

APPLYING A NON-PARAMETRIC BOOTSTRAP TECHNIQUE TO ASSESS THE ACCURACY OF BIOMASS ESTIMATES OF ARGENTINE ANCHOVY (*Engraulis anchoita*) NORTHERN STOCK (34° S-41° S) WITH THE DAILY EGG PRODUCTION METHOD. 1993-2008 PERIOD*

by

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RESUMEN

Aplicación de una técnica de remuestreo para evaluar la precisión de la estimación de biomasa del stock bonaerense (34° S-41° S) de anchoíta (*Engraulis anchoita*) con el Método de Producción Diaria de Huevos. Período 1993-2008. Se estimó la biomasa reproductiva (SSB) del efectivo bonaerense de *Engraulis anchoita* durante el período 1993-2008 aplicando el Método de Producción Diaria de Huevos (DEPM) y se evaluó la precisión de dichas estimaciones con una técnica de remuestreo. Los datos básicos se obtuvieron en distintas campañas de evaluación llevadas a cabo a fines de la primavera, en el pico de la temporada reproductiva. El objetivo del presente trabajo fue construir intervalos de confianza confiables alrededor de las estimaciones de la biomasa reproductiva media aplicando un método de remuestreo no paramétrico. Esos métodos se han convertido en una herramienta estadística muy utilizada por su robustez y por no requerir la presunción de una distribución particular de los datos básicos, características especialmente relevantes cuando se trata de estimar las variables reproductivas del DEPM cuyas distribuciones se desconocen *a priori*. En las estimaciones de SSB del período 1993-2008, de 0,67-3,67 millones de toneladas, se observaron coeficientes de variación de entre 22 y 73%. En cada vuelta del remuestreo se calculó un valor de la tasa de mortalidad embrionaria ajustando un modelo exponencial decreciente a los datos de huevos provenientes de estaciones de plancton aleatoriamente seleccionadas. Del mismo modo, los parámetros reproductivos se estimaron a partir de la extracción aleatoria de peces provenientes de los lances de pesca. Las estimaciones de SSB calculadas utilizando la técnica de remuestreo y el DEPM tradicional arrojaron resultados similares, con un rango de variación de 0,8-1,4.

SUMMARY

Engraulis anchoita Northern stock reproductive biomass (SSB) during the 1993-2008 period was estimated applying the Daily Egg Production Method (DEPM) and accuracy of estimates assessed using a bootstrap technique. The basic data were collected in different research surveys carried out in late Spring, at the peak of the spawning season. The aim of this work was to build reliable confidence intervals around the estimates of the mean reproductive biomass applying a non-parametric bootstrap method. Resampling methods are becoming increasingly popular as statistical tools as

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they are robust and do not require the assumption of any particular distribution of the basic data. This is especially relevant when assessing the DEPM reproductive variables that have an *a priori* unknown distribution. In the SSB estimates corresponding to the 1993-2008 period, of 0.67-3.67 million tons, coefficients of variation that ranged 22-73% were observed. At each loop of the bootstrap an embryonic mortality rate value was calculated fitting an exponential decay model to eggs data derived from randomly chosen plankton stations. Likewise, the reproductive parameters were estimated based on fish from fishing trawls randomly extracted. The SSB estimates calculated with the bootstrap technique and the traditional DEPM showed similar results, with a 0.8-1.4 ratio range.

Palabras clave: *Engraulis anchoita*, DEPM, biomasa desovante, remuestreo, fecundidad, frecuencia de puesta.

Key words: *Engraulis anchoita*, DEPM, spawning stock biomass, bootstrap, fecundity, spawning frequency.

INTRODUCTION

Argentine anchovy *Engraulis anchoita*, the most abundant fish resource in the Southwest Atlantic Ocean, has a vast latitudinal distribution (24° S-48° S) that extends from shallow coastal areas to the continental slope (Ciechomski and Sánchez, 1988; Sánchez and Ciechomski, 1995). The species plays a key role in the pelagic ecosystem of Argentine waters and constitutes the main component of the diet for different important commercial fish species, mammals and seabirds (Angelescu, 1982). South of 34° S there exist at least two populations of *E. anchoita*, the Northern and Southern stocks, separated at approximately 41° S (Hansen *et al.*, 1984). The species spawning pattern presents a large seasonal variability throughout the study area. During Spring, the Northern stock is found in coastal waters, mostly shallower than 50 m, off the Buenos Aires Province where massive spawning occurs (Sánchez and Ciechomski, 1995; Pájaro, 1998). On the contrary, Southern population spawn along the coast, coinciding with a series of frontal systems of different intensity (Sánchez *et al.*, 1996). Research surveys to assess abundance of adult fish, eggs and fecundity of *E. anchoita* were conducted between 1966-1990 (Sánchez, 1991). Since 1993, to estimate Argentine anchovy biomass the Daily Egg Production Method (DEPM) and the acoustic method were applied simultaneously (Sánchez *et al.*, 1996).

The DEPM, a direct method to assess small pelagic fish abundance, was developed at the Southeast Fisheries Center, California, for Northern anchovy *E. mordax*, a species that, as most clupeoids, has indeterminate fecundity (Lasker, 1985). The method has been successfully applied worldwide to a variety of anchovy, sardine and mackerel species such as *E. mordax* (Bindman, 1986; Hunter and Lo, 1997; Lo *et al.*, 2001), *E. ringens* (Santander *et al.*, 1984; Ayón and Buitrón, 1997), *E. capensis* (Armstrong *et al.*, 1988), *E. encrasicolus* (Motos *et al.*, 1991; Santiago and Sanz, 1992; Motos, 1994; Somarakis *et al.*, 2004), *Sardina pilchardus* (García *et al.*, 1992), *E. anchoita* (Sánchez *et al.*, 1996; Pájaro *et al.*, 2009) and *Scomber scombrus* (Priede and Watson, 1993).

The reason to use the DEPM is to assist in the management of stocks providing fisheries independent data on biomass to be incorporated or used to inform on the stock assessment process. According to Bernal *et al.* (2012) there are three main ways to incorporate the DEPM in the assessment process: 1) direct use as an index of biomass assessment; 2) monitor of trends or status of the target stocks with no direct incorporation of information in the assessment; 3) provider of information about spatial or temporal shifts in distribution.

