

## Distribution and abundance of early life stages of *Sardina pilchardus* in the Gulf of Tunis (Central Mediterranean Sea) in relation to environmental and biological factors

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**SUMMARY:** Four seasonal surveys were carried out in the Gulf of Tunis between summer 2002 and spring 2003 to study the abundance and distribution of *Sardina pilchardus* eggs and larvae in relation to environmental parameters. In the Gulf of Tunis, *Sardina pilchardus* begins spawning in autumn (23 eggs/10 m<sup>2</sup>) and attains its peak in winter (257 eggs/10 m<sup>2</sup>) when the mean SST is lowest (13.4°C). Sardine reproduction seems to be triggered by the decrease in the SST. In winter, the main spawning areas were located to the south of Zembra Island and the north of Cape Bon. Larvae were more abundant in winter (38 larvae/10 m<sup>2</sup>), while lower densities were collected in autumn and spring (1 larva/10 m<sup>2</sup>). The highest abundance of larvae (288 larvae/10 m<sup>2</sup>) was recorded southwest of Zembra Island. Eggs and larvae were mainly concentrated in the relatively warmer and saltier waters with high zooplankton abundance and, inversely, with a low concentration of nitrate and chlorophyll *a* and a low diatom abundance.

**Keywords:** *Sardina pilchardus*, eggs, larvae, Gulf of Tunis, spawning seasonality, spatial distribution.

**RESUMEN:** DISTRIBUCIÓN Y ABUNDANCIA DE LOS PRIMEROS ESTADIOS DE DESARROLLO DE *SARDINA PILCHARDUS* EN EL GOLFO DE TÚNEZ (MEDITERRÁNEO CENTRAL) EN RELACIÓN CON LOS FACTORES AMBIENTALES. – Se llevaron a cabo cuatro campañas en el golfo de Túnez, entre verano del 2002 y primavera del 2003, con el objetivo de estudiar la abundancia y distribución de los huevos y larvas de *Sardina pilchardus* en relación con parámetros ambientales. En el golfo de Túnez *Sardina pilchardus* inicia la freza en otoño (23 huevos/10 m<sup>2</sup>), alcanzando el pico de puesta en invierno (257 huevos/10 m<sup>2</sup>), cuando la temperatura superficial media (SST) es mínima (13.4°C). La reproducción de la sardina se dispara al parecer por el descenso de la SST. En invierno, las principales áreas de puesta se localizaron al sur de la isla de Zembra y en el norte del Cabo Bon. Las larvas fueron más abundantes en invierno (38 larvas/10 m<sup>2</sup>); mientras que se recogieron menores densidades en otoño y primavera (1 larva/10 m<sup>2</sup>). La mayor abundancia larvaria (288 larvas/10 m<sup>2</sup>) fue observada en el suroeste de la isla de Zembra. Los huevos y larvas se concentraron principalmente en las aguas relativamente más cálidas y salinas, con altas abundancias de zooplancton, e inversamente bajas concentraciones de nitrato, clorofila *a* y abundancia de diatomeas.

**Palabras clave:** *Sardina pilchardus*, huevos, larvas, golfo de Túnez, estacionalidad de la puesta, distribución espacial.

## INTRODUCTION

Sardine (*Sardina pilchardus*) is the most abundant small pelagic species in Tunisian waters. It represents 39.4% (13197 t) of the small pelagic production and 14.3% of the total Tunisian fleet landings (DGPA, 1999-2004). It is currently considered underexploited because only 38% of the exploitable biomass, estimated at 35000 t (Ben Abdallah *et al.*, 2004) is being fished. Small pelagic fisheries show high inter-annual fluctuations of abundance and distribution, which are related to environmental variables affecting early life stages and hence recruitment (Jacobson and Maccall, 1995; Borja *et al.*, 1996; Alain *et al.*, 2001; Brander and Mohn, 2004).

The reproductive cycle of the Tunisian sardine was studied by Kartas (1981), Gaamour *et al.* (2004) and Khemiri (2006) and its reproductive seasonality agrees fairly well with sardines from the Alborán Sea (Rodríguez, 1990), the NW Mediterranean (Olivar *et al.*, 2003; Palomera *et al.*, 2007), the Adriatic (Karlovac, 1967) and the E Mediterranean (Somarakis *et al.*, 2006). However, to date no studies have focused on its early life stages.

This study was carried out in the Gulf of Tunis (Fig. 1), which opens at its northern boundary to the central Mediterranean, reaching maximum depths of 130 m. Northeast of this gulf we find the Sardinia Channel, and towards the northwest the Sicilian Strait, which channels the water mass exchange between the Western and Eastern Mediterranean basins. In the Strait of Sicily, surface Modified Atlantic Water (MAW), with low salinity, flows into the Eastern Mediterranean, while the Levantine Intermediate Water (LIW) enters the Western Mediterranean. Inflowing currents from the MAW enter the Gulf of Tunis, reaching the Bay of Tunis in the south (Kouki, 1984). The Gulf also receives the outflow of the most important river in Tunisia: the Majreda River situated on the western coast.

This study presents the first information on the spatio-temporal distribution of sardine eggs and larvae in relation to environmental bio-physical parameters: temperature, salinity, nitrate, chlorophyll *a* and nitrate concentration and diatom and zooplankton abundance. It was carried out within the framework of the ESSATEL research project, whose general objectives were to study the complete life cycle of small pelagics and estimate their biomass in order to determine a sustainable exploitation of these resources.

## MATERIAL AND METHODS

Four ichthyoplankton surveys (named ESPOIRS) were carried out on board the R/V HANNIBAL. They were conducted seasonally, in summer (26-30 August 2002), autumn (21-25 October 2002), winter (13-17 February 2003) and spring (11-15 April 2003). Samples were taken from 29 stations covering the whole Gulf of Tunis (Fig. 1): 27 over a grid of 4.8 x 6 nautical miles in east-west and north-south directions, respectively, and 2 near the Majreda and Meliane river estuaries.

Surface temperature and salinity were measured for each station by means of a WTW probe, and surface seawater samples were obtained with a Niskin bottle. Water samples for nitrate and chlorophyll *a* were filtered through Whatman GF/C filters, which were kept frozen ( $-20^{\circ}\text{C}$ ) until being assessed by colorimetric analysis. The filtered water was analysed to determine the nitrate concentration using the method of Strickland and Parsons (FAO, 1975). Phytoplankton water samples were preserved in 2% buffered formalin until analysis. Diatoms were identified and counted by means of a reversed microscope following the Utermöhl method (Thronsen, 1995).

