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Batch fecundity of *Sardina pilchardus* (Walb.) off the Atlantic Iberian coast

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ABSTRACT

Sardina pilchardus *is a serial spawner releasing several batches of eggs per spawning season. The Hydrated Oocyte Method was used for the first time to estimate sardine fecundity on the Atlantic Iberian coast in 1988. The mean batch fecundity of the Atlantic Iberian sardine in March/April 1988 was 30 227.3 (standard error = 1 342.6) and the mean relative fecundity (number of hydrated oocytes per gram of female weight) was 426.54 (standard error = 18.95). The sardines' relative fecundity was higher than that of other clupeoid species. Sardine batch fecundity (F) was expressed as a linear function of ovary-free weight (W) based on both Portuguese and Spanish fecundity data:* $F = -1\,184.7 + 443.26\ W$.

Key words: Batch fecundity, sardine, Iberian Peninsula.

RESUMEN

Fecundidad parcial de *Sardina pilchardus* (Walb.) de las costas atlánticas de la península Ibérica.

La especie Sardina pilchardus *es un reproductor del tipo múltiple, por tanto, realiza varias puestas parciales en cada período reproductivo. En el año 1989 se utilizó por primera vez en las costas atlánticas de la península Ibérica el Método de Ovocitos Hidratados para el cálculo de la fecundidad de esta especie. El valor medio de la fecundidad parcial de la sardina atlántica de la península Ibérica calculado en marzo y abril de 1988 fue 30 227.3 (error = 1 342.6) y el valor medio de la fecundidad relativa (número de ovocitos hidratados por gramo de peso de hembra) fue 426.54 (error = = 18.95). Este valor es más alto que el determinado para otras especies de clupeidos. La fecundidad parcial de la sardina (F) se expresa como una función lineal del peso vivo de las hembras sin ovario (W) basada en los datos obtenidos por Portugal y España:* $F = -1\,184.7 + 443.26\ W$.

Palabras clave: Fecundidad parcial, sardina, península Ibérica.

INTRODUCTION

Fulton (1898) reported that before spawning, ovaries take up a fluid of low specific gravity and considerably increase in size (hydration process). Hydrated oocytes are microscopically distinguishable from the rest of oocytes in the ovary, and can be easily counted. Hunter and Goldberg (1980) developed the Hydrated Oocyte Method to determine batch fecundity of the northern anchovy, *Engraulix mordax*. Fecundity estimates were only based on hydrated females caught a few hours before spawning, because hydrated oocytes can be easily separated from all other oocytes in the ovary for oncoming spawning. The total number of hydrated oocytes in the ovary corresponds to female batch fecundity. The Hydrated Oocyte Method is advantageous because it is accurate, and considerably reduces the time required to obtain fecundity estimates

(Hunter, Lo and Leong, 1985). In sardine, the increase of ovary volume after hydration may attain 264 % (Andreu, 1955), and hydrated eggs are easy to identify; thus, we chose the Hydrated Oocyte Method to determine the batch fecundity of sardine. This paper presents our experience of using this method to estimate the fecundity for *Sardina pilchardus*.

MATERIAL AND METHODS

Sardine were collected off the Atlantic Iberian coast by Portuguese and Spanish research vessels during a joint cruise undertaken in March/April 1988. Just after they were caught the sardines' body cavities (from anus to pectoral fins) were opened, and sex was determined. Ovary-free (Spain) and gutted (Portugal) female bodies were immediately frozen. Excised ovaries were preserved; the fixative used was:

- 4 % buffered formaldehyde solution (Hunter; Lo and Leong, 1985) (Spain).
- Bodian's AAF (Lillie and Fullmer, 1976) (Portugal) modified according to the following formula:
  - Alcohol 50 % 65 ml
  - Formalin 40 % 32 ml
  - Glacial acetic acid 13 ml

Because fecundity estimates should be given in terms of live wet weight, correction coefficients to convert frozen fish weight to wet weight were needed. A number of 300 fish were used to calculate live wet weight and frozen weight after two months, and the value of correction coefficients estimated was used to estimate the live wet weight of all sample fish. The correction coefficients were computed from samples collected from commercial catches in Portuguese waters and from pelagic trawls executed during the Egg Production Method (EPM) survey in Spanish waters. The total fecundity and the corrected ovary-free fish weight, an estimated live wet weight, were analyzed to model the relationship between fish weight and total fecundity.