The main concern about the DEPM is still the cost-effectiveness of the method that implies expensive survey and laboratory procedures (Bernal *et al.*, 2012). Nevertheless, egg production surveys provide a unique opportunity to evaluate reproductive expenses and potential as well

as distribution and production of eggs which, in turn, can be used as indicators and describers of the ecosystem properties (Dickey-Collas *et al.*, 2012).

The DEPM estimates spawning biomass as the ratio between daily egg production and daily specific fecundity. The second parameter includes information on batch fecundity, spawning fraction, average female weight and sex ratio. The method requires only one egg survey during the mid of the spawning season to determine the peak of egg production and the spawning area. In the last ten years new methodologies were developed to improve estimates and reduce the variance of relevant DEPM parameters such as daily egg production, total spawning area and daily specific fecundity (Lo *et al.*, 2001, 2005; Castro, *et al.*, 2005; Stratoudakis *et al.*, 2006).

The DEPM was modified by Stauffer and Picquelle (1981) who incorporated the Delta method (Seber, 1973) to calculate biomass estimates variance. In recent years, different alternatives were applied to reduce errors associated to the daily fraction of spawning females (S) and daily egg production (Po). Po and S are two key elements to assess spawning biomass (Hunter and Lo, 1997). Uncertainty about those parameters contributes mostly to that of spawning biomass estimates (Picquelle and Stauffer, 1985; Armstrong *et al.*, 1988; Hunter and Lo, 1997).

Lately, computer-intensive techniques that yield robust non linear model estimates and reliable confidence intervals were developed. The bootstrap (Efron, 1979) technique is a computer-based, widely used method to estimate any model bias and standard error associated to the estimates of a given statistic. Thus, Melià *et al.* (2002, 2012) applied non-parametric statistics based on the bootstrap to calibrate the egg mortality model required to estimate the daily egg production. The aim of this work was to build reliable confidence intervals around the mean biomass estimate applying a non-parametric bootstrap method.

MATERIALS AND METHODS

Survey description

Plankton and adult fish samples were collected during eleven research cruises carried out by the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) on board of the “Capitán Oca Balda” and “Dr. Eduardo L. Holmberg” RVs to obtain direct anchovy abundance estimates (acoustic and DEPM-based) in the continental shelf off Argentina between 1993-2008. The cruises were carried out between October and November, that is during the peak of the spawning aggregation period (Table 1). The study area included coastal and shelf sectors off the Buenos Aires Province between 34° S and 41° S at approximately 140 m depth (Figure 1).

Oceanographic (CTD) and ichthyoplankton stations were regularly distributed along the acoustic transects (Table 1; Figure 1). Transects were placed perpendicular to the shore according to a stratified randomized design (Hansen and Madirolas, 1996, 1999). The distance among stations and transect lines was about 10-20 and 15-25 nautical miles, respectively. Seawater temperature at five meter intervals was recorded at every station. The spawning area (where Po values were estimated) was considered to be the one bounded by positive stations (anchovy eggs recorded) and including the few embedded zero stations.

Anchovy eggs were collected in vertical trawls with a 0.225 m diameter and 220 μ m mesh size Pairovet net (Smith *et al.*, 1985). Trawls were set at a point from below the maximum depth of eggs (typically 70 m) to the surface. The filtered water volume was estimated with a mechanical flowmeter. Immediately after the end of each trawl plankton samples were preserved in seawater in 5% formaldehyde. Anchovy eggs from each plankton sample were identified and counted under a binocular-dissecting microscope at the laboratory.

Table 1. Number of oceanographic stations (Pairovet and CTD), trawl stations (TS) and number of fish (W , F , R , S) used to estimate Argentine anchovy spawning biomass. 1993-2008 period. W : mean total weight (g), F : batch fecundity, R : sex ratio, S : spawning frequency, A : spawning area (km²).

Tabla 1. Número de estaciones oceanográficas (Pairovet y CTD), lances de pesca (TS) y número de peces (W , F , R , S) utilizados para estimar la biomasa de anchoíta. Período 1993-2008. W : peso medio total (g), F : fecundidad parcial, R : proporción de sexos, S : frecuencia reproductiva, A : área de desove (km²).

Year	Pairovet	CTD	TS	W	F	R	S	A
1993	106	86	25	3,791	191	3,791	636	96,603
1994	101	90	19	2,457	60	2,457	497	109,640
1995	108	84	24	2,398	95	2,398	489	120,071
1996	101	95	24	2,329	46	2,329	592	125,696
1999	116	88	15	1,712	68	1,712	280	85,710
2001	63	63	16	1,887	63	1,887	389	68,694
2002	79	88	11	1,286	66	1,286	272	88,516
2003	92	85	26	2,565	76	2,565	627	96,603

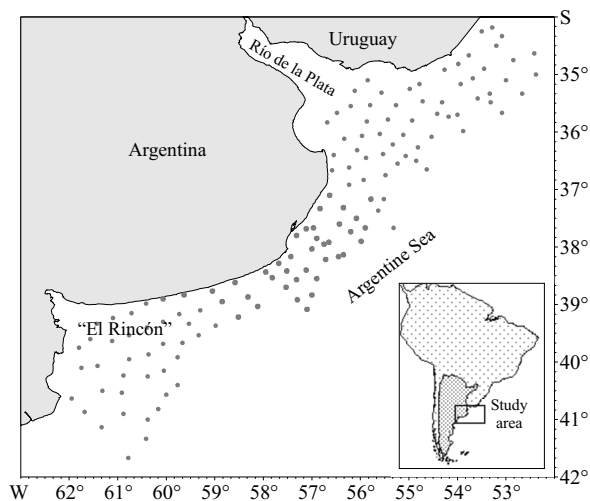


Figure 1. Geographic distribution of the oceanographic (Pairovet and CTD) stations carried out during the research cruises (1993-2008).

Figura 1. Distribución geográfica de las estaciones oceanográficas (Pairovet y CTD) llevadas a cabo durante los cruceros de investigación (1993-2008).

At any time of day or night, during the tracking, whenever the acoustic record showed great quantities of anchovy or mix of species in the echograms needed to be quantified, fishing trawls were performed. Adult anchovies were collected

with a 10 mm mesh size midwater net (Table 1). A raising factor fp_i (where the subindex i stands for the trawl number) was derived to weigh anchovy samples as a function of the acoustic abundance at the corresponding location:

$$fp_i = Ls_i \cdot Ds_i \quad (1)$$

where:

Ls_i : length (nm) of the acoustic track represented for the i -th trawl school of anchovies estimated with the echosound;

Ds_i : school mean anchovy density (tons/square nautical miles) over the segment of length Ls_i (tons/square nautical miles).