Zooplankton and ichthyoplankton were sampled by oblique tows with a Bongo net of 60 cm mouth diameter, fitted with 335  $\mu\text{m}$  mesh nets. The vessel speed was 3 knots. Maximum sampled depth was 100 m, wherever possible, or about 5 m above the bottom where the water depth was shallower. A Hydro-Bios flowmeter was fitted to the net mouth to estimate the volume of water filtered, which ranged from 51 to

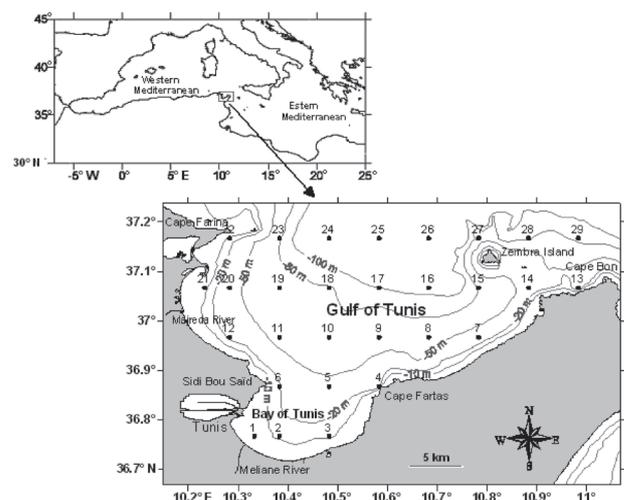


FIG. 1. – Study area and location of the sampling stations.

233 m<sup>3</sup>. At the very shallow stations (depth <15 m) horizontal hauls were performed in order to filter enough water (mean value >126 m<sup>3</sup>). Plankton samples were preserved in 4% buffered formalin. In the laboratory, sardine eggs and larvae were sorted and counted with the aid of a binocular microscope. In parallel, 10% of each sample was taken to count the other zooplankton (>335 µm) organisms.

Eggs and larvae at each station were standardised to numbers beneath 10 m<sup>2</sup> of sea surface area as described by Smith and Richardson (1977), whereas the abundance of other zooplankters was standardised to number of individuals per 10 m<sup>3</sup>. Environmental parameters and sardine egg and larval abundance distributions were mapped using the Surfer software package (Golden Software Inc.), applying the kriging interpolation method and the isotropic linear variogram model. Principal component analysis (PCA) was applied to the data from the winter survey, considering depth, temperature, salinity, nitrate, chlorophyll *a*, diatoms and zooplankton as active variables, and sardine egg and larval abundance as supplement variables. PCA, ANOVA and correlations between parameters were performed using Statistica Software (Statsoft Inc.).

## RESULTS

### Environmental parameters

In summer, the maximum sea surface temperature (25.5°C, SD=0.22) was fairly homogeneous over the survey area (Fig. 2). In autumn, temperature decreased to 21.8°C and the lowest temperature was reached in winter (13.4°C). In winter, positive temperature gradients (4.2°C) from south to north and from west to east were recorded. Colder waters (10.5°C) were located south-east of the River Majreda mouth, and warmer waters (more than 14°C) were observed near Zembra Island and Cape Bon. In spring, there was a mean temperature increase of 2.7°C, reaching an average of 16.1°C, SD=0.86. A slight negative gradient from west to east was observed.

Summer and autumn sea surface salinity (SSS) distributions showed great similarity, with average SSS values of 37.3, SD=0.18 and 37.0, SD=0.16 (Fig. 3) and weak horizontal variations of 0.7 and 0.5, respectively. Winter and spring SSS distributions were also similar. The lowest salinities in these seasons, 22.3 in winter and 29.5 in spring,

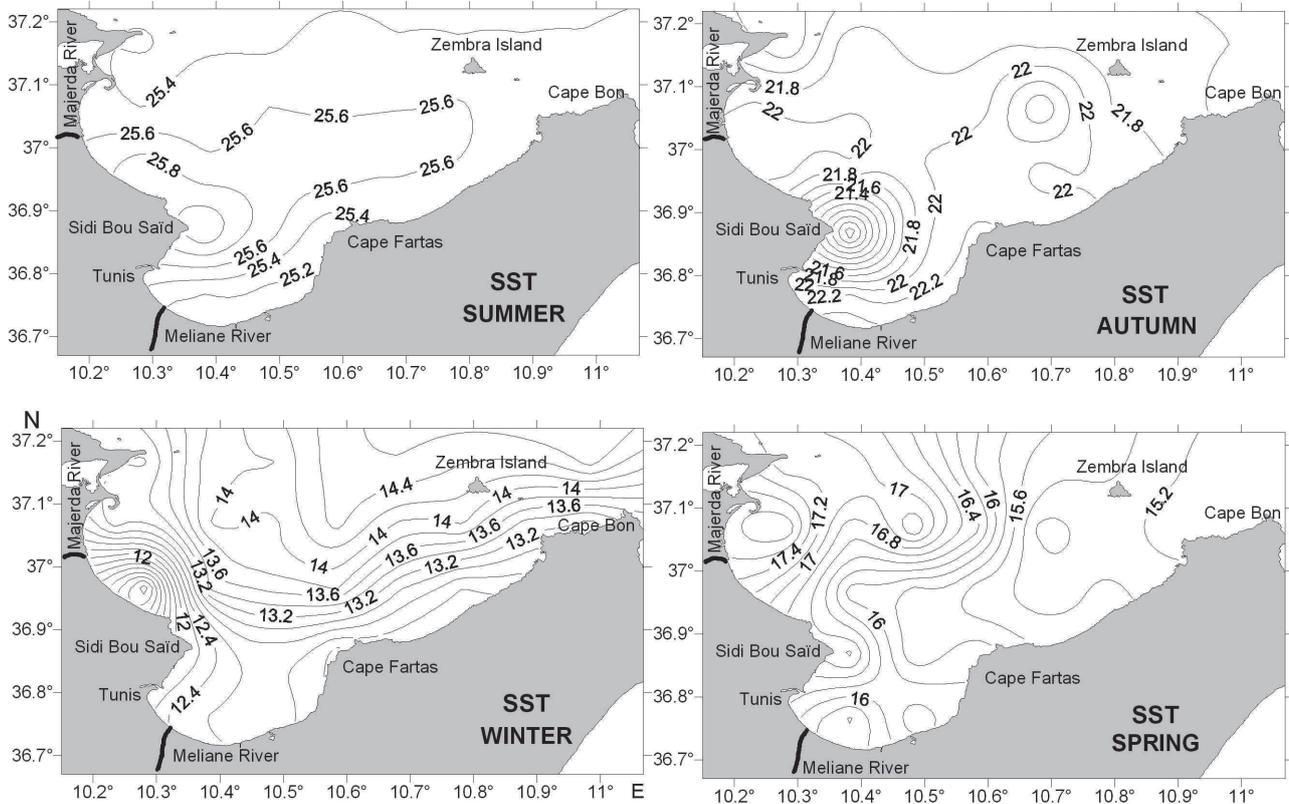


FIG. 2. – Sea surface temperature (SST) maps by season.

