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Anchovy egg development in the Gulf of Cádiz and its comparison with development rates in the Bay of Biscay

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ABSTRACT

Multinomial models were used to analyse anchovy (*Engraulis encrasicholus*, L.) egg development in the Gulf of Cádiz and provide the required egg development model for the application of the daily egg production method in this area. Also, models were modified to allow for a statistical comparison between the egg development in the Gulf of Cádiz and in the Bay of Biscay. The Gulf of Cádiz data was obtained from an incubation experiment carried out during July 2007, where wild adults were collected, an *in vitro* fecundation was immediately performed and viable eggs were maintained in an on-land incubator at five different controlled temperatures (approximately 10, 14, 18, 22 and 26 °C). Eggs were sampled hourly and classified into stages until they developed into larvae. The model of the combined data from the Gulf of Cádiz and the Bay of Biscay show no differences between areas, suggesting that for the same temperatures, the total duration of the egg phase is similar for the Gulf of Cádiz and for the Bay of Biscay.

1. Introduction

Anchovy is a small pelagic fish with a short life span, high reproductive potential and high mortality rates, especially in the early life stages (Freón et al., 2005). Anchovy stocks typically achieve large biomasses in their ecosystems, and show fluctuations at different time scales (short term fluctuations due to variable recruitment and long term fluctuations due to a combination of environmental conditions or so-called regime shifts, e.g. Chavez et al., 2003). Most of the variability for anchovy stocks is generated by processes that affect the survival and growth of the early life stages (eggs, larvae and juveniles) such as predation, competition and settlement (Bakun, 1996). The dynamics of those early life stages in turn control population recruitment, which is the main driver for the fluctuations in stock biomass. Understanding the physiological changes and the dynamics of early life stages of anchovy has therefore been an important objective for their stocks worldwide. Also, estimates of anchovy egg production (number of released eggs in the spawning area) form the basis the daily egg production method (DEPM; Parker, 1980; Lasker, 1985). The DEPM is one of the most common fishery-independent estimators of spawning stock biomass used for anchovy stocks worldwide (see a review of its use in anchovy and other stocks in Barange et al., 2009). In short, the DEPM estimates SSB by comparing the daily total number of eggs produced in the spawning area with the reproductive capacity per individual, hence estimating the number of individuals (or biomass) required to produce a certain number of eggs.

Early fieldwork on egg development of anchovy and other species led to morphological keys that classified the egg phase into different stages (e.g. Bolin, 1936). Laboratory experiments under controlled conditions allow a more detailed analysis of morphological characteristics of the different stages, and have confirmed that several factors, but especially temperature, has a large effect on egg development rates (see a review in Pepin, 1991). These experiments led to the development of temperature-dependent egg development models (Lo, 1985), which are also required to apply the DEPM for species with fast egg development rates (Bernal et al., 2011), as they are needed to infer egg production from the abundance of eggs at different stages.

Early egg development models were based on classical statistical approaches in which either independent models for the duration of each stage were used (Zweifel and Lasker, 1976), or else average age by stage and temperature is first computed from the observed data, and then a common model is used (Lo, 1985). In recent years, multinomial models that utilise the raw data (i.e. the number of eggs by stage at each sampling time, instead of average ages for each stage) from all stages and temperatures from the incubation experiments have been developed (Ibaibarriaga et al., 2007;

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Bernal et al., 2008). Such models can provide a better description of the development process and provide a coherent statistical framework for the analysis of the effects of different variables on the development process.

In this study, multinomial models were used to analyse anchovy (*Engraulis encrasicholus*, L.) egg development in the Gulf of Cádiz (GoC) and provide the required egg development model for the application of the DEPM in this area. The GoC stock is the second largest stock of this species in the Iberian Peninsula, with similar and occasionally larger biomass than the main anchovy stock in the Bay of Biscay (BoB; ICES, 2009). DEPM together with acoustics are used the main fisheries independent biomass assessment tools, although the DEPM has only been recently implemented in the GoC (ICES, 2009), while it has been used in the BoB since the late 1980s (Motos, 1994). Initial applications of the DEPM in the GoC used the egg development model of the BoB anchovy (Ibaibarriaga et al., 2007), although the necessity to test for potential differences in egg development between the BoB and the GoC was recognised by the related ICES Working Group (ICES, 2006). The importance of the fisheries independent methods in both areas have increased with the gradual introduction of biomass differential models as the official assessment tool (Ibaibarriaga et al., 2008), substituting previously used tuned catch-at-age models (ICES, 2009).

In order to analyse egg development in GoC anchovy, a laboratory experiment similar to previous anchovy egg incubation experiments in the BoB (Motos, 1994) was carried out in the area. The results were analysed using multinomial models (Ibaibarriaga et al., 2007; Bernal et al., 2008), and the capability of these models was extended to statistically compare egg development rates in the GoC with that observed in the BoB, and hence deal with the ICES assessment group concerns (ICES, 2006).

2. Materials and methods

2.1. Field samples

Adult anchovy were caught within the main spawning area and season in the Gulf of Cádiz in July 2007, at a location situated at 36°53′N 06°27′W (Fig. 1), using a commercial purse seiner. The haul was performed between 19:30 and 20:00 GMT, near the daily peak spawning time for this species in this area (ICES, 2006). Running females and males were selected and immediately separated from the catch for *in vitro* fertilisation (see below). A representative sample from the total catch was also taken to the laboratory for biological sampling (length, weight and sex distribution).

2.2. In vitro fertilisation

Eggs from 44 hydrated females (size range 10.5–13.0 cm TL) were extracted by abdominal massage, and placed into a 50 ml graduated jar to register the volume. Sperm from 15 ripe males (size range 11.0–12.5 cm TL) was collected using a 1 ml syringe, after drying and massaging the abdominal area. Once the total egg volume was measured, eggs were transferred to a 5 l jar and the sperm was poured over them, adding filtered (1 μm mesh) seawater in the same volume as the eggs (Table 1). The jar was gently mixed and left for 5 min. After this, filtered seawater at ambient temperature (~22 °C) was added up to a total volume of 1 l to allow for the hydration of the chorionic membrane. At this moment, a timer was started to register the elapsed time since fertilisation, and the jar was transported to the incubator located on the laboratory.