Fish samples were obtained through:

- A) Collection of up to 100 hydrated females from each catch, when possible. After a microscopic analysis, calculation of individual batch fecundity and total weight ovary free (W^*) from a set of hydrated but not yet ovulated females was performed at the laboratory (Table 1).
- B) Sampling of 120 randomly selected fish per

positive trawl station. Recording of sex and total weight of every adult fish from the sample (Table 1).

- C) Sampling of 25-30 adult females per positive trawl station. After a microscopic analysis, recording of the number of mature but not resting females was performed at the laboratory (Table 1). The maturity condition was defined by the presence or absence of postovulatory follicles. It should be noted that only day-0 and day-1 POF were considered.

B and C data sets were built from each trawl station. It was assumed that the probability of randomly taking a mature female showing 1-2 day-old postovulatory follicles from the "B sample" of a given trawl station would be equal to taking one from the "C sample" corresponding to the same trawl station. The fish collected on board for the three types of samples were fixed in seawater in 10% formaldehyde; a ventral cut was made in each fish to facilitate penetration of formaldehyde into the body cavity. Prior to gonad removal, each fish was individually weighed at the laboratory.

Individual anchovies batch fecundity (F) was assessed using the hydrated oocyte method (Hunter *et al.*, 1985). The small proportion of trawls including hydrated females did not allow to estimate mean batch fecundity for each trawl station. Even so, as batch fecundity was found to be a linear function of each year ovary-free female mass, a linear regression allowed to predict the population mean from the mean female mass. As the weight of hydrated females increased temporarily due to oocytes hydration a few hours prior to spawning, F is more properly related to the ovary-free weight of females (W^*) by the linear function:

$$F = a + b W^* \quad (2)$$

The sex ratio (R) is the fraction in weight of mature females in the total adult population (Picquelle and Stauffer, 1985) with the value corre-

sponding to each trawl station (R_i) estimated as:

$$R_i = Wf_i / (Wf_i + Wm_i) \quad (3)$$

where Wf_i and Wm_i are the sum of the estimated weights of mature males and females at the i -th fishing station.

The spawning fraction (S) represents the proportion of mature females spawning each day. Since no validated system existed to classify Argentine anchovy postovulatory follicles (POF) per age, they were assigned according to the description made for *E. mordax* by Hunter and Goldberg (1980). The S proportion was estimated from the incidence in the randomized samples of 25-30 females ("C sample") with day-0 (spawning < 24 h) and day-1 (24 h \geq spawning < 48 h) POF separately. The spawning fraction was determined based on the average of percentage of day-0 and day-1 spawning females (Fitzhugh *et al.*, 1993; Pájaro *et al.*, 2011).

DEPM estimate, distribution and confidence intervals

Mortality rate and daily egg production

In every year studied (y), anchovy eggs density was estimated from plankton samples ($j_y = 1, 2, \dots, J_y$). According to the egg developmental stage and seawater temperature, eggs were aged in hours (t) and arranged into four daily groups ($d = 1, 2, 3, D = 4$; where day-1 pooled younger-than 24 h eggs, day-2 between 24 and 48 h, day-3 between 48 and 72 h, and day-4 more than 72 h). Considering sea temperature at 5-10 m depth, for every plankton station eggs were aged according to a 9-stage and 22-substage scale proposed for *E. anchoita* (Ciechomski and Sánchez, 1984). To avoid bias caused by incomplete recruitment of eggs to the plankton, or by hatching, stages 1 and the last substage were discarded. Abundance of each daily cohort in the samples was standardized and egg density expressed as the number of individuals/square meters of sea surface. Zero egg

abundance was recorded anytime egg stages were expected but not observed (zeros were included in the estimates). As spawning was assumed to occur synchronously at 10 p.m. (Pájaro, unpublished data), the eggs ages in hours were recalculated as the time elapsed between 10 p.m. of the daily group assigned and the sampling time. Mean standardized density (H) and mean age in hours (t) were calculated for each of the four daily groups for every plankton station.

Data on total eggs collected were used to assess hourly mortality rate (Z) and Po . A weigh factor (r_j) was calculated for every plankton station giving the data from 20-mile spaced stations twice the weighting of the remaining 10-mile spaced stations.

During the bootstrap the estimate was performed in each of the 3,000 replicates ($q = 1, 2, \dots, Q = 3,000$). A number of J_y plankton stations were randomly chosen, with replacement, and the corresponding egg abundance data (weighed by the station raising factor, r_j) regressed on age by a non-linear least-squares fitting. It was assumed that spawning occurs at a fixed time and that the number of eggs decreases at a constant exponential rate (Alheit, 1985):

$$H_q = \hat{P}o_q \exp(-\hat{Z}_q t_q) \quad (4)$$

where $\hat{P}o_q$ is the estimate of the initial egg production at $t = 0$; H_q is the mean egg density (and t_q is the mean age) corresponding to the four daily groups represented in the randomly chosen stations of the q -th replicate.

Considering that a higher mortality rate would practically result in no survival 36 h after hatching, an hourly $\hat{Z}_q < 0.14$ restriction to fitting was imposed.

