Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (*Gadus morhua*)

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Despite increasing evidence that current exploitation rates can contribute to shifts in life-history traits and the collapse of marine fish stocks, few empirical studies have investigated the likely evolutionary impacts. Here, we used DNA recovered from a temporal series of archived North Sea cod (*Gadus morhua*) otoliths, to investigate genetic diversity within the Flamborough Head population between 1954 and 1998, during which time the population underwent two successive declines.

Microsatellite data indicated a significant reduction in genetic diversity between 1954 and 1970 (total number of alleles: 1954, 46; 1960, 42; 1970, 37), and a subsequent recovery between 1970 and 1998 (total number of alleles: 1970, 37; 1981, 42; 1998, 45). Furthermore, estimates of genetic differentiation (F_{ST} and R_{ST}) showed a significant divergence between 1998 and earlier samples.

Data are consistent with a period of prolonged genetic drift, accompanied by a replacement of the Flamborough Head population through an increased effective migration rate that occurred during a period of high exploitation and appreciable demographic and phenotypic change. Other studies indicate that diversity at neutral microsatellite loci may be correlated with variability at selected genes, thus compromising a population's subsequent recovery and adaptive potential. Such effects are especially pertinent to North Sea cod, which are threatened by continuing exploitation and rising sea temperatures.

Keywords: bottleneck; otolith; genetic diversity; fishing impacts; ancient DNA; match–mismatch

1. INTRODUCTION

Studies that explore the forces shaping genetic structure in wild populations have relied heavily on spatial comparisons among populations, or on temporal studies frequently of short duration, often with only two or three samples (Avice 1994; Frankham *et al.* 2002). Although such approaches have revealed valuable insights into the relative roles of factors such as genetic drift, selection and gene flow on genetic structure, it is often difficult to distinguish informative patterns from short-term fluctuations in gene frequencies. Advances in molecular methodologies, especially the PCR-based recovery of DNA from preserved material (Thomas *et al.* 1990; Hutchinson *et al.* 1999; Nielsen *et al.* 1999; Gómez & Carvalho 2000; Rosenbaum *et al.* 2000), provide new opportunities to explore long-term changes in populations over decades, and even across thousands of years (Limburg & Weider 2002). Where associated ecological data exist, it is possible to investigate the impact of natural and anthropogenic environmental change on population and community structure. One such opportunity is provided by the well-documented Atlantic cod, *Gadus morhua*, where detailed information is available on past demographic change in relation to fisheries-related mortality, together with an

archived collection of otoliths to provide a source of 'ancient' DNA (Hutchinson *et al.* 1999).

Gadus morhua, which is currently listed in the IUCN Red List of threatened species (IUCN 2002), represents a particularly valuable model for testing the hypothesis that highly abundant marine species may be vulnerable to erosion of genetic diversity when undergoing fishery-induced declines. Within the North Sea intense exploitation, exacerbated by rising sea temperatures (O'Brien *et al.* 2000), has contributed to the cod's recent well-documented collapse (Hislop 1996; Serchuk *et al.* 1996; Cook *et al.* 1997). The population is currently undergoing its third and most severe decline in spawning stock biomass (SSB) since the 1920s (figure 1a), and is also exhibiting a significant decrease in median age at maturity, which has been attributed to a fishery-induced selection for earlier maturation (Beacham 1983; Rowell 1993). Consequently, the availability of extensive demographic data and archived otolith samples, collected for managing the North Sea population, offers a unique opportunity to examine past levels of genetic diversity in relation to observed fluctuations in SSB. Cod are especially well suited for such a study as they typically exhibit high microsatellite allelic diversity and heterozygosity (O'Connell & Wright 1997; DeWoody & Avice 2000), and because rare allelic classes are more numerous, the evolutionary impacts of population declines (e.g. drift and inbreeding) are more easily identified (Ryman *et al.* 1995).

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Because endangered species often exhibit decreased levels of genetic diversity (Frankham *et al.* (2002) and references therein), which has been linked to reduced reproductive fitness (Madsen *et al.* 1999; Young *et al.* 2000; Ebert *et al.* 2002), it is important to examine the extent of genetic change in intensely exploited species. Such studies gain particular pertinence because fishery-related mortality has previously been associated with reduced genetic diversity (Pichler & Baker 2000; Jones *et al.* 2001; Hauser *et al.* 2002), though not exclusively so (Nielsen *et al.* 1999; Ruzzante *et al.* 2001).

