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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: MF0159
2. Project title: Genetic structure of cod (Gadus morhua) populations in the southern North Sea and English Channel
3. Contractor organisation(s): Centre for Environment, Fisheries and Aquaculture Science
   Pakefield Road
   Lowestoft
   NR33 0HT
4. Total Defra project costs (agreed fixed price): £ 64794
5. Project: start date ..... 01 December 2006
   end date ..... 31 March 2007

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The southern North Sea is an important area for the exploitation of cod by a number of fisheries (ICES, 2005). Unlike plaice (*Pleuronectes platessa*), for which separate stock assessments are performed, quota management of cod in ICES areas IVc and VIId has been based upon a combined stock assessment since 1996. The stock is currently at historically low levels and subject to a 'recovery plan' involving greatly restricted controls on landings from 2003 onwards. Fishers of the eastern Channel have claimed that the stock is healthier there than in the North Sea with more, and larger fish present. They argue that the Channel cod are a separate population, and that quota allocations should therefore be relaxed. However, recent studies of cod movements (Defra contract MF0158) suggest that cod in the eastern English Channel occasionally move in to the southern North Sea and remain there as adults. The consequences of this movement for the genetic structure of cod populations are not known.

A plethora of population genetic studies have been undertaken on cod and the reported extent of local and large-scale genetic differentiation has differed widely among studies. We undertook a programme of genetic sampling of cod in the eastern Channel and southern North Sea. The programme was different from those conducted previously because the sampling was designed to examine the evidence for stock separation during the spawning season and during the feeding season. The study had the following specific objectives: to provide a line of evidence regarding the mixing of cod stocks between the English Channel and the North Sea; to determine if gene flow is directional; and to combine genetic studies with outputs from studies of fish movements supported by other Defra contracts (MF0158, MF0154).

In total, over 1500 tissue samples were taken from cod in UK waters. Over 800 of these samples were genotyped at 16 gene loci in as balanced a design as possible. In consequence, the current study is the most detailed analysis of the genetics of cod in UK waters to date. The results of the genotyping analysis show that there is very low

genetic differentiation between the tissue samples collected and analysed in the current study. Assuming that the samples were representative of populations in the sampled regions, the results suggests that there is gene flow between the populations investigated in this study, and that there is no evidence for a separate sub-stock of cod in the English Channel. These results are supported by mark-recapture studies that showed that, while many cod tagged in the eastern Channel were recaught close to their point of release many months later, some individuals moved either north into IVc or west into VIIe. Detailed reconstructions of migrations of cod tagged with electronic data storage tags have shown that the northwards movements of some individuals represent pre-spawning migrations into the southern North Sea, and the westwards movements represent the post-spawning migrations of cod that spawned either in the southern North Sea or English Channel.

Together, the results of the tagging and genotyping research suggest that:

1. there is sufficient exchange of fish between the southern North Sea and the English Channel to prevent genetic differentiation between the two areas;

2. there was no evidence to suggest that cod sampled in the eastern Channel comprised sub-stocks, with some being resident individuals and some being migrants;

3. cod tagged in the eastern Channel in spring can undertake extensive migrations to feeding grounds in the western Channel, although many do not;

4. a significant number of the cod tagged in the eastern Channel either moved almost immediately to the southern North Sea (presumably in preparation for spawning) or, after migrating to feeding grounds in the western Channel, migrated eastwards the following year into the southern North Sea in readiness for spawning.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
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   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

Genetic structure of cod (*Gadus morhua*) populations in the southern North Sea and English Channel

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Introduction

Atlantic cod (*Gadus morhua*) are distributed across the continental shelves of the North Atlantic, including the North Sea and English Channel (Graham, 1948; Daan, 1978; Brander, 1994). Similar to many other commercial marine fishes in the North Sea, such as plaice (*Pleuronectes platessa L.*) and sole (*Solea solea),* seasonal changes in the distribution of cod are observed as a result of the migratory behaviour of individuals between feeding and spawning grounds (Graham, 1948; Bedford, 1966; Daan, 1978). In 1971, the ICES North Sea Roundfish Working Group summarised the first 20 years of cod tagging experiments in an attempt to establish the inter-relationships and exchange of cod between the North Sea and surrounding areas (ICES, 1971). Overall, the conclusions of these early analyses suggest that the dispersal of cod from the point of tagging and release in the North Sea is limited to a few hundred km (ICES, 1971; Daan, 1978). Indeed, a recent study of cod tagged in the northern North Sea suggested that average displacement was likely to be less than 100km irrespective of the time at liberty after tagging (Wright et al., 2006). This, in turn, points towards spatial structuring of the North Sea stock because it is unlikely that cod living in the northern North Sea (above 57°N) would mix with cod below 55°N in the southern North Sea (ICES, 1971; Robichaud & Rose, 2004). ICES (1971) identified the following regional groupings of cod: a) the Norwegian side of the Skaggerak; b) the Danish side of the Skagerrak; c) one or several coastal regions, from Flamborough to the Scottish east and north coasts; d) the central North Sea; e) the southern Bight, from the Straits of Dover to latitude 54 N; f) the English Channel, south and west of the Straits of Dover.

The southern North Sea is an important area for the exploitation of cod by a number of fisheries (ICES, 2005). Unlike plaice (*Pleuronectes platessa*), for which separate stock assessments are performed, quota management of cod in ICES areas IVc and VIId has been based upon a combined stock assessment since 1996. Pawson (1995), in re-summarising published cod tagging studies conducted up to 1971, concluded that the data support the movement of cod between the eastern English Channel and North Sea. This is due to a northwards and eastwards dispersal of juveniles from nursery grounds in the eastern English Channel. These fish, as adults, may then move back to spawning grounds in the Channel. However, previous analyses of cod movement in the southern North Sea and English Channel were based on limited sub-sets of the available data (Bedford, 1966; ICES, 1971) and did not distinguish between migrations away from or back to spawning sites. In consequence, cod in the North Sea (IV) and eastern Channel (VIId) have been assessed as one “stock” by the ICES North Sea and Skagerrak working group since 1996. The stock is currently at historically low levels and subject to a‘recovery plan’ involving greatly restricted controls on landings from 2003 onwards. Fishers of the eastern Channel have claimed that the stock is healthier there than in the North Sea with more, and larger fish present. They argue that the Channel cod are a separate population, and that quota allocations should therefore be relaxed. However, recent studies of cod movements suggest that cod in the eastern English Channel occasionally move in to the southern North Sea and remain there as adults. The consequences of this movement for the genetic structure of cod populations are not known.

A plethora of population genetic studies have been undertaken on cod and the reported extent of local and large-scale genetic differentiation has differed widely among studies. Earlier work suggested that all cod stocks mixed except across oceanic scales, whereas more recent

studies have revealed subtle, but significant differentiation, even at local scales. The sub-divisions in cod populations identified by ICES (1971) are supported by the results of recent genetic studies (Hutchinson et al., 2001). Genetically distinct sub-groups were found in four areas: the northern North Sea, the Moray Firth, Flamborough Head and the Southern Bight, broadly corresponding to the ICES stock assessment and management areas IVa, IVb and IVc/ VIId. These areas are large, however, and evidence is increasing that the movement of cod is much more restricted than previously thought (in the North Sea, Righton et al., 2001; Turner et al., 2002; Wright et al., 2006; Metcalfe, 2006; more generally, Green & Wroblowski, 2000; Lawson & Rose, 2000; Morris & Green, 2002; Robichaud & Rose, 2004), which may lead to fine-scale structuring of populations. Gene flow between the southern North Sea and eastern English Channel appears to be largely restricted to populations within the Southern Bight (southern North Sea) and Beachy Head (eastern English Channel). Spatial relationships between the southern North Sea and English Channel thus constitute a tractable and potentially informative geographic scale for more intensive genetic analysis of stock structure.

We have undertaken a programme of genetic sampling of cod in the eastern Channel and southern North Sea. The programme was different from those conducted previously because the sampling was designed to examine the evidence for stock separation during the spawning season and during the feeding season. The study had the following specific objectives:

- to provide a line of evidence regarding the mixing of cod stocks between the English Channel and the North Sea. The evidence is presented to Defra to help improve the management of cod fisheries, whether it supports the current management regime or not.

