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Degeneration of postovulatory follicles of the Bay of Biscay anchovy (*Engraulis encrasicolus* L.)

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SUMMARY: The degeneration of postovulatory follicles (POFs) in time and at different temperatures was studied for the Bay of Biscay anchovy. For this purpose a key of 7 POF stages, solely defined on the basis of their histological degeneration characteristics, was applied. The novelty of this procedure is that it separates staging of POFs from their ageing process. The female gonads, taken from several captivity experiments and field samples, were classified in this way. Water temperature in captivity tanks corresponded to high values (17-21ºC), except for one case in which different day and night temperatures were applied. In addition, 472 field samples (11948 anchovy females) from several cruises were examined; of these, 126 samples (3348 females) were identified as coming from areas of high sea surface temperature (weighted mean =17.76ºC, s.d.=0.84) and 131 samples (3181 females) as coming from areas of low sea surface temperature (weighted mean =14.42ºC, s.d.=0.75). There was close agreement in the succession of POF stages over time after spawning between the experiment and the field samples. The first four stages of POF degeneration occurred in less than 24 h, and by the end of the first day the POFs were mainly in Stage V. Stages VI and VII showed their highest occurrence during the first and second half of the second day after spawning, respectively. Full resorption of POFs was achieved in about 55-60 h. For the range of temperatures examined (13-19ºC), little effect of temperature on the degeneration of POFs over time was noticed. The advanced degeneration stages were found all day round, showing some overlapping periods when successive spawning cohorts co-occurred. The application of these results for ageing POFs is discussed.

Keywords: postovulatory follicles, gonad cycle, spawning frequency, anchovy.

RESUMEN: DEGENERACIÓN DE LOS FOLÍCULOS POSTOVULATORIOS EN LA ANCHOA DEL GOLFO DE VIZCAYA (*ENGRAULIS ENCRASICOLUS* L.). – Se ha estudiado la degeneración de los folículos postovulatorios (FPOs) para la anchoa del golfo de Vizcaya en el tiempo y a diferentes temperaturas. Para este fin se ha utilizado una clave de 7 estadios de FPOs basada únicamente en características de degeneración histológica. La novedad de este procedimiento consiste en la separación entre la clasificación de los estadios de FPOs y su datación. Así, se han clasificado las gónadas de hembras obtenidas a partir de experimentos en cautividad y de muestras de campo. Las temperaturas de los experimentos en cautividad se corresponden con temperaturas altas (17-21ºC), excepto en un caso en el que se aplicaron cambios de temperatura día/noche. Además se clasificaron gónadas de anchoa de 472 muestras de diferentes campañas (11948 hembras de anchoa). De éstas, 126 muestras (3348 hembras) fueron identificadas como procedentes de zonas con temperatura superficial alta (media ponderada =17.76ºC, s.d.=0.84) mientras que 131 muestras (3181 hembras) procedían de zonas con temperaturas superficiales bajas (media ponderada =14.42ºC, s.d.=0.75). Se ha encontrado una gran concordancia en la sucesión temporal de los estadios de FPOs tanto en los experimentos como en las muestras de campo. Los 4 primeros estadios de degeneración de FPOs ocurren en menos de 24 horas y para el final del primer día se alcanza mayoritariamente el estadio V. Los estadios VI y VII son mayoritarios durante la primera y segunda mitad del segundo día tras la puesta, respectivamente. La reabsorción total del folículo se produce en unas 55-60 horas. Para el rango de temperaturas examinado (13-19ºC), la influencia de la temperatura en la degeneración de los FPOs resulta pequeña. Los estadios de degeneración avanzada se encuentran a lo largo de todo el día, mostrando periodos de superposición de cohortes sucesivas. Finamente se discute la aplicación de estos resultados a la datación de los FPOs.

Palabras clave: Folículos postovulatorios, ciclo gonadal, frecuencia de puesta, anchoa.

INTRODUCTION

The daily spawning fraction of fishes can be estimated using the incidence of females with postovulatory follicles (POFs) that remain within the ovaries for a certain time period after spawning, until full resorption (Hunter and Goldberg, 1980; Hunter and Macewicz, 1980; 1985). This is a key parameter for the application of the Daily Egg Production Method (DEPM) (Parker, 1980; Lasker, 1985) devised to estimate the spawning biomass of indeterminate spawners. An understanding of the degeneration process of POFs over time was first achieved for northern anchovy (*Engraulis mordax*) by inducing spawning in aquaria and sampling females at known intervals, following their synchronous spawning (Hunter and Goldberg, 1980; Hunter and Macewicz, 1985). On the basis of this work, POF degeneration levels of the northern anchovy were described and grouped into three daily levels. The description of their degeneration over time, and its correspondence with spawning cohorts in the northern anchovy, has served to guide many of the subsequent applications of the POF method for estimating the spawning frequency, with minor modifications according to specific characteristics of each population (Alheit *et al*., 1984; Armstrong *et al*., 1988; Sanz *et al*., 1992; Palomera and Pertierra, 1993; and Ward *et al*., 2001). All these studies have revealed the ephemeral life of POFs, lasting usually between 15 to 72 h, according to species and spawning temperatures (Hunter and Macewicz, 1985; Clarke, 1987; Fitzhugh and Hettler, 1995).

