

Family relationships and effective population size in a natural cohort of Atlantic cod (*Gadus morhua*) larvae

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Abstract: Sibship relationships within a naturally spawned cohort of Atlantic cod (*Gadus morhua*) larvae on the Western Bank of the Scotian Shelf were investigated by a likelihood ratio method that estimates relationships among individuals using microsatellite (DNA fingerprint) information. We found no evidence of any temporal or spatial family structure among the larvae from seven different sample collections taken at sequential time intervals during a 21-d period of sampling the larval cohort. There was no evidence that larvae were more related within sample collections than across sample collections. Within each sample collection, there was no evidence of a family structure within or among the depths sampled. Similarly, there was no apparent change in the potential occurrence of sibship with time (successive sample collections), or in association with the passage of a storm during the sampling period. This cohort of cod larvae appears to have been a fairly homogeneous mixture of larvae that were not siblings and came from a large genetic pool. The minimum estimate of the inbreeding effective population size is 2800 individual spawners.

Résumé : Nous avons étudié la proximité génétique au sein d'une cohorte de larves de morue franche (*Gadus morhua*) écloses dans la nature sur le banc ouest de la plate-forme néo-écossaise en utilisant une méthode de rapport des vraisemblances qui permet d'estimer les liens entre individus à l'aide de données fournies par les microsatellites (identification par le code génétique). Nous n'avons pas trouvé d'indication de structures familiales temporelles ou spatiales entre les larves de sept ensembles d'échantillons différents, prélevés à des intervalles de temps séquentiels, au cours d'une période de 21 d de prélèvements dans la cohorte de larves. Il n'y avait pas d'indication que les larves étaient plus proches génétiquement au sein d'un même ensemble d'échantillons que par comparaison aux larves des autres ensembles. À l'intérieur de chaque ensemble d'échantillons, il n'y avait pas d'indication d'une structure familiale à une profondeur donnée ou entre les profondeurs de prélèvement. De même, aucun changement apparent dans la probabilité de proximité génétique n'a été observé au fil du temps (prélèvements successifs d'échantillons), ni par suite du passage d'une tempête au cours de la période d'échantillonnage. Cette cohorte de larves de morue semble avoir été constituée d'un mélange assez homogène de larves qui n'étaient pas liées génétiquement, et qui provenaient d'un réservoir génétique important. La valeur estimative minimale du nombre d'individus requis dans une population pour que se manifeste la proximité génétique est de 2 800 géniteurs.

[Traduit par la Rédaction]

Introduction

It has been proposed that new insights needed to predict recruitment variations in Atlantic cod (*Gadus morhua*) and other marine fish should come from studying, among other phenotypic traits, the genetic traits of the individuals that survive and eventually recruit (Taggart and Frank 1990). Proposals of this kind are derived from evidence that suggests that relatively few of the many cohorts spawned annually may be

responsible for ultimate year class strength (e.g., Lambert 1984) and from predictions, based on small scale spatial and temporal heterogeneity in the natural environment, that individual cohorts of larvae should reveal genetic evidence of having been produced by a small part of the potential parental pool (Hedgecock 1994a, 1994b).

Strong selective mortality between male (sire) half-sibship during the critical transition to exogenous feeding has been demonstrated in laboratory-reared cod larvae (Doyle et al. 1995). However, the extent of familial relationship among individual larvae from naturally spawning populations has so far been nearly impossible to measure. This is particularly true for species where mating behaviour cannot easily be observed. Brawn (1961) observed pair matings and male dominance, and unpaired female spawning as well, in cod maintained in aquaria. However, spawning behaviour in large cod aggregations in nature is still largely unstudied.

Several classes of genetic markers (e.g., allozyme, mitochondrial DNA and nuclear DNA markers) are currently used to address various population genetic problems. One class of such nuclear DNA markers, the microsatellites, exhibits

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attributes that make them particularly suitable for fish population genetic research (Wright and Bentzen 1994). Microsatellites are short stretches (10–100 base pairs) of DNA composed of di-, tri-, or tetranucleotide repeats arrayed in tandem (Wright 1993). Each microsatellite locus (i.e., each tandemly arrayed repeat) is flanked by unique DNA sequences. Complementary sequences can be made that hybridize to these unique flanking regions to act as primers for amplification of the tandem array by polymerase chain reaction (PCR). The length of the different size variants of the tandemly repeated array (i.e., the different microsatellite alleles) can then be accurately determined by electrophoresis on a sequencing gel (Wright and Bentzen 1994). Among the attributes that make microsatellites a particularly attractive class of genetic markers are their abundance, their extremely high levels of allelic variation, and the fact that they are codominant markers inherited in a Mendelian way (Wright and Bentzen 1994).

