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BATCH FECUNDITY OF, *Sardina pilchardus* OFF THE IBERIAN PENINSULA

by

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ABSTRACT

Batch fecundity, the number of eggs released per spawning event, was determined for the first time in pilchard *Sardina pilchardus* (Walb.) off the Atlantic coast of the Iberian Peninsula.
The mean relative fecundity (number of hydrated oocytes per gram of female weight was 416 (standard deviation = 112.9).
Different regression models with Portuguese and Spanish data of batch fecundity by weight are presented. A linear model weighted least squares regression is established. The weighted regression presents a homogeneous distribution of the variance, small mean square error of the regression and highest R-squared, according whit this expression:
F = -1260.8 + 444.43 W
Linear regression of batch fecundity by age for Portugal data is:
F = 30811.11 + 3711.88 A

INTRODUCTION

Sardina pilchardus is a serial spawner, (spawns several batches of eggs each year), (Blaxter and Hunter,1982)
During 1988, Portugal and Spain applied the "Egg Production Method", (EPM), (Parker,1985) to estimate spawning biomass of this species off the Atlantic coast of the Iberian Peninsula. The application of this method requires an estimate of batch fecundity, which was made by the application of the "Hydrated Oocytes Method" (Hunter and Goldberg 1980), a technique based on Fulton (1898), who discovered that all oocytes which are to be released in the next spawning batch increase their size by hydration process.
With these data we were also able to compare the fecundity parameters of pilchard in Portugal and Spain waters

METHODS AND MATERIAL

Sardines were collected along the Atlantic Iberian coast by Portuguese and Spanish research vessels on a joint cruise held in March/April 1988. A total of 126 sardines with ovaries in the hydrated condition were collected during the EPM cruise (37 Portugal and 89 Spain).
After they were caught, their body cavity (anus to pectoral fins) was immediately opened and sex was determined. The ovary-free females (Spain) and gutted females (Portugal) were frozen immediately. From all pilchard, the ovaries were excised and preserved. The fixative used on their preservation was different: Spain preserved the ovaries in a 4% buffered formaldehyde solution (Hunter 1985) and Portugal in Bodian's AAF (LILLIE and FULLMER, 1976) modified, according to the following formula:

- Alcohol 50%	65 ml
- Formalin 40%	32 ml
- Glacial acetic acid	13 ml

In the laboratory, females with hydrated oocytes in their ovaries were used to determined batch fecundity.

Since fecundity estimates should be given in terms of live ovary-free weight, correction coefficients were determined. In Spain those were based on epi-pelagic trawl samples collected, during the cruise. In Portugal, those were based on samples collected by the commercial fleet. Sardine total and ovary-free wet weights were determined few hours after fishing, and after 24 hours, it was frozen. Data of live and frozen fish weights were fitted into a regression model which was later used to convert frozen wet weight into live weight.

The relation between female body weight without ovary and female weight with ovary (excluding those with hydrated or immature ovaries) was established using a linear regression model. This regression was used to convert ovary-free weight to total body weight, for Portuguese data.

The ovaries of all hydrated females were preserved. After 1 month each preserved gonad was blotted to remove the excess of fixative and weight. The correction factor is the value of the slope of a regression model used to convert fixed weight to live weight when the intercept is assumed to be zero.

Three pieces of ovarian tissue were taken from each ovaries, in order to prevent biases on the estimates of batch fecundity due to a differential distribution of the hydrated oocytes within the ovary.

Each subsample was weighted and hydrated oocytes were counted under a stereomicroscope. All the ovaries were investigated histologically for the presence of post ovulatory follicles. Their presence is an indication that spawning has already been initiated, and consequently those ovaries can not be used for fecundities estimations since some hydrated oocytes have been already released.

Batch fecundity was estimated based on the average of the three subsamples and determined as a function of female ovary-free weight. Ovary-free wet weights were used since females with hydrated oocytes, temporarily weight more than the average female (HUNTER et al., 1985).

Sampling hydrated females was very difficult at the southern region off Portugal. Biological information and length distribution of sardines along the coast, showed that sardines in higher length classes were concentrated in the south.

Linear regressions of batch fecundity on ovary-free fish weight from both countries were compared. If little difference was found between these two regressions, data of both countries were combined to increase sample size. Different models were fitted to the batch fecundity and ovary-free fish weight data and the model with small mean square errors and highest R^2 (from linear regression) was chosen as the final model.

The number of eggs per batch and age were also used in different regressions, with only Portuguese data.

Relative fecundity (number of eggs spawned/ovary-free female weight) was also estimated for both regions and results compared.