- to determine if gene flow is directional. The benefit of this analysis would be to determine if the Channel or southern North Sea populations act as sources or sinks for adjacent areas. Such knowledge is crucial to implementing recovery plans.

- to combine genetic studies with outputs from studies of fish movements supported by other Defra contracts (MF0158, MF0154). This is an innovative aspect of the research. New insights into spawning behaviour and locations will be made by comparing the results of genetic mixing to individual migration trajectories.

Methods

Genetic analysis

Tissue sampling

Cod specimens collected at fish markets, or directly sampled at sea during the spawning season (January to March) and the feeding season (June to August). Fin clips were taken from the dorsal fin of each specimen and preserved in 100% Ethanol before DNA extraction in the laboratory. Figure 1 and Table 1 summarise the locations and numbers of samples taken.

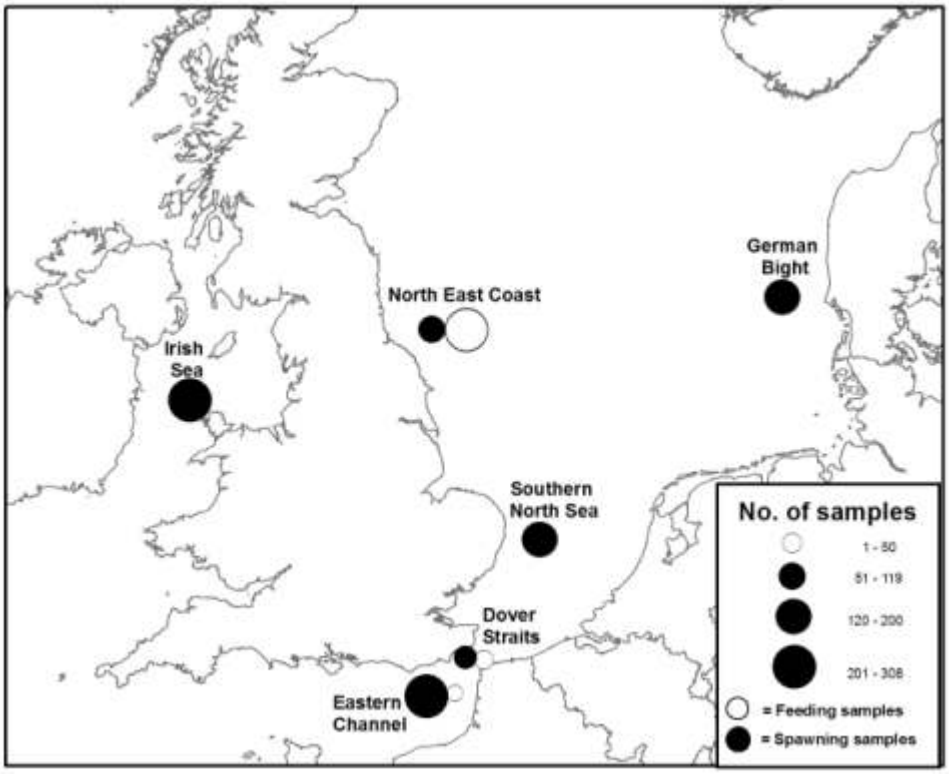

Figure 1. Location of sampling sites.

Region	*Spawning season*	*Feeding season*
North-east coast	110	268
German Bight*	200	
southern North Sea	170	
Dover Straits	119	50
Eastern Channel	308	42
Irish Sea	239	
Western Channel*	27	2
Total	1173	362

*Table 1. Summary of fin-clip samples collected and analysed during the project. *archived samples from previous years.*

Sample selection and DNA extraction

Although the number of spawning/ feeding sites sampled was large, and the number of fin-clips preserved was substantial, the sample set did not provide a balanced design due to inequalities in sample size between different sites and seasons. For this reason, not all samples collected were genotyped.

DNA was extracted in 96-well format from fin tissue using overnight digestion with Proteinase K. Fifty µl of each overnight digest was used for DNA extraction using a modified salt extraction protocol (Aljanabi & Martinez 1997). This resulted in a DNA extract with a concentration of ~20ng/ul. After genotyping, the remainder of the extracted DNA (~95µl) was transferred to a -80°C freezer for long-term storage.

Microsatellite amplification.

All samples were screened for variation at each of 16 microsatellite loci. Eight were dinucleotide loci: PGmo49, PGmo58, PGmo47 (Bjorg et al. 2005), Gmo132, Gmo1, Gmo2 (Brooker et al. 1994), GadM1, GadM2 (Hutchinson et al 2001), three were trinucleotide loci: PGmo32 (Bjorg at el 2005), Gmo35 and Gmo36 (Miller et al. 2000), and 5 were tetranucleotide loci: Tch11 (O'Reilly et al. 2000), Gmo3, Gmo8, Gmo19, Gmo34 (Miller et al 2000). Loci were amplified in 4 multiplex PCR reactions (MPX1, MPX2, MPX3 and MPX4) using D2, D3 or D4 WellRed fluorescently labelled primers on a BIORAD tetrad thermal cycler. 12.5 µl PCRs consisted of 6.25 µl Qiagen multiplex PCR mastermix, 1.25µl Q solution, 1.25µl of primer mix (see Table 2 for primer mixes), 3.25µl ddH_2O, 0.5µl DNA. The following amplification conditions were used 1 cycle of 15 mins at 94°C, followed by 40 cycles of 30 secs at 95°C, 30 secs at A°C (54°C for MPX1, 60°C for MPX2, 57°C for MPX3 and 55°C for MPX4) and 30 secs at 72°C, followed by a final cycle of 20 mins at 72°C.

MPX1			*MPX3*		
Locus	*F primer (µl)*	*R primer (µl)*	*Locus*	*F primer (µl)*	*R primer (µl)*
Gmo3	15	15	Tch11	13	13
GadM1	5	5	Gmo1	3.25	3.25
GMo35	10	10	GadM2	39	39
Gmo36	10	10	H2O	19.5	
Gmo34	5	5			
Gmo8	20	20			
MPX2			*MPX4*		
Locus	*F primer (µl)*	*R primer (µl)*	*Locus*	*F primer (µl)*	*R primer (µl)*
PGmo47	7	7	Gmo2	6.5	6.5
PGmo32	3.5	3.5	Gmo132	13	13
PGmo58	3.5	3.5	Gmo19	5	5
PGmo49	21	21	H2O	81	
H2O	70				

Table 2. Quantities of each primer (10µM) to be added to primer mastermix for each multiplex. Quantities shown (µl) are for 100 reactions (enough for a 96 well plate).

PCR products were then resolved on a Beckman Coulter CEQ8000 automated fragment analyser. Amplification standards and blanks were run on all plates to ensure consistent allele sizing across sizing runs and that no contamination had occurred. Fragment sizes were determined using the Beckman Coulter Fragment analyzer software.

Genotyping analyses

Allele frequencies, observed and unbiased expected heterozygosities under Hardy-Weinberg expectations were obtained using GeneClass2 (Piry et al. 2004). Departures from Hardy-Weinberg equilibrium (HWE) were tested for significance using the probability test implemented within GENEPOP 3.3d (Raymond & Rousset, 1995). Significance levels were determined using the Markov chain method (dememorisation number = 5000, batches=100, iterations = 2000). Genotypes at all pairs of loci were tested for linkage disequilibrium using the exact test implemented in GENEPOP version 3.1d. with significance levels determined by the Markov chain method (dememorisation number = 5000, batches=500, iterations = 10000).

FSTAT 2.9.3 (Goudet 2001) was used to estimate population differentiation using Weir & Cockerham's (1984) F-statistic θ. To estimate the statistical significance of differentiation G-statistics were calculated with 2000 permutations and 2000 bootstraps. Fisher's exact tests were performed (GENEPOP 3.3d) to test for differences in allele frequencies between populations as an additional indicator of population subdivision, with significance levels determined using the Markov chain method (dememorisation number = 5000, batches = 100, iterations = 2000).