The ability to use genetic marker information to infer relatedness among individuals would be a valuable tool to study natural populations, but it has proved to be a very difficult task. Assessment of parent–offspring relationships from analysis of band sharing in multilocus fingerprints requires a detailed knowledge of the study population and DNA samples from both parents and their offspring (Wetton et al. 1987). Precise estimation of individual relatedness is generally impossible (Lynch 1988). Brookfield and Parkin (1993) examined in a theoretical model the use of hypervariable single-locus DNA probes to provide evidence of relatedness of pairs of individuals from a wild population. They concluded that it should be possible to distinguish siblings from unrelated individuals with a realistically small number of loci.

In this paper, a likelihood ratio method is developed to infer possible half-sib or full-sib relationships from single-locus microsatellite data. The existence of sibships among cod larvae was investigated by this approach on a population that was tracked within a well-defined water mass on the Western Bank of the Scotian Shelf. The motivation for the study was two-fold: (1) to infer the mating structure of the spawning aggregation that produced the cohort and to estimate a lower bound for the effective population size of that spawning aggregation; and (2) if families were detected in the larvae, to see if the action of natural selection among sibship could be directly observed in a naturally spawned cohort of larvae over time, as had been observed in the laboratory.

Material and methods

Field operations and larval collections

The field operations of this study were conducted aboard the MV Petrel V from 22 November to 16 December 1992 in the North Atlantic area 4VsW (Western Bank of the Scotian Shelf).

Tracking of the larval cod cohort was enabled through the use of Ocean Probe, a hardware configuration employed to collect and display data from an array of oceanographic instrumentation and enabling the real-time tracking of a particular water mass (Bowen et al. 1995). The operational design allowed us to sample repeatedly the same water mass throughout the sampling period with the exception of a 4-d period (4–7 December) when a storm curtailed sampling.

Larval cod were collected as frequently as every 4–6 h using a 1-m² EZNET (Eastern Marine Marsh), the marketed version of the BIONESS (Bedford Institute of Oceanography Net and Environmental Sampling System; Sameoto et al. 1980). The EZNET was fitted

with 10 opening and closing nets and was deployed to sample 10 discrete depths. When sea state and wind conditions limited the deployment of the EZNET, a Bongo sampler (Posgay and Marak 1980) was deployed using double oblique tows between the surface and ~5 m above bottom to provide depth-integrated samples.

Upon retrieval, all net samples were examined and all cod larvae were removed and counted. When available, a minimum of 10 cod larvae from each net were videotaped for standard length estimates and other morphometric analysis and then individually preserved in liquid nitrogen. The eyeballs of the larvae were later removed and sent to the Marine Gene Probe Laboratory (MGPL) for DNA analyses. Standard length estimates determined from the videotaped images were employed to assign an age to each larva according to an age–length relationship previously established for cod larvae collected on the Scotian Shelf (M. Meekan, Université Laval, Québec, unpublished data). The larval ages were subsequently used to select a subset of individuals that, according to their lengths, would have been born within the same 5- to 6-d period near the beginning of the sampling period.

Additional details of the shipboard data collection, nowcasting system, cruise evolution, and larval collections can be found in Griffin and Lochmann (1993), Bowen et al. (1995), and Taggart et al. (1996).

DNA fingerprinting

Each larva was DNA fingerprinted with six microsatellite probes named Gmo 2, Gmo 4, Gmo 120, Gmo 132, Gmo 141, and Gmo 145. These microsatellite markers are based on dinucleotide repeats and were developed in the MGPL. Details concerning sequences and protocols of PCR amplification for several of these probes can be found in Wright (1993) and Brooker et al. (1994). The microsatellites used in the present study are hypervariable single-locus markers; each larva has two alleles, one inherited from each parent.

A total of 1359 larvae were fingerprinted. Scores were obtained from 1303 larvae for Gmo 2, 1313 larvae for Gmo 132, 1226 larvae for Gmo 120, 1289 larvae for Gmo 141, 1229 larvae for Gmo 145, and 999 larvae for Gmo 4. A total of 857 larvae were successfully scored for all six markers and represented larvae collected among sample dates, depths, and gear (Table 1). Using all of these 857 larvae to build every pairwise comparison would have yielded more than 350 000 likelihood ratios. We thus restricted the analysis to the larvae collected from the seven largest daily consecutive sampling stations before and after the storm that interrupted sampling (Table 1). Stations 57 (29 November), 59 (30 November), 72 (1 December), 81 (2 December), and 90 (3 December) were chosen as they had the largest number of completely scored larvae and represented a 5-d consecutive series of samples collected from the larval cohort prior to the storm period. Stations 123 (10 December) and 126 (11 December) had the largest number of larvae found after the storm. All larval collections from these seven stations were made using the EZNET sampler and thus included depth-structured subsamples. These seven stations had the highest daily success in terms of the number of captured larvae for a given volume of sampled water. There was thus a higher probability that the larvae from these stations had been sampled from the core of the tracked cohort. These stations were then deemed to be the most informative “representatives” of the tracked cohort.

