Integrated stock mixture analysis for continous and categorical data, with application to genetic–otolith combinations

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Abstract: Fish populations or stocks often intermix on fishing grounds, thus posing problems for stock assessors or managers attempting to optimize yields and minimize overexploitation of individual stocks. A Bayesian framework is developed here to simultaneously analyse many of the different data types (e.g., otolith elemental composition, nuclear and mitochondrial DNA) that have been used to identify stock origins of fish in mixed groups and thus take maximal advantage of the available information. Elements of this framework include the capability to analyse each data type either separately or in combination for any number of mixed-group samples, Bayesian credible intervals to evaluate the uncertainty associated with the estimated proportion of the original stocks in the mixed groups, and posterior predictive diagnostics to evaluate the assumptions of the underlying models. The framework was used to re-analyse a subset of otolith elemental composition and microsatellite allele frequency data obtained from the same fish from Atlantic cod (*Gadus morhua*) stocks in the Gulf of St. Lawrence, Canada.

Résumé : Les populations ou les stocks de poissons se mélangent souvent sur les aires de pêche, ce qui présente des problèmes pour les évaluateurs de stocks et les gestionnaires qui essaient d'optimiser les rendements et minimiser la surexploitation des stocks individuels. Nous mettons au point ici un cadre bayésien pour analyser simultanément plusieurs des différents types de données (par ex., composition des otolithes en éléments, ADN nucléaire et mitochondrial) qui servent à identifier l'origine des stocks de poissons en peuplements mixtes, pour ainsi tirer un avantage maximal des renseignements disponibles. Ce cadre comporte plusieurs éléments, dont la capacité d'analyser chaque type de données ou bien séparément ou alors en combinaison pour tout nombre d'échantillons de peuplements mixtes, des intervalles bayésiens crédibles pour évaluer l'incertitude associée à la proportion estimée des stocks originaux dans les peuplements mixtes, ainsi que des diagnostics prédictifs a posteriori pour évaluer les présuppositions des modèles sous-jacents. Nous utilisons le cadre pour analyser de nouveau un sous-ensemble de données de compositions des otolithes en éléments et de fréquences alléliques des microsatellites des mêmes poissons provenant de stocks de morues franches (*Gadus morhua*) du golfe du Saint-Laurent, Canada.

[Traduit par la Rédaction]

Introduction

Trace elemental composition of otoliths is frequently used as a natural tag to identify and track fish aggregations or populations as they move and has been used for identifying and classifying fish in a mixture from various sources (Elsdon et al. 2008) in much the same way as genetic markers are used (Waples et al. 2008). In a number of cases, other material often assumed to be population-specific such as DNA, meristics, and morphometrics are also collected on these populations (e.g., Milton and Chenery 2001; Berg et al. 2005; Miller et al. 2005) and analyzed separately from the otolith data. These different analyses can result in the same fish in the mixed samples being assigned to different source populations depending on the data type. Reconciling the differences in assignment can be difficult when different analytical methods are used and the various kinds of data are analyzed separately. Ideally, one would want to analyse all of the different data types in the same analysis and thus take maximal advantage of the available information.

One of the more common methods for analyzing stock composition data was developed in a maximum likelihood context by Fournier et al. (1984) and then modified by Millar (1987) to use the E-M algorithm. This method begins with a set of known source or base populations for which observations of otolith data, genetic data, or other population-specific measurements are available. A specimen from the mixed samples of unknown origin is assigned to the base population for which the likelihood of their observations and population parameters is a maximum. Pella and Masuda (2001) introduced a Bayesian form of the method

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with specimens from the mixed samples being assigned to base populations based on maximum posterior probabilities.

Classification of a mixture can be based on conditional or unconditional estimation whether one uses a maximum likelihood or a Bayesian approach (Pella and Masuda 2005). In the conditional case, only the parameter estimates from the base populations are used to classify the mixed samples. Examples of this approach are Fournier et al. (1984) and Millar (1987) for maximum likelihood estimation and Munch and Clarke (2008) for a Bayesian formulation. For unconditional estimation, the observations from the mixed samples classified to a base population are used to update the parameter estimates of the base population (e.g., Smouse et al. (1990) for an early maximum likelihood approach and Pella and Masuda (2001) for a Bayesian approach).

The classification model was originally defined to work with continuous random variables such as otolith elemental composition or morphometric measurements, discrete random variables such as frequencies of alleles or meristic measurements, or both continuous and discrete random variables at the same time (Fournier et al. 1984; Millar 1987). However, an integrated analysis has never been reported, in large part due to the absence of an analytical framework. Programs such as HISEA (conditional maximum likelihood; Millar 1990) and Matlab code for conditional Bayesian estimation available from Munch and Clarke (2008) are available for continuous random variables. There are more programs available that are only designed for discrete or genetic data: SPAM (conditional maximum likelihood; Debevec et al. 2000), BAYES (unconditional Bayesian estimation; Pella and Masuda 2001), cBAYES (C++ version of BAYES; Beacham et al. 2005), ONCOR (conditional maximum likelihood; Kalinowski et al. 2007), and Mixstock (unconditional Bayesian estimation in an R package with WinBugs for haplotype data; Bolker 2007). Users can write their own subroutines in FORTRAN to evaluate the likelihood of discrete random variables in HISEA.

At present, the separate and joint analyses of otolith, genetic, or other kinds of data for determining stock composition do not have a coherent and accessible framework for estimation, evaluation, and diagnostics. Although all of the components to do this have been developed, they have never been integrated into a common approach. The objective of this paper is to unify the analysis of these data in a Bayesian framework, thus allowing unknown mixtures to be classified using either otolith elemental or genetic data or both sources combined. Our method is not restricted to these two kinds of data, and the same general Bayesian model can be used with likelihoods specific to the data type. The approach developed herein was applied to a subset of genetic and otolith data collected on Atlantic cod (*Gadus morhua*) from the Gulf of St. Lawrence in 1996 and 1997. These data were originally analysed for stock composition with Campana et al. (1999) using the otolith elemental data and Ruzzante et al. (2000) using the microsatellite allele frequency data. Both of these studies used the conditional maximum likelihood version of the stock composition model. A second objective of the paper is to assess the additional information content available to researchers when multiple data types are analyzed in a single framework.

Materials and methods

Sample collection and data preparation

To characterize the genetic and otolith elemental fingerprints of each of the major cod stocks in and around the Gulf of St. Lawrence, samples of adult cod (35–85 cm) in spawning or near-spawning condition were collected in spring 1996 and 1997 from each of the four known spawning grounds: (1) the southern Gulf of St. Lawrence (NAFO Division 4T), in both the southwest (Shediac Valley) and southeast (off Cheticamp); (2) the northern Gulf of St. Lawrence (NAFO Division 3Pn4RS), primarily near the mouth of St. George's Bay; (3) southern Newfoundland (NF) (NAFO Division 3Ps), both offshore on St. Pierre Bank (in 1996) and inshore in Fortune and Placentia bays (in 1997); and (4) the Scotian Shelf (NAFO Division 4Vs), to both the east (the Gully) and the north (Banquereau Bank) (Table 1; Fig. 1). Each spawning ground was sampled at least twice in any given year, and most spawning grounds were sampled in both 1996 and 1997 (Fig. 1). A minimum of 100 cod was generally collected from each stock, for a total of 1111 fish. The sagittal otolith pair was removed from each fish immediately after capture, as well as a 1 mL sample of blood or a sample of soft muscle tissue immediately posterior to the tongue. Blood and muscle samples were immediately preserved in 95% ethanol, whereas otoliths were stored dry. These samples comprised our base populations, as the stock identity of each of these fish was known with some confidence. Only fish for which matching otolith and genetic assays were available were analyzed in the current analysis.

Several cod stocks are known to overwinter in large aggregations near the mouth of the Gulf of St. Lawrence (Campana et al. 1999). A winter fishery on these mixtures thus poses serious problems for stock assessment and management. A grid sampling design was used to collect the mixed samples of cod in and around the approaches to the Gulf of St. Lawrence each January in 1996 and 1997. Bottom trawl surveys were carried out on 3–22 January 1996 on the CCGS *Wilfred Templeman* (WT182) and on 6–25 January 1997 on the CCGS *Wilfred Templeman* (WT201). The nominal distance between survey samples was 20 km, although some areas were surveyed at 5 km intervals. Otoliths for determination of stock identity were collected from adult cod (35–85 cm) on approximately every second set (Table 1; Fig. 1). A total of 138 successful sets were made in 1996, of which 60 sets were sampled for otoliths (n = 754). Samples from 1997 came from 104 successful sets, of which 49 sets were sampled for otoliths (n = 866). Additional samples (n = 89; 45°50′N latitude, 58°03′W longitude) to the east of the region surveyed by the CCGS *Wilfred Templeman* in 1997 were collected on 15 March 1997 in two sets as part of the CCGS *Alfred Needler* N255 spring survey. Sample processing was as described for the summer samples and once again was restricted to fish for which both genetic and otolith information were available.

