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Performance of finite mixture distribution models to estimate nursery habitat contributions to fish stocks

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Background. Otolith microchemistry applications of finite mixture distribution models (FMDM) describe mixed stocks using three sets of parameters: proportional contributions (pi), baseline parameters (θi) and number of contributing nursery origins (c#). Under ideal scenarios, c# is known and all potential sources are sampled to produce source-based ^θi estimates. Hence, ^pi is the only parameter vector estimated by FMDM from the mixed-data. If some/all nursery areas are unknown or not sampled, some or all θi and c# need to be also estimated from the mixed-data. Our goal here was to assess bias and variability in ^pi, ^θi and ^c# when estimated by FMDM, under a range of data availability scenarios.

Methods. We used a comprehensive Sparus aurata dataset, tat contained otolith elemental ratios from 301 young of the year, sampled at four nursery origins, in three highly contrasting years. Using bootstrap resampling (n=1000) we produced artificial source- and mixed-samples. Source-samples simulated different scenarios where KU=0-4 nursery sources were excluded. We evaluated bias (BI) and variability (VI) in ^pi by fitting FMDM to mixed-samples with true pi=0.1-0.4. Bias and variability in ^θi and ^c# were, instead, assessed on balanced mixed stock-samples (pi=0.25). Estimations of ^c# were obtained by fitting and comparing multiple FMDMs with c#=1-9.

Results. Accurate and precise ^pi estimates (BI<0.03, VI<0.07) were produced by FMDM when samples from all origins were available (KU=0). BI and VI in ^pi tended to increase rapidly as KU increased, yielding unreliable results for KU>1. BI and VI in ^θi were highly heterogeneous among cohorts and less sensitive to KU. Relatively accurate ^θi estimates (BI<0.3) were produced for cohorts 2008 and 2010, but highly biased ones for cohort 2011 (VI>0.53), at all scenarios. Variability in ^θi was relatively low (VI<0.3) and insensitive to KU, across all cohorts. While ^c# tended to underestimate c# (BI=0.05 to -2.06), its variability was relatively high (VI=0.24-1.14) across scenarios and cohorts. Both bias and variability in ^c# were highly sensitive to KU.

Discussion. FMDM estimated accurate and unbiased ^pi and ^θi parameters when all origins were known and sampled. FMDM performance decreased rapidly and all three set of estimated parameters became unreliable when ≥2 origins were missing from nursery-samples. Large differences in BI and VI among cohorts emphasized the need for extensive sampling of nursery origins. Being FMDM one default method for mixed stock analysis, we strongly recommend exploring alternative FMDM implementations and extreme caution when using results from FMDM, under incomplete sampling scenarios.

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ABSTRACT

Background. Otolith microchemistry applications of finite mixture distribution models (FMDM) describe mixed stocks using three sets of parameters: proportional contributions (p_i), baseline parameters (θ_i) and number of contributing nursery origins $(c^{\#})$. Under ideal scenarios, $c^{\#}$ is known and all potential sources are sampled to produce source-based $\hat{\theta}_i$ estimates. Hence, $\hat{p}_i$ is the only parameter vector estimated by FMDM from the mixed-data. If some/all nursery areas are unknown or not sampled, some or all θ_i and $c^{\#}$ need to be also estimated from the mixed-data. Our goal here was to assess bias and variability in $\hat{p}_i$, $\hat{\theta}_i$ and $\hat{c}^{\#}$ when estimated by FMDM, under a range of data availability scenarios.

Methods. We used a comprehensive *Sparus aurata* dataset, tat contained otolith elemental ratios from 301 young of the year, sampled at four nursery origins, in three highly contrasting years. Using bootstrap resampling (n=1000) we produced artificial source- and mixed-samples. Source-samples simulated different scenarios where K_U=0-4 nursery sources were excluded. We evaluated bias (*BI*) and variability (*VI*) in $\hat{p}_i$ by fitting FMDM to mixed-samples with true p_i =0.1-0.4. Bias and variability in $\hat{\theta}_i$ and $\hat{c}^{\#}$ were, instead, assessed on balanced mixed stock-samples (\(p_i\))=0.25). Estimations of $\hat{c}^{\#}$ were obtained by fitting and comparing multiple FMDMs with $c^{\#}$=1-9.

Results. Accurate and precise $\hat{p}_i$ estimates (*BI*<0.03, *VI*<0.07) were produced by FMDM when samples from all origins were available (K_U=0). *BI* and *VI* in $\hat{p}_i$ tended to increase rapidly as K_U increased, yielding unreliable results for K_U>1. *BI* and *VI* in $\hat{\theta}_i$ were highly heterogeneous among cohorts and less sensitive to K_U. Relatively accurate $\hat{\theta}_i$ estimates (*BI*<0.3) were produced for cohorts 2008 and 2010, but highly biased ones for cohort 2011 ($VI_{>}$0.53), at all scenarios.

Variability in $\hat{\theta}_i$ was relatively low (*VI*<0.3) and insensitive to K_U, across all cohorts. While $\hat{c}^{\#}$ tended to underestimate $c^{\#}$ (*BI*=0.05 to -2.06), its variability was relatively high (*VI*=0.24-1.14) across scenarios and cohorts. Both bias and variability in $\hat{c}^{\#}$ were highly sensitive to K_U.

Discussion. FMDM estimated accurate and unbiased $\hat{p}_i$ and $\hat{\theta}_i$ parameters when all origins were known and sampled. FMDM performance decreased rapidly and all three set of estimated parameters became unreliable when ≥2 origins were missing from nursery-samples. Large differences in *BI* and *VI* among cohorts emphasized the need for extensive sampling of nursery origins. Being FMDM one default method for mixed stock analysis, we strongly recommend exploring alternative FMDM implementations and extreme caution when using results from FMDM, under incomplete sampling scenarios.

INTRODUCTION

Evaluating the contribution of different sources to a mixture is a common problem in ecology, biology and natural resource management (Kimura & Chikuni, 1987; Smouse, Waples & Tworek, 1990; Van Dongen, Lens & Molemberghs, 1999; Fleischman & Burwen, 2003; Manel, Gaggiotti & Waples, 2005; Phillips, Newsome & Gregg, 2005; Newman & Leicht, 2007). In fish ecology and fisheries management, for example, researchers are frequently interested in estimating the contribution from different nursery habitats (sources) to adult aggregations, demographic units or stocks (mixtures). This is a task that, beyond its inherent scientific interest, has practical relevance for both management and conservation purposes (Kerr, Cadrin & Secor, 2010). Assessing the accuracy and precision of parameters resulting from such mixture analysis is a fundamental, although often neglected, step, required to facilitate the incorporation of these results into modern management models (Kritzer & Liu, 2014).