In the present study we used an archived collection of North Sea cod otoliths to examine temporal changes in genetic structure using three microsatellite loci. The samples were collected across a period (1954–1998) during which there were two significant population declines associated with intense exploitation.

2. MATERIAL AND METHODS

(a) Sample collection

A series of otolith samples recovered from 72 cod spawned in each of the years 1954, 1960, 1970 and 1981, originating from an archive maintained by the Centre for the Environment, Fisheries and Aquaculture Science at Lowestoft, UK, were compared with a contemporary sample composed of 72 fishes caught in May 2000. The fishes, which were between 2 and 4 years old (table 1), were caught by research vessels or commercial boats fishing off the English Yorkshire coast in the western central North Sea (sampling area four of the ICES North Sea Roundfish Working Group (Jones 1978)) between January and August. The described sampling location was chosen to target specifically the Flamborough Head population (Houghton & Flatman 1981; Hutchinson *et al.* 2001). Although the 2- and 4-year-old fishes collected in May 2000 were spawned in 1998 and 1996, respectively, they will be referred to as the 1998-year class. A single sample of 72 mature cod (4 years old or older) caught in the Southern Bight (southern North Sea) in 1998 were included as a reference sample for estimating levels of divergence between the Flamborough Head temporal samples and the rest of the North Sea.

Untreated otoliths were stored in individual paper envelopes following removal from fishes. Although trace quantities of blood and tissue were evident on the surface of some otoliths, storage in damp warehouse conditions had resulted in significant DNA degradation, and thus ancient-DNA techniques were used to recover and PCR-amplify the DNA (Hutchinson *et al.* 1999).

(b) DNA extraction and microsatellite analysis

DNA was extracted from the contemporary tissue samples using standard phenol–chloroform procedures (Taggart *et al.* 1992), and from the archived otoliths using a specialized otolith technique described in Hutchinson *et al.* (1999).

Levels of genetic diversity within and among samples were assessed through the PCR amplification of three microsatellite loci *Gmo2*, *Gmo132* (Brooker *et al.* 1994) and *GADM1* (Hutchinson *et al.* 2001), using the conditions optimized to amplify DNA recovered from otoliths and the contemporary samples described in (Hutchinson *et al.* (1999, 2001), respectively. Loci that yielded small products (less than 220 bp) were specifically selected owing to the degraded nature of the recovered DNA. The identified microsatellite alleles were sized on 6% polyacrylamide gels using a Pharmacia ALFexpress automatic sequencer.

All sample extractions and preparation of PCR reactions were completed within a dedicated ancient-DNA laboratory, using negative controls throughout the extraction and PCR amplification stages.

(c) Statistical analysis

GENEPOP v. 3.1d (Raymond & Rousset 1995) was used to test conformity of allele and genotype frequencies to Hardy–Weinberg expectations, with the Markov chain method (3000 iterations) estimating levels of significance. Gametic phase disequilibria were tested for using Fisher's exact test (Haldane 1954) and the Markov chain method (3000 iterations).

Several measures were used to assess levels of genetic diversity over the study period. Observed (H_o) and expected (H_e) heterozygosities were estimated using GENEPOP. The effective number of alleles (n_e), a measure of the number of alleles in the population weighted by their frequencies, was calculated for all samples at each of the loci according to Hartl & Clark (1989).

The statistical significance of a decline detected in the total number of alleles across loci (n) between 1954 and 1970 was tested using a Monte Carlo simulation. A regression equation was calculated between time in years and n , and the slope of the regression line (b) was noted. The three samples were subsequently pooled and randomly divided into three sub-samples, prior to recalculating b . The process was repeated 1000 times to test whether the observed fluctuation in alleles represented a significant decline. Statistical tests were limited to the period between 1954 and 1970 because evidence for population mixing in 1981 (heterozygote deficiency in *Gmo2* and *GADM1*), and a significant genetic divergence between 1998 and earlier years (table 3), precluded the use of later samples.