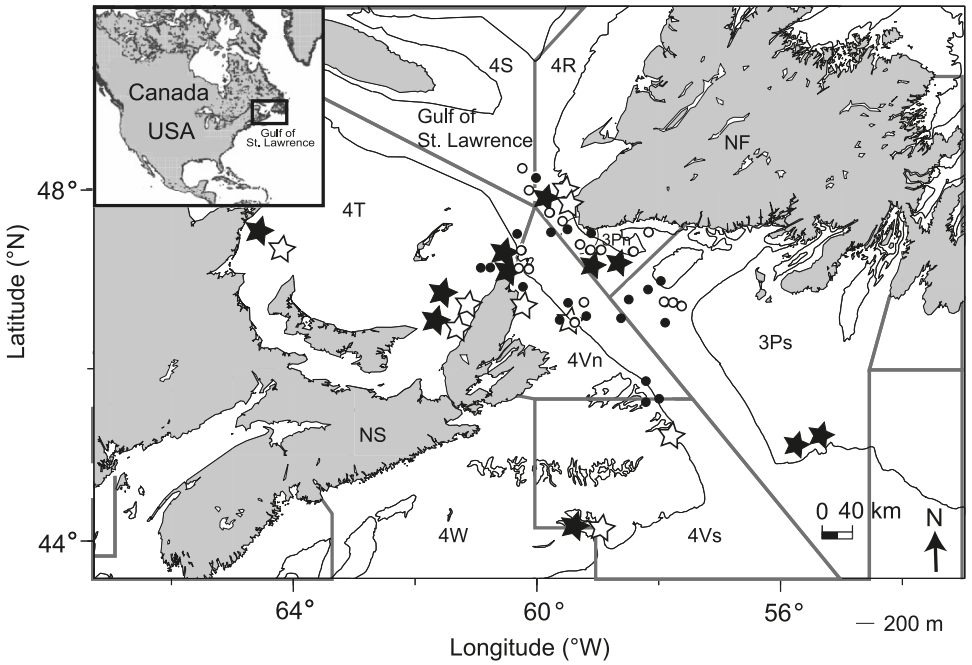
Each of the cod populations off the eastern coast of Canada spawns at a distinct site and thus is of known population identity at the time of spawning (Templeman 1962). Because the otolith elemental fingerprints of the 1996 and 1997 spring spawning aggregations differed significantly among spawning groups (Campana et al. 1999), they were

Table 1. Details on data used in the classification analysis.

		Sample size		No. of alleles					
Year	Location	One	Both	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Gmo151
Base samples									
1996	3Pn4RS	179	130	17	40	32	11	34	43
	3Ps	93	52	13	28	28	7	25	31
	4T	200	179	17	33	34	11	44	45
	4Vs	100	93	15	32	27	16	37	37
	All areas	572	454	22	47	40	19	54	61
1997	3Pn4RS	134	47	10	28	24	8	24	28
	4T	200	64	14	28	29	5	30	38
	4Vn	48	39	11	26	22	7	22	15
	4Vs	150	82	13	31	31	8	28	33
	All areas	532	222	17	40	139	9	40	47
Mixed samples									
1996	3Pn4RS	198	74	13	28	28	10	31	40
	3Ps	169	61	13	33	26	9	32	36
	4Vn	324	126	13	32	32	9	33	41
	4Vs	49	39	10	23	24	8	27	25
	All areas	740	320	16	41	36	11	49	57
1997	3Pn4RS	528	254	16	42	36	9	40	49
	3Ps	255	88	12	31	34	6	28	38
	4T	77	28	11	24	20	8	25	27
	4Vn	152	90	13	29	29	7	33	38
	All areas	1012	460	20	46	38	10	47	59

Note: Location refers to NAFO subarea (see Fig. 1). Sample size in the column "One" indicates number of observations when either otolith elemental composition or allele frequencies were measured. The number of observations with both measurements are given in column "Both".

Fig. 1. Map of study area in eastern Canada. Sample locations for spring spawners (stars) and winter mixed samples (circles) are indicated for 1996 (solid symbols) and 1997 (open symbols). Province of Nova Scotia (NS) and the island of Newfoundland (NF) are labelled on the map.



used as population- and year-specific reference markers against which the winter samples could be compared (Table 1). The elemental fingerprint of each otolith was characterized by a suite of five elements (Ba, Li, Mg, Mn, Sr) using isotope dilution inductively coupled plasma mass spectrometry and procedures described in Campana et al. (1999, 2000). Elemental assays for both spring spawner (known identity, base samples) and winter (unknown iden-

tity, mixed samples) samples from each year were systematically randomized with respect to assay sequence and analyzed together at the same laboratory. As a result, spring spawner and winter samples from any given year are completely comparable. As in previous analysis, the natural log transformation of the observations for the elements of Li and Mn was used to meet the normal distribution assumption for the classification model. Three elements (Mg, Mn, and Sr) were corrected for fish length. Full methodological details, including data transformations and calibration adjustments for fish size, are presented in Campana et al. (1999, 2000).

Nuclear DNA was extracted from the blood of many of the same cod as were analyzed by Campana et al. (1999, 2000), although the total sample size for the DNA analysis was smaller (see Table 1). DNA was analyzed as described in Ruzzante et al. (2000). Briefly, polymerase chain reaction (PCR) was used to amplify six microsatellite loci: Gmo2, Gmo132, Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993), Gmo120 (Ruzzante et al. 1996), and Gmo151 (Ruzzante et al. 2000). The resulting products were size-fractionated using 6.5% denaturing polyacrylamide gels, and alleles were sized by comparison with M13mp18 DNA sequencing reaction products.

Estimates and tests of genetic differentation (F_{ST}) were obtained using the computer package described in Goudet (2001).

Mixture model

The likelihood for the observations from the base tows can be written as

$$(1) \qquad l(\boldsymbol{\theta}) = \prod_{b=1}^{B}\prod_{i=1}^{n_b} f_b(\boldsymbol{x}_{b,i};\boldsymbol{\theta})$$

for observation i from base population b. For the otolith elemental composition data, the distribution function $f_b(\boldsymbol{x}_{b,i};\boldsymbol{\theta})$ was a multivariate normal for the otolith elemental compositional data with parameter set $\boldsymbol{\theta} = (\boldsymbol{\mu}_b, \boldsymbol{\Sigma})$. The distribution function was a multinomial distribution for the allele frequency data with parameter set $\boldsymbol{\theta}$ equal to $(\boldsymbol{N}_{jb}, \boldsymbol{p}_{jb})$, where $\boldsymbol{p}_{jb} = (p_1, p_2, \ldots, p_K)_{jb}$ represents the probability of observing the kth allele ($k = 1, \ldots, K$) at locus j in the bth base population.

The likelihood for the mixture observations over A areas (winter samples), $\boldsymbol{x}'_{am}$, is similarly (for example, see Ripley 1996),

$$(2) \qquad l'(\boldsymbol{\theta}) = \prod_{a=1}^{A}\prod_{m=1}^{M}\prod_{b=1}^{B} w_{amb} f_b(\boldsymbol{x}'_{am};\boldsymbol{\theta})$$

where w_{amb} represents the mixing or the stock composition proportions to be estimated for observations from area a, material m (otoliths or alleles), and base population b. Published applications of stock composition analysis generally concentrate on analysing one mixed sample; however, in our experience, observations are available from a number of sites for which stock compositions may differ. Given that the observations from the mixed samples, once assigned to respective base populations, will be used to estimate the parameters for those base populations, it makes sense to analyse all of the mixed samples at once (see discussion in Bolker et al. 2007).

Note that the two likelihoods given above share the same parameter set $\boldsymbol{\theta}$, implying that parameter estimation will be unconditional. For likelihoods of the joint distribution for the otolith and allele frequency data, $g_b(\boldsymbol{x};(\boldsymbol{\mu}_b, \boldsymbol{\Sigma})) \times h_b(\boldsymbol{x};(\boldsymbol{N}_{jb}, \boldsymbol{p}_{jb}))$ would be substituted for $f_b(\boldsymbol{x};\boldsymbol{\theta})$ in eqs. 1 and 2 above.

The Bayesian model uses the combination of the likelihoods in eqs. 1 and 2 with prior distributions set for w_{amb} and the elements of $\boldsymbol{\theta}$. A Dirichlet distribution, $D(\boldsymbol{\alpha})$, was used as a prior for w_{amb} with $\alpha_b = 1/B \forall b$. Pella and Masuda (2001) recommend setting α_b to $1/B$ for all i so that the mean, variances, and covariances of the resulting posterior distributions reflect the combined increase in the sizes of the base and mixed samples. Noninformative normal priors were used for the otolith element means, with means 0 and variances 10^6. The common variance–covariance matrix was modelled using a Wishart distribution with the parameter set to the rank of the matrix (here equal to five for the five elements used).

Noninformative priors for the $\boldsymbol{p}_{jb}$ can be set using 1 over the number alleles at locus j in base population b as recommended by Rannala and Mountain (1997). However, Pella and Masuda (2001) argue that the prior means should reflect the similarities between the allele frequencies among the base samples and the prior as a whole should allow for a realistic assessment of the uncertainty in the genetic composition of the stock. They developed a pseudo-Bayes estimate for an informative prior based on differences between the allele frequencies for each base population and the mean over all base samples. For those loci with large differences from the mean, the prior will result in the posterior being closer to the observed frequencies. For those loci that are very similar to the mean over all base samples, the posterior will be shrunk towards the prior mean.

Symmetric credible intervals based on the posterior distribution were calculated for the proportion assigned to each of the base groups. Overlap of these intervals was interpreted as indicating equivocal assignment to the base groups.

The classification models were written in WinBUGS (Lunn et al. 2000). The pseudo-Bayes method used here was based on R code from Bolker (2007). Hardy–Weinberg equilibrium was assumed for the allele frequency data. For each model, a total of 10 000 Markov chain Monte Carlo (MCMC) samples (Gibbs sampler) for each of two chains were taken from the posterior distribution, with the first 5000 discarded for burn-in. Convergence to the posterior was evaluated using the Brooks–Gelman test (Brooks and Gelman 1998). An R package (R Development Core Team 2007) for analyzing these kinds of data using the methods presented in this paper is available from the authors.

A natural check on the classification model is to determine if the data observed from the base populations are still plausible under the posterior distribution (Gelman et al. 2004) once the mixed-sample observations have been classified. That is, we can use the posterior distribution to predict the observations that we would expect to see if the data collection was replicated and compare these with those that we had observed in first place.

The fit of the model was evaluated for the otolith data by

Table 2. Proportion of each 1996 mixed sample assigned to each of the 1996 base samples using either otolith elemental composition or allelic frequency data or both kinds of data combined.

