Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability

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Abstract: Polymorphism at six microsatellite DNA loci among cod larvae sampled repeatedly over a 3-week period from an aggregation on Western Bank of the Scotian Shelf provided evidence of several heterogeneous groups within the aggregation. There was strong evidence of heterozygote deficiency and departure from Hardy–Weinberg expectations for the larval aggregation as a whole ($N = 1337$) and for all larvae sampled within a single water mass (CW larvae), but not for a subset of these larvae considered to be part of a single cohort on the basis of age at length. These results suggest that both the entire aggregation and the CW subset originated from several distinct spawning events involving spawners with heterogeneous allelic compositions, but that the larvae forming the cohort originated from a single spawning event. Our results establish a link between the ecological match–mismatch hypothesis and the genetic "sweepstakes" selection hypothesis. There was no evidence that the larvae originated from different populations as measured by $(\delta\mu)^2$ distance, R_{ST}, and F_{ST} estimates among subsets. Additional analyses showed the larval cohort to have greater genetic similarity to adult cod sampled on Western Bank 2 years later than to adult cod sampled on Banquereau Bank (≈150 km away) also 2 years later. These results suggest that the genetic composition of cod on Western Bank remains stable over time.

Résumé : Le polymorphisme observé à six loci de microsatellites de l'ADN chez des larves de morue échantillonnées de façon répétée, pendant une période de 3 semaines, sur le banc Occidental de la plate-forme néo-écossaise a prouvé l'existence de plusieurs groupes hétérogènes dans cette concentration de larves. Les indications étaient fortes d'un défaut d'hétérozygotie et d'un écart par rapport à la loi de Hardy–Weinberg pour l'ensemble de la concentration de larves et pour toutes les larves échantillonnées dans une seule masse d'eau (eau de crête, larves CW), mais non pour un sous-ensemble de ces larves considéré comme faisant partie d'une seule cohorte d'après l'âge selon la longueur. Ces résultats permettent de penser que la concentration entière et le sous-ensemble de larves CW provenaient de plusieurs épisodes distincts de fraye impliquant des géniteurs présentant des compositions alléliques hétérogènes, mais que les larves formant la cohorte provenaient d'un seul épisode de fraye. Nos résultats ont établit un lien entre l'hypothèse écologique bon assortiment – erreur d'assortiment et l'hypothèse génétique de sélection « sweepstake. » Rien n'indiquait que les larves provenaient de populations différentes, d'après les estimations de la distance $(\delta\mu)^2$, de R_{ST} et de F_{ST} entre les sous-ensembles. Des analyses additionnelles ont montré que la cohorte de larves présentait une plus grande similarité génétique avec les morues adultes échantillonnées sur le banc Occidental 2 ans plus tard qu'avec les adultes capturés sur le Banquereau (≈150 km de distance) également 2 ans plus tard. Ces résultats permettent de penser que la composition génétique de la morue du banc Occidental reste stable au fil du temps. [Traduit par la Rédaction]

Introduction

The population genetic structure of a marine species is in part determined by recruitment variation in time and space. Information on the underlying causes of such variation can only be obtained by examining ecological and genetic changes occurring at the spatial and temporal scale of the oceanographic processes (mixing, advection, diffusion, etc.) directly influencing the distribution and abundance of the population under study; i.e., resolving biological processes at or below the scales of the physically driven processes (Roughgarden et al. 1988; Taggart and Frank 1990).

Most marine animals with pelagic eggs or larvae are highly fecund and abundant species that exhibit large variation in recruitment. They also show major discrepancies between observed and effective population size (Hedgecock 1994). The large recruitment fluctuations frequently observed among such species are generally attributed to factors affecting early life stages (Hjort 1914; Cushing 1972; Houde 1987; Peterman et al. 1988; Taggart and Frank 1990). When fertilization is external, its success will depend on oceanographic processes that vary spatially and temporally and thus affect gonad maturation rates, spawning time, and the dispersal of sperm and ova (Levitan and Petersen 1995). These same processes can equally affect the survival of postfertilization stages, for example, by

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directly transporting eggs and larvae to, or away from, nursery grounds or suitable settling areas (Iles and Sinclair 1982; Roughgarden et al. 1988; Possingham and Roughgarden 1990; Farrell et al. 1991).

Several hypotheses have been offered to explain the large discrepancies between observed and effective population numbers (reviewed in Hedgecock 1994). These include (*i*) large disparities between contemporary and historical (or long term) effective population sizes (i.e., bottleneck effects; Hedgecock et al. 1982; Nei and Graur 1984; Avise et al. 1988; Palumbi and Wilson 1990), (*ii*) diversity-reducing selection (Nei and Graur 1984), and (*iii*) large variance in the number of progeny among families (Hedgecock et al. 1982; Avise et al. 1988; Palumbi and Wilson 1990). In particular, Hedgecock (1994) suggested that the large disparity between effective and observed population sizes of very abundant marine animal species results from a large variance in reproductive output among individuals. In extreme cases the entire population may be replaced by the offspring of a small minority of individuals who, by chance ("sweepstakes"), happen to match their reproductive activity with ". . . oceanographic conditions conductive to spawning, fertilization, larval development and recruitment" (Hedgecock 1994, p. 124). Under this hypothesis, which is clearly linked to Cushing's (1972) match–mismatch hypothesis, many individuals fail to contribute to recruitment. This results in high population variance in offspring number and thus in much lower effective than actual population size.

High variance among individuals in their contribution to recruitment can result from varying degrees of synchrony in the release of sperm and ova (which translate into variance in fertilization success), from moderate, or even minor, differences in the timing of spawning among groups of mature individuals, and (or) from differences in access to essential resources by the offspring (i.e., match–mismatch; Cushing 1972). Thus, any biotic, oceanographic, or meteorological event (e.g., changes in wind direction or intensity, changes in the turbulent field) affecting gamete concentration or larval survival has the potential to affect the offspring (potential recruits) of some families but not of others (Levitan and Petersen 1995). At a slightly larger scale this is equally applicable to some cohorts, but not others. Minor timing differences in the production of different cohorts can result in large variance in their contribution to recruitment, and if there are differences in the allelic composition among spawning groups, then there should be detectable genetic differences among their offspring.