RESULTS

Hydrated oocytes preserved in different fixatives, present distinct characteristics. Table I contains a brief description of the morphology of hydrated oocytes preserved in formalin and in Bodian's AAF modified.

The values of weights and of relative fecundity obtained by Portugal and Spain were expressed in terms of ovary-free wet weight. Table II summarizes the correction factors obtained.

Table III presents the values of means, standard deviations of weights and relative fecundities determinated for each country. No significant difference was found in the ovary-free fish weights and the relative fecundities between two countries.

Setting a linear regression model to the existing data sets on fecundity of sardines, showed that variance of batch were not homogeneous distributed, increasing with females weight.

In order to express the relation of female batch fecundity to female weight in terms of a linear model, a weighted least squares regression was used. The unweighted regression was divided into smaller sections in which variance was homogeneously distributed and total variance was calculated based on the females number in its section. The inverse of total variance as the weighting factor to the estimate the new regression model, (Draper and Smith, 1971, in Hunter et al 1985).

According to this proceeding a minimal variance and unbiased estimate of the regression coefficients can be obtained (Hunter et al 1985).

Four models were chosen to fit the data. Two were linear and two were nonlinear. The mean square errors and R^2 from four models were computed. The weighted linear model had small mean square error and the highest R^2. Therefore it was chosen as the final model (Table IV). Fig 1. shows the weighted least squares regression model and linear model.

The relationship of batch fecundity and age of female fish in Portugal waters was also investigated. The linear regression indicated that the variance around the linear was homogeneous. However the nonlinear regression fit the data better than the linear regression. It was also observed that the batch fecundity and fish weight relationship was more pronounced than the batch fecundity and age relationship. Table V. Show the value of batch fecundity to age data for Portugal.

The mean relative fecundity (number of hydrated oocytes per gram of female weight was 416 (standard deviation = 112.9).

CONCLUSIONS

Linear model is usually preferable than other models because its regression coefficients have a simple biological meaning and because for the egg production estimation the fecundity of largest and smallest fish are not as critical as for the fish in the middle range, which is well explained by the simple linear model, (Hunter et al, 1985).

In Atlantic pilchard, a weighted least squares regression model for expressing the relation of female batch fecundity to female ovary-free weight was preferable and were establish for expressing:

$$F = -1260.8 + 444.43\ W$$

If we compare the data of relative fecundity with the value of various clupeiform fish, listed by Alheit, 1988, we can see than the pilchard produce more eggs per gram body weight per batch than all clupeidae, in spite of the weight range of pilchard is smaller than other sardines species.

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REFERENCES

ALHEIT, J.1988. Comparative Spawning Biology of Anchovies, Sardines and Sprats. Early Life History Symposium ICES C.M. 1988 Paper N 16.

BLAXTER, J. H. S., and J. R. HUNTER, 1982. The Biology of the Clupeoid Fishes. Advances in Marine Biology. Vol. 20.

FULTON, T. W. 1898. On the growth and maturation of the ovarian eggs of the teleostean fishes. Annu. Rep. Fish. Board Scotl. 16:88-124.

HUNTER, J. R., and S. R. GOLDBERG, 1980. Spawning incidence and batch fecundity in northern anchovy, <u>Engraulis mordax</u>. Fish. Bull. U.S. 77:641-652.

HUNTER, J. R., 1985. Preservation of the norther anchovy in formaldehyde solution. In R. Lasker (editor). An Egg Prodution Method for Estimatting Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. p. 63-65. U.S. Dep Commer., NOAA Tech. Rep. NMFS 36: 99p.

HUNTER, J.R., N. LO & R. LEONG, 1985. Batch Fecundity in Multiple Spawning Fishes. In R. Lasker (editor). An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. p. 67-77. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36: 99p.

LILLIE, R. D. and H. M. FULLMER, 1976. Histopathologic Technic and Practical Histochemistry (4th edition). McGraw-Hill, Inc., New York, 942.

PARKER, K. 1985. Biomass Model for the Egg Production Method. In R. Lasker (editor).An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy, *Engraulis mordax*. p. 5-6. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36: 99p.

TABLE I

Effects of two different fixatives on hydrated oocytes

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 AAF modified	- large size - unwrinkled contour - translucence appearance similar to unfertil- ized planktonic eggs - yolk granules visible

TABLE II

Corrections factors determined by each country (fW - frozen weight, gW - gutted weight and fWov - fixed weight ovary)

CORRECTION COEFFICIENTS	COUNTRY SPAIN	PORTUGAL
frozen/wet weight bodys	1.0559 * fW	1.044 * fW
gutted/total weight	-	- 0.568 + 1.127 * gW
fixed/wet weight ovary	0.991 * fWov	0.852 * fWov

TABLE III

Mean weight and relative fecundity (no. eggs per gram female) of sardine; standard deviations in brackets. N is number of samples.

