

RESEARCH PAPER

Spatial and Temporal Distribution of the Egg and Larval Stages of the European Anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) in Relation to Environmental Conditions in the South-Eastern Black Sea

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Abstract

Stock assessments are one of the most important stages of fisheries. The study focused on whether this region was a spawning area for anchovy stocks by investigating the abundance of anchovy eggs and larvae in the Southeastern Black Sea. During the 6-month vertical sampling process, a total of 2406 anchovy eggs, including 957 live eggs and 1449 dead eggs while a total of 502 anchovy larvae were also sampled during this process. In the study area, the mean densities were 237.38 ± 141.67 eggs/m² and 49.20 ± 28.97 larvae/m². No anchovy eggs or larvae were encountered during the first ichthyoplankton survey in May. During the sampling period, the most productive month in terms of eggs and larvae was determined as August (591.8 ± 587.99 eggs/m²; 147.5 ± 132.74 larvae/m²), and the highest sea water values were measured for surface water temperature (25.47°C), chlorophyll-a (2.02 µg/L), salinity (18.53‰) and dissolved oxygen (7.94 mg/L). Considering the results obtained, it was concluded that the Southeastern Black Sea is an important breeding ground for anchovy and that the abundance of eggs and larvae increased with rising temperature and nutrient levels.

Introduction

Ichthyoplanktonology is the branch of science that studies fish eggs and larvae. The European anchovy, a planktivorous species found mostly on the continental shelf, is reported to range from the south of the Norwegian coast to South Africa, the Mediterranean, the Sea of Azov and the Black Sea (Raybaud, et.al., 2017; Karataş et. al. 2021).

The Black Sea is one of the most severely destroyed and exploited major marine ecosystems in the world (Zeitsev et al., 2002; Bat et al., 2007; Oğuz, 2017). At the same time, it is always at the forefront of the production of natural resources in the fishing sector in Türkiye. Anchovy always ranks first among the species produced

by fishing in the Black Sea. Moreover, as pelagic, it plays a major role in fishing as well as in the food web of the Black Sea (Daskalov, 2002; Oğuz et al., 2008; Akoğlu et al., 2014; Gücü et al., 2016). In this context, for the sustainability of the anchovy population and its fisheries in the Black Sea, the life cycle of the anchovy population must be known very well.

Research carried out on the anchovy population in the Black Sea; bioecological characteristics (Özdamar, 1991; Kayalı, 1998), reproductive biology (Lisovenko et al., 1996), population and estimation stock abundance by analytical methods (Mutlu, 1996; Mutlu, 2000), recent variations in the population structure age, growth, and mortality rates (Samsun, et al., 2004; Samsun, et al., 2006; Şahin et al., 2008; Sağlam and

Sağlam, 2013), Black Sea anchovy and its fishery (Gücü et al., 2016), population characteristics and stock assessment of European anchovy (Çiloğlu and Şahin, 2022), monthly microplastics change in European anchovy's (Gedik et al., 2023), first maturity size of anchovy and its implications on stock assessment models (Akkuş and Gücü, 2021) studies such as can be listed.

Studies on the effects of egg, larval distribution and environmental conditions in the Black Sea. (Arım, 1957; Demir, 1959; Neirmann et al., 1994; Kideyş et al., 1999; Satılmış et al., 2003; Şahin, and Hacımurtazaoğlu, 2013; Gücü et al., 2017) are quite limited.

In recent years, climate change-driven alterations in environmental conditions have significantly impacted marine habitats in the Black Sea (Tokarev and Shulman, 2007; Bat et al., 2007; Zaitsev et al., 1992). These changes are believed to influence the early life stages of marine organisms, including the reproductive strategies, spatial-temporal distribution of eggs and larvae, and recruitment success. Despite existing studies, there remains a lack of updated and localized data on the ichthyoplankton distribution of European anchovy in the southeastern Black Sea. This study aims to fill this gap by investigating the influence of key environmental factors on the spatial and temporal patterns of anchovy eggs and larvae.

Materials and Methods

This study was carried out monthly at 4 main stations (Akçaabat, Değirmendere, Yomra, and Sürmene) on the Trabzon coast of the Southeastern Black Sea between May and October 2021. Ichthyoplankton samples were carried out vertically from 50 m to 0 m (deep to surface) at a total of 16 intermediate stations determined at 1 mile, 3 miles, and 5 miles from each station (Figure 1).

The study was carried out with the SURAT-ARASTIRMA ship belonging to the Fisheries Central Research Institute. Sampling was carried out using a WP-2 plankton net with a 500 µm mesh size. After the collected samples were buffered with borax and fixed with 5% formaldehyde, species identification (Dekhnik, 1973; Russell, 1976; Yüksek & Gücü, 1994; Çoker, 2003) and counting were performed in the laboratory using a Zeiss 508 microscope. After the identification of anchovy eggs and larvae in the laboratory using morphological criteria defined by Dekhnik (1973) and Russel (1976), the eggs were classified as live, abnormal, or dead. The identification was carried out under a stereomicroscope (2x,5x magnification), based on chorion transparency and, embryonic development. Eggs with intact morphology and visible development were considered live; those with structural deformities