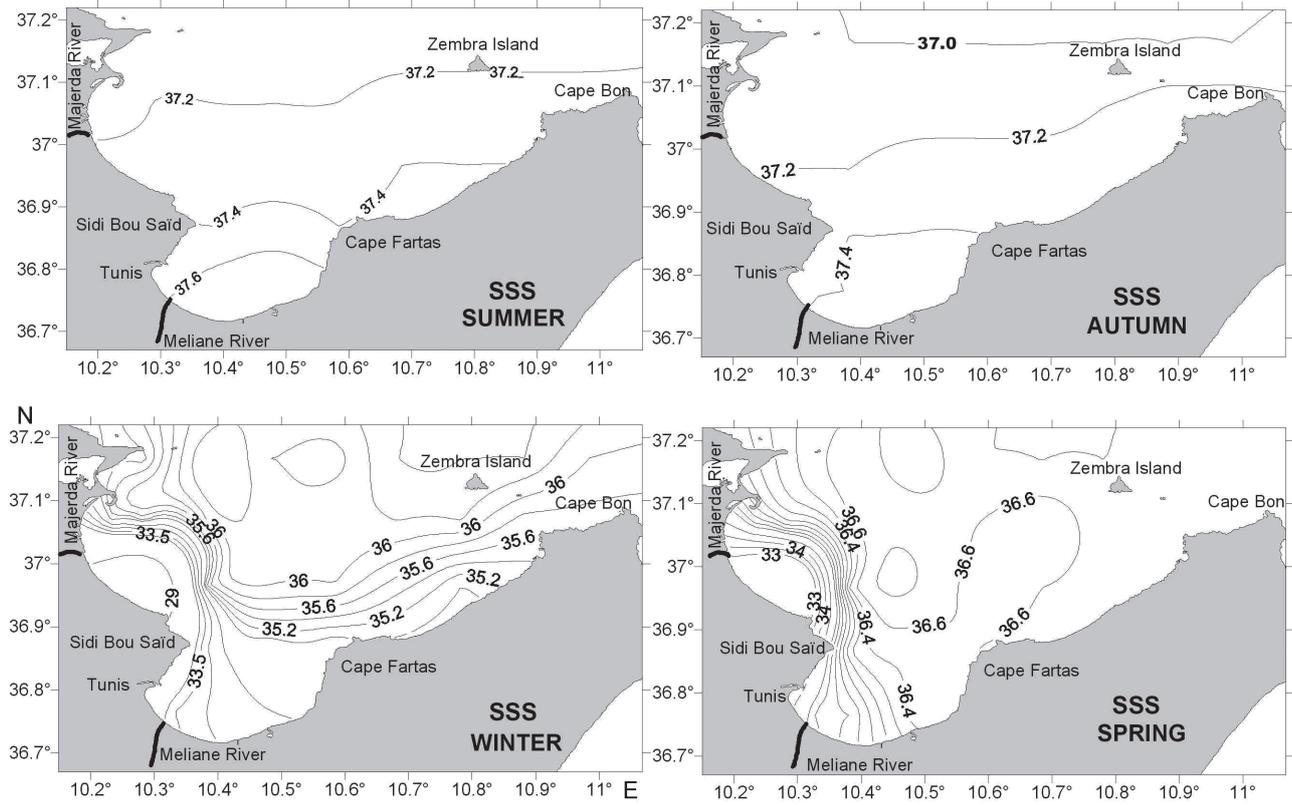


FIG. 3. – Sea surface salinity (SSS) maps by season.

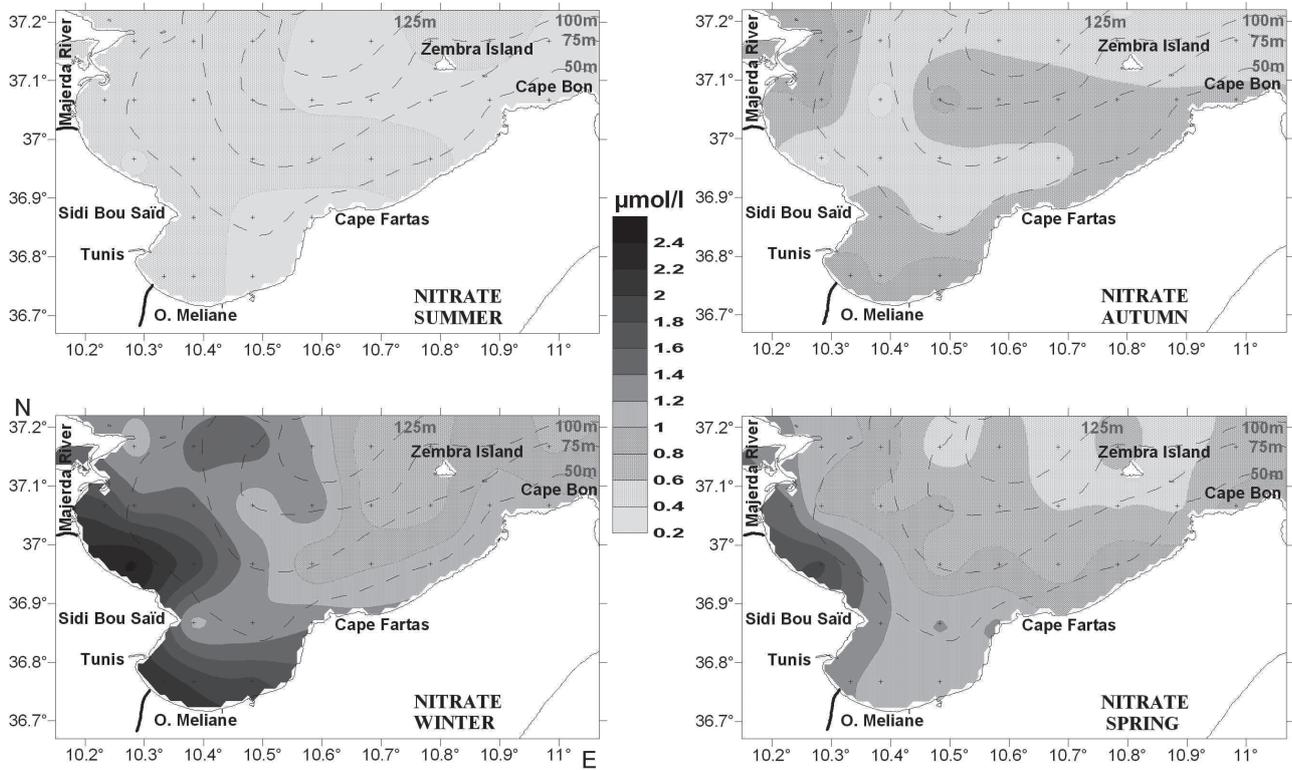


FIG. 4. – Nitrate ( $\text{NO}_3^-$ ) distribution by season.

