Using spatial indicators to investigate fish spawning strategies from ichthyoplankton surveys: A case study on co-occurring pelagic species in the NE Aegean Sea

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Using spatial indicators to investigate fish spawning strategies from ichthyoplankton surveys: A case study on co-occurring pelagic species from the North-East Aegean Sea

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Abstract

Multispecies ichthyoplankton associations are considered adaptive and their objective definition is useful in designating species with similar or contrasting spawning strategies. In this case study, a suite of indicators, recently developed to characterize the spatial patterns of animal populations, are applied to egg abundance data of the summer-spawning European anchovy (*Engraulis encrasicolus*), round sardinella (*Sardinella aurita*) and chub mackerel (*Scomber colias*), in an effort to identify common or contrasting spawning patterns in the North-East Aegean Sea and their relation to the major oceanographic features of the area. There was a considerable increase in abundance and area of presence between the early phase (May) and advanced phase (July) of the 2010 spawning period. The identification of major spatial patches revealed a persistent multispecies spawning location associated with the outflow of Black Sea water. A second major patch was defined for anchovy and chub mackerel in July, clearly associated with the Samothraki gyre (SG). In contrast, round sardinella spawned in coastal waters outside the SG. Surprisingly, in May 2010, the spawning intensity of anchovy was very low in the SG, which was attributed to unfavourable conditions caused by the bloom of mucilage-producing phytoplankton. The levels of aggregation and occupation of space seemed to be adequately described by the indices of Positive, Equivalent and Spreading Area. Conclusively, many spatial indicators applied in this study are potentially important tools for studying egg distributions and the changing priorities of adult fish when selecting spawning sites.

Keywords: Ichthyoplankton; egg; spatial indicators; Northeast Aegean Sea.

Introduction

Ichthyoplankton assemblages in the marine environment include many egg and larval fish species, whose occurrence and abundance varies across spatial and temporal scales. The composition of assemblages is affected by factors such as the initiation of the spawning season of different species, the length of each spawning season, reproductive characteristics of the adults (e.g. fecundity, frequency of spawning), as well as by ambient trophodynamic conditions that influence the development, growth and survival rates of egg and larval cohorts (Houde, 2009). As an adaptation to the seasonality of the marine environment in temperate and sub-tropical ecosystems, fish have evolved to take advantage of optimal environmental conditions for egg maturation and spawning (Winemiller & Rose, 1992; Lowerre-Barbieri *et al.*, 2011). Similar temporal patterns on the occurrence and relative abundance of different egg and larval species

in plankton may indicate similar reproductive strategies (Somarakis *et al.*, 2000; Somarakis *et al.*, 2011a).

The structure and relative composition of ichthyoplankton assemblages can further be influenced by the location of adult spawning in relation to specific topographic and/or oceanographic features (e.g., gyres, fronts) that affect the advection of ichthyoplankton as well as the spatial heterogeneity of larval prey. The spatial distribution of ichthyoplankton can therefore reflect similar or contrasting adaptations of different species to local oceanographic conditions. It is generally believed that fish stocks evolved so that their eggs and larvae develop and recruit within specific physical domains that ensure directed transport or local retention (Houde, 2009). The spawning habitat of many small pelagic fish is characterized by a combination of physical processes, as defined by Bakun (1996) as an "ocean-triad", which include: (i) processes that enrich the ocean (e.g., upwelling, mixing), (ii) concentration processes such as frontal zones, and

(iii) processes that retain or drift the larvae towards favourable habitats.

A suite of spatial indicators have recently been developed to characterize the spatial patterns of animal populations (occupation, aggregation, dispersion) as well as the degree of overlap between different populations (Bez & Rivoirard, 2000a, 2001; Woillez *et al.*, 2009). They have been used to compare age-groups of adult fish (Woillez *et al.*, 2006, 2007) as well as the different life stages of species that represent demersal and pelagic environments (Woillez *et al.*, 2006). These indicators have also been used to describe distributional patterns of adult anchovies, sardines and sprats in the Mediterranean Sea (Saraux *et al.*, 2014; Barra *et al.*, 2015) Finally, these indicators have been implemented within a modelling (particle-tracking) study to describe inter-annual changes in larval dispersal (Huret *et al.*, 2010). Within a comparative framework, the use of spatial indicators to understand the patterns of pelagic egg distributions could facilitate the identification of multispecies spawning associations, including the linkage of egg production to specific oceanographic features. According to Frank & Leggett (1983), multispecies ichthyoplankton associations are adaptive and result from similar responses to the pelagic environment.

The majority of small pelagic fish that inhabit the eastern Mediterranean Sea spawn during spring-summer (Tsikliras *et al.*, 2010). Among those fish, the European anchovy spawns from May to September with a peak in June-July (Somarakis *et al.*, 2004). Chub mackerel and round sardinella also spawn during the summer months, with peak spawning in June-July (chub mackerel in the Adriatic Sea (Cikeš Keč & Zorica, 2012); round sardinella in the Adriatic Sea (Mustać & Sinovčić, 2012); and Aegean Sea (Tsikliras, 2006)). Comparative studies of the egg and larval distributions of these species in the northwest (Sabatés *et al.*, 2009, 2013) and eastern Mediterranean (Somarakis *et al.*, 2002; Isari *et al.*, 2008; Schismenou *et al.*, 2008; Tsikliras *et al.*, 2009; Somarakis *et al.*, 2011a) have mainly focused on inter-annual differences, usually during peak spawning period. However, there are few studies that describe the spawning activity of anchovy and other summer-spawning pelagic species that have covered more extended periods of the spawning season in the Aegean Sea (e.g. Catalán *et al.*, 2010).

In this case study, we test the usefulness of recently developed spatial indicators in describing common or contrasting spawning patterns of three species of pelagic fish: European anchovy (*Engraulis encrasicolus*), round sardinella *(Saldinella aurita)* and chub mackerel (*Scomber colias*). The main objective of the study was to investigate the spawning patterns of those pelagic species in the North-East Aegean Sea (NEA) during an early (May) and advanced (July) phase of the spring/summer spawning period. To the best of our knowledge, this is the first work that describes how changes in oceanographic conditions during the spring-to-summer transition are reflected in the distribution and abundance of ichthyoplankton of the co-occurring anchovy, chub mackerel and round sardinella. We describe the distribution (including aggregation and occupation) patterns of the three species,

their overlap and any changes that occur between early and peak spawning. We discuss these changes in relation to the prominent hydrological structures of the study area as well as the biology of the three pelagic species.

