Estimates of batch fecundity and spawning fraction for the southern stock of horse mackerel (*Trachurus trachurus*) in ICES Division IXa

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Since 1995 the annual egg production method has been applied triennially to the southern stock of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic (ICES Division IXa). This method assumes that fecundity is determinate, but increasing evidence indicates that horse mackerel are indeterminate spawners. The daily egg production method (DEPM) does not rely on the assumption of determinate fecundity, making it the appropriate method for this species. Therefore, we reanalysed samples collected from previous surveys (2002, 2004, 2005, and 2007) to obtain estimates for batch fecundity and spawning fraction, which are important DEPM parameters. The estimates of batch fecundity are around 200 oocytes g^{-1} of female (total ovary-free weight). Several criteria were used to estimate spawning fraction (migratory nucleus stage, hydrated oocytes, and post-ovulatory follicles) and all showed the same trend among years, varying between 0.10 and 0.30 d^{-1}. The estimates were significantly different among methods, but those differences were similar across surveys, indicating that a consistent bias would be reflected in the final spawning-stock biomass (SSB) estimates obtained from the DEPM. Until further information is available regarding the accuracy of the criteria used to estimate spawning fraction, the southern horse mackerel SSB estimates from the DEPM should only be taken as indicative of trends rather than measures of absolute abundance.

Keywords: egg production methods, fecundity, horse mackerel, indeterminate spawner, spawning fraction.

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Introduction

As catches from world fisheries increased rapidly during the 1970s and 1980s, greater importance was placed on understanding the effects of fishing on stock abundance (Bartolino *et al.*, 2008). One of the key objectives of a fish stock assessment is to estimate its abundance. There are several methods to obtain those estimates (e.g. Hilborn and Walters, 1992, for an overview of those methods), and the most reliable ones are based on the use of fishery-independent indices to reach sensible solutions for the equations used to describe population dynamics. Ichthyoplankton-based methods are increasingly being used around the world to estimate biomass of fish stocks and monitor trends in abundance. These include egg production methods (Gunderson, 1993), such as the annual egg production method (AEPM) and the daily egg production method (DEPM), that are commonly used for obtaining fishery-independent abundance indices for fish species with pelagic eggs.

Egg production methods involve ichthyoplankton surveys and samples of adult fish to estimate fecundity, sex ratio, and spawning fraction. Different approaches are followed for estimating the adult parameters. The AEPM requires samples of prespawning females to estimate annual fecundity and atresia to estimate annual potential fecundity (Gunderson, 1993; Hunter, 1997a). However, in horse mackerel (*Trachurus trachurus*), the annual potential fecundity is equivalent to annual fecundity because there is no significant atresia (ICES, 1996). In contrast, the DEPM uses samples of spawning females to estimate batch fecundity [number of hydrated oocytes (HO) released during one spawning event] and random samples of females, independent of maturity, to estimate spawning fraction (number of females spawning per day; Lasker, 1985; Hunter and Lo, 1997; Stratoudakis *et al.*, 2006). The DEPM was developed following the realization that in multiple spawners, the total fecundity at the start of the spawning season (the standing stock of advanced oocytes) bore no relation to the subsequently realized annual fecundity (Parker, 1980).

The AEPM has been applied triennially since 1995 to the western and southern stocks of horse mackerel *Trachurus trachurus* in the ICES Area (Abaunza *et al.*, 2003), assuming that fecundity was determinate. However, several recent studies indicate that this species is most likely an indeterminate spawner, because unyolked oocytes continue to be produced and released throughout the spawning season (Karlou-Riga and Economidis, 1997; Eltink *et al.*, 2000; Costa, 2001; Abaunza *et al.*, 2003; De Oliveira *et al.*, 2006; Gordo *et al.*, 2008). As a result of mounting evidence concerning the lack of determinacy in the fecundity of horse mackerel, a first attempt to implement the DEPM was performed in 2007 for the southern stock of horse mackerel (ICES Division IXa; ICES, 2008). In this paper, we estimate fecundity and

spawning fraction of horse mackerel, which can serve to obtain spawning-stock biomass (SSB) estimates for the assessment of this stock based on the application of the DEPM.

