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First data on batch fecundity and relative fecundity of *Sardina pilchardus* (Walbaum, 1792) (Clupeidae) in the south-western Adriatic Sea

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ABSTRACT

The batch fecundity and relative fecundity of Sardina pilchardus *(Walbaum, 1792) have been determined for the first time in the waters of the Lower Adriatic Sea using the hydrated oocyte method. The samples were collected from the commercial fleet and chartered boats equipped with midwater pair trawls, midwater otter trawls and purse seine during the reproductive period. Various regression models were studied, to express the relationship between the batch fecundity and the weight of ovary-free females. The linear model proved to be the best one, because of its simplicity. Relative batch fecundity (number of hydrated oocytes per gram from ovary-free females) was also studied, and compared with that of other clupeiforms.*

Key words: *Sardina pilchardus,* batch fecundity, relative fecundity, Adriatic Sea.

RESUMEN

Primeros datos sobre la fecundidad parcial y relativa de *Sardina pilchardus* (Walbaum, 1792) en el suroeste del mar Adriático.

Se ha determinado por primera vez la fecundidad parcial y relativa de Sardina pilchardus *(Walbaum, 1792) en las aguas del bajo Adriático con el método de los ovocitos hidratados. Las muestras han sido recogidas de la propia flota comercial y de embarcaciones alquiladas y equipadas con red de arrastre pelágico a la pareja, red de arrastre semipelágico a la pareja o red de cerco con jareta durante el periodo de reproducción. Se han estudiado diferentes modelos de regresión entre la fecundidad parcial y el peso de las hembras sin ovarios. El mejor ajuste corresponde a una regresión lineal. Se ha calculado también la fecundidad relativa (número de ovocitos por gramo de hembra sin ovarios) y se ha confrontado con la de otros clupeiformes.*

Palabras clave: *Sardina pilchardus,* fecundidad parcial, fecundidad relativa, Adriático.

INTRODUCTION

The studies of Hunter and Goldberg (1980) and Hunter and Macewicz (1980) on the northern anchovy *Engraulis mordax* Girard, 1856 have changed previous ideas on the reproductive biology of multiple-spawning fish. This type of fish reproduces by emitting successive batches of eggs during a season. The number of emissions depends on environmental conditions, and can increase in certain years, e.g., when there is more abundance of food (indeterminate number of emissions). Hunter and Leong (1981) have shown that the northern anchovy reproduces on average 20 times a year, a frequency far higher than previously thought. Therefore, the determination of annual fecundity (the total number of eggs deposited in one year by a female) as a function both of the batch fecundity and of the frequency of deposition is too time-consuming; a more practical measure of fecundity can be obtained by counting the number of hydrated oocytes emitted by a female in a single spawning act (batch fecundity).

Hunter and Goldberg (1980), Hunter and Macewicz (1980) and Hunter, Lo and Leong (1985) have developed a new method to determine batch fecundity, based on the number of hydrated oocytes in the ovaries of the fish immediately before spawning. At this time, the volume of eggs ready for emission increases greatly, due to the phenomenon of hydration (Fulton, 1898; Andreu, 1955); this is immediately followed by ovulation and spawning. Sardine spawn at night, usually from dusk until three or four o'clock in the morning; hydration of the eggs begins at least 12 hours before deposition (Alheit, 1989). According to Pérez and Cal (1988), sardines spawn between 19:00 and 21:00 h (GMT) (off Portugal and northern Spain).

This paper reports, for the first time in the Apulian waters of the Adriatic, the batch fecundity of *Sardina pilchardus* (Walbaum, 1792), which is similar to that of other small pelagic fishes, such as sprats and anchovies, characterised by multiple spawning (Blaxter and Hunter, 1982; Pérez, Figueiredo and Lo, 1992). The relative batch fecundity (batch fecundity relative to the wet weight of the ovary-free females) is also reported.

In order to determine batch fecundity, it would be sufficient to go out in a boat, catch the sardines, separate the hydrated females and count the hydrated oocytes in the ovaries. However, sampling for this study was planned in order to assess the spawning biomass according to the Daily Egg Production Method (DEPM) (Lasker, 1985; Parker, 1980; Picquelle and Stauffer, 1985).

