Biocomplexity in a demersal exploited fish, white hake (*Urophycis tenuis*): depth-related structure and inadequacy of current management approaches

Denis Roy, Thomas R. Hurlbut, and Daniel E. Ruzzante

Abstract: Understanding the factors generating patterns of genetic diversity is critical to implementing robust conservation and management strategies for exploited marine species. Yet, often too little is known about population structure to properly tailor management schemes. Here we report evidence of substantial population structure in white hake (*Urophycis tenuis*) in the Northwest Atlantic, perhaps among the highest levels of population structure exhibited by a highly exploited, widely dispersed, long-lived marine fish. We show that depth plays a role in this extensive and temporally stable structure, which does not conform to previously established fisheries management units. Three genetically distinguishable populations were identified, where all straddle several management divisions and two (Southern Gulf of St. Lawrence and Scotian Shelf) overlap in their range, coexisting within a single division. The most highly exploited population in the Southern Gulf of St. Lawrence was also the most isolated and likely the smallest (genetically effective). This work shows that conservation and management priorities must include population structure and stability in establishing effective species recovery strategies.

Résumé : La mise en œuvre de stratégies robustes de conservation et de gestion des espèces marines exploitées requiert une compréhension des facteurs qui génèrent les patrons de diversité génétique. Cependant, il arrive souvent que la structure de la population ne soit pas assez bien connue pour confectionner des schémas de gestion appropriés. Nous présentons ici des données qui montrent une importante structure de population chez la merluche blanche (*Urophycis tenuis*) dans le nord-ouest de l'Atlantique, peut-être l'une des structures les plus marquées présentes chez une population de poissons marins fortement exploitée, à large répartition et à grande longévité. Nous montrons que la profondeur joue un rôle dans cette structure étendue et stable dans le temps, qui ne se conforme pas aux unités de gestion des pêches antérieurement définies. Nous avons identifié trois populations génétiquement distinctes dont deux (du sud de Terre-Neuve et de la plate-forme Néo-écossaise) chevauchent des divisions de gestion et deux (du sud du golfe du Saint-Laurent et de la plate-forme Néo-écossaise) se chevauchent dans leur répartitions et coexistent dans une même division. La population la plus fortement exploitée dans le sud du golfe du Saint-Laurent est aussi la plus isolée et vraisemblablement la plus petite (génétiquement efficace). Notre travail montre que les priorités de conservation et de gestion doivent prendre en compte la structure et la stabilité de la population pour établir des stratégies efficaces de récupération des espèces.

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Introduction

Effective management of key exploited species relies on proper identification of population structure (Ward 2000; Ruzzante et al. 2006; Reiss et al. 2009). Yet, for many harvested aquatic species this basic knowledge is lacking, with management often relying on political or landscape (e.g., oceanographic) information. There are two problems with this approach. First, genetically homogeneous populations can overlap contiguous management zones, which are nevertheless assessed independently, leading to potential misinterpretation of harvest statistics and population trajectories. Second, genetically heterogeneous populations can have overlapping distributions within management zones. Under such circumstances, smaller, less productive populations are more susceptible to local extinction, as they would likely be the first to fall victim to overexploitation (Ward 2000; Bekkevold et al. 2005; Reiss et al. 2009). Such seemingly small losses can have irreparable consequences on the functional role of the species within the ecosystem and ultimately on its own survival, as such populations may offer unique genetic combinations important for future adaptation to changing condi-

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D. Roy and D.E. Ruzzante. Marine Gene Probe Laboratory, Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, NS B3H 4R2, Canada.
T.R. Hurlbut. Fisheries and Oceans Canada, Oceans and Science Branch, Marine Fish Section, Gulf Fisheries Centre, 343 Université Avenue, P.O. Box 5030, Moncton, NB E1C 9B6, Canada.

Corresponding author: Denis Roy (e-mail: denisroy1@gmail.com).

tions. The biocomplexity offered by the network of interconnected locally adapted populations can buffer overall species abundance and survival by allowing different populations to dominate in response to fluctuating environmental conditions (Hilborn et al. 2003; Hutchinson 2008; Schindler et al. 2010). From a practical standpoint, management tactics failing to recognize such biocomplexity can expedite the loss of key populations responsible for the maintenance of metapopulation dynamics and put the long-term viability of the species at risk (Hilborn et al. 2003; Ruzzante et al. 2006; Schindler et al. 2010). Thus, there is a need to identify population structure of exploited marine species and understand what factors shape this structure to properly tailor management strategies for conservation and continued resource use. Unfortunately, identifying such factors and their influence is frequently hampered by the complex nature of population structure in vagile, widely dispersed, and highly abundant marine species (Ward 2000; Palumbi 2003; Reiss et al. 2009).

White hake (*Urophycis tenuis*) is a long-lived (>15 years) demersal gadoid fish with a relatively long history of commercial exploitation. It is mainly distributed in the Northwest Atlantic, but ranges from Icelandic waters to the east coast of Florida (Musick 1974; Fahay and Able 1989; Chang et al. 1999). It is perhaps the most fecund commercially harvested demersal fish, with females producing up to 10 million eggs per spawning season. Spawning times are poorly known and can range from late winter to early fall depending on location (Markle et al. 1982; Fahay and Able 1989; Chang et al. 1999). White hake eggs are small, buoyant, and advectively dispersed. The initial larval stage is followed by a pelagic juvenile stage lasting up to 3 months (Markle et al. 1982; Chang et al. 1999). At 50–60 mm in length, juveniles migrate inshore to nursery areas and subsequently join the demersal stock at about age 1 (Markle et al. 1982; Fahay and Able 1989; Chang et al. 1999). Although such life history traits predict limited population structure (Musick 1974; Markle et al. 1982; Palumbi 2003), knowledge of white hake population structure, genetic diversity, and connectivity remains scant. In the 1960s the Northwest Atlantic Fisheries Organization (NAFO) designated divisions (management units) for recording and reporting catches of managed species within its jurisdiction (http://www.NAFO.int) (Fig. 1). These divisions are largely based on hydrographic features expected to be barriers to fish distribution but are not species specific and do not consider actual population structure and connectivity in setting catch restrictions. White hake population structure inconsistent with NAFO divisions has, however, been suspected for some time based on tagging work (Kohler 1971), ichthyoplankton surveys (Markle et al. 1982; Fahay and Able 1989), limited allozyme data (Clay et al. 1992), and analyses of morphological variation and parasite loads (Hurlbut and Clay 1998; Melendy et al. 2005). Data from ichthyoplankton surveys conducted off the east coast of Canada suggest the presence of two Northwest Atlantic stocks with separate spawning schedules; a shallow-water, summer spawning population in the southern Gulf of St. Lawrence (hereafter SGSL) and on the Scotian shelf (NAFO divisions 4T, 4Vn, 4Vs, 4W, and 4X) and a deep-water, early-spring spawning population occurring in the northeast Gulf (4S and 4R) and possibly extending along the slopes of the Scotian Shelf and further south (Fig. 1) (Fahay and Able 1989).

White hake has been exploited throughout its range and was historically the third most important groundfish fishery in the SGSL (Hurlbut 2005). Following precipitous declines in the early 1990s, a moratorium was declared in 1995 on directed fishing for white hake in the SGSL, which has been in force ever since. Continuous monitoring surveys in the 1990s–2000s found further abundance declines, culminating in the current review of white hake conservation status in Atlantic Canada by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Hurlbut 2005). This predicament highlights the need to properly identify white hake population structure, size, stability, and connectivity within the Northwest Atlantic. Here, we tested the hypothesis of panmixia in white hake and assessed whether NAFO divisions adequately identify population structure. We identified underlying factors that may shape population structure by interpreting genotype variation in light of various concurrently measured environmental variables. We estimated connectivity and effective population size(s) and related our findings to current white hake conservation status in the Northwest Atlantic, with particular emphasis on the SGSL and surrounding area, the region of its highest exploitation.

