2006). As such, accurate information regarding population size and conservation status of exploited populations is indispensable if stocks are ever to be managed sustainably.

Many marine species are characterized by large population sizes with complex dynamics, and the full extent of their spatial usage/distribution is often unknown, making it difficult for managers and conservation biologists to obtain accurate population size estimates (Hutchings and Baum 2005; Murphy and Jenkins 2010). Moreover, in marine ecosystems, even stocks on the verge of collapse may consist of millions of individuals (Ryman et al. 1995), and hence, the census size of a population (N) may not be the most relevant indicator of long-term stability for an exploited population. This perspective stems from the fact that overexploitation not only reduces abundance, but can also cause genetic effects through drift (i.e. artificially induced bottlenecks), or selective removal of genotypes (Allendorf et al. 2008). The major genetic consequence of a reduction in population size is an increase in the rate of loss of genetic variation which, in turn, may reduce individual fitness and the ability of the population to evolve in the future (Ryman et al. 1995). Consequently, the effective population size (N_e) may be considered a measure of the long-term stability of a population. N_e refers to the number of reproductive individuals contributing to the next generation, and it is typically much smaller than the census population size (Frankham 1995). In marine fish species, N_e is often several orders of magnitude smaller than N, such that the ratio of N_e/N typically varies between 10^{-5} and 10^{-3} (Cano et al. 2008). Species with a small N_e/N ratio—even if N is large—can suffer from loss of genetic diversity, as shown for New Zealand snapper, *Pagrus auratus* (Hauser et al. 2002); red drum, *Scianops ocellatus* (Turner et al. 2002); and the North Sea cod, *Gadus morhua* (Hutchinson et al. 2003). Severe reductions in population size due to exploitation have been accompanied by reductions in N_e and, in many instances, have also led to predictable losses of genetic variability (e.g. Hauser et al. 2002; Hoarau et al. 2005; Palstra and Ruzzante 2008).

The effective population size not only helps to predict the rate of change in genetic diversity and potential for inbreeding, but has also become a criterion for assessing conservation status and extinction risks of species and populations (Frankham 1995). Moreover, although census size of marine fish species may be difficult to determine, use of molecular markers and advances in related statistical techniques have greatly facilitated the estimation of N_e (Hill 1981; Wang and Whitlock 2003; Waples 2005; Fraser et al. 2007; Tallmon et al. 2010; Waples and Do 2010). This has resulted in improved monitoring of the genetic diversity, conservation status and viability of marine species under fishing pressure (Miller and Kapuscinski 1997; Hansen et al. 2002; Hauser et al. 2002). In situations where it is difficult to estimate the census population size directly, long-term genetic monitoring can be a particularly effective strategy as it can provide indirect insights into the scale and periodicity of changes in demographic parameters (Schwartz et al. 2007). To this end, temporal comparisons based on historical DNA samples represent an invaluable resource for population studies (Wandeler et al. 2007; Leonard 2008; Nielsen and Hansen 2008). Appropriate tissues can be found in many historic biological collections (e.g. skin, teeth, feathers), and in monitored commercial fisheries, these may be available in the form of archived scale or otolith samples.

Based on its economic valuation, the European whitefish (*Coregonus lavaretus*) is the second most commercially important fish species in the Gulf of Bothnia (Aronsuu and Huhmarniemi 2004; RKTL 2010). Two sub-species are known to exist sympatrically in the Gulf of Bothnia: the migratory whitefish (*C. lavaretus lavaretus*), and the sea-spawning whitefish (*C. lavaretus widegreni*). The species is heavily exploited in Finnish waters, with approximately 70–80 % of commercial catches consisting of the anadromous *C. lavaretus lavaretus* (Aronsuu and Huhmarniemi 2004). Commercial catches of European whitefish in the Baltic Sea have decreased by ca 56 % since 1953 (Fig. 1; RKTL, unpublished data), and the decline has been particularly pronounced since 1996, despite continued high fishing effort (RKTL 2010). Problems affecting the reproductive success of anadromous whitefish, such as the construction of dams preventing mature individuals from reaching spawning grounds, and reduction in the quality of spawning grounds due to peat mining activities, siltation and eutrophication (Bninska 2000), have led managers to adopt the practice of stocking hatchery reared juveniles to support wild populations. Though stocking may be helpful in maintaining fisheries catches and census population sizes, the practice may not be sustainable for the long-term stability of a species. In natural fish populations, stocking has been associated with decreased growth of wild conspecifics (Bohlin et al. 2002), an increased risk of disease introduction (Arsan and Bartholomew 2008), decreases in effective population size (Ryman and Laikre 1991), and an overall reduction of genetic diversity (Utter 2000; Allendorf et al. 2001), although the scope of these potential negative consequences may be under-appreciated (Laikre et al. 2010).