2.3. Laboratory

An egg incubator that provides five different temperatures was developed for this experiment. The selected range of temperatures was 10, 14, 18, 22, and 26 °C, similar to the range of sea surface temperatures (SST) during the spawning season in the Gulf of Cadiz, but also including a temperature below the observed range (10 °C), in order to test the lower temperature limit for egg development (see Ibaibarriaga et al., 2007; Bernal et al., 2008).

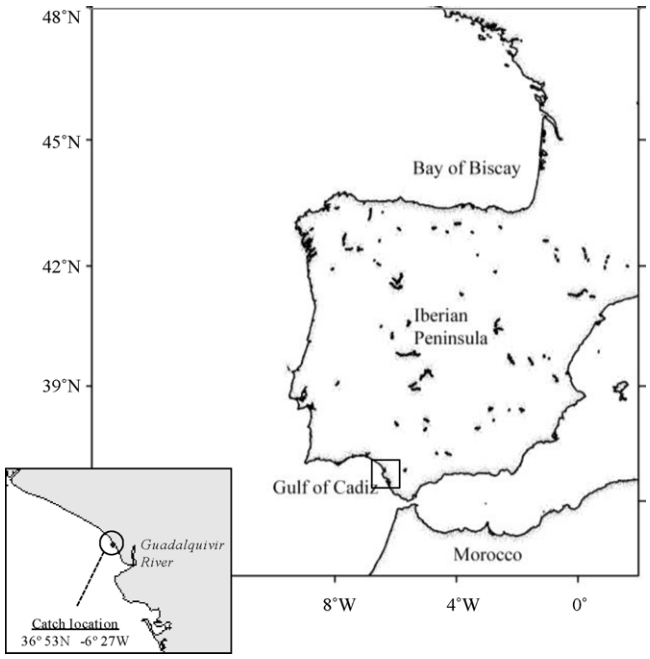

Fig. 1. Location of the fishing haul carried out in the Gulf of Cádiz where the adults employed in the incubation experiment were caught.

The incubator consisted of a series of vessels filled with filtered seawater located inside five different aquaria partially filled with tap water and equipped with different devices to maintain the water temperature at the chosen levels. The overall structure was placed inside a 9 m^2 temperature-controlled room, set at 18 °C. Temperatures below 18 °C were obtained by putting two aquaria inside a water bath of approximately 100 l, partially filled with water refrigerated at 10 °C by a refrigeration device (or cooling coil). One of the aquaria in the refrigerated container was used for incubations at 10 °C and therefore did not require any extra temperature-controlling device. The other aquarium within the refrigerated container was equipped with a thermostat to increase the inner aquarium temperature to 14 °C. The remaining three aquaria, located outside the refrigerated container but inside the temperature chamber, where equipped as follows. One of the aquaria did not have any temperature-controlling device, and therefore remained at (18 °C). The other aquaria were equipped with high precision thermostats set at 22 and 26 °C. All aquaria were equipped with a water circulation system to homogenize the temperature, and all of them were uncovered in order to permit water oxygenation.

Table 1
General information about the incubation experiment carried out in Gulf of Cádiz in July 2007.

Position (Lat–Long)	36°53′N–06°27′W
Time of capture (GMT)	19:32
Time of fertilisation (GMT)	20:50
Time of incubation start (GMT)	23:44
Water temperature at fertilisation (°C)	21–22
N females	44
N males	15
Volume oocytes (ml)	15
Volume sperm (ml)	0.6

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Each aquarium contained a set of four 60 ml vessels filled with filtered seawater and partially submerged. Three of the vessels were used to incubate eggs at the desired temperatures, while the fourth vessel was used to refill any partial loss of water in the other vessels. The purpose of using three vessels with eggs is to minimise the perturbation of the sampling process in the sample, and the three vessels with eggs were not used as "replicates", but else they are treated altogether as the sampling unit, and therefore the results refer to average development in the combined population of the three vessels. Salinity was registered approximately every 4 h during the experiment. Any loss of water from the vessels (by evaporation or by losses when handling the eggs for staging) was replaced using the extra vessel containing filtered seawater at the same temperature. All of the thermostats were initialised 1 day before the experiment was carried out, in order to stabilize the temperatures in each aquarium and inside the vessels.

After approximately 2 h 50 min from fertilisation, viable eggs (identified as those floating on the surface of the water) were transferred from the 5 l fertilisation jar to each of the three dedicated vessel within each aquarium. A similar amount of water and eggs from the jar was transfer to each of the vessels with a pipette, with the objective to deposit a similar amount of eggs in every vessel. Disintegrated or dead eggs (eggs that stayed at the bottom of the jar) were discarded.

Around 50 eggs from each of the temperatures of incubation were sampled every hour. The elapsed time from the beginning of the experiment, the initial and final temperatures of every aquarium and the temperature (initial and final) of the chamber were recorded. Each vessel was only sampled once each three consecutive sampling events. Sampled eggs were immediately staged under a binocular microscope using the morphological keys of Moser and Ahlstrom (1985) (Table 2), and once the staging was finished, eggs were returned to the vessel they were taken from. This means the experiment follows a "sampling with replacement" scheme, adequate to obtain a multinomial distribution of observations (see Section 2.4 below), in comparison with the more commonly used "sampling without replacement" scheme, which would have provided a hypergeometric multinomial distribution (Degroot, 1986). Staging for each sample took around 10–15 min, which means that a given egg stayed outside the incubator for a maximum of 15 min each 3 h (in the improbable case the same egg was resampled in all occasions).

Dead eggs (which can be identified by their unusual appearance and usually sink to the bottom of the vessel) were counted, and removed from the vessel at each sampling interval (1 h). Sampling from each aquarium (or temperature of incubation) was considered completed when all eggs were dead, or when hatched larvae had finished the absorption of the yolk sac. Total egg density in each vessel was estimated at the end of the incubation experiment, by adding the total number of dead eggs and larvae up to the end of the experiment and dividing by the volume of the vessel.