A measure for detecting historic bottlenecks was also calculated. This was based upon the ratio (M) of the number of alleles to the range in allelic size at a given point in time (Garza & Williamson 2001). Garza and Williamson propose that M will decline during a bottleneck event because microsatellite frequency distributions are seldom normally distributed (i.e. rare allelic classes are found throughout the allelic range) and hence the number of alleles will decline faster than the overall allelic range.

The effective population size (N_e) of the Flamborough Head stock was estimated for the periods 1954 to 1960, and 1960 to 1970. The method used (Waples 1989) estimates N_e from the standardized temporal variance in allele frequencies due to drift ('variance effective size'). Confidence intervals (CI) (95%) were calculated according to Waples (1989). The harmonic mean number of spawning cod (N) in the Flamborough Head population between 1960 and 1970 (Houghton & Flatman 1981) was calculated using a corrected mean generation time for overlapping generations (Hill 1979), after adjusting the data to take account of the differing proportions of mature fishes in each age group (Hislop 1988).

Levels of genetic divergence between pairs of samples were estimated by calculating the proportion of genetic variation between and within samples using fixation indices based on the 'infinite allele' (Kimura & Crow 1964) and 'stepwise' microsatellite mutation models (Kimura & Ohta 1978). The computer packages ARLEQUIN v. 1.1 (Schneider *et al.* 1997) and RST-CALC v. 2.2 (Goodman 1997) were used to estimate genetic divergence among samples using $\theta_{F_{ST}}$ (Weir & Cockerham 1984) and $\rho_{R_{ST}}$ (Slatkin 1995), respectively. Significance levels were calculated using 1000 permutations. Where samples were com-

Table 1. Age composition of samples. Each of the yearly samples was composed of varying combinations of 2-, 3- and/or 4-year-old fishes from the same cohort.

year class		1954	1960	1970	1981	1998
age composition of samples	2 year old	23	72	0	32	36
	3 year old	18	0	0	25	0
	4 year old	31	0	72	12	36
	total	72	72	72	72	72

Table 2. Significant deviations from Hardy–Weinberg expectations due to heterozygote deficiencies, calculated using a Markov chain method.

(Fisher's exact test was used where no standard error values are given.)

sample	sample size	locus	F_{IS}	p -value	s.e.
1981: 3 year old	25	<i>Gmo2</i>	+0.068	0.0031	0.0005
1981: 3 year old	25	<i>GADM1</i>	+0.134	0.029	—
1981: combined	72	<i>Gmo2</i>	+0.073	0.0195	0.0019
1981: combined	72	<i>GADM1</i>	+0.084	0.0116	—

Table 3. Pairwise matrix of genetic differentiation between each of the five combined Flamborough Head temporal samples and the single 1998 Southern Bight sample of Atlantic cod (*Gadus morhua*) using θF_{ST} (above diagonal) and ρR_{ST} (below diagonal), with associated levels of significance: (*) $p < 0.05$, (**) $p < 0.01$ before a highly conservative sequential Bonferroni correction, ** $p < 0.01$, *** $p < 0.001$.

	1954	1960	1970	1981	1998	Southern Bight
1954		−0.001	−0.001	0.002	0.005	0.009
1960	0		−0.002	0.001	0.001	0.004
1970	0.011	−0.004		−0.002	0.008(*)	0.009
1981	0.012	−0.002	−0.007		0.017**	0.0154(*)
1998	0.032(*)	0.072**	0.104***	0.096***		0.0056(*)
Southern Bight	0.044(*)	0.088(**)	0.122***	0.111***	−0.002	

posed of multiple year classes (1954, 1981 and 1998) they were analysed both separately and pooled.

Sequential Bonferroni (Rice 1989) adjustments were made to account for multiple simultaneous tests ($\alpha = 0.05$). However, because sequential Bonferroni becomes highly conservative when more than five comparisons are made, thus resulting in an unacceptably high type-II error (Altman 1991), values that became non-significant following Bonferroni correction are displayed in parentheses.

Standard errors were calculated for n , n_e , M , θF_{ST} and ρR_{ST} by bootstrapping individuals within yearly samples across loci (1000 permutations).