MATERIALS AND METHODS

Between November 1992 and March 1993, a total of 110 samples of *S. pilchardus* were collected (26 in November 22 in December 22 in January 18 in February and 22 in March). The samples were mainly collected from the commercial fleet, but also from chartered trips, using midwater pair trawls, midwater otter trawls and purse seine (Fiamozzi *et al.*, 1987), during the period of reproduction. The fishery (figure 1), which is the same for all the clupei-

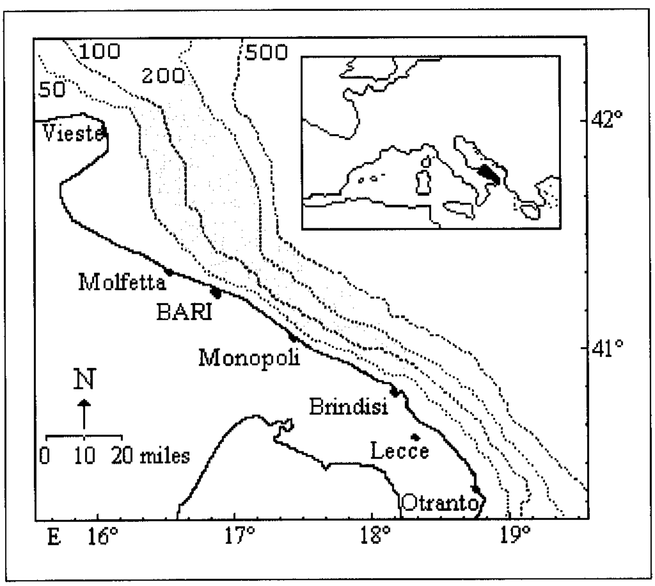

Figure 1. Fishing area.

Table I. Weight in grams and number (in brackets) of *Sardina pilchardus* males and females in the approximately 2 kg samples taken from the different trawls during the period of the study.