Adult parameters and the DEPM spawning biomass equations

A linear function of ovary-free (W^*) on total weight (W) was fitted pooling data from all adult females ($N = 4,494$) examined during the 1993-

2008 period and the estimate of c and d parameters of the following equation performed:

$$W^* = c + d W \quad (5)$$

In the 3,000 bootstrap replicates for each survey, mean values of adult parameters were estimated from samples of adult anchovies collected in the mid-water trawls. The steps to obtain the means corresponding to each q -th replicate were as follows:

- Estimate of the pair of constants $(a, b)_q$ for the batch fecundity-ovary-free weight relation (equation 2) obtained by the least square fitting of the relation to as many randomly chosen pairs $(F, W^*)_q$ as included in the whole "A sample".
- At random choice of as many trawls as originally performed in the survey ($I_q = I_y$), with replacement; for every trawl chosen, as many anchovies as originally present in "B sample" were randomly taken, with replacement; weight means of sex ratio (Rq) and total body weight of females (Wq) corresponding to the current replicate were computed:

$$\bar{R}q = \frac{\sum_{i^*=1}^I fp_{i^*} \cdot \frac{\sum_{b^*=1}^B (w_{fb^*})_{i^*}}{\sum_{b^*=1}^B (w_{mb^*} + w_{fb^*})_{i^*}}}{\sum_{i^*=1}^I fp_{i^*}} \quad (6)$$

where i^* is the randomly chosen trawl, fp_{i^*} the respective raising factor, b^* each of the randomly chosen anchovies in "B sample", and w_{fb^*} and w_{mb^*} the total weight of females and males, respectively.

$$\bar{W}_q = \frac{\sum_{i^*=1}^I f_{i^*} \cdot \frac{\sum_{b^*=1}^B (W_{fb^*})_{i^*}}{f_{i^*}}}{\sum_{i^*=1}^I f_{i^*}} \quad (7)$$

where f_{i^*} is the number of females in the pseudosample corresponding to the trawl randomly taken.

- c) At random choice of as many female anchovies as originally present in “C samples” for every trawl chosen in step (b), with replacement, and computing of the weighted mean of spawning fraction (Sq):

$$\bar{S}q = \frac{\sum_{i^*=1}^I f_{i^*} \cdot \sum_{b^*=1}^B [(POF_{0b^*} + POF_{1b^*}) / (2 \cdot \sum_{b^*=1}^B n_{b^*})]}{\sum_{i^*=1}^I f_{i^*}} \quad (8)$$

where n_{b^*} is the number of active females randomly taken in the pseudosample corresponding to the i^* -th trawl, POF_{0b^*} the number of individuals with age-0 postovulatory follicles, and POF_{1b^*} the number of individuals with age-1 postovulatory follicles.

Spawning biomass bootstrap estimates

The estimates resulting from each bootstrap replicate were included in the main DEPM equation (Parker, 1980) to obtain the q -th spawning stock biomass estimate:

$$SSB_q = \frac{A_y \cdot \hat{P}o_q \cdot \hat{W}_q}{\hat{F}_q \cdot \hat{R}_q \cdot \hat{S}_q} \quad (9)$$

where A_y is the spawning area (km^2) in year y .

To quantify variation of adult data among and within samples a two-step procedure bootstrap was performed (Melià *et al.*, 2012). First, trawl stations were randomly extracted (with replacement) from the overall sample; then, individuals

within each trawl resampled and the resulting pseudo-replicate was used to estimate F , S , R and W adult parameters. The mean and variance of each parameter relating to adult fish were estimated and confidence intervals calculated with the percentile method. Finally, the means and standard deviations of the 3,000 SSB_q estimates were obtained and 95% confidence intervals (0.025 and 0.975 percentiles) around the mean of the spawning biomass estimates built.

RESULTS

The c and d parameters of the linear relation between ovary-free (W^*) and total weight (W) resulted in 0.568 and 0.915, respectively, and the function correlation coefficient in 0.997.

The basic data used to estimate anchovy spawning biomass are shown in Table 1. In the 1993-2008 period total spawning areas ranged 69,000-126,000 km^2 . The estimates of mean sex ratio (R), batch fecundity (F), spawning fecundity (S), total mean weight (W) and the corresponding coefficients of variation (CV) are included in Table 2. The up to 50% S values were the highest mean CV for the period analyzed. Likewise, the rest of adult parameters showed a remarkable interannual variability in sex ratio and average body mass and, consequently, in batch fecundity (Table 2), another parameter with a high CV that averaged 15%. The mean CV of the remaining variables was below 12%. Sex ratio and spawning frequency showed normal frequency distributions (Shapiro-Wilk’s W test; $p > 0.08$ in five and three of the eleven years). Batch fecundity and total weight did not show a normal distribution (Shapiro-Wilk’s W test; $p < 0.026$).

High variations of anchovy eggs density (0-23,500 eggs m^{-2}) were observed in the Pairovet stations (Figure 2). The highest abundance concentrated around the “El Rincón” area, close to the 50 m isobath, in front of the Río de la Plata

Table 2. Reproductive parameters mean values and coefficients of variation (between parenthesis). *F*: batch fecundity, *R*: sex ratio, *S*: spawning frequency, *W*: mean total weight.

Tabla 2. Valores medios y coeficientes de variación (entre paréntesis) de los parámetros reproductivos. *F*: fecundidad parcial, *R*: proporción de sexos, *S*: frecuencia reproductiva, *W*: peso medio total.

Year	<i>F</i>	<i>R</i>	<i>S</i>	<i>W</i>
1993	9,657.4 (0.129)	0.579 (0.056)	0.128 (0.153)	18.4 (0.111)
1994	12,424.8 (0.204)	0.510 (0.117)	0.129 (0.510)	19.7 (0.214)
1995	8,493.3 (0.072)	0.514 (0.071)	0.128 (0.255)	18.4 (0.046)
1996	7,732.2 (0.203)	0.537 (0.076)	0.142 (0.356)	15.0 (0.106)
1999	9,041.6 (0.187)	0.614 (0.093)	0.122 (0.218)	20.5 (0.111)
2001	8,866.0 (0.078)	0.520 (0.068)	0.107 (0.223)	18.8 (0.071)
2002	7,788.0 (0.180)	0.533 (0.053)	0.106 (0.400)	19.8 (0.097)
2003	7,579.3 (0.172)	0.527 (0.083)	0.180 (0.210)	16.7 (0.130)
2004	10,682.9 (0.117)	0.536 (0.078)	0.139 (0.134)	22.6 (0.095)
2006	7,539.5 (0.141)	0.525 (0.046)	0.116 (0.232)	15.8 (0.124)
2008	4,502.7 (0.178)	0.434 (0.104)	0.120 (0.137)	12.0 (0.127)

