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Importance of parental effects on larval survival in *Sardina pilchardus*

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ABSTRACT: A combined field and laboratory study was carried out to determine the importance of parental effects (spatial and temporal variations in spawning, egg size and biochemical composition of the egg) on larval survival in *Sardina pilchardus*. Egg abundance was positively correlated with the seston organic content (SOC; a combination of total protein, carbohydrate and lipid content) of the size particle fraction 20 to 1000 µm. This was interpreted as a reproductive strategy, to spawn more eggs in areas and during periods of enhanced food availability to larvae. Larval survival under starvation conditions was related to temperature, but only when just the larvae in which the yolk-sac was fully absorbed at death were considered. However, if larval survival time was estimated considering all larvae, including those which died before yolk-sac absorption, larval survival time was related to the biochemical composition of the egg. Larval survival time increased as the percentage of protein in the egg increased, indicating the importance of protein under food limiting conditions. Furthermore, percentage of larvae with yolk-sac completely absorbed at death was higher as egg protein percentage increased. Although producing eggs with a higher proportion of proteins in response to diminishing food availability could maximise larval success under starvation conditions, this parental strategy was not exhibited by *S. pilchardus*. There was not a significant relationship between egg protein percentage and SOC. Biochemical composition of the egg seemed to vary according to temperature of the water.

KEY WORDS: *Sardina pilchardus* · Eggs · Survival · Temperature · Food concentration · Biochemical composition · Starvation · Upwelling

INTRODUCTION

The different strategies exhibited by fish populations to increase egg and larval survival may be defined as parental effects. These strategies include spatial and temporal variations in spawning intensity, egg size, yolk volume and egg quality.

Spatial and temporal variability in spawning intensity may affect survival since eggs spawned at different times of day or year, or at different locations, may be exposed to different sources and magnitudes of mortality due to predation, light intensity, feeding conditions, etc. (see Ribeiro et al. 1996). Temporal variability in spawning intensity of sardine in the Iberian peninsula has been shown to be an important factor in minimising the loss of larvae offshore (Robles et al. 1992). Oceanic or coastal spawning grounds of fish are often distant from nursery areas. Fish larvae require appropriate currents and sufficient and suitable food during transit to reach the nursery areas at the proper time, size and condition. Parrish et al. (1981) hypothesised that deviations from 'normal' transport conditions (upwelling and offshore transport) could have profound detrimental effects on a year class by carrying eggs and larvae to areas where there is not enough food to survive.

Egg size may determine larval size and yolk volume at hatching (Baynes & Howell 1996, Kristjánsson & Vøllestad 1996, Pepin et al. 1997). However, it has

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been observed that egg viability and larval fitness can also be determined by the biochemical composition of the egg (Srivastava & Brown 1991, MacKenzie et al. 1996, Pickova et al. 1997).

Although a great amount of work exists on egg biochemical composition, most studies focus on variability in biochemical composition of developing fish eggs and larvae (Rønnestad et al. 1992, Finn et al. 1995, 1996, Rainuzzo et al. 1997) and how biochemical composition of the egg affects vertical distribution of eggs in the water column (Craik & Harvey 1987, Nissling & Vallin 1996, Thorsen et al. 1996, Guisande et al. 1998).

During the transition to exogenous feeding, the amount of yolk remaining and its composition may be important. Starvation is suggested to be one of the major causes of mortality during the period when larvae change from endogenous to exogenous feeding (Canino et al. 1991, Bailey et al. 1995) and has been proposed as a primary agent of mortality in all early larvae (Bisbal & Bengtson 1995, Clemmensen et al. 1997, Jonas & Wahl 1998). However, the significance of starvation in larval mortality can be variable depending on the species and location studied (Theilacker 1986, Bestgen 1996, Theilacker et al. 1996, Rooker et al. 1997, Chícharo 1998, Chícharo et al. 1998).

Larval condition and therefore mortality under food-limiting conditions may also depend on the previous biochemical composition of eggs. While some studies have been conducted to test the importance of egg biochemical composition on larval survival (Srivastava & Brown 1991, Brown & Taylor 1992), they were carried out on eggs that were artificially fertilised in culture tanks. Little is known about what happens under natural conditions. Because the amount of yolk in the egg affects the time that larvae can survive without food, the effect of temperature on absorption rate must also be considered when relating larval survival to egg quality (Hart & Purser 1995, Kucharczyk et al. 1997).

Our aim was to carry out a field study to determine whether *Sardina pilchardus* exhibits any parental strategy to maximise larval survival time. From all possible parental strategies we focused the study on the variations in spatial and temporal spawning intensity, egg size, yolk volume and egg quality.