Materials and Methods

Study area description

The NEA is a highly productive area compared to the generally oligotrophic character of the eastern Mediterranean and the South Aegean Sea (Stergiou *et al.*, 1997; Siokou-Frangou *et al.*, 2010). It is characterized by the existence of two continental plateaus: the Thracian Sea shelf and the Lemnos plateau, which are separated by a deep trench (1550 m depth; Zervakis & Georgopoulos, 2002). Low-salinity Black Sea water (BSW) enters the NEA from the Dardanelles strait and is bifurcated east of Lemnos island into two main branches, one moving north-westward (Lemnos-Imvros Stream, LIS) and one south-westward over the Lemnos plateau (Fig. 1). A large amount of BSW is captured by the Samothraki gyre (SG), an almost permanent anticyclonic gyre that is observed over the Thracian Sea continental shelf (Zervakis & Georgopoulos, 2002; Somarakis *et al.*, 2002; Isari *et al.*,
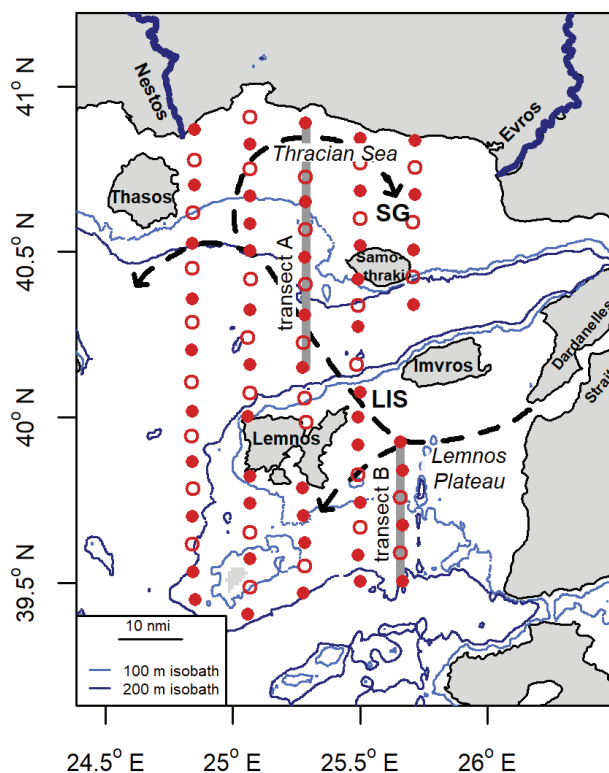
Fig. 1: Ichthyoplankton (shown as open red circles) and CTD sampling stations (shown as filled red circles) during May and July 2010. The isobaths of 100 and 200 m are shown (light and dark blue lines, respectively). Black arrows indicate the main circulation pattern in the area: LIS - Lemnos-Imvros stream, SG - Samothraki gyre (Somarakis *et al.*, 2002). The sampling stations of transects A and B (grey lines) show the vertical structure of the water column in Figure 3.

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2006, 2011). As the BSW enters into the NEA, it collides with highly saline Aegean waters of Levantine origin (Levantine Intermediate Water; LIW), creating a strong salinity front south of Lemnos island (LF). The BSW and inflowing riverine waters flow into the Thracian Sea shelf, enriching the NEA with nutrients and organic matter and substantially increasing the local productivity (Siokou-Frangou *et al.*, 2009, 2010).

Ichthyoplankton sampling

Two sampling cruises were carried out in the NEA during early May (04/05 - 11/05) and July (04/07 - 14/07) 2010 on board the *R/V Philia*. The main objective of the two surveys was to provide comparative information on the characteristics of the spawning grounds of summer spawning pelagic species during the initial (May) and advanced phases (July) of thermal stratification. Ichthyoplankton samples were collected over a predetermined grid of 83 sampling stations (Fig. 1), latitudinally and longitudinally spaced 5 and 10 nmi, respectively. Sampling was conducted with a WP2 sampler (mouth opening: 0.255 m^2, mesh-size: 0.200 mm; flowmeter attached to the mouth opening; towing speed at 1 m s^{-1}). Vertical tows were either made from within 5 m of the bottom at sampling stations shallower than 100 m or from 100 m depth to surface at deep stations. All samples were preserved, immediately after collection, in a 10% borax-buffered formalin solution, as recommended for ichthyoplankton (UNESCO, 1968) and stored until later analysis in the laboratory. In the laboratory, anchovy, round sardinella and chub mackerel eggs and yolk sac larvae were sorted

from the plankton samples. Then, their abundances were counted and standardized to numbers per square meter m^2 (Somarakis *et al.*, 2012).

Environmental data sampling and analysis

Environmental data were collected from 50 predetermined stations along the cruise transects (Fig. 1). Vertical profiles of temperature, salinity and fluorescence were obtained with a Seabird 19 CTD equipped with a Wetstar fluorometer. Fluorescence values were integrated through the water column (0-100 m) and expressed as μg l^{-1}. After sorting and removing all ichthyoplankton, plankton samples were used to estimate mesozooplankton biomass (mg m^{-2} [dry weight]). Specifically, the remaining plankton sample (size fraction: 200–2000 μm) was filtered onto preweighed, glass–fibre filters (Whatman GFA) and dried at 60 ºC for 24 hours before weighing to determine the dry weight (details in Somarakis *et al.*, 2012).

Subsequently, the Local regression method (Loess) was applied in a predictive mode to interpolate temperature, salinity at 10 m depth, integrated fluorescence and mesozooplankton biomass as a function of latitude and longitude, onto a finer grid of 1x1 nmi^2 (based on the weighted least square method and the smallest possible span in order to retain the maximum accuracy R^2>0.94; Cleveland & Devlin, 1988). Then, contour maps of temperature and salinity at 10 m depth and integrated fluorescence and mesozooplankton biomass were created (Fig. 2). Similarly, cross sections of temperature, salinity and fluorescence were created for transects A and B in order to illustrate the vertical structure of the water column
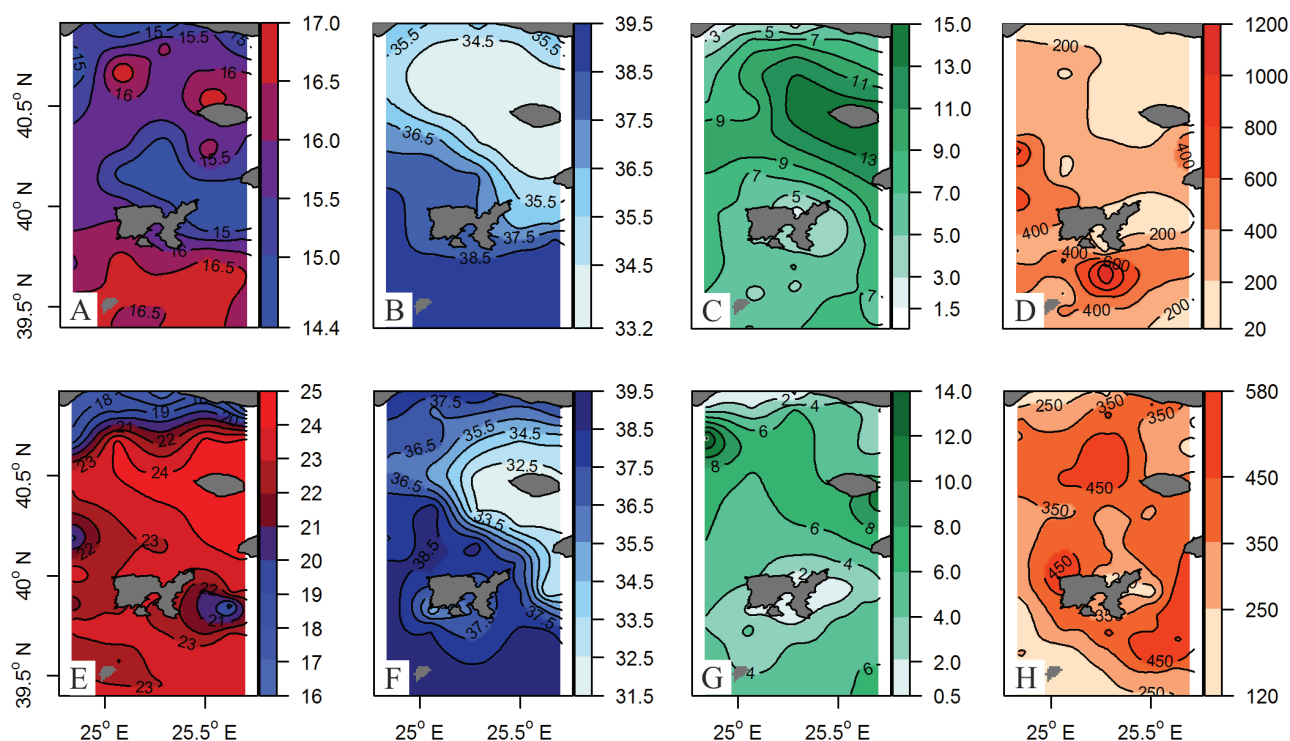