3. RESULTS

(a) Ease of amplification and accuracy of genotyping

PCR-amplification success rates in the three loci were high, ranging from 80% to 100% per sample, showing no correlation with age, size or cleanliness of otolith.

(b) Deviations from Hardy–Weinberg and gametic phase equilibria

All samples conformed to Hardy–Weinberg expectations, with the exception of the 1981 sample that exhibited significant heterozygote deficiencies at the *Gmo2* and

GADM1 loci in the 1981 sample of 3-year-old cod and in the combined 1981 sample (table 2).

There was no evidence of gametic phase disequilibria.

(c) Temporal patterns of genetic diversity

A statistically significant decrease (Monte Carlo, $p = 0.005$) in the total number of alleles across loci (n) was observed between 1954 and 1970, subsequently increasing again between 1970 and 1998 (figure 1*b*). A similar, but delayed, decline and increase occurred in the total effective numbers of alleles across loci (n_e) (figure 1*b*). Fluctuations in mean observed (H_o) and expected heterozygosity (H_e) (not shown) largely reflected the trends seen in n_e , although to a lesser extent. H_o fell below H_e in 1981, yielding significant heterozygote deficiencies at *Gmo2* and *GADM1*.

A consistent loss of alleles from across the allelic distribution resulted in the ratio M declining steadily between 1954 and 1981, before stabilizing in 1998 (figure 1*c*). Garza & Williamson (2001) predict from empirical data that such a decline is indicative of a population undergoing a genetic bottleneck.

(d) Estimation of effective population size

Estimates of the effective population size (N_e) of the Flamborough Head population equated to 69 individuals

Figure 1. Fluctuations between 1954 and 1998 in: (a) SSB (thousands of tonnes) of cod (*Gadus morhua*) in the total North Sea (solid line) and Flamborough Head (dashed line) population (redrawn from Houghton & Flatman 1981; ICES 2001); (b) total number of alleles (n ; triangles) and total effective number of alleles (n_e ; circles) across loci; (c) mean M across loci; and (d) pairwise R_{ST} values between 1998 and earlier Flamborough Head samples ('temporal'; triangles), and between 1998 Southern Bight sample and the temporal series of Flamborough Head samples ('spatio-temporal'; circles). Standard errors were calculated by bootstrapping individuals within yearly samples across loci.

(30–222, 95% CI) for the period between 1954 and 1960, and 121 individuals (51–426, 95% CI) for the period between 1960 and 1970. By contrast, the estimated harmonic mean number of potentially mature cod in the Flamborough Head population (N) between 1960 and 1970 (Houghton & Flatman 1981) equalled 3.1×10^6 individuals, thus giving a N_e/N ratio of 3.9×10^{-5} .

(e) Population differentiation

Estimates of R_{ST} , and to a lesser extent F_{ST} , between the combined yearly samples (table 3) indicate successively greater divergence between the 1998 samples and the 1954, 1960 and 1970 samples, and finally a small decline in the 1998/1981 pairwise value (figure 1d, 'temporal'; F_{ST} , $p = 0.023$ to 0.001 ; R_{ST} , $p = 0.013$ to < 0.0001). A similar pattern is observed in the pairwise estimates of R_{ST} between the Flamborough Head temporal samples and a reference sample collected from Southern Bight in 1998 (figure 1d, 'spatio-temporal'). In tests between the combined yearly samples and individual age groups within years (full data available from authors), the 1981 third year class was significantly divergent from the 1981 second year class (F_{ST} , $p = 0.017$), the 1954 sample (combined classes: F_{ST} , $p = 0.013$; fourth year class: F_{ST} , $p = 0.005$) and the 1960 sample (F_{ST} , $p = 0.013$). Finally, the 1981 fourth year class differed significantly from the 1954 second year class (R_{ST} , $p = 0.028$).

4. DISCUSSION

This study illustrates the value of using archived otoliths for large-scale temporal genetic analysis, and lends weight to calls to use the extensive otolith archives present in many marine institutes (Wirgin & Waldman 1994; Sheridan 1995; Jamieson 1999) to investigate the impacts of long-term selective exploitation and environmental change on levels of genetic diversity and population structuring.