Material and methods

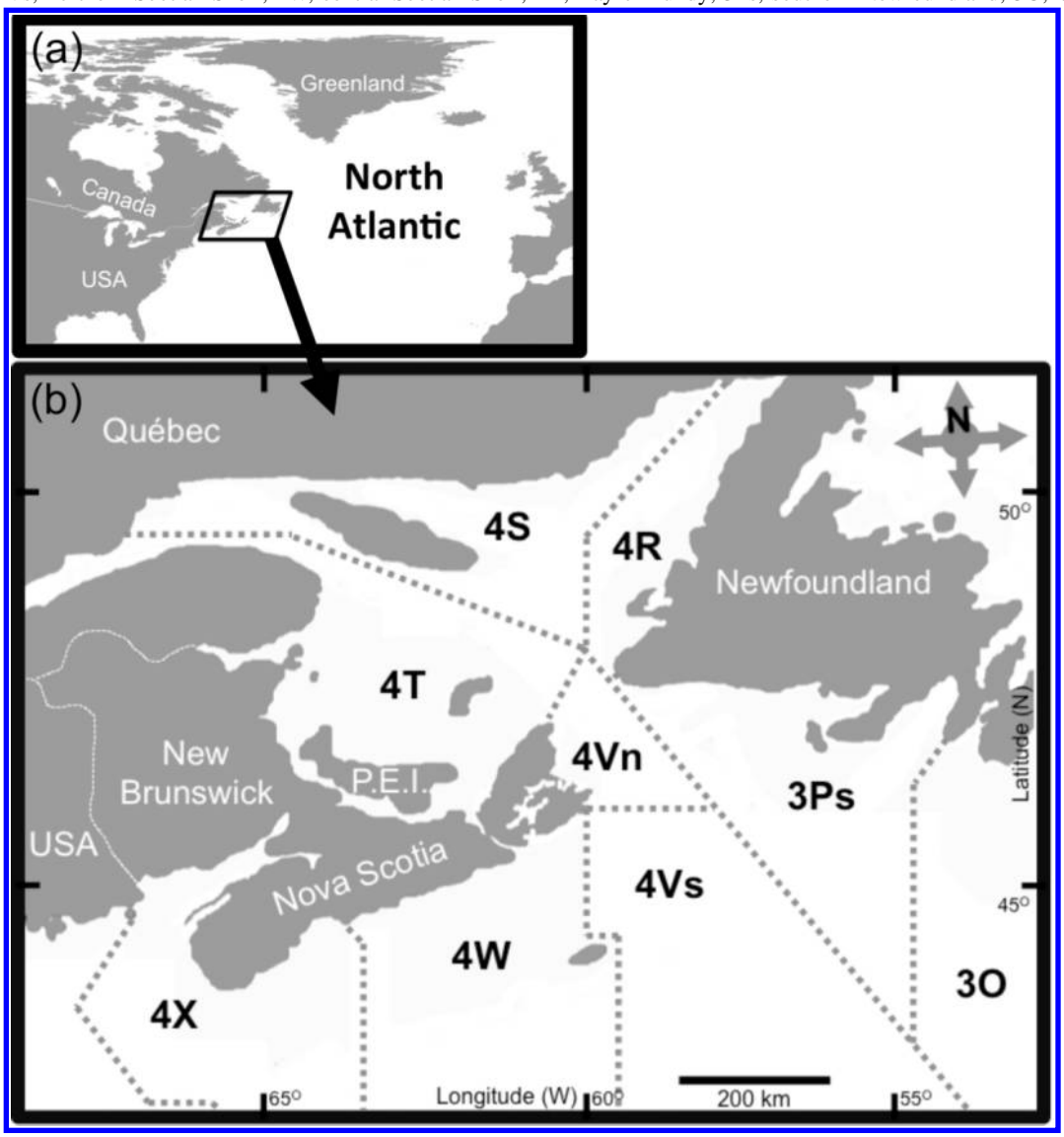
Sampling

Sample collection ($n \sim 2000$) took place between 2003 and 2009 throughout the Northwest Atlantic, including nine different NAFO divisions (Fig. 1; Table 1). The majority of samples were collected during standardized annual bottom-trawl surveys conducted by the Department of Fisheries and Oceans (Chadwick et al. 2007). Fishery observers deployed on commercial vessels acquired additional samples from bottom-trawl or longline fishing gear used by fishers. Sampling targeted spawning or recently spawned individuals but also included individuals in other spawning conditions (Table 1). Sampling periods also coincided with peak pre-moratorium harvest periods (Hurlbut et al. 1996). Consequently, any population structure observed would likely reflect that encountered during peak harvest periods in the past. For each sampled fish, global positioning system coordinates and depth were recorded, and fin clips were taken and stored in ethanol. In several cases, mostly within the SGSL, several other data including temperature and salinity measurements of the water mass in which the fish were collected were also recorded using oceanographic profilers (Table 1).

DNA extraction, amplification, and genotyping

DNA was extracted using a glassmilk protocol set to run on a Perkin Elmer Multiprobe II plus HTEX Robot liquid handling system (Perkin Elmer, Waltham, Mass., USA). Subsamples of each extraction were visualized on 2% agarose gels against a standard reference for quantification. Extracted DNA (1 μL) was used in each of five multiplex polymerase chain reactions (PCRs) amplifying 16 microsatellite loci (Roy et al. 2010). PCR products were diluted 1:5 with RNase-free water, imaged on a bank of eight IR4200-4300 DNA analyzers (LICOR, Lincoln, Nebr., USA), and individual genotypes were scored using SAGA Automated Microsatellite

Fig. 1. (*a*) Geographic location of study area in the North Atlantic Ocean. (*b*) More detailed area showing the geographic delineations of Northwest Atlantic Fisheries Organization (NAFO) management divisions from which white hake samples used in this study were collected during 2003–2009. 4T, southern Gulf of St. Lawrence; 4S, northern Gulf of St. Lawrence; 4R, western Newfoundland; 4Vn, northern Cape Breton Island; 4Vs, northern Scotian Shelf; 4W, central Scotian Shelf; 4X, Bay of Fundy; 3Ps, southern Newfoundland; 3O, Grand Banks.



Software 3.3 (LICOR). Genotypes were verified by eye, grouped by NAFO division and sampling year, and assessed for scoring errors, large allele dropout, and (or) the presence of null alleles using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). No scoring errors or allele dropouts were detected, but evidence of null alleles was discovered in *ute55* and *ute70*. Both loci displayed excess homozygotes at a few alleles, but these were not consistent across groups, suggesting that excess homozygosity may be related to population substructure.

Population structure analyses

To get an unbiased best estimate of population number sampled from all genotyped fish, we used Structurama in a hierarchical approach (Huelsenbeck and Andolfatto 2007). First, the entire data set was used to estimate the number of populations overall. Then the approach was applied separately within each recovered population from the initial analysis. This process was repeated on all recovered populations from every step until no further population structure was detected. Structurama used unsupervised searches to find the most probable number of genetic clusters (*K*), treating it as a random variable with a Dirichlet process prior having a mean set to 1, 5, 10, 15, and 20. Each search ran for 2 000 000 Markov chain Monte Carlo (MCMC) generations using three sampling chains sampled every 200th sample for a total of 10 000 samples. Statistics were calculated after the first 1000 samples were discarded as burn-in. To visualize hierarchical population structure determined by Structurama and to estimate posterior probability of individual admixture proportions overall and within each recovered population, we used STRUCTURE 2.3.3 (Hubisz et al. 2009). In STRUCTURE, the number of populations was set using Structurama results, and the posterior probability of each individual's assignment to each population was assessed through 10 iterations. Each iteration ran for 550 000

Table 1. Summary information for white hake samples used in this study.

NAFO division	Regional code*	Year	*n*	Genotyped	Sampling interval	Lat. (°)	Long. (°)	Depth (m)	Depth range (m)
4T	GSL (S)	2003	120	117	6 Aug. – 18 Oct.	46.888	–61.475	158.92	31–507
4T	GSL (S)	2004	154	152	7–24 Sept.	46.967	–61.907	150.34	18–372
4T	GSL (S)	2005	233	217	11 July – 29 Sept.	47.149	–61.308	181.34	16–377
4T	GSL (S)	2008	227	223	8 July – 20 Sept.	47.956	–61.495	249.90	26–364
4T	GSL (S)	2009	219	210	8 Aug. – 30 Oct.	47.698	–61.402	241.29	25–365
4S	GSL (N)	2008	12	12	6 –18 Aug.	49.256	–61.810	266.50	143–385
4S	GSL (N)	2009	9	8	11– 26 Aug.	49.192	–61.367	299.88	271–377
4R	NFLD (NW)	2008	10	7	30 July – 5 Aug.	49.021	–59.620	241.86	210–271
4R	NFLD (NW)	2009	13	12	4–7 Aug.	48.834	–59.752	282.00	260–314
4Vn	Cape Breton	2008	47	47	25–26 July	46.914	–59.981	190.12	163–311
4Vn	Cape Breton	2009	50	49	19–24 July	46.371	–59.062	247.00	175–366
4Vs	Scotian Shelf (N)	2008	20	14	20–27 July	44.603	–58.447	232.26	99–271
4Vs	Scotian Shelf (N)	2009	9	8	23–25 July	43.873	–59.139	230.54	211–280
4W	Scotian Shelf (C)	2003	190	190	11–23 Sept.	43.794	–62.869	173.94	111–225
4W	Scotian Shelf (C)	2009	50	49	2–27 July	43.850	–61.505	173.66	88–289
4X	Bay of Fundy	2003	100	100	20 Nov.	44.833	–66.350	128	128
4X	Bay of Fundy	2009	81	77	3–7 July	43.529	–65.812	152.60	75–208
3Ps	NFLD (S)	2003	101	101	9–12 Aug.	45.098	–54.794	191.08	148–210
3Ps	NFLD (S)	2005	97	96	3 Aug.	45.150	–54.600	171.18	140–206
3Ps	NFLD (S)	2009	25	17	19 Apr. – 30 May	46.134	–57.142	284.06	265–317
3O	Grand Banks	2005	120	120	3 Aug.	44.030	–52.849	184.74	150–222
3O	Grand Banks	2009	67	59	15 May	44.167	–52.667	198.31	190–206
Total	9	5	1954	1885					

Note: Lat. and long., mean latitude and longitude of sampled individuals. Prop. IM, proportion of immature of genotyped individuals as assessed by visual ±2 standard deviations (±2 SD).