Inferring kinship from microsatellite information

Reference frequencies and probabilities, and estimation of group relatedness

For each single-locus marker, three classes of allele-sharing states can be defined for a pair of individuals: (1) class C_0 : the two individuals in the pair have no common allele for that marker (e.g. genotype AB and CD, or AB and CC), (2) class C_1 : the two individuals have one common allele (e.g., genotype AB and AC, or AB and BB), and

Table 1. Stations, sampling gear, and sampling dates during the tracking cruise.

Station	Gear ^b	Date	No. of larvae ^c	Station	Gear ^b	Date	No. of larvae ^c
28	B	26 Nov.	10	82	E	2 Dec.	23
33	B	26 Nov.	3	87	E	2 Dec.	30
38	B	26 Nov.	8	88	E	2 Dec.	22
41	E	26 Nov.	14	90 ^a	E	3 Dec.	26
44	E	27 Nov.	11	92	B	8 Dec.	1
45	E	27 Nov.	13	95	B	8 Dec.	2
46	B	27 Nov.	16	100	B	9 Dec.	4
54	E	28 Nov.	25	101	B	9 Dec.	6
56	E	29 Nov.	43	108	B	9 Dec.	8
57 ^a	E	29 Nov.	80	109	B	9 Dec.	7
58	E	29 Nov.	44	110	B	9 Dec.	3
59 ^a	E	30 Nov.	61	119	E	10 Dec.	4
60	E	30 Nov.	1	120	E	10 Dec.	3
62	E	30 Nov.	30	122	E	10 Dec.	1
67	B	1 Dec.	17	123 ^a	E	10 Dec.	18
68	B	1 Dec.	1	126 ^a	E	11 Dec.	26
69	B	1 Dec.	1	131	B	12 Dec.	7
71	E	1 Dec.	5	133	B	12 Dec.	5
72 ^a	E	1 Dec.	71	136	B	12 Dec.	9
73	E	1 Dec.	19	137	B	12 Dec.	5
78	E	1 Dec.	54	138	B	12 Dec.	8
79	E	2 Dec.	11	139	B	14 Dec.	5
80	E	2 Dec.	25	142	B	15 Dec.	5
81 ^a	E	2 Dec.	59	146	B	16 Dec.	7

^aChosen representative station.^bB = Bongo, E = EZNET.^cTotal number of larvae for which complete scores from six markers were obtained.

(3) class C_2 : the two individuals have two common alleles (e.g., AB and AB, or AA and AA).

These three classes are essentially similar to those defined by the Cotterman coefficients (Cotterman 1940, discussed in Crow and Kimura 1970; Thompson 1991). For a group of individuals, the observed frequencies of these three classes of allele sharing can be computed over all possible pairwise comparisons in the population, for each single locus marker ($Fr(C_0)$; $Fr(C_1)$; $Fr(C_2)$).

It is also possible to calculate the probabilities of observing the three classes of allele sharing (C_0 , C_1 , and C_2) among individuals with a specific level of relatedness, drawn from a population with the same allelic frequencies as estimated in the sample (Thompson 1991; Brookfield and Parkin 1993). In the present study, three sets of probabilities were calculated for three levels of relatedness (for individuals drawn at random in the population, which are defined to be unrelated individuals, for half-sibs, and for full-sibs): set 1: probabilities of observing C_0 , C_1 , and C_2 among random (unrelated) individuals drawn from a population with the same underlying allelic frequencies ($P(C_0)/R$; $P(C_1)/R$; $P(C_2)/R$), Set 2: probabilities of observing C_0 , C_1 , and C_2 among half-sibs drawn from a population with the same underlying allelic frequencies ($P(C_0)/H$; $P(C_1)/H$; $P(C_2)/H$), and Set 3: probabilities of observing C_0 , C_1 , and C_2 among full-sibs drawn from a population with the same underlying allelic frequencies ($P(C_0)/F$; $P(C_1)/F$; $P(C_2)/F$).

The observed frequencies of the three classes of allele sharing as well as the probabilities of the three classes of allele sharing were calculated for each marker using all the data in the sample (e.g., all 1313 larvae with Gmo 132 scores, all 999 larvae with Gmo 4 scores, etc.).