Evaluations of the spawning frequency of European anchovy (*E. encrasicolus*), spawning at water temperatures of between 13.5 and 23ºC in the Bay of Biscay (Motos, 1994) and between 15 and 26ºC in the Mediterranean (Somarakis *et al*., 2004), have been based upon the application of the POF method defined by Hunter and Macewicz (1985), with minor modifications. However, no complete validation of degeneration of the POFs has been made for this anchovy species.

The application of the POF method to the European anchovy in the Bay of Biscay has faced several challenges which have led the ageing procedures of POFs to be revised. Firstly, the strategy of collecting adult samples from several sources (from adult collections made during the DEPM cruises and/or parallel acoustic surveys or from the commercial fleet), results in a continuous sampling of adults throughout the day. This sampling strategy increases the variety of degeneration states which must be allocated to the same daily spawning cohort; this, in turn, requires a good understanding of the degeneration of POFs over time. The description of the degeneration of POFs for the northern anchovy was more suitable to a discrete sampling period strategy (at night time). Furthermore, the increasing awareness of the effect that different water temperatures might have on the rates of degeneration of POFs (Fitzhugh and Hettler, 1995) made it necessary to examine the potential effect that changes in the average temperature of the sea water on different cruises might have on the degeneration of POFs and on the ageing procedures.

The objective of this study was to assess the degeneration rate of POFs until full resorption is reached within the ovaries of the European anchovy, both in captivity and in the wild and for the temperatures ranges encountered during adult sampling surveys in the Bay of Biscay. The implications of these results for ageing POFs and for the spawning frequency estimation are also discussed.

MATERIALS AND METHODS

The analysis of POFs was carried out following a new classification criteria based on degenerative features similar to those detailed by Hunter and Macewicz (1985), but seprating the staging of the degeneration of POFs from their ageing. The characteristics of the different POF stages used in the present study are summarised in Table 1. Additionally, atresia incidence on the anchovy ovaries was also analysed based on the work of Hunter and Macewicz (1985).

Analysis of captive anchovies

Captivity experiments in live bait tanks

Several experiments were carried out in 1990 on board the "*Divino Jesús de Praga*", a chartered purse-seine vessel equipped with tanks (Table 2, A to E). Anchovies were captured close to the peak spawning time, which is about midnight (Santiago and Eltink, 1988; Santiago and Sanz, 1992b; Motos, 1996). Right after the catch, anchovies were introduced into the tanks and maintained at densities of 50 kg/m^3. The tanks had an open circuit of running water collected from 1 m below the surface around

TABLE 1. – Summary of the morphological characteristics of the 7 POF stages used to characterise the degeneration of POF in the present study.

Stages	I	II	III	IV	V	VI	VII
Size	Large	Large	Large	Large 85% of new POF	Medium 68% of new POF	Medium 50% of new POF	Small 25% of new POF
Look	a)Form loose folds or loops b)Tightly folded	Tightly folded	Slightly reduced	Pronounced degeneration Few folds Regular form	Compact structure No folds	Highly degenerated	Long or polygonal remains between oocytes
Granulosa Cells	Arranged, Columnar Slightly hypertrophied	Marked alignment characteristics	Alignment characteristics still visible	Lost of the linear arrangement	Breakdown of cell walls	Absence of most of the cell walls	Absence of cells
Nuclei	Very large and prominent	Prominent Few of them Pycnotics	Many pycnotics	Mostly pycnotics	Pycnotics	Scarce Pycnotics	Very scarce Pycnotics
Vacuoles	Absence	Small Few	Small Affecting <50% of the granulosa cells	Medium in Massive incidence the granulosa cells	Large High incidence	Large Few	Absence
Theca	Noticeable Separated from the granulosa	With capillaries Separated from the granulosa	Noticeable Adheres to the granulosa	Becomes thinner and more closely adhered to granulosa	Still visible Pycnotic nuclei	Less distinct Incorporating stroma	Not visible
Lumen	Large Irregular with granular material	Large with granular material More regular	Easily visible Granular material still possible	Reduced	Hihgly reduced or absent	Absent	Absent

TABLE 2. – Details of catches of the adult samplings for the captivity experiments. A, B, C, D and E correspond to live bait tanks experiments while F and G correspond to the two aquarium experiments. Catch SST corresponds to sea surface temperature in Centigrade degrees at catch location. Experiment SST range is the range of the water temperatures in the tanks during the experiments in Centigrade degrees (for live bait tanks this is the SST measured in the areas and on the dates of the experiments).

Code	Date GMT	Time GMT (h)	Latitude	Longitude	Catch SST (ºC)	Experiment SST range (ºC)
A	06 May 1990	23:30	43º47'N	01º35'W	17.8	17.0-19.0
B	09 May 1990	04:00	44º46'N	01º38'W	17.7	17.0-19.0
C	05 June 1990	04:30	44º10'N	01º41'W	18.1	17.5-19.5
D	13 June 1990	00:05	43º26'N	03º26'W	17.8	17.0-19.9
E	13 June 1990	01:00	43º28'N	03º27'W	17.8	17.0-19.3
F	28 June 2001	04:20	43º33'N	02º20'W	20.0	20.5-21.6
G	03 July 2001	04:45	43º37'N	02ª19'W	21.0	13.1-21.9

the vessel. Temperatures in the tanks were thus maintained similar to the sea surface temperatures, ranging between 17 and 20ºC in the areas and on the dates when these experiments took place.