Further investigation using Bayesian clustering was undertaken using the software STRUCTURE (Pritchard et al. 2000). These analyses focussed solely on the Eastern Channel and Southern North Sea populations. Two sets of analyses were performed. The first used both the Eastern Channel and Southern North Sea populations. Five runs were performed of each k (where k is the number of hypothesised clusters within the data set) between 1 and 3. Secondly, a single population (Eastern Channel) was investigated using 5 runs at each k between 1 and 3. This was performed to attempt to identify migrant individuals within a population of residents. For both analyses STRUCTURE was run using 200,000 iterations of the Gibbs sampler after a burnin of 100,000 iterations using the admixture model.

The large number of loci used in the study allowed a test for historical genetic bottlenecks in the population to be undertaken using the software Bottleneck (Cornuet & Luikart 1996) using the Infinite Allele Model.

Statistical power

Using a simulation procedure implemented in the software POWSIM we assessed the power for detecting population differentiation for the present set of marker loci. We focused on the probability of obtaining a significant result ($P < 0.05$) in contingency tests when sampling two populations employing sample sizes corresponding to those from our sampling regions (94 and 94 respectively). One hundred simulations, over 10 generations each were performed using the allele frequencies from the current data set as a starting point.

Tagging analyses

Mark-recapture data

We re-evaluated 4336 recaptures (of cod tagged with simple marker tags between 1964 and the present day to assess the seasonal changes in distribution, migration rates and exchange of juvenile and adult cod between the North Sea(ICES area IVc) and eastern English Channel (ICES area VIId). Recapture data for cod tagged spanned mostly between early1960's to mid-1980's. 70% of cod tagged in IVc were recaptured during the 1980's and 66% of cod tagged in VIId were recaptured during the 1970's. Any recaptures of cod that occurred within 90 days of release were excluded. This was to ensure that the movements were those of cod with sufficient time to migrate to different areas between the spawning and feeding periods, and that any same quarter recaptures remaining are of cod that had been at liberty for over a year after their release. Recaptured cod were all released during quarters 1 and 4 (1785 & 255 in IVc and VIId respectively). Only 101 (68 and 33 in IVc and VIId respectively) of the remaining cod recaptures were made during the quarters 1 and 4 in the first year after release. Recapture data were then sub-divided into quarters of the year according to the recapture season; winter and autumn (Q1 & Q4), spring and summer (Q2 & Q3), (see also Bedford, 1966 and ICES, 1971). Finally recaptures were placed into size categories dependent on the length of cod at recapture: smaller than 50cm (classed as 'juvenile') or larger than 50 cm (classed as 'adult').

Recapture positions for cod were plotted for IVc and VIId release areas in ArcView 9.0. The

Animal Movement Analysis Extension to Arcview (AMAE: Hooge and Eichenlaub, 2000) was used to estimate of the extent of geographical range for cod in each area by generating kernel probability density function surfaces (KPDF) for 95%, 70% and 50% volume estimates under the three-dimensional KPDF surface (see Worton, 1987, Seaman and Powell, 1996; Hooge et al., 2000). The KPDF method is more typically used in studies of territoriality and home range (Jones, 2005). However, because tag recapture locations are analogous to the density and distribution of the locations of single individuals over time, the technique is extremely applicable to population level mark-recapture data.

Electronic tagging data

Cod in the eastern English Channel and southern North Sea were fitted with electronic data storage tags between November 2004 and February 2006 using methods described in Righton et al. (2006). Data from all returned DSTs were downloaded and input into a database. Hydrostatic (tidal) data, derived from the sinusoidal pressure cycle recorded in the depth data when a fish is at rest on the seafloor, was used to enable the geographical reconstruction of a cod's movements (termed geolocations). This is referred to as the Tidal Location Method (TLM, as described in Metcalfe & Arnold 1997; Hunter et al 2004). Tidal ranges were extracted using a wave-fitting algorithm. For each day, the best-fitting wave-form was used to calculate the times of high and low water, the tidal range, and an indication of the quality of fit (sum of squares). Estimates of time of high water and tidal range were filtered to obtain the most accurate daily estimate (based on least sum-of squares). These data were then used in the TLM to derive, by day, possible geographic locations where the individual may have been. During the winter and spring, when most of the North Sea and English Channel is vertically mixed, daily average temperatures recorded by the DSTs were then compared to averaged sea surface temperature (taken from Bundesamt für Seeschifffahrt und Hydrographie, Hamburg), and positions at which DST temperature and SST differed by more than 2°C were excluded.

For some DST data, it was possible to trial a new method of geolocation that outputs daily probability fields, rather than unique locations, as estimates of fish position (Pedersen, 2007). The method was developed jointly by Cefas and Difres as a proof of concept, so only a limited number of DST records could be analysed. In consequence, the data returned from cod tagged in the English Channel were prioritised to test the hypothesis that cod in this region do not migrate into the southern North Sea.

Results

Genotyping

Polymorphism

All 16 microsatellite loci displayed high levels of polymorphism. The total number of alleles per locus within a single population varied from 2 to 38 (Table 3). Observed heterozygosity for a single locus within a population varied from 0.085 to 1.0. Observed and expected heterozygosities per population, allele number, size ranges and significant deviations from Hardy-Weinberg expectations are shown in Table 1. Before Bonferroni correction, 15 out of 176 tests revealed significant deviations from Hardy Weinberg equilibrium. After Bonferroni correction, 2 out of 176 tests revealed significant deviations from Hardy-Weinberg equilibrium, both at locus GadM2 (Table 3).

*Table 3a. Sample sizes, numbers of alleles, observed and expected heterozygosity for each population and each locus in the set Gmo3, GadM1, Gmo34, Gmo36, Gm035, Gmo8, PGmo32, PGmo49. Bold indicates a significant deviation from Hardy Weinberg proportions before Bonferroni correction. * indicates a significant deviation after table-wide correction.*

Sample set		Gmo3	GadM1	Gmo34	Gmo36	Gmo35	Gmo8	PGmo32	PGmo49
Collected in spawning season									
North-east coast	**N**	93	92	81	93	91	92	94	94
	Na	5	6	8	6	10	29	6	18
	Ho	**0.194**	0.543	0.642	0.602	0.791	0.891	0.266	**0.670**
	He	0.227	0.536	0.630	0.549	0.824	0.919	0.259	0.774
German Bight 2003	**N**	93	93	93	93	93	91	93	92
	Na	5	6	9	4	8	38	5	17
	Ho	0.172	0.441	**0.624**	0.473	0.882	0.879	0.247	**0.652**
	He	0.163	0.434	0.688	0.534	0.834	0.939	0.253	0.749
Irish Sea 2006	**N**	93	93	93	94	92	63	94	94
	Na	5	5	8	4	10	27	4	12
	Ho	0.194	0.473	0.634	0.574	0.815	0.905	0.266	0.777
	He	0.181	0.496	0.656	0.540	0.827	0.910	0.250	0.786
Dover Straits 2006	**N**	94	94	93	92	92	94	93	93
	Na	4	5	8	5	8	34	6	16
	Ho	0.117	0.479	**0.624**	0.587	0.793	0.915	0.247	0.806
	He	0.122	0.447	0.605	0.560	0.807	0.914	0.235	0.782
Southern North Sea 2004	**N**	92	91	92	91	92	91	92	76
	Na	4	5	8	3	7	31	5	10
	Ho	0.141	0.451	0.543	0.582	0.783	0.989	0.261	0.658
	He	0.144	0.436	0.630	0.509	0.808	0.940	0.235	0.711
Eastern Channel 2005	**N**	87	93	89	95	95	95	94	92
	Na	6	5	8	3	12	36	4	15
	Ho	0.218	0.581	0.584	0.579	**0.821**	0.926	0.234	**0.707**
	He	0.232	0.548	0.644	0.533	0.854	0.934	0.233	0.770
Eastern Channel 2006	**N**	63	64	63	63	64	62	64	62
	Na	5	7	8	4	10	30	5	13
	Ho	0.159	0.516	0.492	**0.349**	0.891	0.968	0.234	0.726
	He	0.179	0.526	0.546	0.419	0.826	0.935	0.241	0.796
Southern North Sea 2005	**N**	32	32	31	31	32	31	32	31
	Na	6	5	7	4	7	20	4	12
	Ho	0.188	0.531	0.581	0.71	0.75	1	0.25	0.806
	He	0.177	0.517	0.67	0.518	0.79	0.922	0.253	0.794
Collected in feeding season									
North-east coast 2006	**N**	86	94	92	94	93	93	89	80
	Na	5	5	8	4	11	35	4	13
	Ho	0.140	0.436	0.663	0.596	0.774	**0.860**	0.281	0.813
	He	0.133	0.426	0.632	0.551	0.830	0.928	0.261	0.789
Dover Straits 2004	**N**	46	46	46	46	46	46	47	46
	Na	5	4	8	5	7	21	4	14
	Ho	0.130	0.478	0.652	0.478	0.783	0.957	0.277	0.696
	He	0.125	0.466	0.642	0.559	0.822	0.917	0.251	0.807
Eastern Channel 2006	**N**	39	41	42	40	39	42	44	40
	Na	4	5	6	3	7	17	5	12
	Ho	0.103	0.439	0.571	0.525	0.744	0.905	0.250	0.800
	He	0.099	0.408	0.520	0.512	0.813	0.860	0.226	0.768