METHODS

Field study. From February 1998 to June 1999 fertilised *Sardina pilchardus* eggs were collected monthly along the Ria de Vigo (NW Spain) in the RV 'Jose María Navaz' at 4 stations: 11 (42° 7.8′ N, 9° 7.5′ W; 148 m depth), 13 (42° 8.5′ N, 8° 57.5′ W; 97 m depth), 14 (42° 12.8′ N, 8° 51′ W; 39 m depth) and 15 (42° 13.3′ N, 8° 47.7′ W; 29 m depth) (Fig. 1).

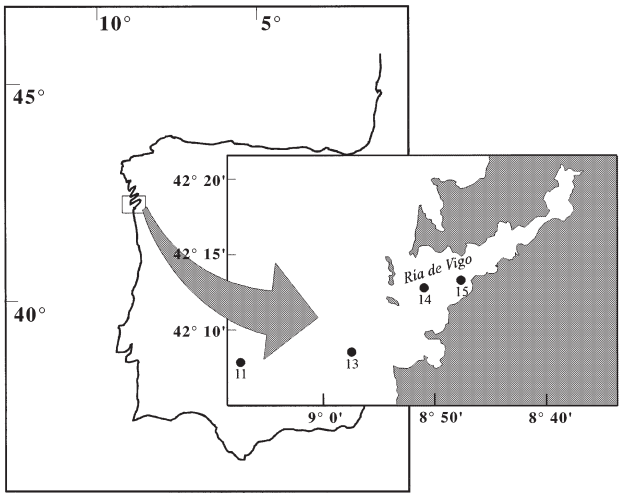

Fig. 1. Map of the study area showing location of sampling stations

Samples were collected by a 50 cm diameter Bongo sampler fitted with 335 µm mesh aperture nets, towed obliquely from about 5 m off the bottom to the surface at approximately 2 knots. Volumes filtered were obtained from flowmeters positioned in the mouth of the nets. Each sample was transported to the laboratory in 30 l of seawater, where samples were analysed within 4 h of collection.

In order to know the quantity and quality of the seston, vertical net hauls using a 20 µm mesh net (equipped with flowmeter) were towed at each station from the same depth as the egg samples. The fraction 20 to 1000 µm was resuspended in 1 l of filtered seawater then subsamples taken for subsequent biochemical analyses.

CTD (Seabird 25) profiles were carried out at all stations. An upwelling index was calculated using the method described by Bakun (1973), which has been used before in the Galician coast by Lavin et al. (1991).

Survival time study. Larval survival time was performed on eggs collected in 1998. In the laboratory, eggs were identified and measured using a binocular microscope fitted with an ocular micrometer. Subsequently, eggs were placed individually in assay tubes with 10 ml of filtered seawater (from the stations where eggs were collected) through 0.45 µm of GF/F (glass microfibre filters) and stored at different temperatures (9, 12, 15 and 18°C) under a 12:12 h light:dark cycle. Every month, the number of eggs incubated at each temperature ranged between 4 and 20. Eggs were inspected every 6 h to record hatching time and time when larvae died. Larval death was determined using a binocular microscope.

Biochemical analysis. When sufficient eggs were collected they were analysed for protein, carbohydrate and lipid content. After staging and measuring, 2 eggs of similar size and at the same developmental stage were transferred to an ultracentrifuge plastic tube with 600 µl of bidistilled water. Samples were homogenised using a pipette tip adapted to fit the shape of the vial. 200 µl of each egg homogenate were used for protein analysis, 200 µl for carbohydrate analysis and 75 µl for lipid analysis. Every month the number of replicates, with 2 eggs in each, ranged between 16 and 43, with the exception of December 1998 when there were only 3 replicates.

The method describe by Lowry et al. (1951) and modified by Maxwell et al. (1978) was used to analyse the protein content of the eggs. To 200 µl sample volumes, 50 µl of NaOH (0.5 N) (prepared just before the test) and 750 µl of solution C were added. Solution C was prepared shortly before the beginning of the analysis and was composed of Solutions A and B in a proportion of 100A:1B. These solutions were as follows: 'Solution A', Na_2CO_3 (2%), NaOH (0.4%), NaK $(COO)_2(CHOH)_24H_2O$ (0.16%), SDS (1%); 'Solution B', $CuSO_45H_2O$ (4%). The samples were shaken and maintained at 30°C for 15 min. Then 75 µl of diluted 1:1 Folin-Cioccalteus were added to each sample. Subsequently, the samples were incubated for 30 min at the same temperature. Absorbance was read at 660 nm and compared with bovine serum albumin (BSA) standard.

Egg carbohydrate content was measured by the phenol-sulphuric acid method (Dubois et al. 1956). To sample 200 µl of homogenate and 200 µl of bidistilled water, 10 µl of 81% phenol was added and, after gently shaking, 1 ml of concentrated sulphuric acid was added. The sample was shaken again and maintained at room temperature for 30 min. Finally, the absorbance was read at 485 nm. A standard curve was established using reagent-grade glucose.