This study investigates potential genetic effects associated with declines in stock biomass and subsequent stocking in anadromous whitefish (*C. lavaretus lavaretus*) populations from two Finnish rivers, Tornionjoki and Kiiminkijoki. To this end, we analyse genetic variability in a collection of scale samples, and investigate the temporal genetic variation in 21 microsatellite loci from samples collected 20–25 years (ca 5–6 generations) apart. The

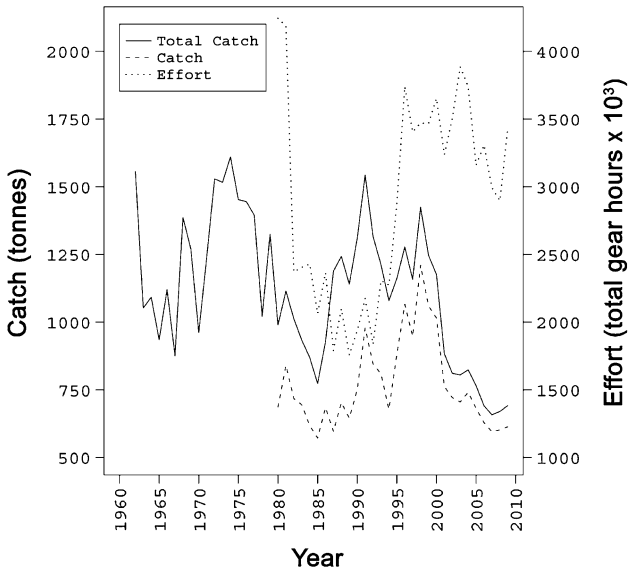

Fig. 1 Commercial landings of whitefish in the Baltic Sea, combining ICES subdivisions 29 (Archipelago Sea), 30 (Bothnian Sea), 31 (Bothnian Bay) and 32 (Gulf of Finland). Total catch corresponds to total annual landings from all gear types, for many of which effort (e.g. seine netting) is unknown. Catch corresponds specifically to landings from selected gear types (i.e. trap nets, gill nets <36 mm mesh size, and 36–60 mm mesh gill nets) for which fishing effort data are also available. Effort is calculated by multiplying total amount of gear used by the number of fishing days. Data from 1980 are reported in annual, national compendia of statistics (e.g. RKTL 2010); pre-1980 data were compiled by the Finnish Game and Fisheries Research Institute (P. Söderkultalahti, personal communication)

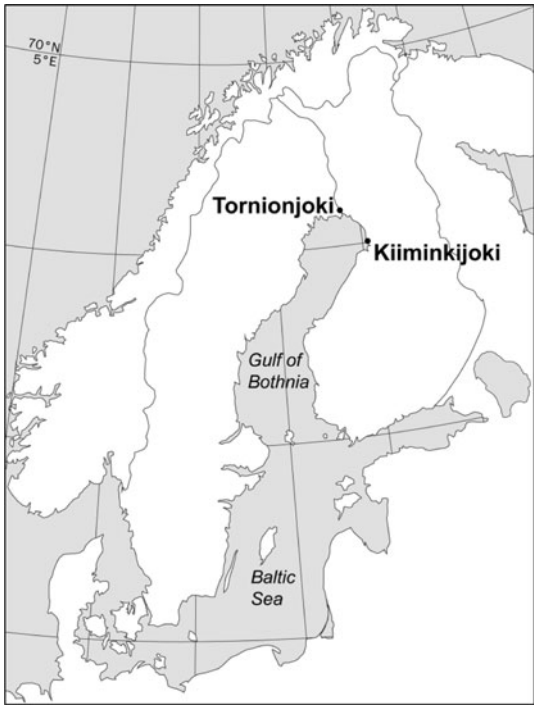

Fig. 2 Locations of outflows for the rivers Tornionjoki and Kiiminkijoki. Latitude and longitude lines are in 5° intervals

study focuses primarily on demographic information particularly germane to management, namely: (1) an evaluation of contemporary population structure within and among rivers; (2) changes in population genetic parameters, particularly N_e, over a 25 year period; and (3) inference into the timing of any severe reductions in effective population size.

Material and methods

Sampling

Anadromous whitefish scales have been collected by the Finnish Fisheries and Game Research Institute (RKTL) annually, from 1981 to 2006. Samples originate from spawning migrants captured at the outflows of the rivers Kiiminkijoki and Tornionjoki, both of which discharge into the northern Gulf of Bothnia in the Baltic Sea (Fig. 2). Although no exact estimates of stock (i.e. population) size exist for these rivers, it was assumed that commercial catch records also reflect the status of whitefish in these rivers. There are no man-made barriers to migration in these rivers so no associated changes have occurred with respect to their fish populations. In the Tornionjoki, groups of whitefish start to ascend the river as early as June-July (the summer run) and this continues into the spawning season in October. Whitefish in the Kiiminkijoki have a much shorter ascent period, occurring only in the autumn, just prior to spawning. The Tornionjoki whitefish population was divided into two groups defined by different temporal sampling. Summer ascending individuals were collected from June until August in Tornionjoki. Autumn ascending samples were collected from both rivers from September to October (Table 1). In this study, we use the most temporally distant samples available for each of the ascending groups to infer changes in genetic and demographic parameters over a 20–25 year time period (ca 5–6 generations).