Mixture analysis in fish ecology and other disciplines relies heavily on the use of artificial and natural tags suitable for tracking or identifying the different sources (origins) contributing to a mixture (Gillanders, 2009). Within natural tags, the elemental and isotopic composition of teleost fish otoliths has been an increasingly common choice for this type of studies during the last decades (Kerr & Campana, 2014). They grow throughout lifetime by a regular deposition of calcium carbonate and protein layers, which, unlike bones, are not reabsorbed (Panfili et al., 2002). While calcium can be partially replaced by other metals (including Sr, Mn and Ba), dominant carbon and oxygen isotopes (^{12}C and ^{16}O) can be replaced by their less frequent alternatives ^{13}C and ^{18}O. When these substitutions are under weak internal control, they may reflect environmental and/or physiological variability (Panfili et al., 2002), and the elemental/isotopic otolith signatures can be considered "fingerprints" for the water masses inhabited by fish at carbonate deposition time (Elsdon et al., 2008). As layering time can be often inferred from the same otolith through ageing techniques, a retrospective identification of nursery or feeding habitats, demographic units (~stocks) and/or migration patterns becomes possible (Campana & Thorrold, 2001; Rooker & Secor, 2004; Elsdon et al., 2008; Barnett-Johnson et al., 2008; Arkhipkin, Schuchert & Danyushevsky, 2009; Darnaude et al., 2014; Niklitschek et al., 2014).

Two main statistical approaches are commonly used to estimate the contribution of different sources to a mixture: discriminant functions (DF) and finite mixture distribution models (FMDM) (Millar, 1990a; Koljonen, Pella & Masuda, 2005). DF include linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), multinomial regression (MNR) and random forest analysis (RM), among several others (Edmonds, Caputi & Morita., 1991; Elsdon & Gillanders, 2003; Pella & Masuda, 2005; Mercier et al., 2011). DF focus on developing discriminant algorithms, which are fit ("trained") using samples from known origins (sources), and then applied to assign putative origins to new individuals sampled from the stock mixture of interest. As mixing proportions derived from DF are not model parameters, they lack of asymptotic standard errors. FMDM approaches focus, instead, on the estimation of mixing proportions, which are explicit model parameters whose standard errors can be derived from their likelihood profile. Moreover, the source baseline parameters that describe each component are also explicit and fundamental parameters in FMDM, which increases the scientific interest for using this method in mixed stock analysis.

Described in detail by Everitt & Hand (1981), FMDM were probably introduced into fisheries science by Cassie (1954). Applications to mixed stock analysis were first presented by Fournier et al. (1984) and increased largely after the HISEA software was made available by Millar (1990b). Recent applications and extensions to mixed stocks combining otolith chemistry and other natural markers have been presented by Smith & Campana (2010) and Niklitschek et al. (2010). FMDM models follow the general form (Everitt & Hand, 1981),

$$f(x) = \sum_{i=1}^{c^{\#}} p_i g(x; \theta_i)$$

which is defined by three groups of parameters: the number of components or sources ($c^{\#}$), the mixing proportions (p_i) and the set of source baseline parameters θ_i that characterize each source *i*, given the probability distribution function *g*(). As this function is frequently, although not necessarily, assumed multivariate normal, θ_i can be decomposed in a vector of means (μ_i) and a covariance matrix (Σ_i) for the response variables considered in the analysis. Translating this terms into the lexicon of otolith chemistry-based analysis of mixed stocks, $c^{\#}$ corresponds to the number of nursery or spawning sources, p_i to the proportional contribution made by each of these sources to the mixed stock, and θ_i to the source baseline parameters describing the distribution of the elemental or isotopic ratios of interest, at each nursery source *i*.

Traditional FMDM applications to mixed stock analysis tend to focus on estimating p_i, given all potential nursery sources have been previously identified (i.e. *c* is known) and sampled to produce *ex-ante* θ_i estimates (examples provided by Hamer, Jenkins & Gillanders, 2005; Crook & Gillanders, 2006; Schloesser et al., 2010; Secor, Gahagan & Rooker, 2012). This approach requires the capability to collect juveniles at each nursery source (source-data), before they emigrate and mix with fish from other nursery sources (mixed-data). If such a sampling or a suitable surrogate to it, results impossible or incomplete, a simultaneous FMDM estimation of both p_i and (some or all) θ_i parameters, is needed to be performed using the mixed-data (examples in Smouse, Waples & Tworek, 1990; Niklitschek et al., 2010; Smith & Campana, 2010). Moreover, if the total number of sources ($c^{\#}$) is also unknown, all three sets of parameters (p_i, θ_i and $c^{\#}$) may need to be estimated from the mixed-data. Such a simultaneous estimation, within a single FDMD fit, may lead however to identifiability issues (Everitt & Hand, 1981). Under this scenario, a model comparison approach can be used, instead, to evaluate a range of plausible $c^{\#}$ values by Akaike's (1973), Schwarz (1978)'s Bayesian or some other information criterion, as done, for example, by Niklitschek et al. (2014).

Although mixed-data based estimations of p_i, θ_i and/or $c^{\#}$ may contain important risks of bias and spurious conclusions, this approach may represent the only option available to gain some information about mixed stocks suffering of incomplete identification and/or sampling of their sources. The magnitude of such risks has seldom been assessed (Wood et al., 1987) as no reference data exists to contrast the parameters estimated by the model. Indirect assessment approaches can be conducted, however, using simulated or empirical datasets whose true $p_{i'}$ θ_i and/or $c^{\#}$ parameters were actually known. In this article, we take advantage of a comprehensive spatio-temporal dataset containing individual otolith elemental signatures from young-of-the-year

Sparus aurata collected in four separate nursery habitats (Mediterranean lagoons), in three highly contrasting years (Tournois et al., 2013). By sub-setting and resampling this dataset we evaluate bias and uncertainty in p_i, θ_i and $c^{\#}$ as a function of (i) the number of nursery sources being identified and/or sampled for source baseline parameters estimation, and (ii) the inter-annual variability observed among nursery sources.

MATERIALS AND METHODS

DATA SET DESCRIPTION

Tournois et al.'s (2013) data set included 301 otolith samples from young-of-the-year YOY *Sparus aurata,* collected in three different years (=cohorts): 2008, 2010, and 2011, from four Mediterranean lagoons: Bages-Sigean, Mauguio, Salses-Leucate and Thau, all located in the Gulf of Lions (NW Mediterranean Sea). Collection occurred in late summer, before YOY returned to mix with individuals from nearby lagoons in the open sea. Sampled lagoons differ in size, depth, freshwater input and degree of connection with the sea, leading to physical and chemical differences in the water and, therefore, in otolith signatures (Tournois et al., 2013). Nonetheless, these lagoons are strongly influenced by rainfall, wind and other environmental forces (Sarà, Leonardi & Mazzola, 1999; Martins et al., 2001), leading to high interanual variability in the degree of separation among their otolith signatures (Tournois et al., 2013). As a result, we consider this dataset might be representative of degrees of separation among sources that could be found in other species and populations.