Egg lipid content was determined using the sulfophosphovanillin method (Zöllner & Kirsch 1962). 50 µl of absolute ethanol was added to 75 µl of homogenate, immediately after which the sample was shaken and maintained at 4°C for at least 2 h. Then 375 µl of concentrated sulphuric acid was added and the sample was homogenised by shaking. The sample was then maintained at 100°C for 15 min. After hydrolysis, when the sample had cooled down, 2 ml of vanillin reagent (1.976 g of vanillin with 800 ml of 85% H_3PO_4 and distilled water to 1000 ml) was added. The sample was shaken and incubated at 30°C for 30 min, then absorbance was read at 530 nm.

Total protein, carbohydrate and lipid contents of the seston fraction (20 to 1000 µm) were determined using methods analysis described above. The organic content (total protein, carbohydrate and lipid content) of the total seston size fraction 20 to 1000 µm was used as an indicator of food potentially available to the larvae. As larval body size of larvae that appear in this area ranged between 2.4 and 28 mm, the seston size fraction measured is within the range of prey captured by larvae of this body size (Conway et al. 1991).

Statistical analyses were made with SPSS 8.0 (1998).

RESULTS

Sampling station means (±SD) of egg size, egg stage, total protein, total carbohydrate, total lipid and organic content of the eggs as well as protein, carbohydrate and lipid percentage of the eggs are given in Table 1. Protein proved to be the most abundant organic component of the eggs with a pooled mean ±SD of 69.3 ± 9.6%, whereas carbohydrate and lipid were 15.0 ± 6.3 and 15.7 ± 8.8% of the total organic content of the egg, respectively. The monthly mean abundance of *Sardina pilchardus* eggs at Stns 13, 14 and 15 during the sample period is shown in Fig. 2. Eggs were never found at offshore Stn 11. The main spawning season throughout the sampling area was from December to June (Pérez et al. 1985, Solá et al. 1992). Although a smooth peak is generally observed in this area in autumn (Solá et al. 1992), egg abundance was very low in autumn of 1998.

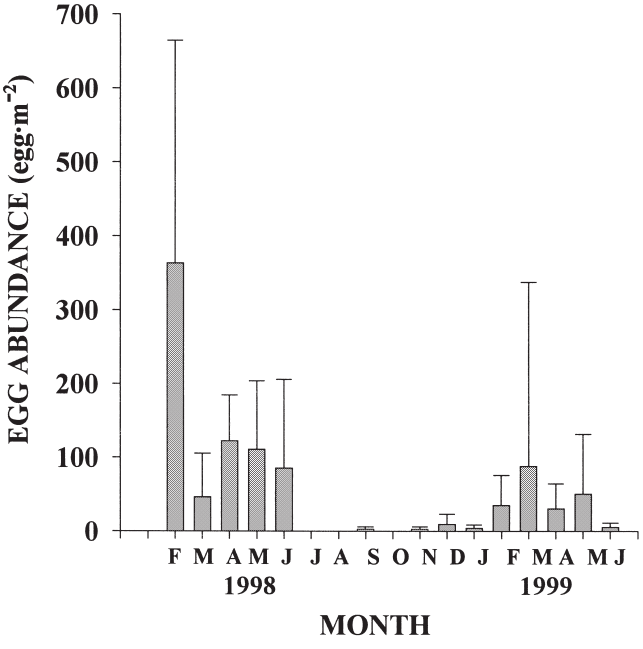

Fig. 2. *Sardina pilchardus*. Monthly means (±SD) of egg abundance at Stns 13, 14 and 15

Table 1. *Sardina pilchardus*. Means (±SD) of egg size (mm), egg stage, protein, carbohydrate, lipid and organic content (µg egg^{-1}) and protein, carbohydrate and lipid percent at each station