Microsatellite analysis

Total DNA was extracted from scale samples by overnight proteinase K digestion, followed by phenol–chloroform extraction (Taggart et al. 1992). All samples were analysed at 21 microsatellite loci: Bwf1^F, Bwf2^H (Patton et al. 1997); Cocl23^T (Bernatchez 1996); Cisco-90^F, Cisco-126^T, Cisco-157^F, Cisco-200^F (Turgeon et al. 1999); Cocl-Lav4^T, Cocl-Lav27^F, Cocl-Lav61^F (Rogers et al. 2004); and ClaTet1^F, ClaTet3^F, ClaTet6^F, ClaTet9^T, ClaTet10^T, ClaTet12^H, ClaTet13^T, ClaTet15^H, ClaTet16^T, ClaTet17^H, ClaTet18^H (Winkler and Weiss 2008). Superscripts (F,H,T) denote 5′-end labelling of each forward primer with

Table 1 Sampling sites, as per Fig. 2, and the number of individuals (*N*) sampled for each year of sampling

Sample (run)	Years	*N*	H_O	H_E	F_{IS} (95 %CI)	Het. excess		*M* Ratio	
						SMM	TPM	M (M_{CRIT})	p Value
Kiiminkijoki	1981	48	0.757	0.818	0.076 (0.048–0.113)	>0.999	0.999	0.6489 (0.7520)	<0.001
(autumn)	2006	48	0.779	0.819	0.047 (−0.004–0.101)	>0.999	0.999	0.6688 (0.7546)	<0.001
Tornionjoki	1981	91	0.768	0.815	0.062 (0.022–0.118)	>0.999	>0.999	0.7345 (0.7646)	0.010
(*both runs*)	2000	96	0.784	0.818	0.042 (0.007–0.071)	>0.999	>0.999	0.7466 (0.7649)	0.018
Tornionjoki	1981	48	0.751	0.808	0.076 (0.020–0.113)	>0.999	>0.999	0.7308 (0.7531)	0.014
(summer)	2000	48	0.774	0.819	0.052 (0.021–0.090)	0.999	0.994	0.6820 (0.7527)	0.001
	2004	48	0.767	0.817	0.058 (0.015–0.106)	>0.999	0.999	0.6748 (0.7546)	<0.001
Tornionjoki	1981	43	0.787	0.819	0.037 (0.005–0.089)	>0.999	>0.999	0.6609 (0.7517)	<0.001
(autumn)	2000	48	0.794	0.815	0.027 (−0.002–0.059)	>0.999	>0.999	0.6647 (0.7546)	0.018

Average observed (H_O) and expected (H_E) heterozygosities are presented for each sample, as calculated using ARLEQUIN. Inbreeding coefficients (F_{IS}) and bootstrapped 95 % confidence intervals were calculated using HIERFSTAT. p values correspond to tests for population bottlenecks based heterozygosity excess (Het. Excess). Simulated equilibrium distributions were based upon a step-wise mutation model (SMM), and a two-phase model (TPM) of microsatellite mutation (see "Materials and methods" section for details). Mean ratio of the number of alleles to the range in allele sizes (M) was evaluated against a simulated distribution of values under equilibrium conditions (M_{CRIT}; see "Materials and methods" section for details)

fluorescent dyes FAM, HEX or TET, used for visualization of PCR products. A GTTT-tail was also added to the 5′-end of each reverse primer to enhance adenylation of the nascent DNA strand (Brownstein et al. 1996). Approximately 20–50 ng of genomic DNA was used for amplification by polymerase chain reaction (PCR), carried out in four multiplex panels with non-overlapping size ranges within each dye. PCRs were performed using QIAGEN Multiplex PCR Kit (QIAGEN) in a final volume of 10μL containing 1 × QIAGEN Multiplex PCR Master Mix, 0.5 × Q-Solution, and 2 pmol of each primer. The PCR cycle began with an initial denaturation step at 95 °C for 15 min, and thereafter the following cycle was repeated 30 times: 94 °C for 30 s, 55 °C for 90 s and 72 °C for 60 s, with a final extension at 60 °C for 5 min. PCR products were visualized with a MegaBASE 1000 automated sequencer (Amersham Biosciences), and fragment lengths were determined based on an ET-ROX 550 size standard (Amersham Biosciences). Alleles were scored manually using Fragment Profiler 1.2 (Amersham Biosciences), and resultant allelic sizes (i.e. migration distances) classified into bins using the FLEXIBIN algorithm (Amos et al. 2007).

Data analyses

GENEPOP 4.0.10 (Raymond and Rousset 1995) was used to test each sample for statistically significant deviations from Hardy–Weinberg equilibrium (HWE), and genotypic linkage disequilibrium. Tests were based on 300 batches of Markov chain derived estimates (3,000 iterations each). Significance levels for multiple tests were adjusted by sequential Bonferroni correction (Rice 1989). FSTAT 2.9.3.2 (Goudet 1995) was used to calculate the number of alleles (A), rarefaction-based allelic richness (Ar, El Mousadik and Petit 1996), and observed and expected heterozygosity. Additionally, we used the R package 'HIERFSTAT' to calculate inbreeding coefficients (F_{IS}) for each sample (Goudet 2005). We determined 95 % confidence intervals for these estimates by a bootstrapping procedure (10,000 iterations).