			Base sample			
Mixed sample	Material	Prior	3Pn4RS	3Ps	4T	4Vs
3Pn4RS	Otoliths		0.879	0.010	0.019	0.093
	Alleles	Pseudo-Bayes	0.336	0.179	0.463	0.023
		Equal proportions	0.377	0.112	0.491	0.019
	Combined	Pseudo-Bayes	0.840	0.012	0.134	0.013
		Equal proportions	0.780	0.013	0.200	0.006
3Ps	Otoliths		0.718	0.248	0.014	0.020
	Alleles	Pseudo-Bayes	0.169	0.175	0.613	0.044
		Equal proportions	0.241	0.095	0.622	0.042
	Combined	Pseudo-Bayes	0.554	0.211	0.220	0.015
		Equal proportions	0.572	0.132	0.280	0.017
4Vn	Otoliths		0.038	0.008	0.939	0.015
	Alleles	Pseudo-Bayes	0.371	0.023	0.578	0.029
		Equal proportions	0.351	0.016	0.610	0.024
	Combined	Pseudo-Bayes	0.195	0.006	0.792	0.007
		Equal proportions	0.218	0.007	0.769	0.006
4Vs	Otoliths		0.140	0.195	0.624	0.041
	Alleles	Pseudo-Bayes	0.129	0.033	0.659	0.179
		Equal proportions	0.185	0.014	0.655	0.146
	Combined	Pseudo-Bayes	0.310	0.074	0.533	0.083
		Equal proportions	0.329	0.026	0.561	0.084

Note: The prior column refers to the prior used for the proportion for each allele for each locus in the base sample.

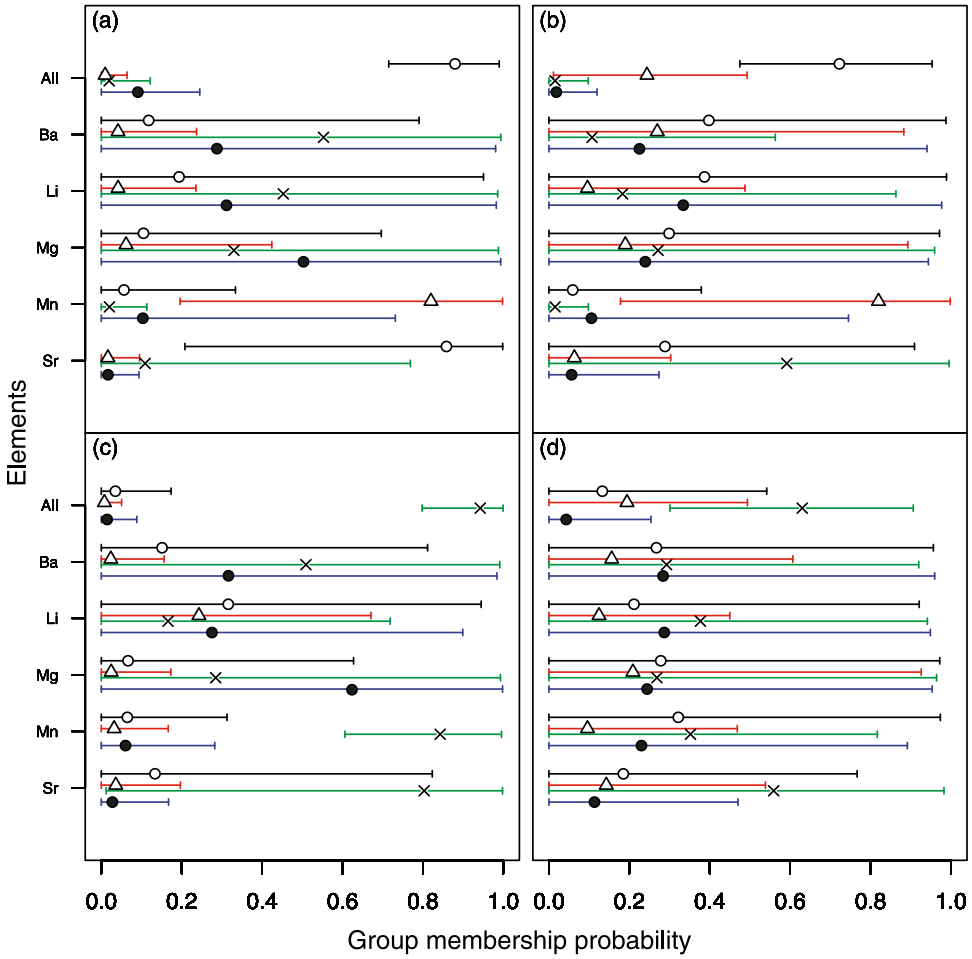
Table 3. Proportion of each 1997 mixed sample assigned to each of the 1997 base samples using either otolith elemental composition or allelic frequency data or both kinds of data combined.

			Base sample			
Mixed sample	Material	Prior	3Pn4RS	4T	4Vn	4Vs
3Pn4RS	Otoliths		0.953	0.038	0.005	0.004
	Alleles	Pseudo-Bayes	0.127	0.252	0.030	0.591
		Equal proportions	0.083	0.195	0.013	0.709
	Combined	Pseudo-Bayes	0.662	0.320	0.013	0.005
		Equal proportions	0.515	0.472	0.008	0.005
3Ps	Otoliths		0.963	0.006	0.009	0.022
	Alleles	Pseudo-Bayes	0.165	0.333	0.017	0.485
		Equal proportions	0.082	0.288	0.015	0.615
	Combined	Pseudo-Bayes	0.723	0.230	0.008	0.039
		Equal proportions	0.477	0.447	0.010	0.066
4T	Otoliths		0.018	0.937	0.028	0.017
	Alleles	Pseudo-Bayes	0.342	0.033	0.029	0.597
		Equal proportions	0.154	0.045	0.023	0.778
	Combined	Pseudo-Bayes	0.088	0.577	0.310	0.025
		Equal proportions	0.448	0.454	0.021	0.076
4Vn	Otoliths		0.014	0.907	0.070	0.009
	Alleles	Pseudo-Bayes	0.253	0.036	0.009	0.702
		Equal proportions	0.198	0.018	0.007	0.777
	Combined	Pseudo-Bayes	0.055	0.547	0.350	0.048
		Equal proportions	0.357	0.490	0.023	0.130

Note: The prior column refers to the prior used for the proportion for each allele for each locus in the base sample.

comparing the means of replicates from the posterior predictive distribution generated at each MCMC iteration with the original means for otolith elements from the base populations. Differences between original and new means were evaluated with a Bayesian version of Student's *t* test by evaluating the probability of obtaining a more extreme mean from the posterior relative to the original mean. We expect that this probability will be between 0.05 and 0.95 if the posterior distribution is a reasonable model for the base tows. Observed probabilities less than 0.05 would indicate

Fig. 2. Credible intervals (95%) for the posterior estimates of the proportions of cod in the mixed samples assigned to the different base groups using otolith elemental data from 1996. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 3Ps; green line (×), 4T; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4Vn, and (*d*) 4Vs.



that the posterior mean was considerably smaller than was originally observed, whereas probabilities greater than 0.95 would imply the reverse situation.

The test statistic for the genetics data was based on χ^2 statistics for both replicate data from the posterior predictive distribution and the original allele frequency data for the base tows. That is,

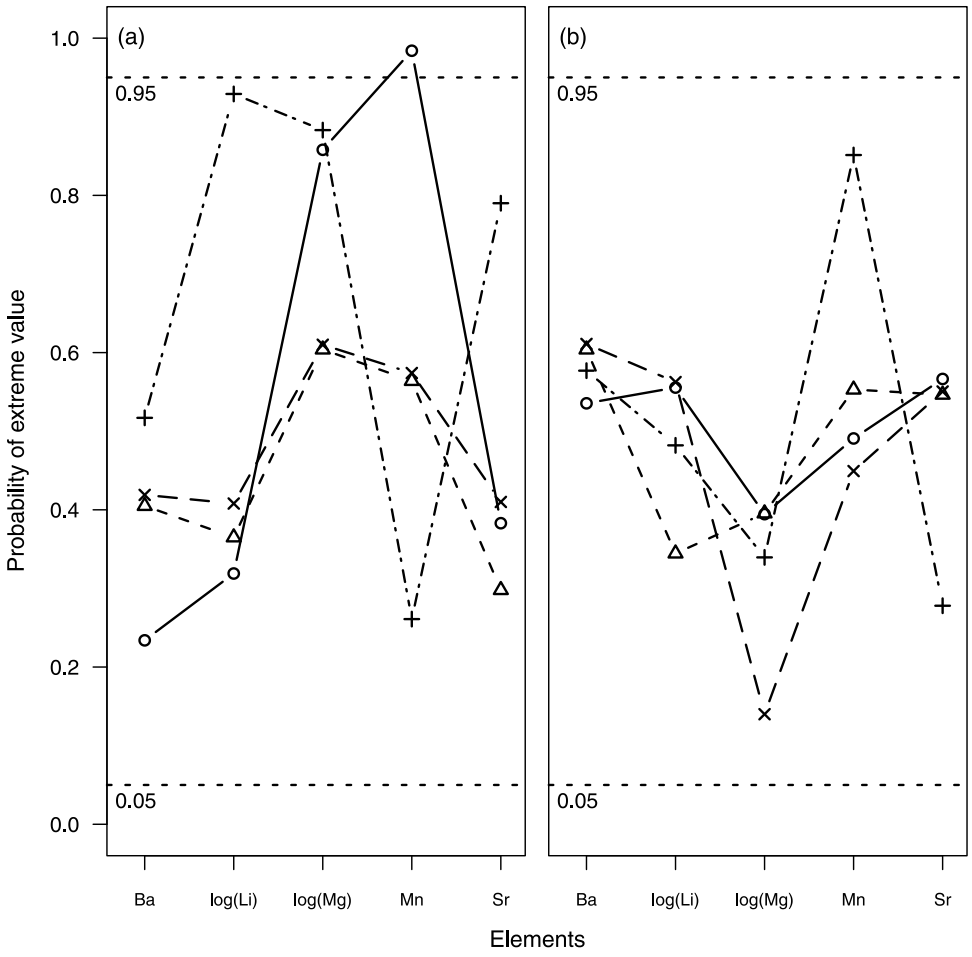
$$X^2 = \frac{\sum_{kjb}(x_{kjb}^{\text{rep}} - N_b\boldsymbol{\theta}_{kjb})^2}{N_b\boldsymbol{\theta}_{kjb}} \quad (3)$$

where x_{kjb}^{rep} is the replicated frequency of allele j at locus k from the current MCMC iteration from the posterior predictive distribution for total sample size N_b and base tow b. The probability associated with allele j from locus k, $\boldsymbol{\theta}_{kjb}$, is also for the current iteration. The test statistic for the original frequencies for each loci for the base tows is constructed by substituting x_{kjb}^{obs} for x_{kjb}^{rep} in eq. 3. These test statistics are evaluated in terms of the probability that X_{rep}^2 is greater than or equal to X_{obs}^2 determined over a series of MCMC iterations. Probabilities less than 0.05 would be interpreted as indicating that the goodness-of-fit of the original observations to the posterior distribution was less than that for the combination of the base and mixed-sample observations. That is, the mixed-sample observations have modified the distribution such that they were more likely to arise from the posterior distribution than the base observations. When the probability was greater than 0.95, the base observations were more likely to have come from the posterior than from the combination of the base and mixed-sample observations.