The chemical composition of otolith samples performed by Tournois et al. (2013) was based in Solution Based Inductively Coupled Plasma Mass Spectrometry, including ^{43}Ca and another 11 elements. We selected seven of them for the current analysis series: ^{7}Li, ^{11}B, ^{25}Mg, ^{85}Rb, ^{86}Sr, ^{89}Y and ^{138}Ba. All concentrations were expressed as elemental ratios to Ca, and standardized to mean=0, and SD=1 to scale all elements equally and facilitate bias analysis. Three obvious outliers were discarded, working with a depurated sample size of 298 otoliths. Data was normalized using a multivariate Box-Cox (1964) 's transformation although it failed to fully normalize three of the seven elemental ratios.

RESAMPLING AND GENERAL SIMULATION PROCEDURES

All analyses described below were conducted repeating the following general procedure: (1) a variable number (K_U=0-4) of nursery sources was selected to be excluded from the source sample to simulate five data availability scenarios where these sources were considered as “unknown”; (2) within each cohort mixed stock-data, a random bootstrap sample of 25 otoliths was selected from each of all “known” nursery sources to produce a balanced “source-sample”, which represented pre-migratory juvenile fish; (3) a similar bootstrap procedure was used to mix data from all sources into an artificial “mixed -sample” (n=300), which represented the mixed-stock formed once juvenile fish had emigrated from nursery sources; (4) Mixing proportions in the mixed-sample varied as detailed below; (5) the source-sample was used to estimate θ_i for all “known” nursery sources; (6) FMDM was used to estimate p_i and θ_i for “unknown” sources and/or $c^{\#}$, depending upon the simulation goal (see below). This six-steps sequence was labelled as a resampling run and was repeated 1000 times for each analysis, cohort and scenario. The bootstrap resampling approach was adopted here to reduce potential sample-size effects affecting conclusions about FMDM performance.

MIXING PROPORTIONS OF KNOWN NURSERY SOURCES

We evaluated the performance (bias and variability) of FMDM when used to estimate mixing proportions ($\hat{p}_i$) of each nursery source *i* within mixed-samples (n_j=100 for each cohort *j*) where Bages-Sigean, Mauguio, Salses-Leucate and Thau were set to contribute arbitrary proportions of

0.1, 0.2, 0.3 and 0.4, respectively. FMDM performance here was evaluated assuming the total number of nursery sources was known ($c^{\#}$=4), under five different data availability scenarios (K_U =0-4 "unknown" sources). As a result, the FMDM was forced to estimate not only the four $\hat{p}_i$, but also the K_U missing source baseline parameters directly from the mixed-sample. Bias in $\hat{p}_i$ was indexed (*BI*) as the difference between estimated and true mixing proportions corresponding to each nursery source, within each cohort and data availability scenario. Variability in $\hat{p}_i$ (*VI*) was computed as the empirical standard error of $\hat{p}_i$ out of the 1000 resampling runs corresponding to each nursery source, cohort and scenario.

SOURCE BASELINE PARAMETERS

Under the assumption of multivariate normal distribution, each estimated source baseline $\hat{\theta}_i$ was composed by a vector of means $\hat{\mu}_i$ and a covariance matrix $\hat{\Sigma}_i$, which described the multivariate distribution of the seven chemical elements measured in the otoliths included in the dataset. Assessing bias and variability in $\hat{\Sigma}_i$ results a complex task which, we considered that exceeded the scope of this paper. Therefore, all bias and variability measures provided hereafter for $\hat{\theta}_i$ are strictly referred to $\hat{\mu}_i$, although we will not emphasize this point further to avoid making even more complex the current structure and nomenclature of this article.

Bias and variability in FMDM-based $\hat{\theta}_i$ were assessed by producing multiple source-samples, to which all but K_U “unknown” nursery sources contributed equally. Therefore, $\hat{\theta}_i$ corresponding to each of these K_U=1-4 "unknown" origins were estimated by FMDM from the mixed-data, which included equal proportions of all nursery sources, with n_{ij}=25, for each nursery *i* and cohort *j*. Bias for $\hat{\theta}_i$ was indexed (*BI*) as the square root of the euclidean distance between the vectors of predicted ($\hat{\mu}_{ij}$) and true (μ_{ij}) means for all seven elemental ratios, within each nursery source (*i*) and cohort (*j*). As all elemental ratios were previously standardized, bias units were equivalent to standard deviations. Variability in $\hat{\theta}_i$, was indexed as $VI = \det(\hat{\Sigma}_{ijk})^{1/2Q}$, where $\hat{\Sigma}_{ijk}$ was the covariance matrix of all $\hat{\mu}_{ij}$ within each nursery source (*i*), cohort (*j*) and scenario (*k*), and $Q=7$ was the number of elemental ratios being described by $\hat{\theta}_i$.

NUMBER OF CONTRIBUTING NURSERY SOURCES

Within each resampling run, we compared a range of FMDMs defined by a variable number of contributing nursery sources ($c^{\#}$), which ranged between a minimum ($c^{\#}_{min}$=4-K_U), naturally constrained to be equal or greater than K_U , and a maximum defined arbitrarily as $c^{\#}_{max}$=9. K_U was set to range between 0 and 4 unknown sources, whose source baselines were estimated directly from the mixture data at each FMDM fit. Source- and mixed-samples were built as described for the assessment of source baseline parameters. Schwarz (1978)'s Bayesian Information Criterion (BIC) was used to select the most informative $c^{\#}$ within each resampling run, which we addressed as the “predicted number or nursery sources” ($\hat{c}^{\#}$). Bias was computed as $BI=\hat{c}^{\#}-4$, and variability (*VI*) as the standard error of $\hat{c}^{\#}$ computed from all resampling runs corresponding to each cohort and sampling scenario.

FMDM PARAMETER ESTIMATION

All $\hat{p}_i$ and mixed-derived $\hat{\theta}_i$ were estimated by maximum likelihood, using the Expectation-Maximization (EM) algorithm (Dempster, Laird & Rubin, 1977). FDMD solutions were constrained to produce definite positive covariance matrices, with $\det(\Sigma)>10^9$. Source-derived $\hat{\theta}_i$ were computed directly as the vector of means and the covariance matrix of elemental ratios found at each source-sample. Starting values for mixed-derived $\hat{\theta}_i$, when needed, were obtained trough a semi-supervised partition-among-centroids clustering procedure, implemented using the

R-package “vegclust” (De Cáceres, Font & Oliva, 2010). This clustering method allowed us to combine fixed centroids, that represented means from “known” nursery sources (present in the source-sample) and mobile centroids, that represented “unknown" nursery sources, only present in the mixed-sample.