In order to analyze the hydrated oocyte distribution between left and right ovaries and between lobes of the same ovary, we took four ovarian tissue subsamples from fish sampled in Spanish waters: one at the center and the other near one ovary edge, in both ovarian lobes. A total of 206 ovary tissue samples were examined. As to Portuguese samples, three ovarian tissue subsamples were taken from each ovary: one at the center and the other two at about one-third of the distance from each edge of only one ovarian lobe, and a total of 114 ovary tissue samples were examined. Egg number per unit ovary weight was calculated for each tissue subsample. Analysis of variance used to test differential distribution of hydrated oocytes inside the ovary and in between lobes was carried out separately for each country.

All the ovaries were histologically examined to detect postovulatory follicles. Their presence was an indication that spawning had been initiated, and that some hydrated oocytes had probably already been released. In such cases, ovaries were not used in fecundity estimates.

From each fish sampled, three tissue samples were examined. From each subsample, an estimated total oocytes was computed as the number of hydrated oocytes per g times the wet gonad weight. The batch fecundity estimate was the average of three estimated total hydrated oocytes (Hunter, Lo and Leong, 1985). As total fecundity increases with fish weight, batch fecundity was determined as a function of female weight, i.e., $EY = f(W)$, where W is the ovary-free wet weight. Ovary-free fish weight was used to prevent upward bias in weight, as females with hydrated oocytes temporarily weigh more than the average female (Hunter, Lo and Leong, 1985).

Simple linear regression was first fitted to the batch fecundity and fish weight data from each of the two countries separately. An analysis of covariance was performed to determine whether the adjusted mean batch fecundities (for a given ovary-free female weight) from the two countries were similar. If so, then data from both countries would be combined to evaluate the batch fecundity-fish weight relationship.

Several regression models for fitting batch fecundity and ovary-free weight data were established. All models included egg

number per batch (F) and ovary-free weights (W) recorded for each female sampled in Portuguese and Spanish waters. In addition to the fecundity-fish weight relationship, we also examined the fecundity-fish age relationship, using Portuguese data for comparison purposes.

RESULTS, DISCUSSION AND DISCUSSION

A total of 126 sardines with hydrated ovaries were collected during the EPM cruise (37 from Portugal and 89 from Spain). Hydrated oocytes were preserved with either formalin or Bodian's AAF modified. The resulting distinct characteristics of hydrated eggs are presented in table I. The correction factors used to convert frozen fish weight and preserved ovary weight to live fish weight and wet ovary weight were also computed for both countries (table II). We also examined the effect of location of hydrated oocytes within ovaries and the effects of right and left ovaries on egg density. The analysis of variance indicated no significant difference in egg density within ovaries and between ovaries at 0.05 level of significance (tables III and IV). Thus, tissue samples from any location, from any one ovary, should give an unbiased egg density estimate. We took three tissue samples from each fish for modelling sardine batch fecundity and fish weight relationship. More tissue samples per fish would be necessary if the variance within the ovary was high compared to the variance around the regression line (Hunter, Lo and Leong, 1985).

A weighted analysis of covariance was used to compare the adjusted batch fecundity for the average ovary-free fish weight between the two countries (table V), where the weight is the inverse of variance [1]. The analysis of covariance indicated that the adjusted batch fecundity from the two countries was not different, at 0.05 level of significance ($F = 0.02$, with $d.f. = 1\,123$). The adjusted batch fecundity for fish of 25.86 g (a weighted average ovary-free wet weight) was 10 308.54 for Portugal and 10 146.63 for Spain. Thus both Portuguese and Spanish data sets were used to establish a representative regression model of batch fecundity for female weight on the Atlantic-Iberian coast. A weighted least squares regression was used to model the fecundity and fish weight relationship (fig. 1) (Draper and Smith, 1981 in Hunter, Lo and Leong, 1985):

$$F = -1184.7 + 443.56 \cdot W^{1}$$

where F is the batch fecundity and W is the ovary-free wet weight.