2.4. Data analysis

The proportion of eggs by stage at a given combination of elapsed time from the start of the experiment and temperature was modelled as a multinomial distribution with k ordered categories (Ibaibarriaga et al., 2007), where k is the number of egg stages plus a larvae stage (i.e. twelve). In order to model all stages together, an extended continuation ratio model (ICES, 2004; Stratoudakis et al., 2006; Bernal et al., 2008) was used. This model uses the number of eggs in category i ($i = 1,\ldots,k$) and above ($i+$) as the number of successes of a binomial distribution, and the number of eggs having reach the previous stage and above ($i-1+$) as the number of trials. For example, for stage 3 at a given sampling time, the number of trials is the sum of the number of eggs in stages 2 and above (2–12) while the number of successes is the sum of the abundance of eggs in stages 3 and above (3–12). The chosen variables to model Gulf of Cádiz egg development were temperature, stage and age (hours since fertilisation), as in Ibaibarriaga et al. (2007), and the full model used allowed for first order interactions between them:

$$\text{Logit}\,(P[i+|(i-1)+]) = \alpha_1\,\text{Age} + \alpha_2\,\text{Temp} + \alpha_3\,\text{Stage} + \alpha_4(\text{Age}:\text{Temp}) + \alpha_5(\text{Age}:\text{Stage}) + \alpha_6(\text{Temp}:\text{Stage}) \quad (1)$$

where Temp is water temperature at sampling time and (var_1:var_2) indicates the interaction between two variables (e.g. Age:Temp indicates the interaction between age and temperature), and α_j (for j in 1,. . .,6) are the model parameters. The age referred above is defined as the elapsed time from the beginning of the incubation experiment until the sampling event. A "quasi-binomial" distribution (dispersion parameter estimated from the data) with a probit link was used to relate the response to the linear predictor, and model selection from the full model shown in Eq. (1) was carried out using Chi-square tests (as the dispersion is not constant and therefore likelihood ratio test cannot be used).

In order to compare the development of GoC and BoB anchovy eggs, the model represented by Eq. (1) is extended to allow for different model parameters for each region. In order to do that, all model parameters α_j are allowed to interact with a strata factor with two levels, BoB and GoC, and the observations from the incubation experiments carried out in both areas within a common temperature range were analysed together. The common temperature range was 18–22 °C, and includes observations from three temperatures in the BoB (18, 20 and 22 °C; Ibaibarriaga et al., 2007) and two observations from the GoC (18 and 22 °C). Model selection was also carried out as described before from the derived full model to check for the significance of stratum and the other variables in the model.

All analysis was carried out using R (R Development Core Team, 2010) and the R libraries "egg" and "eggsplore" (Bernal et al., 2008; https://sourceforge.net/projects/ichthyoanalysis).

3. Results

3.1. Incubation experiment

Temperature levels remained approximately constant through the experiment, although there were differences between the incubators in which precision thermostats were used and the ones in which the temperature was maintained directly through the temperature chamber temperature regulator (18 °C) or the refrigeration device (10 °C; Fig. 2). Average egg density was similar for all

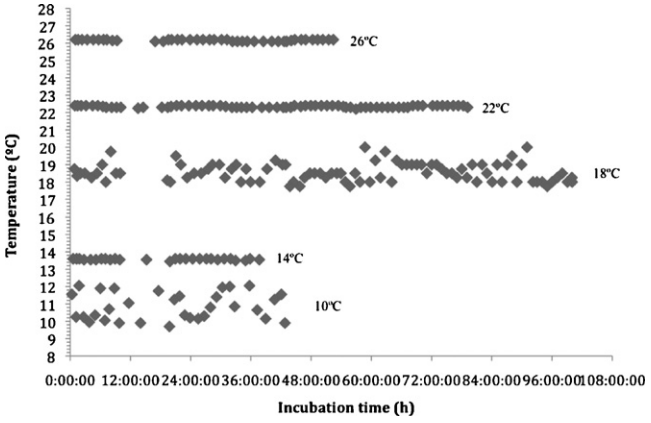

Fig. 2. Temperature (°C) observations in the different incubators during the Gulf of Cádiz anchovy egg development experiment.

Table 2
Anchovy egg stages; morphological key (adapted from Moser and Ahlstrom, 1985 and modified for Atlanto-Iberian anchovy stocks in ICES, 2002).

Stage	Description
I	The cell division has not begun. The cytoplasm of the single cell appears as a clear hemisphere at one pole, the cytoplasm may be displaced to other locations around the periphery of the yolk mass. The unfertilised eggs are included in this stage.
II	*START*: Cell division starts (initially small bubble-like structures are often visible). The cytoplasm divides into 2, 4, 8, 16, 32..... cells. After the cytoplasm has divided into 32 cells it is very difficult counting them, the blastodisc has a mulberry-like appearance. *END*: Blastula: cells are very small but it is still possible to distinguish them individually.
III	*START*: When it has the appearance of tissue rather than of a collection of individual cells. The segmentation cavity is visible. *END*: The blastodermal capal cap is ≤1/3 down the yolk mass and the bilateral nature of the primordial embryo is apparent.
IV	*START*: The blastodermal cap is >1/3 of the yolk mass. *END*: The blastodermal cap is ≤2/3 of the yolk mass.
V	*START*: The blastodermal cap is >2/3 of the yolk mass until complete enclosure of the yolk. *END*: Closure of the blastopore; the tail lies flat against the polar region of the yolk and continues the line of the yolk mass. In this stage there is a rapid differentiation resulting in the formation of several myomeres in the mid-region of the embryonic axis.
VI	*START*: The blastopore is always closed and the tail thickness is apparent. *END*: The tail remains against the yolk mass. The angle formed between the tail and the yolk is ≥90° During this stage the myomeres are apparent along the entire body axis (except at the caudal portion).
VII	*START*: The tip of the tail is free from the yolk and mostly rounded. *END*: The length of the free tail is ≤1/2 the length of the head. The angle formed between the tail and the yolk is <90°
VIII	*START*: The length of the free tail is >than 1/2 the head length. *END*: The tail length is ≤1/4 the yolk sac length. In this stage, the tail becomes pointed and begins to bend away from the axis of the body.
IX	*START*: The tail length is >1/4 the yolk sac length. *END*: When the tail ≤ 1/2 the yolk sac length. The curvature of the tail is evident in this stage. The gut is now apparent along the ventral surface of the tail and the fin-fold is now considerably wider than in the previous stage. From this stage on, it is consider the free portion of the tail from the body and not from the fin-fold. From this stage on, the end of the embryo is considered as the end of the fin-fold.
X	*START*: The tail length is >1/2 the yolk sac length. *END*: The tail length is ≤3/4 the yolk sac length.
XI	*START*: The tail length is >3/4 the yolk sac length *END*: Hatching.

temperatures except for the 14 °C incubation vessels, where, once all eggs were sampled, density was confirmed to be double the one in the rest of the temperatures (Table 3).