*Table 3b. Sample sizes, numbers of alleles, observed and expected heterozygosity for each population and each locus in the set PGmo47, PGmo58, Tch11, Gmo1, Gadm2, PGmo2, PGmo123, PGmo19. Bold indicates a significant deviation from Hardy Weinberg proportions before Bonferroni correction. * indicates a significant deviation after table-wide correction.*

Sample set		PGmo47	PGmo58	Tch11	Gmo1	Gadm2	PGmo2	PGmo132
Collected in spawning season								
North-east coast	**N**	81	94	93	92	93	94	93
	Na	4	8	19	8	25	14	25
	Ho	0.383	0.628	0.914	0.196	0.860	0.830	0.957
	He	0.387	0.551	0.929	0.203	0.913	0.851	0.921
German Bight 2003	**N**	92	93	94	94	93	93	90
	Na	2	7	23	7	29	16	24
	Ho	0.424	0.527	0.904	0.170	**0.817***	0.806	0.967
	He	0.419	0.528	0.927	0.161	0.914	0.838	0.921
Irish Sea 2006	**N**	91	94	94	94	94	93	91
	Na	4	8	20	6	23	18	26
	Ho	0.473	0.543	0.947	0.181	0.915	0.742	0.890
	He	0.482	0.543	0.923	0.180	0.908	0.857	0.924
Dover Straits 2006	**N**	92	94	94	94	94	94	91
	Na	2	7	22	7	23	13	28
	Ho	**0.283**	0.596	0.947	0.085	0.883	0.830	0.967
	He	0.375	0.645	0.924	0.093	0.899	0.858	0.925
Southern North Sea 2004	**N**	90	92	92	92	91	92	92
	Na	2	6	21	7	24	15	27
	Ho	0.378	0.630	0.880	0.152	**0.802***	0.826	0.946
	He	0.420	0.606	0.931	0.154	0.906	0.861	0.925
Eastern Channel 2005	**N**	84	94	96	95	95	93	94
	Na	3	7	19	6	24	17	26
	Ho	**0.310**	0.564	0.865	0.158	0.863	0.806	0.957
	He	0.411	0.546	0.924	0.150	0.920	0.871	0.934
Eastern Channel 2006	**N**	57	64	64	64	63	64	63
	Na	3	7	18	5	22	10	23
	Ho	0.421	0.578	0.984	0.125	0.889	0.734	**0.937**
	He	0.438	0.535	0.916	0.120	0.912	0.850	0.903
Southern North Sea 2005	**N**	27	32	32	32	32	32	32
	Na	2	6	18	3	20	12	14
	Ho	0.519	0.594	0.969	0.125	0.750	0.844	0.906
	He	0.417	0.572	0.919	0.119	0.895	0.837	0.906
Collected in feeding season								
North-east coast 2006	**N**	84	94	90	90	84	93	93
	Na	3	8	19	7	24	13	27
	Ho	0.333	0.500	0.911	0.144	0.869	**0.699**	0.871
	He	0.332	0.581	0.917	0.148	0.918	0.853	0.926
Dover Straits 2004	**N**	43	47	49	48	48	48	45
	Na	2	6	19	11	20	12	18
	Ho	0.256	0.574	0.980	0.271	**0.875**	0.938	0.867
	He	0.392	0.525	0.920	0.285	0.910	0.845	0.908
Eastern Channel 2006	**N**	38	44	38	44	39	43	38
	Na	2	7	17	6	17	12	17
	Ho	0.421	0.773	0.974	0.159	0.923	0.814	0.974
	He	0.332	0.600	0.925	0.172	0.904	0.839	0.862

Linkage disequilibria

Using exact tests, for genotypic disequilibrium, no loci showed evidence of linkage across populations ($P>0.05$), i.e. each locus is independent.

Statistical power

For the 16 loci scored, the number of alleles per locus, their frequency distributions, and the sample sizes used provide a statistical power sufficient for revealing population structure at an FST = 0.0025. The simulations indicated a true FST = 0.0025 would be detected with a probability of 0.96%, demonstrating the utility of the loci selected for detecting population structure in cod.

Evidence for genetic population structure between Southern North Sea and Eastern Channel populations.

Using Fishers exact tests, at each locus, and combined over all samples using Fishers (1954) method, indicated a single locus (Gmo35) showed a significant difference in allele frequencies before correction for multiple tests (P=0.031; Table 4). No significant difference was detected in allele frequencies between Eastern Channel and Southern North Sea after either sequential or non-sequential Bonferonni correction. No significant difference was found in allele frequencies between these populations when combining all loci (P=0.312).

An identical pattern was obtained using single locus comparisons of $F_{ST.}$ Fifteen of the 16 comparisons indicted F_{ST} was not significantly different from zero. A single locus (Gmo35) showed an F_{ST} significantly greater than zero (P=0.0288). However, after Bonferroni correction, none of the loci displayed an F_{ST} significantly greater than zero.

Locus	*Fishers exact tests (P-value)*	*Single locus F_{ST} (P-value)*
GadM1	0.17049	0.382
Gadm2	0.69803	0.1654
Gmo34	0.35675	0.3654
Gmo36	0.12863	0.1224
Gmo35	**0.03132***	**0.0288***
Gmo8	0.27266	0.1356
PGmo32	0.17775	0.1764
PGmo49	0.073	0.1158
PGmo47	0.63299	0.6362
PGmo58	0.44148	0.452
Tch11	0.46478	0.3612
Gmo1	0.86196	0.8614
Gmo2	0.92686	0.7046
Gmo3	0.45379	0.94
Gmo132	0.91443	0.9256
Gmo19	0.79952	0.8702
Over all loci	0.31188	0.4588

Table 4. P values from exact tests of allele frequencies and single locus F_{ST}s in Eastern Channel and Southern North Sea populations. indicates significant difference in allele frequencies before Bonferroni correction. No loci showed significant differences in allele frequencies after correction.*

Population structure- across all populations

There were no pairwise F_{ST} estimates that were significantly different from zero after Bonferroni correction (which is used to control the effect of sample size, and prevent the rejection of a correct null hypothesis) suggesting very low levels of population structure in cod populations surrounding the UK (Table 4). However, care needs to be taken when interpreting post-Bonferroni results, and so some consideration needs to be given to the significant differences *before* Bonferroni correction. These were found between the North-east coast (feeding) population and North-east coast (Spawning), West Irish Sea (spawning), Eastern Channel (spawning), Eastern Channel (feeding) and Eastern Channel 2006 (spawning). A difference was also found between Eastern Channel (feeding) and Eastern Channel 2006 (spawning) (Table 5).