RESULTS

MIXING PROPORTIONS

While relatively unbiased and accurate $\hat{p}_i$ were obtained under the best two scenarios, bias in $\hat{p}_i$ increased rapidly as K_U increased (Table 1, Figure 1). When all nursery sources were known and sampled (K_U=0), *BI* below 0.03 and *VI* below 0.07 were observed across all cohorts. When one nursery source was excluded from nursery samples (K_U=1), bias and variability in $\hat{p}_i$ increased to *BI* ranges of 0.05-0.11 and *VI* ranges of 0.04-0.12, depending upon the cohort (Figure 1). However, for all K_U>1 scenarios very unreliable and variable $\hat{p}_i$ were produced. Under such scenarios, even the rank order of $\hat{p}_i$ was often incorrect among nursery sources, within scenarios (Table 1). Moreover, bias in $\hat{p}_i$ at K_U>2 scenarios were 1.3-2.5 higher than those computed for K_U=0 (Figure 1). Variability in $\hat{p}_i$ also showed a rapid increase up to K_U=2, becoming high, but relatively stable afterwards.

Overall, bias and variability in $\hat{p}_i$ tended to be lower for cohort 2008, and higher for cohort 2011 (Figure 1), matching the smallest and greatest degree of overlap in otolith elemental signatures among all four nursery sources (Figure 2). There was also an evident trend to observe positive bias at lower p_i values, and negative bias at higher p_i values, which was more pronounced as K_U increased (Table 1)

SOURCE BASELINE PARAMETERS

Source baseline parameters estimated by FMDM from mixed-samples showed to define multivariate normal distribution models useful to describe source-data, at least for cohorts 2008 and 2011, under different data availability scenarios (Figure 2). However, matching the greater overlap among sources observed in 2011, the centroids, dispersion and orientation of predicted distributions for this cohort exhibited a much poorer fit to the actual data of this subset (Figure 2). Bias in $\hat{\theta}_i$ was highly variable among cohorts, in terms of both their magnitude and their response to data availability scenarios (Figure 3). As found for $\hat{p}_i$, bias in $\hat{\theta}_i$ was lower for cohort 2008, where *BI* remained below 0.35 across all scenarios, and higher for cohort 2011, where *BI* exceeded 0.53 across all scenarios. Bias in $\hat{\theta}_i$ tended to increase with K_U in cohorts 2008 and 2010, but failed to follow any evident trend in cohort 2011 (Figure 3). Variability in $\hat{\theta}_i$ was much less sensitive to data availability (K_U) than bias in either $\hat{\theta}_i$ or $\hat{p}_i$, showing a moderate increase with K_U, in cohorts 2008 and 2010, while a slightly decreasing trend in cohort 2011. Variability in $\hat{\theta}_i$ was higher for cohorts 2010 and 2011 (*VI*>0.2) than for cohort 2008 (*VI*<0.15), across all treatments.

NUMBER OF CONTRIBUTING NURSERY SOURCES

FMDM-based $\hat{c}^{\#}$ tended to underestimate the true value of $c^{\#}$ under most data availability scenarios (Figure 4). Only under the ideal scenario (K_U=0), where all sources were represented in source-samples, the true value of $c^{\#}$ was predicted correctly in most simulations (76-94%) . Although a small (positive) bias (0.05-0.26) was produced at K_U=0 by a few cases where $\hat{c}^{\#}$=5, no predictions above 5 were observed. As for the remaining scenarios, the percentage of correct estimations decreased to 34-41% at K_U=1, and to 6-21% at K_U=4, i.e. the worst case scenario. Negative bias tended to increase as K_U increased (Figure 4), and, therefore, as $c^{\#}_{min}$ decreased.

Following such trend, the negative bias in $\hat{c}^{\#}$, that ranged between 0.24 and 0.56 at K_U=1, became 0.6-2.1 at K_U=4. Overall, bias tended to be higher at cohort 2011 (*BI*=-2.60-0.26) and lower at cohort 2008 (*BI*=-1.19-0.06). Variability in $\hat{c}^{\#}$ was relatively high (*VI*=0.63-1.14) across all cohorts, increasing with K_U, from a range of *VI*=0.24-0.49, at K_U=0, to a range of *VI*=0.90-1.14 at K_U=4, depending upon the cohort being analysed. Following the general pattern observed in other parameters, variability in cohorts 2010 (*VI=0.45-1.14*) and 2011 (*VI=0.49-1.0*) tended to be greater than in cohort 2008 (VI=0.24-0.90), particularly when the number of "unknown" sources was low.

DISCUSSION

In this article, we had the opportunity to evaluate FMDM performance when used to estimate fundamental mixed stock parameters, under a range of data availability scenarios. Combining bootstrap resampling with a real-world dataset, whose parameters were known or manipulated, we managed to simulate multiple scenarios of interest. Using this particular dataset implied, however, some risk that our results might not be transferable to other stocks, affected by relevant differences in the number, degree of separation and/or data distribution of their mixed components. Nonetheless, we believe the large interanual variability observed in our dataset represented an important part of the variability that could be found in other stocks and geographical areas.

Mixing proportions estimated by FMDM showed high precision and accuracy for all cohorts under ideal scenarios where all nursery sources were known and previously sampled for juvenile fish in order to produce nursery-based source baseline parameters, which were then input as exogenous parameters to the FMDM. Sensitivity to incomplete sampling of nursery habitats was high, leading to unreliable results when two or more of the nursery areas were not included in source-samples, and their corresponding baseline parameters were estimated from the mixed-sample. Thus, while our results confirmed the suitability of FMDM for estimating mixing proportions at ideal or nearly ideal sampling situations, they provided a warning against extending the use of FMDMs to infer potential contributions when multiple nursery sources are “unknown" or had not been sampled to produce reliable source baseline parameter estimates. We have not considered here the intermediate case where some small level of sampling exist for some/all nurseries, for which an unconditional maximum likelihood or Bayesian approaches would be more adequate (Pella & Masuda, 2001).