Results

Classification of the 1996 mixed samples using otolith elemental composition resulted in the largest proportion of fish in the 3Pn4RS and 3Ps mixed samples being assigned to the 3Pn4RS base group, whereas cod in the other two mixed samples (4Vn and 4Vs) were mainly assigned to the 4T base group (Table 2). However, the results of the classification based on the frequency of alleles split the majority of the fish in the 3Pn4RS mixed sample between 3Pn4RS and 4T. The larger proportion of the 3Ps, 4Vn, and 4Vn mixed samples were assigned to 4T. Results were similar for either choice of prior for the allele frequencies. The combination of otoliths and alleles produced classifications closer to those of otoliths alone than those of alleles alone.

Fig. 3. Probability of observing a more extreme value for the mean of each of the otolith elements from the marginal posterior distribution relative to the distribution from the base samples in 1996: (*a*) classification of samples using all of the elements; (*b*) classification of samples using one element at a time. Bounds for 0.90 probability limits are indicated on each panel. Pseudo-Bayes prior is used for allele frequencies. Results are coded by base groups: solid line (open circle, 3Pn4RS; short-dashed line (triangle), 3Ps; dashed–dotted line (cross), 4T; and long-dashed line (×), 4Vs.



Again there was not a great deal of difference between the two priors for the allele frequencies.

There were more differences between otolith and genetic classification results for the 1997 data (Table 3). As was the case for the 1996 mixed sample, the majority of the fish from the 1997 mixed samples were assigned either to the 3Pn4RS group (3Pn4RS and 3Ps mixed samples) or to 4T (4T and 4Vn mixed samples) using the otolith data. The larger proportion of fish from all of the mixed samples was assigned to the 4Vs base group by the genetics data. The combination of otoliths and alleles resulted in only small proportions of the mixed samples being assigned to the 4Vs base group. For the model using the pseudo-Bayes prior for the allele frequencies, the larger proportion of mixed samples was assigned to the 3Pn4RS base group for the 3Pn4RS and 3Ps mixed samples, similar to the otolith only results. For the other two mixed samples, the larger proportions were assigned to the 4T and 4Vn base groups. However, the equal probability prior resulted in a similar split between the 3Pn4RS and 4T base groups for all of the mixed samples.

The credible intervals for the proportions assigned to the base samples in 1996 when using otolith elements alone indicate that only the proportion assigned to 3Pn4RS in the 3Pn4RS mixed sample and the proportion assigned to 4T from the 4Vn mixed sample differed significantly from proportions assigned to the other base groups at the 95% level (Fig. 2). When fish in the mixed samples were classified using each otolith element separately, the only case in which credible regions do not overlap was for the proportion assigned to 4T in the 4Vn mixed sample based on manganese. In contrast to the combined results, the only case in which a large proportion of cod was assigned to 3Pn4RS when individual elements were used was for strontium in the 3Ps4RS mixed sample.

The posterior predictive check on the mixture model for the 1996 data shows that the mean for manganese in the posterior distribution for 3Pn4RS was far lower than that observed for the original base group such that it is unlikely (probability of 0.016) that we would observe the original sample based on the parameters of the posterior (Fig. 3*a*). The means for lithium and magnesium for the 4T posterior distribution were also much lower than the means of the original base samples with associated probabilities <0.12.

Fig. 4. Credible regions (95%) for classification of mixed samples by base groups of allelic frequency data from 1996. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 3Ps; green line (×), 4T; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4Vn, and (*d*) 4Vs.

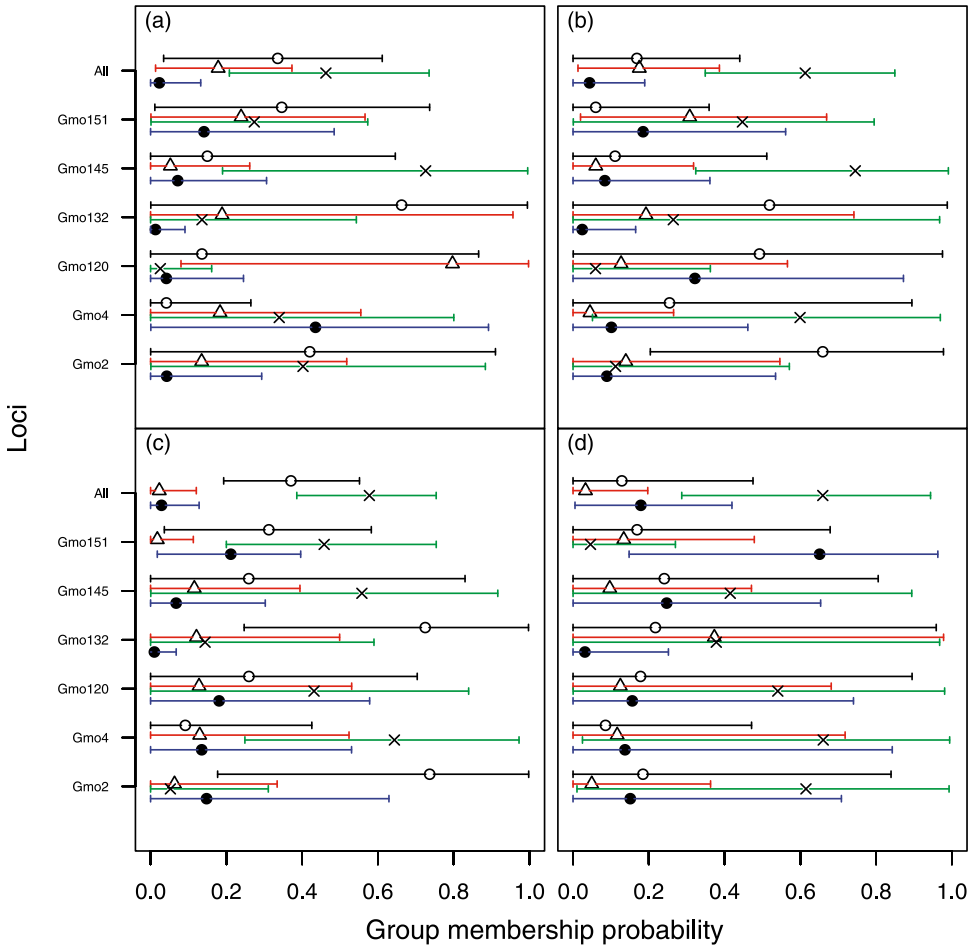


Table 4. Single locus and overall estimates of F_{ST} among the four base groups.

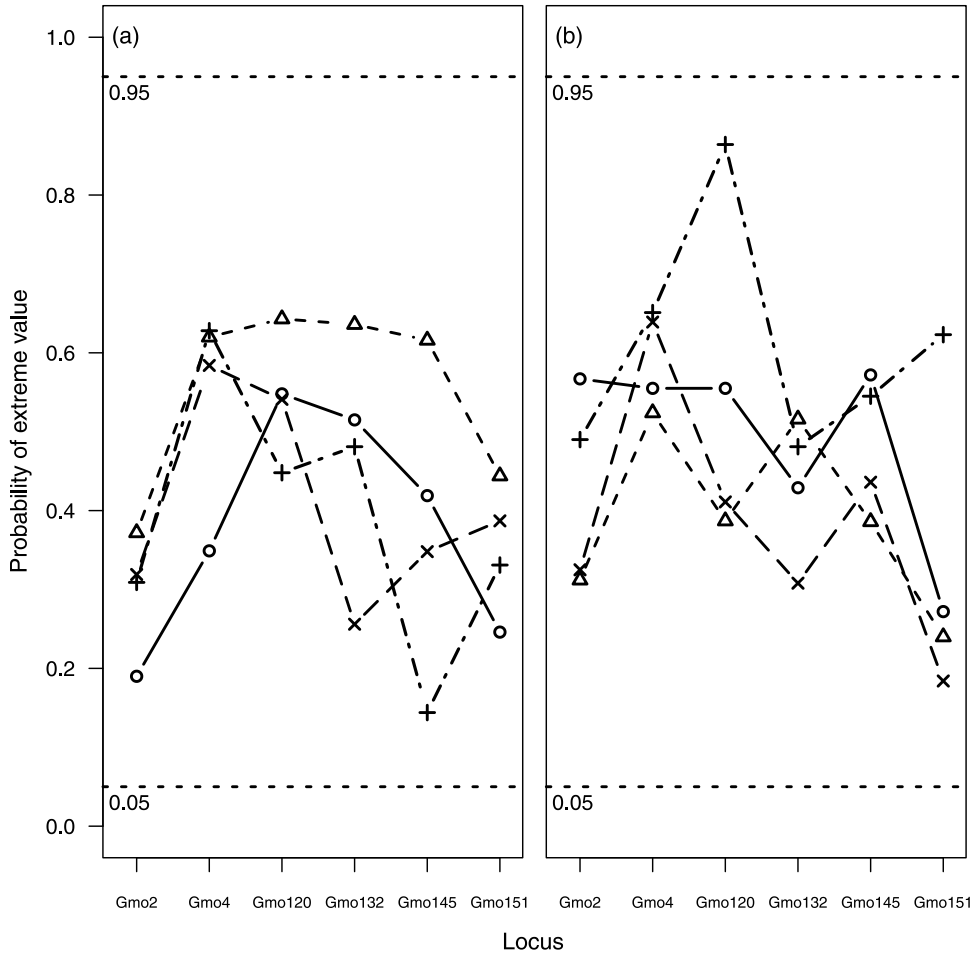
	Locus						
Year	Gmo2	Gmo4	Gmo120	Gmo132	Gmo145	Gmo151	Overall
1996	–0.0009	0.0001	–0.00001	0.0080*	0.0025	0.0037*	0.0020*
1997	–0.0011	–0.0007	–0.00060	0.0300*	0.0040	0.0103	0.0062*

Note: An asterisk indicates p level for test statistic < 0.0083 (= 0.5/6).