Table I.—Effects of two different fixatives on hydrated oocytes.

Tabla I.—Efectos sobre los ovocitos hidratados de los dos diferentes líquidos conservantes.

Preservative solution	*Morphological characteristics of the hydrated oocytes*
4 % buffered formaldehyde	— large size — wrinkled appearance (yolked but nonhydrated oocytes usually retain their smooth contour) — translucence (nonhydrated oocytes are relatively opaque)
Bodian's AFF modified	— large size — unwrinkled contour — translucence apperarance similar to unfertilized planktonic eggs — yolk granules visible

[1] The variance was computed as follows: A simple linear regression of batch fecundity on fish weight was obtained for each country and residuals were obtained. Fish were grouped by 10 g increments and the variance of the residuals within each group was computed.

Table II.—Correction factors determined by each country (fW: frozen fish weight; gW: gutted fish weight, and fWov: preserved ovary weight).

Tabla II.—Factores de corrección calculados por cada país (fW: peso del pez congelado; gW: peso eviscerado, y fWov: peso del ovario fijado).

	Spain	*Portugal*
Wet fish weight	1.0559 (fW)	1.044 (fW)
Total fish weight	—	−0.568 + 1.127 (gW)
Wet ovary weight	0.991 (fWov)	0.852 (fWov)

Table III.—Effect of location of ovarian tissue samples from sardine on the number of eggs per unit sample weight (g), using Spanish data. Effects evaluated by taking tissue samples from two positions (middle, I and edge, II) and both from 103 right and 103 left ovaries.

Tabla III.—Efecto de la situación de las muestras de ovocitos del ovario de sardina, sobre el número de huevos muestreado por unidad de peso (g), con datos españoles. Efectos calculados para dos posiciones (centro I y extremo II) y para 103 muestras en cada uno de los ovarios, derecho e izquierdo. En un ovario sólo se realizaron dos muestras.

MEAN N.° EGGS/g OF OVARY									
Position of sample in ovary	*RIGHT OVARY*			*LEFT OVARY*			*BOTH*		
	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*	*n*
I	2080.6	58.42	52	2143.5	64.38	52	2112.0	43.36	
II	2060.9	55.26	51	2092.9	60.85	51	2076.6	40.86	

TWO-WAY ANALYSIS OF VARIANCE OF EGGS/g OF OVARY TISSUE				
SS due to	*df*	*SS*	*MS*	*F*
Right vs. left ovary	1	113806.28	113806.28	.620
Position within ovary	1	63066.12	63066.12	.344
Interaction	1	12303.82	12303.82	.067
Residual	202	37073891	183534.11	
Total	205	37264681		