After being introduced into the tanks, sets of females were sacrificed every 4 or 6 h. On board the vessel, anchovies were sized, their body cavities were opened, and they were fixed in a 10% buffered formaldehyde solution. Females were subsequently taken to the laboratory, where they were weighed and the gonads were extracted and processed using standard histological procedures (Hunter and Macewicz, 1985; Motos, 1994).

Captivity experiments in the aquarium

In two successive weeks in the early summer of 2001, two samples of adult spawning anchovies were collected on board a commercial purse-seine, between 04:00 and 05:00 h GMT. Anchovies were immediately introduced into tanks on board the ship. Four hours later they were taken to the land-based aquarium, consisting of a 3500 l tank equipped with a sea water circulation system. Water was introduced from the sea surface after filtration by mechanical and UV processes. Excess water overflowed through the top of the tank, where an egg collector was placed

in order to detect the occurrence of spawning.

Two different experiments were carried out in order to observe the histological evolution of the gonads (Table 2). In the first experiment (F), anchovies were introduced into a tank with unregulated water temperature, around 21ºC. For the second experiment (**G**), the reproductive behaviour of anchovies was taken into account. Anchovy descend in the water column during daylight hours, moving up to surface waters during the night for spawning (Massé, 1996; Motos, 1996). Therefore, during the day water temperature was lowered to about 13.5ºC whereas at night it was raised to 22ºC. In practice, however, the lowering of water temperature in the tank took longer than expected, and the target temperature was not fully achieved until 15:00 h or 17:00 h. Warming was achieved faster in the evening, so the changing temperature cycle was in practice mitigated, with shorter periods of low water temperature than experienced by anchovies in the wild.

The first samplings took place 8 h after the catch, i.e. roughly 12 h after the peak of spawning. Subsequent sacrifices of about 50 anchovies were carried out every 12 h for 80 h. Females found in each sampling were separated and processed in the same way as in the live bait tank experiments. The egg collector was checked every 12 h.

Analysis of field samples

In order to validate the results obtained from the tank experiments, 472 adult field samples (11948 anchovy females) collected and processed for the anchovy DEPM surveys from 1990 to 2005 were examined in relation to their time of collection and the daily spawning period. These surveys take place every year from May to June in the area of the Bay of Biscay (from the Cantabrian Coast up to 47º39'N and from the French Coast to 5ºW) (Motos *et al*., 1996; Motos *et al.,* 2005).The potential effect of temperature on the rate of POF degeneration was addressed by analysing two subsets of samples for which sea surface temperature (SST) at capture location was precisely known (6529 females, covering a range of SST 12.4-19.8ºC). The first set of samples was captured at locations with SST above 16.5ºC for a total of 126 samples (3348 females) having a weighted mean SST of 17.76ºC (SD=0.84), i.e. a temperature close to those associated with captivity experiments. The second subset of samples came from locations with SST below 15.5ºC (131 samples corresponding to 3181 females) having a weighted mean SST of 14.42ºC (SD= 0.75). In both cases weighting factors were the number of females per sample. To compare the two sets, mean age by stage from the field samples was deduced from the average age after peak spawning time (assumed to be at midnight) of each stage over a 24-h cycle weighted by the incidence of the stage, at two hour intervals. The daily cycles used for averaging the ages for stages I to IV were their non-overlapping periods, while for the later stages the daily cycles started and ended at the midpoint of their overlapping periods, detailed in Table 6 of the results.

RESULTS

Captive anchovy results

For simplicity of presentation, only the results of three experiments are detailed; experiment A from the live bait experiments and the two aquaria experiments F and G (Table 2). Experiment A (Table 3) corresponds to an almost pure spawning cohort entering the tank, which was successfully followed for several days. The first aquarium experiment (F, Table 4) was the one carried out with the highest temperatures, while the second one (G, Table 5) was the only experiment with controlled daily changing water temperature throughout the day.

Spawning incidence

There was some minor spawning in experiment A during the first and second nights after the catch, as revealed by the presence of a few females with hydrated oocytes and/or POF Stages I to III (Table 3) after the spawning peak. In the aquarium experiments, the presence of eggs in the collector indicated the occurrence of some spawning during the first night in the tank.

POF occurrences

The first sampling, performed immediately after the catch of live bait in experiment A (Table 3), took place at 23:30 h near the spawning peak and showed that most of the females were in recent spawning conditions (POFs in Stages I and II). The females spawning on the night of capture thus constituted the modal spawning cohort in the tank. The

TABLE 3. – Results of the gonad analysis of the females from captivity experiment A (SST range 17-19°C), presented by sacrifices, date and time (GMT) of each sacrifice and elapsed time after assumed modal spawning time.