There are two ways in which a pair of individuals can have a common allele. They may have inherited it from a common ancestor, in

which case the common allele is "identical by descent" in the two individuals. Alternatively, they may have the same allele simply by chance because there is not an infinite number of alleles in the population. The average coefficient of genetic relatedness of a group of individuals can be defined as the average percentage of genes identical by descent in that group. It can be easily shown that for unrelated individuals, the probability of having 0, 1, or 2 common alleles identical by descent should be (1, 0, 0); it should be (0.5, 0.5, 0) for half-sibs and (0.25, 0.50, 0.25) for full-sibs (Thompson 1991). In the present study, the group average relatedness of each of the seven sample collections was estimated by ($Fr(C_2) + 0.5 \cdot Fr(C_1)$), the frequencies being calculated in each of the sample collections. We used only the markers that showed sets of probabilities ($P(C_i)/R$ or H or F) close to the rate of allelic identity by descent for the three categories of relatedness, respectively.

Likelihood ratio

For a pair of individuals, the actual realized pattern of allele sharing for the six available markers is easy to observe. For example, such a pattern could be individuals X and Y have 0 alleles common for markers Gmo 2, Gmo 141, and Gmo 4, one allele common for markers Gmo 120 and Gmo 145, and two alleles common for marker Gmo 132. If the markers are located on different chromosomes and thus segregate independently, it is then easy to calculate what would be the overall probabilities of observing this particular pattern among two unrelated individuals (overall probability = R) or among half-sibs (overall Probability = H) or among full-sibs (overall probability = F) simply by multiplying the appropriate probabilities ($P(C_i)/R$, or $P(C_i)/H$ or $P(C_i)/F$) for each single locus marker. The fact that the six markers used in the study segregate independently was verified in this and several other cod populations using the

genotypic linkage disequilibrium testing capability of the GENEPOP population genetic software (available from M. Raymond and F. Rousset, Laboratoire de Génétique et Environnement, Institut des Sciences de l'Evolution, 34095 Montpellier cedex 05, France).

Two likelihood ratios can then be constructed. Likelihood (H/R) = H/R = odds of observing the pattern of allele shared if the two individuals are half-sib versus the two individuals are drawn at random from the population (unrelated). Likelihood (F/R) = F/R = odds of observing the pattern of alleles shared if the two individuals are full-sib versus the two individuals are drawn at random from the population (unrelated). High likelihood (H/R) or (F/R) thus indicates a pair of individuals with patterns of allele sharing that are more likely to be seen if two individuals are half-sib or full-sib than if they are randomly drawn (unrelated). These likelihood ratios were constructed for all pairs of larvae with complete scores within each of the seven representative sample collections (e.g., every larva from station 57 compared with every other larva from that same station). Likelihood ratios were also constructed for all pairs of larva among some collections (i.e., every larva from station 57 compared with every larva from stations 59 and 81; every larva from station 59 compared with every larva from station 81).

Significance of the likelihood ratio and estimation of individual pairwise relatedness

There are three classes of allele sharing for each locus and six loci were used in the present study. There are thus $3^6 = 729$ possible combinations of allele sharing for any pair of individuals. The two likelihood ratios F/R and H/R can be calculated for each of the 729 possible combinations. It is also possible to calculate the frequencies at which this particular combination should be observed by chance in the population simply by multiplying the six observed frequencies of allele sharing from the six markers ($Fr(C_i)$). For each value of the likelihood ratio, one can therefore calculate the probability of observing a likelihood ratio as high or higher by chance alone, i.e., the significance of that likelihood ratio. This approach is essentially similar to a bootstrap approach where the scores would be randomized among the various larvae and a large number of likelihood ratios would be constructed from the permuted data to estimate the significance of the observed likelihood ratio.

With the present data, there is no extraneous information available that would allow a priori pinpointing of specific pairs of potentially related larvae among all possible pairs of larvae. Our approach is therefore to build the likelihood ratios (H/R) and (F/R) and their associated significance for every pair of larvae within the groups of interest. This generates a large number of likelihood ratios, of which some will appear highly significant by chance alone.

Consider a group of 100 unrelated larvae. A total 4950 likelihood ratios (H/R) or (F/R) can be constructed from every pairwise comparison. One can expect 247.5 ratios (5% of 4950) to have a 5% significance level, 49.5 ratios (1% of 4950) to have a 1% significance level, and so on. In this study, the potential presence of relatedness in the group was analyzed by comparing the number of ratios found to be significant at 5 and 1% with what could be expected by chance alone, and testing whether departures from expectations were statistically significant. The significance of individual pairwise likelihood ratios was also tested using the Bonferroni approximation, i.e., in our example, to test at 5%, a likelihood ratio should be significant at $0.05/4950 = 0.00001$.