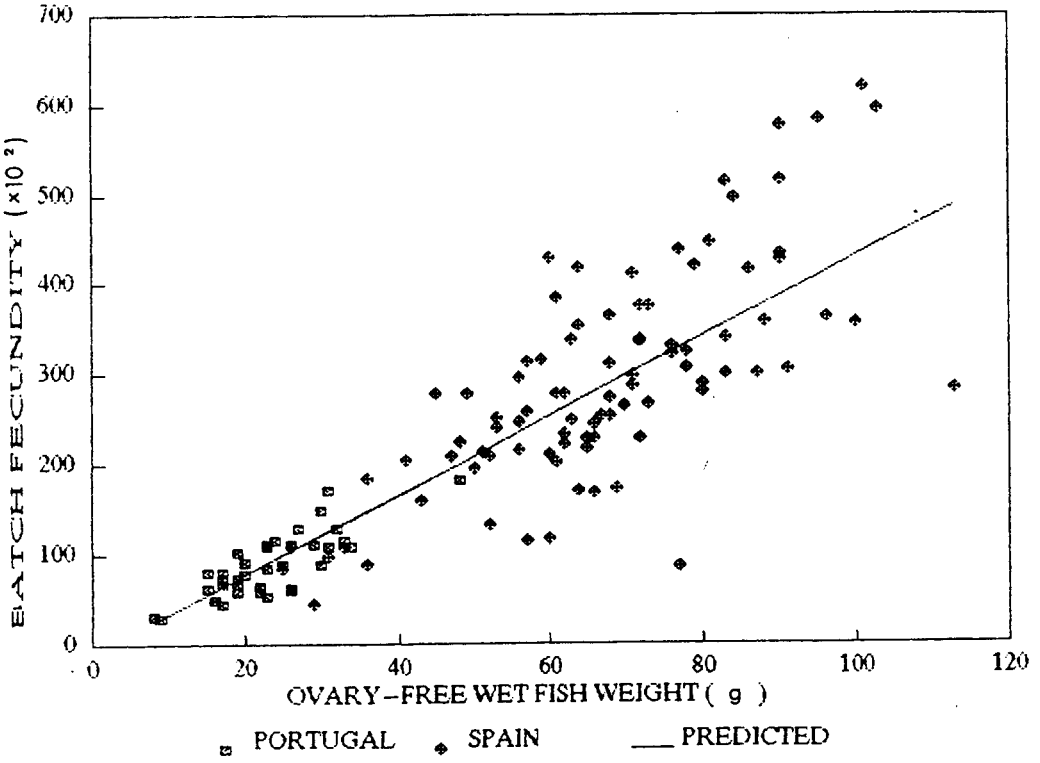

Fig. 1.—Linear regression of batch fecundity ($\times 10^2$) of *Sardina pilchardus* on the ovary-free wet fish weight (g). Data were from Portugal and Spain waters off the Atlantic Iberian coast.

Fig. 1.—Regresión lineal entre fecundidad parcial ($\times 10^2$) de *Sardina pilchardus* y el peso vivo de las hembras sin ovario. Los datos, aportados por Portugal y España, corresponden a las costas atlánticas de la península Ibérica.

The mean batch fecundity was estimated from equation (1) by replacing W with the mean ovary-free fish weight from the survey, which was converted from the mean fish weight based on a fish weight and ovary-free fish weight equation given in García *et al.* (1992). The mean batch fecundity estimate of *Sardina pilchardus* was 30 227.3 (standard error = 1342.6). We also computed the mean relative fecundity estimate as the mean fecundity divided by the mean ovary-free fish weight (426.53, standard error = 18.95). When compared with other clupeoids (Lo, Alheit and Alegre, mate as the mean fecundity divided by the mean ovary-free fish weight (426.53, standard error = 18.95). When compared with other clupeoids (Lo, Alheit and Alegre, 1986; Alheit, 1988), *Sardina pilchardus* produces more eggs per body weight than most other Clupeidae (187-413/g) (table VI).

Although linear regression was chosen to model fish weight and fecundity relationships, two nonlinear models were also presented for comparison purposes (table VII).

Expression of batch fecundity by age group was based only on Portuguese data (table VIII). Comparison of linear regression models indicated that fecundity increases more rapidly with age than with fish weight (tables VII and VIII). This may be a consequence of the age data used, mainly composed of younger sardines (age group 1 and 2) which present higher growth rates than older ones.

Table IV.—Effect of location of ovarian tissue samples from sardine on the number of eggs per unit sample weight (g), using Portuguese data only. Effects evaluated by taking tissue samples from three positions (middle, I and at one-third distance from each edge, II and III).

Tabla IV.—Efecto de la situación de las muestras de ovocitos del ovario de sardina, sobre el número de huevos muestreado por unidad de peso (g), con datos portugueses. Efectos calculados para tres posiciones (centro I y a un tercio de los extremos II y III).