Fig. 2: Temperature (ºC, 10 m depth; A, E), salinity (10 m; B, F), integrated fluorescence (μg Chl-a l^{-1}, 0-100 m; C, G) and mesozooplankton biomass (mg m^{-2}; D, H) during May (A-D) and July 2010 (E-H) in the study area.

and the position of salinity fronts along the Thracian and Lemnos plateaus (Fig. 3). Furthermore, box-and-whisker plots (Fig. 4) were created for surface temperature and salinity (10 m), integrated fluorescence and integrated mesozooplankton biomass in order to visualize the conditions prevailing at three groups of stations located at: 1) the Lemnos Plateau, 2) the offshore pelagic area north of Lemnos (>200 m depth) and 3) the continental shelf of the Thracian Sea.

Statistical analysis

Statistical analysis included: a) seasonal comparison of the biotic variables as recorded in each sampling station b) multiple comparisons between groups of sampling stations for abiotic (temperature, salinity) and biotic parameters (integrated fluorescence and mesozooplankton biomass) within each season and c) spatial indicators applied to the abundance of eggs of the examined species: anchovy, round sardinella and chub mackerel.
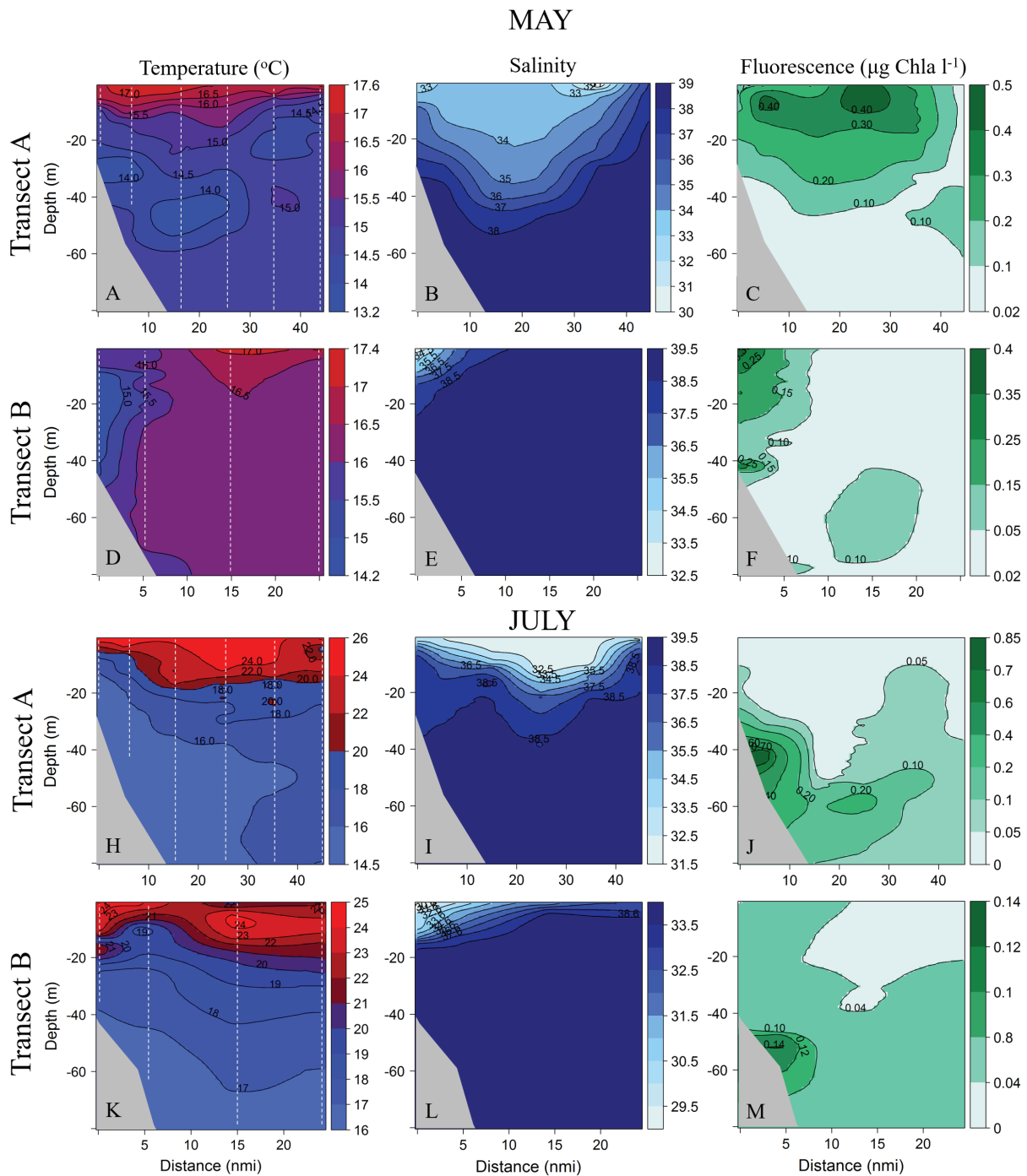
Fig. 3: Contour maps of the vertical distribution of temperature (°C; left column), salinity (middle column) and fluorescence (µg Chlα l⁻¹; right column) during May 2010 along transect A (A-C) and transect B (D-F), and during July 2010 along transects A (H-J) and B (K-M). Y-axis: depth of the water column; X-axis: distance (nmi) from the northern sampling station (0 nmi) to the southern station. The position (distance, nmi) of the sampling stations along the transects are shown in white, dashed lines on top of the temperature contour maps.