The effects of incomplete sampling of nursery habitats upon $\hat{p}$ tended to bias the most extreme mixing proportions towards intermediate values. Therefore, the smallest nursery contributions appeared overestimated, while the largest ones underestimated. This behaviour might be an artefact of the EM algorithm, which may have converged, sometimes, to unsatisfactory local maxima (Marin, Mengersen & Robert, 2005). While Bayesian versions of FMDM (Pella & Masuda, 2001) are an obvious option to be explored, few methods alternative to FMDM seem to be available for estimating mixing proportions under incomplete sampling scenarios. While unsupervised clustering, followed by discriminant analysis, has been used in absence of direct source-data (Arkhipkin, Schuchert & Danyushevsky, 2009; Schuchert, Arkhipkin & Koenig, 2010), semi-supervised clustering (De Cáceres, Font & Oliva, 2010) could be an obvious extension to cases where some source-data is available. Nonetheless, no independent assessments of bias and variability seem to be available for clustering approaches applied to mixed stock analysis under incomplete sampling scenarios.

Baseline parameter estimates were relatively unbiased and accurate for two out of the three evaluated cohorts (2008 and 2010), particularly when no more than two of the four nursery sources were simulated as “unknown”. Highly biased, although not so variable, baselines were estimated, in contrast, for cohort 2011 at all data availability scenarios. This largely heterogeneous situation among cohorts was likely related to large environmental variability in the study area (Tournois et al., 2013), reflected in highly variable source-data distribution patterns among cohorts (Figure 2). These findings suggest that, under scenarios of large overlapping among nursery sources, such as the one observed for cohort 2011, classical FMDM approaches (as the one implemented by us) can be unsuitable to get reliable baseline parameter estimations.

The performance of FMDM models when used to estimate the number of nursery sources was poor, exhibiting an evident trend to underestimate its true number in all cohorts. Since the magnitude of the underestimation was constrained by the number of known nursery sources, it results obvious that enhancing our knowledge about the minimum number of existing nursery sources would be a way to reduce the risk of underestimating their total number. Despite of this negative evaluation, FMDM might be still useful to estimate a lower limit for the number of contributing sources, and to provide a conservative evaluation of the potential existence of additional nursery sources, remaining to be identified in relatively well studied mixed stock. These later applications seem to be supported by our findings that indicate FDMD was rather robust to overestimation, even when all nursery sources were known and the model was not allowed to consider less than 4 nursery sources.

The large variability in otolith elemental signatures we found in this dataset reflected large interanual variability in nursery habitats (Tournois et al., 2013), which may be common to most shallow water and estuarine nursery areas (Secor, 2015). The high sensibility of FMDM model results to this variability highlights the need to assure true independence among individual samples being used to build baseline parameter estimates. This is the needed to avoid confounding variability among nursery sources with variability among years, schools, sampling events or other sources of correlation, commonly neglected in fisheries and ecological studies (Zuur, Ieno & Smith, 2007). Moreover, the effects of these sources of correlation should be formally modelled and corrected through mixed models or equivalent approaches (Bolker et al., 2009) in order to gain new insights about the mixed stock composition. A very intuitive step here would be, for example, to combine data from all three cohorts to improve the estimation of the total number of sources, using either mixed model or Bayesian approaches.

In conclusion, our scrutiny of FMDM performance under incomplete sampling of nursery sources yielded rather discouraging results. Large sensitivity to incomplete sampling scenarios was found, producing highly biased and/or variable results when more than one out of four nursery sources were missing from source-samples. This sensitivity exceeds what would be needed for applying FMDM to many mixed stocks where the number and location of their nursery habitats remain unknown, or where juvenile sampling remains logistically impossible. We have applied here a conditional maximum likelihood approach, based upon the EM algorithm, which is the most common method used in mixed stock analysis after Millar (1987). It must be acknowledged, however, that there is a large variety of FMDM implementations, existing multiple options for improvement, within the FMDM framework (Marin, Mengersen & Robert, 2005). These options include the use of conditional approaches (see Pella & Masuda (2001) for definitions), optimization algorithms alternative to EM, such as the Metropolis–Hastings approximation, as well as the implementation of fully Bayesian approaches (Pella & Masuda, 2001; Marin, Mengersen & Robert, 2005; Smith & Campana, 2010). Although none of these avenues can assure higher degrees of success, the urgent need to enhance our understanding of exploited and threatened fish populations should be a powerful incentive to continue improving, developing and evaluating the analytical methods required to achieve this goal.

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Table 1(on next page)

True and predicted values for the proportional contributions

True and predicted values for the proportional contribution (p_i) of four different nursery origins to three simulated mixed stocks (=cohorts) used to evaluate the performance of Finite Mixture Distribution Models (FMDM). FMDM performance was evaluated under five different data availability scenarios, defined by the number of “unknown” nursery origins (N_U), which were excluded from the nursery-samples used to define FMDM baseline parameters.

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	Nursery	True	Predicted values per scenario				
Cohort	Origin	Value	N_U=0	N_U=1	N_U=2	N_U=3	N_U=4
2008	Bages-Sigean	0.10	0.10	0.13	0.16	0.17	0.20
	Mauguio	0.20	0.21	0.21	0.20	0.21	0.24
	Salses-Leucate	0.30	0.29	0.29	0.30	0.33	0.34
	Thau	0.40	0.40	0.37	0.34	0.29	0.22
2010	Bages-Sigean	0.10	0.09	0.12	0.17	0.22	0.28
	Mauguio	0.20	0.21	0.23	0.25	0.25	0.23
	Salses-Leucate	0.30	0.30	0.29	0.27	0.24	0.26
	Thau	0.40	0.40	0.36	0.31	0.28	0.24
2011	Bages-Sigean	0.10	0.11	0.15	0.20	0.27	0.32
	Mauguio	0.20	0.21	0.23	0.27	0.27	0.27
	Salses-Leucate	0.30	0.27	0.27	0.25	0.23	0.23
	Thau	0.40	0.40	0.35	0.28	0.23	0.18

1

Bias and variability in proportional contribution estimates

Bias (upper panel) and variability (lower panel) in estimated proportional contributions of four nursery origins to artificial mixed stocks of *Sparus aurata,* in three contrasting cohorts. The number of unknown nursery origins (K_U) represent nursery origins being excluded from source-samples to evaluate incomplete sampling effects upon bias and variability.