Date	Stn	Size	Stage	Protein	Carbohydrate	Lipid	Organic	% protein	% carbohydrate	% lipid
February 98	13	1.52 ± 0.11	7.0 ± 2.2	18.2 ± 4.9	5.1 ± 1.9	2.0 ± 1.9	23.9 ± 6.1	71.3 ± 6.7	21.6 ± 6.8	7.2 ± 6.2
	14	1.50 ± 0.05	6.7 ± 0.5	16.7 ± 3.7	3.8 ± 2.2	6.1 ± 3.6	26.6 ± 7.4	64.5 ± 12.4	13.9 ± 6.3	21.6 ± 10.4
	15	1.47 ± 0.11	6.9 ± 2.0	18.2 ± 3.1	5.8 ± 1.6	3.7 ± 1.5	27.1 ± 4.5	65.3 ± 6.2	21.5 ± 5.3	13.2 ± 4.6
March 98	14	1.51 ± 0.13	4.3 ± 1.6	12.8 ± 4.4	2.6 ± 0.9	3.6 ± 1.4	19.4 ± 5.0	66.8 ± 8.5	13.9 ± 4.5	19.3 ± 7.6
	15	1.44 ± 0.10	5.1 ± 1.9	13.1 ± 2.4	2.9 ± 0.5	3.1 ± 1.3	19.2 ± 3.6	68.6 ± 5.1	15.5 ± 2.7	15.9 ± 4.3
April 98	13	1.50 ± 0.07	7.7 ± 3.1	16.4 ± 2.4	2.8 ± 0.4	4.3 ± 2.7	23.6 ± 4.5	70.6 ± 8.8	12.1 ± 1.7	17.3 ± 8.8
	14	1.51 ± 0.12	5.9 ± 2.1	16.7 ± 3.4	3.3 ± 1.1	1.8 ± 1.1	22.4 ± 4.3	77.9 ± 3.3	14.6 ± 4.0	7.4 ± 3.8
	15	1.55 ± 0.07	5.9 ± 1.4	16.9 ± 2.4	3.7 ± 1.2	3.4 ± 1.8	23.9 ± 4.0	70.7 ± 6.5	15.4 ± 4.0	14.0 ± 6.6
May 98	13	1.51 ± 0.04	7.0 ± 0.0	17.6 ± 0.2	2.0 ± 0.0	4.2 ± 1.3	23.8 ± 1.2	74.2 ± 4.5	8.2 ± 0.3	17.6 ± 4.7
	14	1.50 ± 0.07	4.8 ± 2.7	17.4 ± 1.9	2.7 ± 0.5	3.5 ± 2.6	23.5 ± 2.9	74.4 ± 7.6	11.5 ± 3.2	14.0 ± 8.8
	15	1.52 ± 0.03	6.7 ± 1.0	14.7 ± 3.2	2.1 ± 1.0	2.2 ± 1.0	19.0 ± 4.7	78.1 ± 6.1	10.2 ± 3.2	11.6 ± 4.7
June 98	14	1.48 ± 0.06	5.3 ± 2.5	13.0 ± 3.2	2.0 ± 0.9	4.3 ± 3.5	19.4 ± 7.0	69.7 ± 9.3	10.5 ± 3.6	19.9 ± 9.41
	15	1.46 ± 0.06	4.0 ± 1.2	12.0 ± 4.8	2.2 ± 1.1	1.7 ± 2.3	15.9 ± 6.8	75.3 ± 10.8	13.4 ± 4.2	11.3 ± 13.7
December 98	15	1.46 ± 0.06	2.0 ± 0.0	19.9 ± 2.5	7.2 ± 2.4	7.2 ± 2.2	34.3 ± 4.9	58.1 ± 2.2	21.0 ± 5.9	20.9 ± 4.5
February 99	14	1.38 ± 0.17	4.9 ± 1.8	9.9 ± 1.6	4.3 ± 2.2	3.7 ± 3.6	17.9 ± 4.0	58.2 ± 16.1	23.9 ± 11.0	17.9 ± 14.9
	15	1.44 ± 0.02	3.5 ± 2.1	10.6 ± 1.9	2.4 ± 1.4	1.0 ± 0.3	14.0 ± 0.8	75.5 ± 9.5	17.6 ± 11.1	7.0 ± 1.7
March 99	14	1.53 ± 0.08	7.3 ± 2.1	11.9 ± 2.0	3.0 ± 2.1	4.0 ± 2.9	18.9 ± 4.5	65.0 ± 11.5	15.1 ± 7.7	19.9 ± 10.6
	15	1.47 ± 0.08	5.1 ± 3.2	13.0 ± 2.3	2.6 ± 1.4	2.6 ± 1.2	18.1 ± 2.8	71.8 ± 8.5	14.1 ± 7.3	14.1 ± 7.0
April 99	14	1.49 ± 0.08	4.2 ± 2.5	11.4 ± 1.7	3.5 ± 1.7	4.0 ± 1.9	18.9 ± 4.0	61.9 ± 9.7	17.9 ± 5.5	20.3 ± 7.3
	15	1.47 ± 0.08	6.0 ± 0.0	10.8 ± 1.1	1.6 ± 0.4	3.0 ± 1.2	15.3 ± 2.5	70.7 ± 5.3	10.6 ± 1.7	18.6 ± 5.1
May 99	14	1.44 ± 0.09	5.9 ± 3.0	12.4 ± 2.6	2.6 ± 0.9	2.8 ± 1.5	17.8 ± 3.9	70.4 ± 6.6	14.5 ± 3.4	15.1 ± 7.0
	15	1.40 ± 0.11	10.5 ± 0.7	11.9 ± 5.3	4.3 ± 1.9	2.3 ± 0.1	18.5 ± 3.3	62.9 ± 17.2	24.7 ± 14.7	12.4 ± 2.5

Egg abundance varied significantly both temporally and spatially (Table 2). A high proportion of the variation observed in egg abundance was explained by changes in seston organic content (SOC). A stepwise regression showed that considering temperature and SOC, only a significant relationship between SOC and the abundance of eggs was observed (slope different from zero with $p < 0.001$, $r^2 = 0.7$, $F_{1, 32} = 75.4$; Fig. 3).