Results

Overall relatedness within groups

All six markers are hypervariable with 29–63 alleles observed per locus in the sample (Table 2). For every marker and every class of allele sharing, there is a close fit between the observed frequency ($Fr(C_i)$) and the probability among unre-

lated individuals in a population with the same underlying allelic frequencies ($P(C_i)/R$) (Table 2). This indicates that most pairs of larvae in the sample are behaving as if their alleles were drawn at random in the gene pool (i.e., most pairs appear unrelated).

The four most variable markers, Gmo 120, 141, 145, and 4, appear suitable to estimate the average coefficient of genetic relatedness as outlined in the Material and methods section. The calculated $P(C_0)/R$; $P(C_1)/R$ and $P(C_2)/R$ probabilities for Gmo 120, 141, 145, and 4 are not very far from the expected rate of allelic identity by descent (1, 0, 0) for unrelated individuals (Table 2). Similarly, the probabilities among half-sibs and full-sibs are not very far from the expected ((0.5, 0.5, 0.0) and (0.25, 0.50, 0.25)), respectively (Table 2).

With each of the four markers, the average genetic relatedness as estimated by ($Fr(C_2) + 0.5 \cdot Fr(C_1)$) will be slightly overestimated (particularly for unrelated individuals) compared with the true values that would be obtained if the markers would only detect identity by descent. The average overestimation for the three classes of relatedness (random/unrelated, half-sib, full-sib) can be calculated simply by substituting $Fr(C_1)$ and $Fr(C_2)$ by $P(C_1)/R$ and $P(C_2)/R$ or by $P(C_1)/H$ and $P(C_2)/H$ or by $P(C_1)/F$ and $P(C_2)/F$, respectively, in the equation: average genetic relatedness = ($Fr(C_2) + 0.5 \cdot Fr(C_1)$). The four markers will overestimate by 0.025–0.04 the true average genetic relatedness of full-sibs (0.5), by 0.035–0.06 the true average genetic relatedness of half-sibs (0.25), and by 0.066–0.11 the true average genetic relatedness of unrelated individuals (0.0) (Table 3, upper portion).

With each of the four markers, the estimated average genetic relatedness levels among the larvae from the seven sample collections are essentially the levels that would be expected among individuals drawn at random (unrelated) (Table 3, lower portion). There was thus no indication that any of the groups of larvae from the cohort sampled at different times were groups of related larvae.

Likelihood ratios and individual relatedness

Likelihood ratios and their significance were constructed for every pair of larvae within each of the seven sample collections, as well as across some stations (57 and 59; 57 and 81; 59 and 81). Examining the distribution of likelihood ratios to look at individual pairwise relatedness is more sensitive to the existence of family structure than the overall calculation of relatedness within groups as given above.

No pairs of larvae were found with a significant level of relatedness using the Bonferroni adjusted significance levels.

There was no significant difference between the number of likelihood ratios (H/R) and (F/R) significant at 5 and 1% and what could be expected by chance alone, both within and among sample collections (Table 4 illustrates this with the comparisons within station 81 and across stations 57 and 59). There was no evidence of finding more sibling relationship within sample collections than across collections. Similarly, there was no apparent change with time (in successive station collections) or in association with the passage of a storm during the sampling cruise. In every instance, the numbers of likelihood ratios (H/R) and (F/R) significant at 5 and 1% matched fairly closely what could be expected by chance alone among individuals drawn at random (unrelated). No significant deviation was observed in any of these comparisons.

Table 2. Observed frequencies and calculated probabilities of observing C_0 , C_1 , and C_2 classes of allele sharing in the sample.

Marker	No. of larvae analyzed	No. of alleles observed	Class of allele sharing	Observed frequency $Fr(C_i)$	Probability among random fish $P(C_i)/R$	Probability among half-sibs $P(C_i)/H$	Probability among full-sibs $P(C_i)/F$
Gmo 2	1303	29	C_0	0.443	0.436	0.218	0.109
			C_1	0.492	0.497	0.645	0.521
			C_2	0.066	0.067	0.137	0.370
Gmo 132	1313	31	C_0	0.458	0.450	0.225	0.113
			C_1	0.477	0.488	0.643	0.521
			C_2	0.065	0.062	0.132	0.367
Gmo 120	1226	43	C_0	0.819	0.823	0.411	0.206
			C_1	0.176	0.173	0.562	0.519
			C_2	0.005	0.005	0.026	0.275
Gmo 141	1289	63	C_0	0.888	0.885	0.443	0.221
			C_1	0.110	0.113	0.542	0.513
			C_2	0.002	0.002	0.016	0.266
Gmo 145	1229	48	C_0	0.790	0.792	0.396	0.198
			C_1	0.203	0.202	0.572	0.522
			C_2	0.007	0.006	0.032	0.281
Gmo 4	999	62	C_0	0.847	0.849	0.424	0.212
			C_1	0.150	0.148	0.554	0.517
			C_2	0.004	0.003	0.022	0.271

Table 3. Estimation of average genetic relatedness among the larvae from seven representative sampling stations.

