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## Distribution of *Engraulis encrasicolus* eggs and larvae in relation to coastal oceanographic conditions (a south-western Adriatic Sea case study)

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### Abstract

The identification of European anchovy (*Engraulis encrasicolus*) spawning and nursery areas is a key step in the management of an essential resource for fisheries and pelagic trophic webs. Eggs and larvae collected in ichthyoplankton surveys (2012 - 2015) carried out in the south-western Adriatic Sea were examined to investigate the mechanisms that control their distribution. Egg and larval abundance varied through the years, the highest values being recorded in 2012 and the lowest in 2014, and their abundance correlated positively with the zooplankton biomass. Application of quotient analysis to identify their relationships with environmental and biological variables demonstrated egg preferences for depths between 91 - 120 m and avoidance of depths between 11 - 30 m and for high values of chlorophyll-a ( $> 0.52 \text{ mg m}^{-3}$ ) and low values of zooplankton biomass ( $< 199 \text{ mg m}^{-2}$ ). The habitat preferences and avoidances of larvae were characterized by a preference for depths between 11 - 60 m and for high zooplankton biomass values ( $> 1000 \text{ mg m}^{-2}$ ) and by avoidance of depths  $> 150 \text{ m}$  and of low biomass values ( $< 299 \text{ mg m}^{-2}$ ). These correlations and quotient value analysis suggest that the distribution of eggs and larvae in the south-western Adriatic is principally driven by food availability and depth.

**Keywords:** *Engraulis encrasicolus*, spawning habitat, spatial distribution, zooplankton biomass, single-parameter quotient analysis, Adriatic Sea.

### Introduction

Small pelagic fishes such as anchovies and sardines (Clupeiformes) play a significant role in shaping the structure of marine ecosystems and constitute an intermediate trophic level in the marine food web (Coll *et al.*, 2007). Since they act as “wasp-waist” species, exerting top-down control on zooplankton and bottom-up control on top predators, strong fluctuations in their populations have the ability to modify ecosystem structure and functioning (Cury *et al.*, 2000).

Small pelagic fish species are characterized by a short lifespan and a reproductive strategy that involves production of large quantities of pelagic eggs over an extended season; such features make them susceptible to environmental variability, food availability, and physical processes (Bakun, 1996; Cushing, 1996; Giannoulaki *et al.*, 2013).

These species also have an important economic role, since they are target species worldwide; for instance, in 2009 anchovies accounted for 22% of total marine catches (FAO, 2014). Most of these catches come from upwelling zone areas in the eastern Atlantic and eastern Pacific Ocean, where the landing of small pelagic fishes is dominated by a limited number of species (Fréon *et al.*, 2005).

The Adriatic Sea is rich in small pelagic fish. It is one of the richest basins in the Mediterranean and by far the most important fishing ground among the Italian seas, providing more than 66% of the landings of small pelagic species of Italian fisheries (IREPA Onlus 2012). The chief species are European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*), Horse mackerel (*Trachurus* spp.), Atlantic mackerel (*Scomber scombrus*), and Chub mackerel (*Scomber japonicus*). Anchovy and sardine account for than 40% of the total catch of the Adriatic Sea (FAO, 2010).

*E. encrasicolus* is a coastal, pelagic and euryhaline fish with a broad distribution in the Mediterranean and adjacent seas (Black Sea and Sea of Azov) and in the eastern Atlantic, from Scandinavia to the Gulf of Guinea and South Africa (Whitehead *et al.*, 1988; Bellido *et al.*, 2000). It is characterized by high growth rates and early maturity, which is attained at the end of the first year of life (Sinovčić & Zorica, 2006). The species is a serial batch spawner with indeterminate fecundity; in the Adriatic Sea, it reproduces in coastal areas from April to October, egg production peaking in June-July (Regner, 1996; Morello & Arneri, 2009).

Data on anchovy egg and larval distribution have been reported by several studies in a number of areas. In the Bay of Biscay spawning occurs close to the coast, in highly stratified river plumes, or at the shelf break, and seems to be constrained by temperature, especially bottom temperature (Planque *et al.*, 2007). In the Benguela ecosystem, where the species shows a spatially structured spawning pattern, habitat selection is also driven by environmental conditions, most often temperature (van der Lingen *et al.*, 2001). In the north-western Mediterranean Sea anchovy prefer areas under the influence of the continental water runoff; the eggs and larvae show relatively low abundance in shallow waters close to the coast (< 50 m) and are densest near the edge of the shelf (ca. 200 m) (García & Palomera, 1996; Palomera *et al.*, 2007). In the Strait of Sicily egg abundance was maximum within a depth of 100 m, where shallow waters with upwelling

(low temperature, high water density, high chlorophyll-a concentration) and moderate current speed appear to be preferred for spawning (Basilone *et al.*, 2013). In the Gulf of Tunis spawning areas and larval abundance seem to be related to the central part of the area beyond the 75 m isobaths, where productivity is enhanced by rivers, and the main factor controlling spawning intensity seems to be sea surface temperature (Zarad *et al.*, 2006). In the eastern Mediterranean anchovy spawning occurs in river plumes and/or upwelling areas, which may be less saline and more productive than other areas and eggs and larvae are associated with a broad range of salinity, temperature, and water density (Somarakis & Nikolioudakis, 2007).