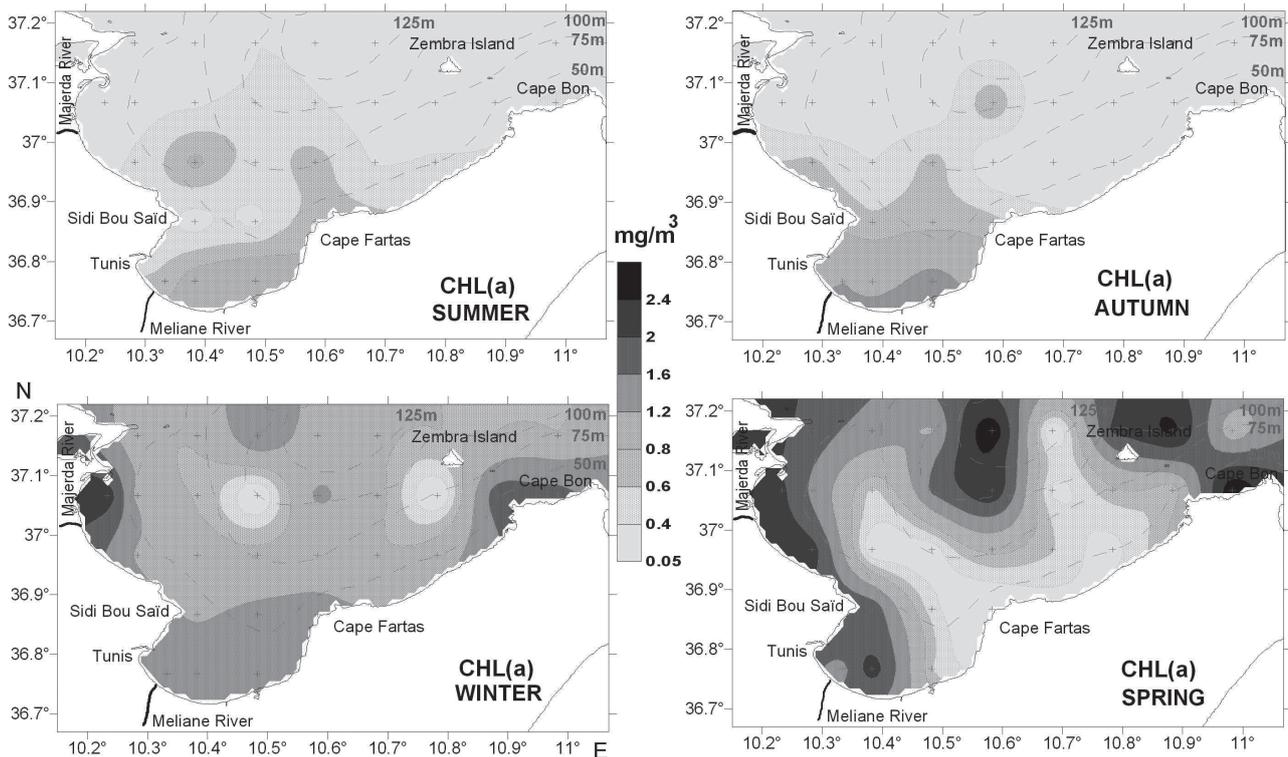


FIG. 5. – Chlorophyll *a* distribution by season.

were located south the River Majreda estuary and positive gradients were recorded in the same direction (west-east).

Nitrate concentrations were low in summer and autumn, showing some heterogeneity, but were higher in winter ( $1.26 \mu\text{mol/l}$ ,  $\text{SD}=0.47$ ) and spring ( $0.90 \mu\text{mol/l}$ ,  $\text{SD}=0.33$ ), in both of which the highest concentration ( $>1.80 \mu\text{mol/l}$ ) was detected to the south of the Rivers Majreda and Meliane (Fig. 4). As observed for salinity, nitrate concentrations showed a negative gradient from west to east.

In consonance with nitrate concentrations, the lowest concentrations of chlorophyll *a* were recorded in summer ( $0.41 \text{ mg/m}^3$ ,  $\text{SD}=0.27$ ) and autumn ( $0.43 \text{ mg/m}^3$ ,  $\text{SD}=0.34$ ), when relatively high concentrations were observed to the south of the Bay of Tunis (Fig. 5). During winter and spring, concentrations increased significantly to average values of  $1.11 \text{ mg/m}^3$ ,  $\text{SD}=0.46$  and  $1.27 \text{ mg/m}^3$ ,  $\text{SD}=0.88$ , respectively. The highest concentrations ( $>1.7 \text{ mg/m}^3$  in winter; and  $>2.7 \text{ mg/m}^3$  in spring) were observed near the Majreda estuary and Cape Bon. However, in spring there were also high concentrations in the centre of the Gulf and north of Zembra Island.

### Biological factors

Diatoms were most abundant in winter ( $1159 \text{ cells/l}$ ,  $\text{SD}=1482$ ) and spring ( $1044 \text{ cells/l}$ ,  $\text{SD}=1505$ ) than in summer ( $300 \text{ cells/l}$ ,  $\text{SD}=322$ ) and autumn ( $552 \text{ cells/l}$ ,  $\text{SD}=467$ ). The highest concentrations were localised in winter near the Majreda estuary ( $5900 \text{ cells/l}$ ) and Cape Bon ( $4700 \text{ cells/l}$ ), and in spring ( $>5000 \text{ cells/l}$ ), also near Cape Bon and at Zembra Island (Fig. 6).

Zooplankton abundance was low in summer ( $451 \text{ ind./}10 \text{ m}^3$ ,  $\text{SD}=318.9$ ) and autumn ( $636 \text{ ind./}10 \text{ m}^3$ ,  $\text{SD}=448.7$ ) and high in winter ( $856 \text{ ind./}10 \text{ m}^3$ ,  $\text{SD}=620$ ) and spring ( $1096 \text{ ind./}10 \text{ m}^3$ ,  $\text{SD}=921.2$ ). In summer and autumn the highest abundances were observed close to the coasts (Fig. 7), whereas in winter and spring they were detected offshore, in the centre of the Gulf in spring and south of Zembra Island in winter.