Sacrifice code	Sacrifice date	Sacrifice time (GMT)	Estimated hours after modal spawning	No. of females sacrificed	% of females showing									
					POF I	POF II	POF III	POF IV	POF V	PO VI	POF VII	POF 0	Atretic state 1 (active)	Atretic state 2 (inactive)
Initial	06 May 1990	23:30	0	21	43%	21%	7%	-	4%	4%	7%	14%	-	-
1	07 May 1990	03:15	4	12	17%	58%	25%	-	-	-	-	-	-	-
2	07 May 1990	09:30	10	6	-	-	33%	67%	-	-	-	-	-	-
3	07 May 1990	14:30	15	12	-	-	50%	8%	33%	-	8%	-	-	-
4	07 May 1990	20:30	21	12	-	-	-	-	58%	25%	17%	-	-	-
5	08 May 1990	02:30	27	12	-	-	-	-	17%	58%	25%	-	-	-
6	08 May 1990	07:00	31	12	-	-	8%	-	25%	42%	25%	-	-	-
7	08 May 1990	12:45	37	12	-	-	-	-		67%	33%	-	-	-
8	08 May 1990	20:15	45	12	8%	-	-	-	8%	33%	42%	8%	-	-
9	09 May 1990	02:15	51	12	-	17%	17%	-	8%	17%	42%	-	-	-
10	09 May 1990	05:30	54	12	-	-	-	17%	-	17%	42%	25%	-	-
11	09 May 1990	12:30	61	8	-	-	-	25%	-	-	-	75%	-	-
12	09 May 1990	19:45	68	8	-	-	-	-	-	-	-	100%	13%	13%
13	10 May 1990	03:30	76	8	-	-	-	-	-	-	-	100%	-	13%

time and date of the spawning event of the modal spawning cohort is hereafter simply named as modal spawning. In 10 h most of the females were in Stages III and IV. In 21 h the degeneration of POFs had advanced to Stages V and VI. The maximum of Stage VI occurred between 27 and 37 h after modal spawning. Between 45 and 54 h after modal spawning, the gonads revealed that most of the POFs were in Stage VII, and some of them showed no POFs. 61 h after modal spawning, full resorption of POFs had occurred since no females showed signs of recent spawning.

Most females of the initial sacrifices in both aquarium experiments (Tables 4 and 5) appeared to be in recent spawning conditions, with POFs in Stages III and IV and some in Stage V. According to the time elapsed since peak spawning time (12-13 h), and by analogy with experiment A, the females

TABLE 4. – Results of the gonad analysis of the females from Aquarium experiment F, presented by sacrifices, date and time (GMT) of each sacrifice and elapsed time after assumed modal spawning time.

Sacrifice code	Sacrifice date	Tank T(ºC) at sacrifice time	Sacrifice time (GMT)	Estimated hours after modal spawning	No. of females sacrificed	% of females showing									
						POF I	POF II	POF III	POF IV	POF V	PO VI	POF VII	POF 0	Atretic state 1 (active)	Atretic state 2 (inactive)
1	28 June 01	21.6	13.20	14	29	-	-	10%	48%	21%	3%	17%	-	-	-
2	29 June 01	21.1	08.40	33	29	-	-	-	-	3%	34%	55%	7%	28%	7%
3	29 June 01	20.9	20.00	44	22	-	-	-	-	-	-	45%	55%	46%	14%
4	30 June 01	21.2	08.00	56	27	-	-	-	-	-	-	11%	89%	26%	59%
5	30 June 01	21.3	19.50	68	12	-	-	-	-	-	-	8%	92%	33%	67%
6	01 July 01	21.3	08.20	80	14	-	-	-	-	-	7%	28%	64%	36%	64%

TABLE 5. – Results of the gonad analysis of the females from aquarium experiment G (controlled temperature), presented by sacrifices, date and time (GMT) of each sacrifice and elapsed time after assumed modal spawning time. Daily minimum temperatures were registered between 15:00 and 17:00 h every day, being 13.9ºC on the first 2 days of the experiment and 13.1ºC on the third day.

Sacrifice code	Sacrifice date	Tank T(ºC) at sacrifice time	Sacrifice time (GMT)	Estimated hours after modal spawning	No. of females sacrificed	% of females showing									
						POF I	POF II	POF III	POF IV	POF V	PO VI	POF VII	POF 0	Atretic state 1 (active)	Atretic state 2 (inactive)
1	03 July 2001	21.0	12:20	13	28	-	-	21%	36%	25%	7%	-	11%	-	-
2	03 July 2001	18.7	21:30	22	27	-	-	4%	4%	56%	37%	-	-	4%	-
3	04 July 2001	21.9	08:40	33	14	-	-	-	-	7%	43%	50%	-	14%	-
4	04 July 2001	16.1	19:45	44	18	-	-	-	-	-	22%	72%	6%	73%	-
5	05 July 2001	21.9	08:20	56	16	-	-	-	-	-	-	33%	67%	66%	13%
6	05 July 2001	18.4	19:30	68	17	-	-	-	-	-	-	18%	82%	30%	59%
7	06 July 2001	21.7	08:40	80	16	-	-	-	-	-	-	-	100%	19%	69%

in Stages III, IV and V should belong to the youngest spawning cohort originated at the peak spawning time on the night of capture. Females in Stages VI, VII or 0 of the first sacrifice should belong to older spawning cohort(s). There was a marked parallelism between A and the aquarium experiments, particularly with the controlled experiment (G): on the evening of the first day (22 h after modal spawning) most POFs were already at Stage V and 33 h after modal spawning the majority of POFs had reached Stage VI and VII. About 44 h after modal spawning most of the POFs were already at Stage VII, while 56 or more hours after modal spawning the majority of the females had no POFs. The development recorded in F is similar except for the earlier predominance of females with no POFs from 44 h after assumed modal spawning. This suggests either a slightly faster degeneration due to the higher temperatures or an effect of a higher mixture of older cohorts entering the tank.