When larval survival time at different temperatures was estimated only considering the larvae in which the yolk-sac was fully absorbed at death, a 2-way ANOVA showed that there were not significant differences in larval survival time from hatch to death between months and sampling stations (Table 3). A stepwise regression showed that, considering all the variables (egg size, egg stage, egg biochemical composition and temperature), only temperature was significantly related to larval survival time (Fig. 4; slope different from zero with $p < 0.001$, $r^2 = 0.94$, $F_{1, 7} = 104.9$). February and March 1998 are not shown because all larvae died before yolk-sac absorption in February and only 1 larva died with yolk-sac fully absorbed in March.

However, larval viability under starvation conditions seems to be also affected by egg biochemical composition. Fig. 5 shows that the percentage of larvae that had a completely absorbed yolk-sac at death was higher as the percentage of egg protein increased (slope different from zero with $p = 0.014$, $r^2 = 0.9$, $F_{1, 4} = 27.1$). Moreover, larval survival time results were different when all larvae, those in which the yolk-sac was fully absorbed at death and those which died before yolk-sac absorption, were considered. A 2-way ANOVA (Table 4) showed that there were significant differences in larval survival time between the different months but not between the different sampling stations. In this case, a stepwise regression showed that, considering all the variables mentioned above, larval survival time was related to changes in egg protein percentage (slope different from zero with $p < 0.001$, $r^2 = 0.77$, $F_{1, 11} = 36.2$) (Fig. 6).

Table 2. *Sardina pilchardus.* Results of ANCOVA of the egg abundance (eggs m^{-2}) with sampling station as factor and month as covariate

Source of variation	df	MS	*F*	p
Sampling station	3	138924.0	3.5	0.021
Month	1	47895.4	4.2	0.044
Interaction	1	39509.1	12.3	0.001
Error	63	111312.1		
Total	68			

Table 3. *Sardina pilchardus.* Summary of 2-way ANOVA comparing larval survival under starvation conditions as a function of sampling station and month. Only larvae in which the yolk-sac had been fully absorbed were considered

Source of variation	df	MS	*F*	p
Sampling station	2	920.7	0.3	0.743
Month	2	883.1	0.3	0.752
Interaction	2	583.6	0.2	0.827
Error	13	3025.7		
Total	20			

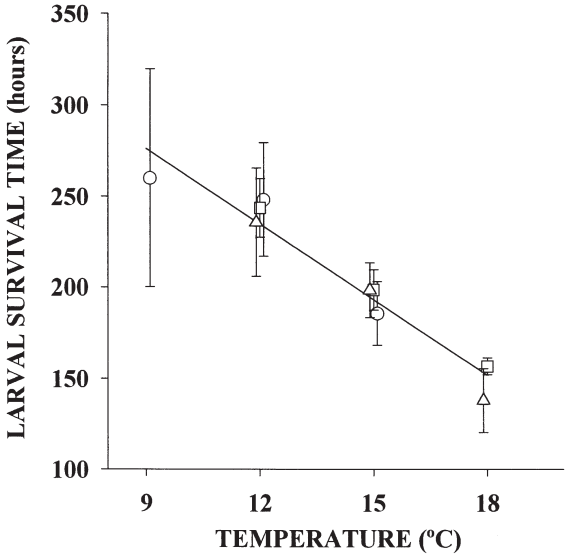

Fig. 4. *Sardina pilchardus*. Relationship between monthly means (±SE) of larval survival (time after hatch) under starvation conditions and temperature from larvae collected in April (○), May (□) and June (△) 1998. Larval survival was estimated only considering larvae in which the yolk-sac was fully absorbed

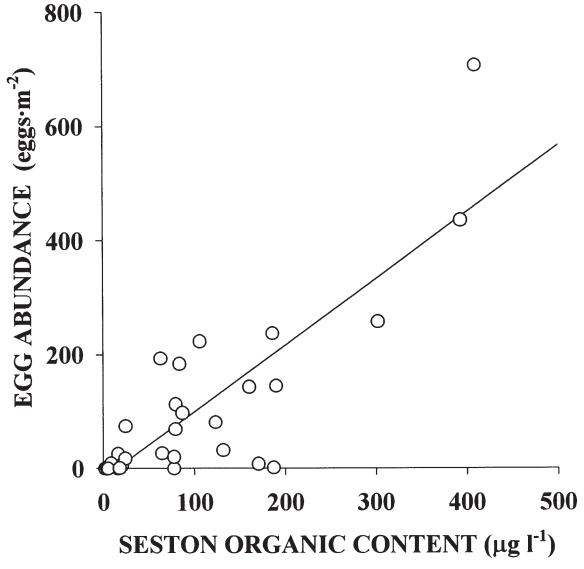

Fig. 3. *Sardina pilchardus*. Relationship between egg abundance and total organic content of the seston size fraction 20 to 1000 µm obtained at the sampling stations