Estimated average genetic relatedness	Gmo 120	Gmo 141	Gmo 145	Gmo 4
Among unrelated fish	0.090	0.060	0.110	0.075
Among half-sibs	0.305	0.285	0.310	0.300
Among full-sibs	0.535	0.525	0.540	0.530
Within station 57	0.105	0.060	0.120	0.085
Within station 59	0.115	0.060	0.105	0.080
Within station 72	0.105	0.060	0.105	0.080
Within station 81	0.080	0.060	0.080	0.085
Within station 90	0.075	0.075	0.095	0.085
Within station 123	0.120	0.065	0.135	0.065
Within station 126	0.090	0.055	0.115	0.090

The same result was obtained when the analysis was restricted to the subset of larvae estimated to have been born during the same 5- to 6-d time window ("same-day" larvae) (Table 5). For example, the number of significant likelihood ratios (H/R) and (F/R) at 5 and 1% among the 32 "same-day" larvae from station 59 and the 27 "same-day" larvae from station 72 was not significantly different from what could be expected by chance alone (Table 5).

Within stations, there was no evidence of a spatial family structure among and across samples collected by individual nets at various depths (Table 6). Within station 81 for example, the 74 and 87 pairs found with 5% level likelihood ratios

(H/R) and (F/R), respectively (Table 4), were as often found among the same nets as across nearby nets or across more distant nets (Table 6).

Discussion

The null hypothesis in this study is that none of the larvae sampled are half- or full-sib (i.e., that they have no common parents, they are unrelated at the first generation level), while the alternative hypothesis is that at least some of the individuals are siblings. Of course, it is possible that some of the pairs of larvae that exhibited high likelihood ratios in this study were indeed true full- or half-sib, but overall we could not reject the null hypothesis that the occurrence of every pair of larvae with high likelihood ratios observed in this study could be explained by chance alone.

Ultimately, the power of such an approach to detect family structure rests, on one hand, with the number of independently segregating markers that can be used and with the informativeness (i.e., allelic variability) of these markers and, on the other hand, with the actual level of family structure in the sample. If the tested population has a high level of family structure and consists of a collection of half- and full-sibs (a fairly extreme form of the alternative hypothesis), the method described here will generally be powerful at detecting that there are more high likelihood ratios than what can be explained by chance alone. For example, this approach has been used in salmonid aquaculture populations with known family structure and has proven successful at identifying siblings versus more distantly related individuals (Doyle et al.

Table 4. Expected and observed numbers of significant likelihood ratios (*H/R*) and (*F/R*) within station 81 and across stations 57 and 59.

	Within station 81	Across stations 57 and 59
No. of pairwise comparisons	1711	4880
No. of observed likelihood ratios (<i>H/R</i>) at 5%	74	231
No. of observed likelihood ratios (<i>F/R</i>) at 5%	87	245
Expected no. of likelihood ratios significant at 5% by chance alone	85	244
No. of observed likelihood ratios (<i>H/R</i>) at 1%	17	46
No. of observed likelihood ratios (<i>F/R</i>) at 1%	20	52
Expected no. of likelihood ratios significant at 1% by chance alone	17	49

Table 5. Expected and observed numbers of significant likelihood ratios (*H/R*) and (*F/R*) within "same-day" larvae from stations 59 and 72.

	Within "same-day" larvae, station 59	Within "same-day" larvae, station 72
No. of pairwise comparisons	496	351
No. of observed likelihood ratios (<i>H/R</i>) at 5%	19	16
No. of observed likelihood ratios (<i>F/R</i>) at 5%	22	14
Expected no. of likelihood ratios significant at 5% by chance alone	25	17.5
No. of observed likelihood ratios (<i>H/R</i>) at 1%	2	2
No. of observed likelihood ratios (<i>F/R</i>) at 1%	4	2
Expected no. of likelihood ratios significant at 1% by chance alone	5	3.5

1995). In a recent double-blind experiment, 57 of 60 California chinook salmon (*Oncorhynchus tshawytscha*) were correctly classified as full-sib, half-sib, or unrelated using six markers considerably less informative (four alleles per loci in average) than the ones used here (D. Hedgecock, University of California at Davis, Davis, Calif., personal communication). If, however, the tested population should consist mostly of unrelated individuals but include a few siblings (a more subtle form of the alternative hypothesis), the approach described here will not easily distinguish the few true siblings from pairs of individuals that exhibit similar DNA fingerprint by chance alone. However, the true siblings will exhibit increasing likelihood ratios, while the false ones will exhibit decreasing ratios as additional DNA markers are used. Ultimately, when enough markers are used, the true siblings (however few) will stand out and will exhibit significant Bonferroni-adjusted likelihood ratios. That has not happened in the present study.