The Adriatic Sea is characterized by a cyclonic circulation with two branches, the East Adriatic Current (EAC) flowing northward along the eastern coast (Marini *et al.*, 2010) and the West Adriatic Current (WAC) flowing southward along the western coast (Artegiani *et al.*, 1997a; Marini *et al.*, 2002), which flushes nutrient-rich water out of the northern Adriatic (Hopkins *et al.*, 1999; Marini *et al.*, 2008; Grilli *et al.*, 2013). Other important features are local gyres like the South Adriatic Gyre (SAG), which may enhance water mixing and foster its enrichment by concentrating nutrients (Marini *et al.*, 2015); the gyres may act as inshore “retention traps” for egg and larvae, limiting their dispersal and making this area highly suitable for spawning (Specchiulli *et al.*, 2016) (Fig. 1). These oceanographic features would also explain the genetic subdivision of local populations along

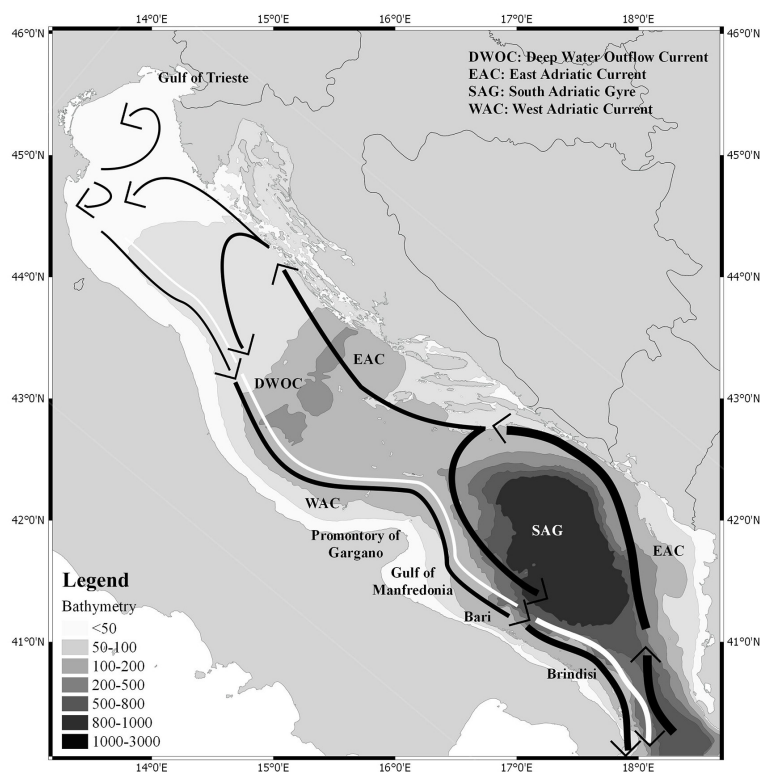


Fig. 1: Adriatic Sea surface circulation (redrawn from [https://commons.wikimedia.org/wiki/File:Adriatic\\_Sea\\_Currents\\_2.svg](https://commons.wikimedia.org/wiki/File:Adriatic_Sea_Currents_2.svg)).

the Croatian coast and at the Po river estuary (Ruggeri *et al.*, 2016b).

Anchovy eggs in the Adriatic have been described at temperatures between 11.6 and 28 °C and at salinity values ranging from 9.1 to 38.7; spawning peaks between 18 and 28 °C, and egg abundance is inversely proportional to salinity (Morello & Arneri, 2009 and references therein). Vucetic (1975) found that variations in egg abundance in the central Adriatic closely correlated with food availability. These findings were confirmed by Gamulin & Hure (1983), who found that egg distribution throughout the Adriatic correlated closely with zooplankton distribution. Regner (1985) suggested that temperature and salinity were proxies for hydrographic conditions favouring production, and that food availability governs the spawning dynamics of anchovy in spatial and temporal terms. During the spawning season, eggs have been detected throughout the Adriatic within a depth of 200 m, but not at greater depths (Gamulin & Hure, 1983; Regner, 1985). The main spawning areas have been identified in coastal waters along the western coast, from the Gulf of Trieste to the Gargano Promontory (Casavola & Marano, 1985; Regner, 1996). In the south Adriatic Casavola (1998) reported that egg abundance ranged from 0 to 100 eggs m<sup>-2</sup>, with a greater abundance between Bari and Brindisi (50 - 100 eggs m<sup>-2</sup>); in the Gulf of Manfredonia abundance ranged between 0 - 20 eggs m<sup>-2</sup>, and was greater between 50 and 100 m. Melia *et al.* (2002) described an egg abundance between 0 - 300 m<sup>-3</sup>, with peaks (>100 m<sup>-3</sup>) off Bari between 100 and 200 m, and a lower abundance in the Gulf of Manfredonia (0 - 100 m<sup>-3</sup>), peaking at a depth of 100 m. In the Gulf of Manfredonia Marano *et al.* (1998) reported a large number of larvae, post-larvae, and juveniles but relatively low egg abundances. Other spawning areas have been identified in the eastern Adriatic, between Susak Island and the Jabuka pit as well as around Palagruža Island, even though spawning intensity in these areas was lower (Sinovčić, 1978, 2000; Piccinetti *et al.*, 1979; Regner, 1996).

Since the last ichthyoplankton surveys in the Adriatic were conducted in the 1980s and 1990s, this study was performed to identify the spawning and nursery areas of *E. encrasicolus* in the Southern Adriatic, update their boundaries, and assess by single parameter quotient analysis the environmental and biological factors affecting the choice of spawning areas during a four-year period (2012 - 2015).

## Materials and Methods

### Sampling method and data collection

Ichthyoplankton samples and oceanographic data were collected on board R/V “G. Dallaporta” during four acoustic surveys carried out in June and July 2012-2015 in the framework of project MEDIAS (pan MEDiterranean Acoustic Surveys) (Leonori *et al.*, 2011; 2012) and Flag Project RITMARE. The area covered by the survey

was along the western continental shelf of Geographical Sub-Area (GSA) 18 and measured about 2510 nm<sup>2</sup> (square nautical miles). The ichthyoplankton sampling design was the same for all surveys. Sampling was performed at intervals of 5 nm along parallel transects perpendicular to the coastline up to the 200 m bathymetry; the distance between transects was 7-10 nm (Fig. 2). If at least one egg was found in the last station of the transect, the sampling was extended for another 5 nm. A total number of 232 samples were collected at 58 stations.