Of equal importance, and directly related to the issue of temporal and spatial genetic heterogeneity within a large planktonic larval aggregation, is the question of whether or not there is population structure, and if so, at what scale it is resolved. Although different techniques vary in their ability to detect population structure, no technique applied thus far to cod (i.e., allozymes, mitochondrial DNA, nuclear DNA restriction fragment length polymorphisms) has consistently, and in a complementary manner, shown evidence of population subdivision at other than large geographic scales, such as across ocean basins, or across deep oceanic channels (see Cross and Payne 1978; Mork et al. 1982, 1985; and Dahle and Jørstad 1993 for evidence using allozymes; Smith et al. 1989; Carr and Marshall 1991*a*, 1991*b*; Árnason and Rand 1992; Árnason et al. 1992; Pepin and Carr 1993; and Carr et al. 1995 for evidence using mitochondrial DNA; and Pogson et al. 1995 for evidence using nuclear DNA restriction fragment length polymorphism, RFLP).

In this study we describe the temporal changes at six microsatellite loci in a larval cod (*Gadus morhua*) aggregation tracked and sampled repeatedly on Western Bank of the Scotian Shelf over 3 weeks in November and December 1992. We demonstrate the existence of genetically heterogeneous groups (cohorts) of larvae within the aggregation. This suggests that the aggregation was composed of larvae that originated from different spawning events involving individuals with different allelic compositions. We then compare five microsatellite loci among larvae, which on the basis of size were presumed to form part of a single cohort, and adult cod collected locally in the same general area as the larvae and in a neighbouring location (≈150 km away) on the Scotian Shelf (Banquereau Bank). We show that it is possible to distinguish the cohort larvae as distinct from adult cod sampled on Banquereau Bank, but not as distinct from adult cod sampled locally on Western Bank, providing evidence that the genetic composition of cod on Western Bank remains stable over time.

Materials and methods

Field collections and laboratory procedures

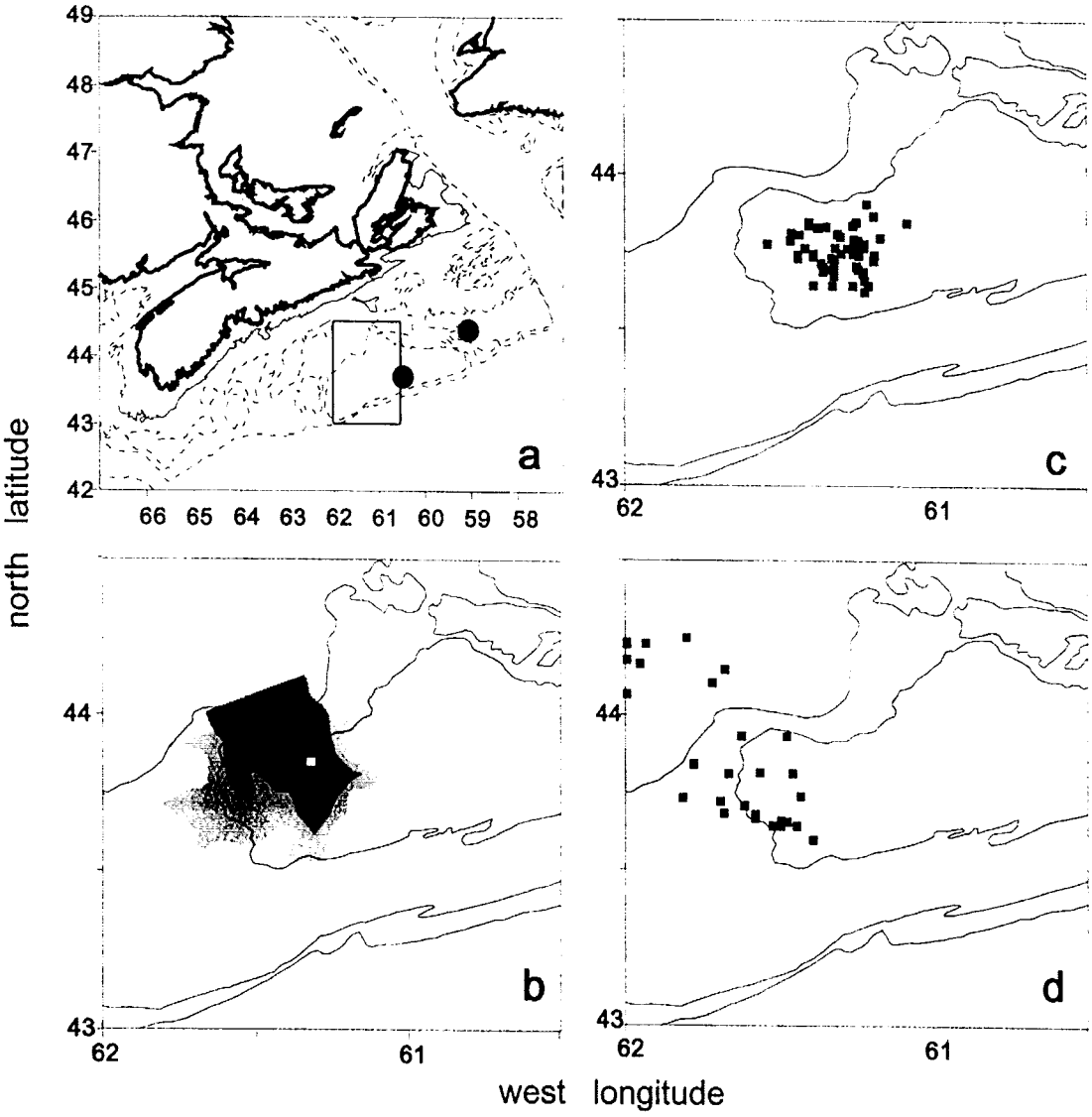
Larval cod samples were collected during the *Petrel V* cruise 31 on Western Bank (Fig. 1*a*) during the period (Coordinated Universal Time (UTC)) 23 November to 16 December 1992 (Griffin and Lochmann 1993). Three sampling transects were conducted across the crest of Western Bank on 23–25 November to ascertain the distribution and abundance of cod larvae (Fig. 1*b*) and collect hydrographic data. This survey revealed several water masses in the region (Griffin and Thompson 1996; Taggart et al. 1996): (*i*) crest water (hereafter CW), on the crest of the bank, which was relatively well mixed and of intermediate temperature (9.1°C) and salinity (31.6 practical salinity units (psu)), (*ii*) a convergent thermohaline front on the eastern and southeastern margin of the CW (hereafter FRONT), and (*iii*) a well-stratified water mass having a surface lens of relatively cold fresh water surrounding the CW (hereafter OUT).