	November		December		January		February		March	
Trawl	F	M	F	M	F	M	F	M	F	M
01	1 432 (43)	546 (16)	1 641 (32)	343 (7)	1 254 (39)	769 (22)	1 620 (45)	388 (11)	1 246 (45)	768 (27)
02	517 (14)	1 492 (53)	1 125 (30)	907 (26)	1377 (41)	632 (18)	1 083 (29)	902 (28)	545 (17)	1 493 (52)
03	1 205 (36)	762 (26)	1 040 (28)	991 (29)	947 (28)	1 055 (31)	1 255 (36)	770 (25)	860 (24)	1 156 (36)
04	637 (18)	1 349 (47)	833 (23)	1 162 (32)	1 038 (29)	972 (29)	976 (29)	1 022 (32)	1 129 (32)	883 (28)
05	1 594 (45)	374 (14)	960 (28)	1 016 (25)	1 151 (27)	880 (26)	1 245 (37)	771 (26)	1 271 (35)	774 (24)
06	1 402 (39)	571 (20)	1 504 (30)	527 (17)	1 012 (25)	1 010 (31)	1 191 (36)	808 (27)	1 114 (29)	914 (29)
07	551 (15)	1 460 (53)	1 768 (45)	260 (9)	1 578 (41)	431 (13)	1 275 (38)	743 (25)	985 (26)	1 019 (32)
08	943 (27)	1 055 (35)	662 (14)	1 321 (39)	584 (14)	1 404 (47)	946 (27)	1 068 (37)	1 267 (37)	735 (23)
09	1 177 (60)	828 (27)	1 068 (22)	937 (28)	860 (22)	1 157 (41)	1 041 (32)	974 (35)	1 268 (36)	729 (24)
10	1 224 (35)	781 (26)	1 264 (26)	746 (22)	1 132 (32)	851 (29)	1 210 (38)	816 (29)	1 277 (37)	773 (25)
11	1 095 (33)	914 (30)	990 (20)	1 039 (31)	1 229 (34)	808 (28)	1 148 (35)	888 (29)	974 (30)	1 066 (34)
12	972 (30)	1 024 (36)	997 (27)	1 013 (28)	1 078 (31)	937 (34)	900 (25)	1 087 (38)	1 292 (19)	733 (23)
13	1 368 (44)	613 (22)	1 298 (35)	750 (18)	1 194 (36)	849 (30)	687 (21)	1 346 (47)	1 152 (38)	897 (29)
14	1 280 (39)	743 (27)	1 249 (34)	780 (25)	1 058 (32)	953 (36)	1 255 (37)	785 (28)	1 664 (83)	357 (12)
15	1 363 (26)	607 (20)	950 (28)	1063 (32)	710 (23)	1 287 (42)	1 470 (44)	544 (19)	925 (26)	1 077 (35)
16	923 (21)	1 061 (37)	1 065 (20)	952 (33)	1 328 (43)	666 (22)	729 (22)	1 267 (45)	676 (20)	1 331 (42)
17	1 321 (32)	704 (24)	1 258 (30)	737 (24)	1 420 (36)	551 (18)	1 134 (34)	885 (30)	927 (27)	814 (26)
18	1 081 (27)	937 (32)	845 (22)	1129 (34)	815 (22)	1 177 (39)	579 (18)	1 431 (52)	916 (25)	1 095 (34)
19	1 837 (41)	190 (6)	617 (14)	1357 (38)	700 (21)	1 337 (46)			1 248 (36)	745 (24)
20	659 (14)	1 359 (46)	1 136 (30)	874 (21)	1 034 (28)	951 (35)			1 543 (41)	481 (16)
21	618 (13)	1 384 (50)	1 465 (30)	546 (16)	595 (17)	1 416 (48)			1 098 (28)	870 (27)
22	897 (24)	1 102 (27)	834 (18)	1 152 (37)	1 297 (39)	705 (25)			988 (28)	1 005 (32)
23	903 (24)	1 155 (32)								
24	1 325 (36)	675 (16)								
25	1 661 (47)	350 (9)								
26	1 095 (21)	871 (29)								

forms, partially coincides with the area of reproduction (Casavola *et al.*, 1981; 1986; 1993).

A 2 kg sample was selected at random after every trawl (table I). Moreover, in order to increase the number of hydrated females in the 2 kg samples, a number of hydrated females were added to these. In the results, when we refer to mature females, we mean those in the 2 kg sample, while hydrated females include both those in the sample and those caught during the trawl. While still on board, and within two hours of capture, the specimens were numbered and opened in order to remove the mature ovaries, which were immediately fixed in 10 % formaldehyde (Hunter, 1985). The ovary-free bodies were then put on ice and transported to the laboratory for biometric measurements: total length, total weight, gonad-free weight and gutted weight.

After at least a month each ovary was reweighed. Three pieces of tissue totalling 100 mg (± 0.01 mg) were removed at random from each ovary in order to count the hydrated oocytes (Pérez *et al.*, 1989); this procedure is described in Casavola, Marano and Rizzi (1996). No difference was observed in the number of hydrated oocytes in each of the three-piece samples, either within the same gonad or between the right and left gonads. All the ovaries were histologically studied in 5-6 µm sections stained with haematoxylin and eosin to detect any postovulatory follicles, which indicate that spawning had either begun or taken place. In order to calculate the batch fecundity, after the histological examination, those females whose gonads contained new postovulatory follicles were rejected because they had already begun spawning, and thus their inclusion in the sample would have led to an underestimation of fecundity values.

Of the 228 hydrated females caught during the expeditions, 177 (49 collected in November, 36 in December, 28 in January, 26 in February and 38 in March) were used to estimate the number of hydrated oocytes. In particular, translucent ones were chosen (oocytes with nucleus migration were observed by 7:30 a.m., while hydrated oocytes were not observed before 4:00 p.m.), without new postovulatory follicles. Moreover, the samples were chosen so that the weight distribution of the hydrated ovary-free females was as similar as possible to that of all the mature ovary-free females (figure 2).