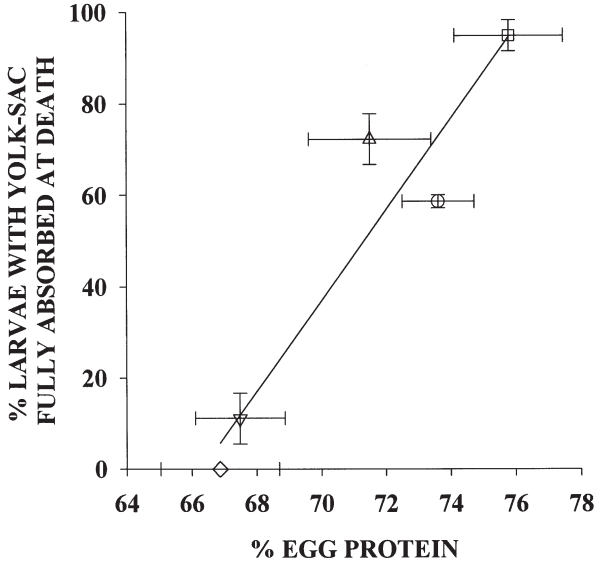

Fig. 5. *Sardina pilchardus*. Relationship between monthly means (±SE) of percentage of larvae with yolk-sac completely absorbed at death under starvation conditions and the percentage of protein in the egg, from larvae collected in February (◇), March (▽), April (○), May (□) and June (△) 1998

Table 5 shows that egg protein percentage varied significantly between sampling stations and months. However, despite the importance of egg protein percentage on subsequent larval survival time under food-limiting conditions, there was not a significant relationship between egg protein percentage and SOC ($p = 0.267$, $r^2 = 0.064$, $F_{1, 19} = 1.3$). The variation in egg biochemical composition was correlated with changes in the temperature of the water (Fig. 7). A stepwise regression showed that in 1998 the variation in egg

Table 4. *Sardina pilchardus.* Summary of 2-way ANOVA comparing larval survival under starvation conditions as a function of sampling station and month. All larvae, whether or not the yolk-sac was absorbed, were considered

Source of variation	df	MS	*F*	p
Sampling station	2	349.7	0.139	0.880
Month	4	19192.4	7.1	0.002
Interaction	3	383.5	0.1	0.934
Error	17	2717.4		
Total	27			

Table 5. *Sardina pilchardus.* Summary of 2-way ANOVA comparing egg protein percentage as a function of sampling station and month

Source of variation	df	MS	*F*	p
Sampling station	2	377.1	4.5	0.013
Month	5	426.8	5.0	0.000
Interaction	6	85.0	1.0	0.423
Error	214	84.5		
Total	228			

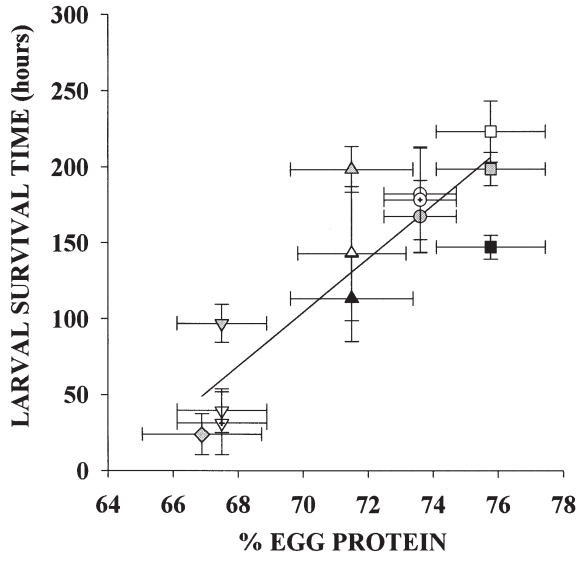

Fig. 6. *Sardina pilchardus.* Relationship between monthly means (±SE) of larval survival (time after hatch) under starvation conditions at different temperatures (9°C: symbols with plus, 12°C: open symbols, 15°C: shaded symbols, and 18°C: filled symbols) and the percentage of protein in the egg from larvae collected in February, March, April, May and June 1998. Larval survival was estimated considering all larvae. Symbols same as in Fig. 5