Results indicate marked genetic changes in sampled cod across a period during which the population also exhibited major fluctuations in SSB and directional shifts in life-history traits (Beacham 1983; Rowell 1993), characteristics often linked to intensive commercial fishing. Although the contemporary estimate of genetic diversity in the Flamborough Head population is comparable to fishes spawned in 1954, the allelic and genotypic compositions of fishes spawned in 1998 were markedly different. Exploitation may therefore result not only in selective genetic changes and reduced recruitment (Cushing 1995; Law 2000), but also in detectable fluctuations in genetic diversity and population integrity, even in a marine species with purportedly large population size. Below, we discuss the underlying genetic and demographic forces that may promote such temporal change, followed by a consideration of the evolutionary implications.

(a) The validity of using age-structured samples for temporal analyses of genetic diversity

Pooling of different ages of fishes was required to obtain suitable sample sizes, and because cod can become mature from the age of 2 years, these samples were composed of varying proportions of pre- and post-recruit fishes. It is unlikely, however, that age-related selection pressures could result in samples composed of older fishes (e.g.

1970) having a lower allelic diversity. Linkage between the microsatellite loci and selected loci, if present, is likely to be very weak, and the exceptionally high mortality rates observed in the first 2 years of the cod's life are substantially reduced in later years (Macer & Easey 1988). Furthermore, there is no evidence of a significant difference between pre- and post-recruit fishes within years.

(b) Change in genetic composition owing to enhanced genetic drift

(i) Decline in number of alleles

Analysis of the total number of alleles (n) across the three loci indicates a significant decline (Monte Carlo, $p = 0.005$) in allelic diversity between 1954 and 1970. However, the detected loss of alleles occurs during a period when the SSB for the total North Sea exhibited a limited twofold decline, and subsequently increased to a greater level of 253 000 tonnes in 1970. It is perhaps unexpected that this fluctuation in population size could result in the observed decline in allelic diversity, though several forces could operate to affect such a loss.

First, because large populations at mutation–drift equilibrium contain higher numbers of alleles than smaller populations, allelic loss is much more severe when suffering a proportionally comparable decline in population size, although the impact on heterozygosity would be minimal until significant inbreeding occurs (Ryman *et al.* 1995).

Second, the Flamborough Head population has undoubtedly undergone a greater reduction in size than that calculated for the total North Sea. Although regional demographic data are collected for most areas within the North Sea, estimates of SSB are typically only calculated for the total North Sea because of the assumption that the North Sea is a single panmictic population. However, panmixia is now questionable, as tagging studies (Bedford 1966; Daan 1978) and genetic analyses (Hutchinson *et al.* 2001) indicate the presence of up to four distinct cod populations in the North Sea, including the Flamborough Head population. Limited SSB data for the Flamborough Head population are available for the period between 1968 and 1977 (Houghton & Flatman 1981), which indicates a fivefold decrease in the SSB during this time, compared with a twofold decline in the combined North Sea data. Moreover, Houghton & Flatman (1981) noted that cod off Flamborough Head had experienced considerably higher levels of exploitation than other proximate populations, and thus the decline in SSB prior to 1968 is likely to have been greater than that observed in the combined North Sea data. Because catchability was seen to increase with declining SSB between 1968 and 1977 (Houghton & Flatman 1981), as observed prior to the 1990s Newfoundland cod collapse (Hutchings & Myers 1994; Walters & Pearse 1996), it is probable that the Flamborough Head population continued to decline at a greater rate than the total North Sea following this period.

Finally, it is generally accepted that the effective population size (N_e) in highly fecund marine teleosts, such as cod, might be between two and five orders of magnitude smaller than the census spawning size (N) (Hedgecock 1994; Grant & Waples 2000). For example, Hauser *et al.* (2002) detected a significant loss of genetic diversity in the New Zealand snapper (*Pagrus auratus*) during a 48-year decline, from which they estimated an N_e/N ratio of