*Regional codes: GSL, Gulf of St. Lawrence; NFLD, Newfoundland; (C), Central; (N), Northern; (NW), Northwestern; (S), Southern.

†Mean temperature at depth ±2 SD for sampled individuals.

‡Mean salinity at depth ±2 SD for sampled individuals (ppt, parts per thousand).

MCMC generations, with an additional 55 000 as burn-in. Results from all STRUCTURE iterations were amalgamated into a single population assignment estimate using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007). To visually assess the fit of our data to current NAFO divisions, each individual georeferenced fish was assigned to its most probable population based on amalgamated results from CLUMPP and embedded in a high-resolution three-dimensional relief model of the Northwest Atlantic created using ArcGLOBE tools in ArcGIS 10 (ESRI, Redlands, Calif., USA). Data for terrestrial and submarine topography were obtained from the ETOP01 Global Relief Model available online (http://www.ngdc.noaa.gov/mgg/global/global.html) using specified coordinates and a 1 min resolution.

Estimating population structure of highly vagile, migratory species with wide distributions that are known to migrate can be challenging, as spawning resident individuals need to be distinguished from transient migratory ones. We thus repeated the population genetic structure analyses described above using only individuals in spawning condition (hereafter spawners).

Because the population number and structure recovered from unsupervised searches using either the complete data set ($n = 1885$) or just the spawners ($n = 574$) was congruent (see Results and Discussion), and substantially reduced from that expected (i.e., 9 NAFO divisions), all subsequent analyses considered only fully genotyped individuals, reducing the total number of samples to $n = 1353$, of which nearly 30% ($n = 409$) were in spawning condition.

Population genetic statistics

We tested for marker neutrality using the LOSITAN selection workbench (Antao et al. 2008), which applies an F_{st} outlier detection algorithm to identify loci possibly under either diversifying or balancing selection. Analyses used both stepwise and infinite allele mutation models using 200 000 permutations and a sample size reflecting the smallest population ($n = 331$). Most loci were within 95% confidence range for candidate neutral loci regardless of mutation model, the exceptions being *ute55* and *ute70*, both of which exhibited signs of diversifying selection (Antao et al. 2008). Based on these findings, we performed all subsequent analyses using all 16 loci and repeated them using only the 14 loci that showed no evidence of departure from neutrality (excluding *ute55* and *ute70*). Results were very similar, and so we focus on those using 16 loci.

Linkage between loci within each population (using 10 000 permutations) and the adherence of loci and populations to Hardy–Weinberg expectations (HWE; using 1 000 000 permutations and 100 000 dememorization steps) were assessed using Arlequin 3.5.1.2 (Excoffier and Lischer 2010), with results evaluated using sequential Bonferroni correction. The same software was used to estimate population-specific allele frequencies and both observed (H_o) and expected (H_e) heterozygosities. Allelic richness (AR), proportion of private alleles (with rarefaction, P_a), and population-specific inbreeding coefficients (F_{is}) were estimated using GenAlEx 6.4 and FSTAT (Goudet 1995; Peakall and Smouse 2006). To assess patterns of genetic differentiation among recovered populations, we

Temp. (°C)[†]	Salinity (ppt)[‡]	Length (cm) (±2 SD)	Age (years) (±2 SD)	Prop. IM	Prop. act. spn
6.14±3.07	32.05± 4.58	51.42±12.18	4.41±1.59	0.27	0.19
11.10±11.91	31.61± 5.53	52.07±14.53	6.50±4.80	0.06	0.29
6.37±7.40	33.28± 4.32	50.89±13.61	4.25±1.43	<0.01	0.39
5.29±2.62	34.23± 2.29	48.02±11.58	4.26±1.71	0.04	0.41
5.15±1.47	34.26± 1.90	46.13±14.06	4.14±1.79	0.10	0.38
3.89±5.27	—	59.80±13.13	4.33±2.00	<0.01	0.42
5.23±0.15	—	47.00±16.84	4.25±2.33	<0.01	0.75
5.42±0.09	—	55.87±12.74	5.00±<0.01	<0.01	0.71
5.16±0.18	—	50.75±15.90	4.33±2.00	0.08	0.08
4.39±1.81	—	40.30±14.90	3.23±2.14	—	—
4.64±2.09	—	48.12±11.02	4.31±1.59	0.12	0.31
6.99±0.28	—	37.73±9.47	3.00±1.79		—
8.45±3.41	—	45.13±7.36	3.86±0.70	<0.01	0.25
—	—	56.79±18.21	—	<0.01	0.28
8.53±2.65	—	46.12±19.14	4.10±2.19	0.24	0.16
—	—	76.82±36.19	—	0.09	0.43
8.07±2.1	—	46.84±26.51	4.18±2.91	0.31	0.27
—	—	77.60±12.53	—	<0.01	0.87
—	—	—	—	—	—
6.59±2.32	—	48.24±15.99	4.65±3.87	0.70	0.29
—	—	—	—	—	—
—	—	—	—	—	—

inspection. Prop. act. spn, proportion of genotyped individuals in active spawning condition. Numerical values report means, ranges, or

performed an analysis of molecular variance using Arlequin (Excoffier and Lischer 2010). Genetic differentiation was also evaluated between all possible pairs of populations using both the classical F_{st} estimate from 100 000 permutations of the MCMC algorithm, as implemented in MSA 4.05 (Dieringer and Schlötterer 2003), and the more robust and recently derived D_{Jost}, as implemented in DEMEtics (Gerlach et al. 2010) using 1000 bootstrapping iterations to calculate significance. All population genetic statistics described above were derived in parallel using both all (fully genotyped) samples and spawners only. Owing to the similarity of patterns, we used the entire data set in subsequent analyses only ($n = 1353$).

Size and connectivity

Contemporary effective population sizes (N_e) were estimated for each recovered population using the linkage disequilibrium method (LDNe) based on the integration of several moment-based estimates as described in Waples and Do (2008, 2010). We recognize the high uncertainty associated with moment-based $\widehat{N}_e$, which can be subject to upward and downward biases when applied to highly dispersed and long-lived marine species. Nevertheless, this method can be used to gauge relative $\widehat{N}_e$ in recovered genetic populations (Hare et al. 2011, but see Fraser et al. 2007). In efforts to minimize the inclusion of potential migrants in $\widehat{N}_e$ calculations, we filtered our data to include individuals assigned to each recovered genetic population by an admixture coefficient ≥80%. Population-specific $\widehat{N}_e$ was calculated by taking the harmonic mean of LDNe estimates from each year the population was sampled. To standardize assessments among populations, we included only years in which all populations had adequate sample sizes (2003, 2005, and 2009). N_e estimates were generated using alleles with frequencies greater than 0.02 and 0.01, and jacknifing was conducted over loci pairs to estimate 95% credible limits. We present N_e estimates using both minimum allele frequencies to show consistency of relative signal among sampled populations.