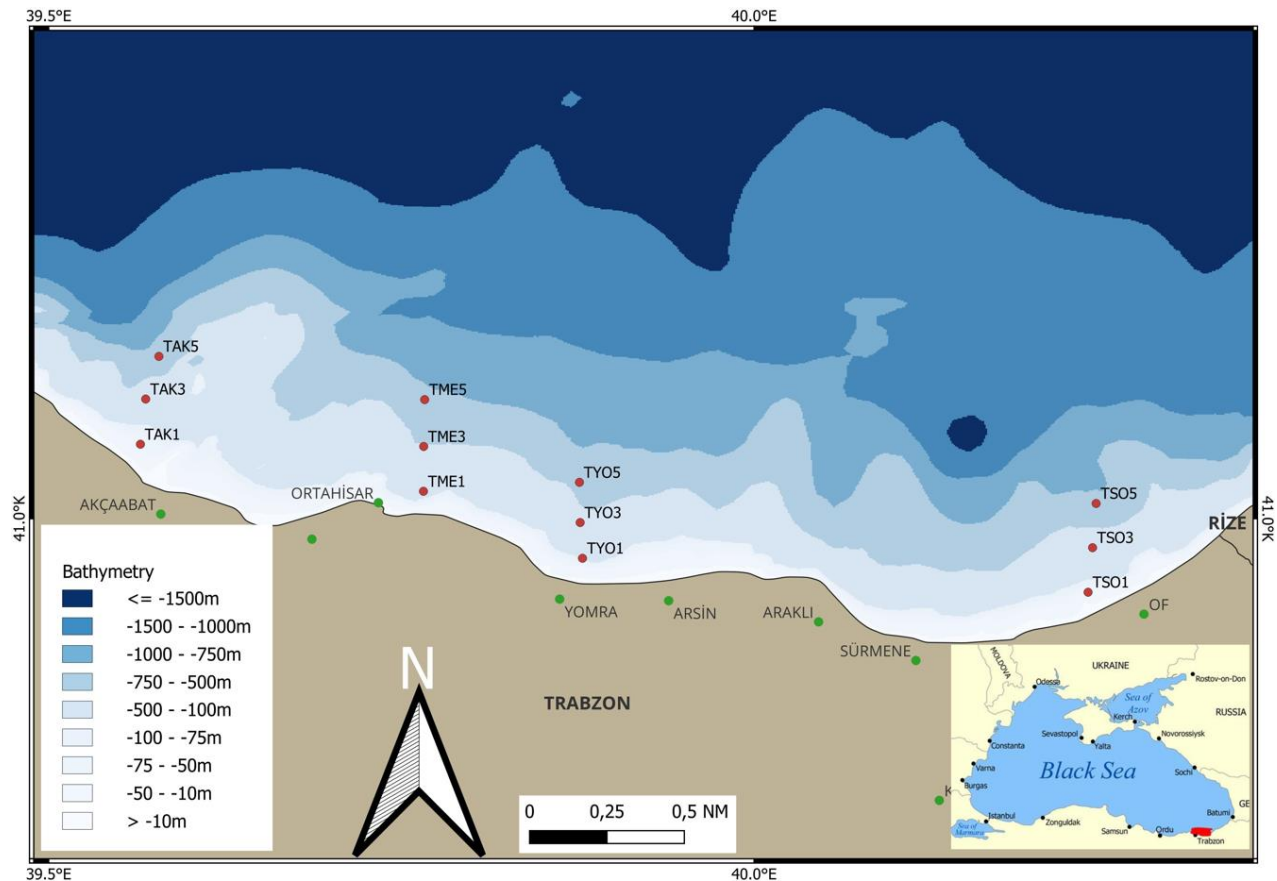


Figure 1. Stations where the study was carried out are marked in Trabzon waters.

were classified as abnormal; and opaque or degraded eggs were defined as dead. Eggs and larvae abundance and distribution were calculated as individuals (ind. m⁻²) per square meter (Smith and Richardson, 1977). Temperature, salinity, dissolved oxygen and chlorophyll-a values were measured at each station between June and October with a Seabird brand CTD device.

The distribution of sampled fish eggs and larvae was calculated as live-dead eggs and larval individuals per square meter. The following relation was used to determine the density of the species according to the stations in the vertical shots and the number of individuals per m².

$$B1 = C1 * (M/V) \quad (1)$$

Where;

B1: Abundance (individuals/m²),

C1: Total number of individuals in the vertical sampling,

M: Depth range at which sampling is done,

V: The volume of filtered water in plankton extraction (m³).

The volume of filtered water during hauls was measured using a Hydro-Bios model 438 115 flow meter. Accordingly, the volume of filtered water calculated with the formula:

$$V = \text{Number of turns} * 0.3 * A \quad (2)$$

Where;

A: the area of the plankton scoop used and calculated from the formula (Smith and Richardson, 1977; Özel, 1998):

$$A = \pi * r^2 \quad (3)$$

Mortality rate in anchovy eggs was calculated separately for live and dead at the time of collection using the following formula. Since the sampling process was carried out instantaneously, this rate does not imply

a "mortality rate" depending on time, but only reflects the instantaneous situation.

$$\text{Mortality (\%)} = (100 * \text{Dead Eggs}) / \text{Total Eggs} \quad (4)$$

In Figure 2 below, live and dead eggs of anchovy eggs taken under the microscope are shown. The first picture is a live anchovy egg; the second picture is a dead anchovy egg. They were classified and calculated in this way according to their condition when they were obtained from the sea. We can easily distinguish whether an anchovy egg is alive or dead by the transparency of the egg cell and the yolk sac. If it is transparent, it is a living egg, if it is opaque, it is a dead egg.