To assess population structuring, we first performed a phenetic analysis on all samples, though using only those markers not exhibiting deviations from HWE expectations (17 of 21 markers; see Supplementary Table S1). PHYLIP 3.69 (Felsenstein 2005) was used to calculate 1,000 bootstrapped matrices of chord distances (D_C) among samples (Cavalli-Sforza and Edwards 1967), and to construct a consensus (majority rule) unrooted neighbour-joining tree. The consensus tree was displayed using TREEVIEW (Page 1996). Additionally, we performed a hierarchical analysis of molecular variance (AMOVA), again using only markers in HWE, in order to quantify genetic differentiation among spawning groups and temporal stability within. We used the HIERFSTAT package to calculate nested divergence estimators, and to evaluate their significance via 10,000 permutations (Goudet 2005).

Finally, we used genetic data to infer both contemporary and historic demographic parameters germane to exploited populations. Since many of these algorithms are based on deviations from equilibrium conditions, we reasoned that markers previously excluded on this basis may be most informative in this regard. Consequently, all subsequent analyses included the full suite of 21 microsatellite markers. Given that our objective was to compare changes in N_e between temporally separated samples, we used the

integrated linkage disequilibrium method (Hill 1981), implemented in NeESTIMATOR 1.3 (Peel et al. 2004), to calculate the effective population size (N_e), as well as the bias-corrected method implemented in 'LDNE' (Waples and Do 2008). These estimates were also contrasted with temporally-based methods of N_e estimation, including Waples' (1989) moment-based estimator, and a likelihood based method implemented as 'MLNE' (Wang 2001). In both it was assumed that six generations separated samples from Kiiminkijoki (1981 and 2006), and five generations separated samples from Tornionjoki (1981 and 2000)—note that the summer sample from 2004 was not used in temporal analyses of N_e.

BOTTLENECK 1.2.02 was used to test for evidence of a genetic bottleneck, applying both the stepwise mutation model (SMM) and the two phase model (TPM) for microsatellite loci: TPM settings followed the authors' suggestions of 95 % single step mutations and 5 % multi-step mutations, with variance among multiple steps set to 12 (Piry et al. 1999). The model was run for 10,000 simulations, and Wilcoxon's test was used to detect heterozygosity excess in all the populations. Additionally, we conducted a second series of tests for bottlenecks based on M, the mean ratio of the number of alleles to their overall range in size (Garza and Williamson 2001). We used the program 'M_P_Val' to calculate the probability of obtaining a value of M less than the observed value based upon 10,000 simulated equilibrium distributions. We also used the program 'Critical_M' to compute the theoretical critical value of M (M_{CRIT}) under equilibrium conditions. For both, initial parameters were based on recommended settings to approximate a two-phase model of microsatellite mutation: $p_s = 0.9$; $\Delta g = 3.5$; $\Theta = 4$ (Garza and Williamson 2001). Finally, we inferred demographic changes by estimating contemporary and historic N_e via Markov chain Monte Carlo (MCMC) simulation, as implemented in the MSVAR program (Beaumont 1999; Storz and Beaumont 2002). Model priors were based on mutation rates typical for microsatellites in teleosts (Yue et al. 2007), and parameter estimates for whitefish populations from the North Sea (Hansen et al. 2008). Priors could be described as only weakly informative, given that the corresponding range of values used spanned at least two orders of magnitude, thereby allowing for potential model convergence indicative of equilibrium population conditions. The range of priors used in the model included: mutation rate (3×10^{-5}–3×10^{-3}), contemporary N_e (100–10,000), historic N_e (10,000–1,000,000) and time since population expansion or contraction (150–150,000 years). We initially performed three independent runs for each sample with an initial number of update steps set at 600,000,000 and a thinning interval of 10,000, thus, yielding 60,000 samples from each Markov chain. However, model convergence was problematic with some runs, and so three additional runs were performed for each sample, each yielding 80,000 Markov chain samples. The final 5,000 samples from each Markov chain were used in parameter estimation. We used the R package 'CODA' to assess convergence within and among chains, and to estimate parameter values. Parameter estimates were based on the modal value of their posterior distribution, bounded by 95 % posterior density interval estimates.

Results

A total of 516 alleles were observed across the 21 microsatellite loci, ranging from 7 to 51 per locus, corresponding to an average of 24.57 alleles per locus (Table S1). Missing genotypes accounted for 1.84 % of the microsatellite data distributed over all loci. Almost 15 % of all alleles were private alleles unique to a single sample. Ascending groups sampled in 1981 had higher numbers of private alleles, except the Tornionjoki summer sample wherein the number of private alleles decreased in year 2000, but increased again in 2004 to fourteen. Out of the 21 microsatellite loci tested, four (Cal Tet-17, Cal Tet-13, Cisco-200 and Cal Tet-16) deviated from Hardy–Weinberg expectation in all samples due to a deficiency of heterozygotes (Table S1). A random sample of 15 % of individuals were re-typed at these loci, yielding the same genotype, suggesting that these results were not due to PCR and/or genotyping artefacts. Averaged over all loci, all temporal samples from the Tornionjoki summer ascending group, in addition to the 1981 samples from the other groups, showed signals of heterozygote deficiency ($F_{IS} > 0$; Table 1).