As discussed by Fraser et al. (2007), the high uncertainty associated with contemporary N_e estimates can be reduced by comparison with estimates obtained with other methods. To get more robust time-integrated estimates of N_e incorporating historical influences in each population, including migration rates, we applied an isolation with migration (IM) model estimating the N_e of two closely related populations in the coalescent (Hey and Nielsen 2004; Hey 2010). IM models estimate long-term N_e by maximizing the posterior probabilities of the population sizes, migration rates, and genealogies (including branch lengths), generating the genetic diversity (θ) of populations included in a phylogeny (Hey and Nielsen 2004; Hey 2010). Since estimated genetic diversity is a function of N_e and the mutation rate (μ) of the markers used to characterize it (i.e., $\theta = 4N_e\mu$; Hey 2010; Hare et al. 2011), it can be used to recover N_e. IM models search parameter space for the most likely estimates using a Bayesian framework assuming random mating within populations, no recombination within but free recombination among selected markers, and that populations are each other's closest rela-

tives not exchanging genes with other nonsampled populations (Hey and Nielsen 2004; Hey 2010). Here, we used IMa2 to generate long-term $\widehat{N}_e$ on a subsample of 40 individuals from each recovered population run in pairwise fashion. The analyses were run pairwise following recommendations concerning the information (i.e., number of marker loci) needed for reliable parameter estimation in studies involving more than two populations (IMa2 manual; Hey 2010). Searches used priors determined from short preliminary runs and were iterated to over 20 000 000 steps, allowing them to reach a stationary distribution from which to sample. Once stationarity was achieved, all runs were allowed to proceed for an additional 10 000 000 steps, with genealogies being recorded every 100th step for a total of ~100 000 genealogies for parameter assessments. All analyses used 100 metropolis-coupled MCMC chains with two heating terms to ensure high swap rates among them, and each pairwise comparison was repeated using the same individuals and priors but with different random seeds to ensure consistency between runs. Long-term $\widehat{N}_e$ was calculated from population-specific θ estimates and using a microsatellite mutation rate of 5×10^{-5}, mid-way between those used in other fish studies (10^{-4} – 10^{-5}; Caldera and Bolnick 2008; Berner et al. 2009). Final population-specific long-term N_e estimates were calculated by taking the mean of all values determined from all pairwise comparisons that included the focal population. For both N_e methods described above, we used only 14 loci to minimize the potential influence of non-neutral markers.

Recent gene flow was estimated using BIMr 1.0 (Faubet and Gaggiotti 2008). BIMr estimates the proportion of resident and migrating individuals among populations and provides a platform to test the influence of external factors on interpopulation migration rates using a Bayesian framework. The sign and magnitude of derived association factors determine the strength and direction of the environmental factors' influence on migration rates among populations (Faubet and Gaggiotti 2008). We tested whether latitudinal, longitudinal, and depth differences (including their first-order interactions) among populations significantly modulated migration rates by including these factors as prior-based influences in our analyses. Because including loci under diversifying selection is expected to underestimate migration rates, we included only the 14 neutral loci in our calculations. Migration rate estimates (m) were generated from 10 replicated MCMC runs, each one starting with 20 000 generation runs used to stabilize acceptance rates of priors, the first 1 000 000 generations set as burn-in and an additional 5 000 000 generations from which to sample. Samples were taken every 100th iteration, resulting in an overall sample size for each run of 50 000. We present results of the run with the lowest Bayesian deviance as suggested by Faubet and Gaggiotti (2008) for data characterized by low population-specific genetic differentiation ($F_{st} \leq 0.01$), but also summarize results from all runs.

Structure and vulnerability of population within the southern Gulf of St. Lawrence

Finally, because all results pointed to population differentiation and structure occurring within the SGSL and the adjacent region off northern Cape Breton Island (NAFO divisions 4T and 4Vn; Fig. 1), we isolated these data from the overall analyses and re-ran the Structurama, STRUCTURE, and CLUMMP analyses described above using the same protocols. We used recovered populations from these analyses to further investigate the likely causes of their differentiation by re-sorting individual admixture proportions as determined in CLUMMP by oceanographic features thought to play a key role in segregating hake morphotypes (Kohler 1971; Hurlbut and Clay 1998; Herder et al. 2005). Georeferenced oceanographic data collected during sampling (temperature and salinity at depth) were incorporated along with sampling date, depth, and location into individual-based models predicting posterior probability of belonging to the "SCOTIAN" population (see Results) as determined from the CLUMPP outputs. Models were built for each sampling year, and for all years pooled together, and were designed to test the ability of environmental variables to predict the population of origin of the individuals sampled within the SGSL (and northern Cape Breton Island). Variables included in the models were based on multicolinearity, and model selection (Akaike information criterion) tests using standard generalized linear modelling scripts run in the base package of R (R Development Core Team 2010).

Results

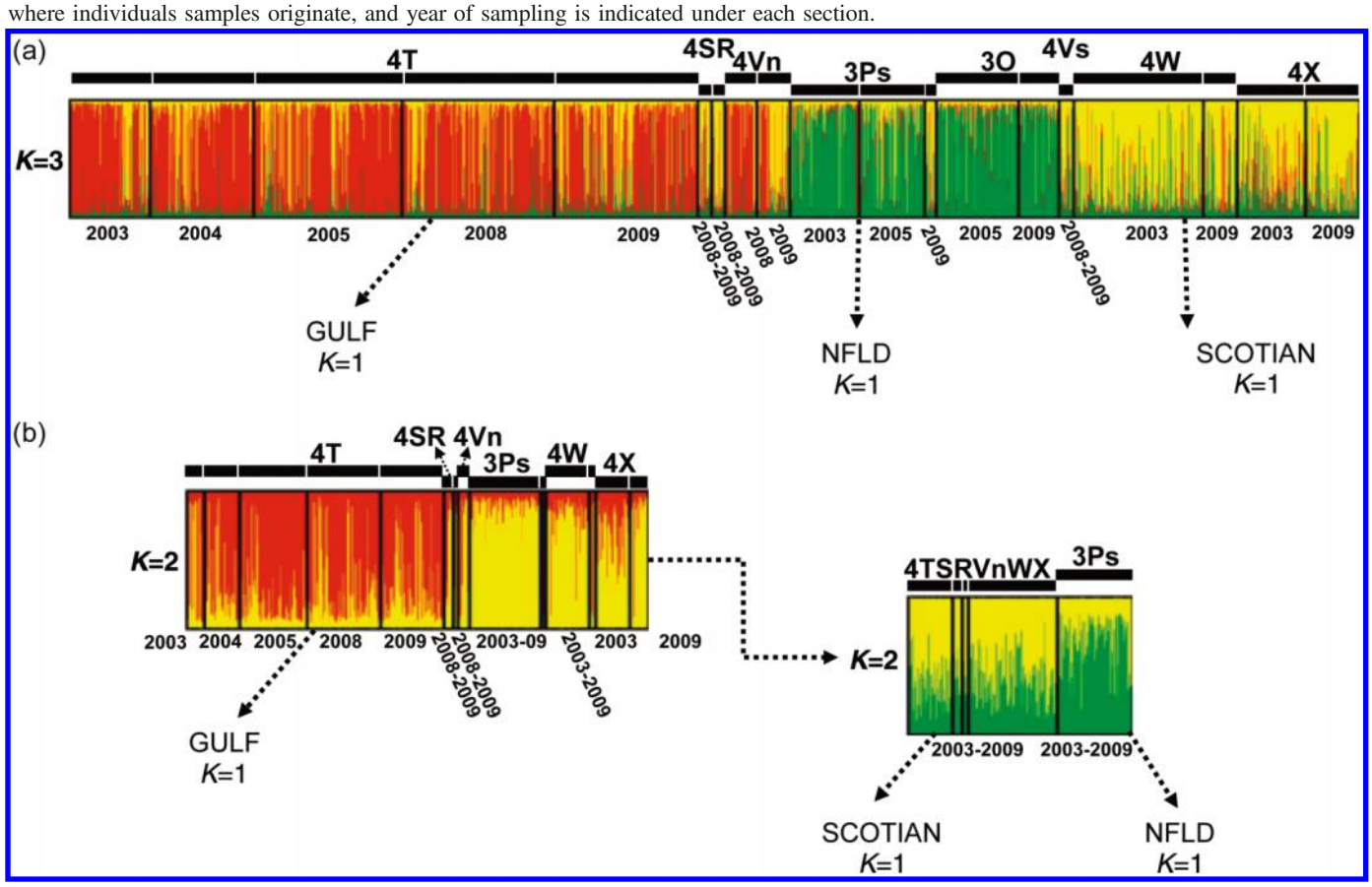
Population structure

Unsupervised searches using Structurama recovered $K = 3$ as the most probable number of genetically distinct populations using the overall data set (Fig. 2*a*; Supplemental Table S1)[1]. Most individuals could be assigned with high certainty to one of these three populations, with only 9.5% of individuals assigned to their most probable population by admixture proportions less than 60%. Recovered populations did not show exclusive relationships to particular NAFO divisions nor to any temporal assessments within them. Nevertheless, plotting individuals on a topographic model of the Northwest Atlantic based on their highest level of admixture proportion revealed a pattern in population structure (Fig. 3*a*). One population (hereafter GULF) is associated with the SGSL, along the southern slope of the Laurentian Channel and extends into the north of Cape Breton Island, encompassing NAFO divisions 4T and 4Vn (Fig. 3*a*). A second population (hereafter NFLD) is predominantly located off southern Newfoundland in NAFO divisions 3O and 3Ps (Fig. 3*a*). Finally, a third population (hereafter SCOTIAN) is located throughout the Bay of Fundy, on the Scotian shelf, but also extends along the Laurentian Channel and into the northern Gulf of St. Lawrence and SGSL (Fig. 3*a*). This last population is the most extensive and includes NAFO divisions 4X, 4W, 4Vs, 4Vn, 4T, 4R, and 4S. Further tests performed on the recovered populations indicated no further population structure within them (i.e., $K = 1$ in each; Fig. 2*a*; Supplemental Table S1)[1].