The CW was contained within a gyrelike feature (approximately 20 km in diameter) that rotated around the crest of Western Bank (Sanderson 1995)[2] and was tracked between 25 November and 16 December (Griffin and Thompson 1996). During this period the resident cod larvae within CW were sampled more or less continuously (Taggart et al. 1996). The water mass was tracked with the Ocean Probe real-time oceanographic monitoring system (see Bowen et al. 1995 for complete details), which included shipboard and moored telemetering instrumentation as well as telemetering Loran-C drifters. The Ocean Probe system delivered oceanographic data in real time to a data-assimilative hydrodynamic model (Griffin and Thompson 1996) that produced time-dependent flow fields within a 60 × 60 km model domain of the study area (Fig. 1). By employing this model (updated at least twice daily) in a Lagrangean frame of reference the flow fields could be used to assess the present, and predict the future, positions of previous sampling locations as they were advected around and across the bank within the moving water mass. Thus, we were able to track the aggregation of larval cod found within the CW.

Cod larvae were collected in the tracked CW and in the surrounding water masses every 4–8 h from 25 November (day 3) to 3 December (day 11, Fig. 1*c*) using either a 60-cm diameter bongo net sampler

[2] Sanderson (1995, p. 6762) erroneously reported his analysis as over the period 5–11 November 1992 when his analyses actually covered the period 25 November to 1 December 1992.

Fig. 1. Bathymetric charts of (*a*) the Scotian Shelf (100- and 200-m isobaths) showing the location of Western Bank (*b*, *c*, and *d* represent the area inside the box in *a*) and the median sampling locations (solid circles) of adult cod on Western and Banquereau banks in 1994. (*b*) Water-column larval cod concentration isopleths (graded grey-scale of 10, 20, and >30 cod larvae/100 m^3 on Western Bank (60-, 100-, and 200-m isobaths) as observed during 23–25 November 1992 and the location (central open square) of the centroid of the drifter cluster at the start of water mass tracking. (*c*) Larval cod sampling locations within the crest water mass during the prestorm period of tracking (25 November to 3 December 1992). (*d*) Larval cod sampling locations within the crest water mass during the poststorm period of tracking (10–16 December 1992).

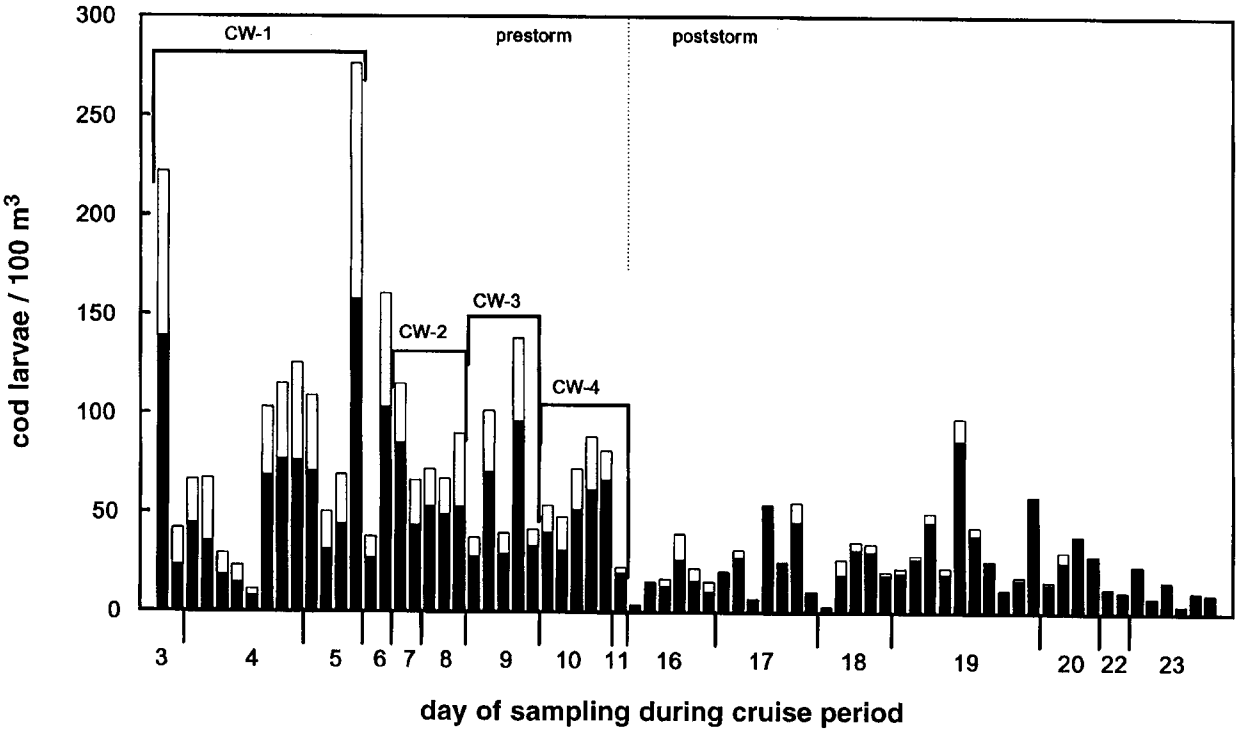


fitted with 333-μm mesh nets (Posgay and Marak 1980) or a 1-m^2 Eastern Marine Marsh EZNET designed after the BIONESS (Sameoto et al. 1980) and fitted with ten 333-μm mesh nets. The bongo was towed at a nominal speed of 1 m/s in a continuous double oblique manner down to 5 or 10 m above the bottom for 10–15 min. The EZNET was also towed at a nominal speed of 1 m/s and was used to sample discrete depth strata at 5-m intervals, down to 35 m, and at 10-m intervals to within 10 m of the bottom. On recovery the nets were rinsed to the cod ends and plankton samples were immediately sorted for cod larvae. At least 10 larvae from each net were videotaped through a dissecting microscope (Miller et al. 1995) for morphometric analysis and individually preserved in liquid nitrogen for biochemical and genetic analyses.