Analysis of replicate samples suggested a strong signal of temporal stability in genetic composition within ascending groups. Temporal samples from Kiiminkijoki clustered together with high bootstrap support (854/1,000 iterations; Fig. 3). Replicates of the autumn and summer ascending forms from Tornionjoki also clustered with their respective historical counterparts. Additionally, we detected no significant differentiation among temporal samples nested within groups (F_{YS}; Table 2). AMOVA results did suggest a small, albeit significant ($F_{ST} = 0.004$; $p = 0.008$), degree of differentiation among all three whitefish groups; however, summer and autumn runs from Tornionjoki did not exhibit significant genetic divergence when analysed separately (Table 2). Consequently, we also pooled Tonrionjoki runs by year (1981 and 2000; 2004 discarded) for all subsequent analyses. AMOVA results based on pooled samples also revealed significant genetic divergence between spawning rivers (Table 2).

BOTTLENECK results suggested no evidence of a recent population bottleneck in the samples: Wilcoxon tests failed to reject the null hypothesis of equilibrium

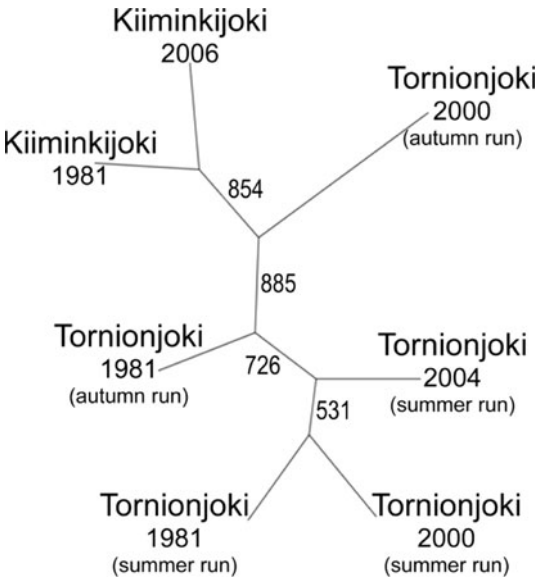

Fig. 3 Unrooted neighbour-joining tree of samples based on Cavali–Sforza and Edwards' chord distance (D_C). Numbers indicate the consensus value of the adjacent node, based on 1,000 bootstrap iterations

heterozygosity in each sample under both the step-wise mutation and two-phase models (Table 1). In contrast, tests based upon the ratio of allele number to size (M) indicated that all samples showed signatures consistent with a population bottleneck (Table 1). MCMC simulations also revealed a significant decrease in effective population sizes: historic N_e estimates were larger than those of contemporary N_e, and the respective 95 % posterior density intervals did not overlap (Table 3). Posterior density intervals of contemporary N_e estimates overlapped between temporally replicated samples within ascending groups (Table 3). No discernible trend was observed in samples from Tornionjoki. However, N_e within the Kiiminkijoki whitefish exhibited a slight, though non-significant, upward trend when comparing both point estimates and their upper bounds (Table 3). Finally, though N_e confidence intervals tended to range over two orders of magnitude, estimates were grossly concordant irrespective of analytical method used (Table S2).

Discussion

This study provides a concrete illustration of the utility of archival scale samples to investigate potential anthropogenic impacts on levels of genetic diversity and population structuring in exploited fish populations. Analyses of DNA extracted from such archival sources were instrumental not only in demonstrating long-term demographic changes, but also in informing our interpretation of the relative timing of these changes. Modelling clearly revealed a significant reduction in the effective population sizes from historic levels, yet despite continued decreases in the census size of whitefish populations in the Baltic Sea, we could not detect any changes in the effective population sizes over the course of the study period. Moreover, analyses of temporally replicated samples suggested that contemporary population structuring may be relatively stable in this system.