Meanwhile, Structurama searches using only the spawners recovered only two genetically distinguishable populations (Fig. 2*b*; Supplemental Table S2)[1]. Although recovering the same population located mainly within the SGSL region, searches recovered only one other population made up of in-

[1]Supplementary data are available with the article through the journal Web site (http://nrcresearchpress.com/doi/suppl/10.1139/f2011-178).

Fig. 2. (*a*) Hierarchical Bayesian posterior probability assignment of white hake sampled from nine different NAFO divisions (n = 1885). Each individual is represented by a bar whose colour pattern indicates its posterior probability of belonging to one of three genetic clusters estimated from Structurama. (*b*) The same analyses repeated using only identified spawning individuals (n = 574). Arrows leading from identified clusters point to results of further cluster-specific analyses. Thick line above admixture charts identifies the NAFO division from where individuals samples originate, and year of sampling is indicated under each section.



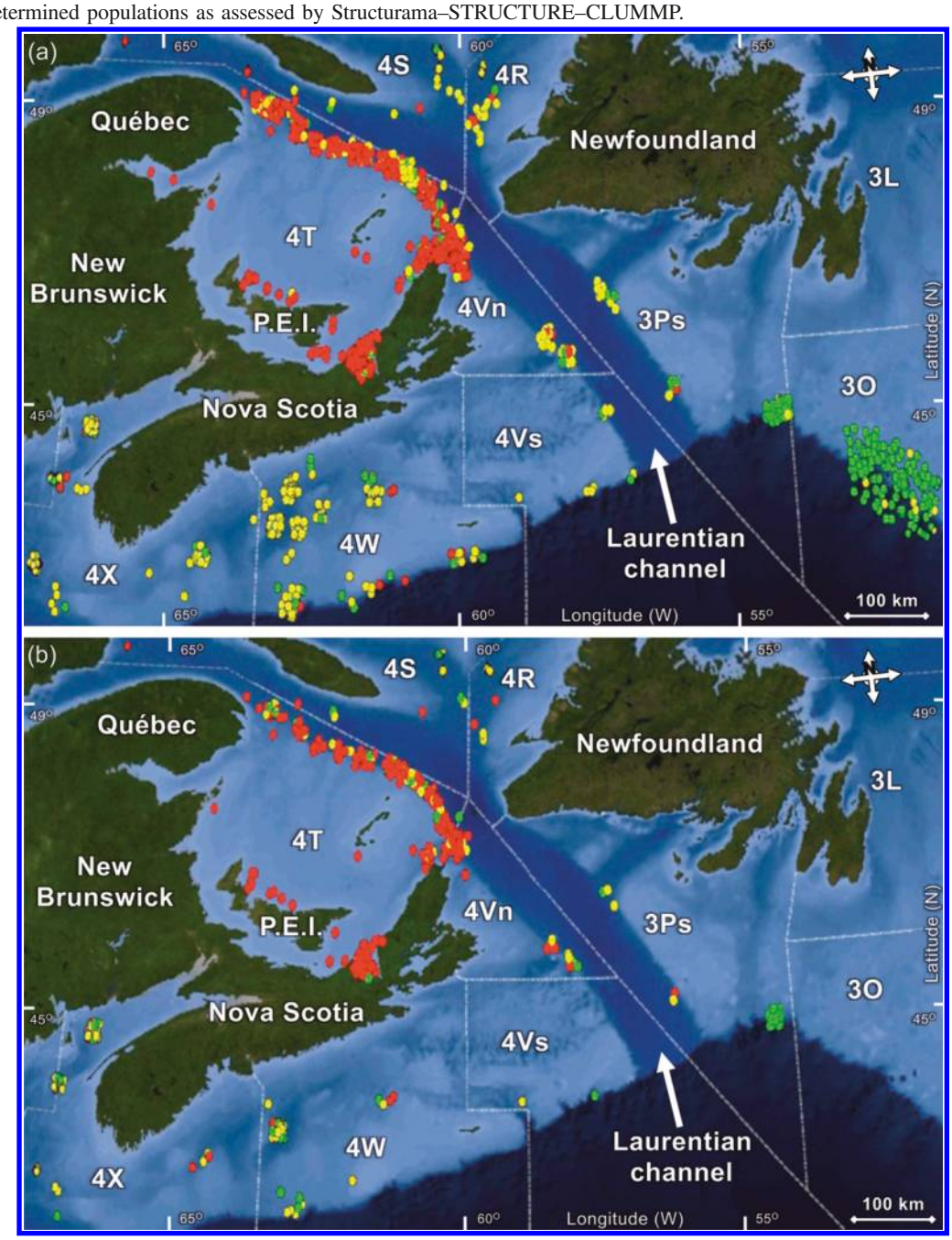
dividuals located primarily outside the SGSL, but not exclusively (Fig. 2*b*). Further tests on the GULF population revealed no further structuring, but that performed on the population found mainly outside the SGSL recovered two populations (Fig. 2*b*; Supplemental Table S2)[1]: one primarily located off southern Newfoundland and the other primarily located on the Scotian Shelf and in the Bay of Fundy. Plotting only spawners on our topographical model of the Northwest Atlantic revealed a very similar distribution pattern of the three genetic populations, consistent with the results described above (Fig. 3*b*). Thus, both the overall data and just the spawners recovered the same three genetically distinguishable populations (i.e., GULF, NFLD, and SCOTIAN).

Statistics of recovered populations

No evidence of linkage among the 16 and 14 loci used within the three identified populations was observed. However, 10 loci–population combinations were out of HWE from a possible 48 comparisons overall samples (Table 2). Four occurred in both the GULF and the SCOTIAN populations, and 6 of these included *ute55* and *ute70*, which showed signs of diversifying selection (see Materials and methods). Using only 14 loci, we found 4 combinations outside HWE out of a possible 42, a number not very far from expected significant results by chance (two). In this latter case, both the GULF and the SCOTIAN populations exhibited two significant deviations, but in both cases loci were not consistent between populations. All genetic diversity variables were generally found to be higher in the SCOTIAN than in the other two populations (Table 2), indicating that the SCOTIAN is the more genetically diverse population. While the NFLD population was the least genetically diverse in terms of AR and P_a followed closely by the GULF, the GULF population also exhibited lower H_e, H_o, and F_{is} than the other two populations, indicating that it also expresses low genetic diversity relative to the other two (Table 2). Population-specific F_{is} were all positive but nonsignificant after sequential Bonferroni correction. Genetic diversity indices followed the same broad trends but with slightly lower values when calculated using 14 loci or when using the spawners data only (Table 2).

An analysis of molecular variance performed on the overall data indicated that 3.62% ($p < 0.001$) of the total genetic variation was due to genetic differences among recovered populations (Supplemental Table S3)[1]. A similar analysis performed on just the spawners showed that a similar and still significant 3.38% ($p < 0.001$) of genetic variance explained by population differences (Supplemental Table S4)[1]. Pairwise genetic distances estimated among recovered populations were significantly different ($p < 0.001$) and indicated that the GULF is the most distinct of the three regardless of whether we used the full complement of 16 loci or only the

Fig. 3. Distribution of (*a*) the complete data set of all sampled white hake used in this study (n = 1885) and (*b*) only spawning individuals (n = 574) in the Northwest Atlantic, where individual colour assignment is based on highest posterior probability of belonging to one of three genetically determined populations as assessed by Structurama–STRUCTURE–CLUMMP.



14 statistically neutral ones (16 loci: GULF–NFLD: F_{st} = 0.053, D_{Jost} = 0.231; GULF–SCOTIAN: F_{st} = 0.037, D_{Jost} = 0.195; 14 loci: GULF–NFLD: F_{st} = 0.043, D_{Jost} = 0.193; GULF–SCOTIAN: F_{st} = 0.030, D_{Jost} = 0.164). The GULF population is genetically more differentiated from the NFLD population than from the SCOTIAN, while the SCOTIAN and NFLD populations are the least differentiated (16 loci: NFLD–SCOTIAN: F_{st} = 0.015, D_{Jost} = 0.116; 14 loci: NFLD–SCOTIAN: F_{st} = 0.011, D_{Jost} = 0.094). The same pattern of population genetic differentiation was recovered using only the spawners but with values that were, on average, 13% lower than those calculated using the complete data set (Supplemental Table S5)[1]. Thus, genetic population structure and differentiation recovered from the fully genotyped spawners (n = 409) was generally weaker than that recovered using all fully genotyped fish (n = 1353).