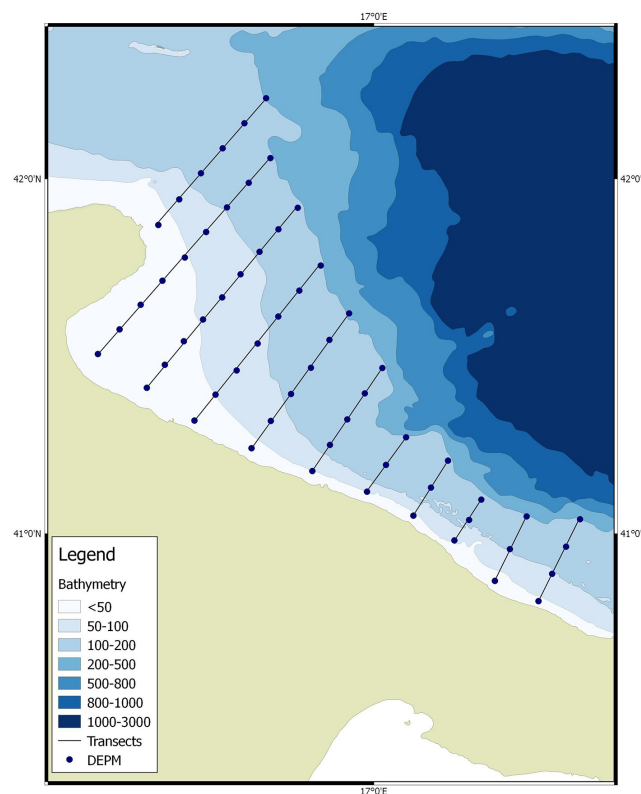


Fig. 2: Sampling area.

Ichthyoplankton samples were collected using a 200-μm mesh size WP2 net. Vertical tows were performed from 3 m above the bottom to the surface; maximum sampling depth was 100 m. Samples were immediately placed in 4% formaldehyde-seawater solution. In the laboratory, anchovy eggs and larvae were identified and sorted under a stereomicroscope; they were counted and their abundance was standardized as number per square metre.

Samples collected in 2014 and 2015 were also used to assess the zooplankton biomass. After subtracting anchovy eggs and larvae, samples were dried in an oven for 24 h at 60° C and weighed; the procedure was repeated until a constant weight was reached (Postel *et al.*, 2000). Possible loss of material due to formalin fixation was corrected by applying a conversion factor obtained by averaging several conversion factors used for different zooplankton taxa (Giguère *et al.*, 1989). The mass loss after drying was estimated to be 31.32% of the total original mass.



In 2012 and 2013, ichthyoplankton and zooplankton samples were collected in parallel. Samples were frozen on board and dried in the laboratory (Postel *et al.*, 2000). Then, the estimated dry mass of anchovy eggs and larvae was subtracted from the total biomass, assuming that an equal number of eggs and larvae was found in the zooplankton and ichthyoplankton samples.

Conductivity, temperature, and depth data were collected with a CTD probe (SeaBird Electronics Model 9, Bellevue, WA, USA) at all 58 stations in 2014 and 2015 and at 39 stations in 2012 and 2013. The missing CTD data were integrated by applying Inverse Distance Weighting (IDW) to the spatial interpolation made with SURFER 12 software (Golden Software, Golden, CO, USA). Sea surface chlorophyll-a satellite data (CHLA 8-day composite version, resolution, 4 km) were extracted based on the sampling grid and dates (<https://ocean-color.gsfc.nasa.gov/>) using SeaDAS software (v.7.4).

### Data analysis

A modified version of single parameter quotient analysis was used to characterize anchovy spawning and nursery areas, using the Shachar package in R (v. 2.15.3, R Core Team 2013) (Bernal *et al.*, 2007). A quotient curve was considered as an explanatory tool describing the relationship between abundance of eggs or larvae and environmental variables, to identify ranges of variables where fish “prefer” or “avoid” spawning (Lluch-Belda *et al.*, 1991; Drapeau, 2005). The variables included in the study - salinity, temperature, depth, chlorophyll-a and zooplankton biomass - were divided into categories to ensure that maximum occurrence per category did not exceed 25% of all measurements. The quotient value (Q) for each category was estimated as:

$$Q(c) = \frac{\% \text{ eggs } \vee \text{ larvae } (c)}{\% \text{ environmental variable } (c)}$$

where (c) is each category of the environmental variable considered; % eggs or larvae is the sum of eggs or larvae per m<sup>2</sup> that were counted in the stations falling into each category, divided by the total number of eggs or larvae per m<sup>2</sup> counted at all the stations sampled in the year considered; and % environmental variable is the ratio of the number of stations falling within a category to the total number of stations sampled. A bootstrap (n = 999 times, with replacement) was used to calculate the confidence intervals (0.025 and 0.975 percentiles) with the simulated random pseudo-surveys. Q values exceeding the upper confidence limit indicated positive habitat selection (preference) and those under the lower confidence interval were considered as negative selection (avoidance). The Q values between the lower and the upper confidence limit was considered as tolerated habitats (Mhlongo *et al.*, 2015).

Mean egg and larval abundance was calculated as the

geometric mean number of eggs and larvae per m<sup>2</sup>:

$$MG = 10^{\frac{1}{n} \sum_{i=1}^n \log Y_i}$$

where *n* is the number of stations where at least one egg or larva was retrieved and *Y<sub>i</sub>* is the number of eggs and larvae per m<sup>2</sup>.

The 95% confidence interval (CI) was calculated as follows:

$$CL_{\pm} = MG \pm \sqrt{\frac{s^2}{n}}$$

where *s*<sup>2</sup> is the variance and *n* the number of stations with at least one egg or larva.

To test the relationships between each environmental variable and the abundance of anchovy eggs and larvae, the physical data were averaged for the first 20 m, assuming that the eggs and larvae were concentrated in this water layer, then non-parametric Spearman's rank correlation test was applied. Principal Component Analysis (PCA) was used to test the presence of structure in the environmental dataset taking into account all the environmental variables. Differences in egg and larval abundance, water salinity, temperature, depth, chlorophyll-a and zooplankton biomass in the four sampling years were subjected to non-parametric Kruskal-Wallis test. A post-hoc test (Dunn's test) was performed to detect differences between years.

### Results

The spatial distribution of eggs showed different patterns in the four years of sampling. Their abundance ranged between 0 – 454.9 egg m<sup>-2</sup>. The percentage of negative stations ranged from 12 to 34%; it was lowest in 2012 and highest in 2015.