Changes in eggs and larvae abundance at stations and monthly were evaluated by applying One-Way ANOVA tests and cluster analysis. Monthly egg and larval abundance data were analyzed using the Bray-Curtis similarity matrix (untransformed, unstandardized) in PRIMER v6. Both hierarchical cluster analysis and non-metric Multidimensional Scaling (nMDS) were applied to assess and visualize temporal similarity patterns (Kruskal & Wish, 1978). In addition, the relationships between temperature, salinity, dissolved oxygen and chlorophyll-a with eggs and larvae distribution and abundance were tried to be determined by Redundancy Analysis (RDA) analyses.

Maps were also produced using QGIS (Quantum Geographic Information Systems) Program version 3.40.2 Bratislava.

Results

At the end of 6 months of ichthyoplankton samplings at 16 stations, a total of 2406 anchovy eggs were counted, including 1449 dead eggs and 957 live eggs. In addition, 502 anchovy larvae were obtained. No anchovy eggs or larvae were found in survey in May. The mean of 237.38 ± 141.67 eggs.m⁻² and mean of 49.20 ± 28.97 larvae.m⁻² were determined from the total eggs and larvae obtained as a result of 5-month sampling at 4 stations, 12 points. Their condition at the

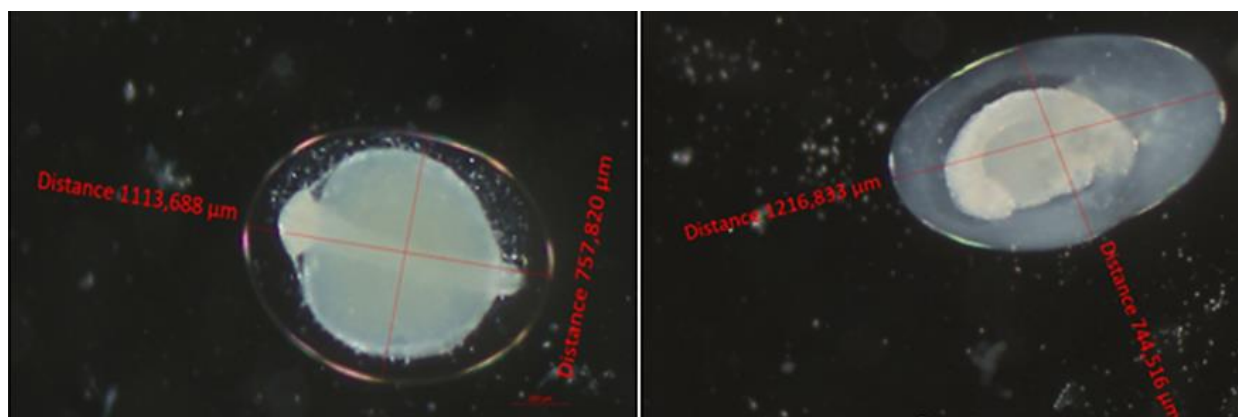


Figure 2. Anchovy (*Engraulis encrasicolus*) Egg (Live egg (A), dead egg (B)).

time of their acquisition was determined as alive or dead. The mean mortality rate in eggs obtained during the sampling period (5 months) was found to be 55.51 ± 12.64 %. Monthly mean values and statistical analyses for eggs and larvae changes are given in Table 1.

In the ichthyoplankton samplings carried out from May to October, when anchovy spawning begins in the Black Sea. August was found to be the most productive month in terms of eggs and larvae production (Table 1). 'In the statistical evaluation of the monthly eggs and larvae abundance distributions, statistically significant differences were observed in August ($P < 0.05$). Dead egg percentages in July and October were found to be statistically significant compared to other months ($P < 0.05$).

When the anchovy eggs abundance between stations was evaluated in 5-month sampling. The most productive stations were found to be (TAK1/50, TY1/50, TY3/50, TY5/50). Here. TAK1/50 station has the highest yield with a value of $2125.79 \text{ egg.m}^{-2}$ in August (Figure 3). It was found that there was no difference in eggs abundance (m^2) at stations 1 mile. 3 miles and 5 miles offshore ($P > 0.05$), but the difference was significant among stations ($F_{(df:19)} = 2.722$; $P < 0.05$). Throughout the monthly sampling period (excluding June, July, and October), larvae were detected at all stations in August and October. The most productive stations were determined as $387.45 \text{ larvae/ m}^2$ (TM1/50) and $354.66 \text{ larvae/m}^2$ (TY5/50) in August (Figure 4). As a result of the statistical analysis. It was found that there was no difference in larvae abundance (m^2) at stations 1 mile. 3 miles and 5 miles away from the coast ($P > 0.05$), but the difference between the stations was significant ($F_{(df:19)} = 3.034$; $P < 0.05$).

By similarity analyzing the temporal distribution (Bray Curtis similarity) of eggs and larvae abundances during the sampling period. It was determined that August and September were similar and separated from the other months (Figure 5A. Figure 5B).

Environmental parameters such as temperature, salinity, dissolved oxygen and chlorophyll-a were obtained with CTD equipment during the sampling period and monthly values were presented in Figure 6.

In May when the temperature was around 15°C . no eggs or larvae were found in the sampling. In June and July. both eggs and larvae were obtained. while no larvae were found in some stations. It was determined that the temperature in these months was above 20°C (Figure 6). In August and September. Eggs and larvae were found in every station. In October. eggs were found in every station. but no larvae were found in 6 stations. In October. The number of larvae was quite low in the stations where larvae were found. In August (Table 1). which is the most productive month in terms of eggs and larvae abundance. the surface water temperature was determined as 25°C (Figure 6).