A total of 7753 eggs were staged during the experiment. Few observations of eggs at stage one were recorded for all temperatures. As sampling only started about 3 h after fertilisation, and eggs were held at approximately 22 °C before the incubation started, most eggs were in stage two when the experimental incubations began. In order to derive the development model (see below), all eggs from all temperatures were assumed to be at stage one at time 0, and the number of observations for stage one at time 0 were assumed to be equal to the average number of eggs sampled by sampling event (Nstage1 = 39 at time 0 for all temperatures in Table 4).

For the upper three temperatures, eggs developed through all stages producing live larvae. None of the eggs incubated at the lowest temperatures (10 and 14 °C) reached hatching (Table 4), and only developed to stages 6 (at 10 °C) and 5 (at 14 °C), respectively. For the 10 °C temperature, a very low percentage of eggs in stages 4, 5 and 6 were observed. A large number of dead eggs were observed in the 14 °C incubator at all sampling events, and no live eggs remained in the vessel after 39 h, forcing to stop the experiment at this temperature (Table 5). Only data from incubation temperatures at which development reached hatching (18, 22, and 26 °C) were used to model egg development.

Stages 2 and 6 were the most frequently observed in eggs incubated at 18 °C, and stages 3 and 6 in 22 °C (Table 4). In 26 °C the stages 7 and 8 were the most frequent. Stage 11 represents the stage with fewer observations registered in all temperatures, with few observations in 18 and 22 °C and only one observation in 26 °C (Table 4). A clear decreasing trend of age by stage for increasing temperatures is observed, indicating that the duration of every stage is smaller for the highest temperatures (Fig. 3). Total incubation time up to hatching decreases with temperature (55 h at 18 °C to 28 h at 26 °C; Table 3, Fig. 3), and an increasing degree of overlapping among stages is observed as eggs approach the hatching

Table 3
Mean temperatures, mean salinities, total incubation, time until hatching and mean number of sampled eggs and larvae for each incubator with the, respectively, standard deviation (sd) in brackets, as well as eggs density per vessel.

Incubation temperatures (°C)	Mean temperature (°C) and (sd)	Salinity (sd)	Time to hatch (h)	Mean number of eggs sampled (sd)	Mean number of larvae sampled (sd)	Egg density/vessel (eggs/60 ml)
10	10.85 (0.81)	35.85 (1.14)	–	38 (19)	–	2256
14	13.57 (0.05)	37.57 (1.7)	–	35 (21)	–	4344
18	18.55 (0.57)	39.09 (2.3)	54.88	41 (20)	14 (13)	2797
22	22.35 (0.05)	41.21 (1.82)	35.97	42 (21)	20 (18)	2007
26	26.17 (0.05)	41.3 (1.64)	28.08	40 (18)	3 (4)	2176

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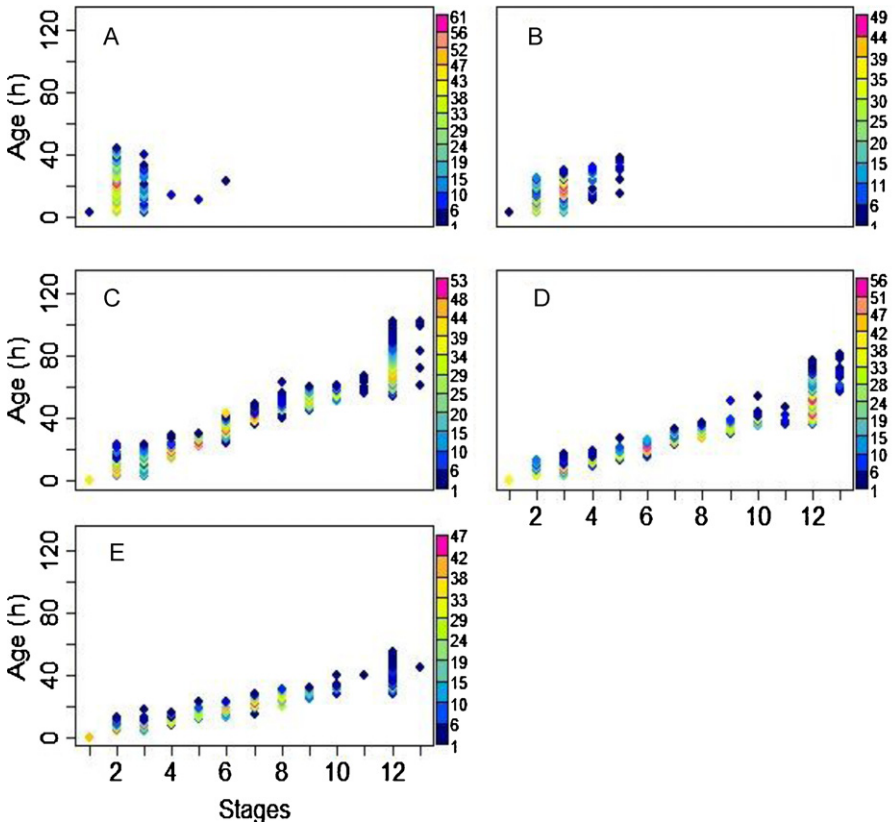

Fig. 3. Observed ages for each stage and temperature in the Gulf of Cádiz anchovy eggs incubation experiment. The colour gradient represents the number of eggs counted in that stage at that temperature and sampling time (the abundance scale is on the right side of each graph). Panels represent the temperatures used in the experiment: (A) 10.85 °C, (B) 13.57 °C, (C) 18.55 °C, (D) 22.35 °C and (E) 26.17 °C.

Table 4
Total number of sampled eggs per stage and temperature. The temperatures correspond to those used in the anchovy incubation experiment. For these temperatures, the abundance of eggs in stage 1 at time 0 was assumed to be equal to the average number of eggs in a sample through all the experiment (39 eggs).

Stage	Temperature (°C)					Total
	10.85	13.57	18.55	22.35	26.17	
1	2	1	39[a]	39[a]	39*	120
2	1167	420	408	199	151	2345
3	237	570	294	241	151	1493
4	4	79	335	147	144	709
5	3	16	259	147	150	575
6	1	0	359	318	140	818
7	0	0	171	153	199	523
8	0	0	106	187	187	480
9	0	0	281	172	59	512
10	0	0	157	103	14	274
11	0	0	9	11	1	21
12	0	0	676	859	75	1610
Total	1414	1086	3094	2576	1310	9480

[a] No eggs in stage 1 were observed for temperatures above 13.57 °C.