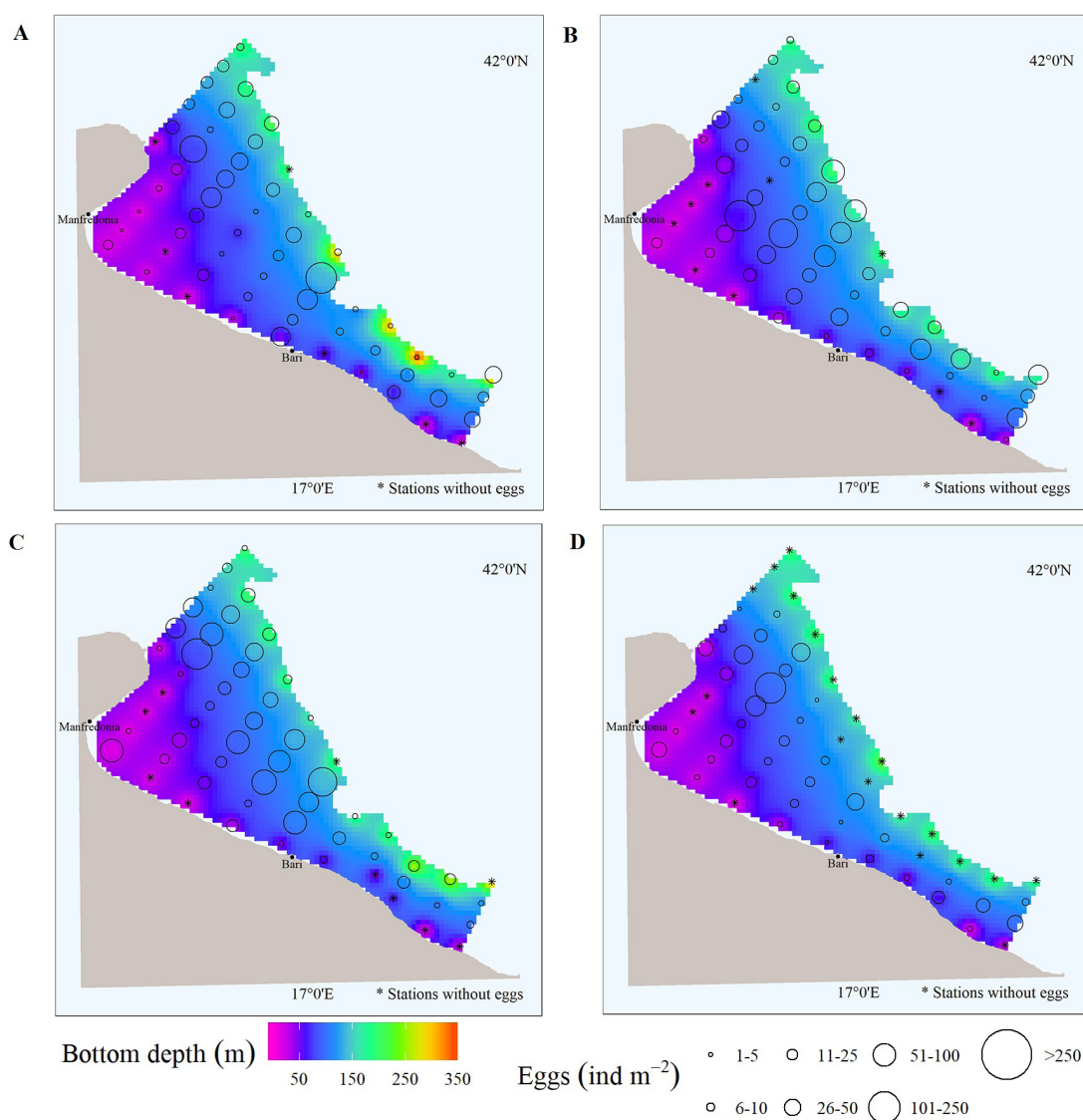
In 2012 the geometric mean of the eggs showed a peak (39.49 egg m<sup>-2</sup>; CI 27.66 – 51.32). Their distribution was wide, with abundance peaks in front of the Gulf of Manfredonia, in front of Bari, and in the southern portion of the study area. The most frequent abundance classes were those with 10 - 25 eggs m<sup>-2</sup> and 50 - 75 eggs m<sup>-2</sup>, which together accounted for 31.6% of total abundance (Fig. 3a). In 2013 the geometric mean was 26.99 eggs m<sup>-2</sup> (CI 21.52 – 32.46); most of the eggs were retrieved in the central portion of the study area, and the most frequent class of abundance was 25 - 50 eggs m<sup>-2</sup>, which accounted for 22.4% of total abundance (Fig. 3b). In 2014 the value of the geometric mean was lowest (20.51 egg m<sup>-2</sup>; CI 14.92 – 26.10), and the eggs were found only in the Gulf of Manfredonia and in the central part of the study area. The most frequent class of abundance was 1 – 5 eggs m<sup>-2</sup> (Fig. 3c). In 2015 the geometric mean was 25.61 eggs m<sup>-2</sup>

(CI 15.73 – 35.49) and the eggs were mostly concentrated in the northern portion of the study area and in the Gulf of Manfredonia; the class with 10 – 25 eggs  $\text{m}^{-2}$  accounted for 20.7% of the total abundance (Fig. 3d).