10^{-5} . This value is comparable with the N_e/N estimates calculated in the present study from the observed variance in allele frequencies between 1960 and 1970. Factors pertinent to cod that may contribute to such a discrepancy include biased sex ratios due to dominant males monopolizing females (Hislop 1988; Bekkevold *et al.* 2002), disproportionate contribution of genotypes through egg production and larval viability increasing with female size (Marteinsdottir & Steinarsson 1998; Trippel 1998), and overlapping generations. Furthermore, it has been proposed that the chance spatial and temporal match or mismatch of spawning with suitable oceanic conditions (Cushing 1975; Hedgecock 1994) could result in the majority of the surviving recruits being spawned by a minority of the resident population. Turner *et al.* (2002) attributed an N_e/N ratio of 10^{-4} in a similarly fecund species, the red drum (*Sciaenops ocellatus*), to a high variance in individual reproductive success and spatial variance in the productivity of the spawning and nursery habitats. Additionally, a direct link has been identified between variations in haddock (*Melanogrammus aeglefinus*) larval survival and the timing of phytoplankton blooms off the Nova Scotian shelf (Platt *et al.* 2003). A similar match–mismatch process may play an important role in cod, where the length of the spawning period has been positively correlated with female age (Marteinsdottir & Steinarsson 1996), and may have been particularly pronounced during the unusually rapid increase in North Sea SSB between 1960 and 1970 (the ‘gadoid outburst’).

(ii) Decline in effective number of alleles

The total effective number of alleles across loci (n_e) declines between 1960 and 1981, lagging behind n . Such a lag might be expected in a declining population undergoing genetic drift. Rare alleles would be lost first, and thus n_e , a measure of the number of high-frequency alleles, will initially be less affected than n , and may even increase as the frequency of the common alleles rises proportionally through the loss of those that are rarer. The observed decline in the ratio M , which is due to rare alleles being lost from within the allelic distribution, is also indicative of a population undergoing a bottleneck (Garza & Williamson 2001).

(iii) Decline in observed heterozygosity

In addition to the loss of allelic diversity, a decline is seen in observed heterozygosity (H_o) between 1960 and 1981, which equates to the weaker trend seen in n . Because the reduction in population size is unlikely to have been sufficiently severe or enduring for major inbreeding to occur (Nei *et al.* 1975; Frankel & Soule 1981), such a disparity between the timing and magnitude of the decline in n and H_o might be expected.

(iv) Increasing divergence from proximate populations

If a sample collected from Southern Bight in 1998 is used as a reference point to calculate F -statistics (figure 1d), the changing composition of the Flamborough Head population is evident from the increasing divergence between 1954 and 1970. Such a trend could not occur through mutation, owing to the limited period of time, but is typical of an isolated population undergoing enhanced genetic drift through a substantially reduced effective population size.

(c) Genetic change owing to increased gene flow

The declines observed in n , n_e and H_o are followed by a subsequent rise between 1970 or 1981 (n , or n_e and H_o , respectively) and 1998, indicating an influx of novel alleles into the population. Such an influx is also concordant with the significant heterozygote deficiency observed in the 1981 sample at the *Gmo2* and *GADM1* loci, which is indicative of a population undergoing increased immigration, and the decreasing divergence between the Flamborough Head samples and the reference Southern Bight sample that occurs between 1970 and 1998.

It is unlikely that the observed changes in allelic composition are due to the sampling of different populations, as samples were collected consistently from the same location. Moreover, tagging studies indicate that the putative stocks along the Eastern English coast are spatially well separated with few individuals undergoing extensive migrations (Bedford 1966; Daan 1978). As such, we propose that as the Flamborough Head population declined substantially in size, occasional immigrants from more resilient genetically divergent populations exerted an increasingly significant impact upon the genetic composition of the recipient Flamborough Head population. Such a scenario is supported by evidence of new alleles and weak sub-structuring in the 1981 sample. By 1998, the Flamborough Head population was largely composed of immigrants from neighbouring population(s), as shown by congruence with the Hardy–Weinberg equilibrium, the higher allelic diversity of the more stable population, and the significant divergence from earlier samples.

The source of the migrants is, however, difficult to ascertain. The most proximate, currently least divergent, spawning aggregations occur around Aberdeen Ground and German Bight. Samples collected from these aggregations, described in an earlier study (Hutchinson *et al.* 2001), were significantly divergent from the 1970 (R_{ST} , $p < 0.05$) and 1981 (F_{ST} , R_{ST} , $p < 0.05$) samples, but not divergent from the 1998 sample. Other spawning aggregations within the North Sea are significantly divergent from the 1998 sample, and are less likely to have contributed migrants owing to the greater migratory distances required, and the limited migration of cod around the North Sea (Daan 1978).