### Distribution of sardine eggs and larvae

Sardine eggs were present only in autumn and winter, with average abundances of  $23$ ,  $\text{SD}=52$  and  $257 \text{ eggs/}10 \text{ m}^2$ ,  $\text{SD}=638$ , respectively. In autumn, two patches of relatively high abundance were lo-

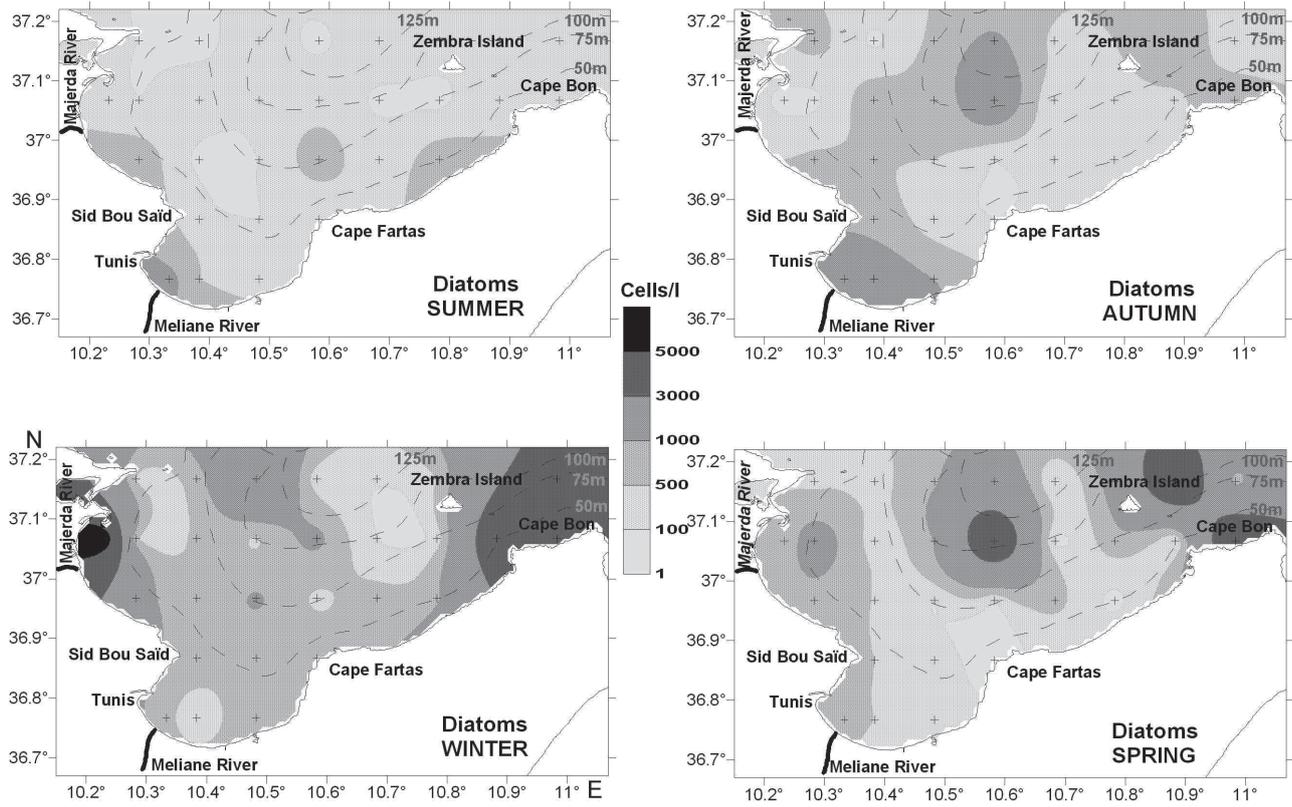


FIG. 6. – Diatom distribution by season.

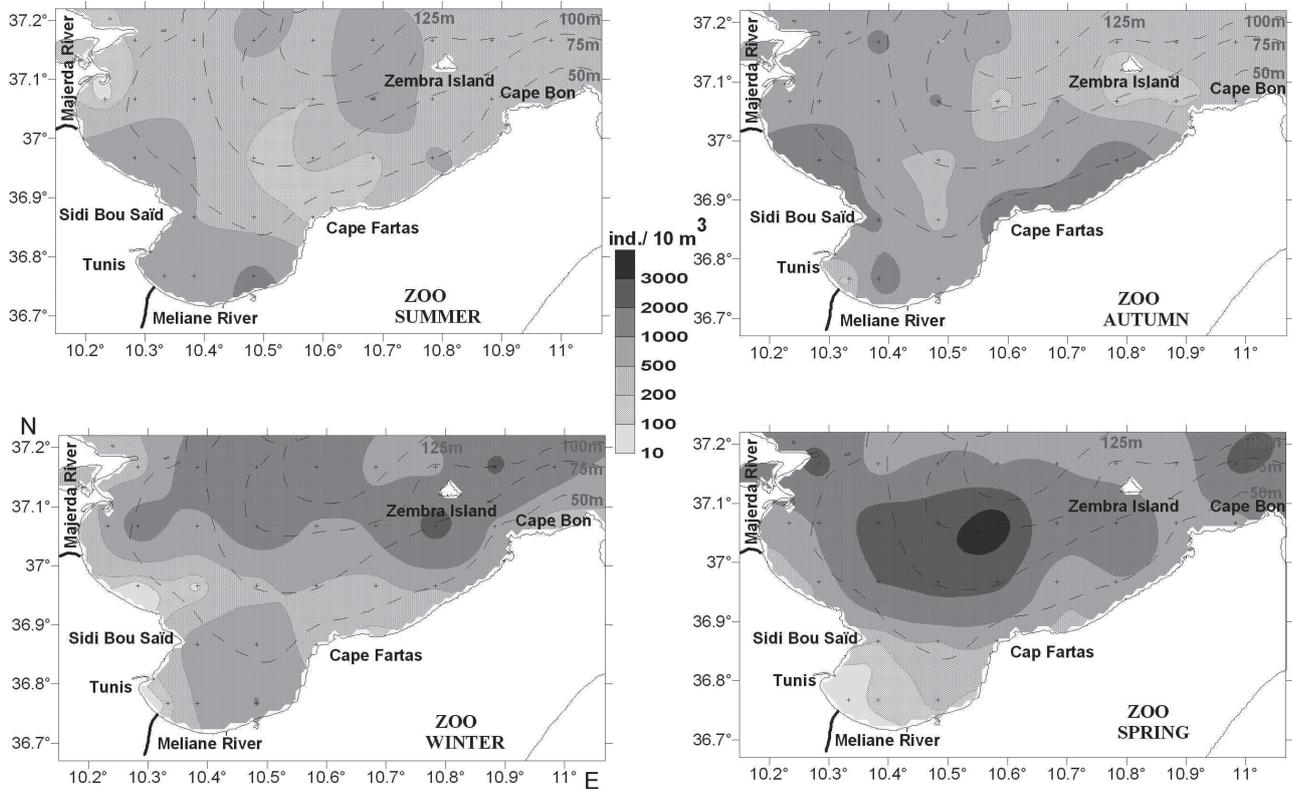


FIG. 7. – Zooplankton distribution by season.