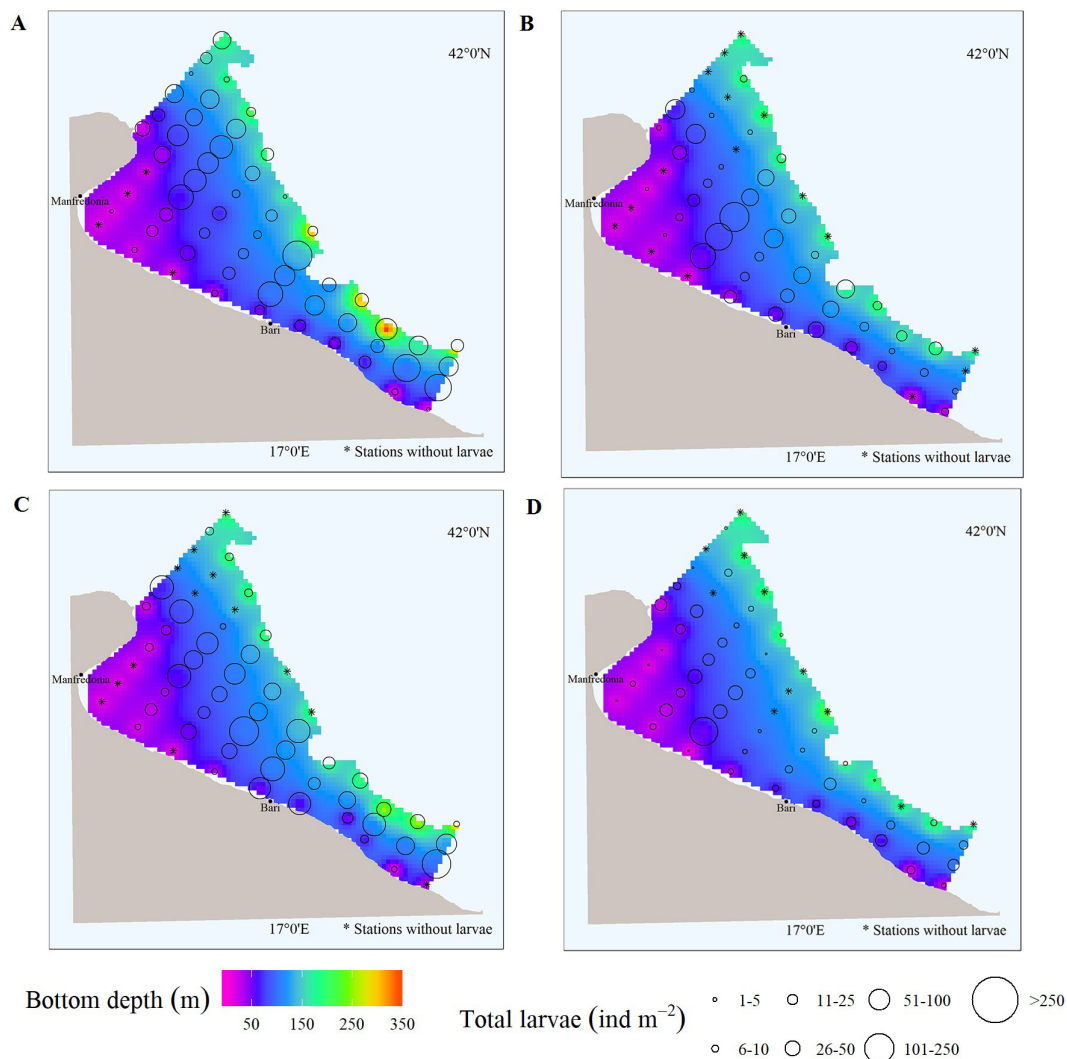
The abundance of larvae ranged between 0 – 1054.9 individuals (ind)  $\text{m}^{-2}$ . Negative stations ranged from 6% in 2012 to 27% in 2013. In 2012 the value of the geometric mean was highest (52.78 ind  $\text{m}^{-2}$ ; CI 43.37 – 62.20) and larvae were found throughout the study area; 20.7% of the total abundance was concentrated in the class with 50 – 75 ind  $\text{m}^{-2}$  (Fig. 4a). In 2013 the geometric mean was 40.04 ind  $\text{m}^{-2}$  (CI 27.52 – 52.56), the larvae were mostly concentrated in front of the Gargano Promontory and in the central part of the study area, and the most frequent abundance class was 25 - 50 ind  $\text{m}^{-2}$  (13.8%) (Fig. 4b). In 2014 the data showed the lowest value of the geometric

mean (24.47 ind  $\text{m}^{-2}$ ; CI 20.04 – 28.89) with no particular abundance hotspots; the most frequent abundance class was the one with 25 - 50 ind  $\text{m}^{-2}$  (22.4%) (Fig. 4c). In 2015 the geometric mean was 44.66 ind  $\text{m}^{-2}$  (CI 21.08 – 68.24), the larvae were concentrated in the northern and southern part of the study areas, and the most frequent abundance class was the one with 25 - 50 ind  $\text{m}^{-2}$  (13%) (Fig. 4d).

Data analysis demonstrated significantly different abundances of eggs and larvae, different environmental conditions, and different chlorophyll-a and zooplankton biomass values in the different years (Table 1). Dunn's post-hoc test highlighted that 2012 was the year when salinity was highest and chlorophyll-a concentrations were lowest and that 2015 was the year with the lowest water temperature. Moreover, 2012 was the year when



**Fig. 3:** Spatial distribution of egg abundance in 2012 (A), 2013 (B), 2014 (C) and 2015 (D) in relation to bottom depth.  
\* stations where no eggs were retrieved.



**Fig. 4:** Spatial distribution of larval abundance in 2012 (A), 2013 (B), 2014 (C) and 2015 (D) in relation to bottom depth stations where no larvae were retrieved..

the abundance of eggs and larvae was highest, with significant differences with 2014 and 2015 for eggs and with 2013 and 2014 for larvae. As regards the biomass of zooplankton, 2014 and 2015 were the years with the lower abundances and were significantly different from the 2013 data. A clear water stratification was detected throughout the study period, with a thermocline between 20 and 40 m (Fig. 5). Spearman's correlation analysis identified significant and positive correlations among egg abundance ( $\rho = 0.38$ ,  $P < 0.001$ ), larval abundance ( $\rho = 0.52$ ,  $P < 0.001$ ), and zooplankton biomass and between depth and salinity ( $\rho = 0.46$ ,  $P < 0.001$ ), and negative correlations between chlorophyll-a and salinity ( $\rho = -0.72$ ,  $P < 0.001$ ), between chlorophyll-a and depth ( $\rho = -0.80$ ,  $P < 0.001$ ), and between temperature and depth ( $\rho = -0.41$ ,  $P < 0.001$ ) (Table 2).