Table 5
Summary of Gulf of Cádiz anchovy eggs and larvae duration (end sampling time in the incubation experiment) per temperature.

Incubation temperatures (°C)	Eggs end sampling time (h)	Larvae end sampling time (h)
10	44.88	–
14	38.63	–
18	67.85	102.83
22	53.96	81.15
26	39.47	54.32

point, especially for larger temperatures. Sampling continued until all larvae died from starvation, with the maximum duration from the beginning of the incubation up to the end being 4 days and a half (103 h at 18 °C) and the minimum time from the beginning of the incubation up to the end being 2 days (54 h at 26 °C) (Table 5). The maximum duration of a larva from the hatching up to its death was almost 1 day and a half (34 h at 18 °C) and the minimum duration was half of a day (15 h at 26 °C).

3.2. Cumulative mortality

Temperatures 10, 14 and 26 °C show higher cumulative mortality rates than 18 and 22 °C, quickly reaching values of near one at around 36 h (Fig. 4). For temperatures 10 and 14 °C, the high mortality rate observed led to the death of all eggs before stage 6, and therefore hatching did not occur for those temperatures. For the temperatures at which hatching occur (18, 22 and 26 °C), mortality rates reduce for older stages and for larvae (in a similar way as reported by Pepin, 1991), so the cumulative mortality curve show a clear asymptote shape. During the larvae stage, mortality is quite low until starvation occurs. For higher temperatures, starvation happens faster (the larvae die first at 26, later to 22, and finally at 18 °C). These results are also coherent with the reported by Pepin (1991) that suggest a reduction of the duration of the yolk-sac larvae stage with the increase of the temperature.

3.3. Multinomial model of GoC egg development

The chosen model only includes stage, age and temperature as covariates, while first order interactions between the covariates were not selected (Tables 6 and 7). The chosen model showed a

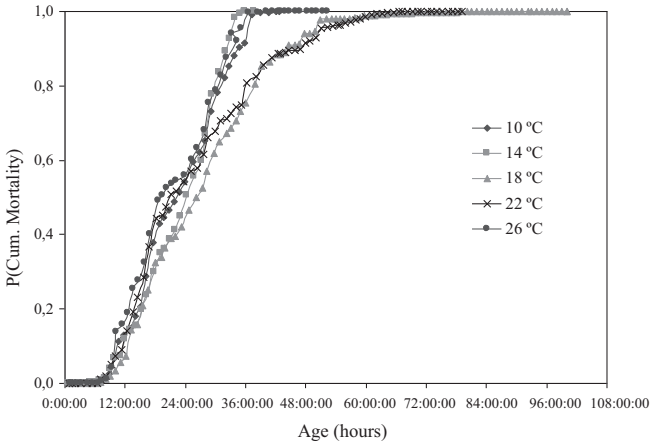

Fig. 4. Gulf of Cádiz anchovy eggs cumulative mortality at each temperature during the incubation experiment.

Table 6
Gulf of Cádiz anchovy egg development backwards model selection from the full used in this work (see Eq. (1) in the text). Each row below the full model row shows the term deleted at each step, the residual deviance of the resulting model and the probability of being equal to the full model.

Model	Null deviance	Residual deviance	*Pr*(Chi)
Full model	67581.8	2860.6	
Stage:Temp		3245.7	1.000
Age:Stage		3873.4	1.000
Age:Temp		8433.6	0.879

high prevalence of eggs in stages 2, 6 and 8 for 18 and 22 °C and 6 and 8 for the 26 °C incubator (Fig. 5). These stages have a longer duration compared with other stages, as indicated by the width of the fitted curves. The modelled curves do not reach the maximum frequencies observed for some stages at some temperatures (e.g. stage 6 at 18 °C). This reflects some lack of flexibility of the models selected, as they have to model the data from all temperatures, and higher temperatures show an increasing degree of overlapping between stages. However, the modal age for each stage is in general well represented by the mode of the fitted curves, indicating that average ages for each stage and temperature are well represented by the model. The probability of finding stages 10 and 11 in the samples is very low, and especially the transition to stage 11 is not clearly marked at the higher temperatures. The fitted multinomial model shows that the total time of incubation up to the hatching as well as the duration of every stage also decreases with the increase of the temperature (Fig. 5).

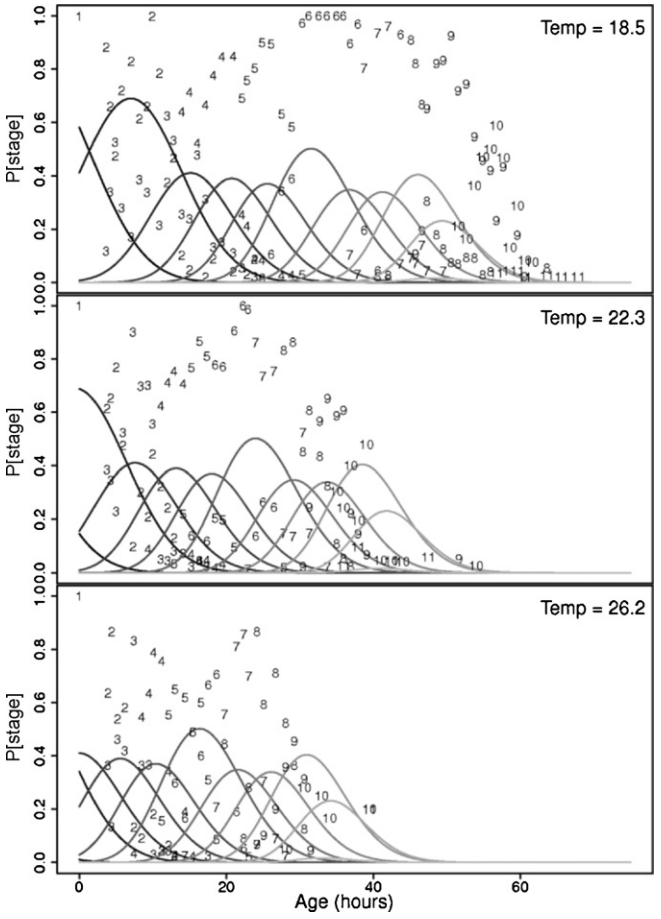

Fig. 5. Observed frequency and modelled probability of anchovy egg stages in the Gulf of Cádiz for each age and temperature used in the incubation experiment (from top to bottom 18, 22 and 26 °C). The numbers (1–11) within each plot represent the observed frequencies of the different stages (stage 1 to stage 11) at each sampling time. Each line represents the evolution of the modelled probability of eggs being in a given stage over age and temperature.