Population size and connectivity

All populations exhibited large and comparable contemporary $\widehat{N}_e$ values, but the GULF $\widehat{N}_e$ was smaller than that of the SCOTIAN and NFLD populations. The SCOTIAN population was the largest, with the NFLD population in between. However, all 95% confidence intervals for population-specific $\widehat{N}_e$ values overlapped, and in all populations upper confi-

Table 2. Population genetic statistics from the genetically identified white hake populations (as per Structurama and STRUCTURE 2.2.3) in the Northwest Atlantic.

Population	$n_{16,\ 14}$	$AR_{16,\ 14}$	$P_{a\ 16,\ 14}$	$H_{o\ 16,\ 14}$	$H_{e\ 16,\ 14}$	$F_{is\ 16,\ 14}$	HWE
GULF	576, 576	20.92, 20.12	1.13, 1.00	0.69, 0.7	0.72, 0.71	0.041, 0.017	4, 1
GULF spawners	194, 201	16.29, 16.31	2.19, 2.07	0.69, 0.69	0.72, 0.71	0.032, 0.023	3, 1
NFLD	331, 331	20.50, 19.64	0.63, 0.57	0.74, 0.75	0.78, 0.77	0.050, 0.034	2, 1
NFLD spawners	103, 115	17.31, 16.40	0.75, 0.64	0.75, 0.75	0.79, 0.77	0.050, 0.027	3, 1
SCOT	446, 446	25.44, 23.63	3.50, 2.93	0.77, 0.77	0.81, 0.80	0.057, 0.027	4, 2
SCOT spawners	112, 116	21.10, 19.79	3.38, 2.86	0.78, 0.77	0.81, 0.79	0.043, 0.030	2, 1

Note: n, sample size. AR, allelic richness, and P_a, private alleles (both calculated with rarefaction). H_o and H_e, observed and expected heterozygosities, respectively. F_{is}, inbreeding coefficient. HWE, number of loci out of Hardy–Weinberg expectations after sequential Bonferroni correction. Results determined using only individuals with complete genotypes for both the overall data and only spawning individuals. Data tabulated for both 16 and 14 loci.

dence limits were indeterminate (Fig. 4*a*). We note here that the relative pattern of population-specific contemporary $\widehat{N}_e$ using this method was consistent regardless of the critical allele frequency used, but those using the larger minimum allele frequency (0.02) were slightly larger (Fig. 4*a*). Long-term $\widehat{N}_e$ based on parameters derived to explain population genetic diversity in the coalescent also showed very similar patterns in estimated effective population sizes, with the GULF and SCOTIAN populations exhibiting the smallest and largest long-term $\widehat{N}_e$, respectively (Fig. 4*a*). The GULF and the NFLD populations exhibited overlapping 95% credible limits on long-term $\widehat{N}_e$, but neither population exhibited overlapping credible limits with SCOTIAN (Fig. 4*a*).

The migration model with the highest posterior probability (all runs) from BIMr included only latitude as a factor negatively related to migration rates (Pr = 13.8%–14.7%). Within the run with the lowest Bayesian deviance, the posterior probability of this model was 13.8% with an association factor $\alpha_{lat} = -1.08$ (–4.89–2.27). The next best model (all runs) included only depth as a variable and was also negatively associated with migration rates (Pr = 8.4%–9.3%; best run Pr = 8.5%; $\alpha_{depth} = -0.901$ (–2.58–4.41)). The third best model (7 of 10 runs) did not include any environmental variables (Pr = 6.6%–7.8%; best run Pr = 7.2%; $\alpha_0 = 0.259$ (–3.65–6.24)), with all subsequent models having lower posterior probabilities. In all models, posterior probabilities were low, suggesting other unknown factors may also be important in modulating migration rates among populations (Faubet and Gaggiotti 2008). Nevertheless, the two best models show that migration rates are negatively associated with increasing latitude and depth differences among genetically differentiated populations. Migration rates between the SCOTIAN and NFLD populations were comparable and relatively high, with exchanges in either direction between 11% and 17% per generation (Fig. 4*b*). Migration rates to and from either the NFLD and (or) SCOTIAN populations into or out of the GULF, in contrast, were generally an order of magnitude lower, indicating that gene flow between the GULF and the other populations is more restricted (Fig. 4*b*).

Population structure within the southern Gulf of St. Lawrence

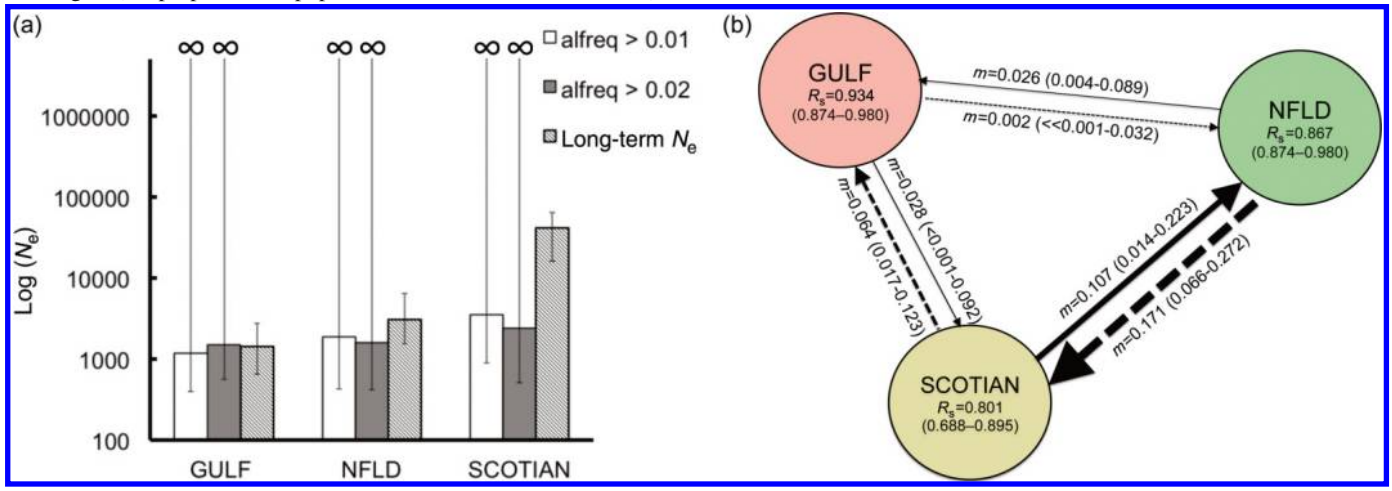
Structurama recovered $K = 2$ as the most likely number of genetic clusters from samples collected within the SGSL and off northern Cape Breton Island (NAFO divisions 4T and 4Vn), consistent with the results of the overall analysis above (Fig. 2, Supplemental Table S6)[1]. Reorganizing individual probability assignments obtained from these areas by various environmental variables showed gradual changes in individual assignments with increasing salinity and depth, while no such patterns were obvious using other environmental variables (Fig. 5). We note here that the cluster most associated with deeper more saline waters in the SGSL is the same genetic cluster as the SCOTIAN population (Figs. 5 and 2*a*). Thus, we hereafter refer to the two clusters apparent within the SGSL and north of Cape Breton Island as the "GULF" and "SCOTIAN" genotypes.

Significant relationships ($R^2 = 0.015$–0.37, $p < 0.001$) were found predicting the posterior probability of white hake belonging to the SCOTIAN population from environmental variables. Generalized linear models quantifying these relationships in each year, as well as over all years, demonstrated that although variable in magnitude and direction, depth was the only consistent factor significantly related to the probability of individuals belonging to the SCOTIAN population within the sampled areas (Table 3; Supplemental Table S7)[1]. Geographic position (longitude), temperature at depth, and sampling date along with some first-order interactions were also significant factors associated with posterior assignment probability. However, these factors were not consistently important through all sampled years (Table 3; Supplemental Table S7). Using the pooled data from all years in the same modelling framework demonstrated that both geographic position and sampling date had significant negative relationships with the probability of belonging to the SCOTIAN population, while depth and temperature at depth were significantly positive. The interactions of geographic position with both depth and sampling date were also significant but in the negative and positive directions, respectively. This overall relationship explained approximately 20% of assignment probability of SGSL and northern Cape Breton white hake (Table 3; Supplemental Table S7) to the SCOTIAN population.