Contemporary population structuring

AMOVA results may be indicative of limited gene flow between spawning sites given the significant, albeit weak, degree of differentiation among all whitefish groups from the rivers Kiiminkijoki and Tornionjoki ($F_{ST} = 0.004$; $p < 0.05$). Additionally, we observed a general pattern of within river/group stability, as indicated by strong signals of temporal stability in genetic structure: phenetic analyses

Table 2 Hierarchical AMOVA comparing total genetic variation among all three spawning groups (F_{ST}) and temporal variation nested within group (F_{YS})

Level	All samples		Tornionjoki		Between rivers	
	F	p Value	F	p Value	F	p Value
F_{ST}	0.0036	0.008	0.0013	0.103	0.0039	0.004
[F_{YS}]	0.0005	0.264	0.0010	0.268	0.0002	0.173
[F_{IS}]	0.0182	–	0.0174	–	0.0437	–
F_{YT}	0.0041	<0.001	0.0023	0.016	0.0061	<0.001
[F_{IY}]	0.0176	–	0.0164	–	0.0416	–
F_{IT}	0.0217	–	0.0187	–	0.0475	–

F_{YT} refers to overall variation among all temporally replicated samples, whereas F_{IY} and F_{IT} partition variance among individuals within samples and in the total dataset, respectively. Results of three analyses are presented: one comparing variation among all spawning groups (all samples), one estimating genetic divergence between the summer and autumn spawning forms from the Tornionjoki, and one estimating divergence between spawning rivers. Note that in the final analysis (between rivers), summer and autumn spawning forms from the Tornionjoki were considered as a single sample. Significance (p value) of the F statistics was evaluated by permutation test (10,000 randomizations)

Table 3 Demographic parameters estimated from Markov chain Monte Carlo simulated gene genealogies, as implemented in MSVAR

Sample (run)	Years	Historic N_e (95 % PDI)		Contemp. N_e	(95 % PDI)	Time	(95 % PDI)
Kiiminkijoki	1981	68,985	(6,425–882,972)	83	(9–1,273)	281	(11–3,503)
(autumn)	2006	86,757	(7,174–877,957)	122	(11–1,774)	353	(18–3,814)
Tornionjoki	1981	61,920	(4,710–809,724)	221	(8–3,473)	243	(22–3,164)
(*both runs*)	2000	104,348	(7,745–1,075,774)	44	(4–621)	263	(27–2,708)
Tornionjoki	1981	94,764	(7,962–966,609)	67	(6–1,008)	236	(10–3,157)
(summer)	2000	91,009	(8,041–952,178)	78	(6–964)	256	(24–3,079)
Tornionjoki	1981	84,851	(5,907–924,109)	70	(5–915)	243	(25–3,311)
(autumn)	2000	98,376	(7,465–965,902)	57	(5–813)	275	(29–3,782)

Parameter estimates are based on the modal value of the posterior distribution, bounded by 95 % posterior density intervals (PDI). Parameters include both historic and contemporary (contemp. N_e) estimates of effective population size, in addition to the number of years (time) between these estimates

clustered replicate samples (Fig. 3), and no significant differentiation among temporal samples, nested within ascending groups, was detected (F_{YS}; Table 2). Such temporal stability in spatial genetic structuring is largely concordant with other studies of exploited marine species—such as Atlantic cod (*Gadus morhua*) and Atlantic herring (*Clupea harengus*)—which have also experienced substantial declines in their census sizes (Ruzzante et al. 2001; Poulsen et al. 2006; Larsson et al. 2010). In the case of migratory whitefish, as with other salmonids, this may be due to a intrinsic homing behaviour, although occasional among river dispersal has been observed from mark-recapture data (Petersson 1966; Lind and Kaukoranta 1974; Huusko and Grotnes 1988).

Despite overall patterns of genetic divergence among the three groups, we detected no significant differences between summer and autumn runs from the River Tornionjoki. This would suggest that structuring of whitefish populations within the Baltic Sea may be driven more by reduced gene flow among, rather than within spawning sites (i.e. rivers), though this remains to be verified in other rivers with heterochronous ascending groups (Säisä et al. 2008). Since run timing is believed to be at least partially genetically determined (Sakamoto et al. 1999), in this context, heterochrony would be predicted to increase genetic differentiation. Yet we were unable to detect any statistically significant genetic differences between summer and autumn ascending groups in Tornionjoki ($p = 0.103$). Indeed, the degree of genetic divergence between runs ($F_{ST} = 0.001$) was comparable to that observed among all temporal samples ($F_{YS} \leq 0.001$). This result is in contrast with a previous study which demonstrated statistically significant genetic differences between these same groups (Säisä et al. 2008). This discrepancy could be due to the greater number of markers used in the current study (17), compared with the relatively few (5) used previously, or due to differences in sampling procedures. However, irrespective of the technical reasons for this discrepancy, its resolution and the identification of any discrete ascending groups within this system will be essential for establishing appropriate management plans for a sustainable fishery. Whilst demographic information specific to spawning runs is essential for the conservation and management of many salmonid (meta)populations, present data do not support the view that heterochronous ascending groups in Tornionjoki represent independent units. Moreover, given that N_e estimates for Tornionjoki varied considerably between pooled and run-specific samples (Tables 3, S2), resolution of their status is particularly germane to future management/conservation plans.