A major storm caused a break in sampling between 4 and 7 December (days 12–15), although real-time monitoring and data assimilation were maintained to continue tracking of the CW (Griffin and Thompson 1996). Following the storm, a second survey of the bank crest area was conducted between 8 and 9 December (days 16–17) and was centred in the area where, according to the assimilated data and model output, a remnant of the CW sampled during the earlier portion of the cruise was to be found. Cod larvae were again collected within the remnant water mass, using the same sampling techniques described above, from 10 to 16 December (days 18–24) when a second storm dispersed the tracked water mass northward and off the bank, ending the study (Fig. 1*d*).

The larval concentration in the water column for each deployment was estimated by dividing the total number of cod larvae collected by all nets in a single deployment by the total volume of water filtered by all nets to produce a single estimate (Fig. 2). The standard lengths (SL, in millimetres) of larvae were measured by the protocol of Miller

Fig. 2. Larval cod concentrations (larvae/100 m^3) observed within the crest water mass on Western Bank during days 3–23 (water mass tracking) of the pre- and post-storm cruise periods. Light shading represents the concentration of larvae that were considered part of the age at length cohort defined for the entire cruise period. CW-1 to CW-4 show concentrations for each of the four consecutive larval groups within the crest water mass (cohort and noncohort). The four consecutive groupings of the age at length cohort (cohort 1 to cohort 4) are subsets of the same four CW groups. Note that the day of sampling interval is proportional to the number of samples taken in a day in the crest water and not to the elapsed period.



et al. (1995). An age (in days post-hatch; d) was assigned to each larva according to an age at length relationship established for cod larvae collected in December on the Scotian Shelf (M. Meekan, Department of Biology, Laval University, Quebec, QC G1K 7P4, personal communication) and was used to select individuals from a common cohort within the tracked CW parcel:

$$(1) \quad d = 1222(6.47 \times 10^{-9})^{\frac{1}{SL}} SL^{-0.63}$$

We also sampled subadult and adult cod in July 1994 from the western end of Banquereau Bank ($N = 48$) in the vicinity of The Gully (median sample location at 44.4°N, 59.09°W; Fig. 1*a*) and in the vicinity of Western Bank ($N = 48$; median sample location 43.57°N, 60.47°W; Fig. 1*a*) during the annual research vessel survey conducted by the Canada Department of Fisheries and Oceans (Anonymous 1994). These cod ranged in size between 23 and 77 cm in fork length (median 37 cm for Western and 39 cm for Banquereau) and were between 2 and 8 years old (median age: 4 years in both locations). The majority of spawners (ages 4+) were recently spent or recovering from spawning at the time of collection.

Tissue collections and processing

Cod larvae initially preserved in liquid nitrogen were thawed and the eyeballs removed for genetic analysis (the remainder of the carcass was used for other phenological and biochemical purposes). The eyeballs were placed in 50 µL of extraction buffer (10 mM Tris, pH 8.3, containing 50 mM KCl and 0.8% Tween 20) and held at –20°C. The DNA was released from the tissue by digestion with proteinase K (200 µg/mL final concentration) at 65°C overnight. Following digestion the samples were incubated at 95°C for 15 min to deactivate the proteinase K and 1–2 µL of this mixture was used directly for polymerase chain reaction (PCR).

The DNA from adult cod (measured for length, weighed, and later aged) was obtained from either blood or soft muscle tissue taken from the tongue. Samples were preserved immediately in 5 mL of 95% ethanol and stored.

Details of DNA extraction from alcohol-preserved tissue are in Bentzen et al. (1990). Briefly, a sample of approximately 100 mg of muscle or 100 µL of blood in ethanol was washed in high TE (100 mM Tris-HCl, 40 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) to remove the ethanol. The samples were then placed in 250 µL of lysis buffer (10 mM Tris-HCl, pH 8.3, 1 mM EDTA, 400 mM NaCl containing 0.8% sodium dodecyl sulphate (SDS) and 400 µg/mL proteinase K). Samples were digested at 65°C for 18 h and transferred to an SST tube (Becton Dickinson). The samples were extracted twice with 2.5 mL of phenol buffered with 0.1 M Tris, pH 8.0, and once with 2.5 mL chloroform. The supernatant containing the purified nucleic acids was transferred to a 1.5-mL Eppendorf tube and precipitated with 0.2 M NaCl and 1 volume of isopropanol. The DNA pellet was resuspended in 100 µL of TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) and adjusted to 25 ng/µL as a PCR template.

Genetic analysis

PCR analysis was as previously described by Brooker et al. (1994). We employed six sets of cod microsatellite primers: Gmo2, Gmo4, Gmo120, Gmo132, Gmo141, and Gmo145 (Table 1). Probes Gmo2, Gmo4, Gmo120, Gmo132, and Gmo141 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). The allele sizes of all loci varied by increments of two base pairs (bp) except for Gmo145. Gmo145 showed occasional alleles that differed by a single bp change. The PCR products were resolved on 6.5%

Table 1. Sequence variants in the *F′* and *R′* ends that define the six different cod microsatellite loci along with their respective annealing temperatures and the primary references for their application.

Locus	Sequence (5′–3′)	Annealing temperature (°C)	Source
Gmo2	$(GT)_x$	51	Brooker et al. 1994
F′	CCC TCA GAT TCA AAT GAA GGA		
R′	GTG TGA GAT GAC TGT GTC G		
Gmo4	$(GT)_x$	57	Wright 1993
F′	ATG TTT AGG CAG ATT TGA ATC AGC		
R′	TGT GGG TAT GTG CAA AGC ATA CAT		
Gmo120	$(GT)_x$	54	Ruzzante et al. 1996
F′	GAG CAA ACA TGC TCA GAG TG		
R′	GAC TGA TCT CCA TGA GAG G		
Gmo132	$(GT)_x$	52	Brooker et al. 1994
F′	GGA ACC CAT TGG ATT CAG GC		
R′	CGA AAG GAC GAG CCA ATA AC		
Gmo141	$(GT)_x$	51	This study
F′	CCG AAT ACT GTT TGA TTG GC		
R′	GAC ATA CTA CTA GGA ACA TAG		
Gmo145	$(G)_x(GA)_x$	50	Brooker et al. 1994
F′	GCA TTG TAG GAA CAA TTA AC		
R′	GTG CAT GTG CTC ATT ATA GC		

Table 2. Levels of genetic variation in an aggregation of larval cod (*Gadus morhua*) on the Scotian Shelf at six microsatellite loci.