PCA demonstrated that the environmental parameters accounted for 75% of the variability captured by the first two axes. The first axis explained 58% of the variance

and correlated highly and positively with chlorophyll-a and negatively with depth. The second axis, which captured 17% of the variability, showed a strong correlation with the zooplankton biomass.

Quotient analysis of egg abundance data indicated that anchovy prefer spawning areas found at intermediate depth (91 - 120 m) and that they avoid shallower waters (11 - 30 m) with high chlorophyll-a concentrations ( $> 0.52 \text{ mg m}^{-3}$ ) and low zooplankton biomass ( $14.82 - 199 \text{ mg m}^{-2}$ ). Quotient analysis of larval abundance highlighted a preference for depths between 11 and 60 m with high zooplankton biomass ( $> 1000 \text{ mg m}^{-2}$ ) and avoidance of deeper water ( $> 150 \text{ m}$ ) with low zooplankton biomass ( $14.82 - 199 \text{ mg m}^{-2}$ ) (Table 3, Fig. 6).

## Discussion and Conclusion

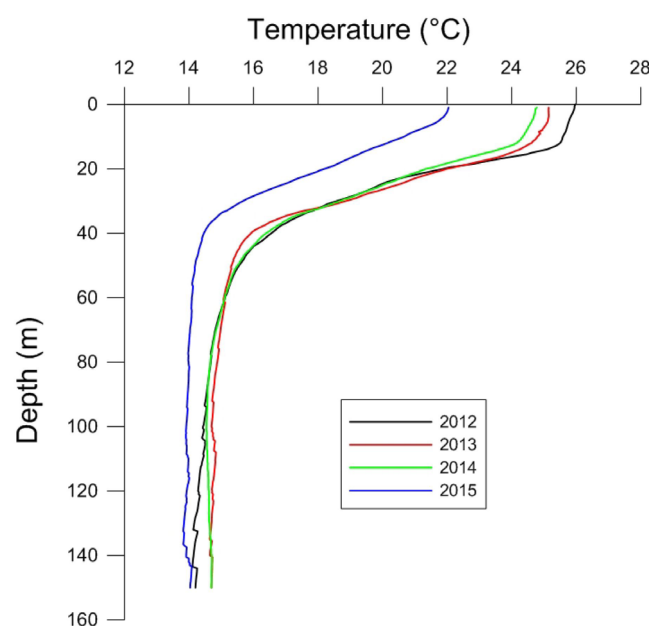
A considerable effort has been made to understand the factors that determine habitat selection by anchovy in

**Table 1.** Temperature and salinity values (averaged for the upper water column, 0-20 m); bottom depth (average), satellite chlorophyll-a, zooplankton biomass, and abundance of eggs and larvae with median values, interquartile ranges (IQR) and Kruskal-Wallis ( $\chi^2$ ) test results.

	2012		2013		2014		2015		X <sup>2</sup>	d.f.
	Average	Median (IQR)	Average	Median (IQR)	Average	Median (IQR)	Average	Median (IQR)		
Temperature (°C)	25.01	25.17 (1.91)	24.52	24.75 (1.00)	23.88	24.33 (1.44)	20.49	20.37 (1.00)	145.7***	3
Salinity (psu)	38.74	38.74 (0.22)	37.67	37.65 (1.14)	37.67	37.77 (0.91)	37.90	37.95 (1.32)	118.72***	3
Depth (m)	105.6	101.75 (87.13)	99.87	103.50 (93.25)	103.34	101.75 (91.25)	101.3	103.00 (91.75)	ns	3
Chlorophyll-a (mg m <sup>-3</sup> )	0.21	0.16 (0.12)	0.36	0.27 (0.22)	0.33	0.25 (0.28)	0.46	0.35 (0.48)	36.32***	3
Zooplankton (mg m <sup>-2</sup> )	780.07	537.57 (692.68)	734.82	680.25 (654.96)	498.87	486.79 (370.17)	543.34	490.29 (516.87)	10.11*	3
Eggs (ind m <sup>-2</sup> )	64.91	43.14 (90.20)	34.55	23.53 (50.98)	30.90	15.69 (48.04)	30.76	9.80 (42.16)	12.84**	3
Larvae (ind m <sup>-2</sup> )	77.35	54.90 (93.14)	52.81	21.57 (79.41)	28.74	19.61 (43.14)	82.55	33.33 (106.86)	17.47***	3

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

ns not significant



**Fig. 5:** Mean profiles of temperature for the 4 years of sampling.

several ecosystems worldwide (e.g., Somarakis & Nikolioudakis, 2007; Palomera *et al.*, 2007; Bonanno *et al.*, 2014; Mhlango *et al.*, 2015; Zarrad *et al.*, 2017). As regards the Adriatic Sea, although it is a key area for small pelagic stocks, the available information is limited and dated. In a study of the Mediterranean Sea, Giannoulaki *et al.* (2013) suggested that the south-western Adriatic was a potentially favourable habitat for adults and juveniles. The present study was directed at identifying suitable anchovy spawning and nursery areas.

The major finding of this work is the significant correlation found between egg and larval abundance and the zooplankton biomass. These data agree with the preference ranges of eggs and larvae for high zooplankton biomass and by the avoidance for low zooplankton biomass values of eggs, highlighted by quotient analysis. Similar findings have been reported in other studies (Regner, 1996; Twatwa *et al.*, 2005; Somarakis & Nikolioudakis, 2007; Zarrad *et al.*, 2012), where the hypothesis was advanced that spawning could occur in areas with high zooplankton concentrations, which provided better conditions for egg and larval survival. Anchovy spawning areas are known to be characterized by a stratified and stable water column, which allows the formation and maintenance of food aggregations. According to Agostini



**Table 2.** Spearman correlation illustrating the possible relationships among abundance of eggs and larvae and environmental variables.