The family structure of a natural assemblage of larvae or juveniles from most marine animals, including cod, is largely unknown. Large interannual variation in cod recruitment is consistent with the notion that some spawning aggregations may be small. Female cod are known to be multiple-batch

spawners characterized by high fecundity (Kjesbu and Holm 1994). Although juvenile and adult cod are widely distributed, spawning is known to occur at certain regular times and restricted locations where large numbers of putative spawners aggregate (Brander 1994). Brawn (1961) observed, pair matings in cod maintained in aquaria. Some aggressive males were able to establish territories and generally one dominant male monopolized most of the reproductive activity. However, female cod were also seen to spawn without the direct presence of males (broadcast spawning) on a few occasions (Brawn 1961). Actual mating behaviour of cod in the spawning aggregations cannot easily be observed and the extent to which broadcast spawning and/or pair matings and/or dominance by some males occurs in large cod aggregations in nature is still largely unknown. A cohort of larvae may represent a very large number of parents if broadcast spawning takes place. Alternatively, if cohorts are derived predominantly from single batches of spawn from several females with pair matings, cohorts would then represent a mixture of relatively few full-sib families. If, in addition, some males dominate the reproductive activity, cohorts may comprise relatively few paternal half-sib families.

It is likely that this cohort of cod larvae was a fairly homo-

Table 6. Expected and observed number of significant likelihood ratios (*H/R*) and (*F/R*) among and across the various nets of station 81.

Distance between nets ^a	No. of pairwise comparisons within station 81	No. of likelihood ratios (<i>H/R</i>) at 5%	Expected distribution of 74 cases among nets	No. of likelihood ratios (<i>F/R</i>) at 5%	Expected distribution of 87 case among nets
0	236	15	10.2	14	12
1	385	15	16.6	18	19.6
2	355	15	15.3	22	18.1
3	285	12	12.3	14	14.5
4	212	8	9.2	9	10.8
5	128	3	5.5	5	6.5
6	110	6	4.8	5	5.6
Total	1711	74	74	87	87
		$\chi^2 = 4.00$ (NS)		$\chi^2 = 2.04$ (NS)	

^a0 means larvae from same net, 1 means larvae from one net apart, etc.

geneous mixture of larvae that were not half- or full-sib. No larval pairs could be conclusively shown to have common parents (i.e., by having exceptionally high levels of allele sharing, relative to expectations from randomly drawn larval pairs). This cohort probably did not originate from a small number of pair matings, and no evidence of male dominance on the reproductive activity could be found. It appears more likely that this cohort originated from unpaired matings (broadcast spawning) of a parental population that was at least twice as large (males + females) as the number of larvae analyzed.

However, one cannot completely disregard the possibility that the absence of family structure in this cohort simply reflected considerable oceanic mixing occurring immediately after spawning. Yet the larvae were still very young (5–9 d old) at the time of the first analyzed sampling (station 57), and the ship “nowcasting system” appears to have convincingly tracked the same water mass over the cruise period (Bowen et al. 1995).

Assuming that the larvae analyzed in detail here (315 individuals from the seven collections) were indeed representative of the 1400 or so larvae collected and fingerprinted in the present study, we can estimate that a minimum of 1400 parent cod of each sex (i.e., 2800 parent cod in total) contributed to this cohort, since no two larvae appeared to have common parents. This “minimum parental population”, inferred from the observation that the larvae are not siblings and each has two parents, is equivalent to a lower bound on the inbreeding effective population size, N_e , defined as $N_e = 4 \cdot N_f \cdot N_m / (N_f + N_m)$ (Hartl and Clark 1989, p. 86). This is, to our knowledge, the first time that a lower bound estimate of effective population size in a naturally mating cod population has been obtained, without requiring extraneous assumptions about genetic marker mutation rates. The actual number of individual parents present in the spawning aggregation would be larger than this estimate, particularly if there is large parental variation in fecundity and offspring survival.

The second goal of this study was to see if the action of natural selection among sibship could be directly observed. This could not be done in the present study, since the pres-

ence of sibship was not detected. No first-generation family structure was detected, and thus, family-specific mortality could not be observed, even if it did happen in this cohort as it had in laboratory-reared cod larvae (Doyle et al. 1995).