	Scar (S)	GB (S)	WIS (S)	Folk (S)	Low (S)	East (S)	Scar (F)	Folk (S)	BeH (F)	Eas2 (S)	Low2 (S)
Scar (S)	0.000	0.000	0.002	0.001	0.001	-0.001	0.0014*	-0.001	0.005	0.000	-0.001
GB (S)		0.000	0.000	0.001	0.000	-0.001	0.002	-0.001	0.003	0.001	-0.003
WIS (S)			0.000	0.002	0.001	0.000	0.0041*	-0.001	0.0083*	0.000	-0.001
Folk (S)				0.000	0.000	0.001	0.001	0.001	0.004	0.001	0.000
Low (S)					0.000	-0.001	0.001	-0.001	0.005	0.002	0.000
East (S)						0.000	0.0013*	-0.002	0.0042*	0.000	-0.002
Scar (F)							0.000	0.000	0.0041*	0.0027*	0.001
Folk (S)								0.000	0.003	0.000	-0.003
BeH(F)									0.000	0.004	0.000
Eas2(S)										0.000	0.000
Low2(S)											0.000

*Table 5. Pairwise FST values between all population pairs. (S)=spawning sample, (F)=feeding sample. * significant before Bonferroni correction.*

Winter vs summer sampling.

Significant differences (before Bonferroni correction) were found between spawning aggregations and feeding aggregations at North-east coast, and between the North-east coast feeding sample and several other sites (Table 5; Figure 2).

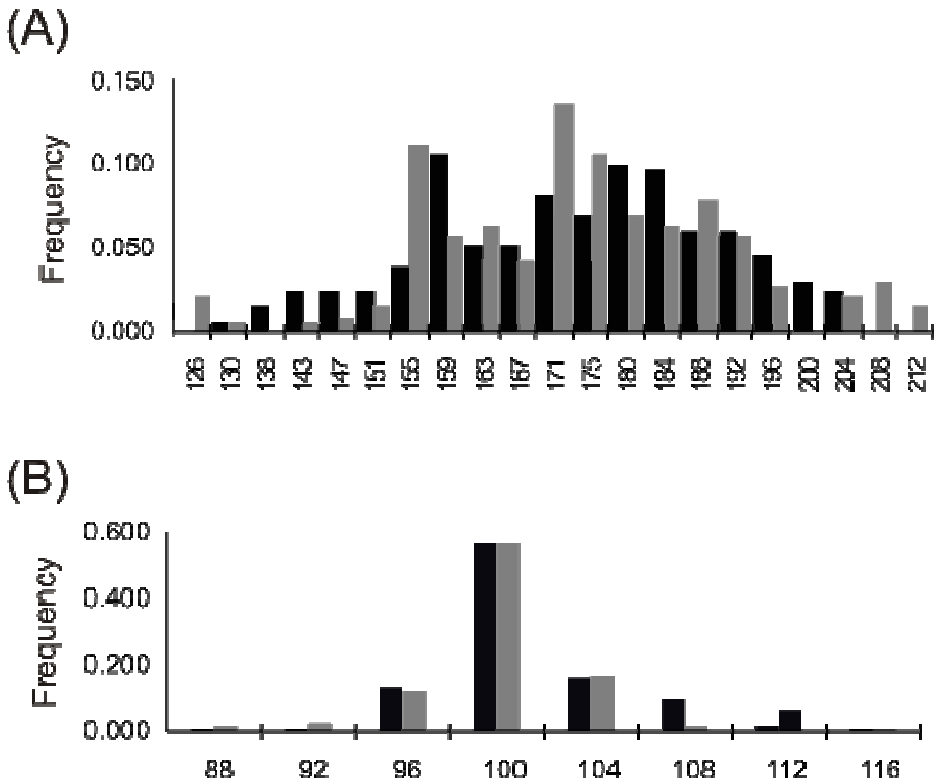

Figure 2. Example of allele frequency differences at North-east coast (spawning season- black bars) and North-east coast (feeding season-grey bars) at two loci: (A) Tch11 and (B) Gmo34.

Bayesian clustering

For the two different sets of analyses, similar outcomes were obtained. For the Eastern Channel and Southern North Sea samples, Log likelihood (Ln P(D)) scores ranged from -10062.8 to -10084.4 for k=1, -10069.3 to -10259.6 for k=2, and -10823.5 to -11949.0 for k=3. These results indicated the number of clusters within the data with the highest log likelihood was k=1.

When investigating a single population (Eastern Channel), Log likelihood (Ln P(D)) scores ranged from -5220.3 to -5235.6 for k=1, -5227.3 to -5269.6 for k=2, and -5249.9 to -5584.3 for k=3. These results again indicated the number of clusters within the data with the highest log likelihood was k=1. Thus, we could not identify immigrant individuals into the Eastern Channel population using STRUCTURE.

Population movements

Mark-recapture experiments

In both ICES areas, cod were recaptured further away from the point of release during summer than during winter (Table 6). In IVc, adult and juvenile recaptures were restricted to the coast close to their release sites during winter (Figure 3). In contrast, it was evident that both juvenile and adult cod mixed over a much wider area of the central North Sea during summer, after dispersing north away from the coast (Figure 3B&D; Table 6), with some adult cod dispersing as far as the Danish coastline. The recapture area for adult cod was therefore relatively large (Table 6). juvenile cod were more restricted in their dispersal compared to adult cod during summer and the size of the recapture area of 'juveniles' was similar for winter and summer (Figure 3A&C; Table 6). The average distances travelled (in km), and dispersion coefficients were both lower for juvenile cod compared to that of adult cod in IVc and emphasises the difference in their seasonal movements.