Atresia incidence (Tables 3, 4 and 5)

Anchovies entering the tanks showed minimal or no signs of atresia. Atresia appeared about 68 h after the modal spawning in the live bait experiments. In the aquarium experiments early signs of atresia (atretic state 1, indicating some decrease in the spawning potential) appeared 22 h after modal spawning; anchovies became inactive after 56 h.

Given the general consistency throughout the above experiments and also with the other live bait experiments (not shown here), a visual summary of them all is shown in Figure 1. The sacrifices of the different live bait and aquarium experiments are merged on the basis of the estimated time elapsed (in 2 h steps) after the assumed modal spawning time. The total number of females sacrificed in the same time frame is thus added and new percentages by stages are inferred. This procedure provided a quasi-continuous overview of the degeneration of POFs throughout the stages in time.

Field sampling results

POF occurrences (Figs. 2, 3 and 4)

The analysis of the 11948 females showed that, for the total set of anchovies (Fig. 2) as well as for both sets of high and low surface water temperature (Figs. 3 and 4), Stages I and II only occurred

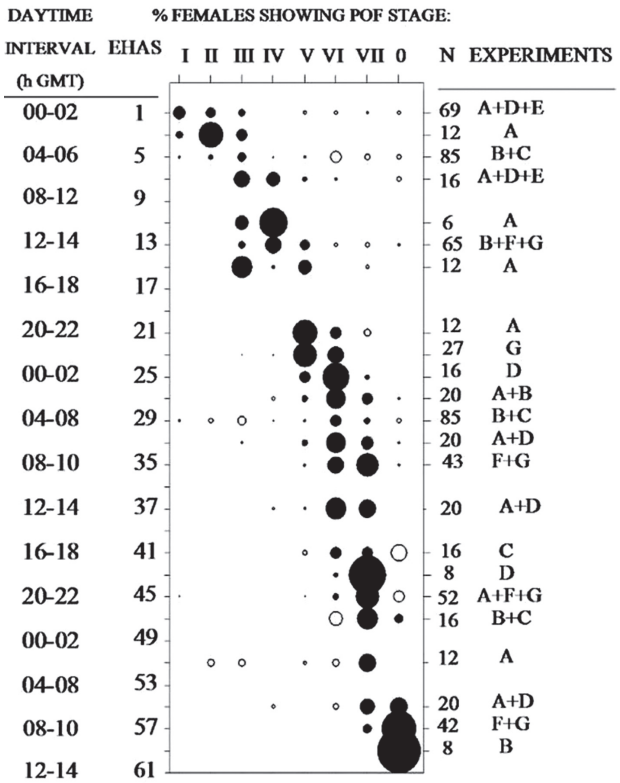

FIG. 1. – Percentages of occurrence of POFs degeneration stages over time, for all the experiments pooled together. EHAS, estimated hours after assumed spawning during two hourly daytime intervals. N, number of females analysed. EXPERIMENTS, codes of the experiments used for the present analysis. The size of bubbles is proportional to the relative abundance (%) per time interval of females showing a determinate POF stage. Black bubbles correspond to the likely progression of the degeneration of POFs during stages, over time, for the modal spawning cohort entering the tanks. White bubbles represent observations considered by the authors as unlikely to correspond to modal spawning cohort, i.e. "noisy" data for the present analyses; right-top observations would arise from a previous spawning cohort entering the tanks whilst left-bottom observations would correspond to later residual spawning taking place in the tanks.

at certain times of the day between the evening and midday. Stages III and IV showed very pronounced minima and maxima, congruent with the indication of a duration shorter than a day provided by the experiments with maximum occurrences between 00:00 h and 16:00 h and during day time respectively. Stages V, VI and VII had a more continuous distribution than the former stages, appearing in significant abundance throughout the day. Nevertheless, some periods of maximum and minimum incidence were still detected. The incidence of Stage V was at its peak from the afternoon until two hours after midnight. Stage VI had a maximum occurrence from the evening until the following midday. POFs at Stage VII showed maxima from morning to midnight. In both cases most of the samples showing no POFs were collected between 12:00 h and 24:00 h.

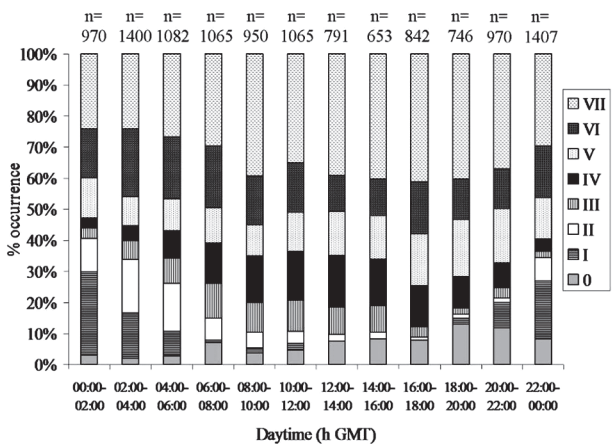

FIG. 2. – POF incidence-time analysis for the total of anchovy females analysed (% of occurrence) during two hourly daytime intervals. N represents the number of females examined at each daytime interval.