None of the posterior predictive probabilities was beyond the limits in Fig. 3*b* when classification was based on each element alone. The probability of exceeding the mean from the original base sample for manganese is now closer to 0.50 as more of the 3Pn4RS and 3Ps mixed samples were assigned to the 3Ps base group (Fig. 2). Comparison of the distribution of manganese for the base and mixed samples shows that the distributions for these two mixed samples were much closer to the 3Ps base sample than to the 3Pn4RS base to which they were assigned when all elements were used. Assignment to the latter base group for the all element case was due to strontium dominating the classification and resulted in large numbers of observations of manganese less than zero (detrended manganese) substantially lowering the mean for posterior distribution of manganese for 3Pn4RS relative to the original base group. Because Mn was the only element requiring statistical removal of a substantive effect due to fish length (Campana et al. 2000), it seems likely that the transformation somehow induced some unintended artifacts. This issue will be explored in later work.

The credible intervals for the proportions of the 3Pn4RS and 3Ps mixed samples assigned to the 3Pn4RS base group and of the 4T and 4Vn mixed samples assigned to the 4T base group do not overlap with the proportions assigned to the other base groups when using only otolith data in 1997. However, the classification results when using all elements appear to be the same as those for manganese alone as all other credible intervals overlap for the other elements. Despite the fact that manganese appears to be driving the classification when all elements are being used, the posterior predictive diagnostics indicate that the means of the poste-

Fig. 5. Probability of observing a more extreme value for the goodness-of-fit statistic for allele frequencies from the marginal posterior distribution relative to the distribution from the base samples in 1996: (*a*) classification using all loci; (*b*) classification using one locus at a time. Bounds for 0.90 probability limits are indicated on each panel. Pseudo-Bayes prior was used for allele frequencies. Results are coded by base groups: solid line (open circle), 3Pn4RS; short-dashed line (triangle), 3Ps; dashed–dotted line (cross), 4T; and long-dashed line (×), 4Vs.



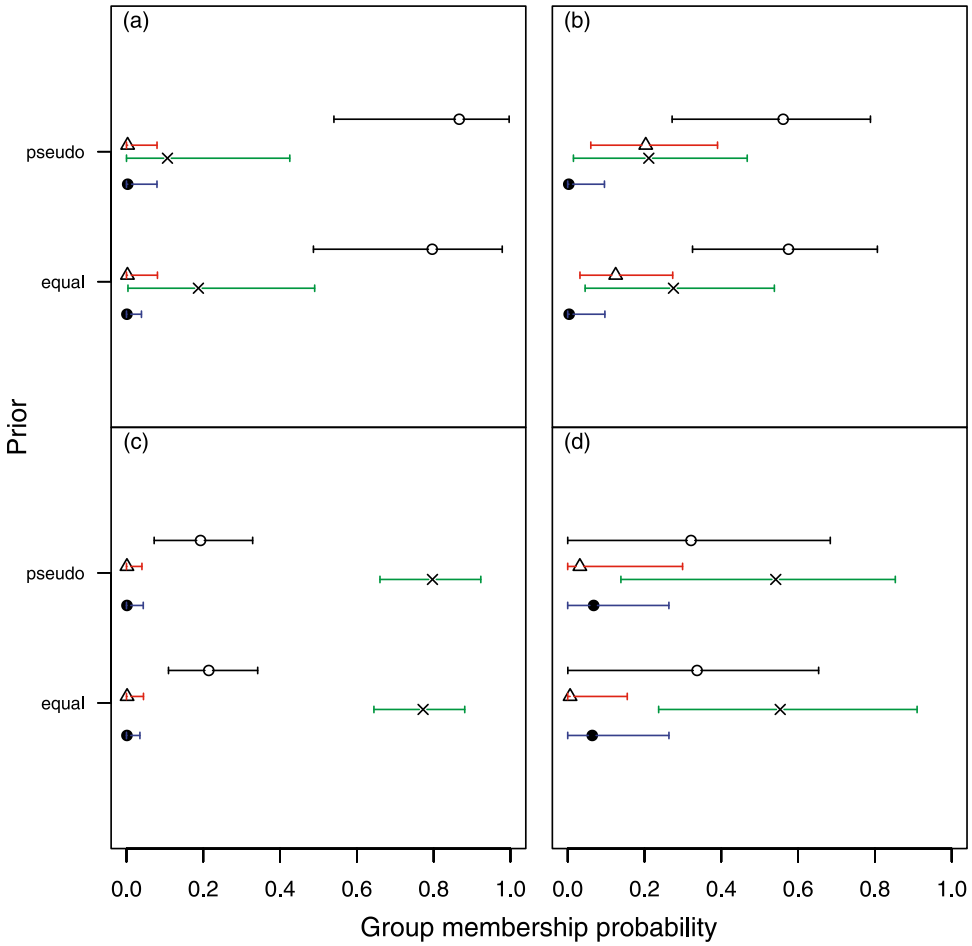
rior distribution for manganese are either higher than expected for the 4T base group or lower than expected for the 3Pn4RS base group. This pattern remains so for manganese even when classification is based on each element alone.

On the other hand, the larger proportion of cod in the 3Pn4RS and 3Ps mixed samples could be assigned to either the 3Pn4RS or the 4Vs base groups, resulting in a decreased mean for the posterior distribution for base group in either case. That the larger proportions of these mixed samples are assigned to 3Pn4RS may be due mainly to the much larger sample sizes in these mixed samples than in the base groups. Together these mixed samples account for 332 observations and can easily swamp the base groups for 3Pn4RS (47 observations) or 4Vs (82 observations) (Table 1). During the initial MCMC iterations, enough of the 3Pn4RS and 3Ps mixed-sample observations could be randomly assigned to the 3Pn4RS base group to lower the mean substantially such that all future iterations will assign the cod from these mixed samples to 3Pn4RS. The smaller sample size for the 3Pn4RS base group relative to the 4Vs base group may result in the former group being more vulnerable to this swamping effect. In fact, trials conducted with starting values for the group membership variable favouring the 4Vn base group for these mixed samples will result in the majority of their observations being assigned to the 4Vn base group. This base group has the smallest number of observations and can be more vulnerable to swamping if enough observations are are assigned to it in the initial iterations.

With the possible exception of the 4Vn mixed sample, the credible intervals all overlap for the classification when using only the allele frequencies in 1996 (Fig. 4). One could also argue that for a shorter interval corresponding to 90% coverage, the larger proportions assigned to 4T in the 3Ps and 4Vs mixed samples (possibly 4Vn as well) could well have credible intervals that do not overlap. However, the intervals for classifications based on a single locus overlap for all of the mixed samples.

The equivocal classification results were not unexpected given the low F_{ST} estimates and minimal genetic structuring observed among the four baseline groups analyzed (Table 4). The posterior predictive diagnostic indicates no serious issues with the allele frequencies for the posterior distribution when all loci are used (Fig. 5*a*) or when each individual locus is used (Fig. 5*b*).

Fig. 6. Credible intervals (95%) for the posterior estimates of the proportions of cod in the mixed samples assigned to the different base groups using both otolith elemental data and allele frequencies from 1996. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 3Ps; green line (×), 4T; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4Vn, and (*d*) 4Vs.



The credible intervals for the proportion assigned to the 4Vs base group for the 3Pn4RS and 3Ps mixed samples were the only intervals not to overlap with the other intervals when only allele data were used to classify the mixed samples from 1997. The results for the 3Pn4RS mixed sample for all loci were very similar to those for locus Gmo145 alone, while classification was equivocal for the rest of the loci in this and the other mixed samples. Again the estimates of F_{ST} indicate little structure for the 1997 data (Table 4). There was no evidence of problems from the posterior predictive estimates when either all loci were used or each locus was used separately.

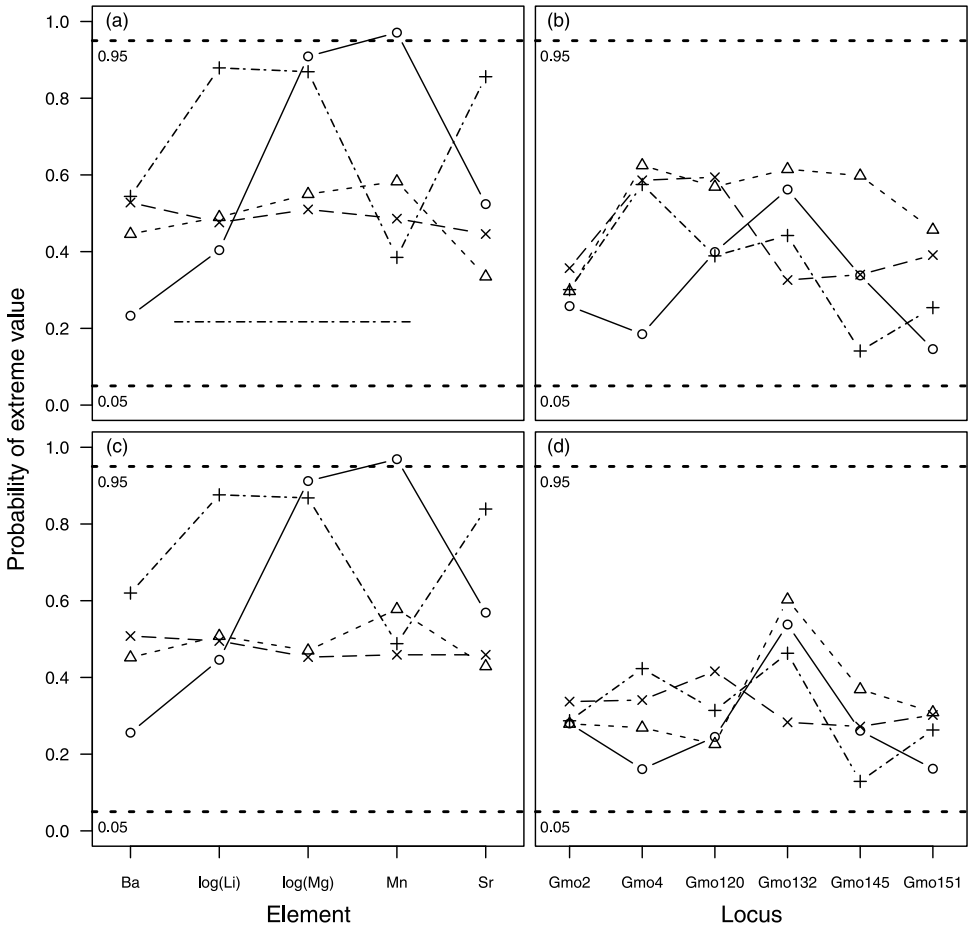
The credible intervals for the combined otolith and allele frequency data in 1996 were almost identical to those for otoliths alone (Fig. 6). There was very little difference in the intervals between the results from the pseudo-Bayes prior and the equal probability prior. The predictive posterior statistics changed little in pattern from the results when either otoliths or allele frequencies were used (Fig. 7).