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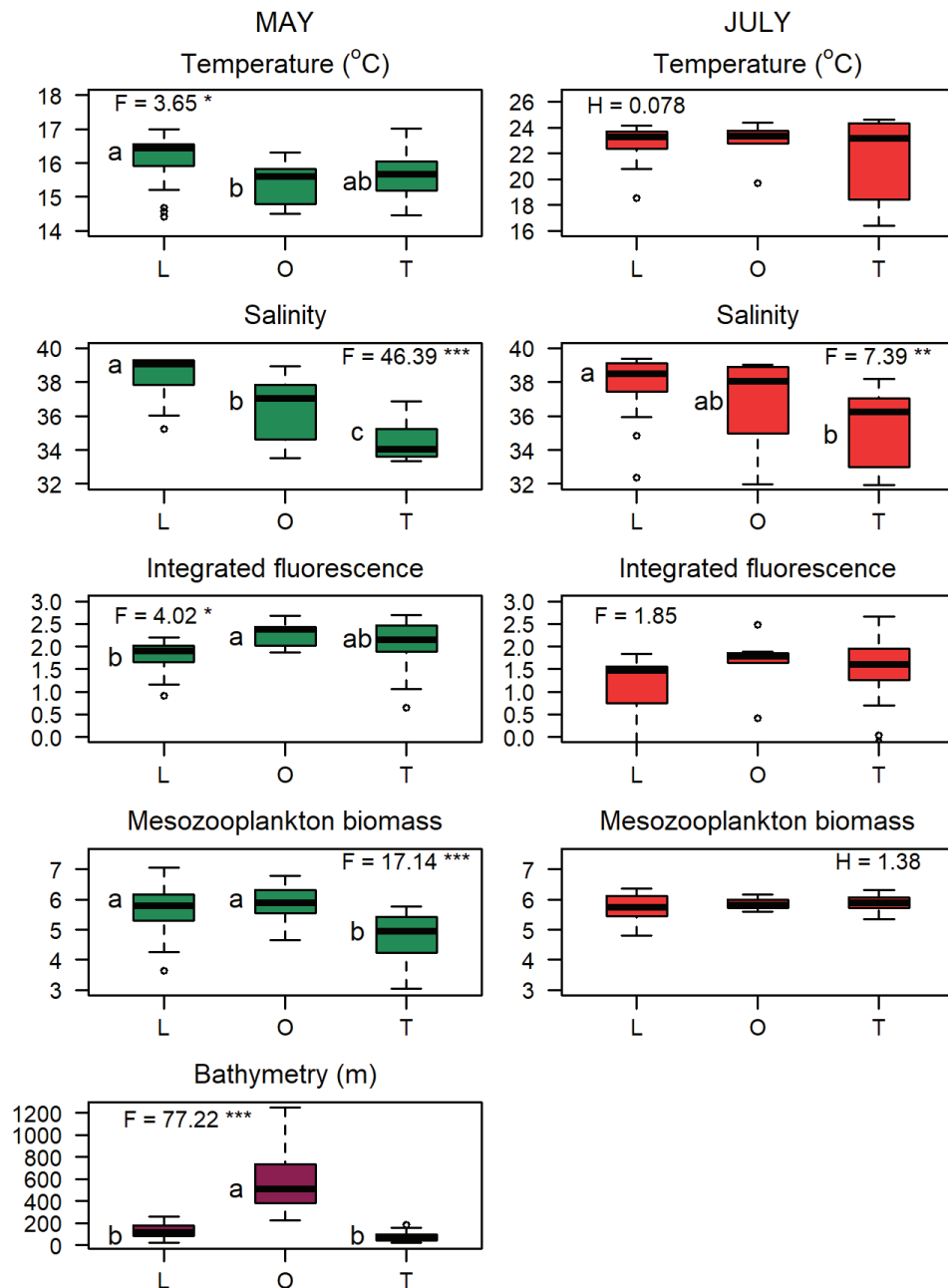
Fig. 4: Box-and-whisker plots for temperature (°C; at 10 m depth), salinity (at 10 m depth), log-transformed integrated fluorescence (μg l^{-1}) and log-transformed mesozooplankton biomass (mg m^{-2}) between three groups of sampling stations: L – Lemnos plateau, O – offshore pelagic area north of Lemnos island, and T- Thracian Sea shelf. Boxes indicate median and interquartile ranges, whiskers delineate full ranges. *F*-values are provided for comparisons of the parameters between the three groups (L, O, T) and within each season. For comparisons that did not meet the assumptions of the analysis of variance, the Kruskal-Wallis statistic *H* is provided. Asterisks indicate significant differences: *p < 0.05, **p < 0.01, ***p < 0.001. Post-hoc multiple comparisons were performed with a Student-Newman-Keul's test, where statistically significant differences among groups are indicated by letters *a, b, c* on the left side of each box. Groups with the same letter do not differ significantly.

Seasonal comparison

Sign tests were applied to evaluate statistically significant differences of the biotic components of the NEA ecosystem. Specifically, the integrated fluorescence, the mesozooplankton biomass and the abundance of eggs of the examined species between May and July, as recorded at each sampling station, were compared by means of sign tests (Zar, 1999). The sign test is a non-parametric statistical method to test for consistent differences be-

tween pairs of observations; it makes few assumptions about the nature of the distributions under test. All variables were log-transformed prior to analysis. Statistically significant differences were accepted at p< 0.05.

Group comparison

Differences in the environmental parameters (i.e. temperature and salinity values at 10 m depth and on log-trans-

formed integrated fluorescence and log-transformed integrated mesozooplankton biomass) among three groups of stations (Lemnos, Offshore, Thracian) were tested by Analysis of Variance (ANOVA), or the Kruskal-Wallis non-parametric test when ANOVA assumptions were not met. Student-Newman-Keul's post-hoc test was used for the parametric multiple comparisons to identify homogenous and non-homogenous groups (Zar, 1999). Statistically significant differences were accepted at p< 0.05.

All statistical analyses on seasonal and group comparisons were performed with R software v3.4.3 (R Core Team, 2017) using the *stats* package implemented in R basic functions, the *BSDA* package for the sign-test and the *agricolae* package for multiple comparisons.

Spatial indicators

The aggregation patterns of eggs of the three species were investigated through a set of spatial indicators (Table 1), calculated on the density values (eggs m^{-2}) of each species. All indices were calculated with a set of R functions under R v. 3.4.3 (available in electronic form at www.alr-journal.org; see Woillez *et al.*, 2009, appendix S1). An exhaustive description about the derivation of each indicator and the functions used are in Woillez *et al.* (2009).