3.4. Comparison with BoB egg development

The selected model of the combined data from the Gulf of Cádiz and the Bay of Biscay did not show any regional variability in any of the fitted parameters (all interactions with area were rejected, Table 8). Therefore, for the same temperatures, the egg development process in the Gulf of Cádiz and the Bay of Biscay can be described using a common model.

Table 7
Summary of the chosen anchovy egg development multinomial model for the Gulf of Cádiz.

	Estimate	Std. Error	*t*-Value	*Pr*(>|*t*|)
Age	0.1664	0.0090	18.53	0.0000
Temp	0.3309	0.0239	13.87	0.0000
Stage 1	−1.0214	132.4458	−0.01	0.9938
Stage 2	−6.3470	0.5485	−11.57	0.0000
Stage 3	−8.2615	0.5818	−14.20	0.0000
Stage 4	−9.0007	0.6194	−14.53	0.0000
Stage 5	−9.8306	0.6580	−14.94	0.0000
Stage 6	−10.5824	0.6978	−15.17	0.0000
Stage 7	−11.8625	0.7458	−15.91	0.0000
Stage 8	−12.4152	0.7850	−15.82	0.0000
Stage 9	−13.1130	0.8143	−16.10	0.0000
Stage 10	−14.0552	0.8489	−16.56	0.0000
Stage 11	−14.1863	0.8710	−16.29	0.0000
Stage 12	−12.7390	0.9188	−13.86	0.0000

Table 8
Summary of the chosen anchovy egg development multinomial model for the combined data from the Bay of Biscay and Gulf of Cádiz.

	Estimate	Std. Error	*t*-Value	*Pr*(>|*t*|)
Age	0.1941	0.0068	28.41	0.0000
Temp	0.5996	0.0261	22.93	0.0000
Stage 1	−5.9112	75.9138	−0.08	0.9379
Stage 2	−11.2033	0.5372	−20.86	0.0000
Stage 3	−13.6071	0.5786	−23.52	0.0000
Stage 4	−14.5768	0.6096	−23.91	0.0000
Stage 5	−15.5764	0.6391	−24.37	0.0000
Stage 6	−16.5975	0.6746	−24.60	0.0000
Stage 7	−18.4260	0.7218	−25.53	0.0000
Stage 8	−19.0067	0.7547	−25.18	0.0000
Stage 9	−19.7548	0.7807	−25.30	0.0000
Stage 10	−20.9229	0.8059	−25.96	0.0000
Stage 11	−21.2073	0.8237	−25.74	0.0000
Stage 12	−20.2226	0.8335	−24.26	0.0000

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4. Discussion

Models relating fish egg development rates to biological and environmental parameters are a pre-requisite for the estimation of egg production, and in principle should be species and ecosystem specific, as potentially both intraspecific and interspecific differences may occur (Lo, 1985). Sources of variability in egg development include temperature (Pepin, 1991), as well as various maternal effects (Brooks et al., 1997), including indirect environmental effects of food availability and energy content in egg quality (see a review in Chambers and Leggett, 1996). Ecological differences between the Bay of Biscay and the Gulf of Cádiz ecosystems are therefore potential sources of variability in the development of anchovy eggs in both areas, and the validity of the existing egg development models for the Bay of Biscay (Ibaibarriaga et al., 2007) in the context of the DEPM application cannot be taken for granted (ICES, 2006).

Differences in both seasonal temperature range and temperature range around spawning time exist comparing the Gulf of Cadiz and the Bay of Biscay. Average temperatures during the spawning season are higher in the Gulf of Cádiz than in the Bay of Biscay, so based on previous studies on the effect of temperature on egg development (see review in Pepin, 1991 and results for Bay of Biscay anchovy in Ibaibarriaga et al., 2007) the duration of the egg phase is expected to be shorter in the GoC. Differences in ecosystem productivity and dynamics also exist between both areas (Bellier et al., 2007; García Lafuente and Ruiz, 2007), although the anchovy dynamics in both areas is related to river outflow as one of the main drivers of recruitment success and habitat distribution (Allain et al., 2007; Bellier et al., 2007; Ruiz et al., 2009).

A shorter duration of the egg phase for the average temperature in the spawning season is confirmed by the incubation experiment carried out in this work. However, the results also show that for similar temperatures, the duration of the egg phase is similar between areas, suggesting that other variables affecting egg development (maternal effects and other environmental drivers) have a relative smaller effect. This is coherent with some recent works for other anchovy stocks, where temperature was found to explain a large percentage of egg development variability for the same species in different ecosystems (Llanos-Rivera and Castro, 2006; Tarifeño et al., 2007), as well as with the general temperature driven trend for various species shown in Pepin (1991).

The minimum temperature level at which eggs were not able to reach the larva stage described for the Bay of Biscay (Ibaibarriaga et al., 2008) also seem to be comparable with the results from the present study, although problems with the high density of eggs at one of the lowest temperature of incubation (14 °C) in the experiment presented here prevented this being rigorously tested. What seems to be clear is that at 10 °C eggs from both areas show a stagnation process, which can be induced by a thermal shock due to the difference between the ambient temperature at which the eggs were fertilised and the incubator temperature, or by a thermal tolerance minimum (Motos, 1994). Unfortunately, eggs incubated at 14 °C in the present experiment also suffered stagnation and did not hatch, but in this case the source of mortality may not have been temperature, but some other effect related to egg density in the incubator. An early work by Houde and Palko (1970) in a different clupeoid species already show that excessive densities of incubated eggs may led to high mortalities and slower growth. Also Blaxter (1956, 1962) and Motos (1994) showed that hatching success depends on the density of incubated eggs, associating high densities with higher mortalities, due to a decrease of oxygen, and an increase in ammonia and bacterial development.