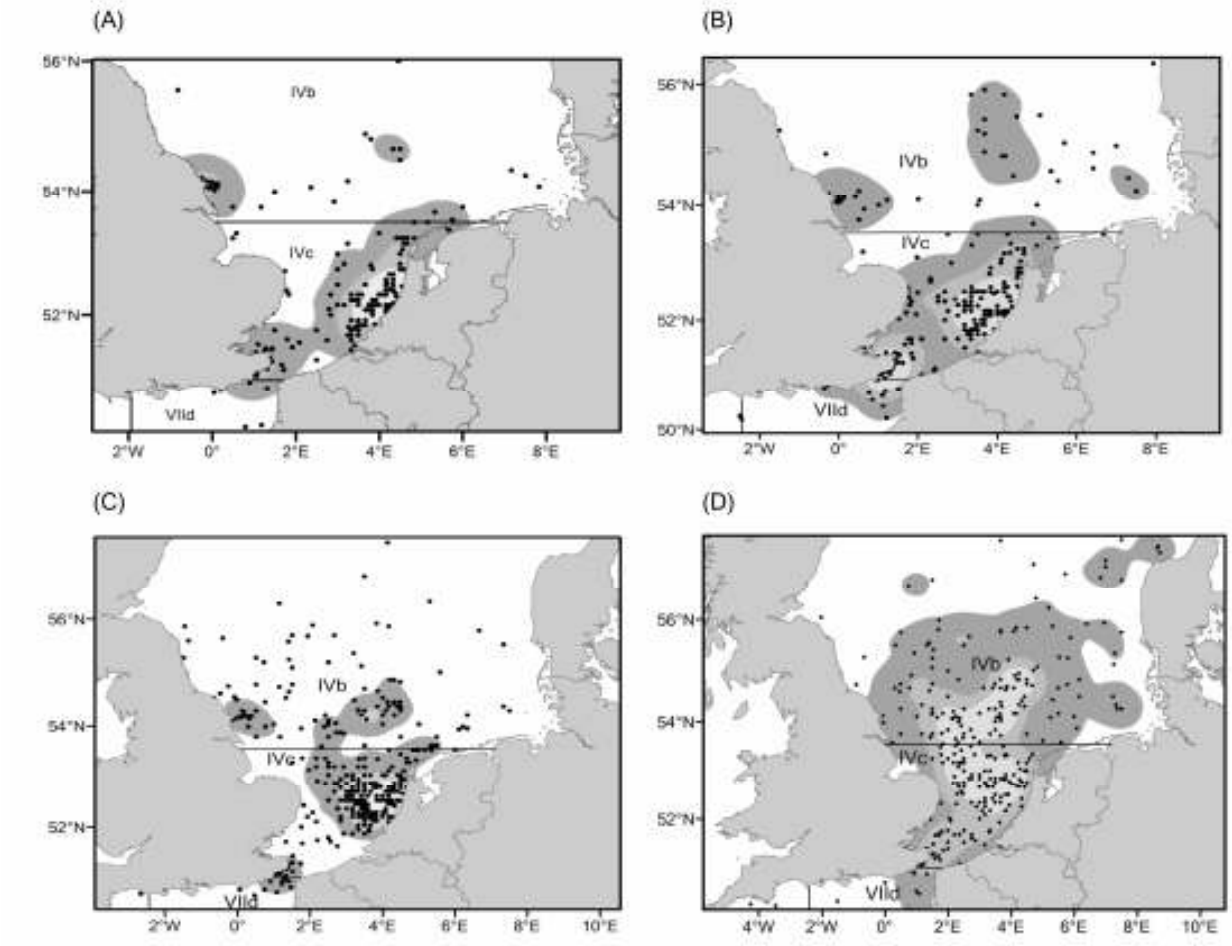

Figure 3. Recapture positions of cod released in ICES area IVc. Solid symbols show exact recapture locations, while shading shows the probability density surfaces for 50% (lightest grey), 75% (mid grey) and 95% (dark grey) of the recaptures. Data shown are for (A) 'juveniles' recaptured during the winter; (B) 'adults' recaptured during the winter; (C) 'juveniles' recaptured during the summer; and (D) 'adults' recaptured during the summer.

	'Juveniles'		'Adults'	
IVc:- North Sea	Winter	Summer	Winter	Summer
Total number of fish	319.0	715.0	349.0	402.0
Average time at liberty	271.0	154.0	441.0	383.0
Average distance (km)	82.0	125.0	114.0	222.0
Dispersion coefficient (a^2)	28.4	71.6	47.1	150.5
VIId:- English Channel	Winter	Summer	Winter	Summer
Total number of fish	67.0	43.0	86.0	59.0
Average time at liberty	252.0	157.0	588.0	457.0
Average distance (km)	53.7	57.0	151.0	213.0
Dispersion coefficient (a^2)	19.3	44.3	85.7	99.8

Table 6. Population movement parameters for each area of release, split by season of recapture (Autumn and winter Q1 & Q4; Spring and summer Q2 & Q3) and length class ('Juveniles': recaptures <50cm; 'Adults' recaptures >50cm).

Cod tagged in VIId did not exhibit such a clear pattern of movement (Figure 4). juvenile cod were generally caught close to their point of release within VIId at all times of the year with little eastwards or northwards movement (Figure 4A&C). Similarly 'adult' cod released in VIId were generally recaptured close to the area of release, but were also captured within the southern North Sea during Q4 & Q1 (Figure 4B) and throughout the central North Sea during Q2 & Q3 (Figure 4D). As for cod in IVc, juvenile cod did not move or disperse as far as 'adult' cod at any time of the year (Table 6). The difference in northwards movement between the size classes was particularly pronounced: 'adult' cod were recaptured approximately ten times further north than juvenile cod.

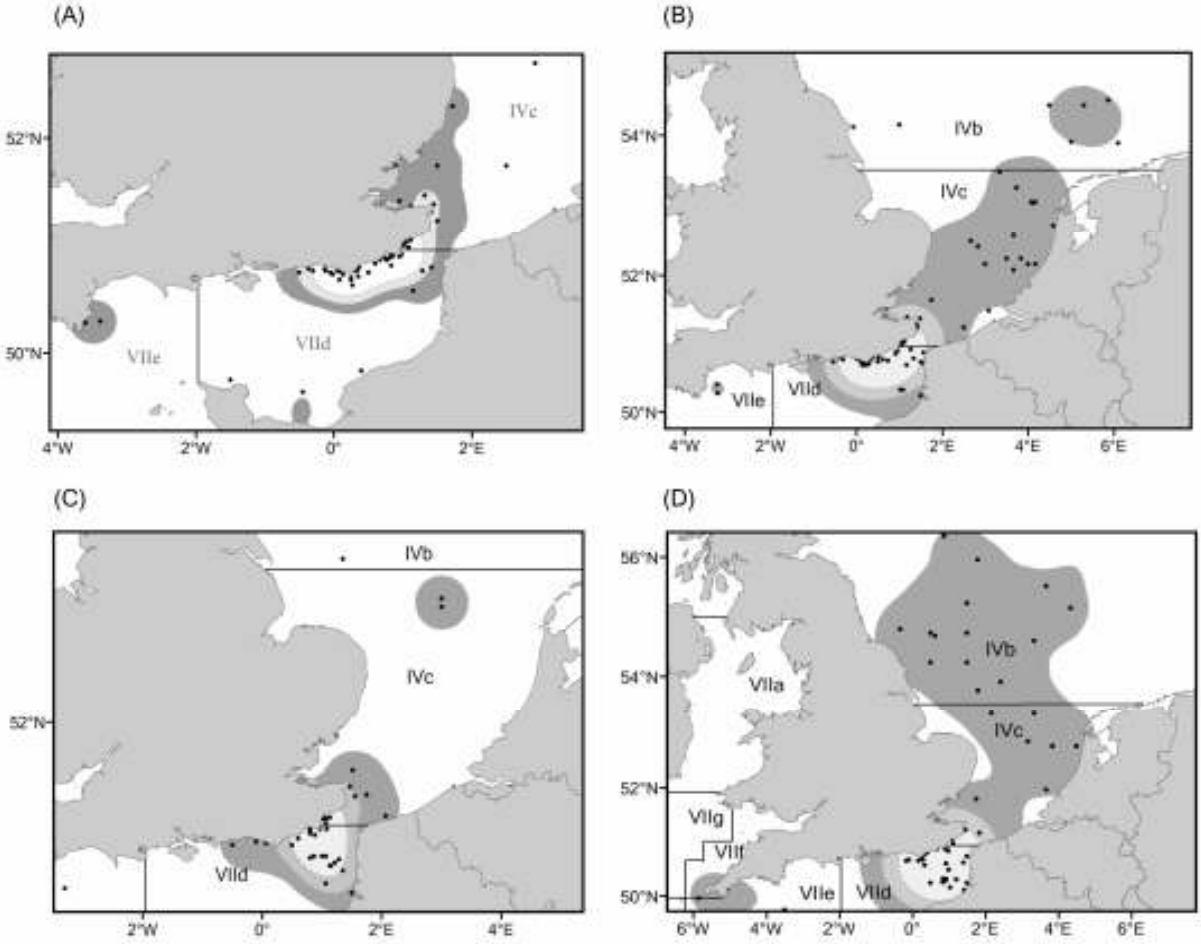

Figure 4. Recapture positions of cod released in ICES area VIId. Solid symbols show exact recapture locations, while shading shows the probability density surfaces for 50% (lightest grey), 75% (mid grey) and 95% (dark grey) of the recaptures. Data shown are for (A) 'juveniles' recaptured during the winter; (B) 'adults' recaptured during the winter; (C) 'juveniles' recaptured during the summer; and (D) 'adults' recaptured during the summer.

Individual migrations

Of the 9 cod returned after release in IVc, six could be geolocated between release and recapture using the tidal location method (TLM). Four of these cod were at liberty <75 days. Two of these cod migrated away from their release position on a north-eastwards bearing towards the Brown Bank. In their short time at liberty, these cod displayed evidence of selective tidal stream transport (STST; as described by Arnold et al., 1994). The other two cod stayed within 18km of their release position and spent the majority of their time close to the seabed, and occasionally ascended into mid-water at night to feed. Two cod (ID12237 and ID234) were at liberty for longer than 3 months. Figure 5A shows the migration of cod 12237. After an initial post-tagging movement north-east the cod undertook a period of rest off Knoll Deep for 14 days before continuing its migration north-eastwards. For the week following the 25th March 2005, the cod moved into mid-water at 13 h intervals, behaviour that is consistent with STST. On the 5th April 2005 STST was abandoned for 18 days as the cod consistently swam at depths between 5 - 20 m. In the two weeks between 23rd April and recapture on the 4th May, the cod exhibited further evidence of STST as it moved towards the Horn North Ground off the coast of Denmark. Figure 5B shows the southwards migration of Cod 234. The cod displayed a moderate and continuous pattern of vertical movement activity as it migrated south within shallow coastal waters towards South Falls. From the 1st June 2005 the cod increased its active movement with evidence of STST to aid southeast movement towards French coastal waters where it was recaptured on the 15th July 2005.