Material and methods

Data were obtained from samples collected by bottom trawl during four surveys carried out along the Portuguese coast, Gulf of Cadiz, and the west coast of Galicia. The 2004 survey was planned for the horse mackerel AEPM, the 2002 and 2005 surveys were for the sardine DEPM, and the 2007 collections were the first ones planned for the southern horse mackerel DEPM. The AEPM surveys were divided into three periods, covering the onset (January), peak (February), and end (March) of the main spawning period in this region (Borges and Gordo, 1991). Table 1 shows the sampling effort and sample size for each survey. The total length (cm), total weight (g), sex, and maturity stage of all sampled fish were recorded. Gonads from mature females were extracted and preserved in 4% buffered formalin. Histological slides were prepared using standard procedures and interpreted with a binocular microscope. The histological staging of ovaries followed the maturity key of Costa (2001; Table 2).

Batch fecundity (F) is the number of eggs released per fish during a single spawning event and is estimated by identifying and quantifying the cohort of HO in the gonad. Estimates of F were obtained using the ovaries of spawning females (maturity stage 4; Table 2). Hydrated ovaries containing new post-ovulatory follicles (POFs) were not used to estimate batch fecundity. Batch fecundity was estimated for each fish as described in Lasker (1985): three pieces of ~0.10 g each were cut from one lobe of the ovary, weighed, and the HO counted. This procedure assumes there are no significant differences in the number of HO per unit weight between the left and the right ovaries, as described by Sanz and Uriarte (1989) for anchovy. The mean number of HO per gramme was then extrapolated for the ovary-free weight of the female [see Zwolinski *et al.* (2001), for a detailed description]. Pairwise comparisons between the batch fecundity estimates of the different surveys were performed using Tukey's honestly significant differences (HSD) test (Miller, 1981; Yandell, 1997) using R (R Development Core Team, 2008).

Table 1. Number of stations sampled, number of fish collected, number of mature females (2–6 maturity stages), and number of spawning females (4 maturity stage), for each survey.

Year	Month	No. Stations	No. fish	No. mature females	No. spawning females
2002	January	32	755	120	9
2004	January	12	411	87	2
2004	February	8	433	77	5
2004	March	7	1 272	179	114
2005	March	43	916	214	15
2007	February	14	1 021	531	38

Table 2. Microscopic maturity stage key for horse mackerel females (Costa, 2001).

Maturity stage	Microscopic charactheristics
1 (immature)	Small ovaries with unyolked oocytes
2 (early ripening)	Bigger unyolked oocytes and yolked oocytes
3 (late ripening)	MN stage oocytes
4 (ripening)	HO
5 (partly spent)	POFs and atresia
6 (spent/recovering spent)	Atretic and unyolked oocytes

To estimate the spawning fraction, random samples of mature females (stages 2–6; Table 2) were collected from each haul. Three different criteria were applied to estimate this parameter based on the presence/absence of the following characters: oocytes at the migratory nucleus (MN) development stage, HO, and POFs. The proportion of gonads in spawning condition, as indicated by the presence of each of those structures, was recorded for each sample. The incidence of atresia was also registered, although it was not used directly in the estimation of spawning biomass using the egg production method, but it is important mainly because the late atretic stages may be confused with POFs (Lasker, 1985). The estimates of spawning fraction per day (S) were obtained by dividing the proportion of females in spawning condition, according to each criterion, by the time (d) that each of those structures lasts in the gonad. Although for several species of the genus *Trachurus* there are indications that the MN lasts ~1 d (Eltink, 1991; Macewicz and Hunter, 1993), there is currently no information on the duration of either HO or POFs in horse mackerel. However, HO are known to last for 12 h in anchovy (Hunter and Macewicz, 1985), and the POFs can last from 36 to 60 h in several other species (e.g. Hunter and Macewicz, 1985; Fitzhugh and Hettler, 1995; Ganias *et al.*, 2003). Therefore, for illustrative purposes, we calculated estimates for S assuming a 12 h duration for HO and two possible approximate durations for POFs: 2 and 3 d.