MEAN N.° EGGS/g OF OVARY			
Position	$\bar{x}$	*s.e.*	*n*
I	2733.3	115.37	38
II	2060.9	136.63	38
III	2734.2	114.46	38
Total			114

TWO-WAY ANALYSIS OF VARIANCE OF EGGS/g OF OVARY TISSUE					
SS due to	*df*	*SS*	*MS*	*F*	*Sig.lev.*
Between groups	2	5220	2609.92	0.005	0.9954
Within groups	111	63380485	570995.36		
Total	113	63385705			

Table V.—Analysis of covariance for the effect of country on the adjusted mean batch fecundity. The covariate is ovary-free fish weight.

Tabla V.—Análisis de covarianza del efecto de los países sobre el ajuste de la fecundidad parcial media. Covariante es peso de las hembras sin ovario.

ANALYSIS OF COVARIANCE FOR DEPENDENT VARIABLE - FECUNDITY						
Source	*Sum of squares*	*D.F.*	*Mean square*	*F*	*Tail prob.*	*Regression coefficients*
Country	0.01749	1	0.01749	0.02	0.8995	
fwt	246.866008	1	246.86608	230.51	0.000	4.4628
Error	131.72915	123	1.07097			

ADJUSTED MEANS FOR DEPENDENT VARIABLE		
	Portugal	*Spain*
Fecundity ($\times 10^2$)	103.08544	101.46636

Table VI.—Relative fecundity of various clupeiod fish species. (Adapted from Alheit, 1988.)

Tabla VI.—Fecundidad relativa de varias especies de clupeidos. (Adaptada de Alheit, 1988.)

Group	*Species*	*Area*	*Relative fecundity*	*n*	*Authors*
Clupeidae					
Sardines	*Sardinops caerulea*	California	263		MacGregor, 1957
	Sardinops sagax	Perú	283	91	Lo, Alheit and Alegre, 1986
	Sardinops sagax	Chile	255	168	Retamales and González, 1983
	Sardinops ocellatus [1]	SW Africa	265		LeClus, 1987
	Sardina pilchardus	Portugal	427	127	Present paper
Sardinella	*Sardinella brasiliensis*	Brazil	356	23	Isaac-Nahum *et al.*, 1988
	Sardinella aurita	Senegal	400		Connand, 1977
Sprat	*Sprattus sprattus*	Kiel Bay. Baltic	232	46	Heidrich, 1925
	Sprattus sprattus [2]	Southern North Sea	413	41	Alheit, 1987
Others	*Clupea bentincki* [3]	Chile	350	126	Mújica and Rojas, 1984
	Herklotsichthys quadri maculatus	Hawaii	236	46	Williams and Clarke, 1983

[1] For a female of 120 g. [2] At peak of spawning. [3] Recalculated.

Table VII.—Models used to express the relationship between female batch fecundity (Y) and female ovary-free weight (X).

Tabla VII.—Modelos usados para expresar la relación entre fecundidad parcial (Y) y peso de las hembras sin ovario (X).

Model	a *(se)*	b *(se)*	*MSE* (10^{-6})	R^2 [3]
$Y = a + bX$	−2291 (1488)	471.15 (24.94)	50.1	0.74
$Y = a + bX$ (weighted)	−1184.7 (535.9)	443.26 (17.45)	0.0106	0.83
$Y = a \cdot X^b$	252.48 (85.6)	1.12 (0.77)	50.0	0.74
$Y = e^{(a + bX)}$	8.9614 (0.0914)	0.0185 (0.0012)	58.4	0.70

[3] R^2 is computed as the regression sum of squares divided by the total sum of squares.

Table VIII.—Models used to express the relationship between female batch fecundity (Y) and female age (X).

Tabla VIII.—Modelos usados para expresar la relación entre fecundidad parcial (Y) y la edad de las hembras (X).

Model	a (*se*)	b (*se*)	*MSE* (10^{-6})	R^2
$Y = a + bX$	3081 (806.85)	3711.88 (470.089)	4.7	0.64
$Y = a + X^b$	6696.24 (392.818)	0.68884 (0.0742)	4.2	0.68
$Y = e^{(a + bX)}$	8.66856 (0.8061)	0.25835 (0.0343)	6.2	0.52

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