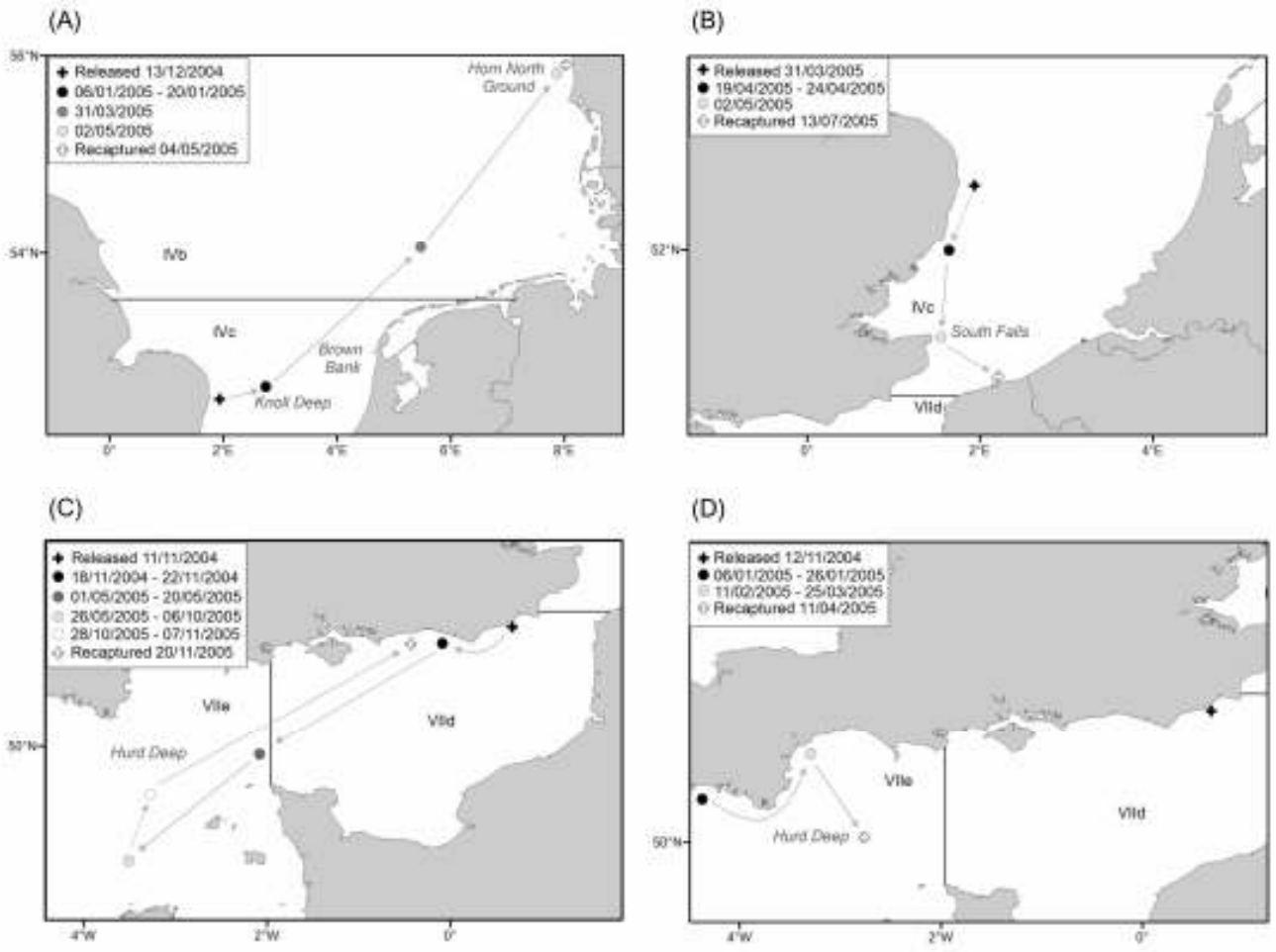

Figure 5. Migrations of cod tagged in ICES area IVc and VIId. (A) cod 12237, (B) cod 234 (C) cod 6448 and (D) cod 6433.

37 cod were returned after release in VIId. Ten of these cod were at liberty less than 92 days and stayed within 10km of their release position close to the seabed (<40m), while 11 moved east into the southern North Sea and were caught between Folkestone and Oostende. Two other cod moved just southwest of their release position into deeper waters (<60m), where periods of high activity were observed during the hours of darkness. Both of these cod moved back into shallower waters before being recaptured within 7km from their release position. One other cod undertook a south-west migration into deeper waters towards the edge of Hurd Deep (an area within the neighbouring ICES management area of VIIe), and was recaptured just to the east of the Hurd Deep after 8 months at liberty. Two cod (ID6448 & ID6433) migrated considerable distances during their time at liberty. Figure 5C & D show the reconstructed migrations of these cod. Figure 5C shows a year-long return migration by cod 6448. The cod initially remained within shallow waters (<40m) close to the seabed with occasional bouts of nocturnal activity until the end of April 2005. From 1st May 2005 the cod migrated south-west into deeper waters (60 – 70m). On the 7th November 2005 it began to migrate back towards coastal waters before being recaptured off Hastings on 20th November 2005 16km away from its original release position. Figure 4D shows the southwest migration by cod 6433. The cod initially migrated southwest into deeper waters (<60m) until the end of November then moved on towards shallower waters on 1st December (<30m) until the end of December 2004. Throughout January 2005 the cod resided off the Cornish coastline. From the 4th February 2005 the cod began to move back round the coast off Exmouth between the 11th February – 25th March 2005. From 27th March 2005 the cod migrated away into deeper waters (<60m) before being captured within the area of the Hurd Deep.

The migrations of cod ID6448 (2+ years old at capture) and cod ID1186 (3+years old) have been reconstructed on a probabilistic basis using a newly developed method. Both cod moved gradually west after release and arrived at feeding grounds in the Hurd Deeps in May 2005 (Figure 6 & 7). Cod ID6448 moved into ICES area VIIe, off the coast of Britanny (Figure 5). Cod ID1186 travelled much further west and into the Celtic Sea (ICES VIIh; Figure 6). Both cod remained on the feeding grounds for over six months before migrating rapidly east towards their release position in November. Cod ID6448 was caught close to its point of release in November 2005 (Figure 6), while cod ID1186 survived into January 2005 when it was caught in the southern North Sea. Reconstructions of tags ID6440, ID6433 and ID6423 also suggest movement into the southern North Sea during their time at liberty. More detailed results from the geolocation method will be presented as part of Defra-funded project MF0154.