Discussion

Results presented here allow us to reject the hypothesis of panmixia in white hake and show that this species exhibits a degree of population structure among the highest yet recorded for a widely distributed and abundant marine species under exploitation, including other gadoids. More importantly, population subdivision in this species does not follow previously defined management units (NAFO divisions) but,

Fig. 4. (*a*) Contemporary effective population sizes (N_e) estimates determined using the linkage disequilibrium method with a minimum allele frequency of 0.01 (empty) and a minimum allele frequency of 0.02 (grey), and long-term N_e estimated using isolation with migration models run in IMa2 (hatched). Error bars indicate 95% confidence intervals around estimates, where those running off the chart are not finite. (*b*) Migration rates (*m*; determined from BIMr) connecting the three identified population with associated 95% highest posterior density intervals framing *m* (R_s, proportion of population estimated as resident fish).



and especially in one of the regions examined, is a function of geographic position and depth. Within the SGSL and its surrounding area (north of Cape Breton Island), individuals collected in the late summer – early autumn from the inshore relatively shallow areas characterized by warmer temperatures are genetically distinguishable from the majority of those collected in mid-summer from the deeper colder waters associated with the slope of the Laurentian Channel, but both groups are present in this region during all sampling periods. Overall, three genetically distinguishable groups of white hake have been identified, and they differ in their effective population sizes and connectivity. White hake experienced sharp population declines over the last decades, particularly in the SGSL, initially resulting in the closure of the fishery in 1995 and more recently in its impending consideration for listing as a threatened species by the Committee On the Status of Endangered Wildlife in Canada (COSEWIC). Below, we examine these issues in detail and assess the genetic and conservation implications of the identified population structure in this species.

High population structure for a widely distributed marine species

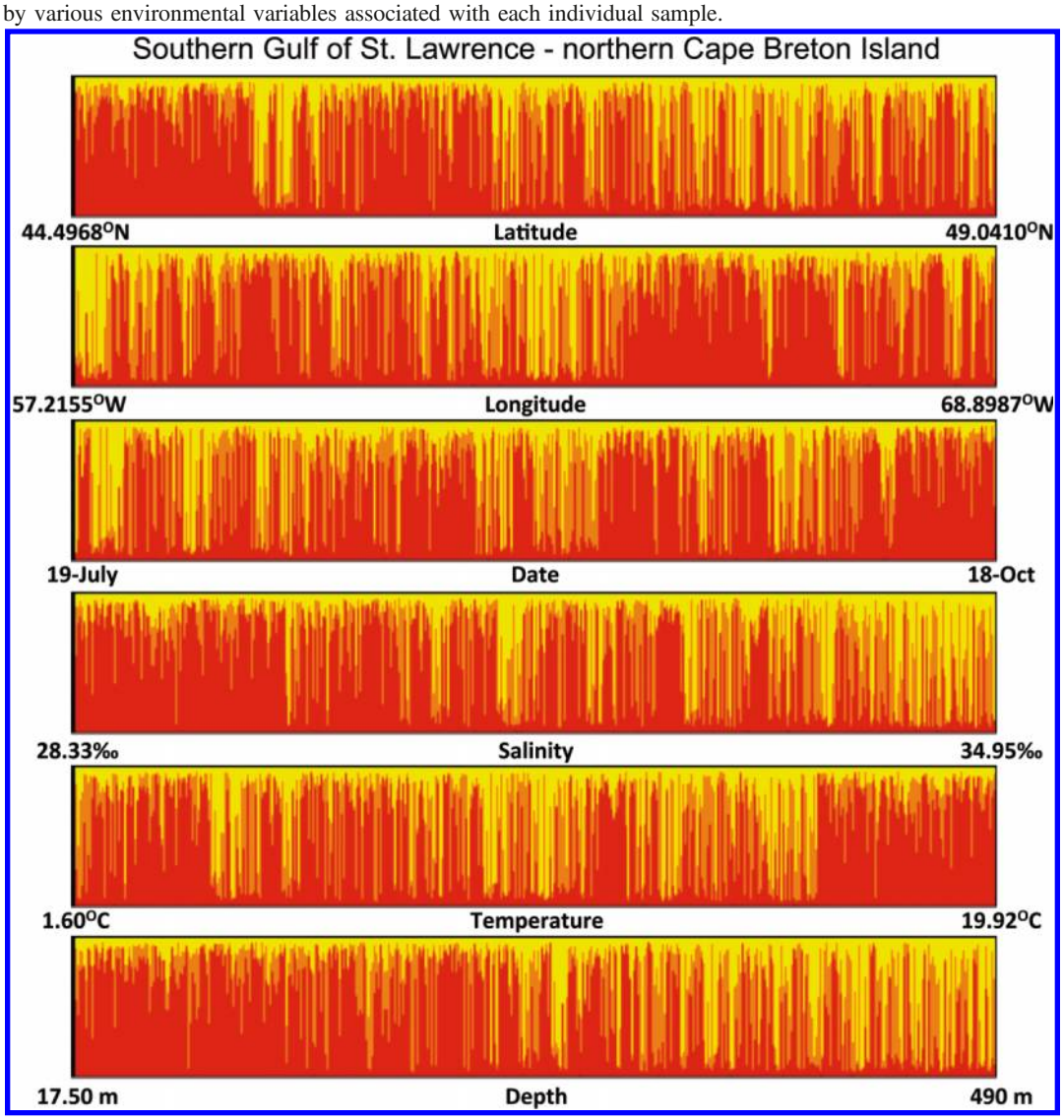
White hake population structure is much more pronounced than recorded for other gadoids (e.g., cod, Ruzzante et al. 1999; Pampoulie et al. 2006) and many other marine fishes (Saillant and Gold 2006; Gonzalez et al. 2008; White et al. 2011). In fact, population structure in this species seems to be, on average, an order of magnitude higher than has been generally recorded for cod (Ruzzante et al. 1999; Pampoulie et al. 2006) and herring (Bekkevold et al. 2005). This is an unexpected result, particularly given that other long-lived, widely distributed marine species demonstrate little to no population structure throughout their distributions as assessed from extensive microsatellite surveys as conducted here (Gonzalez et al. 2008; White et al. 2011, but see Stefánsson et al. 2009).

Population structure reported in other marine species has been related to oceanographic processes such as advective dispersal by currents of eggs, larvae, and even adults (Kenchington et al. 2006; Pampoulie et al. 2006; Galindo et al. 2010). The population structure of white hake, however, shows no obvious relationship to large-scale oceanographic currents, thought to be the major means of dispersal for this species (Musick 1974; Markle et al. 1982, but see Fahay and Able 1989). For example, the SCOTIAN population, mostly located on the Scotian Shelf, also extends along the Laurentian Channel and into the northern Gulf. Predominant currents in this area move southward down the Strait of Belle Isle (between Quebec and Newfoundland), mix with freshwater inputs from the St. Lawrence River, and cause cyclonic flushing of the SGSL, moving water masses both onto, and off, the Scotian Shelf (Han et al. 1999; Kenchington et al. 2006). The extensive presence of SCOTIAN genotypes in the Gulf suggests that such currents do not impede the SCOTIAN distribution. Moreover, population connectivity estimates based on migration rates from BIMr also conflict with current-based dispersal hypotheses. Even though predominant currents in the Northwest Atlantic vary seasonally in strength, they are directionally stable throughout the year, making large-scale reversals unlikely (Han et al. 1999; Kenchington et al. 2006). Inconsistencies in population distributions from those expected based on advective dispersal attest to the directed movement of white hake in establishing population-specific distributions during sampled periods.

Population structure inconsistent with management spatial scale

The white hake population structure observed during our sampling periods reflects that of pre-moratorium peak harvest times and is not consistent with NAFO management units. First, genetically homogenous populations straddle several NAFO divisions (e.g., SCOTIAN in 4W-4X; NFLD in 3Ps-3O). Second, genetically distinguishable populations are found within single divisions (GULF and SCOTIAN in 4T and 4Vn), wherein they occupy preferentially different regions but also exhibit overlapping distributions. Thus NAFO management units do not appear to adequately represent cur-

Fig. 5. Bayesian posterior probability assignment of individual white hake (n = 902) sampled from the southern Gulf of St. Lawrence and northern Cape Breton Island (NAFO divisions 4T and 4Vn) to either the GULF (dark) or SCOTIAN (light) populations. Posterior probabilities are sorted by various environmental variables associated with each individual sample.



rent population structure. Nonetheless, estimating population structure of such a highly mobile and extensively distributed species is challenging, as many individuals may engage in seasonal migrations. As mentioned previously, white hake population structure in the Northwest Atlantic is not well known, nor are white hake spawning times or locations. This raises the possibility that the population structure we have detected may in part reflect migratory patterns of transient populations moving through the sampled areas, a likely scenario given that 60%–70% of our data correspond to nonspawning individuals. Our results, however, do not support such a premise, as we recover the same population structure using only spawners and, indeed, individuals in spawning condition assigned to the SCOTIAN population can be found in the SGSL region. Perhaps more important, however, is that population differentiation using only spawners is actually weaker than that estimated with the entire data set. Such results have three important implications. First, population structure estimated from the complete data set does indeed reflect actual breeding populations. Consequently, using the entire data set resulted in a better delineation of the three genetically distinguishable populations than using just spawners. Second, although quite broad and extending over several hundred kilometres, recovered populations are distinguishable to a level not often observed among marine species, and a high proportion of individuals within them are residents. Third, population differentiation occurs despite partially overlapping spawning distributions.