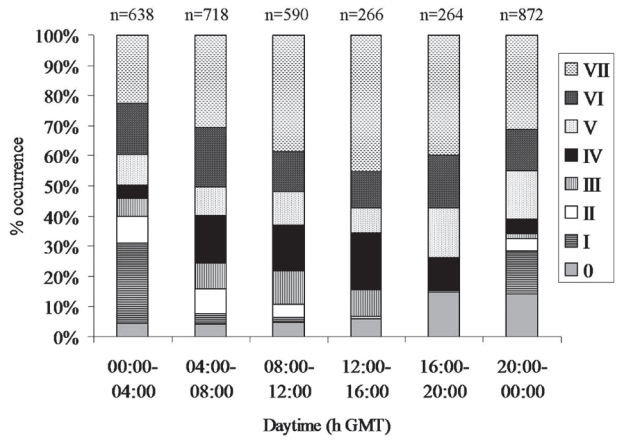

FIG. 3. – POF incidence-time analysis for the high water temperature dataset (% of occurrence), during four hourly daytime intervals. N represents the number of females examined at each daytime interval.

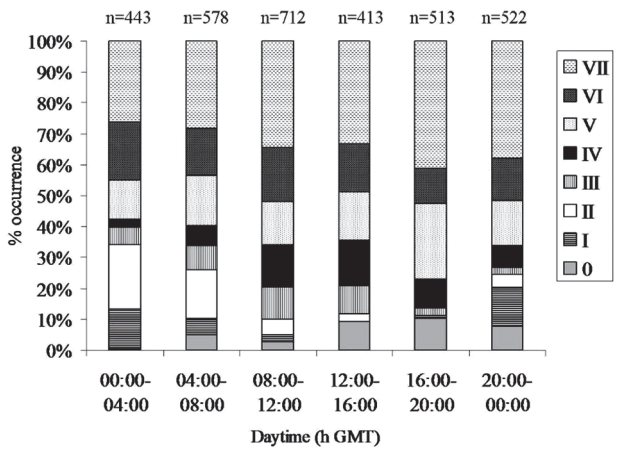

FIG. 4. – POF incidence-time analysis for the low water temperature dataset (% of occurrence), during four hourly daytime intervals. N represents the number of females examined at each daytime interval.

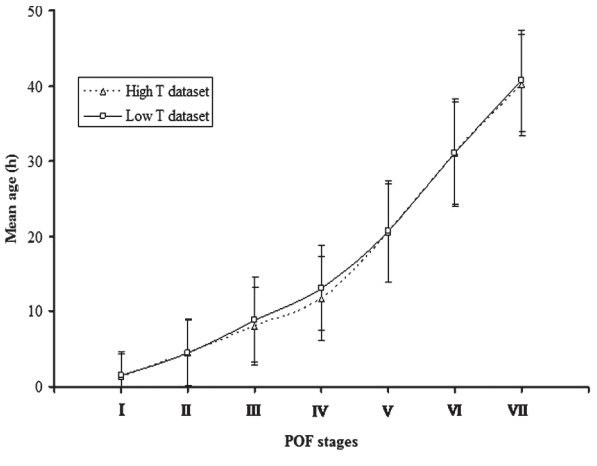

FIG. 5. – Mean ages in hours of POF stages obtained from the low and high water temperature sets of samples. Vertical bars correspond to one standard deviation of data of POF ages in hours.

Few changes were detected in the occurrence of POFs during the daily cycle according to the two sets of samples with different SST examined. Visually, in the low temperature dataset, the maximum occurrence of Stages IV, VI and VII seems to have been reached slightly later than in the high water surface temperature dataset. This suggests a global delay in the degeneration of POFs of about 4 h (Figs. 3 and 4). However, in the comparison of the average age of the stages during a complete day cycle, such differences are not evidenced (Fig. 5). Occurrence of females with no POFs (Stage 0) shows maxima in all cases in the afternoon and evening, but a higher incidence of this stage for the high temperature dataset than for the lower one is noticeable. Mean incidences in the period 16:00 to 04:00 h were 10.8 and 6.4% respectively. This difference is statistically significant with a likelihood ratio (G test) for heterogeneity of P(G) =1.12E-05 (Sokal and Rohlf, 1997).

Atresia incidence

In field samples, the incidence of atresia was in general minimal. In the samples corresponding to high temperature dataset, atresia signs were observed in 76 of the 3348 gonads examined, and only 10 of them corresponded to inactive females. The samples corresponding to a low temperature dataset revealed similar impact levels of atresia.

A summary of all the above results from the experiments and survey sampling concerning the occurrence in time of the different stages and their most likely ages is presented in Table 6.

TABLE 6. – Summary of time of occurrence and respective ages of the different POF stages based on the results obtained from both the captivity experiments and the field-survey samples.