The relationships between parameters such as temperature, salinity, dissolved oxygen, chlorophyll-a and the abundance of eggs and larvae were revealed by RDA analysis (Figure 7).

The effects of environmental variables. especially temperature and chlorophyll-a. on eggs and larvae abundance were found to be statistically significant ($F = 9.6$, $P = 0.002$).

Discussion

Ichthyoplankton survey is an important component in determining the spawning season. Breeding grounds and fishery-independent spawning stock (Bernal, 2012). Anchovy is the most important industrial fish species in Türkiye and its production is constantly debated due to its fluctuating decline. Due to the changes that have occurred in the Black Sea ecosystem in the last fifty years. The hypothesis that the spawning grounds of anchovy had changed has come to the fore (Neirman 1994; Kideys 1999; Gordina 2001; Gücü, 2017).

Some of the reasons for the decline in anchovy stocks and changes in spawning areas can be determined through egg survey research. In this context, in this research. Monthly eggs and larvae sampling was carried out from May to October at the stations determined from the shore to the offshore on the Trabzon coast. Although sampling started in May. The first eggs and larvae were found in June. The abundance of eggs and larvae peaks in August (Table 1).

Table 1. Monthly minimum, maximum, mean numbers of dead eggs, live eggs, total eggs, larvae and their abundances per m^2

Parameters	June	July	August	September	October
Range of dead	1-17	5-36	10-227	1-76	1-17
Mean	7.66 ± 6.01	17.66 ± 10.22	64.33 ± 68.28	24.75 ± 20.84	6.33 ± 5.24
Range of Live	0-21	2-20	12-58	2-40	2-33
Mean	6.75 ± 85.78	6.58 ± 6.15	27.16 ± 13.68	23.00 ± 13.75	16.25 ± 11.64
Range of Total	1-173	9-44	38-270	3-104	3-49
Mean	14.41 ± 11.29	24.25 ± 13.20	91.50 ± 79.81	47.75 ± 26.32	22.58 ± 15.75
Mean per m^2 s	85.5 ± 64.58^c	151.3 ± 91.40^{bc}	591.8 ± 587.99^a	267.2 ± 141.24^{ab}	123.5 ± 90.16^{bc}
Mortality (%)	56.1 ± 15.94^{ab}	72.7 ± 14.96^a	63.1 ± 14.67^{ab}	48.5 ± 20.64^b	28.8 ± 13.33^c
Total Larvae (n)	0-5	0-12	4-61	2-44	0-6
	2 ± 1.85	3.08 ± 3.47	23.75 ± 21.55	11.00 ± 11.57	2 ± 2.25
Mean per m^2 Larvae	12.6 ± 12.47^b	19.2 ± 22.25^{bc}	147.5 ± 132.74^a	62.7 ± 65.54^{ac}	10.5 ± 11.8^{bcd}

Note: Different superscript letters on the same line indicate that the difference is statistically significant ($P < 0.05$).