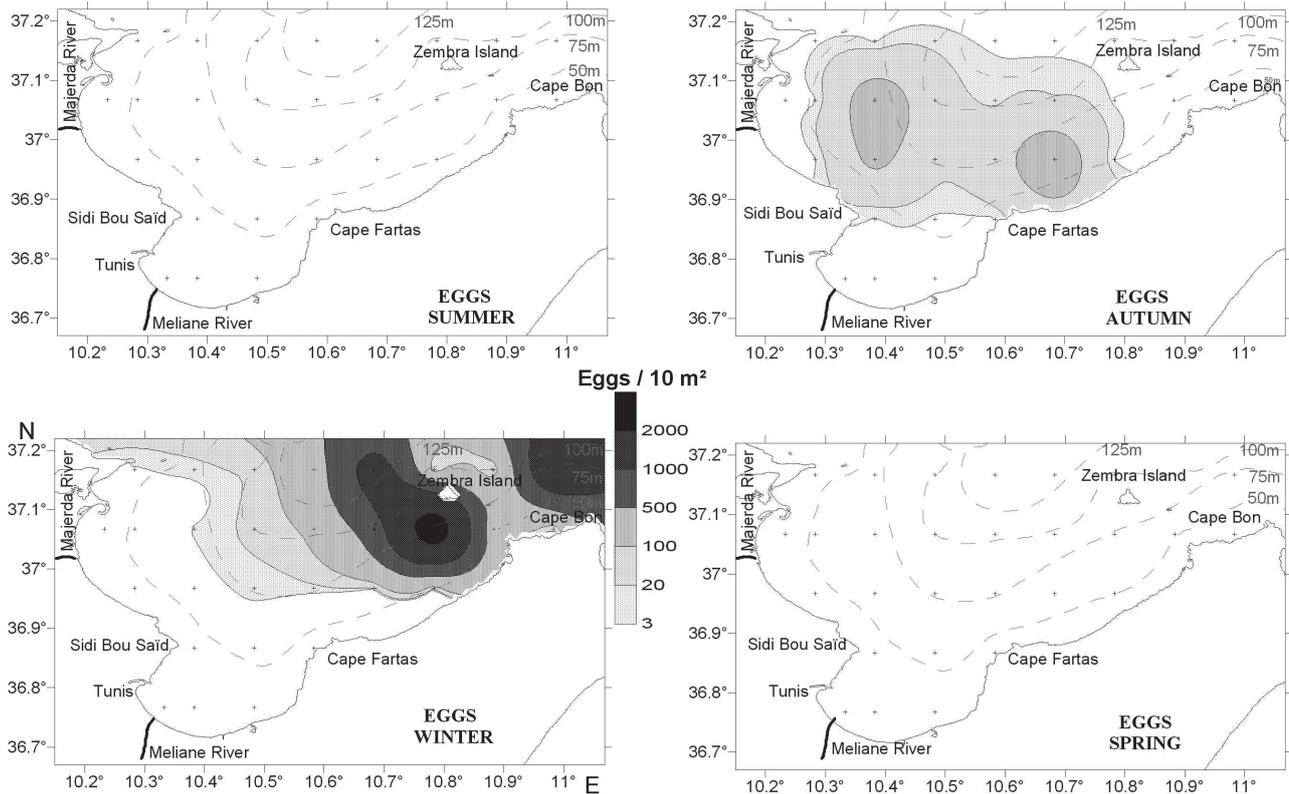


FIG. 8. – *Sardina pilchardus* egg distribution by season.

cated northwest of Cape Fartas (192 eggs/10 m<sup>2</sup>) and north of Sidi Bou Saïd (around 150 eggs/10 m<sup>2</sup>) at depths of between 50 and 75 m (Fig. 8). In winter, eggs were concentrated in the northwestern part of the gulf, with a maximum abundance near Zembra Island (2805 eggs/10 m<sup>2</sup>) and north of Cape Bon (1733 eggs/10 m<sup>2</sup>). No eggs were found in the southern and western parts of the Gulf. Overall, sardine eggs were more abundant in the 75-100 m stratum.

Higher mean abundance of sardine larvae was recorded in winter (38 larvae/10 m<sup>2</sup>, SD=64). The larvae were widely spread over the Gulf (Fig. 9), but the highest abundance (288 larvae/10 m<sup>2</sup>) was located to the south of Zembra Island, where the highest abundance of eggs was also found. Autumn and spring abundances were lower than 1 larva/10 m<sup>2</sup> and larvae were only caught at 2 and 3 stations, respectively. In autumn, larvae were found northwest of the gulf and northeast of Cape Fartas, whereas in spring they were mainly concentrated near the estuaries of the Rivers Majerda and Meliane.

In winter, sardine eggs and larvae represented 39% and 41% of total pelagic eggs and larvae, respectively. PCA of the winter survey data (Fig. 10A) showed that temperature, nitrate concentration,

depth, salinity and zooplankton abundance in PCA Factor 1 and diatoms and chlorophyll *a* in PCA Factor 2 were the variables helping to explain the PC axis. The two main axes accounted for 52.31% and 21.06% of the total variance, respectively (a sum of 73.37% of the total). Sardine egg and larval abundance was positively correlated with zooplankton abundance and negatively with nitrate and chlorophyll *a* concentrations (Fig. 10B, Table 1). The examination of the similarity between stations (Fig. 10B, Table 2) allowed 3 groups to be identified:

- I: Characterised by higher depths, warmer and saltier waters, low concentrations of nitrate and chlorophyll *a* and higher egg abundance (778 /10 m<sup>2</sup>) (Table 2).

- II: Showing intermediate environmental conditions.

- III: Characterised by shallower depths, lower temperature, salinity and zooplankton abundance and higher concentrations of nitrate, chlorophyll *a* and diatoms.