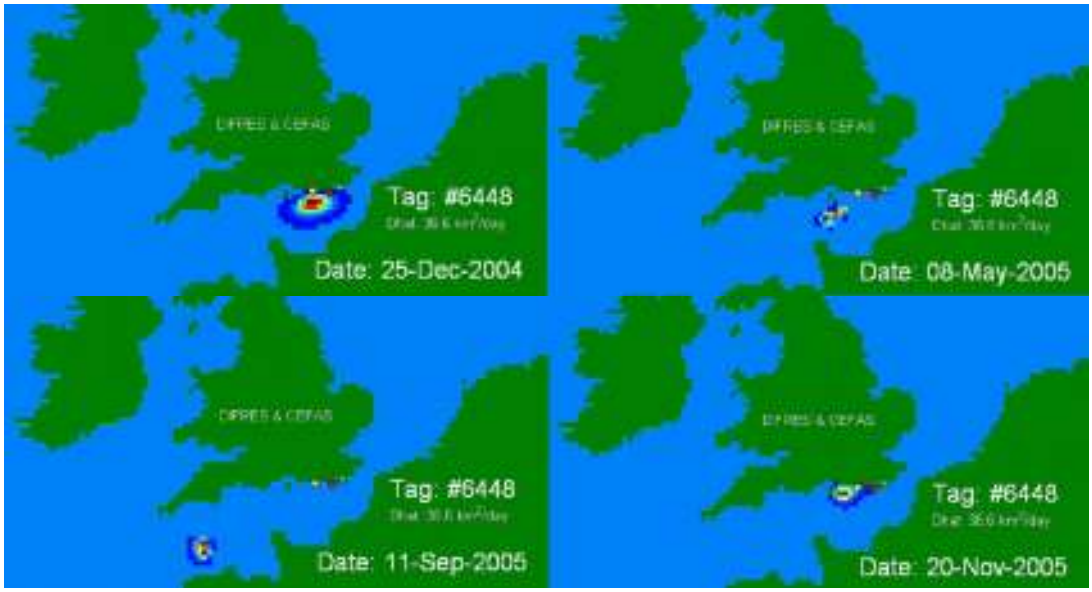

Figure 6. Migration of cod ID6448. Probability of location is shown by the shaded area, where red shows the most certain, and dark blue the least certain. The green triangle shows the release position, the red triangle the reported recapture position, and the yellow triangle the recapture position estimated from the geolocation technique.

Figure 7. Migration of cod ID1186. Probability of location is shown by the shaded area, where red shows the most certain, and dark blue the least certain. The green triangle shows the release position, the red triangle the reported recapture position, and the yellow triangle the recapture position estimated from the geolocation technique.

Discussion

Fish tagging and genetic methods have been applied to the study of stock structure in cod in the past, but have never been integrated in this way. Our combination of genotyping, mark-recapture analysis, and the detailed reconstruction of individual migrations is a first step towards aligning the sometimes contradictory evidence that can result from studies that attempt to discriminate stocks using samples collected in different ways, at different times and in different locations. The genetic and behavioural results of our study strongly support the interchange of spawning fish between the English Channel and the southern North Sea, and identify the eastern Channel as a migration 'highway' between the two areas in the spawning season.

Significance of the genotyping results

The simulations undertaken using POWSIM indicate that the number of individuals and the numbers of loci used in the present study provide a high power to identify genetic structure at very low levels. As a result of these we are confident that genetic structure would have been identified if it were present. The current study has used more samples and more loci than any other study of cod in UK waters.

There were no significant pairwise F_{ST} values after correction for multiple tests indicating very low genetic structure between the cod populations investigated in the current study. Additionally, none of the spawning samples showed a significant FST value before Bonferroni correction. The spawning season should be the period of maximum stock integrity, and this suggests there is gene flow between the populations investigated in this study. Of particular relevance, there were no differences between the Eastern Channel samples (English Channel) and the Southern North Sea samples (southern North Sea). However, some small differences (before, but not after Bonferroni correction) were found between the North-east coast feeding

sample, the North-east coast spawning sample and some other populations. Immigration from populations not sampled in the current study during the summer months into the North-east coast area is one explanation that may explain these findings.

Bayesian clustering was attempted on the microsatellite data, however, the levels of differentiation between populations were very low to non-existent, and was too low for the algorithm to detect, even though we have used a high number of microsatellite loci in the analysis.

Overall, the results suggest that:

1. The genetic analysis of 16 loci was sufficient to detect genetic structure;

2. There was no genetic differentiation between samples collected in the southern North Sea and eastern English Channel;

3. There was no evidence of genetic structure between populations sampled at any sites during the spawning season;

4. There was evidence of weak but significant structure between North-east coast feeding population and most other sites;

5. There was evidence of genetic differentiation between tissue samples collected at North-east coast in the summer vs those collected in the winter;

6. Outlier analysis suggested that the loci Gmo36 (trinucleotide) shows unusual signal, but not Gmo132.

Significance of the tagging results

The mark-recapture data showed that movement of cod released in IVc resulted in a net movement of cod into ICES area IVb during spring and summer quarters. In contrast, cod in VIId were nearly always recaught within VIId at all times of year, although some individuals strayed either north into IVc or west into VIIe. There were relatively small overlaps between both juvenile and 'adult' areas of recapture. juvenile areas hardly overlapped at all, suggesting discrete nursery grounds in IVc and VIId, but there was overlap between adult spawning areas during the spawning season. Cod tagged in both areas showed fidelity to the area of their release during the autumn and winter quarters and to feeding grounds during the spring and summer quarters, with no cod from the southern North Sea moving down into the English Channel or vice versa. Cod tagged in IVc moved away from their release position on a predominantly north-east bearing, whilst cod tagged in VIId moved westwards from their release position into deeper water.

However, it should be acknowledged that the mark-recapture data are limited in two ways. First, the number of recaptures for cod tagged in VIId was relatively small. Thus, while we can be confident that the migratory patterns we describe for cod tagged in IVc are genuine and robust, the results for cod tagged in VIId are less so. Second, very few tagging experiments have been conducted during summer on cod in the North Sea or the English. Hence, while we are able to determine the movements of cod between winter and summer, we were less able to quantify with any certainty, for example, whether cod tagged in IVc during summer might move into VIId to spawn during the autumn and winter or vice versa. For this reason, the detailed reconstructions of migrations of cod tagged with electronic data storage tags are very valuable, which show that cod tagged in the Channel in spring may make return migrations into the southern North Sea in readiness for spawning.

Overall, the tagging results suggest that:

1. cod tagged in the eastern Channel in spring can undertake extensive migrations to feeding grounds in the western Channel, although many do not;

2. a significant number of the cod tagged in the eastern Channel either moved almost immediately to the southern North Sea (presumably in preparation for spawning) or, after migrating to feeding grounds in the western Channel, migrated eastwards the following year into the southern North Sea in readiness for spawning;

3. mixing of sub-stocks may occur during the feeding season, but limited sample sizes, sample areas and uneven sample sizes hinder the significance of this analysis.

Conclusions

Together, the results of the tagging and genotyping research suggest that:

1. There is sufficient movement and exchange of fish between the southern North Sea and the English Channel during the spawning season to prevent genetic isolation, as demonstrated by the lack of genetic differentiation between the two areas;

2. there was no evidence to suggest that cod sampled in the eastern Channel comprised sub-stocks, with some being resident individuals and some being migrants;

3. The potential for rapid movement of adult cod during the spawning season makes the design of sampling efforts extremely difficult. New studies that integrate tagging and the genotyping of tissue samples from all life history stages (eggs, larvae, juveniles and adults) will enable more definitive statements to be made on the true nature of genetic structure of cod stocks in UK waters.

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Actions resulting from the work

1. One paper of direct relevance to the work is in press with the Journal of the Marine Biological Association (UK).

Righton, D., Quayle, V. A., Hetherington, S., and Burt, G. (2007). Movements and distribution of cod (*Gadus morhua* L.) in the southern North Sea and English Channel: results from conventional and electronic tagging experiments. Journal of Marine Biological Association, UK, 87, 5464/1-15.

2. This SID5 report will be redrafted as a manuscript for peer-review with the addition of more detailed reconstructions of individual cod migrations. The manuscript will be submitted to Marine Ecology Progress Series, and presented at the AFBI/Cefas/FRS mini-symposium on cod stock structure at AFBINI in May 2007.

Gary Carvalho, Victoria Quayle, David Righton, Martin Taylor. Genetic structure of cod (*Gadus morhua*) populations in the southern North Sea and English Channel.

5. An article for Fishing News on the results of the latest Cefas DST lottery is being published in Fishing News. This article contains details of the cod tagging programme in the English Channel. A second article on the genetic/ tagging work will be prepared for Fishing News when the SID5 has been approved by Defra.

5. The results of work will inform the development of a proposal to the Defra-NERC marine bioresources programme.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Righton, D., Quayle, V. A., Hetherington, S., and Burt, G. (2007). Movements and distribution of cod (*Gadus morhua* L.) in the southern North Sea and English Channel: results from conventional and electronic tagging experiments. Journal of Marine Biological Association, UK, 87, 5464/1-15.

Righton, D. Channel fisherman nets £1,000 for single cod. Fishing News, in press.