Demography at multiple temporal scales

Bayesian modelling (MSVAR) provided strong inferential support for substantive declines in the effective size of whitefish populations in the Baltic Sea. Similarly, *M* ratios were significantly lower than equilibrium expectations, and thus, corroborate the inference of a relatively recent bottleneck. Although Bayesian estimates can yield a false signal of a population bottleneck if samples from structured populations are mixed (Chikhi et al. 2010; Peter et al. 2010), no such statistical artefact should be affecting our results given that both spawning groups were analysed separately. Estimates of historic levels were on average 1,000 times greater than those of contemporary populations (Table 2). Furthermore, despite the wide range of parameter estimates—a problem typical for demographic inferences based on molecular data—posterior density intervals of historic and contemporary N_e exhibited no overlap. Moreover, estimates indicated that changes have occurred on a contemporary, rather than geological, time scale. Point estimates from the posterior distribution suggest that significant population declines most likely occurred within the past 250 years. More importantly, lower bounds date firmly

to the Holocene (ca 3,000 years). Since whitefish could not have colonized the Baltic Sea prior to Fennoscandian deglaciation, ca 10,000–7,000 years ago (Eronen et al. 2001), reductions in N_e cannot be attributable to population contraction within a glacial refuge. This is largely in accord with previous analyses of *C. lavaretus* mitochondrial DNA (mtDNA) from the Gulf of Bothnia which suggested the absence of a severe, historic population bottleneck, based on high contemporary mtDNA variability (Bernatchez et al. 1989).

In light of such convincing support for declines in effective population size, the discrepancy with results from BOTTLENECK (i.e. groups did not differ significantly from mutation-drift equilibrium) was somewhat surprising. We contend, however, that inference based on Bayesian estimates of demographic parameters (MSVAR) should be favoured. MSVAR estimates were derived from multiple, independent modelling runs, with random differences in starting parameter values. Moreover, all runs were formally evaluated for statistical convergence, and parameter estimates were averaged over all model runs. In contrast, BOTTLENECK is less flexible in its operation, and estimates were derived from a single model run. Furthermore, given observed marker variability, and assuming Bayesian estimates of demographic parameters, BOTTLENECK software may be limited in its power (<0.2) to detect a population bottleneck in this system (Cornuet and Luikart 1996). Moreover, recent empirical work has shown that BOTTLENECK often fails to detect signals of population decline that are revealed through inference on the *M*-ratio (Hundertmark and Van Daele 2010; McEachern et al. 2011; Shama et al. 2011), a trend similarly seen in this study.

The discrepancy between inferential methods does, however, raise an interesting perspective. Namely, do estimated declines from historical N_e represent a true population bottleneck? In other words, what fraction of a breeding population must be lost to significantly alter population genetic parameters? In a conservation genetics context, a minimum N_e of 500–5,000 has been suggested as necessary to secure populations from losing their evolutionary potential (Franklin and Frankham 1998), although it should be noted that empirical evidence supporting this contention is largely lacking, and any 'true' minimum viable N_e is likely to be largely defined by a given species' life-history. Nevertheless, in the present study, point estimates from both single-sample and temporal methods suggest contemporary effective population sizes of ca 100–1,000 individuals (Table S2), which falls within the lower threshold of sustainable N_e, and may be sufficient to explain why BOTTLENECK analyses failed to detect population declines.

The exploitation of migratory whitefish—most of which currently occurs on the feeding grounds—has a long history in Fennoscandia. Fisheries records for the specific rivers (i.e. spawning groups) studied here are unavailable, but may be inferred from catch and effort data for the Baltic Sea given that 70–80 % of commercial landings consists of anadromous whitefish (Aronsuu and Huhmarniemi 2004). Catch per unit effort data clearly show decreasing trends which should reflect concomitant declines in local census population sizes (Fig. 1). During the period studied (1981–2006), there has been a decrease of approximately 38 % in the landings from the Baltic Sea, despite a general increase in fishing effort. This led us to hypothesize that the effective population size of migratory whitefish might also be declining, as census size is generally considered to be proportional to effective population size (Tallmon et al. 2010). However, results showed no compelling evidence for a decrease of N_e over the study period (Tables 2, 3). The reasons behind this remain unclear, but below we discuss the possible effects of supplemental breeding and stocking.

Potential stocking effects

Given the drastic decrease in census population sizes, the IUCN has classified anadromous whitefish as an endangered species (Urho et al. 2010). In order to compensate for fisheries induced losses and support wild populations, approximately 50–60 million newly hatched fry and 6–8 million one-summer-old whitefish are released annually into the Gulf of Bothnia (Leskelä et al. 2004). Beginning in the 1970s, stocking also occurred in both rivers. In the Tornionjoki, intense and systematic stocking lasted until the end of 1990s, but has almost completely ceased in recent years. Stocking effort in the Kiiminkjoki has always been lower (E. Jokikokko, unpublished data). Stocking plans in the these two rivers have varied slightly from year to year, but have generally either used native broodstock collected each year from the rivers, or by stripping brood fish kept in a local hatchery. Multiple females are stripped for eggs which are then randomly fertilized with milt from hundreds of males in an effort to achieve adequate genetic diversity. Use of native broodstock may be a good management practice to conserve locally adapted genes, as a similar stocking program for Pacific herring, underway for the past 20 years, has shown no adverse genetic impacts (Kitada et al. 2009). However, for the most part, the long-term effects of stocking program on wild populations, particularly their effects on N_e, remain untested (Araki et al. 2007; Araki and Schmid 2010; Laikre et al. 2010).