Group	Mean sample size per locus (individuals)	Total alleles	Mean H_{obs}	Mean H_{exp}	Deficiency	
					Mean *D*	*p*
Pool	1226.8	276	0.895	0.902	–0.009	0.015
Pool before	968.3	267	0.893	0.902	–0.011	0.005
CW	939.8	268	0.894	0.902	–0.009	0.018
CW before	705.7	256	0.891	0.901	–0.013	0.008[a]
CW-1 (days 3, 4, 5)	132.0		0.909	0.900	0.011	0.273
CW-2 (days 7, 8)	197.2		0.888	0.905	–0.020	0.023
CW-3 (day 9)	203.0		0.892	0.896	–0.005	0.210
CW-4 (days 10, 11)	213.0		0.889	0.898	–0.011	0.083
Cohort	304.2	216	0.898	0.899	–0.003	0.279
Cohort before	283.3	212	0.897	0.898	–0.004	0.263

Note: Pool, the pool of larvae collected in the tracked crest water (CW), in the thermohaline front (FRONT), and in the surrounding water mass (OUT); pool before, the pool of larvae (CW + FRONT + OUT) collected before the storm that interrupted sampling; CW-1 to CW-4, the four consecutive subsets of CW larvae collected before the storm; cohort, the subset of CW larvae defined to be part of a cohort on the basis of age at length and sampling date information; cohort before, cohort larvae collected before the storm; mean D, deficiency (if <0) of heterozygotes averaged over six loci. Deficiencies of heterozygotes for individual loci are presented for the CW before group (see footnote *a*).

[a]Deficiency values for individual loci were as follows: Gmo2, –0.035; Gmo4, 0.016; Gmo120, 0.010; Gmo132, –0.032; Gmo141, –0.036; Gmo145, –0.001.

sequencing gels and the alleles were sized relative to a sequence ladder generated from M13mp18 (Yanisch-Perron et al. 1985).

Biological data

Cod larvae were classified according to sampling date and the water mass from which they were collected (Taggart et al. 1996), i.e., (*i*) inside the well-mixed crest water (CW), (*ii*) at the front (FRONT), or (*iii*) in the surrounding stratified water mass (OUT).

We examined the genetic variation at the six microsatellite DNA loci in a maximum of 1337 cod larvae (Table 2), and at five of these loci in the 96 adult cod samples. The genetic analysis of the larval aggregation proceeded at several scales of temporal and spatial resolution. We first analyzed the pooled data set (i.e., all larvae from CW, FRONT, and OUT collected over the whole time period) as well as the fraction of the pooled data (CW + FRONT + OUT) collected only prior to the storm that interrupted sampling. Subsequent analyses focused only on samples from the CW (the tracked water mass) where we subdivided the CW larvae that were collected before the storm (Fig. 2) into four temporally sequenced groups of similar sizes (CW-1 to CW-4; Table 2). These four groups generally corresponded to different larval concentration maxima observed in the crest water over the prestorm period (Fig. 2).

To assess the temporal changes in the genetic profile of a single cohort within the tracked CW we considered a subgroup of these larvae, i.e., those that hatched during a specific period. On the basis of the age at length relationship described in (*i*), larvae that were at least 1 day old (post-hatch) and no more than 6 days old on day 3 were considered part of the initial cohort. This hatch window was then stepped through time, such that a larva collected 3 days later (day 6) was considered part of the same cohort if its length indicated it to be between 4 and 9 days old. Larvae from this cohort collected before the storm were further subdivided into four consecutive groups (cohort 1 to cohort 4, Fig. 2) that were drawn from the four subsets of CW larvae (Fig. 2).

Table 3. Single locus statistics.

Locus	N (individuals)	n (alleles)	Size range (bp)	H_{obs}	H_{exp}	Probability of homogeneity of allele frequency distribution: Pool before – pool after	CW before – CW after	CW, 4 groups	Cohort, 4 groups
Gmo2	1303	29	102–204	0.774	0.794	0.042	0.061	0.029	0.051
Gmo4	1000	61	111–269	0.970	0.960	0.003*	0.006*	0.449	0.234
Gmo120	1227	44	108–230	0.958	0.952	0.111	0.084	0.290	0.159
Gmo132	1315	31	101–187	0.777	0.798	0.003*	0.008*	<0.001**	0.006*
Gmo141	1285	63	103–243	0.945	0.970	0.180	0.266	0.006*	0.027
Gmo145	1231	48	137–233	0.943	0.942	0.113	0.112	<0.001**	0.019

Note: N, number of individuals; n, number of alleles; size range, allele size range in base pairs (bp); H_{obs}, observed heterozygosity; H_{exp}, expected heterozygosity. The last four columns provide probabilities of homogeneity of allele frequency distributions between or among the groups listed. As expected for microsatellite DNA, heterozygosity estimates were high and ranged from 0.794 for a locus with a low number of alleles (e.g., Gmo2) to 0.970 for a locus with a high number of alleles (e.g., Gmo4).

*$p < 0.008$ with Bonferroni adjustment for six simultaneous comparisons.

**$p < 0.002$ with Bonferroni adjustment for six simultaneous comparisons.

Data analysis

We conducted tests for heterozygote deficiency or excess as well as for the probability of homogeneity of allele frequency distribution between and among the samples collected on different dates during the sampling period. Tests for Hardy–Weinberg equilibrium (HWE) were conducted using the goodness of fit and log-likelihood ratio tests following Weir (1990, pp. 84–85). We estimated $(\delta\mu)^2$ (Goldstein et al. 1995). Estimates of subpopulation structure were obtained using R_{ST} (Slatkin 1995), and for comparative purposes we calculated F_{ST} values following Weir and Cockerham (1984).