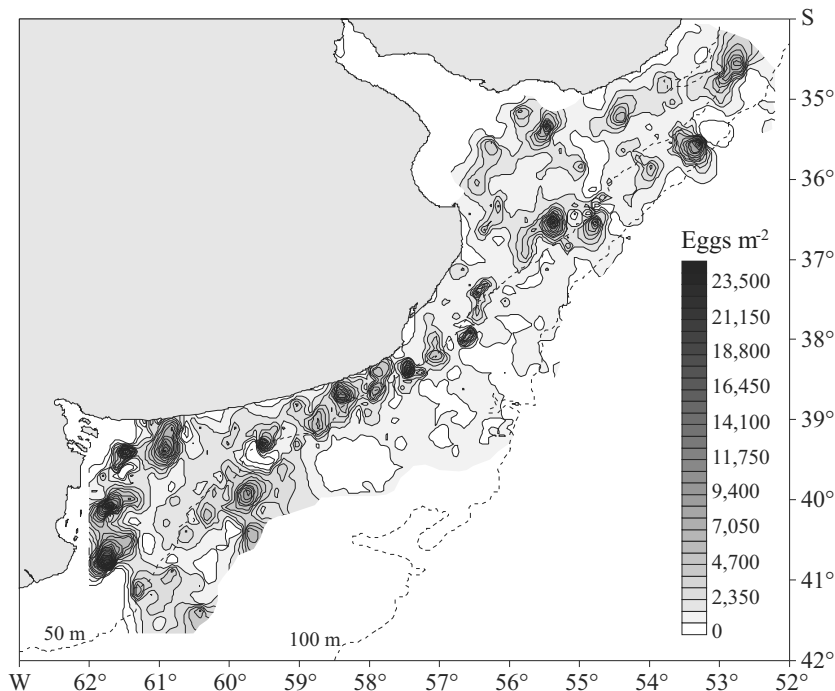


Figure 2. Distribution and abundance of *Engraulis anchoita* Northern stock eggs in Spring, 1993-2008 period. Data were considered as a whole.

Figura 2. Distribución y abundancia de huevos del stock bonaerense de *Engraulis anchoita* en primavera, período 1993-2008, considerando todos los datos en conjunto.

Table 3. Hourly mortality rate (Z), daily egg production (Po) and weighted sea surface temperature (SST) as a function of *Engraulis anchoita* Northern stock egg abundance per Pairovet station. Between parenthesis: coefficient of variation.
 Tabla 3. Tasa de mortalidad horaria (Z), producción diaria de huevos (Po) y temperatura de superficie ponderada (SST) en función de la abundancia de huevos del stock bonaerense de *Engraulis anchoita* por estación Pairovet. Entre paréntesis: coeficiente de variación.

Year	Po (eggs m ⁻²)	Z	SST (°C)
1993	329.0 (0.229)	0.0214 (0.211)	12.62
1994	194.4 (0.442)	0.0057 (0.427)	12.19
1995	306.1 (0.198)	0.0115 (0.378)	12.31
1996	474.3 (0.254)	0.0229 (0.305)	13.74
1999	724.9 (0.374)	0.0176 (0.387)	13.48
2001	552.2 (0.248)	0.0179 (0.156)	14.09
2002	752.5 (0.279)	0.0144 (0.336)	13.57
2003	855.7 (0.331)	0.0271 (0.196)	14.81
2004	772.4 (0.345)	0.0195 (0.275)	13.18
2006	463.3 (0.266)	0.0230 (0.180)	15.09
2008	351.7 (0.145)	0.0203 (0.115)	14.57

River and northeastwards. Although a high egg density was usually found over the 50 m isobath, the highest value was recorded at 20 m depth.

Mean Z and Po estimates with the respective CV and weighted sea surface temperature as a function of eggs density are shown in Table 3. Mean daily egg production ranged 194-856 eggs m⁻² and the CV 15-44%. High daily egg production values were observed in 1999 and during the 2002-2004 period; the lowest Po corresponded to 1993-1995 and 2008 (Figure 3). Expansion of the Po survey means to the corresponding spawning areas allowed to record the highest daily production, of 97 million eggs, in 2004 (Figure 3).

The Argentine anchovy Northern stock spawning biomass (SSB) was estimated at 0.67-3.67 million tons, with confidence intervals (95%) ranging 0.17-1.35 (lower confidence interval) and 1.43-9.42 (upper confidence interval) million tons (Table 4). The mean SSB bootstrap estimates and those calculated using Parker (1980) and Stauffer and Picquelle (1981) traditional methodology had a similar 0.8-1.4:1 ratio range (Figure

4). 1994, 1996 and 2002 showed the largest differences between both estimates (> 11%), the highest CV of the bootstrap mean and the largest spawning frequency dispersion.

Most years, the bootstrap estimates means were higher than the non-bootstrap traditional DEPM estimates; nevertheless, the medians calculated with both methods were similar (Table 4).

DISCUSSION

The traditional method, as described by Parker (1980) and modified by Stauffer and Piquelle (1981) and Armstrong *et al.* (1988) has been used to date. Since the 90's, Argentine anchovy abundance direct estimates are obtained with acoustic methods and stocks spawning fraction assessed with the DEPM (Sánchez *et al.*, 1996; Pájaro *et al.*, 2006).

A bootstrap technique to obtain confidence intervals around the DEPM spawning biomass

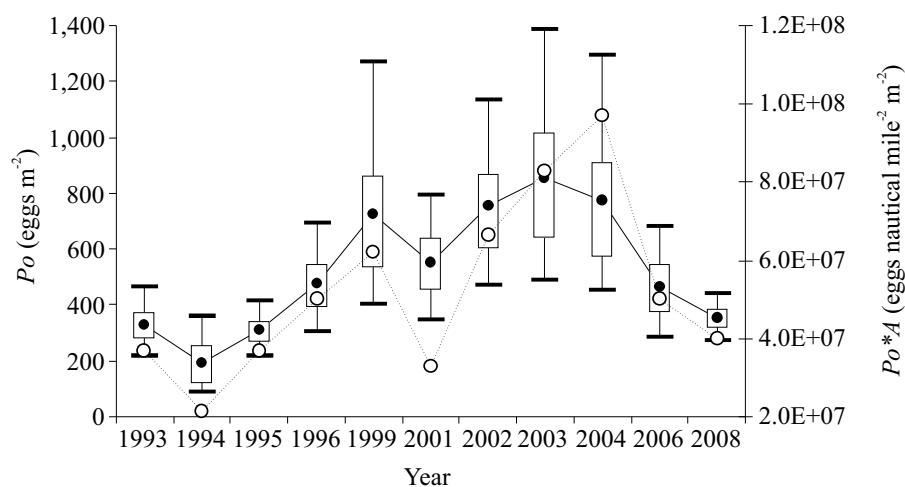


Figure 3. Box-and-whisker plots of *Engraulis anchoita* Northern stock daily egg production (P_o) showing interquartile ranges (boxes), mean values (black circles) and 90% confidence intervals (whiskers) and daily egg production estimated for the total spawning area (P_o*A) in the 1993-2008 period (full circles and dotted line).