As previously suggested by Ibaibarriaga et al. (2007) and Bernal et al. (2008), the use of multinomial models provides a detailed description of the development process as observed in the laboratory. Differences in the prevalence of each stage in the samples are obvious on the distribution of stages in the samples, and the sequence of one stage to the next one is well captured by the multinomial model used. The prevalence of stages observed in this experiment, corrected by the decrease of abundance with age due to mortality is coherent with the distribution of eggs in the samples (large relative proportion of stages 2 and 6; results not shown). These details are not obvious in most of the traditional egg development models (e.g. Miranda et al., 1990), as sampling times are aggregated to estimate average ages for each stage. Also, the extension of multinomial models to allow model parameters to interact with other variables provides a statistical framework for comparison of egg development between areas, and can also be extended to compare egg development between species, etc. Despite these advantages, the multinomial models used in this work show some lack of flexibility to model the frequency of any given stage in the samples, obvious in the difference between observed maximum frequency and predicted probability of a given stage in the samples (for example stage 6 at around 35 h at 18.5 °C). Although the mode of the distribution of stages by age at any given temperature (which represent the mean age by stage at a given temperature if interactions between model variables are not used) is well represented in the model, stage duration is overestimated (by the tales of the modelled distribution), generating larger predicted overlaps between stages than in the observations. This problem is also obvious in the anchovy egg development multinomial models used by Ibaibarriaga et al. (2007) but not in the sardine egg development model used by Bernal et al. (2008), suggesting that depending on the species egg development process and the experimental design some extra flexibility on the models used may be required. The analysis performed in this work suggest that including interactions between the variables used in this particular experiment only improves the models at the expenses of overfitting. Alternatively, some extra assumptions, like the use of truncated distributions to describe the evolution of each stage may be desirable.

Apart from the observed differences in egg densities between incubators, there is a number of simplifications and shortcomings of this particular incubation experiment that should be highlighted. First, eggs and sperm from various spawning anchovy individuals have been pooled together, therefore maternal effects cannot be considered in this work. Also, fertilisation percentage was not initially measured, and eggs stayed for 2.5 h at ambient temperature before being introduced in the various water baths at different temperatures, therefore initial development was not carried out at the incubation temperatures, and eggs suffer an initial and instantaneous thermal change. Eggs also suffered some thermal changes when they were observed through the incubation experiment and returned to the vessels. Finally, the partition of eggs incubated at a given temperature in various vessels may introduce some extra variability in the development, although the observations do not provide any indication of differences in egg development between jars. The temperature variations described above affect the experiment in two different ways: (i) by introducing perturbations in the temperature-controlled experimental design, or (ii) by directly affecting the egg development process. In relation to the first effect, changes in the temperature introduce some bias in the observed temperature-mediated development rates, i.e. for the 18 and 26 °C incubators, eggs are exposed to 22 °C for 2.5 h at the beginning of the experiment (which represent between 4 and 9% of the total incubation time at 18 and 26 °C, respectively) and to a variable ambient temperature (due to sampling; outside chamber temperature between 19 and 25 °C) for a maximum of around 8% of the rest of the total incubation time. Average egg development rate at 22 °C is around 35% faster than at 18 °C, and around 30% slower than at 26 °C. Assuming that average ambient temperature at sampling

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is also around 22 °C, bias on total egg development time directly due to temperature effect on development would be a maximum of 4–5% for the 18 and 26 °C incubators, respectively. This value will be a rough estimate of the maximum bias due to direct temperature effects, as it will require that all eggs sampled from a given jar in the first occasions are re-sampled in each of the following occasions. However, this estimate does not include any other potential indirect lethal and sub-lethal effects of temperature changes on development. Both lethal and sub-lethal effects of thermal variation, egg handling and other causes such as salinity variation and bacterial concentration in the survival, development rates and viability of fish egg in incubators have been described for various fish species (see a review in Brooks et al., 1997). Unfortunately, most of this works have been carried out in culture species (Brooks et al., Opus cit.) and very little comprehensive information is available on sub-lethal effects on small pelagic fish eggs in laboratory (an example is the early work of Houde and Palko, 1970). For the purpose of the experiment carried out in this work, egg mortality alone should not directly affect the egg development rates obtained, as the multinomial model uses only alive eggs to analyse the trends in the probability of any given stage as age increases. However, sub-lethal effects on egg development may exist, and in that case the development rates obtained may have some extra bias. As explained above, sub-lethal effects on development have been rarely studied in the context of DEPM incubation experiments, and cannot be studied with the crude incubation design used in this work.

Finally, a number of variables that may affect development were not monitored through the experiment: oxygen, bacterial concentration, etc. In general, the various shortcomings highlighted above indicate that the Gulf of Cádiz incubation experiment design is crude, and a better experimental design would have allowed to extract more ecological information, such as parental effects, relative influence of various environmental conditions on egg viability, etc.

In conclusion, the analysis presented in this work provides a first estimate of anchovy egg development rates in the Gulf of Cádiz, as well as a methodological framework and the first comparison between development rates in the area and in the Bay of Biscay. Results obtained allow assuming that anchovy temperature-mediated egg development rates in the area are similar to those in the Bay of Biscay, and provides the minimal required temperature-dependent egg development model to apply the DEPM to this stock (ICES, 2008). However, an improved egg development experiment, together with some refinements in the assumptions in the multinomial model used will be desirable to improve the precision of the resulting egg development model, and to evaluate other issues of ecological importance for this species in the area.

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References

Allain, G., Petitgas, P., Lazure, P., 2007. The influence of environment and spawning distribution on the survival of anchovy (*Engraulis encrasicolus*) larvae in the Bay of Biscay (NE Atlantic) investigated by biophysical simulations. Fisheries Oceanography 16, 506–514.

Bakun, A., 1996. Patterns in the Ocean. Ocean processes and marine population dynamics. Scientia Marina 61 (4), 527.

Barange, M., Bernal, M., Cercole, M.C., Cubillos, L., Cunningham, C.L., Daskalov, G.M., de Oliveira, J.A.A., Dickey-Collas, M., Hill, K., Jacobson, L., Köester, F.W., Masse, J., Nishida, H., Ñiquen, M., Oozeki, Y., Palomera, I., Saccardo, S.A., Santojanni, A., Serra, R., Somarakis, S., Stratoudakis, Y., Uriarte, A., van der Lingen, C.D., Yatsu, A., 2009. Current trends in the assessment and management of small pelagic fish stocks. In: Checkley, D.M., Roy, C., Alheit, J., Oozeki, Y. (Eds.), Climate Change and Small Pelagic Fish. Cambridge University Press, Cambridge, UK, xx + 372 pp.

Bellier, E., Planque, B., Petitgas, P., 2007. Historical fluctuations in spawning location of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in the Bay of Biscay during 1967–73 and 2000–2004. Fisheries Oceanography 16, 1–15.