The possibility of using different criteria to estimate S raises the question of which criterion would provide the most accurate estimates. To determine if there were significant differences in the estimates of S provided by the three criteria, we fitted the generalized linear model to our data:

$$\mathcal{F}(y_{ijkl}) = \mu + \alpha_i + \beta_j + \gamma_{k(j)} + (\alpha\gamma)_{ik(j)} + \epsilon_{(ijk)l},$$

in which y_{ijkl} is the observation whether the lth individual (in the ith survey, jth sampling station according to the kth method) is in spawning condition or not, y a binary variable, α the survey effect, β the sampling station effect, γ the effect of the method within station (given that all methods were applied to all fish sampled at each station), and $(\alpha\gamma)$ the interaction effect between survey and method. A logit link function $\mathcal{F}(\cdot)$ was used, and the sampling station was included as a random effect in the model. This generalized linear mixed-effects model was fitted using function `glmer` in the `lme4` package of R (Pinheiro and Bates, 2000; R Development Core Team, 2008). The significance on the main effects and interaction term was tested with likelihood-ratio tests (Pinheiro and Bates, 2000).

Results

Batch fecundity estimates (F) for all surveys are shown in Figure 1 and Table 3. In January and February 2004, it was not possible to estimate F, as a result of the small number of samples collected during poor weather conditions (Table 1). Consequently, a large number of samples was collected in March 2004 in an attempt to compensate for the lack of samples in the previous two surveys. We did provide an estimate of F despite the small

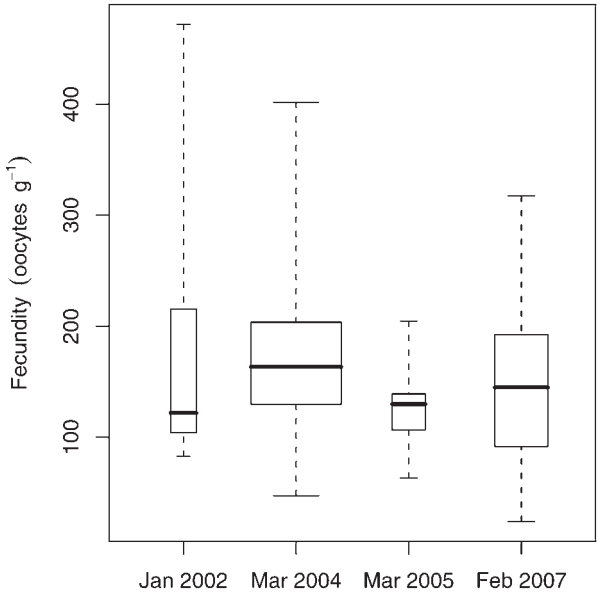

Figure 1. Batch fecundity estimates (number of eggs released per gramme of ovary-free female weight) from four surveys. The boxplots show the quartiles, with the bars extending to ± 1.5 the interquartile range. The box width is proportional to the number of samples in each month (Table 1).

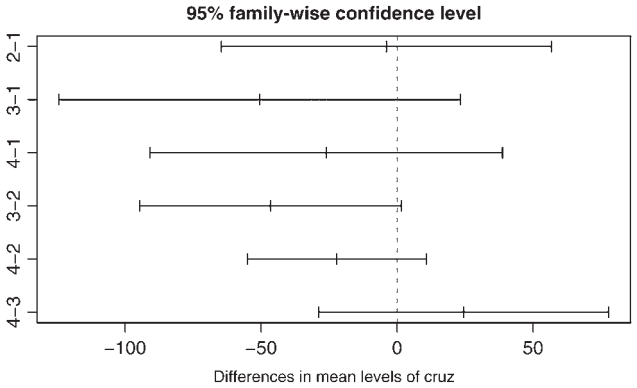

Figure 2. Differences between the mean batch fecundity estimates from different surveys at January 2002 (1), March 2004 (2), March 2005 (3), and February 2007 (4) (Tukey's HSD test). The vertical line at zero is the reference line used to determine no-significant differences between group means of the different surveys in the analysis of variance.

number of samples, to have at least one estimate of batch fecundity for each year of the study.

Average fecundity was lowest in March 2005 (124.4 ± 32.9) and highest in January 2002 (174.9 ± 122.9). Also, the highest variance in the estimates of F was obtained in January 2002, mainly because fewer fish in spawning condition were sampled during that survey than in any other (Table 1). The mean batch fecundity was around 170 in the 2002 and 2004 surveys, whereas in 2005 and 2007 the values decreased to 124 and 148, respectively. Despite these apparent differences, Tukey's HSD test revealed no significant differences at a 95% confidence level (Figure 2).