Our analysis revealed no change in the contemporary effective population size over a 25 year period (Tables 1, 3), which would suggest no deleterious effects to N_e can be attributed to the large scale stocking which has occurred. Similar results were shown by Hedrick et al. (1995; 2000)

wherein releases of endangered winter-run Chinook salmon (*Oncorhynchus tshawytscha*) appeared to stabilize the overall effective population size. In contrast, reductions in N_e have been observed in stocked populations of Atlantic salmon, *Salmo salar* (Tessier et al. 1997), and anadromous brown trout, *Salmo trutta* (Hansen et al. 2002). Interestingly, we observed a trend towards reduced inbreeding in the later temporal samples, as evidenced by decreasing point-estimates of F_{IS} (Table 1). However, this must be interpreted cautiously since this indicator of inbreeding is a measure of homozygosity which, in turn, could be affected by genotyping errors associated with allelic drop outs in old samples due to degradation in DNA quality.

The temporal groups analyzed in the present study can be viewed as reflecting short- versus long-term impacts of the release of artificially reared stocks in the study rivers. Pairwise F_{ST} comparisons of spawning groups did reveal a trend towards decreasing genetic differentiation over time (Fig. 4). This could suggest that immigration or straying has increased in the two rivers as a consequence of stocking (Quinn 1993), a trend also observed in Atlantic salmon populations of the Baltic Sea (Vasemägi et al. 2005). Interestingly though, no such trend was observed in temporal comparisons between the summer and autumn ascending groups of the Tornionjoki (Fig. 4). However, these trends must also be interpreted with some caution

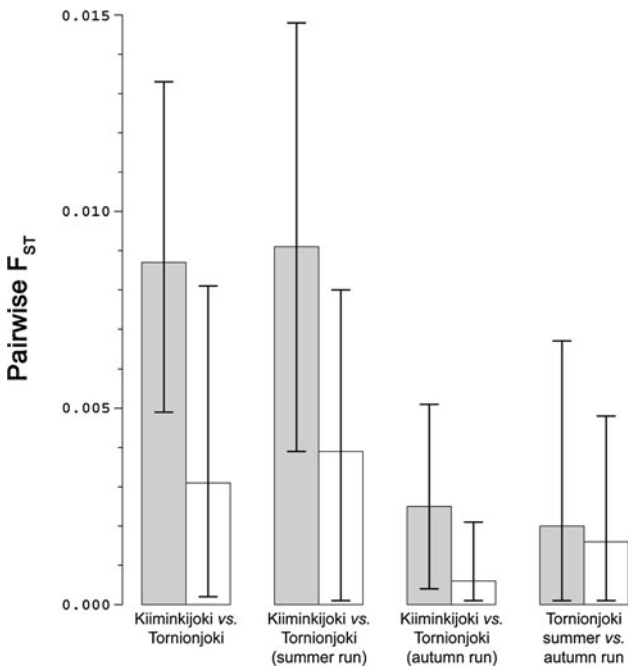

Fig. 4 Pair-wise comparisons of F_{ST}. Grey bars denote comparisons based on 1981 data; white bars represent data from 2000 (Tornionjoki, both runs) and 2006 (Kiiminkijoki)—note that in the first comparison, summer and autumn runs are considered as a single sample in Tornionjoki. Whiskers denote 95 % confidence intervals of the estimates, obtained from bootstrapping (10,000 iterations)

given that they are based on single point-samples from the different decades, and as such, they may also reflect typical inter-annual variation within this system.

Hatchery stocking had been adopted as a conservation effort to compensate declining populations, yet ironically, stocking has also been associated with deleterious genetic effects in many wild populations (Cross and King 1983; Allendorf and Ryman 1987; Hindar et al. 1991; Ryman and Laikre 1991; Waples 1991). In this respect, salmonid fishes have been the most studied species since 1950, and many such deleterious effects have been well documented through genetic and ecological studies, although some cases have also been observed where few effects were found (Araki and Schmid 2010). Stocking management practices largely depend on the size of the population, stocking intensity, genetic diversity of populations, released progenies, and gene flow among populations (Kitada et al. 2009). Maintaining genetic diversity of stocked populations through advanced breeding technologies may minimize genetic risks; however, stocking should not be assumed as a panacea for stock improvement. In this study we could not detect any noticeable change in the effective number of breeders between the two temporal groups representing short versus long-term impacts of stocking, despite a probable concomitant decline in the census population size over the same period. Whilst this could indicate that the stock enhancement program has helped to maintain N_e, we have no reference from a 'control' system to confirm this. Moreover, even if stocking were beneficial in the context of maintaining N_e, concomitant changes in pair-wise genetic divergence between spawning sites suggest that this benefit may come at the cost of decreasing population structure in the future. The extent to which this may negatively influence the long-term evolutionary potential and stability of the system remains an even greater uncertainty, but not one that should be ignored in light of current concerns over potential climate induced ecosystem changes.

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