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References

- Bowen, A.J., Griffin, D.A., Hazen, D.G., Matheson, S.A., and Thompson, K.R. 1995. Shipboard nowcasting of shelf circulation. *Continental Shelf Res.* **15**: 115–128.
- Brander, K.M. 1994. Patterns of distribution, spawning, and growth in North Atlantic cod: the utility of inter-regional comparisons. *ICES Mar. Sci. Symp.* **198**: 406–413.
- Brawn, V.M. 1961. Reproductive behaviour of the cod (*Gadus Callarias* L.). *Behaviour*, **18**: 177–198.
- Brooker, A.L., Cook, D., Bentzen, P., Wright, J.M., and Doyle, R.W. 1994. Organization of microsatellites differs between mammals and teleost fishes. *Can. J. Fish. Aquat. Sci.* **51**: 1959–1966.
- Brookfield, J.F.Y., and Parkin, D.T. 1993. Use of single-locus DNA probes in the establishment of relatedness in wild populations. *Heredity*, **70**: 660–663.
- Cotterman, C.W. 1940. A calculus for statistico-genetics. Ph.D. thesis, Ohio State University. *In Genetics and social structure. Benchmark papers in genetics. Edited by P. Ballonoff. Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pa.* pp. 1975. 157–272.
- Crow, J.F., and Kimura, M. 1970. An introduction to population genetics theory. Harper and Row, New York. pp. 132–136.

- Doyle, R.W., Herbinger, C.M., Taggart, C.T., and Lochmann, S.E. 1995. Use of DNA microsatellite polymorphism to analyze genetic correlations between hatchery and natural fitness. *Am. Fish. Soc. Symp.* **15**: 205–211.
- Griffin, D.A., and Lochmann, S.E. 1993. *Petrel V* cruise 31 to Western bank, 22 November – 16 December 1992. OPEN Rep. 1993/1, Department of Oceanography, Dalhousie University, Halifax, N.S.
- Hartl, D.L., and Clark, A.G. 1989. Principles of population genetics. 2nd ed. Sinauer Associates, Inc., Sunderland, Mass.
- Hedgecock, D. 1994a. Does variance in reproductive success limit population sizes of marine organisms? *In Genetics and evolution of aquatic organisms. Edited by A.R. Beaumont.* Chapman and Hall, London. pp. 122–134.
- Hedgecock, D. 1994b. Population genetics in marine organisms. *U.S. Globec News*, **6**: 7–11.
- Kjesbu, O.S., and Holm, J.C. 1994. Oocyte recruitment in first-time spawning Atlantic cod (*Gadus morhua*) in relation to feeding regime. *Can. J. Fish. Aquat. Sci.* **51**: 1893–1898.
- Lambert, T.C. 1984. Larval cohort succession in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). *Can. J. Fish. Aquat. Sci.* **41**: 1552–1564.
- Lynch, M. 1988. Estimation of relatedness by DNA fingerprinting. *Mol. Biol. Evol.* **5**: 584–599.
- Posgay, J.A., and Marak, R.R. 1980. The MARMAP bongo zooplankton samplers. *J. Northwest Atl. Fish. Sci.* **45**: 91–99.
- Sameoto, D.D., Jarosynski, L.O., and Fraser, W.B. 1980. The BIONESS — new design in multiple net zooplankton samplers. *J. Fish. Res. Board Can.* **37**: 722–724.
- Taggart, C.T., and Frank, K.T. 1990. Perspectives on larval fish ecology and recruitment processes. Probing the scales of relationships. *In Large marine ecosystems: patterns, processes, and yields. Edited by K. Sherman, L.M. Alexander and B.D. Gold.* AAAS Selected Symposium Series. pp. 151–164.
- Taggart, C.T., Thompson, K.T., Mailler, G.L., Lochmann, S.E., and Griffin, D.A. 1996. Abundance distribution of larval cod (*Gadus morhua*) and zooplankton in a gyre-like water mass on the Scotian Shelf. *In Survival strategies in early life stages of marine resources. Proc. Int. Workshop, Yokohama. Edited by Y. Watanabe, Y. Yamashita, and Y. Oozeki.* Balkema Press, Rotterdam, The Netherlands. pp. 155–173.
- Thompson, E.A. 1991. Estimation of relationships from genetic data. *In Handbook of statistics. Vol. 8. Edited by C.R. Rao and R. Chakraborty.* Elsevier, Amsterdam, The Netherlands. pp. 255–269.
- Wetton, J.H., Carter, R.E., Parkin, D.T., and Walters, D. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature (Lond.)*, **327**: 147–149.
- Wright, J.M. 1993. DNA fingerprinting of fishes. *In Biochemistry and molecular biology of fishes. Vol. 2. Edited by P.W. Hochachka and T. Mommsen.* Elsevier, Amsterdam, The Netherlands. pp. 57–91.
- Wright, J.M., and Bentzen, P. 1994. Microsatellites: genetic markers for the future. *Rev. Fish Biol. Fish.* **4**: 384–388.