Significance values for all tests (i.e., heterozygote deficiency, HWE, homogeneity of allele frequency distributions, genetic distances, and estimates of subpopulation structure) were determined by Monte Carlo simulations and the bootstrap method (at least 1000 resampling trials per individual comparison, Manly 1991). In all cases, bootstrapping was conducted by reshuffling alleles across individuals and populations for each locus separately. Bootstrapping each locus independently allows comparison of estimates across loci. Bootstrapping across individuals and populations allows estimation of significance values for the test under consideration (e.g., HWE, R_{ST}). This method addresses the variance associated with statistical sampling but not that associated with genetic sampling, which occurs each new generation during reproduction (Weir 1990). The standard method of accounting for the effects of genetic sampling is to bootstrap across loci, but this method is meaningful only if a large number of loci is available. We have assumed that the six microsatellite DNA loci examined here constitute a random sample of the genome. Estimates of R_{ST} and F_{ST} combined over loci were calculated by first averaging numerators and denominators across loci and then taking ratios, as suggested by Weir and Cockerham (1984) and Slatkin (1995).

Results

The average sample size per locus for the pooled data was 1226.8 larvae (Table 2) with a minimum of 1000 for Gmo4 and a maximum of 1315 for Gmo132 (Table 3). The majority of these larvae were sampled in the crest water (CW) mass (mean sample size per locus: 939.8 larvae; Table 2) and most of these were sampled before the storm (days 3 through 11 of the cruise period, mean sample size per locus: 705.7, Table 2). There were a total of 276 alleles in the pooled data and the majority were present in the subset of larvae collected in the CW prior to the storm ($n = 256$ alleles, Table 2). As expected for microsatellites, observed and expected heterozygosities were high and ranged from 0.878 to 0.909 and from 0.896 to 0.905, respectively (Table 2).

Temporal changes in microsatellite DNA variation

There were significant deficiencies of heterozygotes in the larval collection as a whole (CW, FRONT, and OUT) and in the subset collected in the crest water mass (CW) when the entire sampling period was considered, as well as when only the period prior to the storm was considered (Table 2). In contrast, there was no evidence of heterozygote deficiency among the subset of CW larvae that were considered to be part of a single cohort (Table 2).

Similar analyses within each of the four temporally sequenced groups of CW larvae (regardless of cohort affinity) also showed evidence of heterozygote deficiency for three of the four groups (although the results are significant for only one group ($p = 0.023$) and marginal for a second group (Table 2). In contrast, we found no evidence of heterozygote deficiency in any of the four temporal groups that constituted the age at length cohort (cohort 1 to cohort 4, $p > 0.123$).

Tests of HWE using the goodness of fit and the log-likelihood ratio tests were consistent with the above results. The analyses indicated departure from equilibrium ($\alpha' = 0.008$ with Bonferroni adjustment for six loci) with both tests for the six loci for all larvae (pool) as well as for all larvae collected before the storm (pool before). Larvae collected in the CW prior to the storm showed evidence of departure from HWE with both tests for five loci, and with one test for the remaining locus (Gmo4). In contrast, both tests showed that for all but one locus (Gmo2) the larval cohort as a whole was in HWE.

Microsatellite DNA variation among loci

Analyses based on genetic variability at individual loci did not allow rejection of the null hypothesis that the larval cohort originated from a single group of spawners. However, the same analysis with all of the CW larvae (regardless of cohort affinity) resulted in rejection.

Allele frequency distributions were heterogeneous for two of the six loci (Gmo4 and Gmo132) when all larvae collected before the storm were compared with all larvae collected after the storm (Table 3: pool before – pool after). The same was true for the CW subset (Table 3: CW before – CW after), suggesting

Table 4. Probability of homogeneity of allele frequency distributions among three groups: the entire larval cohort or each of the four consecutive subsets collected before the storm (cohort 1 to cohort 4), and adult cod collected on Banquereau Bank ($N = 48$), and on Western Bank ($N = 48$).

Locus	Entire cohort ($N = 316$)	Cohort 1 ($N = 79$)	Cohort 2 ($N = 86$)	Cohort 3 ($N = 74$)	Cohort 4 ($N = 77$)
Gmo2	0.017	0.007*	0.005*	0.014	0.038
Gmo4	0.692	0.831	0.264	0.315	0.782
Gmo120	0.576	0.434	0.151	0.578	0.269
Gmo132	0.003*	<0.001**	0.001**	<0.001**	0.004*
Gmo145	0.115	0.052	0.061	0.165	0.007*

Note: The values in the table are for comparisons among three groups (cohort, Banquereau Bank adults, Western Bank adults).

*$p < 0.010$ with Bonferroni adjustment for five simultaneous tests.

**$p < 0.002$ with Bonferroni adjustment for six simultaneous comparisons.

that the composition of the larval aggregation, even that of the subset collected within a single water mass, changed during the storm period. Differences were also significant among the four consecutive CW larval subsets (CW-1 to CW-4) collected before the storm, i.e., these subsets were different at three loci (Gmo132, Gmo141, Gmo145; Table 3: CW, 4 groups). By contrast, the four consecutive cohort subsets (cohort 1 to cohort 4) were different at only one locus (Gmo132; Table 3: cohort, 4 groups).

Genetic distances and population structure in the larval aggregation

Although the above results based on allele and genotype frequency distributions suggest that the entire larval aggregation in the CW originated from different spawning groups, results based on $(\delta\mu)^2$ distances and on estimates of population structure (R_{ST}) suggest that the different spawning groups may not have come from different populations but instead may have been different components of a single population.

None of the six pairwise comparisons among the subsets of the CW larvae or of the cohort larvae indicated significant differences ($\alpha' = 0.008$ after Bonferroni adjustment for six simultaneous groups) when measured with $(\delta\mu)^2$ distance ($p \geq 0.106$ for comparisons between the CW subsets, and $p \geq 0.047$ for comparisons between the cohort subsets). Similarly, there was no evidence of significant population structure between or among any of the subsets of larvae when measured with R_{ST} ($p \geq 0.245$) or with F_{ST}.