POF Stages	Occurence after spawning (hours)	Daytime of maximum occurrence (GMT)	Modal ages (hours)	Mean ages Field data (hours) (Std. dev)	Ageing of POFs				
					Non-overlapping period		Overlapping period		
					Daytime (GMT)	Age of the cohort (hours)	Daytime (GMT)	Age of the cohorts (hours) Youngest	Oldest
I	-06 +06	18:00-06:00	-06 +06	1.1 (3.1)	16:00-16:00	-08 +16	-	-	-
II	-04 +12	22:00-10:00	-02 +10	4.6 (4.5)	18:00-18:00	-06 +18	-	-	-
III	-04 +20	00:00-16:00	00 +16	8.8 (5.6)	20:00-20:00	-04 +20	-	-	-
IV	00-24	04:00-22:00	04 +22	12.4 (5.6)	00:00-24:00	00 +24	-	-	-
V	06-36	10:00-02:00	10 +26	20.4 (6.8)	12:00-06:00	12 +30	06:00-12:00	06-12	30-36
VI	14-46	22:00-12:00	22 +36	30.4 (6.5)	22:00-14:00	22 +38	14:00-22:00	14-22	38-46
VII	24-60	06:00-24:00	30 +48	40.5 (6.7)	10:00-24:00	34 +48	00:00-10:00	24-34	48-58
0	> 44	06:00-24:00	> 56	NA	-	-	20:00-20:00	44-68	> 68

DISCUSSION

Degeneration of POFs and gonad cycle

None of the experiments carried out in this study could uniquely follow a single spawning cohort of females either because several past spawning cohorts entered the tanks or because there was some incidence of additional spawning in the subsequent days after captivity. For these reasons, there is a certain level of "noise" in the analyses undertaken on the results of the tanks on board the vessel and in the aquarium experiments (represented by white bubbles in Fig. 1). Nevertheless, all these results show a general agreement in the degeneration of POFs throughout the stages over time which allows the process to be tracked. The succession of POFs is a continuous degeneration process with some overlapping among contiguous POF Stages; this is indicative of the natural individual variability in the degeneration of POFs and probably of the variability in the spawning time.

The time of appearance of the maximum frequencies for the POF stages for the total set of wild samples, as well as for both subsets of high and low SST, and for captive anchovies (Figs. 1, 2, 3 and 4, and Table 6), is concordant; as expected, Stages I to IV show gaps or pronounced minimum occurrences in the sea samples throughout the 24-hour cycle since, according to the experiments, they last for less than 24 h. Furthermore, the correlative maximum occurrence of these four stages (18:00 h - 06:00 h, 22:00 h - 10:00 h, 00:00 h -16:00 h and 04:00 h - 22:00 h, respectively) fits well with the maximum occurrence in the experiments (Fig. 1). Stage V may last for about 24 h, showing maxima in the sea-collected females in the afternoon up until midnight, as occurs in the experiments. However, the occurrence of Stage V in the field data seems broader than in the experiments. As expected, Stages VI and VII, which appeared to last slightly over a day, occur throughout the day in field samples. However, the relative maxima shown by Stage VI during the night and the morning (22:00 - 12:00 h), and by Stage VII during the day and the evening (06:00 - 24:00 h) for the whole and for the high and low water temperature datasets of field samples are consistent with the maxima shown in the experiments. These consistencies prove that the duration and succession of POFs over the time shown in the experiments are valid and applicable to the field data, as considered in previous tank experiment studies (Hunter and Macewicz, 1985; Pérez *et al*., 1992; Fitzhugh and Hettler, 1995).

In the field samples, the number of females with no indication of recent spawning (absence of POFs) increased during daytime, showing their maxima in the afternoon and evening. This observation is consistent with the tank experiment results, because full resorption of POFs was completed within 56 and 60 h after spawning. The minimum detections of females without POFs during the night may be due either to the oversampling of active spawning females (Santiago and Sanz, 1992a) or to the direct recruitment of the females which had just fully reabsorbed their POFs into a new spawning activity.

Influence of temperature on POF degeneration

Most of the experiments were performed at high water temperatures (between 17 and 21ºC). The second of the aquarium experiments (G) attempted to simulate the circadian changing temperature of the environment inhabited by anchovies in the wild. However, the reduction of temperature did not reach the expected values. In fact, the weighted mean temperature in G experiment was about 18.5ºC, very

close to A and to most of the onboard experiments. Therefore, the degeneration of POFs in that tank was very close to that of the other experiments in high temperature waters (compare Tables 3 and 5) and in both cases full resorption of POFs was seen as predominant around 56-60 h after spawning. Consequently, no noticeable delay effect on the degeneration rates of POFs could be attributed to circadian changing water temperature in that tank.