The parameters for the sardine were estimated using averages weighed ($\bar{\bar{Y}}$) and variances ($Var\bar{\bar{Y}}$) (Cochran, 1963):

$$\bar{\bar{Y}} = \frac{\sum_{i=1}^{n} \left(\frac{m_i}{\bar{m}}\right) \bar{Y}_i}{n} \quad (1)$$

$$Var\,(\bar{\bar{Y}}) = \frac{\sum_{i=1}^{n} \left[\left(\frac{m_i}{\bar{m}}\right)^2 (\bar{Y}_i - \bar{\bar{Y}})^2 \right]}{n\,(n-1)} \quad (2)$$

where m_i is the number of fish in the i-th trawl; $\bar{m}$ is the average number of fish per trawl; n is the number of trawls; $\bar{Y}_i = \sum_{j=1}^{n} \frac{Y_{ij}}{m_i}$ is the average value for the i-th trawl and Y_{ij} is the value of the j-th fish in the i-th trawl.

RESULTS

The relationship between the values of batch fecundity, F, for each female and the weight of hydrated ovary-free females, W_h^*, collected every month during the survey (table II), was analysed statistically.

Both linear and non-linear regression models were selected from the statistical interpolation methods available, and their coefficients are reported in table III. The regression models used were parabolic and exponential, as well as straight-line. It is well known that a sound criterion to test the validity of regression is to estimate the value of the determination coefficient, R^2. Obviously, in the case of non-linear interpolating functions, R^2 refers to the linearised and transformed function (Draper and Smith, 1981). The coefficients of determination estimated for all the months with