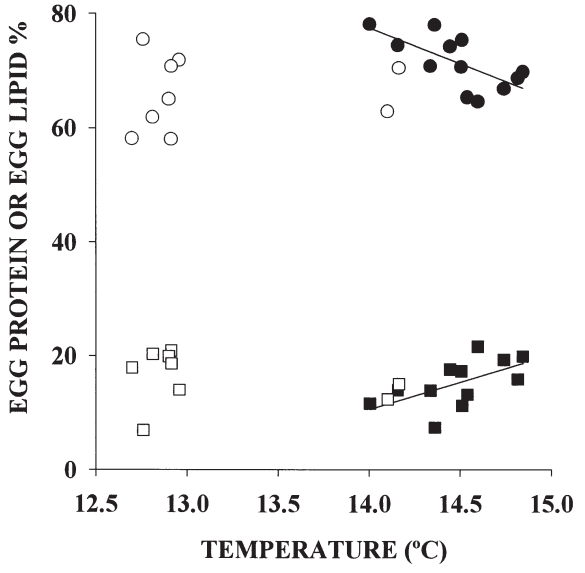

Fig. 7. *Sardina pilchardus.* Relationships between egg protein percentage in 1998 (●) and 1999 (○) and egg lipid percentage in 1998 (■) and 1999 (□) and mean temperature of the whole water column

protein percentage was explained by changes in the mean temperature of the water column (slopes different from zero with p = 0.015, r^2 = 0.46, $F_{1, 10}$ = 8.6 for egg protein percentage and p = 0.046, r^2 = 0.34, $F_{1, 10}$ = 5.2 for egg lipid percentage), but there was not a significant relationship with temperature in 1999 (p = 0.817, r^2 = 0.01, $F_{1, 7}$ = 0.05 for egg protein percentage and p = 0.501, r^2 = 0.07, $F_{1, 7}$ = 0.5 for egg lipid percentage). The lack of a relationship between egg biochemical composition and temperature in 1999 might be due to the atypical temperature values of 1999 (Fig. 8). During the spawning season there is normally a decrease in the temperature of the water from December to March (mean ± SD of 1.6 ± 1.0°C obtained for 1995 to 1998), whereas in 1999 the reduction was only 0.3°C. These atypical temperatures during the spawning season in 1999 could be explained by an upwelling process during February and March (Fig. 9).

DISCUSSION

One of the aims of this study was to gain an insight into the importance of temporal and spatial variability of spawning intensity. Our assessment was dependent on the assumption that reproductive strategy of females could be crucial in determining larval survival time. A relationship was established between egg abundance and SOC and provides a frame of reference for evaluating the importance of spatial and temporal variations in spawning. The reproductive strategy exhibited by adults of *Sardina pilchardus*, probably to

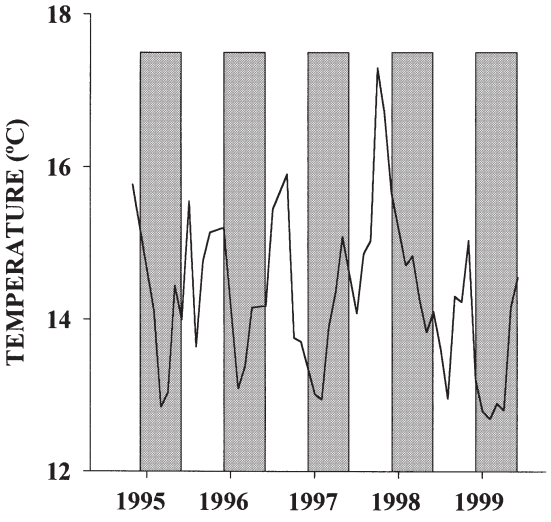

Fig. 8. Temporal variation in the mean temperature of the whole water column at Stn 14. Shaded bars show the spawning period in this area for *Sardina pilchardus*

assure reproductive success, seemed to be to produce a greater number of eggs in those locations and time of year in which food was more abundant. However, it is necessary to point out that eggs and seston co-occurrence observed in this study could not be due to a parental strategy, but to just some physical aggregating mechanisms. Food limitation during the larval period has been frequently hypothesised to be a major regulator of larval survival time (Cushing 1975). Canino et al. (1991) demonstrated that when spawning is not linked to the abundance of food, food densities are too low for optimal growth of first feeding larvae and this will have significant effects upon rates of feeding, growth and subsequent survival. In general agreement with this conclusion, Kucharczyk et al. (1997) and Kuyawa et al. (1997) stated that survival of larvae appears to be related to coincidence with optimal food concentrations rather than coincidence with optimal temperatures.

It has been shown that the main spawning period of sardine in Galician coastal waters is usually outside the upwelling period to minimise the loss of larvae offshore. Dickson et al. (1988) and López-Jamar et al. (1995) showed an inverse relationship between upwelling conditions and recruitment of sardine in Galician waters. The upwelling process in early spring 1999 (Fig. 9) may be responsible for the low number of eggs found at the stations that year, and survival might have been reduced by transport of eggs offshore, where food is less available (Robles et al. 1992).

There is clear evidence that during the transition to exogenous feeding, temperature is a key determinant of development and time-to-yolk absorption rates in marine fish larvae (Miranda et al. 1990, Hart & Purser 1995, Watanabe et al. 1995, Kuyawa et al. 1997). Time-to-yolk absorption defines the period during which larvae must rely on endogenous energy reserves. Increasing temperature reduces the duration of the yolk-sac stage, which results in an earlier initiation of exogenous feeding. Our results show that when only the larvae in which the yolk-sac was fully absorbed were considered, temperature appeared to be the most important factor affecting larval survival time (Fig. 4).