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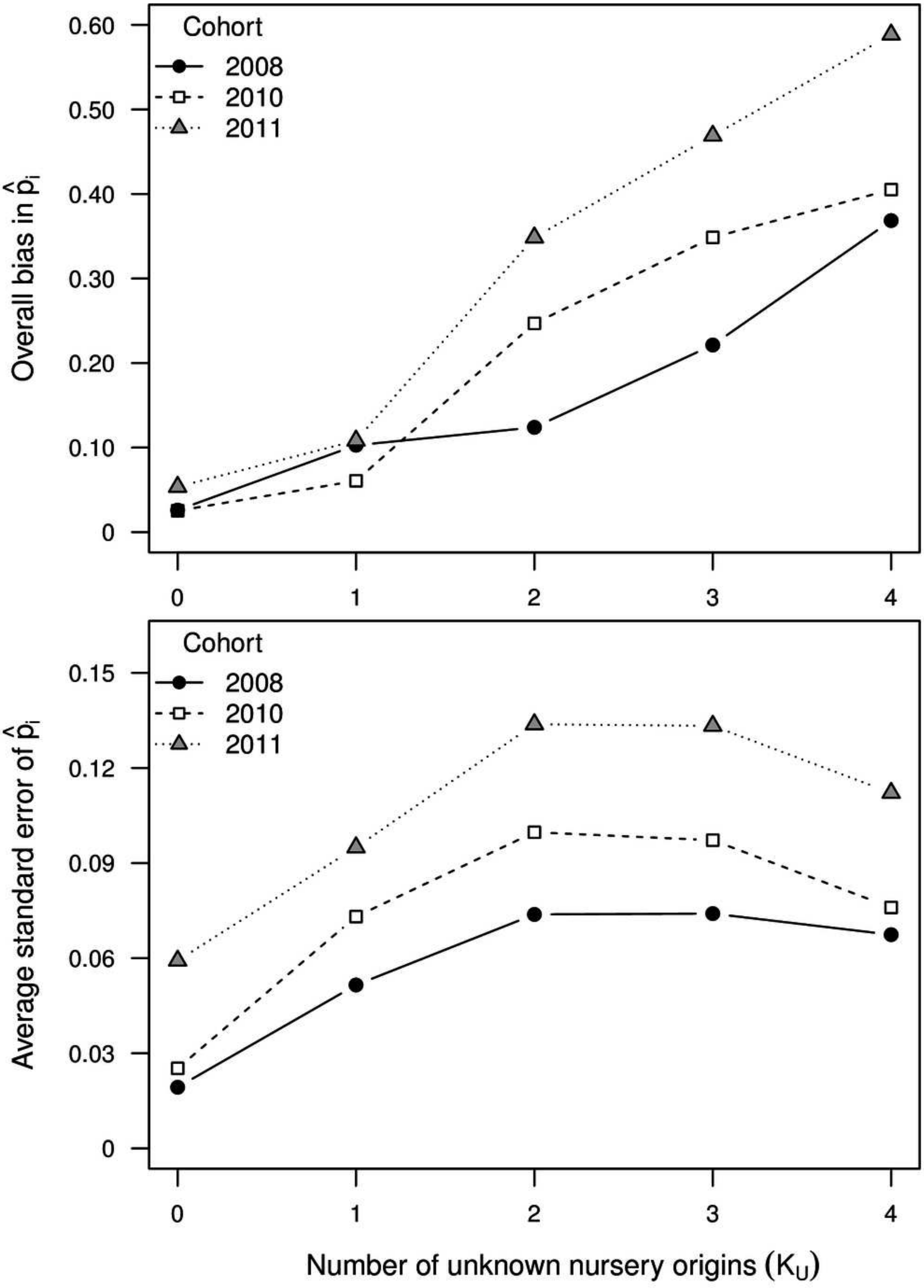
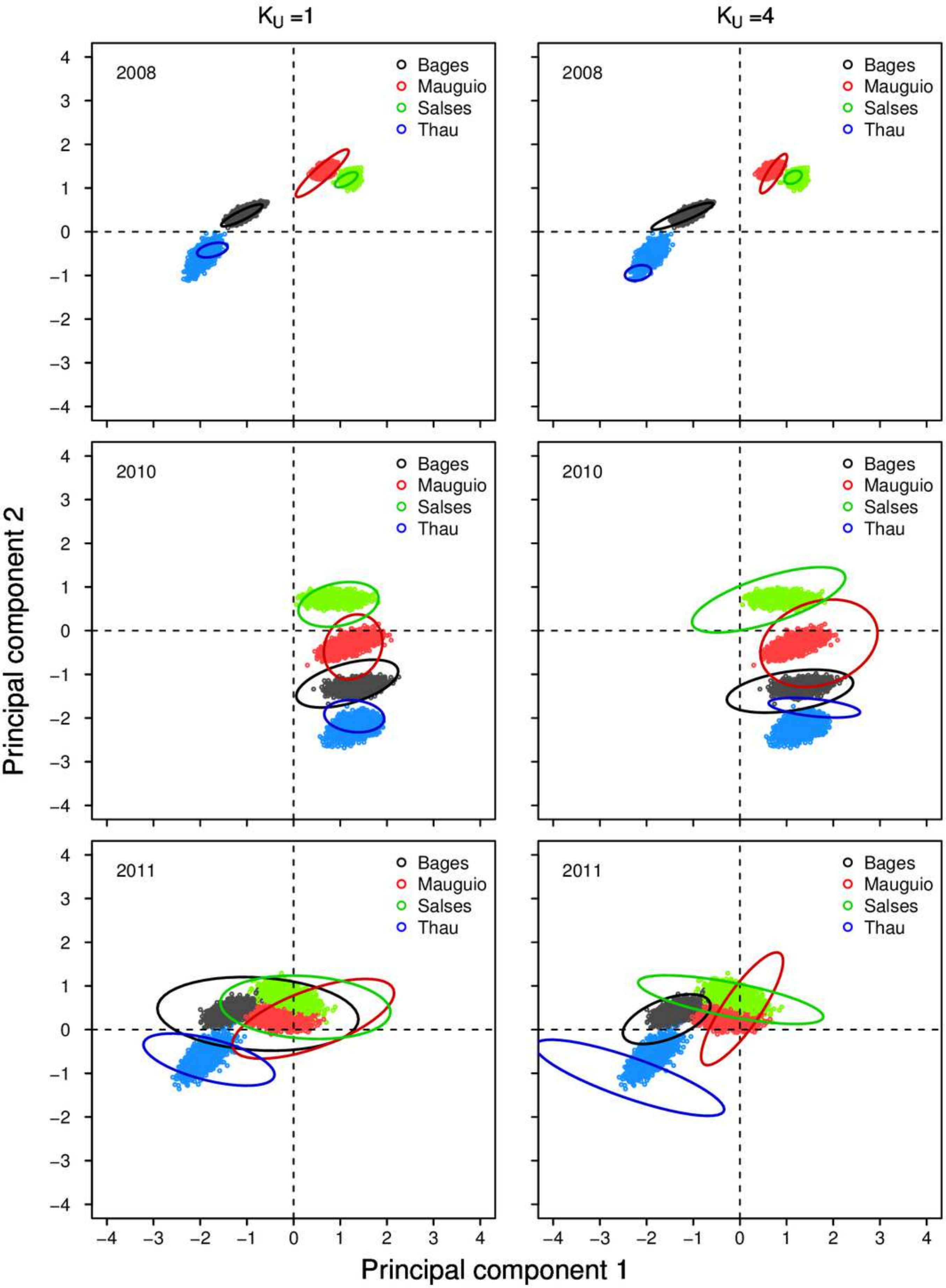