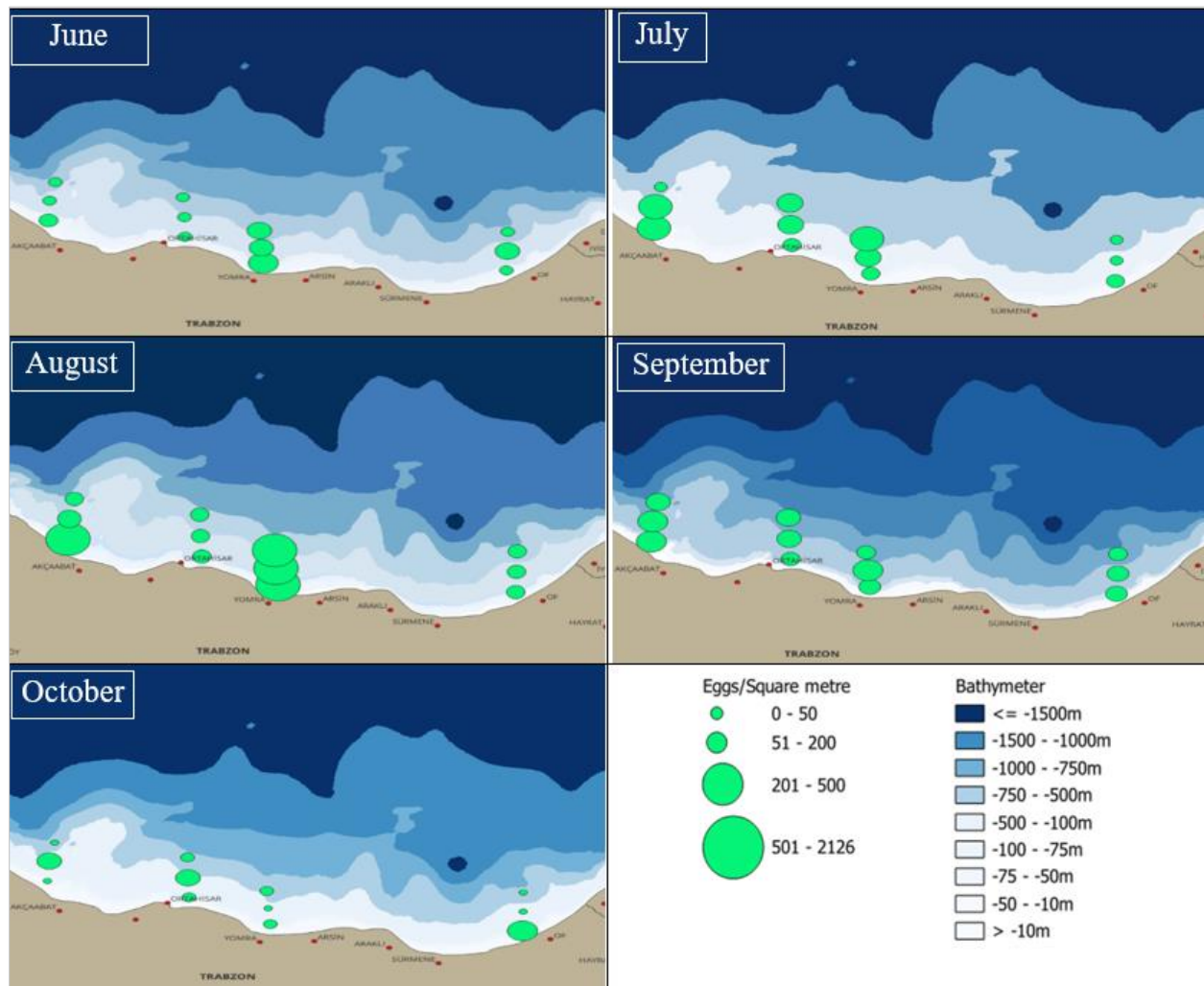


Figure 3. Monthly variation in the density of the European anchovy eggs in the South-Eastern Black Sea.

Indicating that the majority of the spawning stock sheds eggs in this month. In the statistical evaluation (Table 1). The fact that August differed from other months in terms of egg abundance, and August and September differed from other months in terms of larva abundance supports the conclusion that the egg shedding of the anchovy spawning stock in the Southern Black Sea reached its peak in the year the study was conducted. Although eggs and larvae were found until October. There is a significant decrease in abundance. Considering the eggs and larvae productivity at the stations. It is seen that there are monthly differences in Figure 3 and Figure 4. The most productive stations are seen as Yomra (TYO1, TYO3, TYO5) and Akçaabat (TAK1, TAK3, and TAK5) stations. These stations may have been preferred by the breeding stock as a better breeding habitat than other stations. When the mortality rate in eggs was evaluated monthly. The highest was determined in July and was found to be statistically significant (Table 1). After August significant decreases were observed in mortality rates. This shows that the eggs laid by the spawning stock in September and October have a higher chance of survival. In some

studies conducted in the Black Sea. Mortality rates were determined as 96.9% by Satılmış, 2005 81.20% by Şahin et al. (2013). 50%-90.43% by Çakır, 2006 in Edremit Bay of the Aegean Sea and 12% by Daban, 2020 in the Northern Aegean Sea. These mortality rates differed from the mean value determined in the study. It can be said that these differences are due to regional differences. Sampling periods and the effects of environmental factors in that period.

Therefore, in this study carried out on the Southern Black Sea (Trabzon) coast. It was possible to say that anchovy used this region as a spawning ground. The eggs and larvae abundance results in this study support previous studies in the region (Table 2).

Considering the results of the studies in Table 2. The Southern Black Sea Region can be defined as one of the main spawning areas for anchovy. There are discussions that the Southern Black Sea is preferred as a spawning ground due to changes in the ecosystem of the Northwestern continental shelf of the Black Sea, which is the classical spawning ground of anchovy (Gordina et al. 2001; Gücü et al. 2001, 2016). Although anchovy makes its classical spawning migration in the