The Centre of Gravity (CG) represents the mean geographic location of the population (Bez & Rivoirard, 2000b). The number of spatial patches was estimated with the threshold distance set to 25 nm (i.e. minimum distance from sample to patch centre). The number of spatial patches represent those patches with abundance >10% of the overall abundance. Inertia (I) describes the dispersion of the population around its CG (i.e. the mean square distance between a random individual and the CG of the population) and isotropy (I$_s$) describes whether such dispersion is identical in every spatial direction around CG. The Positive Area (PA; nmi^2) corresponds to the area occupied by the population, considering low abundance values but no zero values. The Spreading Area (SA; nmi^2) measures specific aspects of aggregation and expresses the area occupied by the population after accounting for density values. The Equivalent Area (EA; nmi^2) is another index of aggregation, also related to PA, and expresses the area of occupation where mean sample density is weighted by the number of individuals per sample (Bez & Rivoirard, 2001); it takes values between 0 and PA.

The spatial relations between more than one species can be described with indices of collocation. The Global Index of Collocation (GIC) is a measure of the degree of spatial overlap between different species. The GIC takes values between 0 (each population is concentrated in a single but different location) and 1 (the two CGs coincide; Woillez *et al.*, 2009).

Results

Environmental conditions

There was a southward increase in temperature in May. Temperature at 10 m depth ranged between 14-17 °C (Fig. 2A), with lower values recorded at the coastal area of the Thracian Sea and in the area directly influenced by the outflow of BSW. South of Lemnos island, the temperature was higher (16.5-17 °C). In July, the surface temperature ranged between 16-25 °C (Fig. 2E). Colder waters were again observed at the coastal area

Table 1. Summary table reporting the spatial indices (based on Woillez *et al.*, 2009) used to characterize the spatial patterns of anchovy, round sardinella and chub mackerel eggs.

Indicator	Short name	Units/Range	Associated population feature	Description
Centre of Gravity	CG	Degree	Location	Mean location of population
Number of Patches	NP	>0	Patchiness	Patchiness, hotspots of biomass
Inertia	I	nmi^2	Dispersion	Dispersion of the individuals around the CG of the population
Isotropy	I$_s$	0 to 1	Dispersion	Elongation of the population
Positive Area	PA	nmi^2	Occupation	Area of presence
Spreading Area	SA	nmi^2	Aggregation	Area occupied by the population accounting for density values
Equivalent Area	EA	0 to PA nmi^2	Aggregation	Area that would be covered by the population if all individuals had the same density, equal to the mean density per individual
Global Index of Collocation	GIC	0 to 1	Overlap	Overlapping degree of two populations

of the Thracian Sea, which is influenced by river runoff and by the weak upwelling created from the anticyclonic circulation of the BSW (SG: Samothraki Gyre). The SG is quite prominent in the salinity maps (Fig. 2B, F) as a tongue of low salinity water that surrounds Samothraki island.

In May, the integrated fluorescence ranged between 2 to 15 μg l^{-1}, and was remarkably high over the Thracian Sea (11-15 μg l^{-1}), within the SG. These high fluorescence values were clearly associated with the presence of BSW (Fig. 2C). Zooplankton biomass exhibited a contrasting pattern when compared to fluorescence, with the lowest biomasses over the eastern Thracian Sea and highest south of Lemnos (Fig. 2D). In July, the integrated fluorescence ranged between 0.5 to 14.5 μg l^{-1}, with the highest values (10-14.5 μg l^{-1}) recorded at stations close to Thasos and Samothraki islands (Fig. 2G). The highest values of zooplankton biomass in July occurred in the Thracian Sea, in association with the SG, and over the Lemnos plateau, in the area directly influenced by BSW. Integrated fluorescence, as well as mesozooplankton biomass differed significantly between May and July (fluorescence: $Z = 7.556$, $p < 0.001$; mesozooplankton: $Z = 3.284$, $p = 0.001$). Fluorescence was higher in May than in July (means of 8.2 μg l^{-1} vs. 5.1 μg l^{-1}, respectively) and mesozooplankton biomass was higher in July that in May (means of 354 mg m^{-2} vs. 299 mg m^{-2}, respectively).

The vertical section of transect A (Fig. 1 and Fig. 3) shows the water column characteristics in the Thracian Sea continental shelf and adjacent offshore stations during May and July 2010. The SG could be identified by the deepening isotherms and isohalines at its centre (Fig. 3B, E). In the northern (coastal) area, the anticyclonic water movement associated with the SG promotes a weak upwelling, i.e. the shallowing of the pycnocline. The SG is fed by the LIS (Fig. 1) which advects the low salinity BSW into the system. The LIS flows at the southern (offshore) edge of the SG, about 35-45 nmi along transect A (Fig. 3I). In May 2010, fluorescence values in the low salinity waters of the SG were particularly high (Fig. 3C), in contrast to July 2010 (Fig. 3J) when the highest phytoplankton concentrations were observed in subsurface waters of the coastal (northern) stations.

Transect B was located in the eastern part of the Lemnos plateau (see Fig. 1), where BSW meets the Aegean Sea water, creating the salinity front (LF). The low surface salinity water at the northern end of transect B indicates the presence of the newly entered BSW, which is separated from the southern Aegean waters by a strong salinity front (Fig. 3E, L). In May 2010, the low salinity BSW carried large amounts of phytoplankton as evidenced by its high fluorescence values (Fig. 3F). The same was observed in July 2010, where the surface BSW presented relatively higher fluorescence; however, the highest fluorescence values were recorded in deeper waters (deep chlorophyll maxima formation) (Fig. 3M).

The difference in bathymetry between the deep "offshore pelagic area" north of Lemnos island, the Lemnos plateau and the Thracian Sea shelf is highlighted in Figure 4. In addition, the ANOVA test showed significant differences in the environmental variables between the three groups of sampling stations in May (Lemnos, Offshore, Thracian; Fig. 4). During that period, warmer and more saline waters were found in Lemnos plateau compared to the "offshore pelagic area". The lowest salinity waters were recorded over the Thracian Sea shelf. The Thracian Sea shelf also presented high integrated fluorescence values combined with lower mesozooplankton biomass, as opposed to the "offshore pelagic area", where high integrated fluorescence values were combined with high values of mesozooplankton biomass. During July, salinity was the only environmental variable that showed statistically significant differences, with lower salinity values recorded over the Thracian Sea shelf.

Distribution and abundance of ichthyoplankton

During May, the abundance of anchovy eggs was generally low (maximum: 98 eggs m^{-2}). The main egg distribution grounds were located in the western coastal area of the Thracian Sea and east of Lemnos in front of the Dardanelles Strait (Fig. 5A). During July, abundances were high (maximum: 1115 eggs m^{-2}) and eggs were widely distributed over the surveyed area (Fig. 5C). Highest abundances were observed over the Thracian Sea shelf and Lemnos plateau and at pelagic stations north and west of Lemnos island (Fig. 5C). The abundances of eggs and yolk sac larvae of anchovy (Table 2) were significantly lower in May (eggs: $Z = 5.875$, $p < 0.001$; larvae: $Z = 8.254$, $p < 0.001$) when no anchovy larvae were found in the Thracian Sea.