Cohort
2008
2010
2011
Overall bias in $\hat{p}_i$
0.60
0.50
0.40
0.30
0.20
0.10
0
0
1
2
3
4
Average standard error of $\hat{p}_i$
0.15
0.12
0.09
0.06
0.03
0
Number of unknown nursery origins (K_U)

2

Empirical and predicted distributions of elemental ratios in juvenile *Sparus aurata* otoliths

Principal component diagrams representing the distribution of otolith elemental ratios in juvenile *Sparus aurata.* Coloured points represent empirical means per nursery source and cohort, corresponding to 1000 bootstrap samples (size=25). Ellipses represent 67% confidence intervals defined by multivariate normal baselines estimated through finite mixture distribution modelling (FMDM) under two data availability scenarios. Left panel shows predicted distributions when only the nursery source of interest was estimated by FMDM. Right panel shows predicted distributions when all nursery sources were simultaneously estimated by FMDM



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3

Bias and variability in source baseline estimate

Bias (upper panel) and variability (lower panel) in estimated source baseline estimates for four nursery origins contributing to artificial mixed stocks of *Sparus aurata*, in three contrasting cohorts. The number of unknown nursery origins (K_U) represent nursery origins being excluded from source-samples and evaluated by finite mixture distribution modelling from mixed-samples

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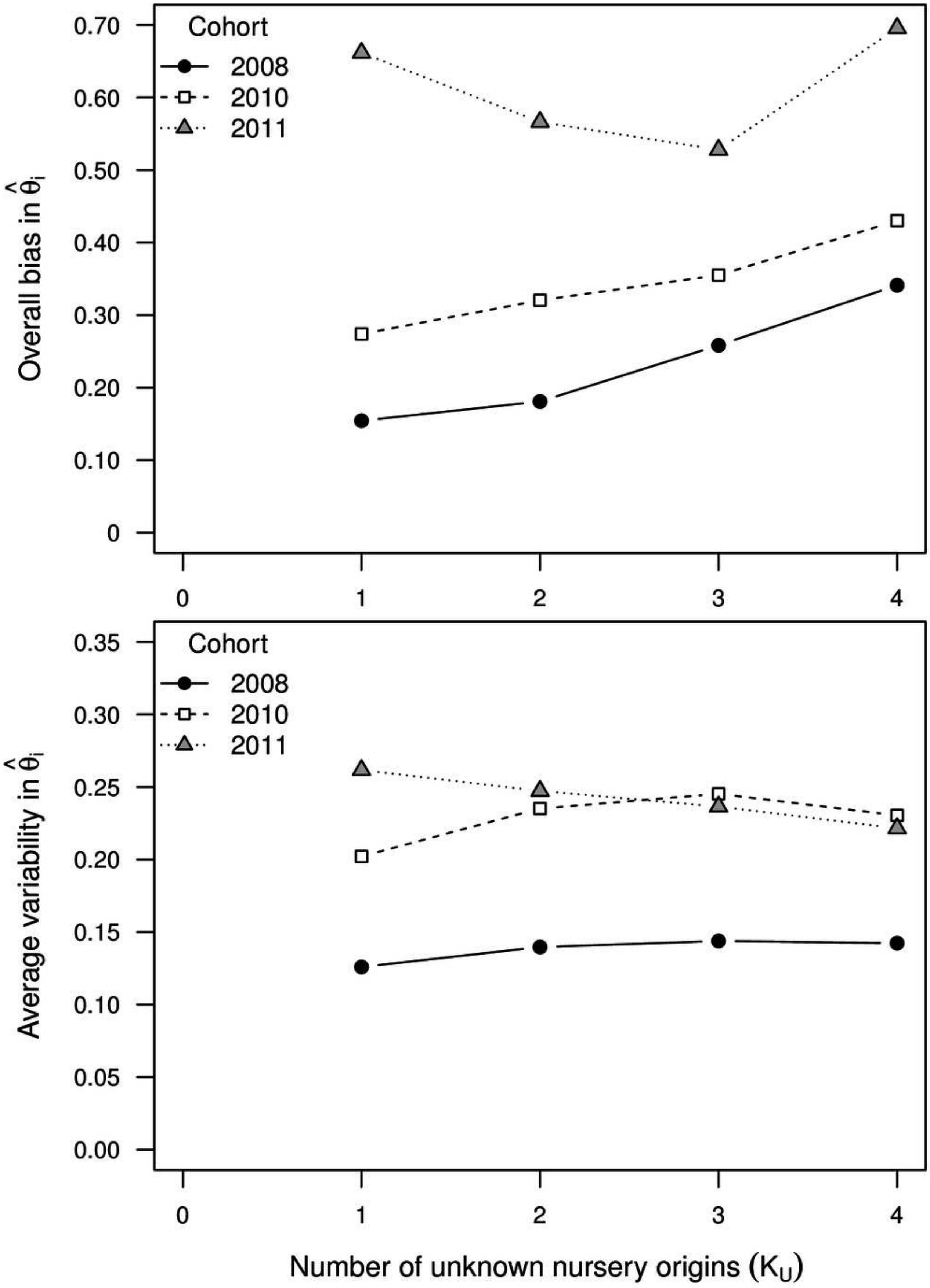
Cohort
2008
2010
2011
Overall bias in $\hat{\theta}_i$
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
0
1
2
3
4
Cohort
2008
2010
2011
Average variability in $\hat{\theta}_i$
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
Number of unknown nursery origins (K_U)

4

Predicted number of contributing sources

Predicted number of sources (nursery origins) contributing to simulated mixed-stock of *Sparus aurata* ($c^{\#}$). Predictions obtained by comparing alternative finite mixture distribution models (FMDM) that considered a range of $c^{\#}$=1-9. Different data availability scenarios excluded K_U=0-4 nursery sources from source-samples and forced FMDM to estimate all parameters from mixed-samples. Relative frequencies computed after 1000 resampling runs from available data (n=298), per tested scenario