Figura 3. Gráficos de caja y bigote de la producción diaria de huevos (P_o) del stock bonaerense de *Engraulis anchoita* indicando rangos intercuartiles (cajas), valores medios (círculos negros) e intervalo de confianza del 90% (bigotes) y producción diaria de huevos estimada para la totalidad del área de desove (P_o*A) en el periodo 1993-2008 (círculos y línea punteada).

Table 4. Bootstrap estimates of means and medians of the spawning stock biomass (t), confidence intervals and comparison with the estimates performed with the traditional method described by Parker (1980). CV: coefficient of variation, iqr: interquartile range.

Tabla 4. Estimación con remuestreo de las medias y medianas de la biomasa del stock desovante (t), intervalos de confianza y comparación con las estimaciones realizadas con el método tradicional descrito por Parker (1980). CV: coeficiente de variación, iqr: rango intercuartiles.

Year	Bootstrap mean (CV)	Lower confidence interval 95%	Upper confidence interval 95%	Bootstrap median (iqr)	Traditional mean (CV)	Ratio between both methods (means)
1993	842,302 (0.30)	462,863	1,428,606	808,84 (325,944)	804,864 (0.51)	1.0
1994	672,121 (0.73)	172,015	1,997,104	525,243 (498,470)	494,474 (1.27)	1.4
1995	1,303,547 (0.35)	678,141	2,427,400	1,214,532 (523,679)	1,175,870 (0.68)	1.1
1996	1,831,895 (0.57)	688,551	4,478,233	1,525,737 (1,135,832)	2,342,715 (0.45)	0.8
1999	2,006,287 (0.46)	849,872	4,429,041	1,811,328 (1,060,618)	1,818,663 (0.76)	1.1
2001	1,538,710 (0.38)	728,414	2,931,025	1,439,581 (688,713)	1,414,862 (0.99)	1.1
2002	3,666,293 (0.59)	1,327,080	9,422,628	3,057,207 (2,344,016)	2,973,851 (0.96)	1.2
2003	2,047,005 (0.43)	891,001	4,161,210	1,871,732 (1,040,300)	1,866,802 (0.47)	1.1
2004	2,840,202 (0.40)	1,354,160	5,745,938	2,578,918 (1,359,062)	2,744,583 (0.76)	1.0
2006	1,847,413 (0.38)	827,566	3,569,194	1,731,805 (878,987)	1,706,390 (0.62)	1.1
2008	2,103,410 (0.22)	1,351,299	3,122,226	2,053,191 (585,713)	2,281,831 (0.51)	0.9

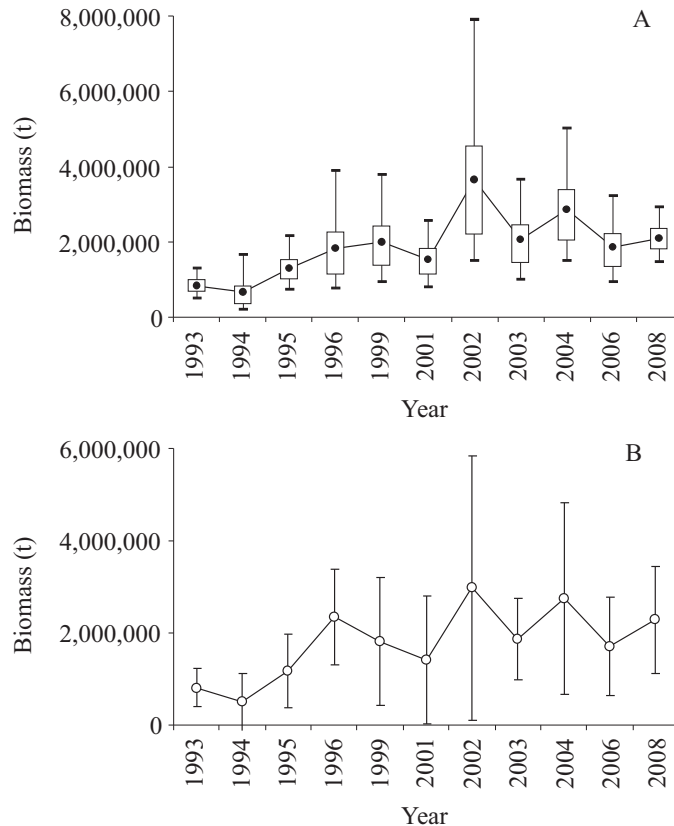


Figure 4. A) Box-and-whisker plots of daily *Engraulis anchoita* Northern stock spawning biomass estimated with the Daily Egg Production Method calculated with non-parametric bootstrap showing interquartile ranges (boxes), mean values (black circles) and 90% confidence intervals (whiskers). B) Mean (open circles) and standard deviation (bars) of spawning biomass estimated with the traditional method (Parker, 1980).

Figura 4. A) Gráficos de caja y bigote de la biomasa desovante diaria del stock bonaerense de *Engraulis anchoita* estimada con el Método de Producción Diaria de Huevos calculada con remuestreo no paramétrico mostrando rangos intercuartiles (cajas), valores medios (círculos negros) e intervalo de confianza del 90% (bigotes). B) Media (círculos vacíos) y desviación estándar (barras) de biomasa desovante estimada con el método tradicional (Parker, 1980).

estimates for Argentine anchovy Northern stock was employed for the first time in this work. The technique allows, mainly, to measure the uncertainty associated to the estimate of the parameters involved (Efron, 1979). One of the advantages of the non-parametric bootstrap is that it does not require any *a priori* assumption regarding the statistical properties of the model (Melià *et al.*, 2012). Bootstrap procedures were used to deal with the DEPM biomass estimates of snapper *Pagrus auratus* (Zeldis and Francis, 1998; Jackson and Cheng, 2001), sardine *Sardinops sagax* (Ward *et al.*, 2011)

and european anchovy *E. encrasicolus* (Melià *et al.*, 2012). Bullman *et al.* (1999) built bootstrap-based confidence intervals around blue grenadier (*Macruronus novaezelandiae*) spawning stock biomass estimate off western Tasmania. Lo *et al.* (2001) conducted bootstrap simulations to indicate the possible bias and estimate the standard error of P_0 and Z under an adaptive allocation sampling scheme of surveys that had Pacific sardine (*Sardinops sagax*) as a target species.