Spawning fraction (S) estimates, according to each criterion (MN, HO, POFs2, and POFs3), varied between 0.10 and 0.30, with the estimates obtained with the HO criterion being generally lower than the others and the average POFs2 estimates being the highest ones in most surveys (Figure 3; Table 3). The likelihood-ratio tests showed that all main effects were significant ($p < 0.01$), but the interaction between survey and method in the generalized linear mixed-effects model fitted to the spawning fraction data was not significant ($p = 0.58$; Table 4).

Discussion

The batch fecundity values obtained during this study, around 200 eggs released per gramme of female ovary-free weight, are close to the values previously estimated for horse mackerel from the southern stock (180–200 eggs g^{-1} gutted female weight; Abaunza *et al.*, 2008) as well as from the Gulf of Saronikos, Greece (205 oocytes g^{-1} of female ovary-free weight; Karlou-Riga and Economidis, 1997). These similarities in fecundity values together with the fact that no significant differences were found among surveys support the assumption that batch fecundity of horse mackerel may be relatively stable across spawning seasons (De Oliveira *et al.*, 2006). Our batch fecundity estimates are higher than the values estimated for the western and North Sea stocks of horse mackerel, owing to the decreasing trend in batch fecundity with increasing latitude in the Atlantic (Abaunza *et al.*, 2008), but lower than those estimated for the horse mackerel population off the coast of Mauritania (240 eggs g^{-1} gutted female weight; Abaunza *et al.*, 2008). Other species of the genus *Trachurus*, such as the jack mackerel (*Trachurus symmetricus*) from southern Californian waters (Macewicz and Hunter, 1993) and *Trachurus murphyi* off the coast of Chile (Canales, 2007), had lower batch fecundities, 112 and 115 oocytes g^{-1} of female ovary-free weight, respectively.

Table 3. Mean and variance, by survey, of batch fecundity per gramme of female less ovary weight (F), spawning fraction determined with the MN stage criterion (S_{MN}), HO criterion (S_{HO}), POFs criterion assuming a 2-d duration (S_{POFs2}), and POFs criterion assuming a 3-d duration (S_{POFs3}).

	F		S_{MN}		S_{HO}		S_{POFs2}		S_{POFs3}	
Survey	**Mean**	**Variance**	**Mean**	**Variance**	**Mean**	**Variance**	**Mean**	**Variance**	**Mean**	**Variance**
January 2002	174.9	122.9	0.091	0.021	0.108	0.023	0.203	0.006	0.136	0.003
January 2004	160.3	95.9	0.168	0.022	0.174	0.016	0.278	0.020	0.186	0.004
February 2004	175.9	50.0	0.339	0.041	0.076	0.012	0.227	0.009	0.152	0.004
March 2004	171.0	64.5	0.244	0.059	0.206	0.042	0.232	0.020	0.155	0.009
March 2005	124.4	32.9	0.276	0.030	0.333	0.019	0.309	0.025	0.206	0.003
February 2007	148.9	68.5	0.198	0.015	0.130	0.007	0.147	0.004	0.098	0.001