Round sardinella exhibited low spawning activity in May as well, with eggs and larvae present in low abundances only over the Lemnos plateau (4-12 individuals m^{-2}; Fig. 5B). In July, the highest abundance for round sardinella eggs reached 45 eggs m^{-2}, and eggs were distributed within the coastal waters of the Thracian Sea as well as at the Lemnos plateau (Fig. 5D). The abundances of round sardinella eggs and larvae were significantly lower in May as compared to July (eggs: $Z = 4.051$, $p < 0.001$; larvae: $Z = 3.600$, $p < 0.001$). Finally, no eggs and larvae of chub mackerel were collected in May. In July, chub mackerel eggs (10-60 eggs m^{-2}) were mainly found over the Lemnos plateau and the eastern Thracian Sea (Fig. 5E).

With the exception of May, when no yolk sac larvae of anchovy were caught over the Thracian Sea, the distribution patterns of the larvae of the three species were similar to those of their respective eggs (not shown).

Spatial indicators

The CGs for anchovy eggs were consistently located southwest of the Samothraki island in both May and July, yet main egg aggregations were more widely distributed in May as compared to July (inertias of 914 and 568 nmi^{2}, respectively). The positive area for anchovy eggs exhibited a three-fold increase in July as compared to May (1005

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Species	Month	Eggs mean abundance	Eggs maximum abundance	Larvae mean abundance	Eggs - positive stations	Larvae - positive stations	Sea surface temperature range	Sea surface salinity range
anchovy	May	4 (±13)	98	6 (±18)	21 (0.25)	28 (0.34)	16.0-17.8	32.6-38.7
	July	126 (±206)	1110	140 (±132)	60 (0.73)	76 (0.93)	21.1-24.9	31.6-38.2
round sardinella	May	0.3 (±2)	12	0.3 (±1)	3 (0.04)	5 (0.06)	16.0-16.7	33.4-37.7
	July	4 (±9)	45	4 (±10)	22 (0.27)	22 (0.27)	22.6-24.5	31.6-36.8
chub mackerel	July	5 (±10)	60	4 (±12)	28 (0.34)	24 (0.29)	21.1-24.9	31.6-37.9

to 2890 nmi^2, respectively), accompanied by an increase in both the spreading and equivalent areas, as well as in the number of patches (Table 3). Egg abundances were similar among the stations during May, whereas there were larger differences (higher aggregation) recorded during July (spreading area increased 2-fold from May to July; Fig. 5A, C).

The position of the CG of round sardinella eggs changed from May to July due to the absence of reproductive activity in May over the Thracian Sea plateau (Fig. 5B, D; Table 3). The population exhibited an almost five-fold increase in the positive area and an eight-fold increase in inertia but exhibited similar anisotropic behaviour in July as compared to May (PA: 203 and 1110 nmi^2; I: 110 and 888 nmi^2; I$_s$: 0.46 and 0.42 for May and July, respectively). In addition, there was a four-fold increase in the equivalent and spreading areas and a higher number of patches from May to July, indicating higher egg abundances and wider spawning areas (Table 3, Fig. 5B, D).

The CG of chub mackerel eggs in July was located north-east of the Lemnos island. The positive area of eggs was 1414 nmi^2, while the equivalent and spreading areas were half of the estimated positive area, indicating high aggregation of eggs (Table 3, Fig. 5E). Furthermore, egg aggregations exhibited anisotropic behaviour (I$_s$: 0.32; Table 3).

During May, anchovy and round sardinella eggs had a moderate degree of overlap (GIC = 0.795; Table 4), due to the absence of round sardinella eggs at the coastal stations of the Thracian Sea (CGs in Table 3, Fig. 5B). During July, anchovy and round sardinella eggs highly overlapped (GIC = 0.995). The overlap of chub mackerel eggs with both anchovy and round sardinella eggs was lower (GIC was 0.828 and 0.844 respectively) (Table 4). In addition, the CG of chub mackerel eggs had a more southward location as compared to the other two species (Table 3), since chub mackerel eggs were not present at the coastal stations of the Thracian Sea (Fig. 5E).

In July, eggs of round sardinella occupied the small-

Table 3. Estimates of spatial indicators for the eggs of the three species. XCG and YCG: coordinates of centre of gravity. The number of the major patches with egg abundance >10% along with the relative abundance (%) of each major patch for each species and period are shown.

Species	Month	Positive Area (nmi²)	Equivalent Area (nmi²)	Spreading Area (nmi²)	XCG	YCG	Inertia	Isotropy	Number of Patches	Relative abundance (%)
anchovy	May	1005	403	505	25.5	40.2	914	0.27	2	64, 33
	July	2890	1040	1170	25.4	40.4	568	0.52	3	62, 17, 11
	May	203	170	155	25.5	39.9	110	0.46	1	100
round sardinella	July	1110	682	661	25.3	40.4	888	0.42	3	
										34, 49, 14
chub mackerel	July	1414	712	787	25.5	40.1	599	0.32	2	55, 34
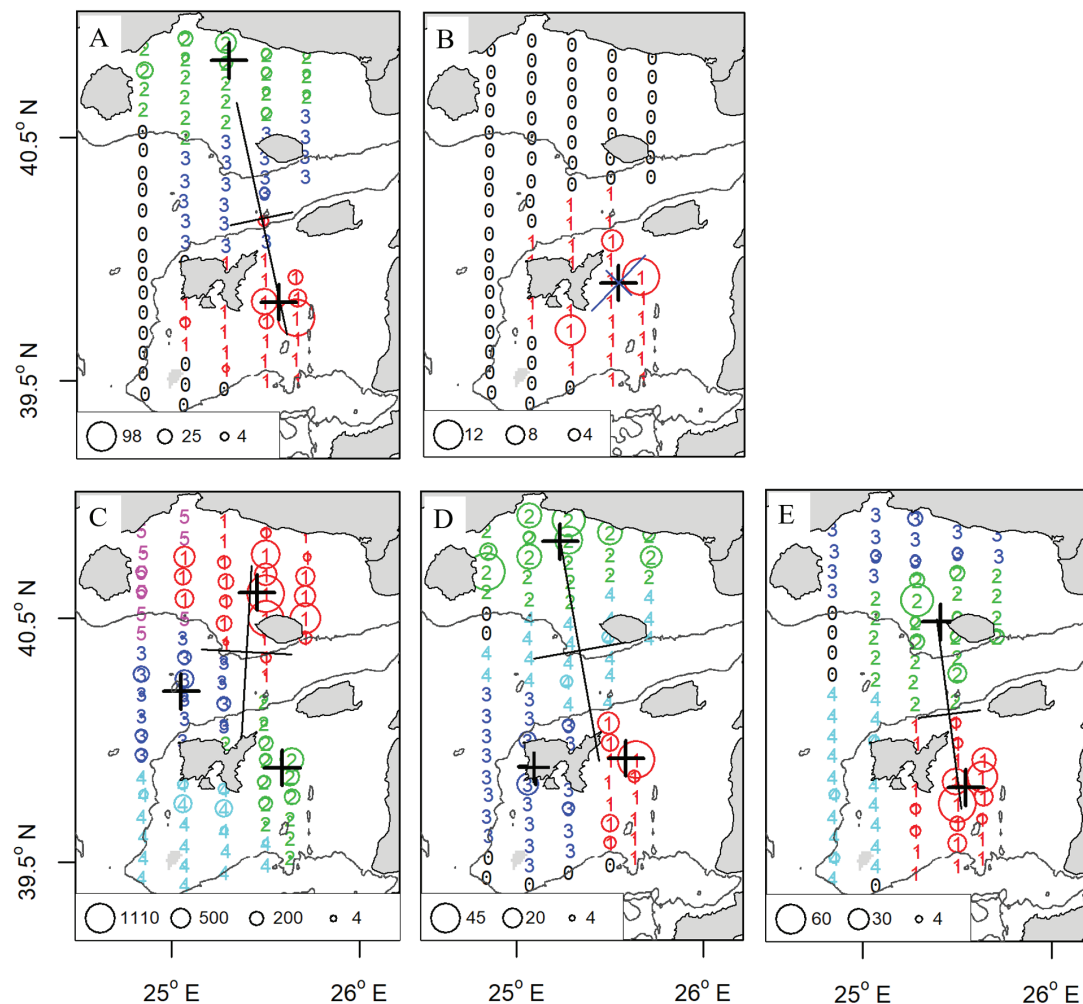
Fig. 5: Distribution and abundance maps of eggs during May (A, B) and July 2010 (C-E), in the NE Aegean Sea. The major patches are indicated by different colours, while the size of the circles is scaled by the maximum abundance per species and period (Table 2). The number of each patch indicates the ranking according to the abundance of individuals in each patch. The small black cross symbols show the centres of gravity (CG) of each major patch (i.e. those patches having more than 10% of the overall abundance; Table 3). The large cross indicates the CG of the population. The length of the cross axes indicate the isotropy of the egg distribution. The 200m isobaths are also shown (dark contour line).