Results presented here confirm the presence of two genetically distinguishable populations within the SGSL during harvesting periods, as suspected from previous work. For instance, Kohler (1971) showed extensive phylopatric behaviour of tagged white hake within the SGSL. Clay et al. (1992) showed weak allozyme differences, and Hurlbut and Clay (1998) and Herder et al. (2005) showed morphological bimodalities and length–depth relationships in samples collected from the shallow and deep areas of the SGSL. Herder et al.'s (2005) results were, however, temporally variable and were thus attributed to changes in population demographics imposed by harvesting. Melendy et al. (2005), meanwhile,

Table 3. Generalized linear modelling coefficients of determination (slopes and intercepts) predicting the posterior probability that fish sampled from the southern Gulf of St. Lawrence (NAFO 4T) and northern Cape Breton Island (NAFO 4Vn) belong to the SCOTIAN population based on selected environmental variables (determined by model selection using Akaike information criterion).

Year	Long	Depth	Temp	Date	Long × temp	Long × depth	Long × date	Depth × date	Depth × temp	Temp × date	Intercept	R^2	F	df	P
2003	3.148	0.001	37.855	—	–0.614	—	—	—	—	—	–193.711	0.372	15.878	4, 107	<0.001
2004	–0.050	–0.005	–0.032	—	—	—	—	—	0.001	—	3.557	0.325	16.701	4, 139	<0.001
2005	0.129	–0.049	—	–0.006	—	—	—	<0.0001			–6.189	0.280	18.734	4, 193	<0.001
2008	—	0.002	–0.889	–0.021	—	—	—	—	—	0.004	4.636	0.150	8.792	4, 200	<0.001
2009	–0.848	0.002	–0.050	–0.212	—	—	0.004	—	—	—	51.413	0.184	10.712	5, 237	<0.001
All years	–0.394	0.016	0.008	–0.110	—	–0.0002	0.002	—	—	—	24.132	0.204	38.217	6, 895	<0.001

Note: Long, longitude in decimal degrees (latitude significantly correlated with depth within each sampled year and overall; $r^2 = 0.69–0.87$, $p < 0.001$); depth, depth at which fish were collected (in m); temp, temperature of the water at which fish were caught (°C); date, ordinal date of catch.

were generally able to assign white hake samples from the SGSL to either shallow or deeper regions based on parasite prevalence and abundances. Thus, population structure in the SGSL has been suspected for some time, however, no previous study had conclusively demonstrated temporally stable genetic population structure of white hake in either the SGSL or throughout the northwest Atlantic during peak harvest times as we have done here.

Effective sizes and connectivity among populations

The three genetically distinguishable white hake populations identified using genetic clustering algorithms differ in their effective sizes and connectivity. The GULF appears to be smaller than the other two populations based on contemporary estimates, but has rather wide credible limits around $\widehat{N}_e$. Therefore, these results should be interpreted cautiously. More accurate $\widehat{N}_e$ values using the LDNe method for systems with overlapping generations like white hake can be obtained by considering individual cohorts. This approach represents the effective number of breeders ($\widehat{N}_b$). The product of the harmonic mean of $\widehat{N}_b$ and species generation time typically confers a more adequate $\widehat{N}_e$ for such populations. The harmonic mean of several $\widehat{N}_e$ values estimated this way can give a running average over time (Waples and Do 2008, 2010). In this study, however, the ages were not available for most individuals. Thus we estimated N_e values using all available individuals regardless of age, which may have downwardly biased our estimates (Fraser et al. 2007; Hare et al. 2011). In addition, a recent simulation study shows that although the influence of migration on $\widehat{N}_e$ using the LDNe method is generally negligible, in instances of high and sustained migrant exchanges among large populations ($\widehat{N}_e >$ 500), the inclusion of potential migrants can lead to an overestimation of $\widehat{N}_e$ by reducing the allelic linkage disequilibrium among included individuals (Waples and England 2011). Consequently, estimates made on populations experiencing high gene flow, such as the populations in our study, could be subject to upward bias (Fraser et al. 2007; Hare et al. 2011; Waples and England 2011). Although we minimized the potential source of migration-related bias in our estimates of N_e by considering only individuals with high assignment probabilities (>0.80; see Methods), it is still possible for our estimates of contemporary $\widehat{N}_e$ to exhibit some degree of upward or downward bias. Nevertheless, the consistency of results using two allele frequency minimum thresholds demonstrates that the relative size differences among sampled populations are likely real. This, in conjunction with long-term $\widehat{N}_e$ obtained using the IM approach (whose assumptions our data meet more adequately), which shows a similar pattern (but with larger values) and corroborates that the GULF population is likely the smallest of the three.

The GULF population also appears the most isolated, as migration rates into and out of it are generally an order of magnitude lower than those between the NFLD and SCOTIAN populations. This is unexpected considering the proximity of the SCOTIAN and GULF individuals observed along the southern slopes of the Laurentian Channel, versus the disjoint distributions of the NFLD and SCOTIAN indi-

viduals across the Laurentian Channel. Thus, although distances among populations are important in limiting population connectivity, other factors are likely also influential. One such factor is depth, where depth differences were also (to a lesser extent than geographic position) negatively associated with migration rates. Depth has increasingly been shown to be an important dimension structuring marine fishes (Pampoulie et al. 2006; Stefánsson et al. 2009; Ingram 2011), and our study adds to this body of evidence. Since geographic position and depth only explained a limited proportion of estimated migration rate variation, however, other unidentified factors may also influence connectivity among white hake populations. Further investigations more directed on the connectivity among white hake populations are needed to better identify more important spatio-temporal factors constraining gene flow in this species.

Population structure in the Southern Gulf

Similar to results concerning the entire Northwest Atlantic, we found that population structure within the SGSL was linked to depth and sometimes to geographic position, temperature, and sampling date. Our results are consistent with those of studies examining population structure in other gadoids, including Norwegian, Icelandic, and North Sea cod (*Gadus morhua*) (Case et al. 2005; Pampoulie et al. 2006; Skarstein et al. 2007), suggesting that similar oceanographic features may drive population structure in marine fishes sharing comparable life history characteristics or phylogenetic history. Population structure in marine fishes along an environmental gradient, and in particular depth, may thus be indicative of a more general depth-related niche axis along which divergence occurs through local adaptation to diverse feeding habitats, light conditions, spawning sites, or other ecological factors (Stefánsson et al. 2009; Sivasundar and Palumbi 2010; Ingram 2011). The magnitude and direction of influence of depth, location, temperature, and date in our study was variable and only partially explained variation in population assignment both on an individual-year basis and considering all years combined. These findings suggest that other unidentified factors may also contribute to SGSL population structure.

Studies of cod have shown that population structure is related to depth, temperature, and salinity based on allele frequency differences at the pantophysin (*Pan* I) marker, a nuclear locus purported to be under diversifying selection (Case et al. 2005; Pampoulie et al. 2006; Skarstein et al. 2007). Jakobsdóttir et al. (2011) also showed differences in allele frequency at the *Pan* I locus associated with size at maturity. These authors showed that the loss of older, late-maturing, slow-growing individuals over a six-decade period of intense fishing resulted in significant changes in allele and genotypic frequencies at this functional marker, while no temporal changes were observed in a suite of neutral microsatellite markers (Jakobsdóttir et al. 2011). Although we showed here that 2 of the 16 loci exhibit signs of diversifying selection, their contribution to population structure was not stronger than that of other loci; population structure recovered using only 14 loci was essentially the same. Nonetheless, it remains a possibility that reduced population sizes within the SGSL from overharvesting may have led to changes in population structure related to functional loci that are not fully observed in a mostly neutral marker survey (André et al. 2011).

Population structure within the SGSL has been suspected for some time, and our data are consistent with this hypothesis. We also found that the shallow GULF population may be the smallest and is the most isolated of the three populations identified. This is also the population that has been most extensively exploited in the past (Hurlbut 2005). Currently, the conservation status of white hake in Atlantic Canada is under COSEWIC revision. The information provided from this work is expected to help refine conservation efforts for the SGSL population and possibly for the species as a whole. Based on our analyses, collection of white hake in the SGSL should be avoided, particularly in the late summer – early fall in the shallower, less saline, warmer basins.

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