Stations 12 and 21 were rather different from the rest of the stations. Station 12 had the lowest temperature and salinity, a high nitrate concentration and a low chlorophyll *a* concentration and zooplankton

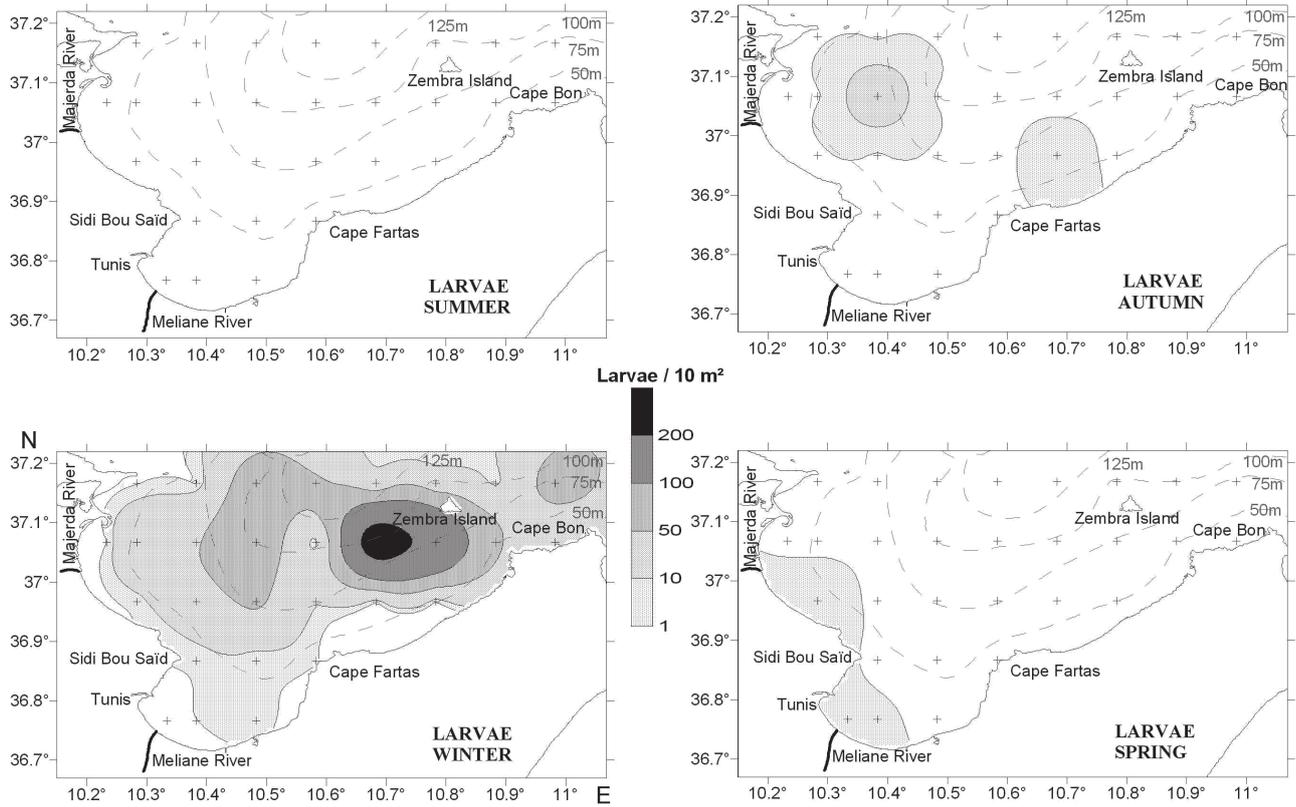


FIG. 9. – *Sardina pilchardus* larval distribution by season.

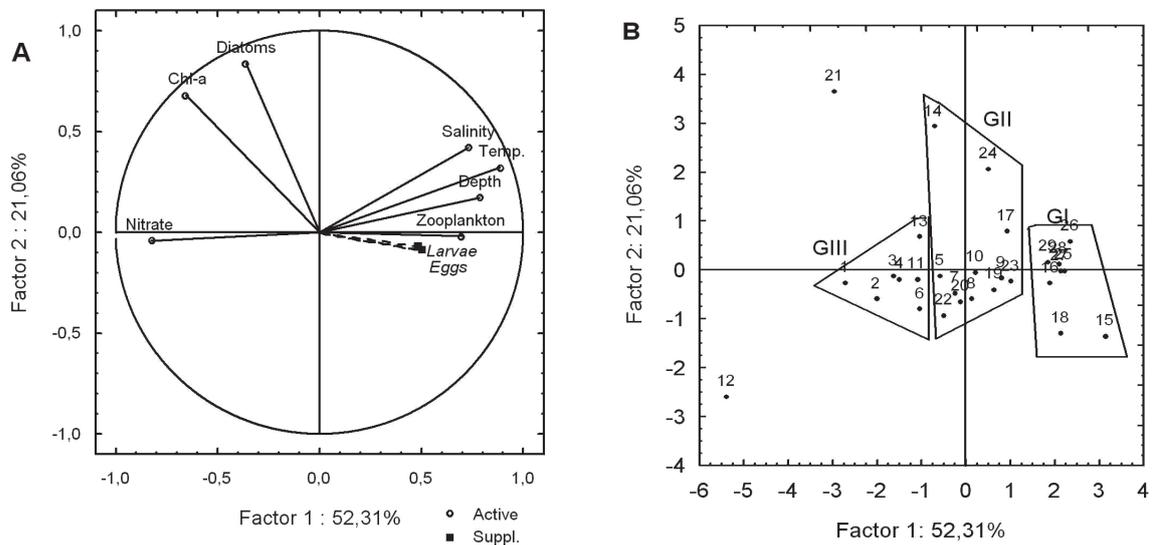


FIG. 10. – PCA of environmental parameters with supplement variables: egg and larval abundances. A: correlation circle of variables; B: projections of stations in the factorial plan.

abundance. Station 21 had the shallowest depth and the highest chlorophyll *a* and diatom concentrations. The ANOVA analysis of groups (I, II and III without taking into account stations 12 and 21) showed significant differences for all parameters, except for diatom concentrations (Table 3).

DISCUSSION

The hydrographical situation showed two clearly distinguishable periods. The first, summer-autumn, was characterised by high temperatures and salinities and low chlorophyll *a* and zooplankton concentra-

TABLE 1. – Spatial correlations between sardine egg and larval abundance and environmental parameters in winter; r: correlation coefficient and p: probability with n.s. (p&gt;0.05); \* (p&lt;0.05); \*\* (p&lt;0.01); \*\*\* (p&lt;0.001).

Parameter		Depth	T	NO <sub>3</sub> -	Chl- <i>a</i>	Zooplankton	Sardine eggs
Sardine eggs	r	0.34	0.39	-0.48	-0.43	0.53	-
	p	n.s.	*	**	*	**	-
Sardine larvae	r	0.43	0.35	-0.40	-0.38	0.46	0.57
	p	*	n.s.	*	*	*	***

TABLE 2. – Mean values of environmental parameters and sardine egg and larval abundance by group and min-max for all stations.