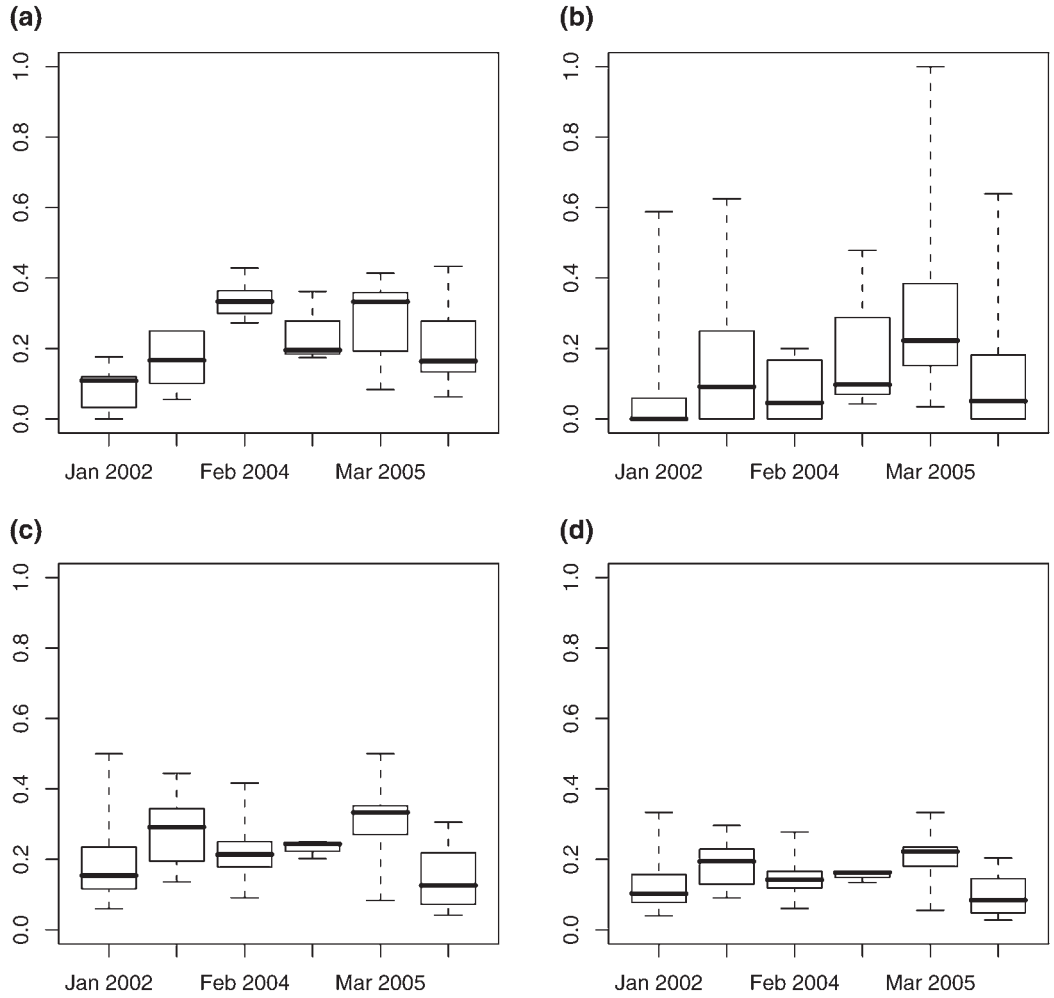

Figure 3. Spawning fraction estimated with the following methods: (a) MN; (b) HO; (c) POFs assuming degeneration time of 2 d (POFs2), and (d) POFs assuming a degeneration time of 3 d (POFs3) and their variation among surveys. The boxplots show the quartiles, with the bars extending to ± 1.5 the interquartile range.

Table 4. Results of the generalized linear mixed-effects model fitted using function `glmer`, the significance on the main effects and interaction term was tested with likelihood-ratio tests.

Models	d.f.	AIC	BIC	logLik	Chisq	Chi d.f.	Pr(>Chisq)
Main effects (survey, method)	10	3 761.5	3 824.0	−1 870.8			
Main effects + interaction	20	3 773.0	3 897.9	−1 866.5	8.518	10	0.58

The fraction of spawning females (S) is one of the most sensitive parameters in the calculation of SSB. Our estimates for S, applying the three methods to the same samples, varied between 0.10 and 0.30. The variation in S among surveys was expected, given that this parameter can change during the season and between years (Hunter, 1997b). However, the three methods should, in principle, be able to provide similar estimates of S as long as the duration of each oocyte development stage and POF deterioration stage can be taken into account. That information is still incomplete for this species. Previous studies have shown that the MN stage of *T. trachurus* (Eltink, 1991) and *T. symmetricus* (Macewicz and Hunter, 1993) lasts for ∼24 h. There are no data for horse mackerel on the duration of the HO, but for northern anchovy (*Engraulis mordax*), they last for 12 h (Hunter and Macewicz, 1985). For POFs, their duration depends on the fish species and also on the water temperature. The Atlantic menhaden (*Brevoortia tyrannus*), for example, spawns at 15–20°C, with the POFs duration ranging from 36 to >60 h (Fitzhugh and Hettler, 1995). At similar temperatures (13–19°C), northern anchovy (*E. mordax*), Pacific sardine (*Sardinops sagax*), and Atlantic sardine (*Sardina pilchardus*) have POFs that last ∼48 h (Hunter and Macewicz, 1985; Ganias *et al.*, 2003).