est area (PA) as compared to anchovy and chub mackerel eggs; they also had higher dispersion (i.e., Inertia) compared to the other species (Table 3).

A permanent egg patch was observed east of Lemnos island during both periods that corresponded to all three species. The position of the patch was related to the frontal zone at the Lemnos plateau. During May, this was the unique patch for round sardinella eggs (100% relative abundance, Table 3). Besides this major patch of anchovy eggs (64% relative abundance), the species presented a second patch (with 33% relative abundance) positioned at the coastal area of the Thracian Sea, at the periphery of the gyre. In July, the second patch of anchovy eggs was at the centre of SG at the Thracian Sea (increasing up to 62%

Table 4. Global index of Collocation (GIC) for the eggs of anchovy, round sardinella and chub mackerel in May and July 2010.

Month	Species	GIC
May	anchovy - round sardinella	0.795
July	anchovy - round sardinella	0.995
July	chub mackerel - anchovy	0. 828
July	chub mackerel - round sardinella	0.844

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in relative abundance), while a third patch (with 11% relative abundance) was positioned at the offshore stations north of Lemnos island, an area that is influenced by the LIS. Two more round sardinella egg patches appeared in July, in addition to the one east of Lemnos island (with 34% relative abundance). A second patch was found in the coastal area of the Thracian Sea shelf at the periphery of the gyre (with 49% relative abundance), while a third patch was found at the coastal area southwest of Lemnos island (with 14% relative abundance). In May, it was clear that, for both anchovy and round sardinella, the patch with the highest relative abundance was the one east of Lemnos island, whereas in July the patch with the highest relative abundance formed over the Thracian Sea shelf. Finally, chub mackerel had two patches: one permanent patch east of Lemnos (55% relative abundance) and the other one located within the SG close to the shelf break of the Thracian Sea (34% relative abundance).

Discussion

In the present study, we investigated the temporal and spatial spawning patterns of three co-occurring summer-spawning pelagic species. We assessed their association with specific hydrological features, in an effort to detect similarities in the selection of peak spawning areas/periods and to test the usefulness of spatial indicators in capturing such patterns when using abundance data of newly spawned eggs.

The timing, location and intensity of spawning in fishes is believed to be adapted to the characteristics of the annual cycle of biological production and the existing circulation features, so that eggs and larvae develop and grow within an oceanographic and trophodynamic domain that favours offspring growth and survival (Houde, 2009; Lowerre-Barbieri et al., 2011). The similarities or differences in the response of different species to the pelagic environment, i.e. the timing or location of spawning, reflect similar or contrasting adaptations to a common environmental setting (Somarakis et al., 2000; Houde, 2009). In the Aegean Sea, spring to early summer is a period of high zooplankton abundance and many fish species synchronize the initiation of their annual spawning with this productive season (Tsikliras et al., 2010). This is reflected in the richness of the ichthyoplankton community during early summer, as reported by many studies conducted in the Greek Seas (Somarakis et al., 2000, 2002; Isari et al., 2008; Somarakis et al., 2011a, b).

In terms of average values, we recorded a significant increase in egg abundance from spring (May) to mid-summer (July) for both anchovy and round sardinella. Concerning chub mackerel, eggs of this species were only caught in the July survey. Past studies in the North Aegean Sea reported the presence of chub mackerel larvae in June (Somarakis et al., 2000, 2002; Isari et al., 2008). According to Cengiz (2012), chub mackerel spawn between June and August in the NEA. This delay in the onset of the spawning period of chub mackerel when compared to anchovy and round sardinella could be attributed to the chub mackerel's higher temperature threshold for the initiation of the annual spawning period (Somarakis et al., 2011a). Finally, in July 2010, the abundance of anchovy eggs was markedly higher when compared to the other two pelagic species. This difference could be attributed to differences in spawners' biomass and/or in other reproductive parameters, such as the daily specific fecundity, which is particularly high for anchovy in the NEA (Somarakis et al., 2012).

Our study area is largely influenced by the BSW that enters into the NEA from the Dardanelles Strait, generating the LF south of Lemnos and supplying the SG in the north. The general pattern of horizontal hydrological structure observed in our surveys was in agreement with previously published circulation patterns (Somarakis et al., 2002; Zervakis & Georgopoulos, 2002; Isari et al., 2006, 2007; Somarakis & Nikolioudakis, 2007). The outflow of BSW into the NEA and the associated LF and SG, which are permanent features in the area during the thermally stratified period, induce high biological heterogeneity and have a strong influence on mesozooplankton distribution and abundance (Isari et al., 2006, 2007, 2011; Siokou-Frangou et al., 2009). The BSW is rich in organic material and positively affects planktonic productivity in the area (Siokou-Frangou et al., 2002, 2009; Giannakourou et al., 2014; Souvermezoglou et al., 2014). Furthermore, river outflows enrich the northern coastal area of NEA with nutrients, particularly inorganic nitrogen and phosphorus, which enhances productivity along the coast (Tsiaras et al., 2014).