The statistical properties of quantities estimated from stratified randomized surveys are diffi-

cult to predict. This is true even when relatively simple parameters such as mean fish density from trawl stations is estimated. Confidence intervals around the means are often built on the basis of normal distribution; nevertheless, in many actual surveys few yearly observations can be made. Fish distributions are usually contagious and skewed. As a result, very wide and frequently nonsensical intervals such as negative lower limits are constructed. To face the problem and deal with this kind of data another type of distribution (Poisson, Delta-lognormal) was proposed, but the approaches suggested may result in biased estimates of the population means and variance (Smith, 1990). Considering that building bootstrap confidence intervals does not require a distributional assumption, bootstrap methods are used in a number of fisheries survey applications to model estimates distribution.

Smith (1997) compared different methods to bootstrap fish density data from complex survey designs. Given the fact that a mean bootstrap estimate is an unbiased estimate of the stratified mean and that variance derived from a simple (“naïve”) bootstrap procedure is an inconsistent estimate of the true variance, those methods were suggested to find the most consistent estimate of the variance (Rescaled bootstrap, Rao and Wu, 1988; Mirror-match bootstrap, Sitter, 1992). Smith (1997) also compared three ways to compute percentiles for confidence intervals from bootstrap estimates called percentile, bias-corrected, and bias-corrected and accelerated methods. Results confirmed that the simplest bootstrap procedure actually underestimated the stratified variance and, hence, slightly narrower than expected confidence intervals around the means were built. Nevertheless, the differences did not seem to be large enough so as to recommend the adoption of a more sophisticated, computer-intensive bootstrap method.

When applying the DEPM to assess Argentine anchovy biomass, the estimate is obtained combining two sources of information. The first is *Po*

derived from the number of eggs at different sea locations (Pairovet samples) that has an often contagious distribution. The second consists of fish reproductive parameters estimated analyzing samples from the scarce number of trawls usually performed. Said parameters often show rather complicated distributions; thus, derivation of a consistent estimate of the variance is expected to become a cumbersome matter that justifies the use of resampling techniques. As it is not clear how other resampling procedures recommended by Smith (1997) would perform when estimating biomass ratios such as the reproductive parameters *R* and *S* (Zeldis and Francis, 1998), a naïve bootstrap may be an appropriate way to evaluate the DEPM as an indicator of fish biomass.

Inaccuracy of *E. anchoita* spawning biomass estimates is the result of the large variance usually associated to the daily spawning females fraction (*S*). The high costs associated to labour and shortage of financial resources are the main problems encountered worldwide when trying to use the DEPM to estimate adult parameters and, particularly, *S* (Hunter and Lo, 1997). The authors concluded that about 50 randomized samples containing 20-25 active females each and a histologic analysis of over 1,200 ovaries per survey are required to produce accurate spawning rates estimates. As an example it should be mentioned that Pacific sardine spawning fraction represents less than 20% of mature females (Lo *et al.*, 2010) which implies a large number of samples (> 60) to obtain precise data (Bernal *et al.*, 2012).

Northern Argentine anchovy eggs spatial distribution pattern was quite similar throughout the years analyzed. However, differences in the location of the highest egg density were observed. The four main spawning areas identified were near the uruguayan coast; in front of the Río de la Plata River; south of 37° S, along the 50 m isobath; and in the “El Rincón” area, at 39° S-41° S, to the west of 59° W (Pájaro *et al.*, 2008). The Río de la Plata River and “El Rincón” are environments with frontal regions limited by salinity

(Acha *et al.*, 2004; Martos *et al.*, 2005). The area south of 37° S is characterized by the presence of a thermal front that extends along the 50 m isobath (Lucas *et al.*, 2005).

Following spawning frequency, P_o is the DEPM parameter that causes most of the variability around the estimated biomass. The high P_o variance observed in the 1993-2008 period was the result of the scattered data pattern used to fit the egg mortality relation and of the large variability in egg abundance recorded in the samples. Uncertainty about P_o and Z is the major factor of incertitude as regards spawning biomass estimates (Picquelle and Stauffer, 1985). If the areas where spawning and egg aggregations take place may be anticipated, a stratified sampling scheme will help to improve estimates accuracy (Lo, 1997). Adaptable sampling, addition of larval stages to improve Z precision and continuous underway egg sampling are the ways suggested by Hunter and Lo (1997) to reduce the bias associated to P_o .

The highest mean daily egg production was recorded in 2003. However, when total egg production was considered, the highest value corresponded to 2004, when the high P_o mean coincided with a large spawning area. Pájaro *et al.* (2008) analyzed anchovy eggs density for the

1993-2006 period and concluded that the “El Rincón” region represented the main Argentine anchovy Northern stock spawning area. In this work, the lowest P_o CV was not recorded the year when more Pairovet stations were performed. It is possible that the lowest P_o CV related to a similar abundance of eggs over the total spawning area.

As the mean batch fecundity estimated in each survey depended on the corresponding total weight mean, batch fecundity (F) was another adult parameter that showed high CV values, mostly due to inter-annual differences in total weight (Figure 5). The bootstrap performed in this work tried to resemble the fecundity-mass relation variability caused by sampling (Picquelle and Stauffer, 1985). It is worth noting that the highest F CV estimate was observed in 1994 when the highest total weight annual mean CV was also calculated.