Given the lack of knowledge on the duration of the oocytes and POFs development and deterioration rates, we tested two null hypotheses: one that the differences between methods were similar across surveys, and the other that there were no differences among methods (all methods would provide similar estimates). The first hypothesis was not rejected, according to the results of

the likelihood-ratio test of the interaction between survey and method. However, the second hypothesis (that the estimates were similar across methods) was clearly rejected at a 95% significance level, as shown by the high significance of the method main effect in the generalized linear mixed-effects model. This indicates that, although the overall level of the estimates was different among methods, this difference remained relatively stable across surveys. Figure 3 shows that the medians of the estimates obtained with each method do not vary exactly the same way across surveys; however, the lack of a significant interaction between method and survey can be explained by taking into account the high variability in some of those estimates.

Our results indicate that there are consistent differences among methods to estimate *S* across surveys, so the next step is to determine which method would provide the most accurate and precise estimates. The formation of aggregations of females at spawning time (HO stage or late MN stage), as in clupeoids (Hunter and Goldberg, 1980; Alheit *et al.*, 1984; Zwolinski *et al.*, 2006), together with the relatively short duration of those stages, makes the fish in those stages difficult to sample. This may result in biased estimates of *S* when the sampling effort is insufficient, or in estimates with a large variance, as seems to be the case with our estimates based on HO (Figure 3). The POFs criterion overcomes the problem of female aggregation, because the structures last longer in the gonad and the females are likely to disperse and mingle just after spawning. However, in some DEPM applications, such as for Atlantic sardine, recently formed POFs are not considered for *S* estimation because of the potential aggregation effect and resultant biased estimation (Cunha *et al.*, 1992; Stratoudakis *et al.*, 2006).

In our case, the POFs3 criterion, assuming that a POF lasts on average 3 d, produced the estimates with the lowest variance (Table 3). Hence, the POFs3 one seems a good criterion to estimate *S*, just by taking into account the precision of the estimates. However, achieving minimal bias is as important, if not more, than achieving high precision. To estimate spawning fraction using the POFs criterion, one must be able to estimate with accuracy their rate of deterioration in relation to water temperature (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985), which is a task yet to be accomplished for horse mackerel (and most other species). Alheit *et al.* (1984) developed a method applied to *Engraulis ringens* that permits estimation of the age of POFs by sampling fish at sea through the day and night. However, this method requires a diel rhythm of spawning activity so that the time elapsed from spawning to capture can be used to assign ages to POFs, and such a diel rhythm could be difficult to determine in species without a well-defined daily spawning period, as may be the case of horse mackerel (Farris, 1961; Kaiser, 1973; Karlou-Riga and Economidis, 1997).

In this study, we estimated the batch fecundity of southern horse mackerel, which may be used for estimating SSB using the DEPM. We have also shown that these estimates are significantly different among the four surveys (2002, 2004, 2005, and 2007). However, in terms of the spawning fraction, a second key parameter used in the application of the DEPM, our findings demonstrate that the estimates are significantly different among the three methods available, but those differences are similar across surveys, indicating that some (or possibly all) methods provide consistently biased estimates. These potential biases would be reflected in estimates of SSB obtained with the DEPM and may have two different sources: (i) inaccurate estimates of the duration of the oocyte or POFs stages, and (ii) aggregation of fish in a given reproductive stage. On one hand, the MN criterion is currently the only one that has a known duration in horse mackerel (Eltink, 1991), but it is also one of the most prone to bias as a result of fish aggregation. On the other hand, the POFs criteria provided the most precise estimates of *S* and are the least prone to bias because of fish aggregation (especially the latest stages), but their duration in horse mackerel is currently unknown. Therefore, the degeneration rate of POFs in this species has to be estimated, e.g. by inducing the spawning of fish in the laboratory at different temperatures and measuring the POFs deterioration rate (e.g. Alday *et al.*, 2008). Until such a precise and accurate criterion is available to estimate *S*, and given that all methods provided similar trajectories of *S* across surveys, one has to admit that there will be uncertainty in the potential bias in the final SSB estimates obtained with the DEPM. The current abundance indices for the southern horse mackerel must therefore be treated as being proportional to the true abundance (i.e. a consistently biased measurement), but they should not be used as measures of absolute abundance.

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