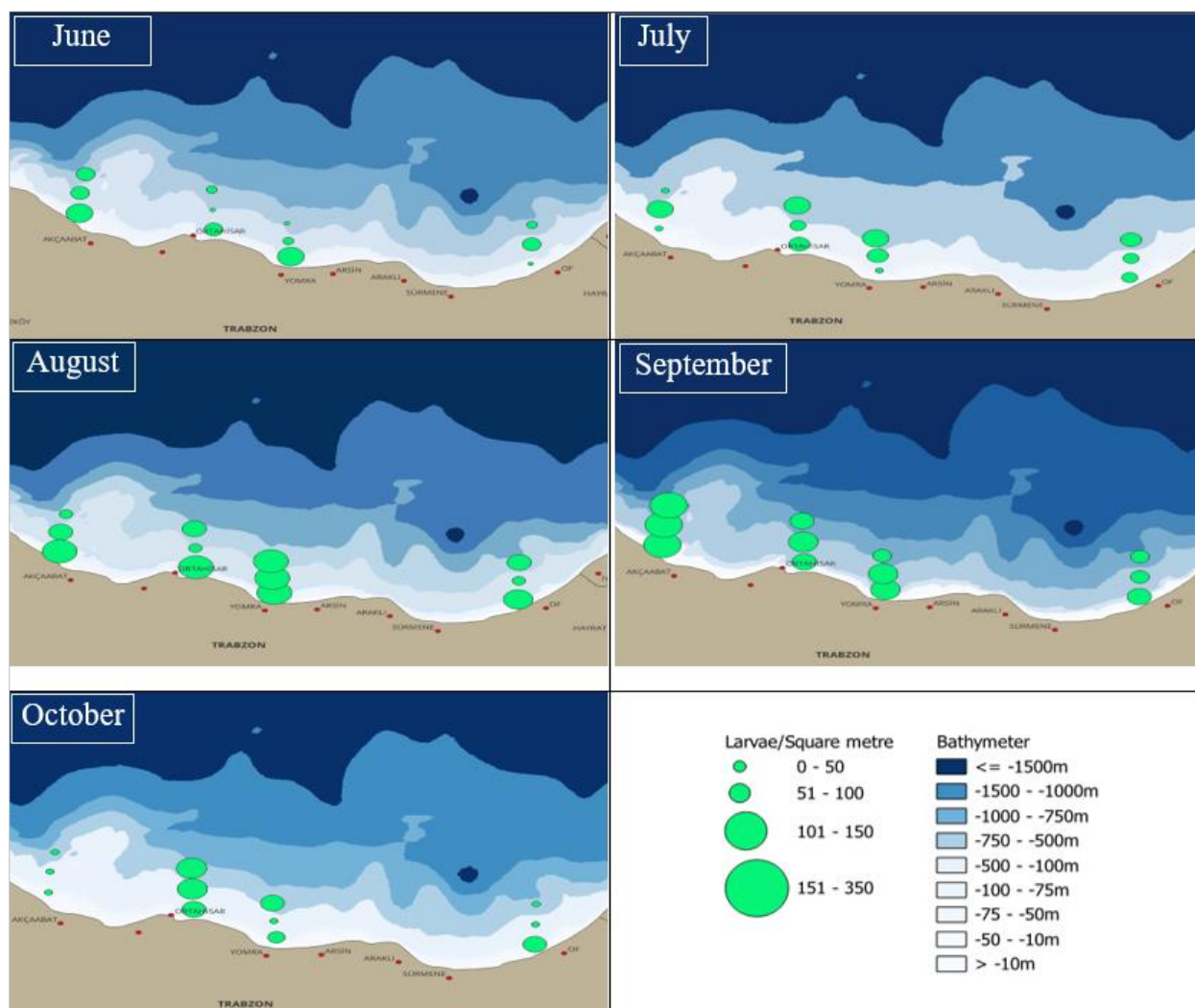


Figure 4. Monthly variation in the density of the European anchovy larvae in the South-Eastern Black Sea.

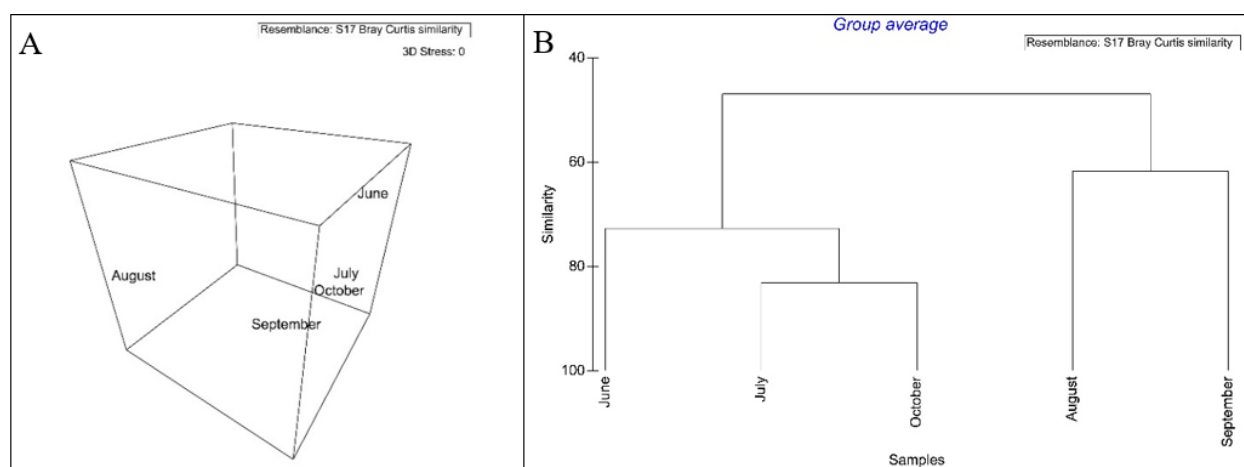


Figure 5. 3D Multidimensional scaling ordination (A) and cluster dendrogram (B) of monthly hauls of mean number of eggs and larvae.