Genetic distances and population structure: the larval cohort and adult cod

We compared the genetic composition of the larval cohort with that of two subadult and adult cod samples collected on the Scotian Shelf in July 1994 (one sample from Western Bank and one from the eastern end of Banquereau Bank). Genetic distances and estimates of population structure (R_{ST} and F_{ST}) consistently indicated that the larval cohort was more similar to the cod collected in the same general location on Western Bank than to those collected on Banquereau Bank.

We compared variation in five of the six microsatellite loci (Gmo141 was not run for the adult cod samples). The allele frequency distributions for Gmo132 were consistently heterogeneous among all three populations, either for the entire cohort sample, or when any of its four consecutive subsets were considered (Table 4). Probabilities that allele frequencies were homogeneous were low and occasionally significant for two other loci: Gmo2 and Gmo145. Similar analyses comparing the cohort larvae separately, with the Banquereau or the Western Bank adult cod samples, indicated that the cohort larvae differed more from Banquereau Bank cod than from Western Bank cod. Only 3 out of 20 possible comparisons (five loci, four subsets) among the larvae and Western Bank cod indicated heterogeneity in allele frequency distribution ($\alpha' = 0.01$ with Bonferroni adjustment for five simultaneous tests, i.e., five loci), whereas 6 out of 20 comparisons indicated heterogeneity between larvae and Banquereau Bank cod (data not shown). This difference is not great. However, $(\delta\mu)^2$ genetic distance estimates also suggest that the larval cohort was more similar to cod from Western Bank than to those from Banquereau Bank (Table 5), and, as well, comparison of R_{ST} and F_{ST} estimates of population structure between the cohort larvae and each of the adult populations (Table 6) also showed that the larvae were consistently more similar to Western Bank cod than to Banquereau Bank cod. Thus, all four measures consistently indicated greater affinity of the larvae to those adults collected on Western Bank.

Table 5. $(\delta\mu)^2$ distances between the entire larval cohort, each of the four consecutive subsets collected before the storm (cohort 1 to cohort 4), and adult cod collected on Banquereau and Western banks.

	Banquereau Bank ($N = 48$)		Western Bank ($N = 48$)	
Group	$(\delta\mu)^2$	p	$(\delta\mu)^2$	p
Entire cohort ($N = 316$)	5.89	0.041	2.25	0.359
Cohort 1 ($N = 79$)	6.22	0.030	4.98	0.070
Cohort 2 ($N = 86$)	5.82	0.033	2.11	0.416
Cohort 3 ($N = 74$)	4.43	0.093	1.31	0.672
Cohort 4 ($N = 77$)	11.81	0.006	6.12	0.097

Note: $p < 0.05$ for comparisons involving the entire cohort and $p \leq 0.012$ for comparisons between each of the four consecutive cohort subsets and adult cod with Bonferroni adjustment for four simultaneous tests. $(\delta\mu)^2$ distance between Banquereau and Western banks = 2.44 ($p \approx 0.450$).

Discussion

We have shown, on the basis of microsatellite DNA variation, that an aggregation of larval cod repeatedly sampled on the Scotian Shelf over approximately 3 weeks was formed by more than one genetically distinct larval group. Furthermore, a subset of the larval aggregation, presumed to be part of a single cohort on the basis of age at length, was genetically more similar to adult cod sampled some 2 years later in the same general area on Western Bank than to adult cod sampled on a neighbouring bank situated approximately 150 km away. These results therefore suggest that (*i*) the aggregation was formed by larvae that originated from different spawning events involving different groups of individuals and (*ii*) the genetic composition of cod on Western Bank remained fairly stable over time. We next address these points and their implications in detail.

Table 6. R_{ST} and F_{ST} estimates of population structure between each of the four consecutive cohort subsets and adult cod collected on Banquereau Bank and on Western Bank.

	Cohort 1		Cohort 2		Cohort 3		Cohort 4	
Group	Estimate	p	Estimate	p	Estimate	p	Estimate	p
				R_{ST}				
Banquereau Bank	0.0322	0.005	0.0318	0.033	0.0149	0.110	0.0599	0.001
Western Bank	0.0180	0.043	0.0160	0.140	0.0012	0.468	0.0419	0.004
				F_{ST}				
Banquereau Bank	0.0010	0.241	0.0067	0.002	0.0027	0.107	0.0029	0.064
Western Bank	0.0011	0.276	0.0022	0.100	≪0.00001	0.438	0.0019	0.142

Note: Sample sizes are as in Table 5.

Heterogeneity within the larval aggregation

The presence of several heterogeneous groups among the larvae collected in the tracked gyrelike water mass on the crest of the bank (CW) was suggested by evidence of heterozygote deficiencies (Wahlhund effect), departures from HWE, and heterogeneity of allele frequency distributions when all larvae sampled in the crest water (CW) mass were considered, but not when similar analyses were restricted to larvae that, on the basis of their estimated age at length, comprised a single cohort (Tables 2 and 3).

HWE is characteristic of large, randomly mating populations. A population is in HWE for a given locus if the probability of observing a given genotype is equal to the product of the probabilities of observing each of the alleles (i.e., the allele frequencies) for a homozygote, or twice this product for a heterozygous genotype. If a population is subdivided, and there are two or more groups that differ in allele frequencies at a given locus, then analysis of the entire population, without considering its structure, will show a deficiency of heterozygotes. This is essentially what our analyses described for the entire larval aggregation and the subset of all larvae sampled in the crest water mass: they appear to have originated from several spawning groups among which there were marked differences in allele frequencies. In contrast, there was no evidence for heterozygote deficiency (and evidence for departures from HWE was weak) among the cod larvae that formed a single cohort within the water mass. Comparisons of allele frequency distributions among possible consecutive subsets of larvae supported these conclusions; there were more significant differences in allele frequencies among the four consecutive subsets of all larvae sampled in the water mass than among the four consecutive subsets of the cohort (Table 3).