In the field samples, some shift of the maxima occurrence in Stages IV, VI and VII in the low water temperature dataset relative to the high temperature dataset appears to indicate a cumulative delaying effect in the degeneration of POFs (Figs. 3 and 4); this is probably a result of the lower temperature of the first dataset. However, Figure 5 does not show any difference in the mean age of occurrence of stages between the two temperatures tested. This result might be the consequence of having produced mean ages over fixed ranges of 24 h by stages, instead of delaying those ranges for the low SST survey dataset. However, it was considered that any shift in the selected range for the latter dataset would have been too subjective. If one accepts that the cumulative delaying effect of lower temperatures on POF degeneration exists, the visual comparison of Figures 3 and 4 suggests that such a delay would be about 4 h. This low effect partly disagrees with those published earlier for other species (Fitzhugh and Hettler, 1995), which could be due to the moderate difference in average sea surface temperature between the two sets of field samples (about 3ºC). Alternatively, the most likely explanation could be the daily vertical migration of anchovy in the wild. During the hours of daylight, the distribution of anchovies close to the bottom makes them experience uniformly low temperatures (of about 12 or 13ºC) (Massé, 1996; Motos *et al*., 2005), without any influence of the SST; this fact will mitigate the average difference in the temperature inhabited by the anchovies from the two sets of selected samples. In fact, the expected differences of the weighted mean temperatures inhabited by the anchovies throughout a 24 h cycle for the two datasets would be half of the differences in SST (so about 1.5ºC in this case). In addition, the similar results obtained in the experiments and the field data endorse the perception of minimal influence of temperature on the degeneration of POFs for this anchovy at the range of temperatures covered in this study (basically between 13 and 19ºC, leaving aside the 21ºC of experiment F). Otherwise, the first aquarium experiment (F, Table 4) could suggest that a uniformly high temperature at about 21ºC would induce faster resorption of POFs, this being already noticeable 44 h after spawning. Though uncertainties arise from the mixture with an older spawning cohort at the start of this aquarium experiment, the higher occurrence of females without POFs between 16:00 and 04:00 h in the high temperature field dataset suggests that some faster resorption rates could actually take place in these field samples. This would imply some resorption of POFs since the evening of the second day after spawning at the highest SST of field data. Therefore, further research would be valuable to further understand the role of water temperature on the degeneration of POFs for this anchovy, particularly above the upper range of the SSTs studied here.

The rate of degeneration of POFs is slower than the rates observed for other small pelagics spawning in waters warmer than 20ºC, which usually achieve full resorption in less than 24 h. This is the case of the Hawaian anchovy (*Encrasicholina purpurea;* Clarke, 1987), the Bay anchovy (*Anchoa mitchilli*; Luo and Musick, 1991), and the Japanese anchovy (*Engraulis japonicus;* Funamoto and Aoki, 2002), occurring between 24-36 h depending inversely upon temperature. However, it is consistent with the rates observed for other small pelagic fishes spawning at temperature ranges of 13-21ºC, such as Peruvian anchovy (*Engraulis ringens*; Alheit *et al.*, 1984), which achieves resorption in around 54 h, Mediterranean sardine (*Sardina pilchardus*; Ganias *et al*., 2003), which achieves it in 47-58 h, and Iberian Sardine (*S. pilchardus*; Perez *et al*., 1992), which achieves it in more than 60 h. An exception to this is the northern anchovy for which full resorption takes longer: about 72 hours or more (Hunter and Macewicz, 1985).

The rapid resorption of POFs in the ovary (in under three days) and the low number of females with no signs of recent spawning (average incidence of Stage 0 of about 6.7%, SD=3.9%) suggest higher spawning frequencies than those reported in the 1990s for the European anchovy (ranging from 20 to 33%; Santiago and Sanz, 1992a; Somarakis *et al*., 2004). This may be due to the slightly faster degeneration rates of POFs for the European anchovy than for the northern anchovy, which had until now been taken as a reference for *E. encrasicolus* (Motos, 1996). But most probably it is due to the difficulties in the praxis of the previous ageing procedures, par-

ticularly in the direct allocation of a large variety of POFs (from a continuous sampling all day around) to the proper daily spawning cohorts. The current approach of separating the staging process of POFs from that of ageing should facilitate and make the latter more objective. All these results point to the need to revise the ageing procedures of POFs, combined with the estimation of the spawning frequency for the Bay of Biscay anchovy, taking into account the likely disturbance that oversampling of the most active spawning females can induce (Santiago and Sanz, 1992a).

The knowledge acquired about the duration of POF stages allows them to be aged over the periods of their major occurrence, according to the difference between the time of spawning and the time of capture, but adding 24 h (or 48 h) for the old stages when they are expected to occur during the second (or partly in the third) day after modal spawning (Table 6). This is particularly easy to apply for the entire period of occurrence of the first four stages of POFs, since they last for less than 24 h. For the older POF stages (Stages V to VII), lasting for about 24 h or more, this approach can also be applied but only for their periods around maximum occurrence when no overlapping between successive daily spawning cohorts can take place. In the overlapping period, some assumptions need to be made to allocate those stages to a spawning cohort. The simplest approach would be to set a single break point time in the middle of the overlapping period, after which all females showing the same POF stage could be assumed to belong to the youngest spawning cohort. This is the approach followed here to estimate the mean age of POFs (Fig. 5). A second approach would be to set symmetrical and gradual transition percentages for the recruiting and leaving spawning cohorts during the overlapping periods. For instance, for Stage V, according to daytime intervals of 2 h each, a 25, 50 and 75% recruiting process for the most recent to the oldest spawning cohort can be devised for the overlapping period, between 06:00 and 12:00 h. Analogous gradual transition vectors for the recruiting spawning cohorts can be designated for the overlapping periods of Stages VI and VII. The assumptions required for the increasing overlapping periods of the most advanced degeneration stages of POFs, as well as the higher affectation of their degeneration rates by the cumulative effect of temperature, lead us to conclude that ageing POFs younger than 24 h is essentially more objective and reliable than ageing older ones. This favours the use of a Day 1 spawning cohort to estimate spawning incidence for this anchovy species.

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