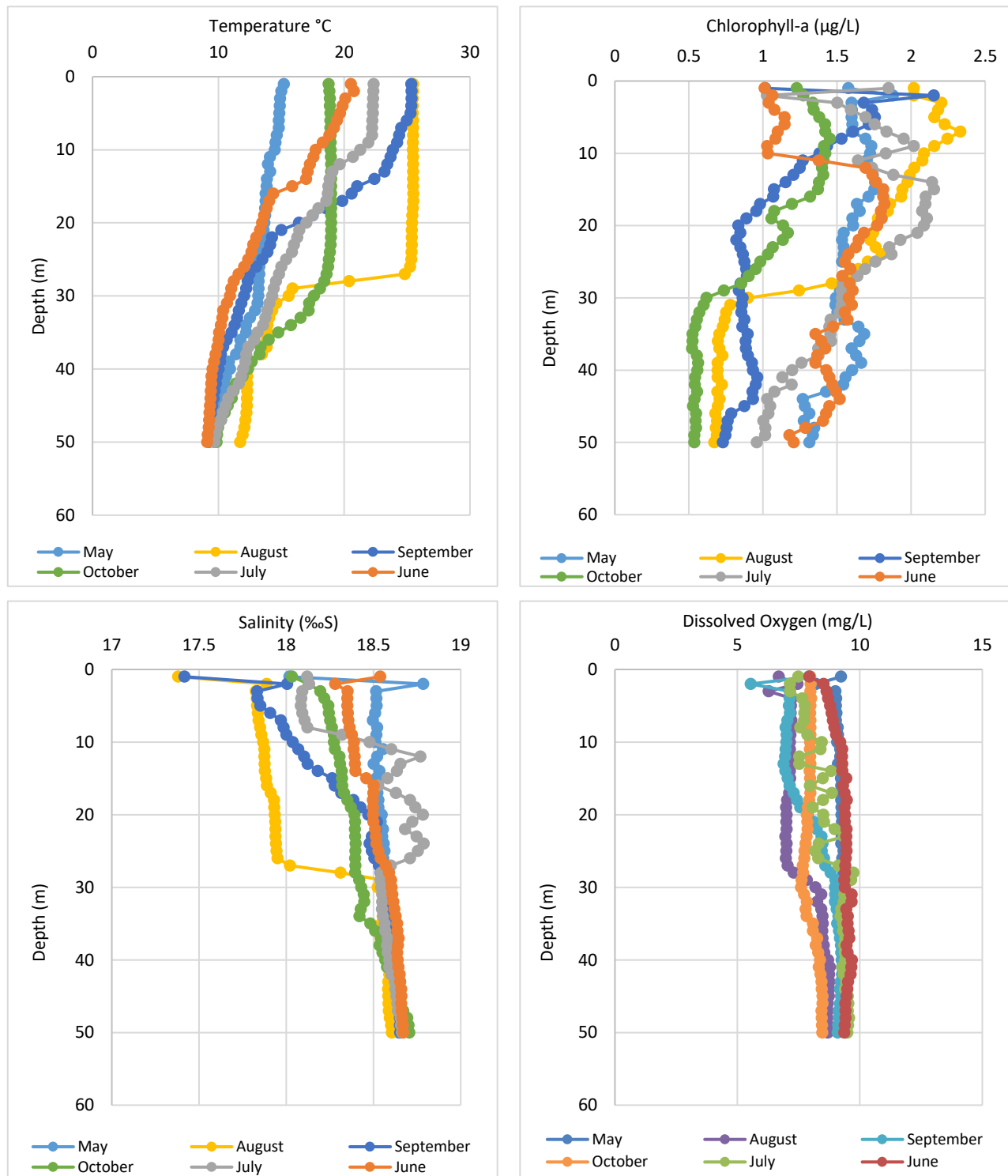


Figure 6. Monthly temperature, salinity and dissolved oxygen distributions.

Black Sea, based on this study and previous studies, it is possible to say that there are also spawning stocks that do not migrate from the Southern Black Sea (Kideys et al., 1999; Gücü et al., 2016). Considering the results of this study and previous studies. It can be said that the Southern Black Sea is an important spawning area preferred by anchovy fish.

Environmental factors play a major role in the distribution and development of eggs and larvae. Some

studies emphasize that temperature and chlorophyll are the main factors that trigger these developments (Bernal et al., 2012; Basilone, 2006; Kideys, 1999). In this study, the effects of temperature and chlorophyll-a as well as salinity and dissolved oxygen on anchovy egg and larva distribution were evaluated. As a result of the statistical analysis, it was observed that there was a positive relationship between egg and larva distributions and temperature and chlorophyll-a, while

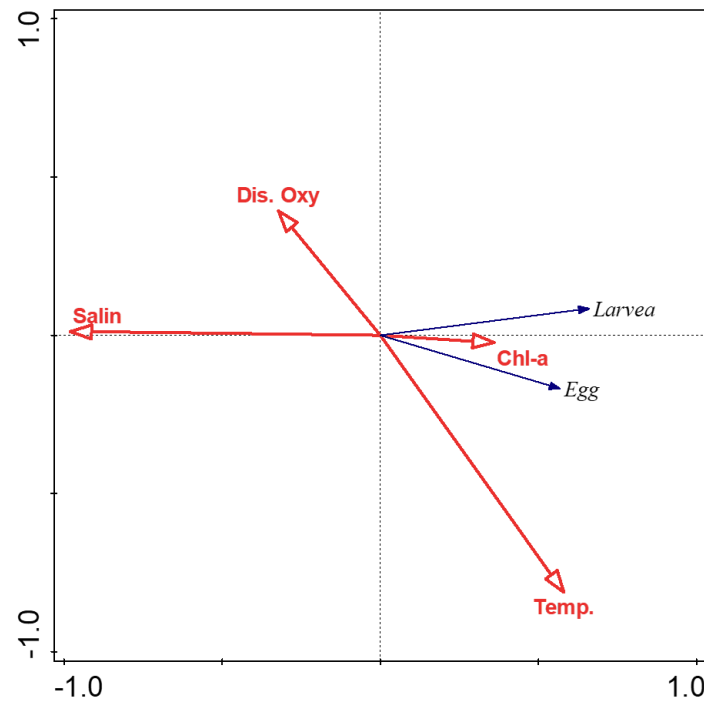


Figure 7. Redundancy Analysis (RDA) results showing relationships between eggs & larvae and CTD variables.