Departures from HWE can result from factors other than population subdivision, such as selection, inbreeding, phenotypic assortative mating, and (or) the presence of null alleles (Devlin et al. 1990; Chakraborty and Jin 1992). Population subdivision, however, is believed to be the most important of these factors for microsatellite (variable number of tandem repeats, VNTR) loci (Lander 1989). Null alleles are unlikely to be the cause of the deficiency of heterozygotes in the pooled data set or the subset of all larvae sampled in the crest water mass (Table 2). If null alleles were responsible for this deficiency it is reasonable to assume that they would be present among the larval cohort as well. The fact that we do not detect a deficiency of heterozygotes within the cohort suggests that, if present at all, null alleles are unlikely to be the sole cause of the departures from HWE or of the heterozygote deficiencies observed at the larger time and space scales of the larger groupings.

Although our results suggest the presence of several genetically heterogeneous groups of larvae within the larval aggregation collected over the sampling period, it is not trivial to estimate how many individuals might have contributed gametes to the formation of each of these groups. In a related study that attempted to infer possible kinship relationships among the same individuals as the ones used here, Herbinger et al. (1997) found no evidence of any temporal or spatial family structure within a subset of 315 individuals analyzed in detail. Assuming that these individuals were indeed representative of the 1400 or so larvae collected and assayed, they estimated the minimum parental population to have been of the order of 2800 individuals (1400 of each sex), since no two larvae could be assured of having two parents in common (Herbinger et al. 1997). We believe that actual population abundance was much higher, perhaps an order or two of magnitude higher. Calculations based on an exponential mortality model for the tracked cohort (Taggart et al. 1996) and the volume of the crest water mass suggest that the number of spawners necessary to produce the observed numbers of larvae may have been no less than 100 000. This conservative estimate assumed an average fecundity of 200 000 eggs per female (fecundity for a 50-cm cod; Scott and Scott 1988; somewhat larger than the cod sampled in the region in 1994, see methods), no egg mortality, complete fertilization, and a 1:1 sex ratio. The true spawning population size was likely higher than this estimate.

Population structure: the larval cohort and adult cod

Collectively, the results based on $(\delta\mu)^2$ distance and on estimates of population structure, R_{ST} and F_{ST} (Tables 5 and 6), suggest a fairly stable genetic composition of cod over time on Western Bank; i.e., the cohort in the larval aggregation was more similar to adult cod from Western Bank collected some 2 years later than to adult cod from the neighbouring Banquereau Bank. The existence of significant genetic differences between the cohort larvae and adult cod sampled only 150 km away in the area of Banquereau Bank is intriguing but perhaps not unique and could be explained as resulting from isolation by distance. The broader implications of these results are that, given moderate sample sizes (no less, and preferably more, than 50 individuals per location, D.E. Ruzzante, unpublished observations), if genetic differences exist in Atlantic cod at scales of hundreds of kilometres (e.g., among neighbouring banks), then they are likely to be detected with microsatellite DNA variation. Two related studies are consistent with these conclusions: in the first of these studies, Ruzzante (1996)

used microsatellite DNA to distinguish cod overwintering inshore in a Newfoundland bay from those overwintering a few hundred kilometres away on the Grand Bank. Differences occurred despite the fact that offshore and inshore overwintering cod intermingle inshore during the summer feeding period. In the second of these studies, Bentzen et al. (1996) described evidence based on microsatellite DNA polymorphism suggesting that genetic differences exist among cod populations in the northwest Atlantic (i.e., among northern cod, Flemish Cap, and Scotian Shelf samples), and between northwestern and southeastern components of the northern cod complex off Newfoundland. Microsatellites, therefore, would seem to allow the detection of population structure at smaller spatial and temporal scales than has been possible to examine in the past with a variety of other genetic markers.

Previous studies on the genetic structure of Atlantic cod populations based on allozymes, mitochondrial DNA, and nuclear DNA RFLP appear not to have the same small-scale resolution afforded by microsatellite DNA polymorphism. One can argue that studies based on allozyme loci have produced ambiguous results because they tend to show significant differences among neighbouring populations when a limited number of blood protein loci are examined (Møller 1968; Jamieson and Otterlind 1971; Jamieson 1975; Cross and Payne 1978; Dahle and Jørstad 1993), but these differences sometimes disappear when a larger number of conventional electrophoretic loci are examined over the distributional range of the species (Mork et al. 1982, 1985). Studies of mitochondrial DNA variation have shown evidence of differentiation across the North Atlantic (Smith et al. 1989; Carr and Marshall 1991*a*; Árnason and Rand 1992), but not at smaller geographic scales (Carr and Marshall 1991*a*, 1991*b*; Árnason et al. 1992; Pepin and Carr 1993; but see Dahle 1991). In a recent study based on RFLP, Pogson et al. (1995) showed that these genetic markers are capable of detecting genetic population structure at ocean basin scales where allozyme loci do not.

Cohort contribution

We have shown that the larval aggregation on Western Bank was composed of genetically heterogeneous groups of larvae that originated from different spawning events. However, a subset of this aggregation, presumed to be part of a single cohort on the basis of age at length, remained genetically homogeneous through time.

Distinct larval cohorts within a large planktonic aggregation are found in other species. For example, Lambert (1984) reported evidence based on polymodal size distributions of a succession of distinct larval cohorts separated by periods of several days in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). Lambert (1984) discussed the possibility that such reproductive strategy might be widespread among marine species with pelagic eggs and suggested it may have important consequences for the survival of early life stages by ". . . spreading the spawning effort over time to take advantage of a variable environment" (Lambert 1984, p. 1562).

The large larval aggregation described in the present study was confined within a relatively small area of coastal ocean involving three different water masses that contained larvae spanning a range of sizes and ages. Considered as a whole, the aggregation was genetically heterogeneous. However, a subset of these larvae, considered to be part of a cohort on the basis of age at size, was genetically homogeneous. Together, this evidence indicates that the larval aggregation as a whole resulted from the contribution of more than one and presumably several spawning groups who's offspring form genetically defined larval cohorts upon which temporally and (or) spatially varying biotic, oceanographic, and meteorological processes can act to effect differential reproductive success among individual spawners and (or) spawning groups. If our interpretation is correct, then moderate, or minor, differences in the timing of spawning among groups of mature individuals or differences in the availability of resources to the offspring (i.e., match–mismatch; Cushing 1972, see also Levitan and Petersen 1995) appear to be the mechanisms that lead to high variance among cohorts in their contribution to recruitment, and thus explain part of the relationship between observed and effective population sizes, as hypothesized by Hedgecock (1994).

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