Using both otoliths and allele frequency data for 1997 resulted in credible intervals indicating weaker support for classification of the larger proportions of the 3Pn4RS and 3Ps mixed samples to the 3Pn4RS and 4T base populations when using the pseudo-Bayes prior than when using otoliths alone. Credible intervals indicated equivocal classification for the 4T and 4Vn mixed samples for the pseudo-Bayes prior and for all mixed samples for the equal probability prior (Fig. 8). The difference between the means for manganese from the original and posterior base populations no longer appears to be an issue when either prior is used for the allele frequencies. However, the goodness-of-fit for loci Gmo120, Gmo132, Gmo145, and Gmo151 has declined as a result (Fig. 9).

Discussion

The data analyzed here represent a subset of the data sets analysed by Campana et al. (1999) and Ruzzante et al. (2000). The reduced data set was a consequence of choosing only cod for which both the otolith elemental composition and allele frequencies had been measured. The more-complete data sets contained more base populations and larger numbers of observations per base and mixed samples when considering only otolith or genetic measurements. In addition, Ruzzante et al. (2000) combined 1996 and 1997 allele frequency data, thus increasing sample sizes for many of the areas. A pooled-year approach was not appropriate for the

Fig. 7. Probability of observing a more extreme value for the mean of each of the otolith elements and for the goodness-of-fit statistic for allele frequencies from the marginal posterior distribution relative to the distribution from the base samples in 1996: (*a* and *b*) results from using the Pseudo-Bayes prior; (*c* and *d*) results from using the equal probability prior. All loci were used in the classification. Bounds for 0.90 probability limits are indicated on each panel. Pseudo-Bayes prior was used for allele frequencies. Results are coded by base groups: solid line (open circle), 3Pn4RS; short-dashed line (triangle), 3Ps; dashed–dotted line (cross), 4T; and long-dashed line (×), 4Vs.



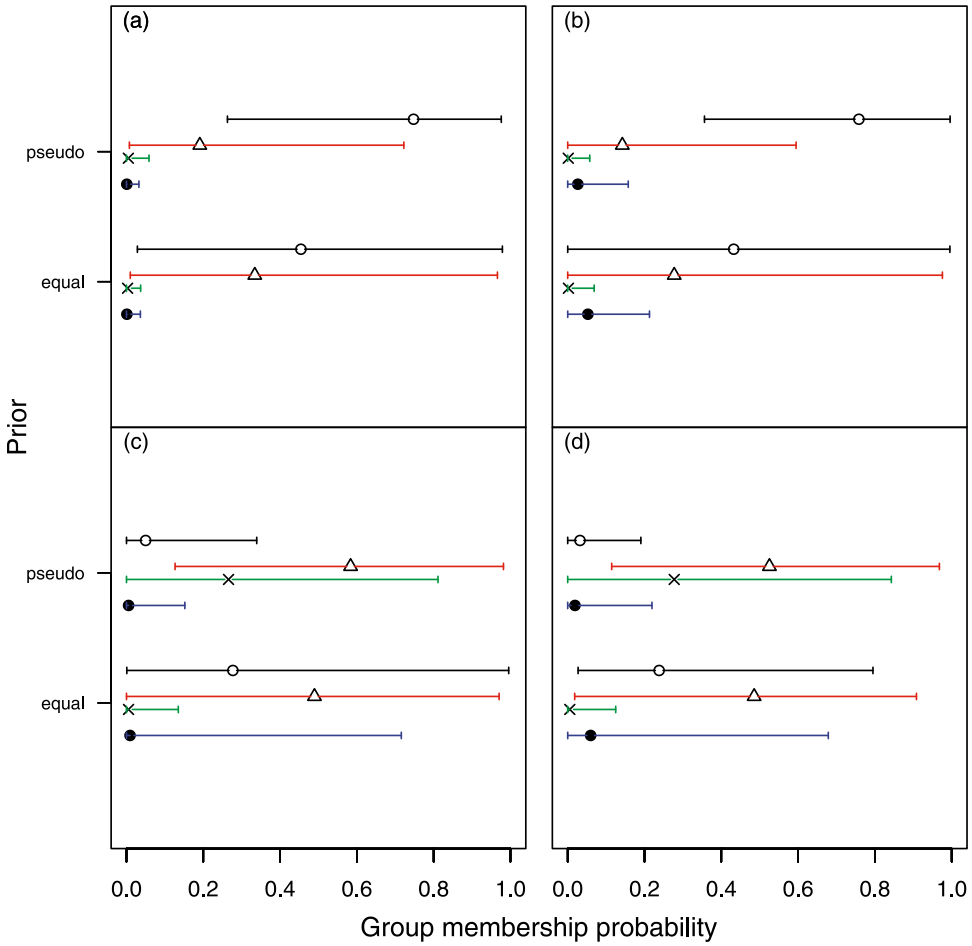
otolith data given that the elemental markers are not necessarily constant across years (Campana et al. 2000).

Despite these differences, our results from the otolith data are broadly comparable with those from Campana et al. (1999). The majority of cod from the mixed samples in 3Pn4RS were classified as belonging the 3Pn4RS base population in both years. Cod from 4T, 4Vn, and 4Vs mixed samples were mainly identified as being from the 4T base population. However, the larger proportion of cod from the mixed sample taken in 3Ps both years were classified as belonging to the 3Pn4RS population, whereas Campana et al. (1999) reported that these fish were classified as coming from 3Ps. The difference may be explained by the fact that our subset of the data contained fewer 3Ps base population records in 1996 and no records for the 3Ps base population in 1997. Confidence intervals were not reported in Campana et al. (1999).

Ruzzante et al. (2000) found that the larger proportion of all of the mixed samples was classified by the allele frequency data as coming from the 4T base population. Classification to the other base populations was equivocal based on the 95% bootstrap confidence intervals presented in their figs. 4 and 5. The only similar results in our case were for the 4Vn and 4Vs mixed samples in 1996.

The Gmo132 locus was identified here as exhibiting significant genetic differentiation. This locus has also been identified as exhibiting high genetic differentiation in cod stocks across the North Atlantic (Bentzen et al. 1996) and off the coast of Norway (Wennevik et al. 2008). This locus also had the least number of alleles of the loci used in our study and in those of both Ruzzante et al. (2000) and Bentzen et al. (1996). Although some authors suggest that the more polymorphic a locus is, the better able it is to differentiate between populations (Bernatchez and Duchesne 2000; Kalinowski 2004), others have found the opposite to be true (e.g., O'Reilly et al. 2004). Nielsen et al. (2006) has suggested that the higher genetic differentiation exhibited by this locus could be indicative of it being subject to hitchhiking selection. They also argue that although this would result in inflated levels of differentiation, these kinds of selected markers would also have increased statistical power for mixed-stock analysis compared with neutral markers. However, in our case, classification by this locus alone was equivocal in our analysis of both the 1996 and 1997 mixed samples.

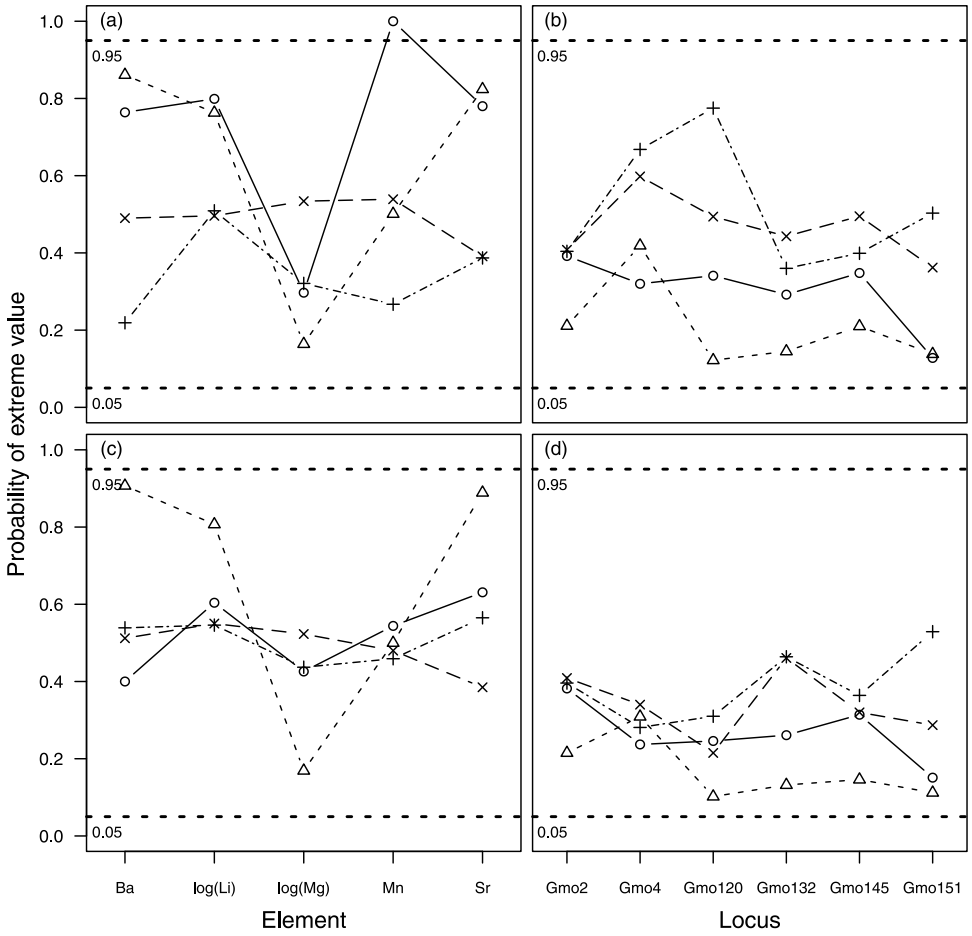
Fig. 8. Credible intervals (95%) for the posterior estimates of the proportions of cod in the mixed samples assigned to the different base groups using both otolith elemental data and allele frequencies from 1997. Credible intervals are coded by base groups: black line (open circle), 3Pn4RS; red line (triangle), 4T; green line (×), 4Vn; and blue line (solid circle), 4Vs. Position of posterior mean is indicated by symbols. Panels correspond to mixed samples (*a*) 3Pn4RS, (*b*) 3Ps, (*c*) 4T, and (*d*) 4Vn.