Melià *et al.* (2012) observed that european anchovy spawning biomass derived from the tradicional DEPM procedure was markedly lower than the bootstrap means and closer to the bootstrap medians. Instead, in this work it was observed that the SSB bootstrap estimate of the different surveys was similar to that obtained

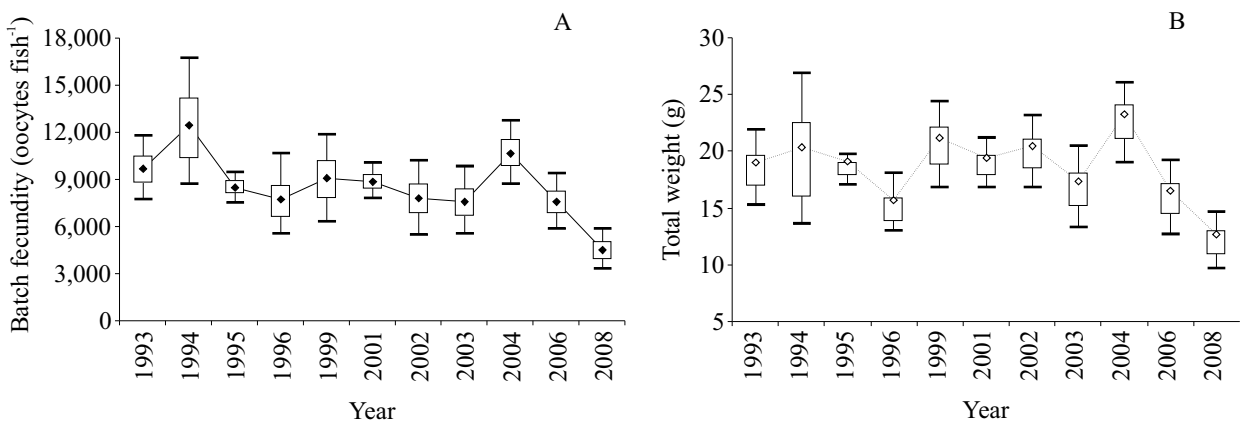


Figure 5. Box-and-whisker plots of *Engraulis anchoita* Northern stock batch fecundity (A) and total weight (B) showing interquartile ranges (boxes), mean values (diamonds) and 90% confidence intervals (whiskers).

Figura 5. Gráficos de caja y bigote de fecundidad parcial (A) y peso total (B) del stock bonaerense de *Engraulis anchoita* mostrando rangos intercuartiles (cajas), valores medios (diamantes) e intervalo de confianza del 90% (bigotes).

with the traditional DEPM (Parker, 1980). The largest difference was observed in 2002 when the mean SSB recorded using the bootstrap technique reached 700 thousand tons, 19% above the results obtained with the traditional method. The largest SSB corresponded to the same year that showed

the most important egg production means and a lower daily specific fecundity (calculated as the ratio between the S , R , F product and total weight) (Figure 6). The CV values of spawning stock abundance bootstrap estimates ranged 22-73% and those of the DEPM based on Parker

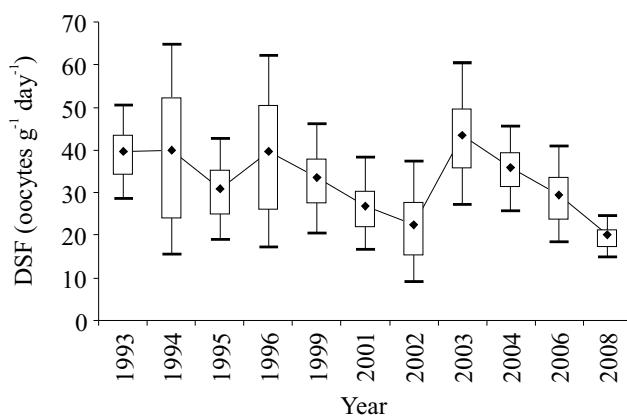


Figure 6. Box-and-whisker plots of daily specific fecundity (DSF) of *Engraulis anchoita* Northern stock showing interquartile ranges (boxes), mean values (diamonds) and 90% confidence intervals (whiskers). The DSF was estimated as the ratio between the reproductive parameters product (batch fecundity, proportion of females and spawning frequency) and total weight.

Figura 6. Gráficos de caja y bigote de la fecundidad diaria específica (DSF) del stock bonaerense de *Engraulis anchoita* mostrando rangos intercuartiles (cajas), valores medios (diamantes) e intervalos de confianza del 90% (bigotes). La DSF se estimó como la relación entre el producto de los parámetros reproductivos (fecundidad parcial, proporción de hembras y frecuencia reproductiva) y el peso total.

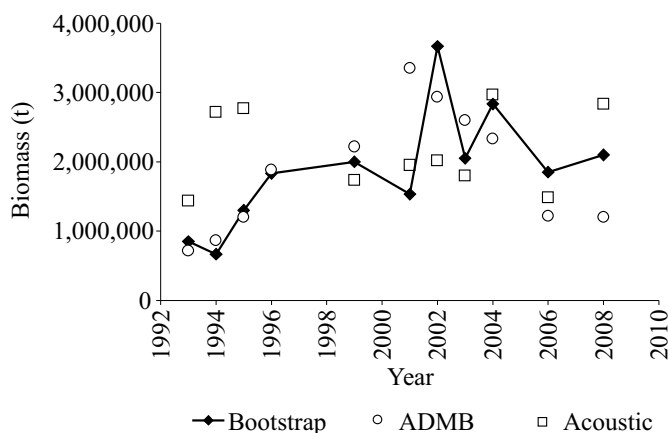


Figure 7. *Engraulis anchoita* Northern stock biomass estimate: spawning biomass estimated with non-parametric bootstrap and the catch-at-age statistical model (ADMB; Hansen *et al.*, 2012) and total biomass estimated with the acoustic method.

Figura 7. Estimación de la biomasa del stock bonaerense de *Engraulis anchoita*: biomasa desovante estimada con remuestreo no paramétrico y el modelo estadístico de captura por edad (ADMB; Hansen *et al.*, 2012) y biomasa total estimada con el método acústico.

model (1980) 45-127%. It is possible that a larger number of trawls are required for the Delta Method (Stauffer and Picquelle, 1981) to be statistically reliable. So, most of times CV of the DEPM parameters calculated with the Parker method were higher than the bootstrap.

Northern Argentine anchovy total biomass acoustically estimated (1993-2008 period) and SSB indirect estimates derived from a catch-at-age statistical model (Hansen *et al.*, 2012) are shown in Figure 7. Both series values, very close to the bootstrap DEPM estimates, were also included within the range of historic assessments on stock abundance (Pájaro *et al.*, 2006; Hansen *et al.*, 2010). However, it should be considered that the DEPM estimates do not include juvenile abundance (young-of-the-year and part of 1 year old fish) that could account for a significant proportion of total anchovy biomass.

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