COUNTRY	MEAN WEIGHT (g)		RELATIVE FECUNDITY
PORTUGAL	23.04 (7.5)	N = 37	382.50 (88.6)
SPAIN	66.90 (17.9)	N = 89	429.41 (118.7)
TOTAL	54.00 (25.3)	N = 126	416.34 (112.9)

TABLE IV

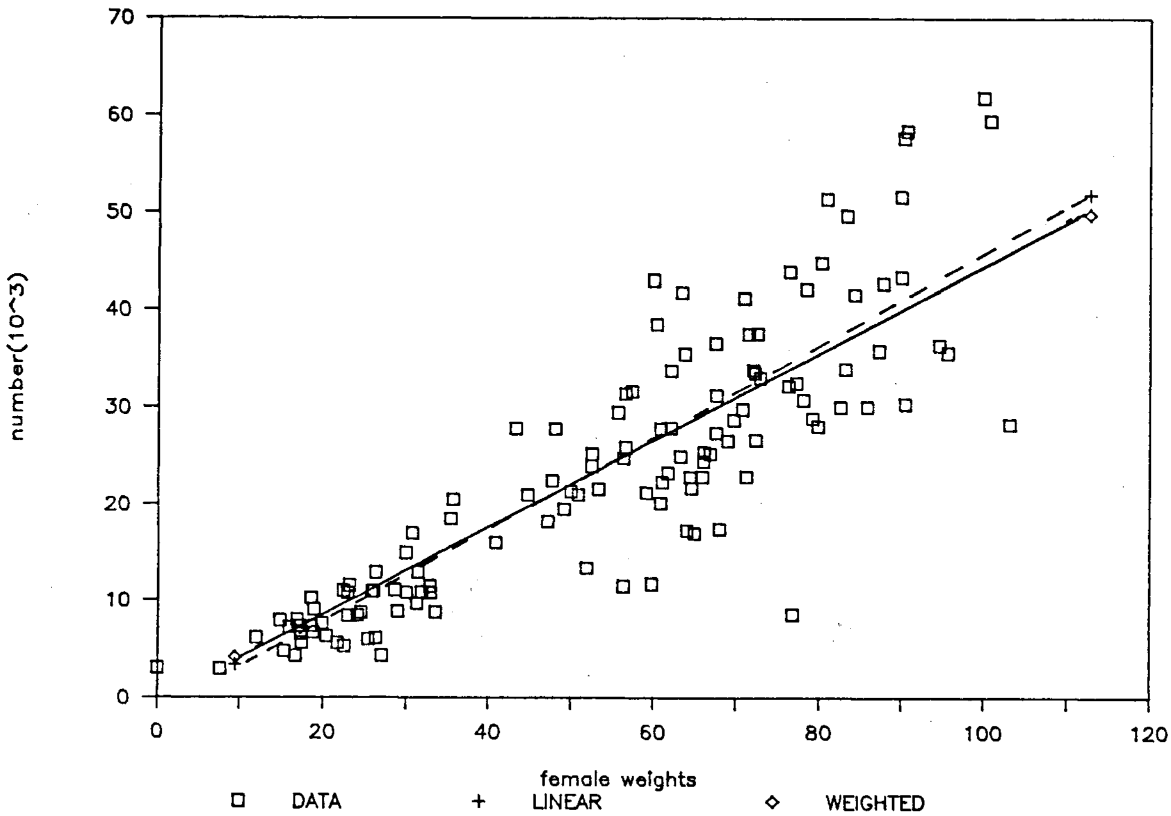
Models used to express the relation of female batch fecundity (Y) and female ovary-free weight (X)

MODEL	a (se)	b (se)	MSE 10^{-6}	R^2
$Y = a + bX$	-2304.51 (1485.9)	471.28 (24.91)	50.1	0.74
$Y = a + bX$ (weighted)	-1260.80 (650.9)	444.43 (18.5)	50.7	0.82
$Y = a * X^b$	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

TABLE V

Model used to express the relation of female batch fecundity (Y) and female age (X)

MODEL	a (se)	b (se)	MSE 10^{-6}	R^2
$Y = a + bX$	3081.11 (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



F.1. Weighted least squares regresion model and linear model.