Our Bayesian credible intervals were wider than the bootstrap limits presented in Ruzzante et al. (2000). These 95% bootstrap limits were constructed by sampling with replacement from both the base and mixed populations a number of times and then obtaining the 0.025 and 0.975 quantiles of the maximum likelihood estimates from each sampling. Bolker et al. (2003) has also reported that Bayesian credible intervals tend to be wider than the bootstrap limits from the maximum likelihood stock composition model for a simulation study, as well as when analysing mitochondrial DNA from sea turtles. From their simulation study, they concluded that the Bayesian credible intervals were more robust than the bootstrap confidence intervals especially when many of the base populations contribute sparsely to some of the mixed samples.

Both Campana et al. (1999) and Ruzzante et al. (2000) used maximum likelihood conditional estimation for their stock composition analysis. As a result, they had limited means by which to evaluate the performance of their classification model. Here the combination of unconditional estimation and the Bayesian predictive posterior distribution provides a direct means for analysis of model performance once the classification of the mixed samples has been completed. For the 1996 otolith data, it appears that classification based on all of the elements results in a distorted distribution of manganese in the posterior distribution for the 3Pn4RS base population. This distortion does not occur when manganese is used by itself to classify the data. It appears that strontium may be driving the allocation of the large number of samples to the 3Pn4RS base group, and this classification was not supported by magnesium. On the other hand, manganese was identified as an issue for the 1997 data, but there, two different mechanisms were in play. First, the mixed samples had much larger sample sizes than the base population samples, and the manganese samples from the 3Pn4Rs and 3Ps mixed samples could overwhelm the parameter estimates for any of the base populations. Second, the distribution of manganese measurements in these two mixed samples were atypical compared with any of the base populations, possibly indicating that one or more actual base populations were missing from the analysis. The distribution of manganese in the 3Pn4Rs and 3Ps mixed samples more closely resembled that for the 3Ps base population in the 1996 data than the distribution in the 3Pn4RS base population in either 1996 or 1997. Recall that a 3Ps base population was not identified in the subset of the 1997 data used here, although they were present in the otolith study of Campana et al. (2000). Similarly, the larger

Fig. 9. Probability of observing a more extreme value for the mean of each of the otolith elements and for the goodness-of-fit statistic for allele frequencies from the marginal posterior distribution relative to the distribution from the base samples in 1997: (*a* and *b*) results from using the Pseudo-Bayes prior; (*c* and *d*) results from using the equal probability prior. All loci were used in the classification. Bounds for 0.90 probability limits are indicated on each panel. Results are coded by base groups: solid line (open circle), 3Pn4RS; short-dashed line (triangle), 4T; dashed–dotted line (cross), 4Vn; and long-dashed line (×), 4Vs.



proportion of the mixed samples from 4T and 4Vn in 1997 was allocated to the 4T base group, but the posterior mean was also distorted compared with that of the base population. Again, disparate sample sizes were probably an issue, but this pattern may also point to a case of missing base populations in the analysis. Methods for classification when base populations are missing are presented in Corander et al. (2006), Pella and Masuda (2006), and White et al. (2008).

Our analysis integrating otolith and genetic markers did not result in enhanced classification success relative to either method by itself, although it produced classifications similar to those of the otoliths. The explanation for this perhaps counterintuitive result appears to be linked to the poor discriminatory power of the genetic markers for cod in and around the Gulf of St. Lawrence. Presumably, the poor genetic discrimination was due to the occasional mixing of cod spawners among the populations, in keeping with previous tagging results (Taggart et al. 1995). The integration of the weaker genetic discriminations with the stronger otolith-based classifications then degraded the overall classification success. Needless to say, this pattern is not necessarily representative of other population mixtures, where genetic assays may well provide superior discriminatory power over other methods. In this particular system, however, the genetic assays were not particularly informative in discriminating among populations.

The possibility that the different time scales represented by the genetic and otolith elemental techniques contributed to some inconsistencies between their results seems unlikely but cannot be excluded. Population-level differences in genetic markers usually evolve over many generations, whereas otolith elemental differences reflect environmental differences during the lifetime of the individual. In most situations, one would expect genetic differences also to be reflected in environmental differences due to the different habitats being occupied by each population (Begg and Waldman 1999). Although the reverse is not necessarily true, there is no reason to expect incompatibilities between the methods. In principle, as long as there are significant and stable differences in population markers among each of the source groups, the nature of the marker is irrelevant. In a marine ecosystem, genetic markers usually have the advantage of being stable across cohorts, whereas environmentally driven markers such as otolith elemental fingerprints usually have the advantage of producing larger differences among

groups of fish. The key to using them both in an integrated analysis is that known-origin reference groups exist for each.

A number of different materials and methods have been used on their own for stock identification (e.g., mark–recapture, parasites, otolith characteristics such as shape and composition, genetic material including mitochondrial and nuclear DNA), with each having its own assumptions and shortcomings. Begg and Waldman (1999) have recommended that more robust stock identifications could be obtained if using more than one kind of these materials provided similar stock identifications. On the other hand, issues arising from only using one method would be evident if the different methods resulted in different stock affinities. Although this recommendation is important to keep in mind when trying to identify putative base stocks, it is critical to the evaluation of the identification of the proportion of each base stock in a mixed sample.

We know of three published studies in which more than one kind of stock identification data were used to identify the proportion of base groups in mixed samples. In all three cases, different methods were used to analyse the different kinds of data. Bradbury et al. (2008) reported a conflict between the results of a maximum likelihood stock composition analysis of allele frequency data and a discriminant analysis of otolith elemental composition data for resolving the degree of early life history dispersal of the anadromous rainbow smelt, *Osmerus mordax*, in southeastern Newfoundland. In a second study, base populations were defined by otolith shape analysis or by a geographic criterion in Wennevik et al.'s (2008) analysis of the distribution of coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, northern Norway. These authors used the BAYES computer program (Pella and Masuda 2001) to analyse the allele frequency data but simply tabulated the proportion of coastal-type or oceanic-type otoliths in the mixed samples. Thorrold et al. (2001) used the conditional maximum likelihood approach on otolith elemental composition data to estimate the proportion of adult weakfish (*Cynoscion regalis*) spawning in their natal estuary based on the base population of juveniles in each estuary. Genetic data were available but were not used in the analysis because a separate study concluded that there were no significant differences between populations based on an analysis of molecular variance (published in Cordes and Graves 2003). Both Thorrold et al. (2001) and Cordes and Graves (2003) suggested that there was sufficient exchange among the base populations (estuaries) to prevent genetic divergence.

Our framework allows for the evaluation of classifications based on one or more concurrently measured stock identification data. Our Bayesian model allows for the same base populations to be used to classify the same mixed samples for these different kinds of data. For example, the question about limited dispersal of rainbow smelt (Bradbury et al. 2008) could have been more precisely answered by using our model and the same base populations to analyse both the genetic and otolith data. The cod otolith shape data (Wennevik et al. 2008) could have been modelled as a multinomial random variable and analysed both separately and combined with the allele frequency data to investigate the distribution of the two base populations in the mixed samples. Finally, a combined analysis of the otolith and genetic data for the weakfish (Thorrold et al. 2001) could provide insights into the distributions of the genotypes assuming that the otolith data dominates the classification of the base groups. In each of these examples, a more powerful analysis of the available data would have been possible using an integrated statistical analysis.

The ready availability of Bayesian credible limits for separate or combined analysis of stock identification material allows for the assessment of the degree of uncertainty with respect to stock composition. These limits could in turn provide the basic information for assessments of the risk of using the resultant classifications when developing harvest advice or rebuilding plans for depleted or at-risk stocks. Similarly, the diagnostic tests presented here offer the means to evaluate the assumptions of the analysis such as identification of all of the base populations. The identification of missing base populations is an ongoing problem in stock composition analysis (Pella and Masuda 2006). Although this problem is not completely solved in this framework, the diagnostic tests do provide a valuable tool for recognizing the existence of missing base populations.

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References

Beacham, T.D., Candy, J.R., Mcintosh, B., Macconnachie, C., Tabata, A., Kaukinen, K., Deng, L., Miller, K.M., Withler, R.E., and Varnavskaya, N. 2005. Estimation of stock composition and individual identification of sockeye salmon on a Pacific Rim basis using microsatellite and major histocompatibility complex variation. Trans. Am. Fish. Soc. **134**(5): 1124–1146. doi:10.1577/T05-005.1.