Bernal, M., Ibaibarriaga, L., Lago de Lanzós, A., Lonergan, M., Hernández, C., Franco, C., Rasines, I., Valdés, L., Borchers, D., 2008. Using multinomial models to analyse data from Iberian sardine egg incubation experiments; a comparison with traditional techniques. ICES Journal of Marine Science 65, 51–59.

Bernal, M., Stratoudakis, Y., Wood, S., Ibaibarriaga, L., Uriarte, A., Valdés, L., Borchers, D., 2011. A revision of daily egg production estimation methods, with application to Atlanto-Iberian sardine. 1. Daily spawning synchronicity and estimates of egg mortality. ICES Journal of Marine Science 68.

Blaxter, J.H.S., 1956. Herring rearing. III. The effect of temperature and other factors on development. Marine Research Scotland 5, 19.

Blaxter, J.H.S., 1962. Herring rearing. IV. Rearing beyond the yolk-sac stage. Marine Research Scotland 1, 18.

Bolin, R.L., 1936. Embryonic and early larval stages of the California anchovy *Engraulis mordax* Girard. California Fish and Game 22 (4), 314–321.

Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries 7, 387–416.

Chambers, R.C., Leggett, W.C., 1996. Maternal influences on variation in egg sizes in temperate marine fishes. American Zoologist 36, 180–196.

Chavez, F.P., Ryan, J., Lluch-Cota, S., Ñiquen, C., 2003. From anchovies to sardines and back: multidecadal change in the Pacific Ocean. Science 299 (5604), 217–221.

Degroot, M.H., 1986. Probability and Statistics. Addison Wesley Publishing Company, 723 p.

Freón, P., Cury, P., Roy, L.Shannon yC., 2005. Sustainable exploitation of small pelagic fish stocks challenged by environmental and ecosystem changes: a review. Bulletin of Marine Science 76 (2), 385.

García Lafuente, J., Ruiz, J., 2007. The Gulf of Cádiz pelagic ecosystem: a review. Progress in Oceanography 74, 228–251.

Houde, E.D., Palko, B.J., 1970. Laboratory rearing of the clupeid fish *Harengula pensacolae* from fertilized eggs. Marine Biology 5, 354–358.

Ibaibarriaga, L., Bernal, M., Motos, L., Uriarte, A., Borchers, D.L., Lonergan, M.E., Wood, S.N., 2007. Characterization of stage-classified biological processes using multinomial models: a case study of anchovy (*Engraulis encrasicolus*) eggs in the Bay of Biscay. Canadian Journal of Fisheries and Aquatic Science 64, 539–553.

Ibaibarriaga, L., Fernández, C., Uriarte, A., Roel, B.A., 2008. A two-stage biomass dynamic model for Bay of Biscay anchovy: a Bayesian approach. ICES Journal of Marine Science 65, 191–205.

ICES, 2002. Report of the study group on the estimation of the spawning biomass of sardine and anchovy. ICES CM 2002/G:01, 59 pp.

ICES, 2004. The DEPM estimation of spawning-stock biomass for sardine and anchovy. ICES Cooperative Research Report 268, 91 pp.

ICES, 2006. Report of the working group on acoustic and egg surveys for sardine and anchovy in ICES Areas VIII and IX (WGACEGG). November 27–December 1, 2006, Lisbon, Portugal, ICES CM 2006/LRC:18, 169 pp.

ICES, 2008. Report of the working group on acoustic and egg surveys for sardine and anchovy in ICES Areas VIII and IX (WGACEGG). November 24–28, 2008, Nantes, France. ICES CM 2008/LRC:17, 183 pp.

ICES, 2009. Report of the working group on acoustic and egg surveys for sardine and anchovy in ICES areas VIII and IX (WGACEGG). November 16–20, 2009, Lisbon, Portugal. ICES CM 2009/LRC:20, 181 pp.

Lasker, R. (Ed.), 1985. An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*). NOAA Technical Report NMFS 36.

Llanos-Rivera, A., Castro, L.R., 2006. Inter-population differences in temperature effects on *Engraulis ringens* yolk-sac larvae. Marine Ecology Progress Series 312, 245–253.

Lo, N.C.H., 1985. A model for temperature-dependent northern anchovy egg development and an automated procedure for the assignment of age to staged eggs. In: Lasker, R. (Ed.), An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Application to the Northern Anchovy (*Engraulis mordax*), 36. NOAA Technical Report NMFS, pp. 43–50.

Miranda, A., Cal, R.M., Iglesias, J., 1990. Effect of temperature on the development of eggs and larvae of sardine *Sardina pilchardus* (Walbaum) in captivity. Journal of Experimental Marine Biology and Ecology 14, 69–77.

Moser, H.G., Ahlstrom, E.H., 1985. Staging anchovy eggs. In: Lasker, R. (Ed.), An Egg Production Method for Estimating Spawning Biomass of Pelagic Fish: Applica-

tion to the Northern Anchovy (*Engraulis mordax*), 36. NOAA Technical Report NMFS, pp. 37–41.
Motos, L., 1994. Estimación de la biomasa desovante de la población de anchoa del golfo de Vizcaya, *Engraulis encrasicolus*, a partir de su producción de huevos. bases metodológicas y aplicación. Ph.D. Thesis, University of the Basque Country, Spain.
Parker, K., 1980. A direct method for estimating northern anchovy, *Engraulis mordax*, spawning biomass. Fishery Bulletin 78, 541–544.
Pepin, P., 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Canadian Journal of Fisheries and Aquatic Science 48, 503–518.
R Development Core Team, 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, http://www.R-project.org.
Ruiz, J., González-Quirós, R., Prieto, L., Navarro, G., 2009. A Bayesian model for anchovy (*Engraulis encrasicolus*): the combined forcing of man and environment. Fisheries Oceanography 18, 62–76.
Stratoudakis, Y., Bernal, M., Ganias, K., Uriarte, A., 2006. The daily egg production method (DEPM): recent advances, current applications and future challenges. Fish and Fisheries 7, 35–57.
Tarifeño, E., Carmona, M., Llanos-Rivera, A., Castro, L.R., 2007. Temperature effects on the anchoveta *Engraulis ringens* egg development: do latitudinal differences occur? Environmental Biology of Fishes 81, 387–395.
Zweifel, J.R., Lasker, R., 1976. Prehatch and posthatch growth of fishes—a general model. Fishery Bulletin 74, 609–621.

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