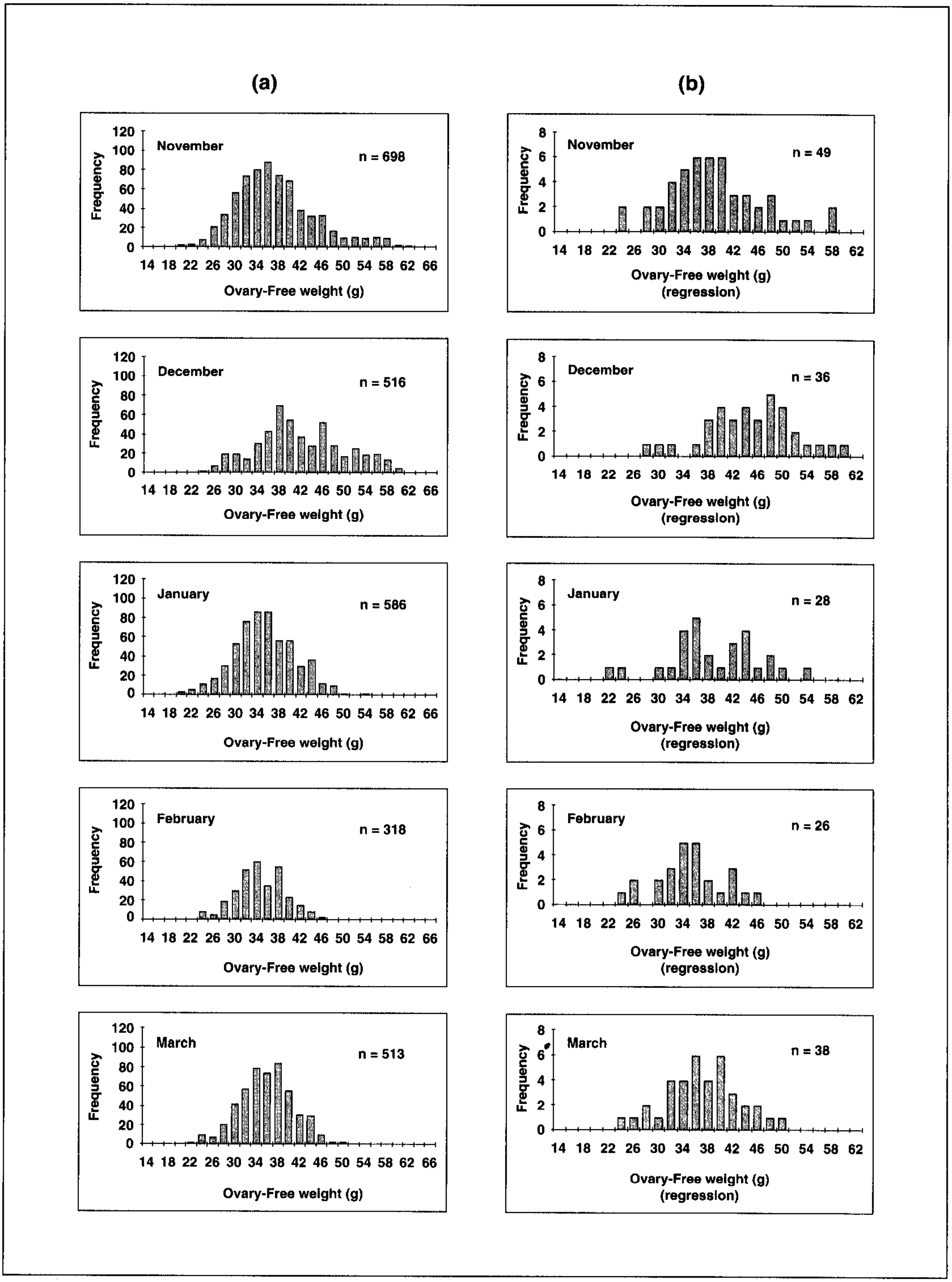

Figure 2. (a): Monthly frequency distributions of ovary-free weight of females from 2 kg samples; and (b): monthly frequency distributions of ovary-free weight of females with hydrated oocytes to estimate the batch fecundity.

Table II. Canonic equations used to express the relation between the batch fecundity F and the weight of ovary-free hydrated females W_h^* for each period of the survey; includes both the hydrated females in the sample and the others caught in each trawl.

Equation		a (SE)	b (SE)	MSE (× 10^3)	R^2
$F = a + bW_h^*$	November 1992	−3 682.6 (1 713.9)	463.5 (44.0)	5 629.492	0.70
	December 1992	−4 754.1 (2 434.0)	498.1 (54.8)	5 767.303	0.71
	January 1993	−5 167.6 (2 474.8)	517.5 (64.1)	6 101.122	0.72
	February 1993	170.3 (1 084.5)	260.0 (31.5)	741.421	0.74
	March 1993	−7 619.1 (2 457.5)	581.1 (66.9)	5 589.120	0.68
$ln\,F = a + b\,lnW_h^*$	November 1992	4.67 (0.392)	1.33 (0.108)	0.000024	0.76
	December 1992	4.78 (0.533)	1.31 (0.141)	0.000022	0.72
	January 1993	4.85 (0.593)	1.29 (0.163)	0.000032	0.71
	February 1993	5.42 (0.430)	1.04 (0.122)	0.000010	0.75
	March 1993	4.17 (0.578)	1.48 (0.161)	0.000026	0.70
$ln\,F = a + bW_h^*$	November 1992	8.19 (0.121)	0.034 (0.003)	0.000028	0.72
	December 1992	8.34 (0.153)	0.031 (0.003)	0.000023	0.71
	January 1993	8.12 (0.172)	0.037 (0.004)	0.000030	0.73
	February 1993	8.02 (0.137)	0.031 (0.004)	0.000012	0.72
	March 1993	7.92 (0.164)	0.042 (0.004)	0.000025	0.72
$F = a + b\,lnW_h^*$	November 1992	−49 607.5 (6 024.9)	17 565.7 (1 661.1)	5 593.461	0.70
	December 1992	−59 668.9 (8 887.3)	20 381.1 (2 357.1)	6 187.6861	0.69
	January 1993	−49 047.6 (8 962.1)	17 565.3 (2 474.5)	7 289.4546	0.70
	February 1993	−21 132.7 (3 499.4)	8 579.8 (994.5)	695.22377	0.76
	March 1993	−57 748.9 (8873.5)	19 900.8 (2 477.6)	6 191.2089	0.64

different interpolating functions range from 0.68 to 0.76.