	Salinity (psu)	Temperature (°C)	Depth (m)	Chlorophyll-a (mg m <sup>-3</sup> )	Zooplankton (mg m <sup>-2</sup> )	Eggs (ind m <sup>-2</sup> )	Larvae (ind m <sup>-2</sup> )
<b>Salinity</b>	1.00	-0.004	0.46***	-0.72***	0.03	0.09	-0.04
<b>Temperature</b>		1.00	-0.41***	0.14	0.08	0.04	0.06
<b>Depth</b>			1.00	-0.80***	0.15	0.12	-0.04
<b>Chlorophyll-a</b>				1.00	-0.09	-0.15	0.04
<b>Zooplankton</b>					1.00	0.38***	0.52***
<b>Eggs</b>						1.00	0.49***
<b>Larvae</b>							1.00

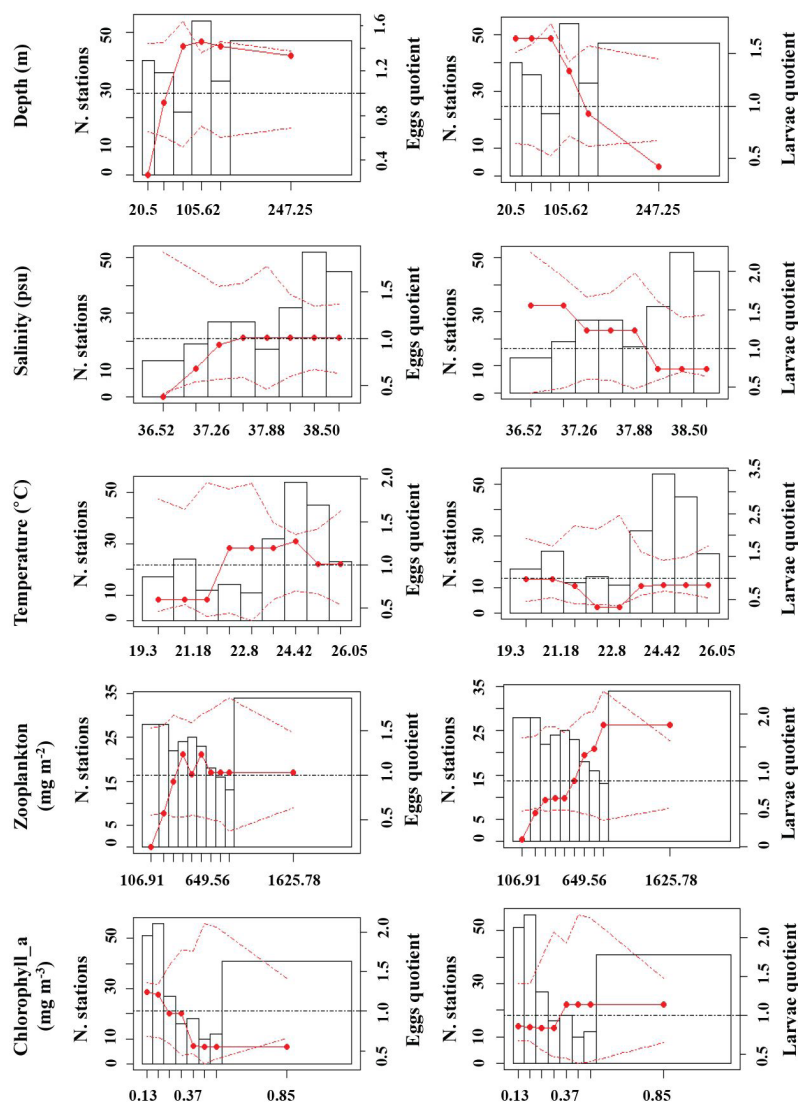
\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001

**Table 3.** Results of single parameter quotient analysis for anchovy eggs and larvae.

		Parameter range	Preference range	Tolerance range	Avoidance range
<b>Eggs</b>	<b>Temperature (°C)</b>	18.80 - 26.47		18.80 - 26.47	
	<b>Salinity (psu)</b>	36.24 - 39.00		36.24 - 39.00	
	<b>Depth (m)</b>	11 - 399	91 - 120	31 - 90 and > 120	11 - 30
	<b>Chlorophyll-a (mg m<sup>-3</sup>)</b>	0.11 - 1.65		0.11 - 0.52	> 0.52
	<b>Zooplankton (mg m<sup>-2</sup>)</b>	14.82 - 5410.37		199 - 5410.37	14.82 - 199
<b>Larvae</b>	<b>Temperature (°C)</b>	18.80 - 26.47		18.80 - 26.47	
	<b>Salinity (psu)</b>	36.24 - 39.00		36.24 - 39.00	
	<b>Depth (m)</b>	11 - 399	11 - 60	61 - 150	> 150
	<b>Chlorophyll-a (mg m<sup>-3</sup>)</b>	0.11 - 1.65		0.11 - 1.65	
	<b>Zooplankton (mg m<sup>-2</sup>)</b>	14.82 - 5410.37	> 1000	299 - 999	14.82 - 299

& Bakun (2002), processes that favour retention in optimal areas for egg and larval survival are present in the southern Adriatic. Here, fronts are created by the clash between riverine inputs and the more saline waters of the Mediterranean sea; these factors, combined with limited mixing of the water column and with a high degree of stratification (due to mild wind action in summer), create an area where nutrients and zooplankton are densely concentrated. The seasonal cyclonic gyre (SAG) also exercises a strong influence, promoting retention mechanisms for zooplankton and ichthyoplankton (Artegiani *et al.*, 1997b; Bakun, 2006; Specchiulli *et al.*, 2016).

Quotient analysis also highlighted an avoidance range of egg deposition in correspondence with high levels of chlorophyll-a, even though several studies have reported a positive correlation between the abundance of different anchovy life stages and chlorophyll-a concentrations, both in highly productive systems like the Humboldt Current ecosystem (Bertrand *et al.*, 2008) and in less productive ecosystems like the Strait of Sicily and the north Aegean Sea (Bonanno *et al.*, 2014). However, our chlorophyll-a data come from satellite measurements, which explore only a few centimetres from the surface, where chlorophyll-a concentrations are lower than in the deep-



**Fig. 6:** Results of quotient analysis. Histograms represent the number of stations comprised in each category of the environmental variables. The continuous line represents egg (left) and larval (right) quotient values, the dashed lines represent the upper and lower confidence intervals. The horizontal line represents the null hypothesis (uniformly distributed eggs or larvae), with quotient value=1.

er layers. Indeed, it is well established that sub-surface maxima of phytoplankton productivity and biomass occur especially during summer stratification (Ninčević *et al.*, 2002). The Deep Chlorophyll-a Maximum (DCM) is found at the lowest depth where the light makes it possible for phototrophic populations to exploit nutrient advection from sub-euphotic depths. Since in the middle and southern Adriatic a DCM is consistently detected between 50 and 75 m (Ninčević *et al.*, 2002; Alcaraz *et al.*, 2016), it is conceivable that avoidance of egg deposition in presence of high chlorophyll-a concentrations is an artefact due to the collinearity between chlorophyll-a and depth.