Table 2. Some studies on ichthyoplankton in the Black Sea and some other seas

Authors	Egg abundance (m ² or m ³)	Larva (m ² or m ³)	Regions
Neirmann, et al. 1994	18-991 (ind/100 m ³)	0-110 adet (ind/100 m ³)	Black Sea
Kideys, et al. 1999 (1992,1993,1996 years)	0-1167 (ind/m ²) 0-718 (ind/m ²) 0-577 (ind/m ²)	0-55 (ind/m ²) 0-39 (ind/m ²) 0-44 (ind/m ²)	Southern Black Sea
Satılmış, et al. 2003	2,86- 39,84 (ind/m ²)	2,5 (ind/m ²)	Black Sea (Sinop)
Gordina et al. 2001	10,2- 31,6 (ind/m ²) 68,7 (ind/m ²)	0,7-1,2 (ind/m ²) 1,9 (ind/m ²)	Black Sea (Ukrainian) Black Sea (Türkiye)
Zarrad et al. 2006	1038-1255 (ind/10m ²)	16-445 (ind/ 10m ²)	Mediterranean Sea (Gulf of Tunis)
Şahin, et al. 2013	0,97-2272,29 (ind/m ³)	0,33-37,84 (ind/m ³)	Black Sea (Trabzon-Rize)
Gücü, et al. 2016	60-3051 (ind/m ²)	3-359 (ind/m ²)	Southern Black Sea
Zarrad, et al. 2018 (2005,2009 years)	1189,6±3958(ind/10m ²) 352,2±1675(ind/10m ²)	230,3±1139(ind/10m ²) 13,4±52(ind/10m ²)	Mediterranean Sea (Gulf of Gabe's)
Şahin, et al. 2019	2,2-1935,1 (ind/100m ³)	69,9-180,3 (ind/m ³)	Southern Black Sea (Giresun)
Klimova, et al. 2024	3,4-290 (ind/m ²)	3,6-90 (ind/m ²)	Azov Sea and Northern Black Sea (Caucasus)
Zorica et al. 2018	2,61-1040,24 (ind/m ²)	2,73-611,14 (ind/m ²)	Adriatic Sea
Casaucio et al. 2021	(16,2-87.9 (ind/m ³)	-	Atlantic
This research	5,60-2125,79 (ind/m ²) 237,38±141,67(ind/m ²)	0-,387,45 (ind/m ²) 49,20±28,97 (ind/m ²)	Southern Black Sea (Trabzon)

there was a negative relationship with salinity and dissolved oxygen. This means that with the increase in temperature and food the abundance of eggs, the abundance of larvae and the survival rate of larvae increase. Studies conducted in the Black Sea emphasized that the spawning temperature range of anchovy is between (14-26°C) and the spawning productivity and the survival rate of larvae peak between 20-22.5°C, but the productivity decreases when the temperature drops below 20°C (Nierrmann et al., 1994; Kideys at. al., 1999; Şahin & Hacimurtezaoğlu, 2013). In studies conducted in the Western Mediterranean. Northwestern

Mediterranean and Adriatic Sea. Although the spawning temperature ranges of anchovy vary, it has been reported that it is between 15-28°C and the most productive temperature is between 19-23°C (Motos, 1996; Garcia and Palomera, 1996; Palomera et al., 2007; Zorica et al., 2018).

Even though, the spawning temperature (21-26 °C) and chlorophyll-a (1.01-2.02 µg/L) ranges in the field where the research was carried out are within the ranges determined for the Black Sea in the literature, the most productive temperature for egg and larva productivity (25.47 °C) is above the value determined in

the literature, indicating the tolerance of anchovy to temperature. Temporal changes in temperature also affect the spawning period (Basilone et. al., 2006; Palomera et. al., 2007). In some studies, conducted in the Black Sea in recent years, it has been stated that there has been an increase in water temperatures and that this situation affects the dynamics of biotic and abiotic structures in the ecosystem (Ağırbaş et. Al., 2010). In the study, the spawning period was determined as June-October, while in some studies conducted in the region it was determined as April–September (Satılmış 2005), June–August (Şahin & Hacimurtezaoğlu, 2013). It can be said that the differences are due to the temperature differences of the periods of the studies.

Conclusion

This study confirms that the Southeastern Black Sea coast functions as a significant spawning ground for *Engraulis encrasicolus*, based on the spatial and temporal distribution patterns of eggs and larvae. These findings are consistent with previous ichthyoplankton surveys conducted in the region. To support the long-term sustainability of anchovy stocks, future management strategies should incorporate assessments of daily spawning yield, identification of active spawning stocks, and estimations of recruitment success within spawning areas. Such biological metrics are critical for effective fisheries regulation. Although temperature and chlorophyll-a were revealed as the main environmental factors affecting the egg abundance, larval abundance, distribution and survival rates of anchovy in the early life period in the study, other dynamics of environmental factors affecting early life should also be taken into account in future studies.

It is known that the climatic changes occurring in the world also negatively affect the Black Sea ecosystem. Effective management plans must incorporate scientific insights into how climate-induced changes affect the embryonic and larval stages of marine organisms.

Ethical Statement

Not applicable.

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Author Contribution

Tugba Kaya: Conceptualization, Writing -review and editin Investigation, Methodology, Visualization and Writing -original draft; Cemalettin Sahin: Supervision;

visualization and writing, Yusuf Ceylan: Statistically analyses, editing, review Ahmet Sahin: Species identification; Writing review and editing; Muhammet Emanet: Collecting Data; Tuğberk Can: Data Editing and review; Recep Parlak: Writing-review and editing.

Conflict of Interest

Not applicable.

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