After examining the equations used and remaining within the range of size variability represented in figure 3, we selected the linear one, due to its simplicity. Other functions would be useful in the case of very low or very high female weight values.

It is interesting to compare these results of relative fecundity with those obtained from experimental data (table III), where the relative batch fecundity (F/W_h^*) was cal-

Table III. Average weight of mature ovary-free females; estimated relative fecundity (number of eggs per gram of ovary-free hydrated female) by the method of weighted means; estimated relative fecundity by the line passing through the origin. Coefficients of variation in brackets.

	Nov.	Dec.	Jan.	Feb.	Mar.
Relative fecundity calculated with the data (eggs/g)	362.5 (2.42)	386.4 (2.55)	376.7 (3.50)	264.9 (1.93)	366.0 (3.01)
Relative fecundity estimated from the relevant line (eggs/g)	366.9	389.6	391.3	264.9	370.9
Average weight of ovary-free hydrated females (g)	38.16 (4.06)	43.82 (5.18)	37.94 (4.41)	34.05 (3.51)	36.26 (3.36)

culated for all the ovary-free hydrated females collected during each month of the survey by applying equations (1) and (2). The values shown in table III have been calculated using the slopes of the straight lines passing through the origin, according to

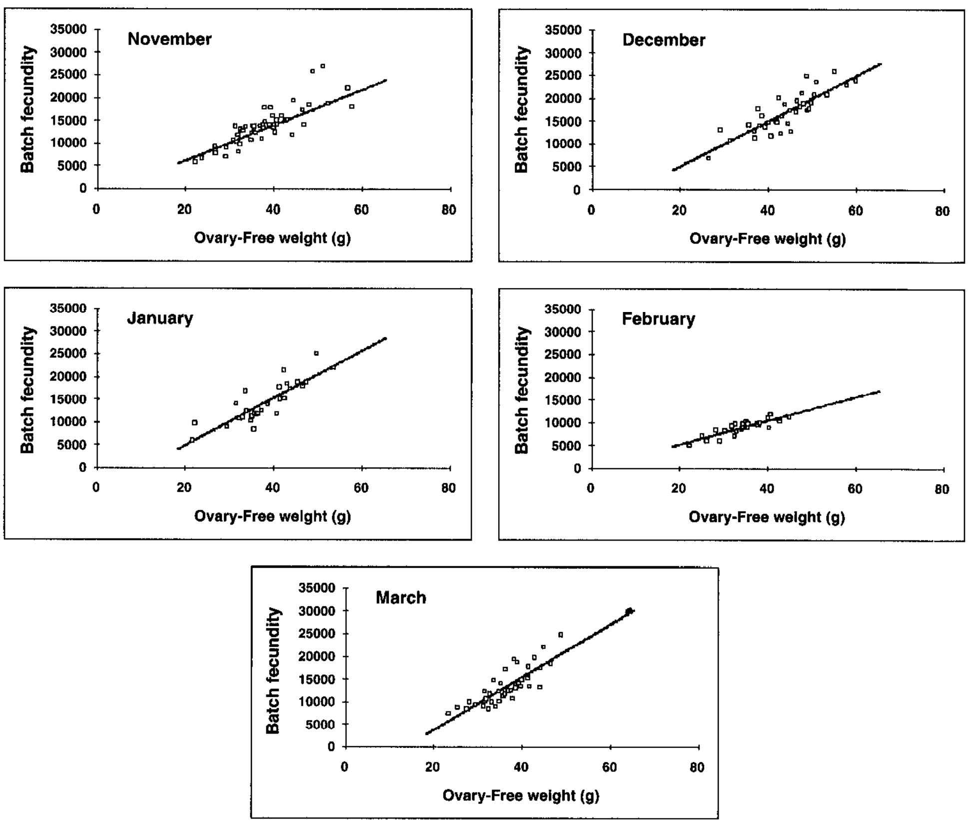

Figure 3. Linear regression of batch fecundity on ovary-free weight of the sardines sampled between November 1992 and March 1993.

standard procedure (Draper and Smith, 1981). The reduction in relative fecundity is visible from both calculated and estimated values in February; during this month, the Student's *t*-test for the intercept indicates a high significance for the passage of the line through the origin ($P < 0.88$; $t = 0.16$). The value of relative fecundity calculated by average weight is fairly similar from November to January, while that obtained from the slope of the representative line shows a modest growth trend; in March there is a net recovery in both the calculated and estimated values of relative fecundity. These results show a variability in the relative fecundity within the reproductive season, in agreement with the literature (Alheit, 1989).