Quotient analysis of depth values highlighted a preference range for egg deposition between 91 - 120 m and avoidance at shallower depths (11 - 30 m); the larvae showed a preference for coastal waters (11 - 60 m) and avoided deeper waters (> 150 m). Several studies have

reported that the selection of spawning and nursery areas is driven by depth (García & Palomera, 1996; Regner, 1996; Somarakis *et al.*, 2004; Somarakis & Nikolioudakis, 2007; Basilone *et al.*, 2013; Giannoulaki *et al.*, 2013; Bonanno *et al.*, 2014). According to these findings the spawning habitat is confined to the continental shelf within the 200 m isobaths and the nursery area is near the coast, where photosynthetic activity can support abundant zooplankton growth and conditions for larval survival are optimal. These data agree with those reported by Giannoulaki *et al.* (2013), who found in the Mediterranean Sea a preference for spawning areas at depths of 40 - 150 m, and with those of Saraux *et al.* (2014), who identified two recurrent areas further from the coast (isobaths of ~70 to 100 m) and unfavourable areas close to the coast in the Gulf of Lion for adult anchovy. The results of the present study show significantly different distributions of

eggs and larvae over the years, in line with the notion that the biomass of small pelagic species undergoes wide fluctuations over time throughout the world (Brander, 2007; Leonori *et al.*, 2009, 2011; Ruggeri *et al.*, 2016a). The PCA data explained the environmental variability mainly in terms of depth and chlorophyll-a, but also stressed the importance of zooplankton abundance. In 2012, the high abundance of eggs and larvae was paralleled by peak values of salinity and by trough concentrations of chlorophyll-a, whereas the years when the abundance of eggs (2014 and 2015) and larvae (2013 and 2014) was lower were also cooler (2015) and characterized by lower zooplankton biomass (2014 and 2015). Thus, there emerged a clear trend relating the environmental parameters to egg and larval distribution; in particular, egg and larval abundance were higher at intermediate depths in areas characterized by low concentrations of chlorophyll-a and high zooplankton abundance.

Anchovy spawning is believed to be related to certain temperature and salinity ranges; for example, in the southern Mediterranean Sea temperatures  $> 25^{\circ}\text{C}$ , depth and water stratification are major factors controlling spawning intensity (Zarrad *et al.*, 2006, 2017). In the Benguela ecosystem the spawning temperature ranges between  $16 - 21^{\circ}\text{C}$  (van der Lingen *et al.*, 2001; Twatwa *et al.*, 2005), whereas in the north-western Mediterranean anchovy eggs are mainly found between  $17 - 23^{\circ}\text{C}$  (Palomera *et al.*, 2007). However, a number of studies have failed to identify a clear relationship; for example, the spawning distribution of Californian anchovy has been reported to be related to temperature in some studies but not in others (Fréon *et al.*, 2005 and references therein). In the Mediterranean Sea, different ranges of preference have been described in relation to salinity. In the western Mediterranean Palomera *et al.* (2007) reported two peaks of preference, one at 35.8 and another at 37.6 psu, whereas in the eastern Mediterranean a broad range of values have been described, spawning being most intense in areas with lower salinity (Somarakis & Nikolioudakis, 2007). These studies have stressed the flexibility of anchovy preferences for spawning habitats, suggesting that they change according to the prevailing ecosystem conditions (Basilone *et al.*, 2013). The data analysed in the present work demonstrate a rising gradient from the coast to the open sea for salinity and an opposite gradient for temperature. However, these data correlated neither with the abundance of eggs or larvae nor with preference/avoidance behaviours according to quotient analysis, suggesting that salinity and temperature are not the oceanographic variables driving egg and larval distribution in the southern Adriatic.

Earlier surveys in the northern Adriatic have found that anchovy eggs were consistently more abundant near the shore (25 – 50 m), that their number declined seawards and southwards with a N-S and W-E gradient, and that abundance was highest ( $> 400 \text{ eggs m}^{-2}$ ) in the low salinity area of the Po River estuary and in the Gulf of Trieste. The larvae were distributed farther offshore,

but the area around the Po estuary is known to favour their survival, due to water column stability conferred by the low salinity of the estuary surface water. These conditions help to maintain the water column stratification, hence the vertical aggregation of food particles (Coombs *et al.*, 2003). A study conducted by Specchi *et al.* (1998) in the northern and central Adriatic found a strong correlation between anchovy egg abundance and mesozooplankton standing crop, whereas no correlation was detected between egg abundance and environmental variables, except a correlation - limited to the Gulf of Trieste - between egg abundance and temperature. Marano *et al.* (1998) reported that in the southern Adriatic most of the eggs were concentrated between Bari and Brindisi at depths ranging between 50 and 100 m; abundance values were between 0 -  $100 \text{ eggs m}^{-2}$ , the lowest values being measured in the Gulf of Manfredonia, which the authors described as an exclusively nursery area. In all past survey conducted in the central and southern Adriatic food availability and bathymetry were described as the key factors affecting spawning dynamics in spatial and temporal terms (Vucetic, 1975; Gamulin & Hure, 1983; Regner, 1985). In the present study, data analysis indicated depth, zooplankton abundance, and water column stability as the factors characterizing favourable spawning and nursery areas in the south-western Adriatic. In conclusion, the present data do not outline a clear spatial distribution pattern of anchovy eggs and larvae in the area; nonetheless, their high abundance found throughout the study area in four successive years, suggests that the entire area can be considered as a favourable spawning and nursery area for anchovy.

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