However, some of the earlier larval stages died before endogenous energy reserves were exhausted. Therefore, yolk-sac reserves were not always sufficient to assure larval survival. It was observed that the percentage of larvae that died before yolk absorption was higher as egg protein percentage diminished (Fig. 5) and, furthermore, considering all larvae, larval survival time was higher as egg protein percentage increased (Fig. 6). Lein et al. (1997) suggested that temperature has a significant influence on morphological development, although variations in temperature do not appear to have any significant effect on larval size and survival. Therefore, it seems that factors other than temperature could determine larval survival (Pepin 1990). Among the hypotheses proposed to explain larval failure to successfully feed, those dealing with interspecific and intraspecific competition for food resources, or with malformations induced during incubation have received most attention (McGurk et al. 1993). Egg size has also been considered as an important factor which affects the endogenous feeding

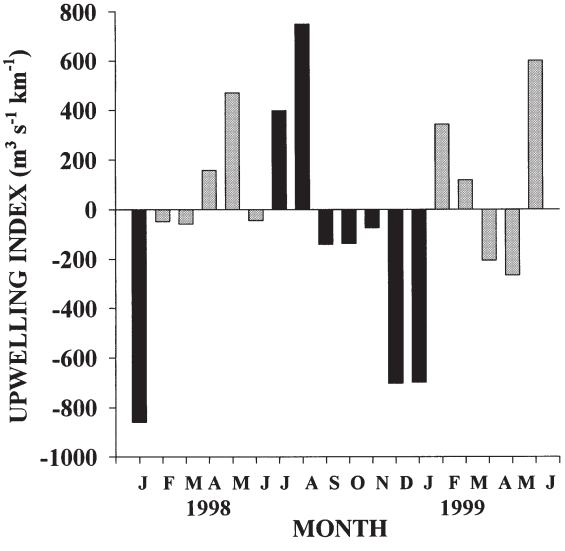

Fig. 9. Temporal variation of the upwelling index in the sampling area during 1998 and 1999. Shaded bars show the peak spawning period in this area for *Sardina pilchardus*. Positive values indicate upwelling and negative values indicate downwelling

phase. It has been observed that larvae hatching from smaller eggs exhibited a poor nutritional condition earlier than those hatched from larger eggs (Baynes & Howell 1996). However, our results show that time-to-yolk absorption is not a function of egg size but a function of egg biochemical composition.

The potential usefulness of biochemical characteristics of eggs for assessing nutritional condition during embryonic and early larval development has already been pointed out by Srivastava & Brown (1991). The use of endogenous energy reserves during fish early-life-history stages has been reported in numerous studies. In general, either lipids (Mourente & Vázquez 1996) or free amino acids (Rønnestad et al. 1992) have been considered to be the most important sources of energy. However, it has also been shown that larval tissues start to be metabolised when yolk-sac reserves are nearly exhausted and metabolic demands are high (Hemming & Buddington 1988). Proteins proved to be the energy reserve being used when limiting food concentrations occurred, since a reduction in larval protein content was observed during the time of yolk-sac exhaustion and the initial stages of exogenous feeding (Canino et al. 1991). In turbot *Scophthalmus maximus* embryos and yolk-sac larvae, body proteins were metabolised after exhaustion of free amino acids (Finn et al. 1996).

In this study, proteins proved to be the limiting factor for sardine larval survival time, not in absolute terms but when compared to the other biochemical components of the egg (i.e. lipids and carbohydrates). Survival rates under starvation conditions were observed to increase with protein percentage in the egg and this increase was not temperature dependent (Fig. 6). However, the spatial and temporal variations observed in egg protein percentage seem to be explained by changes in temperature conditions (Fig. 7) rather than by changes in food available for the larvae. Changes in larval biochemical composition according to prevailing temperature and salinity conditions have been observed before in *Sardina pilchardus* (Guisande et al. 1998). As egg buoyancy has been shown as an important factor affecting recruitment in fish (Page et al. 1989, Nissling & Westin 1991), the trade-off observed in *S. pilchardus* between protein and lipid content of the egg could be interpreted as a mechanism to achieve optimal larval buoyancy. The redistribution of the egg biochemical components could also be related to achieving an optimal egg development time according to prevailing temperature conditions. The lack of a relationship between egg biochemical composition and temperature in a year with an atypical evolution of water temperature (1999) caused by an upwelling process during February and March (Fig. 9) could indicate a fixed pattern of egg biochemical composition change over the spawning season in this area, where the upwelling process normally takes place between April and September (Lavin et al. 1991). However, a longer study should be necessary to corroborate the hypothesis that egg biochemical composition variation over the spawning season is a response to the normal evolution of temperature during this period in the area.

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