Group /Station	I	II	III	12	21	mean	min	max
Number of stations	8	12	7	1	1	-	-	-
Depth (m)	96.8	67.0	22.7	22.2	12.0	59.2	9.0	130.0
Temperature (°C)	14.2	13.5	12.8	10.5	13.1	13.4	10.5	14.7
Salinity (psu)	36.1	35.7	34.5	22.3	35.0	35.1	22.3	36.4
Nitrate (µmol/l)	0.86	1.25	1.72	2.45	1.86	1.29	0.62	2.45
Chlorophyll <i>a</i> (mg/m <sup>3</sup> )	0.58	1.10	1.33	1.14	2.30	1.11	0.09	2.30
Diatoms (cells/l)	280	1291	650	1400	5900	1159	100	5900
Zooplankton (ind./10 m <sup>3</sup> )	1534	766	584	16	579	856	16	2503
Eggs (eggs/10 m <sup>2</sup> )	778	46	0	0	0	256	0	2805
Larvae (larvae/10 m <sup>2</sup> )	130	27	9	4	0	38	0	288

TABLE 3. – ANOVA of environmental parameters and sardine eggs and larval abundance by group (GI, GII and GIII), F: statistical test, p: probability with n.s. (p&gt;0.05); \* (p&lt;0.05); \*\* (p&lt;0.01); \*\*\* (p&lt;0.001).

Parameter	F	p
Depth	17.80	***
Temperature	20.22	**
Salinity	16.70	***
Nitrate	14.68	***
Chlorophyll <i>a</i>	9.87	***
Diatoms	0.06	n.s.
Zooplankton	10.78	***
Sardine eggs	6.65	**
Sardine larvae	5.03	*

tions. The second, winter-spring, showed the opposite characteristics. In the later period, the increase in nitrate concentration, particularly in the upper layer, seems to be related to river enrichment and mixing processes. As a result, in this period chlorophyll *a* and zooplankton concentrations increase. The described situation is in agreement with the results obtained previously by Daly Yahia-Kéfi (1998) and Daly Yahia-Kéfi *et al.* (2005) in the Bay of Tunis, showing that the main bloom of phytoplankton, in particular diatoms, takes place in winter, and that higher zooplankton abundances occur during the winter-spring period, as Daly Yahia (1998) observed. We noted that the spatial distribution of both groups was different, with phytoplankton being more abundant near the coast and zooplankton being concentrated in the deeper areas of the Gulf.

Regarding the spawning seasonality of sardine, from our observations we can infer that the species begins to spawn in autumn and attains its peak in winter, as reported for other areas of the Western Mediterranean (Rodriguez, 1990; Palomera and Olivar, 1996; Alemany *et al.*, 2006). In spring, although the SST (16.1°C) was lower than that of autumn (21.85°C), no sardine eggs were found. Spawning seems to be triggered by the effect of water cooling (Aldebert and Tournier, 1971; Palomera *et al.* 2007) and spawning intensity decreases as temperature increases (Solà *et al.*, 1992).

Previous studies in Tunisian waters based on gonadosomatic index (GSI) also showed that sardines spawn in winter (Kartas, 1981; Gaamour *et al.*, 2004; Khemiri, 2006), which is in agreement with our results and with those reported for other areas of the Mediterranean and Atlantic. Indeed, the spawning season of sardine extends from October to May, with a spawning peak occurring in January-February (Gómez Larrañeta, 1960; Palomera and Olivar, 1996; Olivar *et al.*, 2003). In the Adriatic Sea, (Dulcic and Grbec, 2000) sardines spawn when temperature ranges between 11.4 and 19.3°C (Karlovac, 1967). In the estuary of Vigo (northern Spain) sardines spawn from November to July, with temperatures of 10 to 16°C (Ferreiro and Labarta, 1984). Off Portuguese coasts, spawning takes place predominantly from November to April (Figueiredo and Santos, 1989; Ré *et al.*, 1990; Zwolinski *et*

*al.*, 2001). However, the Moroccan Atlantic sardine spawns all year round, but maintains a winter peak (Amenzoui *et al.*, 2006).

The occurrence of the highest numbers of early life stages of sardine in the Gulf of Tunis in winter is opposite to the situation of anchovy, which mainly spawns in spring and summer (Zarrad *et al.*, 2006). This will reduce competition between the two species throughout their whole life cycle. This alternating occurrence of anchovy and sardine eggs and larvae, associated with the inversion of the thermal features and water column state, agrees with observations reported for other Mediterranean regions (Olivar *et al.*, 2001). The spatial pattern for sardine spawning in the gulf of Tunis, in waters deeper than 50 m, is similar to what has been reported on the Portuguese and Greek coasts, where the maximum abundance of sardine eggs was found from approximately the 50 to the 100 m isobath (Afonso, 1991; Cunha *et al.*, 1992; Somarakis *et al.*, 2006).

The main reproductive period of sardine in the Gulf of Tunis coincides with periods of greatest diatom and zooplankton abundance. The synchrony with this period of greater food availability ensures high larval developmental success, an adaptation which is particularly important in oligotrophic waters such as those of the Gulf of Tunis. The positive correlation between the abundance of eggs and zooplankton in winter could result from the preference of adults for choosing a suitable spawning habitat which enhances larval survival. Chícharo *et al.* (2003) demonstrated that the mean RNA/DNA ratios for sardine larvae during winter off northern Portuguese coasts were relatively high, indicating that almost all larvae collected were in good condition—concomitantly with the high microzooplankton biomass and high daily egg production of the copepod *C. helgolandicus* recorded during the same period.

However, our results show negative spatial correlation between eggs and chlorophyll *a*. In the main spawning areas, chlorophyll *a* concentration was the lowest. In spite of the high chlorophyll *a* concentration in the coastal area, sardine eggs were scarce or absent. It could be hypothesised that sardine schools do not find a suitable feeding or reproductive habitat in these shallower waters, preferring deeper areas with a high abundance of zooplankton, like those found south of Zembra Island. Sardine shows a high plasticity in its feeding behaviour, since it can switch between the selective particle-feeding and the non-selective filter-feeding mode (Van der Lingen, 1994,

2002; Bode *et al.*, 2004) depending on the availability of plankton resources. This omnivorous species selects sites with increased mesozooplankton or phytoplankton biomasses as an adaptation to the large spatial and temporal variability of plankton in the Mediterranean Sea in order to sustain growth and reproduction (Somarakis *et al.*, 2006).

In conclusion, in the Gulf of Tunis sardine spawning is centered in winter and at depths of over 50 m located mainly south of Zembra Island. The spawning habitat and the area of larval concentration are characterised by relatively warmer and saltier waters, low nitrate and chlorophyll *a* concentrations and high zooplankton abundance.

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