To summarize, we propose that as the Flamborough Head population declined in size, genetic drift resulted in significant erosion of allelic diversity until a time between 1970 and 1981 when migration of genetically divergent individuals into the population became the dominant process, with a subsequent increase in allelic diversity. Although allelic diversity returned to levels comparable with 1954, such a process would have resulted in an effective loss of the Flamborough Head population, which comprised approximately one-tenth of the total North Sea cod prior to the 1970s decline (Houghton & Flatman 1981). Combined with the substantially higher levels of exploitation and greater decline, it may have been particularly vulnerable to such a displacement.

The validity of the above argument is based on variation at three microsatellite loci. While other studies may have employed more loci, there are several features that strengthen our interpretation of the data from the three loci. First, the information content of each locus, in terms of numbers of alleles across loci and the high frequency

of rare alleles, was high. It is well established that rare alleles are sensitive indicators of genetic drift (Frankel & Soulé 1981), especially in large marine populations comprising millions of individuals (Ryman *et al.* 1995). Second, three forms of genetic data provided congruent patterns indicating genetic change and possible population replacement: fluctuations in the numbers of alleles (n), changing allelic distributions (n_e , M , H_o and N_e) and the changing relationship between the Flamborough Head samples and the reference Southern Bight sample evident from the F -statistics (F_{ST} and R_{ST}). Such concordance across loci and statistics, in combination with contemporary and historical data on cod genetic structure (Hutchinson *et al.* 2001), demographic (ICES 2001) and tagging data (Bedford 1966; Daan 1978), provide support for the temporal changes postulated.

A similar temporal study by Ruzzante *et al.* (2001) found no evidence of a change in the pattern of genetic structuring or levels of genetic diversity in the collapsed Newfoundland cod. However, the authors suggest that although the populations declined by one to two orders of magnitude, final population sizes were still of sufficient size that any genetic effects were unlikely to be observed. Although the decline in the Flamborough Head population was not as great, the minimum size of the population is likely to be in the order of one magnitude lower than that of the Newfoundland cod. Furthermore, the use of equal sample sizes and five temporal sampling points afforded greater statistical power than was available in the Ruzzante *et al.* (2001) study. These factors may, in part, explain the discrepancy between the two studies.

(d) Evolutionary implications

The nature and extent of genetic changes observed here suggest that fluctuations in the SSB of cod are underlain by significant changes in genetic composition and population integrity. Estimates of genetic differentiation among populations appear to be highly correlated between molecular markers (e.g. F_{ST}) and adaptive traits (e.g. Q_{ST}) (Merilä & Crnokrak 2001), suggesting that, in certain circumstances, molecular estimates may be useful surrogates for estimating local adaptation (Carvalho *et al.* 2002). Although the relationship between molecular and adaptive genetic variability is complex and often unpredictable (Reed & Frankham 2001), loss of molecular allelic diversity may coincide with a loss of variation in alleles at neutral and weakly selected genes (Crow & Kimura 1970). In a study of 34 published datasets, Reed & Frankham (2003) found that population fitness was significantly positively correlated with heterozygosity, population size and quantitative genetic variation. Such processes may impact on a population's short-term survival and the long-term adaptive potential under changing environmental conditions. The apparent replacement of the 1954 Flamborough Head population through a combination of reduced SSB and immigration by genetically distinct fishes may thus have altered the range of adaptive potential in North Sea cod. Such a scenario would, however, depend crucially on factors including population size, selection pressures and levels of gene flow (Karhu *et al.* 1996). These processes are of particular relevance to a vulnerable species experiencing continued environmental change such as global warming (O'Brien *et al.* 2000).

The availability of archived biological collections (e.g. otoliths and fish scales), as well as natural repositories (e.g. resting eggs and skeletal materials), provide a valuable temporal dimension when examining evolutionary processes. Not only can historical genetic data provide insights into the impact of environmental change on population persistence and diversity, but importantly they also provide a basis for developing predictive estimates on the nature and extent of adaptability and evolutionary potential.

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