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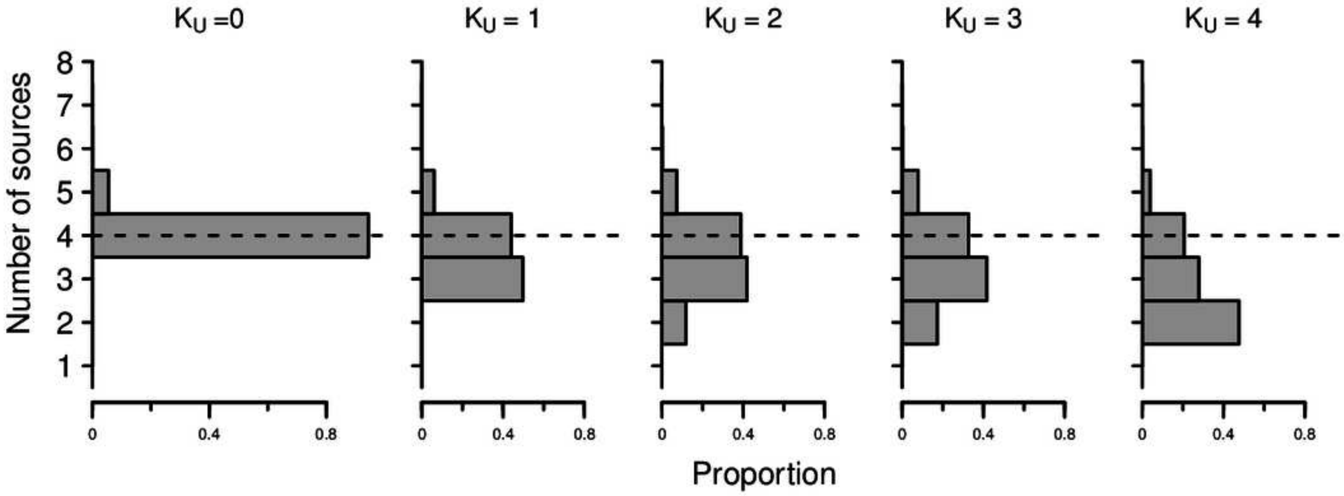
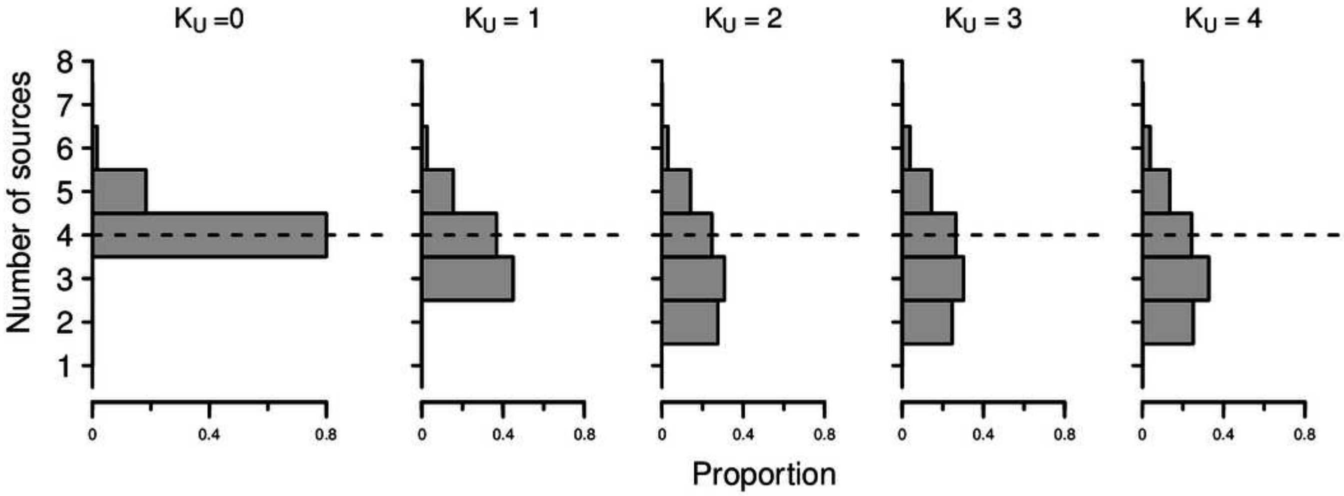
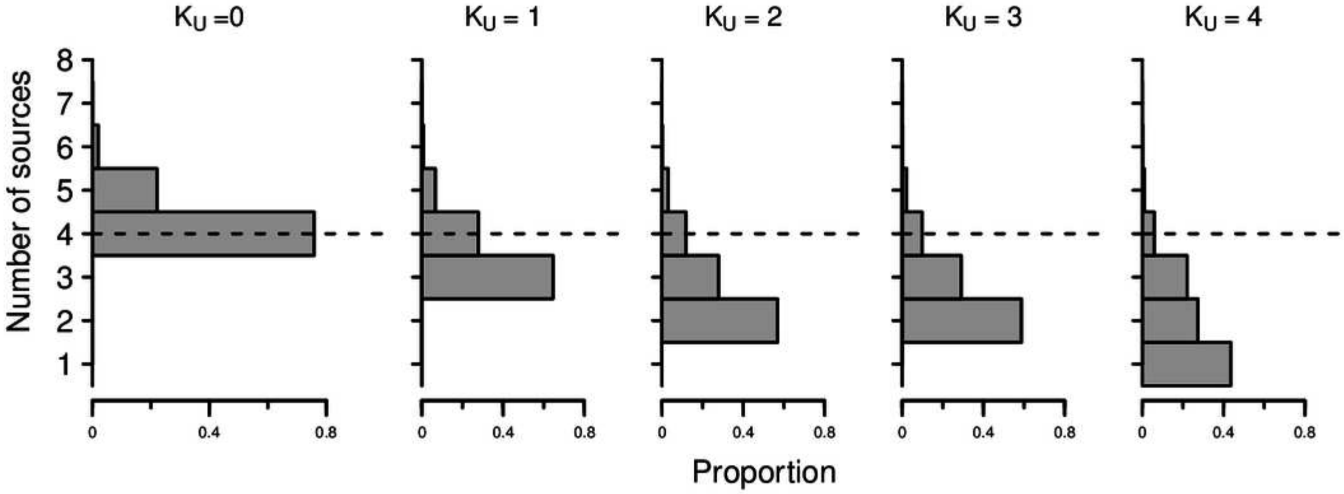

K_U =0
K_U = 1
K_U = 2
K_U = 3
K_U = 4
Number of sources
8
7
6
5
4
3
2
1
0
0.4
0.8
Proportion


K_U =0
K_U = 1
K_U = 2
K_U = 3
K_U = 4
Number of sources
8
7
6
5
4
3
2
1
0
0.4
0.8
Proportion


K_U =0
K_U = 1
K_U = 2
K_U = 3
K_U = 4
Number of sources
8
7
6
5
4
3
2
1
0
0.4
0.8
Proportion