Begg, G.A., and Waldman, J.R. 1999. An holistic approach to stock identification. Fish. Res. **43**(1–3): 35–44. doi:10.1016/S0165-7836(99)00065-X.

Bentzen, P., Taggart, C.T., Ruzzante, D.E., and Cook, D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **53**(12): 2706–2721. doi:10.1139/cjfas-53-12-2706.

Berg, E., Sarvas, T., Harbitz, A., Fevolden, S., and Salberg, A. 2005. Accuracy and precision in stock separation of north-east Arctic and Norwegian coastal cod by otoliths — comparing readings, image analyses and a genetic method. Mar. Freshw. Res. **56**(5): 753–762. doi:10.1071/MF04172.

Bernatchez, L., and Duchesne, P. 2000. Individual-based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles? Can. J. Fish. Aquat. Sci. **57**(1): 1–12. doi:10.1139/cjfas-57-1-1.

Bolker, B. 2007. mixstock: functions for mixed stock analysis. R package version 0.8.4.2. Available from http://www.R-project.org.

Bolker, B., Okuyama, T., Bjorndal, K., and Bolten, A. 2003. Sea turtle stock estimation using genetic markers: accounting for sampling error of rare genotypes. Ecol. Appl. **13**(3): 763–775. doi:10.1890/1051-0761(2003)013[0763:STSEUG]2.0.CO;2.

Bolker, B.M., Okuyama, T., Bjorndal, K.A., and Bolten, A.B. 2007. Incorporating multiple mixed stocks in mixed stock analysis: 'many-to-many' analyses. Mol. Ecol. **16**(4): 685–695. doi:10.1111/j.1365-294X.2006.03161.x. PMID:17284204.

Bradbury, I.R., Campana, S.E., and Bentzen, P. 2008. Estimating contemporary early life-history dispersal in an estuarine fish: integrating molecular and otolith elemental approaches. Mol. Ecol. **17**(6): 1438–1450. doi:10.1111/j.1365-294X.2008.03694.x. PMID:18321254.

Brooker, A.L., Cook, D., Bentzen, P., Wright, J.M., and Doyle, R.W. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. Can. J. Fish. Aquat. Sci. **51**(9): 1959–1966. doi:10.1139/f94-198.

Brooks, S., and Gelman, A. 1998. General methods for monitoring convergence of iterative simulations. J. Comput. Graph. Statist. **7**(4): 434–455. doi:10.2307/1390675.

Campana, S.E., Chouinard, G., Hanson, J.M., and Fréchet, A. 1999. Mixing and migration of overwintering Atlantic cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **56**: 1873–1881. doi:10.1139/cjfas-56-10-1873.

Campana, S.E., Chouinard, G., Hanson, J.M., Fréchet, A., and Bratty, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res. **46**(1–3): 343–357. doi:10.1016/S0165-7836(00)00158-2.

Corander, J., Marttinen, P., and Mäntyniemi, S. 2006. A Bayesian method for identification of stock mixtures from molecular marker data. Fish. Bull. (Washington, D.C.), **104**: 550–558.

Cordes, J.F., and Graves, J.E. 2003. Investigation of congeneric hybridization in and stock structure of weakfish (*Cynoscion regalis*) inferred from analyses of nuclear and mitochondrial DNA loci. Fish. Bull. (Washington, D.C.), **101**: 443–450.

Debevec, E.M., Gates, R.B., Masuda, M., Pella, J., Reynolds, J., and Seeb, L.W. 2000. SPAM (version 3.2): statistics program for analyzing mixtures. J. Hered. **91**(6): 509–510. doi:10.1093/jhered/91.6.509. PMID:11218094.

Elsdon, T.S., Wells, B.K., Campana, S.E., Gillanders, B.M., Jones, C.M., Limburg, K.E., Secor, D.H., Thorrold, S.R., and Walther, B.D. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanogr. Mar. Biol. Annu. Rev. **46**: 297–330. doi:10.1201/9781420065756.ch7.

Fournier, D.A., Beacham, T.D., Riddell, B.E., and Busack, C. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic and electrophetic characteristics. Can. J. Fish. Aquat. Sci. **41**(3): 400–408. doi:10.1139/f84-047.

Gelman, A., Carlin, J.B., Stern, H.S., and Ruben, D.B. 2004. Bayesian data analysis. 2nd ed. Chapman and Hall/CRC, Boca Raton, Florida.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices. Version 2.9.3. Available from http://www.unil.ch/dee/page36921_en.html (accessed 31 March 2010).

Kalinowski, S.T. 2004. Genetic polymorphism and mixed-stock fisheries analysis. Can. J. Fish. Aquat. Sci. **61**(7): 1075–1082. doi:10.1139/f04-060.

Kalinowski, S.T., Manlove, K.R., and Taper, M.L. 2007. ONCOR: a computer program for genetic stock identification. Department of Ecology, 310 Lewis Hall, Montana State University. Available from http://www.montana.edu/kalinowski/Software/ONCOR.htm (accessed 31 March 2010).

Lunn, D.J., Thomas, A., Best, N., and Spiegelhalter, D. 2000. WinBUGS — a Bayesian modelling framework: concepts, structure, and extensibility. Stat. Comput. **10**(4): 325–337. doi:10.1023/A:1008929526011.

Millar, R.B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. **44**(3): 583–590. doi:10.1139/f87-071.

Millar, R.B. 1990. A versatile computer program for mixed stock fishery composition estimation. Can. Tech. Rep. Fish. Aquat. Sci. No. 1753.

Miller, J., Banks, M., Gomez-Uchida, D., and Shanks, A. 2005. A comparison of the population structure in black rockfish (*Sebastes melanops*) as determined by otolith microchemistry and microsatellite DNA. Can. J. Fish. Aquat. Sci. **62**(10): 2189–2198. doi:10.1139/f05-133.

Milton, D., and Chenery, S. 2001. Can otolith chemistry detect the population structure of the shad hilsa *Tenualosa ilisha*? Comparison with the results of genetic and morphological studies. Mar. Ecol. Prog. Ser. **222**: 239–251. doi:10.3354/meps222239.

Munch, S.B., and Clarke, L.M. 2008. A Bayesian approach to identifying mixtures from otolith chemistry data. Can. J. Fish. Aquat. Sci. **65**(12): 2742–2751. doi:10.1139/F08-169.

Nielsen, E.E., Hansen, M.M., and Meldrup, D. 2006. Evidence of microsatellite hitch-hiking selection in Atlantic cod (*Gadus morhua* L.): implications for inferring population structure in non-model organisms. Mol. Ecol. **15**(11): 3219–3229. doi:10.1111/j.1365-294X.2006.03025.x. PMID:16968266.

O'Reilly, P.T., Canino, M.F., Bailey, K.M., and Bentzen, P. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. Mol. Ecol. **13**(7): 1799–1814. doi:10.1111/j.1365-294X.2004.02214.x. PMID:15189204.

Pella, J., and Masuda, M. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fish. Bull. (Washington, D.C.), **99**: 151–167.

Pella, J., and Masuda, M. 2005. Classical discriminant analysis, classification of individuals, and source population composition of mixtures. *In* Stock identification methods: applications in fishery science. Chapter 25. *Edited by* S.X. Cadrin, K. Friedland, and J.R. Waldman. Elsevier/Academic Press, Burlington, Mass. pp. 517–552.

Pella, J., and Masuda, M. 2006. The Gibbs and split-merge sampler for population mixture analysis from genetic data with incomplete baselines. Can. J. Fish. Aquat. Sci. **63**(3): 576–596. doi:10.1139/f05-224.

R Development Core Team. 2007. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org. ISBN 3-900051-07-0

Rannala, B., and Mountain, J.L. 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. U.S.A. **94**(17): 9197–9201. doi:10.1073/pnas.94.17.9197. PMID:9256459.

Ripley, B.D. 1996. Pattern recognition and neural networks. Cambridge University Press, Cambridge, UK.

Ruzzante, D.E., Taggart, C.T., and Cook, D. 1996. Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. Can. J. Fish. Aquat. Sci. **53**(12): 2695–2705. doi:10.1139/cjfas-53-12-2695.

Ruzzante, D.E., Taggart, C.T., Lang, S., and Cook, D. 2000. Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. Ecol. Appl. **10**(4): 1090–1109. doi:10.1890/1051-0761(2000)010[1090:MSAOAC]2.0.CO;2.

Smouse, P.E., Waples, R.S., and Tworek, J.A. 1990. A genetic

mixture analysis for use with incomplete source population data. Can. J. Fish. Aquat. Sci. **47**(3): 620–634. doi:10.1139/f90-070.

Taggart, C.T., Penney, P., Barrowman, N., and George, C. 1995. The 1954–1993 Newfoundland cod-tagging database: statistical summaries and spatial–temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. 2042.

Templeman, W. 1962. Divisions of cod stocks in the Northwest Atlantic. ICNAF Redbook, 1962, Part III. pp. 79–123.

Thorrold, S.R., Latkoczy, C., Swart, P.K., and Jones, C.M. 2001. Natal homing in a marine fish metapopulation. Science (Washington, D.C.), **291**(5502): 297–299. doi:10.1126/science.291.5502.297. PMID:11209078.

Waples, R.S., Punt, A., and Cope, J. 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish Fish. **9**: 423–449.

Wennevik, V., Jørstad, K.E., Dahle, G., and Fevolden, S.-E. 2008. Mixed stock analysis and the power of different classes of molecular markers in discriminating coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway. Hydrobiologia, **606**(1): 7–25. doi:10.1007/s10750-008-9349-5.

White, J.W., Standish, J.D., Thorrold, S.R., and Warner, R.R. 2008. Markov chain Monte Carlo methods for assigning larvae to natal sites using natural geochemical tags. Ecol. Appl. **18**(8): 1901–1913. doi:10.1890/07-1792.1. PMID:19263887.

Wright, J.M. 1993. DNA fingerprinting of fishes. *In* Biochemistry and molecular biology of fishes. Vol. 2. *Edited by* W. Hochachka and T. Mommsen. Elsevier Science, Amsterdam, the Netherlands. pp. 57–91.