During May 2010, integrated as well as surface values of fluorescence were exceptionally high over the Thracian shelf, particularly within the low salinity BSW entrapped in the SG (Fig. 2, 3). In addition, these high fluorescence waters held high amounts of mucilage, which was even obvious to the naked eye and likely the reason for the difference in the spawning intensity of anchovy in the Thracian Sea (the area occupied by the SG) between late spring and mid-summer. Mucilage formation in the North Aegean Sea between the winter and spring of 2009-2010 negatively affected the breeding success of the European shag in 2010 (Thanou, 2013 and references therein). Furthermore, there were many anecdotal reports from local fishermen that this phenomenon impeded their fishing operations. The phenomenon of massive blooms of certain phytoplankton species and the formation of mucilage was observed in the Adriatic Sea during the 1980s, leading to unfavourable conditions for anchovy spawning. In particular the anchovy spawning population exhibited low spawning intensity and avoided the traditional spawning grounds in the northern Adriatic because of the high concentration of mucous (Dulčić, 1995; Regner, 1996). Similarly, unfavourable conditions in the study area during May 2010 might have led the anchovy to avoid the main SG-associated spawning ground, limiting its activity in the coastal area (inshore patch) and the Lemnos plateau.

The temporal and spatial changes in the spawning distribution of the three species was further investigated in this study through a suite of spatial indicators that were

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recently developed to describe the aggregation and spatial occupation patterns of fish populations (Bez & Rivoirard, 2000a; Woillez et al., 2009). The complex topography and bathymetry of the NEA, with a deep trench separating the two extended continental shelves, allowed us to evaluate the suitability of the given spatial indicators to efficiently capture existing spawning spatial patterns. Certain spatial indicators revealed spatial patterns and underlined the importance of local hydrographic structures for spawning. During the early spawning period, the positions of the CG of anchovy and round sardinella eggs were located north and east of Lemnos island, respectively. As the spawning season came to its peak in July, eggs of both species became more abundant and increased their area of presence (PA), extending to the Thracian shelf. As a result, the position of the CGs shifted northwards as compared to late spring. Moreover, during the peak of spawning, the CG of anchovy and round sardinella eggs were located close to each other and in close association with the SG. The location of the CG for chub mackerel, however, was more south; this result was primarily due to the absence of major patches of chub mackerel eggs at the coastal area of the Thracian Sea, and the apparent association of spawning with LF and SG. However, the CG usually fell in the offshore pelagic area, where no major egg patches were found, implying that CG does not constitute an appropriate indicator to describe the spawning distributions in areas with such complex topography and bathymetry. In this case, the high inertia (I) values were more informative, supporting the increased aggregation of high egg concentrations away from the CG. The GIC is also dependent on the position of CGs of the different species and as a result was not as useful as expected. Even though all species had a high degree of overlap according to GIC, during both periods, this overlap was not reflected in other indices like the occupation (PA) and aggregation (SA) area (see below). The isotropy index (I_s) was also less informative. For example, similar values were obtained for round sardinella both in the case of a single centre of spawning (in May) as well as two distant centres of spawning, i.e. the continental shelves of the Thracian Sea (north) and Lemnos island (south) in July.

The level of aggregation of anchovy and round sardinella eggs did not change between the two periods, as the spreading area (SA) took values smaller (roughly 50% smaller) than the occupation area (PA), indicating that half of that area was occupied by high egg abundances. Therefore, a comparison of these two indices (PA, SA) provides information on the degree of patchiness in the spawning distribution of adults. The different aggregation patterns as well as changes in location of major egg patches between early and peak spawning reflected the changing priorities of adult fish when selecting spawning sites.

The number and position of the major patches proved particularly useful in identifying hot spots of egg abundance and in revealing the circumstances in which spawning activity related to specific hydrographic structures. The location of major egg patches revealed both consistent (independent of spawning time or species) and contrasting spawning centres. Specifically, a permanent patch of eggs was located east of Lemnos; this patch was common for all three species during both late-spring and mid-summer. As there was no significant difference in temperature between the Lemnos plateau and the Thracian shelf, this patch was clearly associated with the outflow of BSW and the adjacent frontal area. The LF is generally characterized by high primary and secondary productivity (Isari et al., 2006; Zervoudaki et al., 2006; Siokou-Frangou et al., 2009). As such, enriched BSW entering from the Dardanelles Strait enhances the local productivity. The strong salinity front between the BSW and the Aegean water acts as a convergence zone for mesozooplankton (Isari et al., 2006, 2007, 2011) and ichthyoplankton, limiting their dispersal of larvae southward in the high salinity oligotrophic waters.

A contrasting pattern in spawning site selection appeared for another important oceanographic feature of the study area, the SG. During late-spring, no major egg patch was associated with SG, with anchovy spawning occurring primarily in the coastal area of the Thracian shelf. As previously discussed, the low intensity of spawning in the SG area during May 2010 was most likely related with the presence of mucilage and the associated unfavourable conditions. However, in mid-summer, during the peak of spawning, the first major anchovy egg patch was clearly associated with the SG and an additional anchovy major egg patch was defined offshore, north of Lemnos. The latter was most likely formed from the advection of eggs spawned east of Lemnos by the Lemnos-Imvros Jet (Somarakis et al., 2002), the strong current that entrains outflowing BSW northwards and partly feeds the SG. Indeed, the acoustic surveys carried out in the area have never recorded the presence of anchovy schools in the deep basin north of Lemnos (Somarakis et al., 2012). Chub mackerel, whose eggs were collected mid-summer, were also associated with the SG. The SG anticyclone acts as a retention area, exhibiting high concentrations of mesozooplankton (Isari et al., 2006, 2007, 2011) and ichthyoplankton (Somarakis & Nikolioudakis, 2007; Schismenou et al., 2008). It exhibits characteristics that conform with the 'ocean triad', i.e. enrichment (from BSW), concentration and retention (Bakun, 1996; Agostini & Bakun, 2002).

Contrary to the other species, round sardinella did not select the SG for spawning during mid-summer. It appeared to rather prefer the more productive (in terms of integrated fluorescence) area east of the Thasos island and the coastal waters of the Thracian Sea. This species is known to prefer coastal and productive waters for spawning (Ettahiri et al., 2003; Sabatés et al., 2008, 2009; Zeeberg et al., 2008). Earlier studies have also found that the anchovy and round sardinella spawning grounds are spatially segregated in the North Aegean Sea (Somarakis et al., 2002; Schismenou et al., 2008) and other parts of the Mediterranean Sea (Palomera & Sabatés, 1990; Palomera et al., 2007; Sabatés et al., 2013), with round sardinella's spawning habitat being more coastal than that of anchovy. The spatial separation of the main spawning ground of round sardinella in the Thracian Sea from that of the

other two species would likely reduce inter-specific competition during the peak spawning time in summer.

As shown in the present study, the objective definition of major egg patches is particularly useful in linking spawning activity with local hydrography. The levels of aggregation and occupation of space are adequately described by the indices of *PA*, *EA* and *SA*. Conclusively, many of the indices applied in this case study are potentially important tools for studying the use of space by spawning aggregations (Spedicato *et al.*, 2007; Woillez *et al.*, 2007). For this purpose, the application of spatial indices to ichthyoplankton data should be restricted to the egg stage, which is least affected by physical dispersal. Defining locations which are important for fish spawning could be very useful for setting marine spatial planning schemes (Micheli *et al.*, 2013).

Acknowledgements

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