Table IV. Relative fecundity of various clupeid species (modified from Alheit, 1989). (1): for a female of 120 g; (2): at the peak of spawning; (3): recalculated.

Group	Species	Area	Relative fecundity	n	Authors
Sardines	*Sardinops caerulea*	California	263		MacGregor, 1957
	Sardinops sagax	Peru	283	91	Lo, Alheit and Allegre, 1986
	Sardinops sagax	Chile	255	168	Retamales and González, 1983
	Sardinops ocellatus	SW Africa (1)	265		LeClus, 1987
	Sardina pilchardus	Portugal	427	127	Pérez, Figueiredo and Lo, 1992
	Sardina pilchardus	Italy, Southern Adriatic	362 November 386 December 377 January 265 February 366 March	49 36 28 26 38	This study
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	Scotland	187	68	De Silva, 1973
	Sprattus sprattus (2)	Southern North Sea	413	41	Alheit, 1987
Others clupeids	*Clupea bentincki* (3)	Chile	350	126	Mújica and Rojas, 1984
	Herklotsichthys quadrimaculatus	Hawaii	236	46	Williams and Clarke, 1983

DISCUSSION

Using hydrated eggs in the determination of batch fecundity gives more reliable results than other methods used in the past (Alheit, 1985). These can lead to erroneous conclusions, since they are based on assumptions that have proved unfounded. For example, it has been demonstrated that it was mistaken to maintain that only yolked oocytes or oocytes over a certain size threshold would be emitted during the following spawning or reproduction season (Hunter and Macewicz, 1985). It is not always easy to identify a frequency distribution of the size of oocytes in the ovary (Pinto and Andreu, 1957; MacGregor, 1976; Sinovcic, 1984). Neither is it convenient to try to separate the last mode, also because the last two modes are superimposed. Such a method is less precise and more time-consuming, because more than one day is usually necessary to determine the last batch fecundity of just one female. It is quicker to use hydrated oocytes (usually half an hour per female), as well as offering higher precision.

Among the different equations obtained between the batch fecundity and the weight of ovary-free hydrated females, the linear model has proven to be the most suitable because of simplicity, since the determination coefficients of all the equations are fairly uniform.

Table IV, worked out by Alheit (1989) and modified by us, contains values of the relative batch fecundity for various clupeid species studied using the hydrated oocyte method. Our values fall within the limits of the estimates observed for other clupeids (Heidrich, 1925; MacGregor, 1957; De Silva, 1973; Conand, 1977; Retamales and González, 1983; Williams and Clarke, 1983; Mújica and Rojas, 1984; Lo, Alheit and Allegre, 1986; Alheit, 1987; LeClus, 1987; Isaac-Nahum *et al.*, 1988; Pérez, Figueiredo and Lo, 1992). The relative fecundity values of sprat have been included because they are very similar to those of sardine. However, it can be observed that, of all the clupeids, the sardine fished along the Iberian Peninsula coast spawn more eggs per gram of body weight per batch.

The trend in the sardine relative fecundity, both calculated and estimated, examined in the present paper (progressive increase from November to January, negative peak in February, followed by a marked increase in March), cannot be considered a general characteristic, but rather limited to the period of observation. The relative fecundity of *S. pilchardus* is a parameter that varies over the reproductive season. The reported results prove that it is impossible to use historical data in the DEPM. As a matter of fact, in such a methodology it is recommended not to use the relative fecundity values found in different months or in previous years, in order to estimate the spawning biomass to prove this, the DEPM requires collecting at same time both the eggs and the adults.

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